Atlas of Genetics and Cytogenetics in Oncology and Haematology

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Atlas of Genetics and Cytogenetics in Oncology and Haematology

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Scope

The **Atlas of Genetics and Cytogenetics in Oncology and Haematology** is a peer reviewed on-line journal in open access, devoted to genes, cytogenetics, and clinical entities in cancer, and cancer-prone diseases.
It presents structured review articles ("cards") on genes, leukaemias, solid tumours, cancer-prone diseases, more traditional review articles on these and also on surrounding topics ("deep insights"), case reports in hematology, and educational items in the various related topics for students in Medicine and in Sciences.

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OPEN ACCESS JOURNAL AT INIST-CNRS

Editor

Jean-Loup Huret
(Poitiers, France)

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Atlas of Genetics and Cytogenetics in Oncology and Haematology

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Volume 14, Number 6, June 2010

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Gene Section

Mini Review

ABCB5 (ATP-binding cassette, sub-family B (MDR/TAP), member 5)

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DOI: 10.4267/2042/44768

Identity

Other names: ABCB5alpha; ABCB5beta; EST422562

HGNC (Hugo): ABCB5

Location: 7p15.3

DNA/RNA

Description

The gene encompasses 108081 bp of DNA with 19 exons.

Transcription

ABCB5 encodes a 2784 bp mRNA. The coding region consists of exon 4-19, while exon 1-3 and 3' part of exon 19 are non-coding.

Protein

Description

Only 2 isoforms, ABCB5alpha and ABCB5beta have been studied so far. ABCB5 P-gp (isoform 1, also known as ABCB5beta) contains 812 amino acids (P-gp is short for "permeability glycoprotein"). ABCB5alpha contains only 131 amino acids.

Expression

ABCB5 is reported to be expressed in many different tissues, including brain, intestine, kidney, mammary gland, testis and skin. Besides, ABCB5 has a significantly higher expression level in malignant melanomas than in benign melanocytes.

Localisation

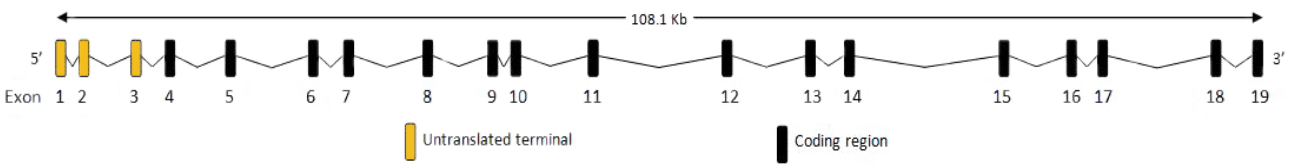
ABCB5 P-gp is located in the plasma membrane, with 5 transmembrane helices flanked by both extracellular and intracellular ATP-binding domains.

Function

ABCB5 belongs to the ATP-binding cassette (ABC) transporter superfamily of integral membrane proteins. These proteins participate in ATP-dependent transmembrane transport of structurally diverse molecules. ABCB5 mediates melanoma doxorubicin resistance via its function as a doxorubicin efflux transporter. In addition, ABCB5 P-gp can regulate progenitor cell fusion. However, ABCB5alpha alone may be non-functional.

Homology

ABCB5 shares 54% and 56% amino acid identity with ABCB1 and ABCB4, respectively.



ABCB5 gene on chromosome 7p.

Implicated in

Malignant melanoma

Note

Tissue microarray showed that primary and metastatic malignant melanomas expressed significantly more ABCB5 protein than benign melanocytic nevi, thick primary melanomas more than thin primary melanomas, and melanomas metastatic to lymph nodes more than primary lesions. Melanoma cell subpopulations identified by expression of ABCB5 were enriched for human malignant-melanoma-initiating cells (MMIC). Besides, ABCB5 also mediates chemoresistance in human malignant melanoma.

Chemoresistance in human malignant melanoma

Oncogenesis

ABCB5 P-gp mediates melanoma doxorubicin resistance via its function as a doxorubicin efflux transporter.

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Atlas of Genetics and Cytogenetics in Oncology and Haematology

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Gene Section
Mini Review

CCRK (cell cycle related kinase)

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Identity

Other names: CDCH; p42; P42; EC 2.7.11.22; PNQALRE

HGNC (Hugo): CCRK

Location: 9q22.1

Local order: 235kb telomeric to cathepsin L1 (CTSL1).

DNA/RNA

Description

Human CCRK gene spans around 8.3kb of genomic DNA on the chromosome 9q22.2 in telomere-to-centromere orientation. This gene locates within the locus tag RP11-350E12.2. A block of hypermethylated CpGs has been identified in the CCRK promoter and is associated with its high expression in adult human brain cortex (Farcas et al., 2009).

Transcription

Four alternative spliced transcript variants of CCRK gene are known. The generic variant 3 (GenBank#: NM_001039803) consists of 8 exons, with the start codon on exon 1 and stop codon on exon 8. Both transcript variant 1 (GenBank#: NM_178432) and variant 2 (GenBank#: NM_012119) have had their exon 5 deleted. Variant 1 also differs from the other variants by an additional 39nt on exon 2. The cardiac splice variant (GenBank#: AY904367) lacks both the exons 5 and 6, and has truncated 5'- and 3'-untranslated regions.

Pseudogene

No pseudogenes for CCRK are known.

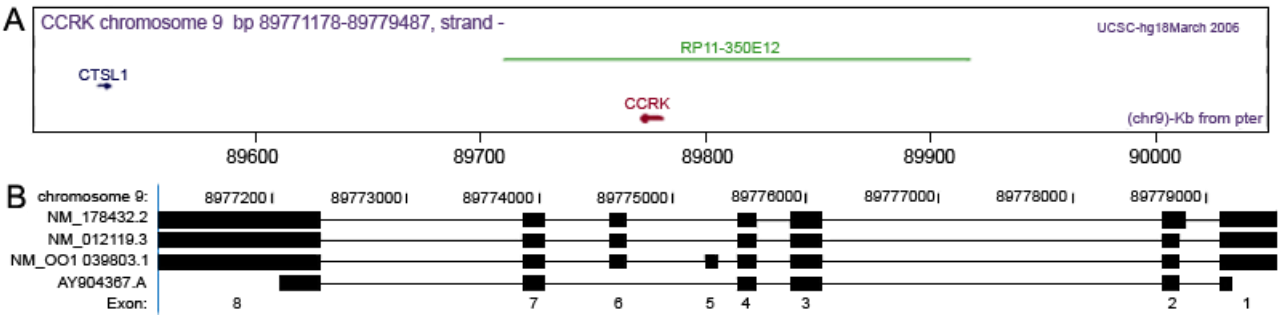
Protein

Note

There has been controversy over whether CCRK functions as a second cyclin-dependent kinase (CDK)-activating kinase (CAK) (i.e., in addition to CDK7). Inconsistent with other studies, Wohlbold and colleagues (2006) reported that monomeric CCRK has no intrinsic CAK activity.

Description

The open reading frame encodes a 346-amino acid



(A) Chromosomal location of human CCRK gene. (B) Genomic organization of four CCRK transcript variants.

protein, with molecular weight of 42kDa. CCRK protein has a protein kinase domain extending from residues 4-288, in which typical ATP-binding region and serine/threonine kinase active site can be identified. Its interacting proteins include CDK2, cyclin H and casein kinase 2.

Expression

In human tissues, the 2.2kb CCRK transcript is expressed predominantly in the brain and kidney, and to lesser extent in the liver, heart and placenta. The cardiac CCRK isoform is detectable only in heart, liver and kidney. CCRK is also widely expressed in cell lines originating from glioblastoma (U87, U118, U138, U373 and SW1088), cervical adenocarcinoma (HeLa), colorectal carcinoma (HCT116), osteogenic sarcoma (U2OS), breast adenocarcinoma (MCF-7), ovarian carcinoma (UACC-1598, UACC-326, OVCAR-3, HO-8910 and TOV-21G), lung fibroblast (WI-38), myoblast (C2C12), and lymphocyte (GM08336).

Localisation

Mainly in nucleus and perinuclear region. Relative low expression in cytoplasm.

Function

CCRK is an important regulator of G1- to S-phase transition in cell cycle and is indispensable for cell growth. It possesses CDK-activating kinase activity that is essential for the phosphorylation of CDK2 at Thr160 (Liu et al., 2004) and male germ cell-associated kinase-related kinase (MRK) at Thr157 in mammalian cells (Fu et al., 2006). CCRK also acts as a negative regulator of apoptosis and may confer cells with drug resistance (MacKeigan et al., 2005). Moreover, CCRK splice variant expressing in the heart has been shown to promote cardiac cell growth and survival (Qiu et al., 2008).

Homology

CCRK belongs to the CDK family. Among the other 10 CDK members, human CCRK shares the highest sequence identity (43%) with a well known CAK, CDK7. Orthologs of CCRK are found in orangutans, Old World monkeys, bovine, dog, boar, mouse, rat, fishes, frog, budding yeast and fission yeast.

Implicated in

Colorectal carcinoma

Note

Knockdown of CCRK inhibits HCT116 cell proliferation (Wohlbold et al., 2006). A small molecule kinase inhibitor (RGB-286147) that targets CCRK has been shown to promote HCT116 cell death in the absence of cell cycle progression (Caligiuri et al., 2005).

Glioblastoma multiforme

Note

In 14 of 19 (74%) human high-grade glioblastoma multiforme patient samples, CCRK mRNA expression levels are more than 1.5-fold higher than those of 3 normal brain tissue samples. By contrast, only 2 of 7 (29%) low-grade glioma samples have elevated CCRK expression. Knockdown of CCRK suppresses glioma tumor growth in mouse xenograft model. CCRK knockdown also inhibits glioblastoma cell proliferation via G1/S-phase arrest and reduction of CDK2 phosphorylation in vitro. Overexpression of CCRK induces malignant transformation of non-tumorigenic glioblastoma cells (U138) both in vitro and in vivo (Ng et al., 2007).

Ovarian carcinoma

Note

By CCRK immunohistochemical staining of CCRK in ovarian tissue microarray, CCRK is overexpressed in 65/122 (53%) invasive ovarian carcinoma patient samples, as compared with 22 normal ovarian surface epithelium samples. In 12 pairs of primary ovarian carcinoma and adjacent normal tissue specimens, CCRK expression is elevated in 6 (67%) ovarian carcinoma samples. Ectopic expression of CCRK promotes tumor growth in vivo and ovarian carcinoma cell proliferation in vitro via upregulation of cyclin D1 (Wu et al., 2009).

Prognosis

CCRK expression is positively correlated with ascending histological grade and advanced clinicopathologic features. It is also an independent biomarker for shortened survival time of patients with ovarian carcinoma.

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Gene Section
Review

CD151 (CD151 molecule (Raph blood group))

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Identity

Other names: CD151 antigen; GP27; MER2; PETA-3; PETA3; PETA3F; RAPH; SFA-1; SFA1; TSPAN24; Tspan-24; Tetraspanin-24

HGNC (Hugo): CD151

Location: 11p15.5

Local order: Telomere--PNPLA2--EFCAB4A--CD151--POLR2L--TSPAN4--Centromere.

DNA/RNA

Note
Information sourced from UCSC Genome Database Mar 2006 Assembly (hg18) RefSeq genes and from analysis of mouse gene organisation (Fitter et al., 1998) and human gene structure (Whittock et al., 2001).

Description

5884 bp, 9 exons (7 coding).

Transcription

mRNA 1574bp (length may vary for utr alternate splicing).

Pseudogene

None in humans.

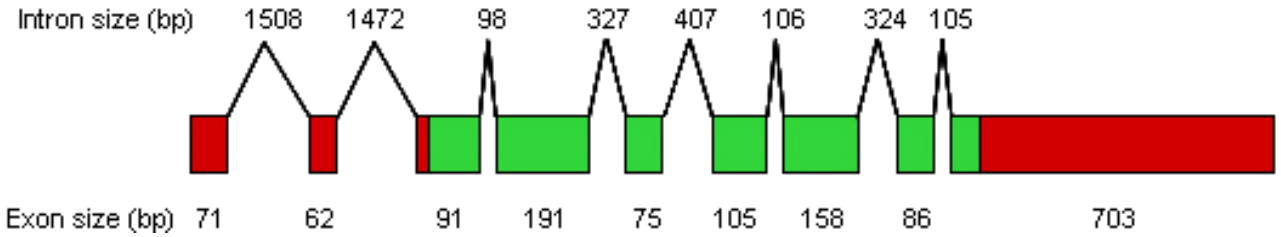
Protein

Description

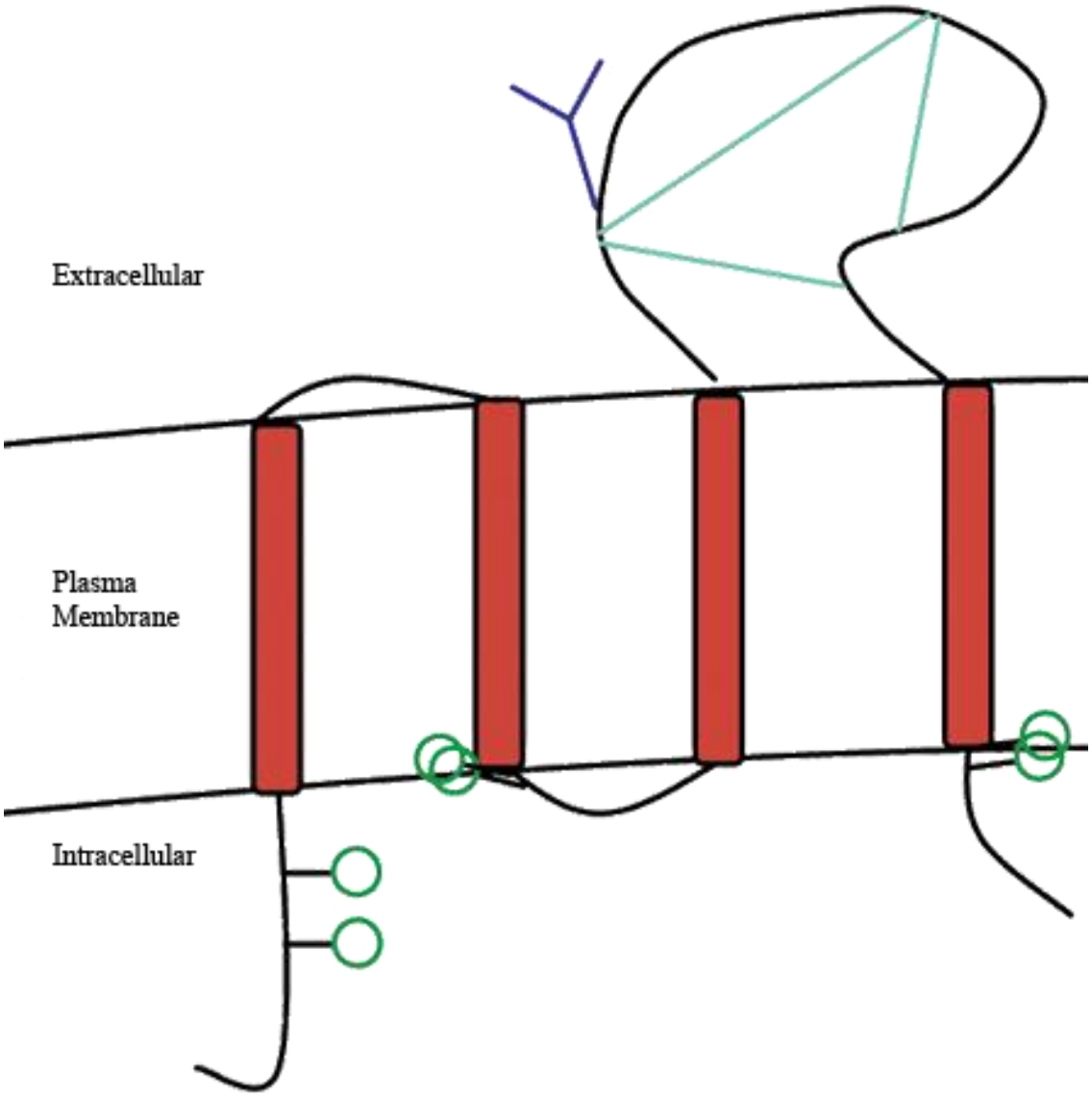
Size: 253 aa, 28247 Da with a mature protein size of 32 kDa; pI: pH 7.44.
Post-translational modifications include disulphide bridges and an N-linked glycosylation site in the large extracellular loop and 6 palmitoylation sites.

Expression

Widely expressed, particularly on epithelial cells, endothelial cells, Schwann cells, muscle cells, megakaryocytes and platelets. Tissues typically display expression restricted to these cell types with lung, kidney, spleen, tonsil and cardiac muscle all having high levels. Low expression detected on fibroblasts, erythrocytes and leukocytes (Sincock et al., 1997).
Highly expressed (mRNA) in: heart, uterus, lung, prostate, liver (adult), spleen, placenta, pancreas.
Low/no expression (mRNA) in: foetal liver, brain, testes, ovaries.



The red bars indicate utr and green bars indicate coding exons. The size of each intron is indicated at the top and each exon below. An alternate transcript may be generated from splicing out exon 2 in the 5'utr as indicated with the blue lines.



The red bars indicate transmembrane regions as predicted by TMHMM (Krogh et al., 2001), with the green circles palmitoylation sites (Berditchevski et al., 2002). The blue Y indicates an N-linked glycosylation site (Fitter et al., 1995) and the light blue lines indicate approximate sites of potential di-sulphide bridges (Seigneuret et al., 2001).

Localisation

Plasma membrane, endosomes, endothelial cell junctions and hemidesmosomes in basal epithelial cells (Sincock et al., 1999; Sterk et al., 2000).

Function

CD151 is a major component of tetraspanin enriched microdomains, which are platforms for assembly of membrane signalling complexes (Hemler et al., 2005; Charrin et al., 2009). CD151 functions in signal transduction through forming direct complexes with integrins particularly alpha3beta1, alpha6beta1, alpha6beta4 and alphaIIbbeta3, thereby influencing a variety of cell functions including motility and adhesion which are outlined further below. CD151 also affects matrix metalloproteinase activity, with overexpression of CD151 in human melanoma cells resulting in increased expression of MMP9 (Hong et al., 2006). CD151 has been shown to interact with pro-matrix metalloptroteinase 7 in osteoarthritic cartilage and regulate its activity (Fujita et al., 2006). In endothelial cells CD151 associates with the matrix metalloproteinase MT1-MMP and regulates its collagenolytic activity (Yañez-Mó et al., 2008).

Homology

Tetraspanin protein family. This protein family has 33 members in humans and is well conserved throughout vertebrates and also present in invertebrates. Key characteristics include the presence of 4 transmembrane domains with both N- and C-terminals in the cytoplasm, conserved cysteine-containing motifs and disulphide bonds in the large extra cellular loop and charged residues in the transmembrane domains.

Mutations

Note
Only 3 mutations have been identified in humans to date, two (G533A and C511T), are predicted not to significantly alter CD151 function and are not associated with disease (Karamatic Crew et al., 2004; Karamatic Crew et al., 2008).

Germinal

Homozygous 1bp insertion, G383, resulting in a frameshift at Lys127 and a truncated protein at codon 140.
Homozygous G533A substitution resulting in an Arg178His mutation.
Homozygous C511T substitution resulting in an Arg171His mutation.

Implicated in

Note
In vitro studies
In vitro assays on Cd151-null keratinocytes, showed lack of migration compared to wild-type keratinocytes (Geary et al., 2008). Over-expression and knock-down studies of CD151 in various cell lines generally show that CD151 promotes migration and adhesion, however these finding are influenced by cell type and extracellular matrix components and primarily appear to be modified by the expression of the integrin alpha3beta1 (Berditchevski et al., 2002; Winterwood et al., 2006; Liu et al., 2007; Yang et al., 2008). CD151 is down-regulated by HIF-1alpha in colon cancer cells and is re-expressed upon normal oxygenation. This is proposed to allow detachment from the primary tumour and re-attachment at sites of metastasis (Chien et al., 2008).

Oncogenesis
Increased CD151 expression may lead to enhanced tumour progression and metastatic capacity based on enhanced motility, migration and adhesion of CD151 expressing cells. Antibodies to CD151 blocked in vivo metastasis in model systems (Testa et al., 1999; Zijlstra et al., 2008). Xenograft breast cancer models involving silencing of CD151 showed a delay in tumour formation (Yang et al., 2008). CD151 expression is increased in metastasis compared to primary tumour site in colon cancer (Chien et al. 2008).

Prostate cancer

Note
Immunohistochemical detection of CD151 in a prostate cancer tissue specimens had greater prognostic value than Gleason grading (Ang et al., 2004).

Prognosis
High CD151 expression was indicative of poor outcome.

Oncogenesis
High CD151 expression indicated poor survival outcome, suggesting a role for CD151 in enhancing tumourigenesis or resistance to treatment. Also refer to 'In vitro studies'.

Gingival squamous cell carcinoma

Note
Real-time PCR analysis of CD151 gene expression compared to GAPDH was analysed (Hirano et al., 2009). Assessment of protein expression by immunohistochemistry correlated with gene expression however no statistical analyses were performed on protein expression.

Prognosis
High CD151 expression was indicative of poor outcome.

Oncogenesis
High CD151 expression indicated poor survival outcome, suggesting a role for CD151 in enhancing tumourigenesis or resistance to treatment. Also refer to 'In vitro studies'.

Colon cancer

Note
Real-time PCR analysis of CD151 gene expression compared to beta-actin was analysed (Hashida et al., 2003). Assessment of protein expression by immunohistochemistry correlated with gene expression however no statistical analyses were performed on protein expression.

Prognosis
High CD151 expression was indicative of poor outcome.

Oncogenesis
High CD151 expression indicated poor survival outcome, suggesting a role for CD151 in enhancing tumourigenesis or resistance to treatment. Also refer to 'In vitro studies'.

Hepatocellular carcinoma

Note
Real-time PCR analysis of CD151 gene expression compared to GAPDH was analysed. Assessment of protein expression by immunohistochemistry and immunoblotting generally correlated with gene expression. CD151 expression was increased in hepatocellular carcinomas compared to normal liver tissues (Ke et al., 2009).
Immunohistochemical analysis of tissue microarrays identified a positive correlation between CD151 expression and aggressive histopathological factors such as vascular invasion and poor tumour differentiation. CD151 expression was also indicative of poor outcome (Ke et al., 2009).

Prognosis
High CD151 expression was indicative of poor outcome.

Oncogenesis
High CD151 expression indicated poor survival outcome, suggesting a role for CD151 in enhancing tumourigenesis or resistance to treatment. Also refer to 'In vitro studies'.

Non-small cell lung carcinoma

Note
Real-time PCR analysis of CD151 gene expression compared to beta-actin was analysed (Tokuhara et al., 2001). Assessment of protein expression by immunohistochemistry correlated with gene expression however no statistical analyses were performed on protein expression.

Prognosis
High CD151 expression was indicative of poor outcome.

Oncogenesis
High CD151 expression indicated poor survival outcome, suggesting a role for CD151 in enhancing tumourigenesis or resistance to treatment. Also refer to 'In vitro studies'.

Breast cancer

Note
Immunohistochemical analysis of CD151 expression in a cohort of invasive ductal carcinoma identified a significantly higher risk of death from breast cancer in CD151 positive tumours compared to CD151 negative tumours. CD151 expression was also positively associated with the involvement of regional lymph nodes. No associations between CD151 expression and other clinical factors including estrogen receptor status were found (Sadej et al.,2009).
Immunohistochemical analysis of CD151 in breast tissue Microarrays identified positive correlations between CD151 expression and high tumour grade as well as negativity for the estrogen receptor. No other associations were identified between CD151 expression and clinical factors (Yang et al., 2008). Associations between CD151 expression and outcome were not able to be made due to unavailability of data.

Prognosis
High CD151 expression was indicative of poor outcome.

Oncogenesis
High CD151 expression indicated poor survival outcome, suggesting a role for CD151 in enhancing tumourigenesis or resistance to treatment. Also refer to 'In vitro studies'.

Pancreatic cancer

Note
Immunohistochemical analysis of pancreatic cancer cell lines and pancreatic tumours identified high CD151 expression associated with tumours/cell lines compared to normal tissue. Tumour stroma also expressed CD151 (Geiserich et al., 2005).

Oncogenesis
Refer to 'In vitro studies'.

Neovascularisation/pathologic angiogenesis

Note
Determined from in vivo studies in Cd151-null mice and in vitro studies of Cd151-null mouse lung endothelial cells (Takeda et al., 2007). Analysis of a rat myocardial ischaemia model also showed that viral delivery of CD151 can promote neovascularisation (Zheng and Liu, 2006).

Disease
Cancer, ischaemia

Oncogenesis
Lack of Cd151 expression resulted in impaired tumour angiogenesis, suggesting that Cd151 may be involved in promoting tumour angiogenesis.

Nephropathy

Note
CD151 is expressed normally in the kidney particularly in the glomerular basement membrane (Sincock et al., 1997).

Disease
Nephropathy in humans (Karamatic Crew et al., 2004). Cd151-null mice develop progressive renal failure on the FVB/N strain but not the C57BL/6 strain (Sachs et al., 2006; Baleato et al., 2008).

Prognosis
Loss of CD151 activity leads to chronic renal failure.

Cytogenetics
Homozygous frameshift mutation causing a premature stop codon (codon 140) due to the insertion of 1bp in exon 5 of CD151 (G383).

Hybrid/Mutated gene
Resultant protein lacks the integrin binding domain and causes null expression of the CD151/MER2 antigen (Karamatic Crew et al., 2004).

Pretibial epidermolysis bullosa

Note
The Nephropathy described above is attributed to the

same mutation in CD151 and occurs in conjunction with pretibial epidermolysis bullosa and deafness (Karamatic Crew et al., 2004).
Wound repair in wild-type mice is associated with an up-regulation of Cd151 in the migrating epidermis at the wound edge (Cowin et al. 2006).

Disease
Pretibial epidermolysis bullosa in humans.
Defective wound repair in Cd151-null mice (Cowin et al. 2006; Geary et al 2008).

Cytogenetics
Homozygous frameshift mutation causing a premature stop codon (codon 140) due to the insertion of 1bp in exon 5 of CD151 (G383).

Hybrid/Mutated gene
Resultant protein lacks the integrin binding domain and causes null expression of the CD151/MER2 antigen.

Deafness

Note
This loss of function of CD151 is attributed to the same mutation in CD151 as that described above for nephropathy and pretibial epidermolysis bullosa, with all 3 disorders occurring in the same patients (Karamatic Crew et al., 2004).

Prognosis
Progressive deafness occurring by early adulthood.

Cytogenetics
Homozygous frameshift mutation causing a premature stop codon (codon 140) due to the insertion of 1bp in exon 5 of CD151 (G383).

Hybrid/Mutated gene
Resultant protein lacks the integrin binding domain and causes null expression of the CD151/MER2 antigen.

Hemostasis

Note
As assessed in Cd151-null mice, loss of Cd151
caused increased bleeding time and decreased clotting ability, suggesting endothelial and/or platelet cell functional defects. Cd151-null mice did not show any overt physiological differences unless challenged (Wright et al., 2004). Further in vitro analysis of Cd151-null platelets showed impaired functions relating to aggregation, spreading and clot retraction (Lau et al., 2004).

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Gene Section
Mini Review

CLIC4 (chloride intracellular channel 4)

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Identity

Other names: MTCLIC; P64H1; CLIC4L; H1; huH1

HGNC (Hugo): CLIC4

Location: 1p36.11

DNA/RNA

Description

CLIC4 gene comprises of 6 exons spanning a region of about 99 kb on human chromosome 1p36.

Transcription

CLIC4 gene codes for a protein of 253 amino acids length corresponding to molecular weight of about 29 kDa. No alternative isoforms of CLIC4 has been reported.

Protein

Description

CLIC4 is a putative chloride channel for intracellular organelles. The human protein consists of 253 amino acids with an N-terminal transmembrane domain and C-terminal nuclear localisation signal.

Expression

Ubiquitous and induced by p53, TNF-alpha and c-myc.

Localisation

It is localised in cytoplasm and mitochondria in primary keratinocytes and translocated to nucleus upon cellular stress.

Function

CLIC4 has been shown to regulate TGF-beta signaling. It has been shown to translocate to the nucleus in a Schnurri-2 dependent manner and nuclear CLIC4 has been shown to subsequently stabilise phospho- Smad2 and Smad3.
CLIC4 has been implicated in angiogenesis. It has been shown to be involved in acidification of vacuoles along the cell hollowing tubulogenic pathway.
CLIC4 has been shown to be expressed in myofibroblasts and inhibit motility of MEF/3T3 cells.
CLIC4 has been implicated in Myc-induced apoptosis. It was identified as a candidate gene after protein expression analysis during Myc-induced apoptosis. Myc has been shown to bind to CLIC4 promotor and activate its transcription.

CLIC4 gene consists of 6 exons. The number between the exons indicate the length in kilo bases of intervening introns.

Domain organisation of CLIC4. TM indicates transmembrane domain and NLS represents nuclear localisation signal.

Homology

CLIC1, CLIC2, CLIC3, CLIC5 and CLIC6.

Implicated in

Various cancer

Note

Expression analysis on a human tumour array has shown that CLIC4 expression is dimished in several tumour types including breast, ovary and kidney. CLIC4 expression has also been shown to be upregulated in some tumours.

In matched tissue arrays, CLIC4 was predominantly nuclear in normal epithelial tissues but not cancers. As tumours progressed CLIC4 expression became undetectable in tumour cells but increased in stromal cells.

Sequence analysis of CLIC4 cDNA of 60 human cancer cell lines (NCI60) and EST database analysis failed to reveal mutations in CLIC4 gene.

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Atlas of Genetics and Cytogenetics in Oncology and Haematology

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Gene Section
Review

CST6 (cystatin E/M)

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Identity

Other names: Cystatin-6; Cystatin-E; Cystatin M; Cystatin E/M

HGNC (Hugo): CST6

Location: 11q13.1

Local order: The human CST6 gene is located on the long arm of chromosome 11 at 11q13.1. It corresponds to a total DNA sequence of about 1,515 bp. Most other human cystatin genes (i.e., the genes for CST1 to CST9 and CST11) cluster on chromosome 20p11.

Note
Misleading annotations:
-CSTB or CSTb (is a different cystatin gene)
-Yeast CST6 (is an unrelated gene encoding a yeast transcription factor)
-Mouse cystatin E1 (mouse CRES-like)
-Mouse cystatin E2 (mouse testatin-like)

DNA/RNA

Note
The human CST6 gene is a tiny gene. Together with its basic promoter, it spans about 2,500 bp and is flanked in the 5' upstream region by an inverted, 290-bp Alu-Sx(Sq) repeat.

Description

Like most cystatin genes, the human CST6 gene is organized into three exons separated by two introns. Exon-1 is 294-bp long, contains the 5'-untranslated region (5'-UTR) and the starting ATG codon of the coding sequence. Exon-2 is 126-bp long. Exon-3 is 188-bp long, contains a TGA stop codon, the 3'-UTR as well as a typical AATAAA polyadenylation signal followed by 20 bp. Intron-1 and intron-2 are 541- and 365-bp in length, respectively.

Transcription

The human CST6 gene is transcribed into a single mRNA species of about 607 nucleotides (nt). There are no alternate transcript species. The transcript is composed of a 5'-UTR of 53 nt, a coding sequence of 447 nt, and a 3'-UTR of 107 nt. A palyndromic structure located some 360 nt downstream of the AUG initiation codon (or 26 codons upstream of the TGA stop codon) seems to be responsible for some sequence variation in that region. Indeed, several expressed sequence tags (ESTs) differ primarily if not solely in that region of the mRNA sequence.

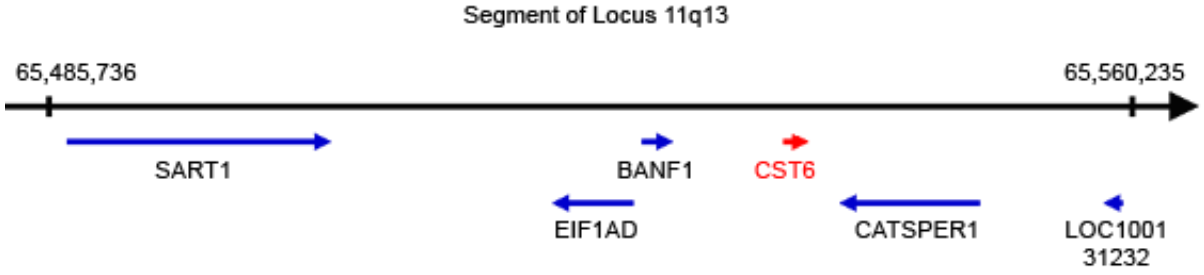

Figure 1: In the above diagram are represented the various genes flanking the human CST6 gene. More information on these genes can be found at: Entrez Gene.

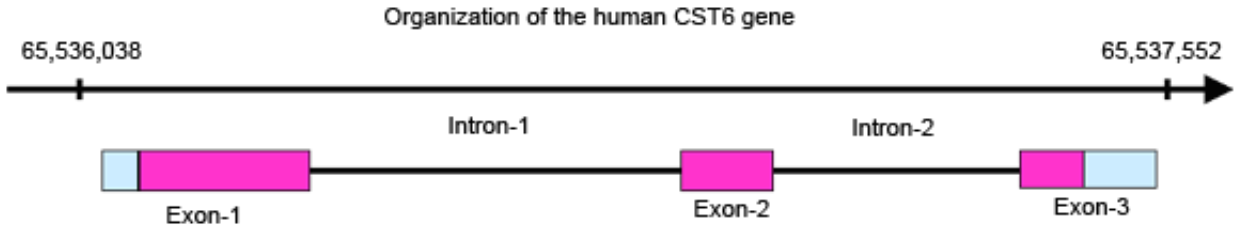

Figure 2: Structure of the human CST6 gene. Exon-1 contains the 5'-UTR (in blue) and the starting ATG codon of the coding sequence (in magenta). Exon-3 contains a TGA stop codon and the 3'-UTR (in blue). More information on the CST6 gene organization can be found at: Entrez Gene.

Transcription from the CST6 gene promoter seems to be both constitutive and regulated. Numerous potential SP1 binding sites (TESS/TransFac database v4.0) in the CST6 promoter may account for a low to moderate basal promoter activity in many tissues.
High expression occurs only in a few tissues such as the skin, placenta, ovary, pancreas and the lungs. A quite widespread expression of CST6 is also supported by data extracted from gene expression libraries (GEO, GeneNote, GNF Symatlas, CGAP, EST, SAGE, and UniGene eNortherns).
However, there are some conflicting data in the literature suggesting that the CST6 mRNA is expressed in a tissue-specific manner mainly if not exclusively in the skin.
Expression from the human CST6 gene is epigenetically silenced in several tumor types (see below). The 5'-end of the CST6 gene including exon-1 has an unusually high (≥ 70%) content in G and C nucleotides. As a matter of fact, a typical CpG island spans across the transcription start site (bp +1) from bp -186 to bp +320 and encompasses all of exon-1. Not surprisingly, treatment of tumor cells by histone deacetylase or DNA methyltransferase inhibitors results in 're-expression' of CST6 at levels similar to those seen in the normal or benign counterparts.
The unusual GC content (~ 80%) of the 5'-UTR of the mRNA suggests that CST6 expression might also be regulated at the translational level by eIF-4E.

Pseudogene

No pseudogenes have been identified.

Protein

Note

The CST6 gene product, Cst6, is a typical secretory protein. It is synthesized as a preprotein with a patent N-terminal signal sequence. The protein is translocated into the rough endoplasmic reticulum where about 30-50% of the nascent Cst6 polypeptides are N-glycosylated. Upon SDS-PAGE, Cst6 harvested from most cell secretions migrates as two major forms, a 14-kDa unglycosylated and a 17- to 18-kDa glycosylated form.

Description

The three-dimensional organization of Cst6 (assuming it is similar to that of chicken egg white cystatin shown in figure 4) is that of a compact five-pleated beta-sheet that partially wraps around a central alpha-helix. It is not clear what role glycosylation of residue N_{137} fulfills. Perhaps, N-glycosylation promotes binding of the protein to cells and entry into the endosomal/lysosomal system where Cst6 can interact with target proteases.

Expression

Cst6 is a cell-secreted protein. In vitro, the majority (> 95%) of the protein accumulates in the media conditioned by the cells. In cells that overexpress Cst6, prominent labeling of the Golgi apparatus can be seen using indirect immunofluorescence cytochemistry.

Localisation

In the human skin, where localisation of Cst6 has been most carefully explored, the protein is detected in the stratum granulosum of the epidermis, in the outer root sheet of hair follicles, in the secretory coil epithelium of sweat glands, and in the inner, mature cells of sebaceous glands.

Function

Protease Inhibitor Function: The most widely accepted function of cystatins is that of protease inhibitors. The name 'cystatin' further reminds us that these endogenous protease inhibitors target cysteine proteases. In contrast to metallo- and serine proteases that are mostly secreted proteases, most cysteine proteases are confined within cells where optimal pH and redox conditions favor their enzymatic activity. Thus, the majority of intracellular cysteine proteases are inactivated by oxidizing conditions outside the cells. Nevertheless, it is believed that cystatins inhibit cysteine proteases much faster than do oxidizing conditions and, thereby, prevent excessive tissue damage during the release of lysosomal enzymes.
Among the various types of intracellular cysteine proteases, cystatins seem to target preferentially endosomal/lysosomal cysteine proteases of the papain family, such as cathepsin B, cathepsin K/O2, cathepsin L, cathepsin L2/V and cathepsin S.

Fig. 3

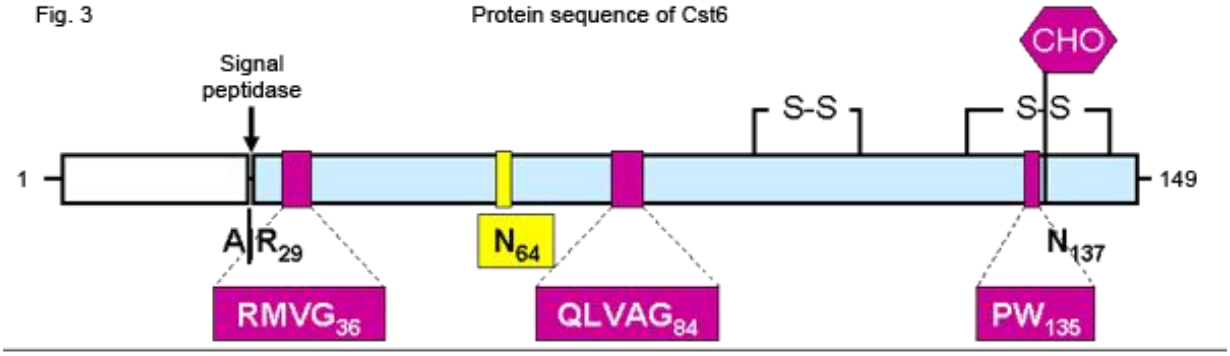

Fig. 4

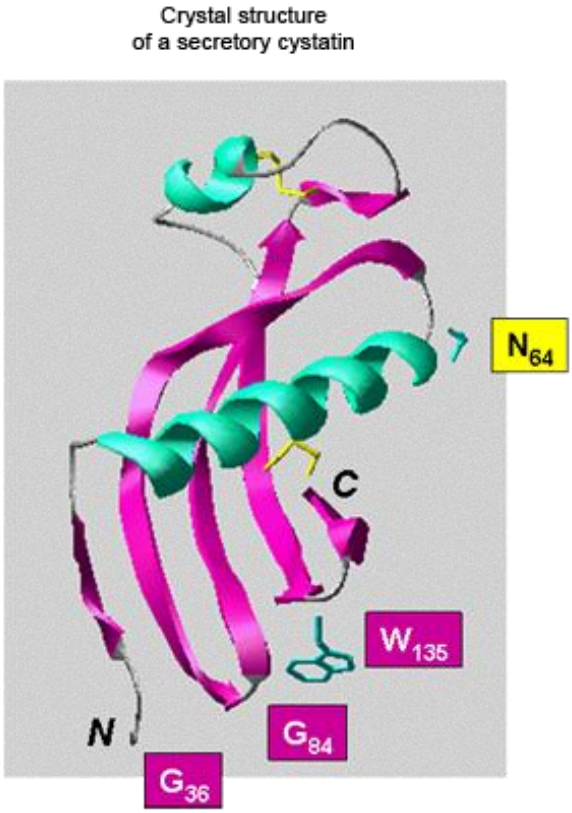

Figure 3: The above diagram depicts the primary structure of the Cst6 precursor. The first 28 amino acids represent a canonical signal peptide. The mature and secreted Cst6 molecule (in blue) contains two disulfide bonds (-S-S-), one N-glycosylation site (N_{137}-CHO), and two distinct binding sites for lysosomal cysteine proteases (purple and yellow boxes). The purple boxes represent the amino acids (RMVG, QLVAG and PW) that are involved in the binding and inhibition of the cathepsins B, K, L, L2/V or S. The yellow box represents the critical amino acid (N_{64}) for binding and inhibition of lysosomal Asn-endopeptidase (AEP or mammalian legumain).
Figure 4: Typical crystal structure of a secretory cystatin. The coordinates for the crystal structure of chicken egg white cystatin (1CEW) were obtained from the PDB database. A 3D-model of the cystatin was then generated using SwissPDB-Viewer. The N- and C-termini of the protein are marked by 'N' and 'C', respectively. The two conserved disulfide bonds are highlighted in yellow. The amino acids that are part of the two distinct binding sites for lysosomal cysteine proteases are labeled by purple and yellow boxes as described in the legend to figure 3. N_{64} and W_{135} are particularly important in this regard and are highlighted in blue. The amino acid numbering refers to that of the Cst6 preprotein, i.e., the protein with a 28-amino acid signal peptide (not present).

Some cystatins such as Cst6 are double-headed inhibitors and have a second inhibitory site, i.e., N_{64} in figures 3 and 4 above. Via this alternate inhibitory site, Cst6 is capable of binding and inhibiting legumain-type cysteine proteases such as AEP/mammalian legumain. Cystatins do not inhibit caspases and calpains seem to be regulated in a different manner. Little is known about the inhibitory potential of cystatins towards other types of intracellular cysteine proteases.

Epithelial barrier function: One important function of Cst6 seems to be in the terminal differentiation of stratified squamous epithelial cells and in the formation of cornified envelops. Indeed, ichq mice with a null mutation in the cst6 gene develop neonatal abnormalities in skin cornification and desquamation that resemble Harlequin ichthyoses in humans. However, no alterations in the CST6 gene were found in the DNA of patients with Harlequin ichthyosis.

In mice, the lack of Cst6 function leads to severe dehydration and neonatal lethality. Before serving as a substrate to transglutaminases and being deposited into cornified cell envelops, Cst6 is believed to be important in fine-tuning the enzymatic activities of endosomal/lysosomal cysteine proteases such as cathepsin L, cathepsin L2/V and AEP/mammalian legumain. Deregulated activity of these proteases could lead to abnormal activation of transglutaminases and disorders in cornification.

Homology

CST6 Gene orthologs:

Species	UniGene ID	Chromosome	Homology
Human	Hs.139389	11q13.1	100%/149 aa
Pig	Ssc.9061	2p16-17	78%/149 aa
Cow	Bt.5468	29	75%/148 aa
Dog	Cfa.23670	18	71%/149 aa
Rat	Rn.9609	1q43	70%/149 aa
Mouse	Mm.36816	19 A (4.0 cM)	69%/149 aa
Worm	Cel.5518	V	13%/143 aa

Mutations

Note

In 2004, CST6 was coined as a novel candidate tumor suppressor gene for breast carcinoma. Since then, this gene has been identified as a tumor suppressor gene for other cancers such as cancers of the breast, prostate, brain, lung, cervix and melanocytes. In most tumor tissues, CST6 seems to be epigenetically silenced rather than deleted or mutated. However, in one case (see below) more profound alterations in the human CST6 gene have been observed.

Cervical cancer: One out of 19 primary tumors revealed homozygous deletion of exon-1 sequences. Six other primary tumors exhibited point mutations in the CDS of the CST6 gene. Two of these mutations (M34T and L131F) occurred in proximity to the consensus binding sites for cathepsins (figure 6) and resulted in diminished affinity of the mutant inhibitor for cathepsin L.

Germinal

No germ-line mutations have been detected.

Implicated in

Cancer progression

Loss of heterozygosity (LOH) affecting the locus 11q13 is quite common in cancers. This locus indeed harbors several tumor or metastasis suppressor genes such as BAD, MEN1, BRMS1, RASGRP2, GSTP1 and CST6.

In a study using differential RNA display it was initially established that human breast cancer cell lines exhibited lack or reduced CST6 expression when compared to immortal or normal counterparts. CST6 was coined a novel candidate tumor suppressor gene for breast cancer on October 1st, 2004.

Cystatins	Cst3	Cst5	Cst6	Cst7	Cst4	Cst2	Cst1	Cst8
Cst3	100	52	32	30	56	54	52	29
Cst5		100	24	29	57	57	57	22
Cst6			100	28	28	27	29	22
Cst7				100	29	29	30	24
Cst4					100	89	89	28
Cst2						100	86	26
Cst1							100	27
Cst8								100

Figure 5: Degree of amino acid homology among human cystatin (in %).
Cst3, cystatin C; Cst5, cystatin D; Cst6, cystatin E/M; Cst7, cystatin F/leukocystatin; Cst4, cystatin S; Cst2, cystatin SA; Cst1, cystatinSN; and Cst8, CRES.

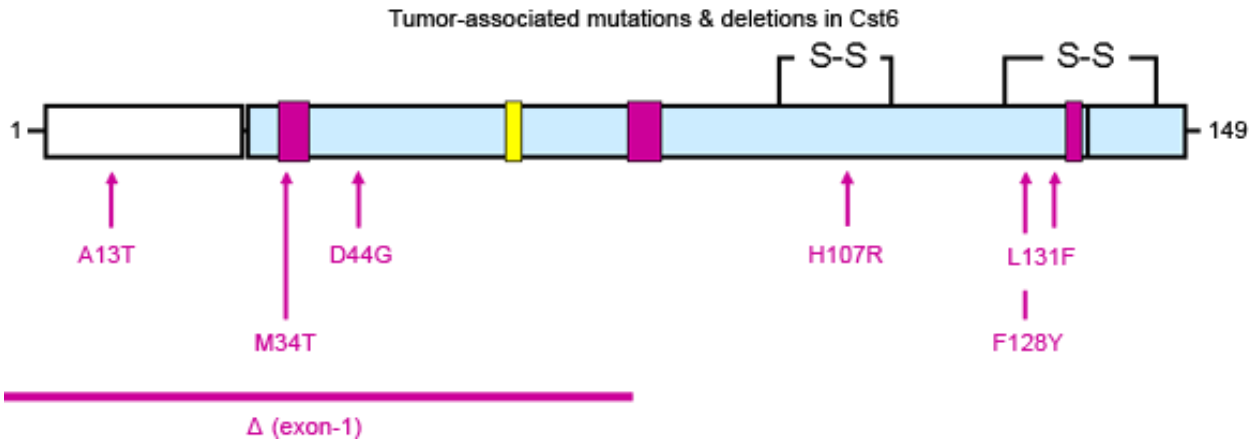

Figure 6: This diagram depicts locations of six point mutations and one deletion affecting the CST6 gene that have been observed in cancer specimens. Amino acid numbering refers to the precystatin sequence as for figure 3.

Since then, several groups have reported on the lack or diminished expression of CST6 in various cancer types (listed below). However, some groups also observed overexpression of CST6 in select cancer types (listed below). One of the challenges in current research on CST6 is to define the proteases targeted by CST6 and their precise role in the progression of the disease.

Cancer types with diminished CST6 expression

Breast cancer

Note

Using various approaches, several groups have independently established that the human CST6 gene promoter is epigenetically silenced in breast carcinomas when compared to normal breast tissue. In one study, 24/40 (60%) breast carcinomas exhibited CST6 promoter hypermethylation as compared to 7/28 (25%) normal breast tissue samples. In another study, 25/45 (56%) of primary tumors and 17/20 (85%) of lymph node metastases expressed reduced levels of CST6 when compared to normal breast tissues. CST6 promoter hypermethylation could be demonstrated in 3/11 (27%) primary tumors and 8/12 (67%) lymph node metastases. In 35% of neoplastic lesions, no association could be established between the loss of CST6 expression and promoter methylation. This suggests that besides promoter hypermethylation other (structural or regulatory) mechanisms might operate to prevent CST6 expression in cancer cells.

Most established breast cancer cell lines also exhibited little or no CST6 expression (21MT-1, MCF-7, T-47D, ZR-75-1, Hs578T, SK-BR-3, MDA-MB-157, MDA-MB-361, MDA-MB-435S, MDA-MB-436, MDA-MB-453, BT-474 and BT-549). Some established breast cancer cell lines expressed moderate levels of CST6 (MDA-MB-231, MDA-MB-415 and MDA-MB-468) and only few (21PT, 21NT, 21MT-2 and BT-20) expressed levels of CST6 similar to normal or immortal counterparts (70N and 80N or 76N, MCF-10A, MCF-10AT and MCF-12A, respectively). Treatment of CST6-negative tumor cells by the histone deacetylase inhibitor Trichostatin A (TSA) or the DNA methyltransferase inhibitor 5-Aza-2'-deoxycytidine (5-Aza) results in 're-expression' of CST6 at levels similar to those seen in the normal or benign counterparts.

Overexpression of CST6 in breast cancer cells (MDA-MB-435S and T-47D) is associated with diminished tumor cell colony formation, proliferation, migration, Matrigel invasion and orthotopic tumor growth in scid mice.

Prostate cancer

Note

In a study of matched pairs of normal/cancer tissues, loss of CST6 expression was observed in 18/20 (90%) prostate cancers. Similarly, only 6% of prostate cancers exhibited strong Cst6 immunohistochemical staining as compared to 63% of normal tissues.

Among prostate cancer cell lines, RWPE-1 and DU-145 express high and moderate levels of CST6, respectively, whereas LNCaP, PC-3 and PC-3M express little to no CST6. Treatment with TSA leads to strong upregulation of CST6 expression in all three cell lines. In contrast, treatment with 5-Aza up to five days had no effect. Further studies using methylation-specific PCR showed that prostate cancer cell lines and tissues had lower promoter methylation than normal tissues. DNA hypermethylation of the CST6 promoter does therefore not account for the silencing of CST6 expression in prostate cancer. Instead, histone deacetylation and chromatin remodeling seem to be responsible for diminished CST6 expression.

Similar to breast cancer cells, forced expression of CST6 in prostate cancer cells (PC-3) leads to diminished tumor cell proliferation and Matrigel invasion. In addition, overexpression of CST6 also selectively reduces expression of the target enzyme, cathepsin B. Conversely, silencing of CST6 expression

in a CST6-positive prostate cancer cell line (RWPE-1) leads to the exact opposite results from overexpression. In mice, orthotopic injection of PC-3 cells overexpressing CST6 resulted in considerably smaller tumors when compared to vector controls. The CST6 tumors expressed reduced levels of cathepsin B.

Lung cancer

Note

Two groups have recently reported on the epigenetic silencing of the CST6 gene in non-small cell lung cancer (NSCLC) using genome-wide expression profiling. In one study, 2/5 (40%) primary tumors and 1/5 (20%) normal lung tissues exhibited CST6 promoter methylation. In the other study, the numbers were respectively 10/19 (53%) and 2/15 (13%).

NSCLC cell lines that express little or no CST6 are the following: A-427, A-549, NCI-H23, NCI-H522, NCI-H1299 and NCI-H460. Three cell lines expressed moderate to high levels of CST6 (NCI-H322, NCI-H358 and NCI-H292). In all nine above cell lines, CST6 expression could be increased to normal levels by a combined treatment of the cells with TSA and 5-Aza.

Overexpression of CST6 in lung adenocarcinoma A-549 cells resulted in a > 50% reduction in colony formation in vitro compared to vector controls.

Cervical cancer

Note

One study recently reported on the lack of CST6 expression in 9/11 (82%) primary squamous cell carcinomas of the cervix, but expression of the gene in 5/5 (100%) normal cervical tissues as well as in normal lung, thyroid, kidney, brain, ovary, uterus, smooth muscle and connective tissues. Two out of 11 (18%) primary tumors (one of which being an adenocarcinoma) expressed low levels of CST6, which might be due to contamination of the tumor material by adjacent normal tissue.

Cervical cancer cell lines such as HeLa (D98/AH-2), C41, SiHa, Caski, HT3 and C33A all lack expression of CST6. Treatment of tumor cells by 5-Aza and/or TSA results in 're-expression' of CST6 at levels similar to those seen in normal tissues. Similar to the situation in prostate cancer cells, some cell lines (SiHa and HT3) respond only to TSA treatment. Caski, C33A and C41 cells exhibit both unmethylated and hypermethylated CST6 promoters whereas HeLa cells has homogenously hypermethylated CST6 promoters.

Overexpression of CST6 in HeLa and SiHa cells leads in both cases to a reduction in the number and size of colonies forming in soft agar and in cell proliferation. Another consequence of the forced expression of CST6 in HeLa cells is a reduction in intracellular levels of the target protease, cathepsin L, possibly explaining the reduced growth of the CST6 overexpressing cells.

Head and neck squamous cell carcinoma (HNSCC)

Note

Comparison of the gene expression profiles (HuFL6800) of two matched pairs of primary and metastatic human oropharyngeal SCC cell lines (MDA-686TU and LN) revealed relative overexpression of CST6 in the metastatic cell line. Immuno-cytochemical analysis further showed that overexpression of CST6 in the metastatic cell line was not homogenous. Instead, small clusters of cells overexpressed the protein whereas the majority of cells expressed little or no CST6. Further studies using RNA interference indicated that loss of CST6 expression in MDA-686LN promoted proliferation of the cells and Matrigel invasion.

In another study, human SCC-25 cells were treated with the vitamin D3 analog EB1089 for various times and the effect of this drug treatment on gene expression analyzed using HuGene FL oligo microarrays. In this study, CST6 expression was found to increase > 30-fold over a 24-hr period. Overall, EB1089 treatment reversed the malignant phenotype of SCC-25 cells and induced keratinocytic differentiation.

Brain cancer

Note

One study reported on downregulation of CST6 expression in 15/17 (88%) brain tumors, which included 7/9 (78%) multiform glioblastomas (MG). Moreover, MSP analysis demonstrated CST6 promoter methylation in 17/30 (57%) brain tumors. These latter included 14/19 (74%) MGs. In comparison to brain tumors, normal brain tissue exhibited only 6% CST6 promoter methylation.

CST6 expression and methylation status was also analyzed in six glioblastoma cell lines: LN-229, LN-18, T98G, DBTRG-05MG, U-87MG and U-118MG. All six cell lines expressed little or no CST6. In addition, all cell lines had quite homogenously hypermethylated CST6 promoters. Re-expression of CST6 could be triggered with 5-Aza alone.

Transfection of T98G, LN-229 and U-87MG cells with a mammalian CST6 expression vector resulted in a modest (20-25%) suppression of T98G and LN-229 cell growth when compared to vector controls. Forced expression of CST6 in U-87MG cells had no effect on their capacity to form colonies and proliferate.

In conclusion, CST6-mediated suppression of tumor cell growth seems to be most pronounced in cells of epithelial origin, i.e., in cells developing multiple cell-to-cell communications and elaborating a basement membrane.

Cancer types with increased CST6 expression

Squamous cell carcinoma of the skin

Note

Squamous cell carcinoma (SCC) of the skin versus psoriasis.

CST6 is highly expressed in the normal human skin, which might explain why no further increase in expression could be detected in SCC. However, a five- to six-fold differential expression of CST6 was observed when SCC was compared to psoriatic skin. Differential expression of CST6 was accompanied by a similar differential expression of one of its target proteases, cathepsin L2/V.

Pancreatic cancer

Note

CST6 was identified as an upregulated gene in several genome-wide expression studies. One study used microarray analysis to profile gene expression in pancreatic adenocarcinomas (T=10), pancreatic cancer cell lines (C=7), chronic pancreatitis (P=5) and normal pancreas (N=5). According to this study, CST6 levels change 20-, 20- and 24-fold in T/N, T/P and C/N, respectively. In another study using a similar approach (oligo microarray) the T/N ratio was found to be 4.4-fold and upregulation of CST6 was not observed using other platforms such as SAGE or cDNA-based microarrays. Instead, among six genes that were consistently overexpressed across all three platforms was one of the major CST6 targets, cathepsin L2/V.

In yet another study using a cDNA microarray, CST6 was found to be overexpressed in 18 microdissected pancreatic ductal adenocarcinomas (PDAC) when compared to normal ductal epithelial cells. Subsequent silencing of CST6 expression in a PDAC cell line (PK-59) reduced colony formation and cell proliferation. Conversely, overexpression of CST6 in a CST6-negative PDAC cell line (KLM-1) promoted tumor growth in nude mice. Likewise, addition of recombinant human CST6 to the growth medium of KLM-1 cells promoted their proliferation in a dose-dependent manner. Engineered CST6 variants lacking either N-glycosylation (N137D, figure 3) or with an altered protease binding site (deletion of MVG38, figures 3 4) did not have any effect on cell proliferation suggesting that both N-glycosylation and protease specificity are required for oncogenic activity of CST6.

Thyroid cancer

Note

Initial immunohistochemical studies found positive staining for CST6 in 80% (8/10) of papillary thyroid carcinomas (PTC) and 73% (11/15) of benign thyroid lesions. Independent studies established a strong correlation between CST6 expression, PTC and BRAF (V600E) mutational status. CST6 expression was also associated with PTC lymph node metastasis.

Ovarian cancer (OvCA)

Note

In order to better define the molecular profiles of the four major histological types of OvCAs (clear cell, mucinous, endometrioid, and serous), a microarray analysis was performed on 113 human specimens. Expression of CST6 was found to be on average 3.8-fold higher in clear cell OvCAs when compared to other histological types. It is interesting to note here that more than one-half of clear cell OvCAs do not exhibit tumor invasion at presentation.

Breakpoints

Note

A 300-kb region flanked by the markers D11S4908 and D11S5023 and harboring the CST6 gene has been identified as the minimal tumor deletion on 11q13 in cervical cancer cell lines and primary cervical tumors. This region was reported to contain a high density of DNA repeats rendering it fragile and prone to potential DNA breaks and carcinogenesis. A rare fragile site FRA11A overlaps indeed with this region.

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Gene Section
Mini Review

DHX9 (DEAH (Asp-Glu-Ala-His) box polypeptide 9)

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DOI: 10.4267/2042/44773

Identity

Other names: DDX9; LKP; NDHII; RHA; NDH2; leukophysin

HGNC (Hugo): DHX9

Location : 1q25.3

DNA/RNA

Description

The gene spans 48.5 kb and is composed of 27 exons.

Transcription

Transcription start is 163 bp upstream of first ATG of the DHX9 ORF. The translation start site is located in exon 2 and there is a sole isoform ubiquitously expressed.

Pseudogene

DHX9 pseudogene (DHX9P) is located at 13q22.

Protein

Description

Monomeric 140 kDa protein. Human DHX9 is 1270 amino acids. It contains an helicase catalytic domain flanked by two double-stranded RNA binding domains (dsRBD) at the N-terminus and an RGG-box at the C terminus. A bidirectional nuclear transport domain is located at the C terminus.

Expression

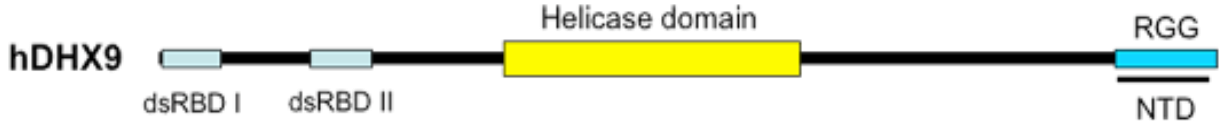
All tissues tested, ubiquitous expression.

Localisation

DHX9 shuttles between the nucleus and the cytoplasm.

Function

DHX9 is a nucleic-acid helicase that unwinds double-stranded DNA and RNA in a nucleotide dependent manner. It acts as a transcriptional coactivator which stimulates transcription by interacting with the transcriptional coactivator CBP/p300, the breast cancer protein BRCA1, the RNA polymerase II and has an important role in the assembly of STAT6 transcriptosome.
DHX9 plays a role in regulating chromatin structure by interacting physically and functionally with topoisomerase IIa.
It mediates the attachment of nuclear ribonucleoprotein complexes to actin filaments, which may be related to RNA processing and transport.



Structure of DHX9. dsRBD, double-stranded RNA binding domain; RGG, arginine and glycine-rich region; NTD, nuclear transport domain.

DHX9 interacts with the survival motor neuron which plays a role in the assembly and regeneration of small nuclear ribonucleoproteins and spliceosomes.
DHX9 acts as a nuclear shuttle protein promoting the export of mRNA transcripts through binding to TAP and HAP95.
In the cytoplasm, DHX9 is preferentially associated with actively translating polyribosomes and is necessary for efficient translation of RNAs that contain a highly structured 5'UTR.
DHX9 might be necessary for maintaining genomic stability as it plays a role in promoting the DNA processing function of WRN. Overexpression of a truncated DHX9 peptide prevents normal BRCA1 function, such as BRCA1 association with nuclear foci following DNA damage. DHX9 associates with gH2AX after DNA damage, suggesting a role for DHX9 in DNA repair.
DHX9 is also necessary for early embryonic development in mice.

Homology

Sequence analysis revealed that DHX9 contains seven helicase core motifs that are conserved among the DEX[D/H] helicase superfamily. DHX9 is highly conserved among man, cow, mouse, worm, and fruit fly.

Mutations

Note
DHX9 truncating mutations were reported to affect the interaction with BRCA1 and RNA polymerase II, and to result in decreased transcriptional activity of BRCA1.
In mammals, DHX9-knockout mice are embryonic lethal for homozygous DHX9 mutants. DHX9 is thus necessary for early embryonic development in mice. It was also suggested that DHX9 is required for the survival and differentiation of embryonic ectoderm.
DHX9 maps to chromosome 1q25 near a major susceptibility locus for prostate cancer.

Implicated in

Lung cancer

Note
DHX9 is over-expressed in tumor samples compared to normal lung tissues. There was a tendency for higher expression levels in small cell lung cancer compared to non-small cell carcinomas.

Prognosis
There was no correlation with tumor stage and survival.

Breast cancer

Note
Involvement of DHX9 in breast cancer susceptibility was analyzed in a cohort of breast cancer individuals from non-BRCA1/BRCA2 French Canadian families. This study did not identify any deleterious truncating mutation or aberrant splicing in the DHX9 gene. It was concluded that studies on much bigger cohorts are needed to fully evaluate the association of variants identified with breast cancer risk.

Systemic lupus erythematosus (SLE)

Note
Anti-DHX9 is a new serologic marker for SLE. The production of anti-DHX9 may depend on a process restricted to early SLE, or it may be highly sensitive to treatment.

Disease
Systemic lupus erythematosus (SLE) is a largely genetically based disease in which environmental factors are also involved. SLE is an autoimmune disease characterized by autoantibody production and involvement of multiple organ systems. Variable manifestations and outcome reflect the clinical heterogeneity of the disease. It is characterized by acute and chronic inflammation of various tissues of the body including joints, kidneys, mucous membranes, and blood vessel walls.

Prognosis
Among patients with SLE, anti-DHX9 was common in young patients and at an early stage of the disease.

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Gene Section
Mini Review

EIF3F (eukaryotic translation initiation factor 3, subunit F)

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Identity

Other names: EIF3S5; eIF-3-epsilon; eIF3-epsilon; eIF3-p47; eIF3f; eIF3 subunit p47; p47

HGNC (Hugo): EIF3F

Location: 11p15.4

Note: eIF3f is one of at least 13 subunits comprising the eukaryotic initiation factor 3 (eIF3) complex.

DNA/RNA

Description

10.82kb long.

Transcription

8 exons; transcript length: 3,228 bps; 1073 bp coding sequence.

Pseudogene

Chromosome 2 from 58,332,079 to 58,333,164 bp (AC007250.3, Ensembl).

Protein

Description

357 amino acids; 37,563.75 Da; Isoelectric point: 5.122; Mov34/MPN/PAD1 domain.

Expression

Ubiquitous.

Localisation

Cytoplasmic. There is also evidence of its existence in the nucleus.

Function

eIF3f is a subunit of eIF3 complex and is highly conserved among species. eIF3f interacts with the caspase-processed isoform of CDK11 ($CDK11^{p46}$), which appears to be a down-stream effector in apoptotic signaling. eIF3f can be phosphorylated at Ser46 and Thr119 by $CDK11^{p46}$ during apoptosis. The phosphorylation of eIF3f contributes to translation inhibition and apoptosis. The expression of eIF3f is significantly decreased in pancreatic cancer and melanoma. Loss of the eIF3f allele has been reported in pancreatic cancer and melanoma. Ectopic expression of eIF3f causes rRNA degradation, inhibits translation and cell proliferation, and induces apoptosis in pancreatic cancer and melanoma cells. On the other hand, knockdown of eIF3f prevents apoptosis in pancreatic cancer and melanoma cells. Recent studies also showed decreased cell growth, cell proliferation, colony formation and increased apoptosis in eIF3f-overexpressing NIH3T3 cells.

Homology

eIF3f is not found in S. cerevisiae; However it is found in Schizosaccharomyces pombe (SPBC4C3.07, 35% identity) and in Drosophila Melanogaster (CG9769, 49% identity).

Mutations

Note

No eIF3f mutations have been reported.

Implicated in

Pancreatic cancer

Disease

Decreased eIF3f mRNA and protein was found in pancreatic cancer.

Oncogenesis

Decreased eIF3f gene expression may result from loss of eIF3f gene allele and down regulation of transcription. Overexpression of eIF3f in pancreatic cancer and melanoma cells can lead to 28S rRNA degradation, decreased translation and increased apoptosis. On the other hand, knockdown of eIF3f attenuates apoptosis in tumor cells.

Melanoma

Disease

Decreased eIF3f mRNA and protein was found in melanoma.

Oncogenesis

See above.

Muscle atrophy

Disease

eIF3f is a key target of MAFbx, an E3 ubiquitin ligase, during muscle atrophy and has a major role in skeletal muscle hypertrophy. During muscle atrophy, MAFbx targets eIF3f for ubiquitination and degradation. Thus, eIF3f appears to be an attractive therapeutic target.

To be noted

Note

eIF3f is a potential tumor suppressor in pancreatic cancer and melanoma. Loss of heterozygosity and decreased expression of eIF3f has been found in most human pancreatic cancer and melanoma specimens. Silencing of eIF3f increases cell proliferation and colony formation. Restoration of eIF3f expression induces cancer cell death.

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Gene Section
Mini Review

EML4 (echinoderm microtubule associated protein like 4)

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Identity

Other names: C2orf2; DKFZp686P18118; ELP120; EMAP-4; EMAPL4; FLJ10942; FLJ32318; ROPP120

HGNC (Hugo): EML4

Location: 2p21

Note: Gene type: protein coding; Member of the EMAP-family.

DNA/RNA

Description

23 exons; DNA-length: 163,173 kb.

Transcription

Two transcript variants.

Protein

Note
Strongly expressed during mitosis.
2 isoforms:
- Isoform a (= variant 1): longer transcript,
- Isoform b (= variant 2): shorter transcript, lacks an alternate in-frame exon.

Description

Weight: 120 kDa.
At least 2 Domains: HELP motif, WD40 repeat.
Exon 2 encodes for coiled-coil domain.

Expression

Ubiquitous.

Localisation

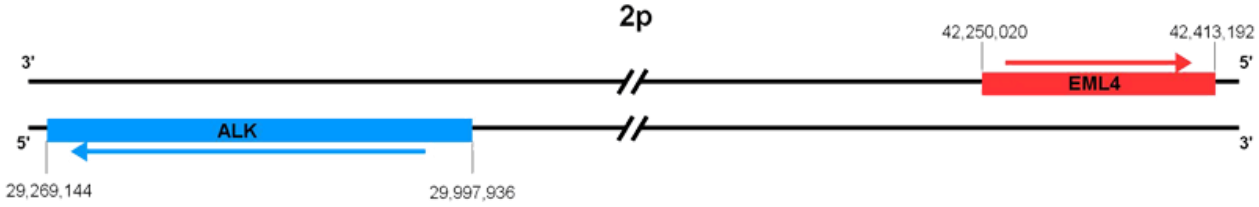
Intracellular.

Function

Microtubule binding.
Necessary for correct microtubule formation (stabilizes microtubules).
May modify the assembly dynamics of microtubules, such that microtubules are slightly longer, but more dynamic.

Homology

Mouse, rat.



2p with location of EML4 and ALK relative to each other.

Implicated in

Non-small cell lung carcinoma (NSCLC)

Note

Mouse models suggest that EML4-ALK fusion might play an essential role in NSCLC carcinogenesis.

EML4-ALK-fusion-transcript is detectable in a low amount (frequency between 2,7% and 6,7%) of NSCLC-samples and NSCLC-cell-lines. So far, the fusion-protein could only be detected by mass-spectrometry. Immunohistochemical detection of the Alk-protein in fusion-transcript-positive primary NSCLC-samples is contentious.

Alk-inhibitors impede proliferation in EML4-ALK-fusion positive lung cancer cell-lines. In experiments with transgenic mice, treatment with ALK-inhibitors resulted in reduced tumor mass.

The role of EML4-ALK-fusion as a specific biomarker for NSCLC remains controversial.

Disease

NSCLC, frequency between 2,7% and 6,7%.

Prognosis

Controversially discussed. Limited informative because of low numbers studied so far.

Cytogenetics

EML4-ALK-Fusion. inv(2)(p21p23), other fusion-mechanisms suggested.

Hybrid/Mutated gene

EML4-ALK.

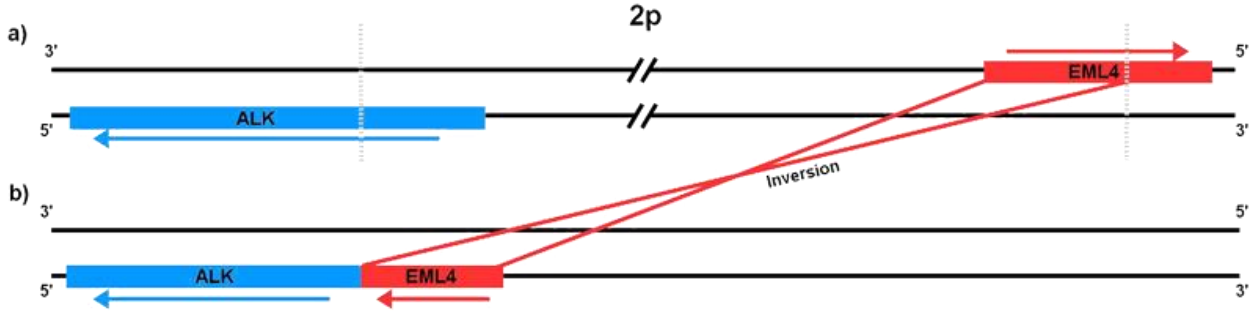
Abnormal protein

EML4-ALK = tyrosine-kinase, which is constitutively dimerized and thus activated.

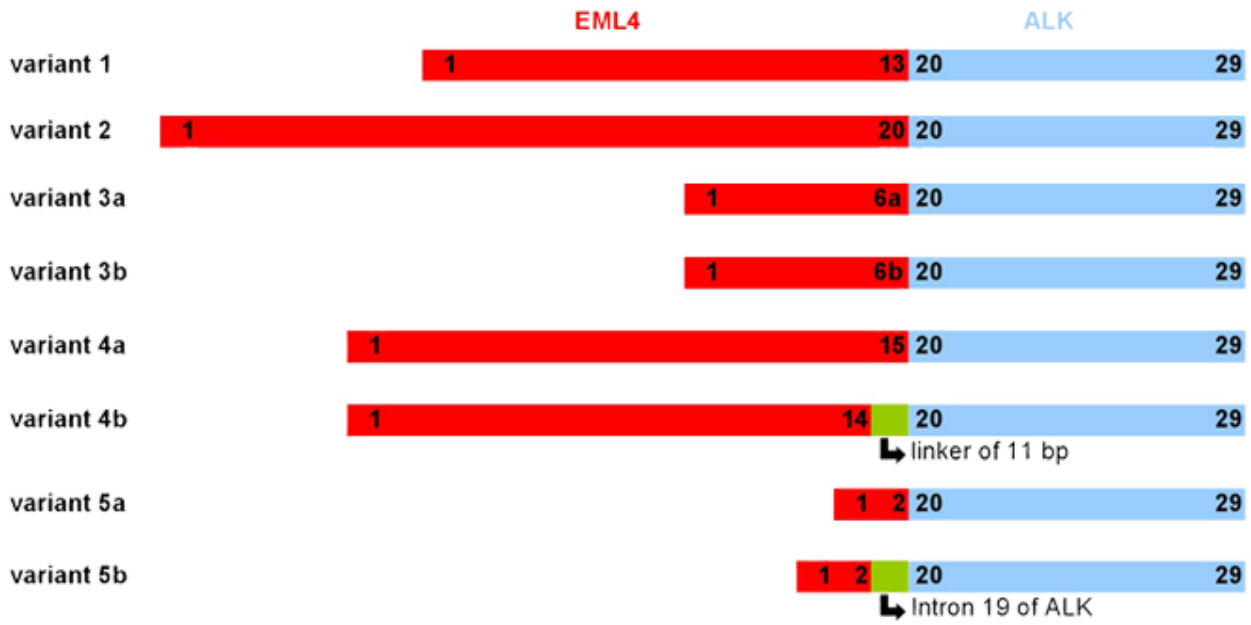
For known fusion partners of ALK, see ALK. For example, TPM3, TFG, MYH9, NPM, ATIC, MSN, ALO17 are ALK partners in anaplasic large cell lymphoma, CLTC in diffuse large cell lymphoma, and TPM4 in inflammatory myofibroblastic tumors.

Breakpoints

Known variants:
Variant 1: exon 1-13 (EML4) + exon 20-29 (ALK)
Variant 2: exon 1-20 (EML4) + exon 20-29 (ALK)
Variant 3a: exon 1-6a (EML4) + exon 20-29 (ALK)
Variant 3b: exon 1-6b (EML4) + exon 20-29 (ALK)
Variant 4a : exon 15 (EML4) + exon 20-29 (ALK)
Variant 4b : exon 14 (EML4) + linker of 11bp + exon 20-29 (ALK)
Variant 5a : exon 2 (EML4) + exon 20-29 (ALK)
Variant 5b : exon 2 (EML4) + intron 19 (ALK) + exon 20-29 (ALK)



Fusion of EML4-ALK. a) wild type EML4 and ALK. b) fused EML4-ALK.



EML4-ALK fusion isoforms (numbers = exon number).

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Gene Section
Review

ESRRA (estrogen-related receptor alpha)

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Identity

Other names: ERR-alpha; ERR1; ERRa; ERRalpha; ESRL1; NR3B1

HGNC (Hugo): ESRRA

Location: 11q13.1

Note: Size: 11,172 bases; Orientation: plus strand.

DNA/RNA

Description

-Sequence length 11,172 bases;
-CDS: 2221;
-Exons: 7.

Transcription

Alternative splicing results in transcript variants, but these have not yet been well-characterized.

Pseudogene

A pseudogene has been reported, ESRRAP, located at 13q12.1. However, it is possible that this pseudogene is not transcribed (Sladek et al., 1997).

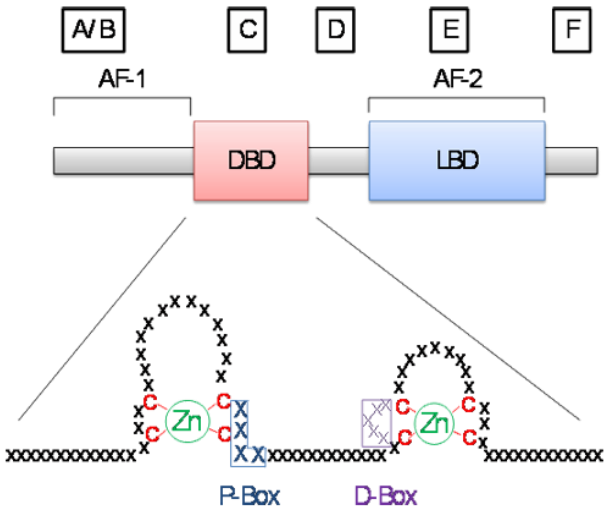
Protein

Description

ERRa is a 45.5 kDa, 423 amino acid orphan nuclear receptor. Although closely related to the estrogen receptors, its transcriptional activity is regulated to any significant degree by estrogens. ERRa binds to specific DNA sequences within target gene promoters as a monomer or homodimer and recruits coactivating proteins, the best known of which is PGC-1a.

Expression

ERRa is ubiquitously expressed throughout development with the highest levels of expression in tissues that oxidize fatty acids such as kidney, heart, cerebellum, intestine and skeletal muscle (nursa).



Schematic of nuclear receptor structure and function.
ERRa is a member of the nuclear receptor (NR) superfamily of transcription factors and is most closely related to estrogen receptor alpha (ERa). The modular structure of NRs consists of seven (A-F) domains. The A/B region, which harbors activation function 1 (AF-1), is not well-conserved across NRs, but regions C and E are highly conserved and harbor, respectively, the DNA-binding domain (DBD) and ligand-binding domain (LBD). ERRa shares with ERa 68% sequence identity within the DBD and 33% within the LBD. The functional regions of the DBD have been finely mapped. In addition to two zinc finger motifs, this domain contains a Proximal-box (P-box) which determines DNA sequence specificity, and a Dimerization-box (D-box), which part of the dimerization interface.

Localisation

ERRa is thought to be predominately nuclear, although recently it has been reported to be perinuclear and cytoplasmic in breast cancer tissue (Jarzabek et al., 2009).

Function

The function of ERRa as a metabolic regulator is supported by the observation that erra-null mice demonstrate impaired fat metabolism and absorption (Luo et al., 2003). It has recently been demonstrated that erra-null mice also have a reduced capacity for adaptation to hemodynamic stressors. Due to this functional deficit, these mice often develop cardiac contractile dysfunction. The cardiac remodeling under stress in ERR-null mice is due to defects in ATP synthesis and reduced phosphocreatine stores, which are both characteristic of pathologic cardiac hypertrophy (Huss et al., 2007). That the expression of ERRa is elevated in exercising muscle and in fasting liver specifically implicates this receptor in beta-oxidation of fatty acids, a metabolic pathway that is highly active under these conditions. On a mechanistic level, several studies have revealed that ERRa is involved in the transcriptional regulation of genes required for mitochondrial biogenesis, oxidative phosphorylation and fatty acid oxidation (Huss et al., 2004; Mootha et al., 2004; Dufour et al., 2007).

Thus far, metabolic studies of ERRa function have mainly focused on its role as the downstream effector of PGC-1a. PGC-1a is a promiscuous nuclear receptor coactivator expressed at low basal levels but induced by fasting and other metabolic stresses (Puigserver and Spiegelman, 2003). PGC-1beta, a related cofactor, may have similar functions, although its expression level is not as acutely regulated by variations in energy demand (Yoon et al., 2001). Rather than being regulated by ligand, the magnitude of ERRa activity is thought to be largely dependent on the presence of transcriptional coactivators such as PGC-1a and beta. Interest in the ERR-PGC-1 regulatory axis was heightened by the observation that there is a decrease in both PGC-1a and PGC-1beta in the skeletal muscle of patients with diabetes and obesity (Mootha et al., 2003).

Homology

Sequence analysis reveals that the ERRs and the classical estrogen receptors share a high degree of homology within their DNA and ligand binding domains. In particular, ERRa shares with ERa approximately 68% sequence identity within the DNA binding domain and 33% within the ligand binding domains. This relationship provides a structural basis both for the conserved nature of DNA binding and the divergence in hormone binding between these two receptors.

Mutations

Note

Although over 80 SNPs have been reported, only one variant has been shown to carry clinical associations.

Laflamme et al. reported a polymoprohic hormone response element within the ESRRA promoter (Laflamme et al., 2005). The variant sequence, present in 11% of the population tested (white, premenopausal women), included an ERRa responsive element within the additional 23-nucelotides. This longer variant was associated with higher bone mineral density measured in the lumbar spine.

Kamei et al. reported that the longer variant is associated with a significantly higher body mass index in their study population of 729 Japanese men and women (Kamie et al., 2005).

Implicated in

Breast cancer

Prognosis

Two independent clinical studies have implicated ERRa in breast cancer progression (Ariazi et al., 2002; Suzuki et al., 2004). In the first study to link ERRa to clinical and pathological characteristics of breast cancer, Ariazi et al. found that ERRa expression is significantly associated with ERa-negative and progesterone receptor-negative tumor status as well as Her2 status. Further exploring the relationship between ERRa and Her2, Barry et al. demonstrated that ERRa transcriptional activity can be enhanced by phosphorylation events downstream of Her2 (Barry and Giguere, 2005). Building on the association between ERRa and negative prognostic biomarkers, Suzuki et al. demonstrated a direct correlation between ERRa expression and unfavorable breast cancer patient outcomes including increased tumor recurrence and decreased survival (Suzuki et al., 2004). Importantly, the predictive value of ERRa expression was shown to be independent of ERa status, confirming that targeting the ERRa pathway may be of therapeutic benefit in patients with either ERa-positive or ERa-negative breast cancer.

Recently, the function of ERRa has been evaluated in xenograft models of breast cancer. Stein et al. demonstrated that ERRa is critical for the growth of ERa-negative breast cancer through use of RNAi (Stein et al., 2008). Furthermore, Chisamore and coworkers found that an ERRa antagonist inhibited the growth of ERa-positive and ERa-negative breast cancer cell lines in a xenograft model (Chisamore et al., 2009).

Ovarian cancer

Prognosis

Sun et al. demonstrated that the ovarian tumors had significantly higher ERRa mRNA levels than normal ovaries and that high ERRa expression correlated with clinically advanced and histologically aggressive disease. Furthermore, ERRa expression was shown to be an independent prognostic factor for poor overall patient survival (Sun et al., 2005).

Colorectal cancer

Prognosis

Analysis of 80 colorectal tumor samples demonstrated that higher levels of ERRa mRNA are expressed in

tumor tissue versus in the surrounding normal mucosa. Furthermore, tumor tissue ERRa mRNA levels are positively correlated with increased tumor stage and histological grade (Cavallini et al., 2005).

Prostate cancer

Prognosis

Cheung et al. investigated the expression patterns of the three ERR family members in normal and malignant human prostate epithelial cells and cell lines (Cheung et al., 2005). The authors also characterized ERR protein expression and localization in normal, dysplastic, and malignant prostate tissue (Cheung et al., 2005). They concluded that ERRbeta and ERRgamma protein expression is reduced in neoplastic prostatic cells versus their non-malignant counterparts and suggested that each is down-regulated in the progression of prostate cancer. The authors went on to measure the effect of overexpressing the ERRs on proliferation of an immortalized prostate cell line and a prostate cancer cell line in vitro and on prostate cancer xenograft growth in vivo (Yu et al., 2007; Yu et al., 2008). They found that ERRbeta and ERRgamma can inhibit proliferation in cells derived from normal and malignant prostate epithelium by inducing a G1-S cell cycle arrest. Furthermore, activation of either ERRbeta or ERRgamma using the agonist DY131 resulted in a decreased rate of prostate tumor growth in a xenograft model.

Endometrial cancer

Prognosis

Gao et al. explored the extent to which the ERRs are involved in ERa-positive endometrial adenocarcinoma (Gao et al., 2006). They measured the expression of each ERR family member in malignant versus normal endometrium and compared the expression levels to clinical and pathologic features. They concluded that the expression of ERRa mRNA was lower in ERa-positive endometrial adenocarcinoma versus normal endometrium. However, they also found that ERRa mRNA expression was positively correlated with tumor stage and myometrial invasion. Additionally Gao et al. found that the expression of ERRgamma mRNA was increased in endometrial adenocarcinoma compared to normal endometrium.

Breakpoints

None.

To be noted

Note

In the absence of known endogenous ligand, considerable effort has been made toward identifying small molecules to modulate ERRa activity. Several ERRa antagonists have been developed and recently a novel antagonist was described that inhibited the growth of breast cancer xenografts (Chisamore et al., 2009).

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Gene Section
Mini Review

KCMF1 (potassium channel modulatory factor 1)

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DOI: 10.4267/2042/44777

Identity

Other names: DEBT91; DKFZP434L1021; FIGC; PCMF; ZZZ1

HGNC (Hugo): KCMF1

Location: 2p11.2

DNA/RNA

Description

DNA size 87.29 kb, mRNA size 7555 bp, 7 exons.

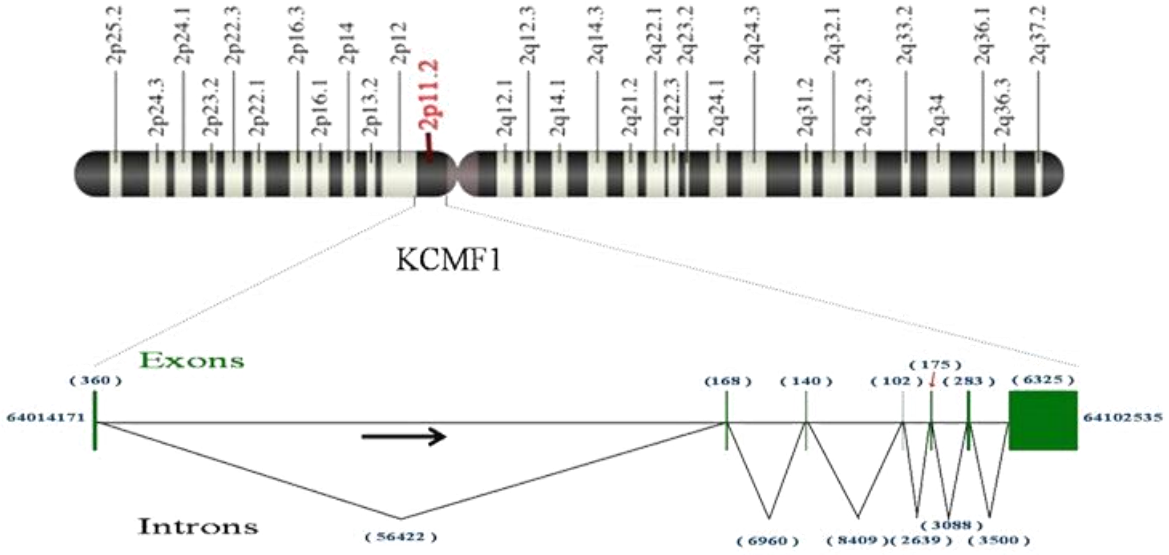
Protein

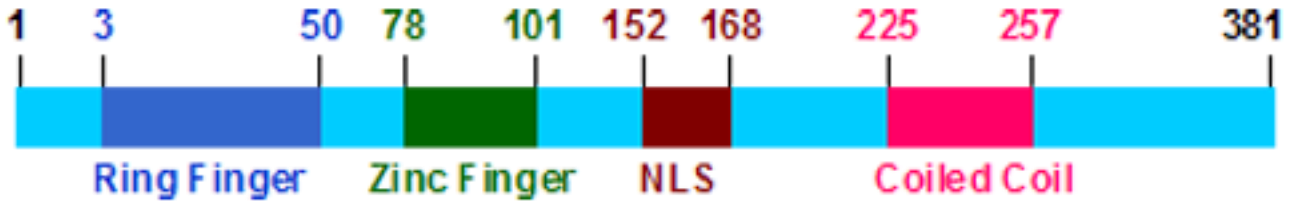
Description

381 amino acids; 41.945 kDa protein.

KCMF1 protein contains ring finger (Zinc finger, ZZ-type) 3-50 (48), zinc finger (C2H2-type) 78-101 (23), nuclear localization signal (NLS) 152-168 (17), and a coiled coil domain 225-257 (33).

Isoforms: Two isoforms that predicted to encode proteins containing the zinc finger domain have been identified; other isoforms are relatively shorter and not well defined.





Isoform bApr07: This partial mRNA is 625 bp long. It is reconstructed from a myeloma cDNA clone. The pre-messenger RNA has 5 exons and covers 74.08 kb. The predicted partial protein has 208 aa (22.9 kDa, pI 5.8) and a very good coding score (7). It contains one Zinc finger, ZZ-type domain, one zinc finger, C2H2-type domain.
Isoform dApr07: The dApr07 mRNA variant is 431 bp long. It is reconstructed from a testis cDNA clone. The pre-mRNA has 4 exons and covers 12.72 kb. The predicted partial protein has 143 aa (15.7 kDa, pI 7.2) and a very good coding score (5). It contains one zinc finger, C2H2-type domain.

Expression

Ubiquitously expressed. High level of expression is in pharynx, thyroid, respiratory tract and larynx; less expressed in female system, uterus and cervix.

Localisation

Nuclear.

Function

KCMF1 is a transcription factor. Basic functions of the KCMF1 gene are (i) early gene up-regulation during growth factor-induced branching tubulogenesis, (ii) ubiquitination through intrinsic E3 ubiquitin ligase activity, and (iii) a possible role in ion channel activity.

Homology

The percent identity below represents identity of KCMF1 over an aligned region in UniGene.
Pan troglodytes: 97 (Percentage Identity)
Canis lupas familiaris: 91
Bos Taurus: 90
Mus musculus: 96
Gallus gallus: 93
Danio rerio: 85.

Implicated in

Ewing's sarcoma family of tumors (ESFT)

KCMF1 is down regulated by high constitutive CD99 (a cell surface glycoprotein) expression in ESFT. KCMF1 expression is inversely correlated with CD99 expression, as seen in a series of 22 primary ESFT. High CD99 expression levels contribute to the malignant properties of ESFT by promoting growth and migration of tumor cells.

Gastric cancer

KCMF1 (also known as FIGC) encode a RING finger protein, has intrinsic E3 ubiquitin ligase activity and promotes ubiquitination. KCMF1 contains a novel C6H2-type RING finger domain at the NH_2-terminal region, consensus sequence $CX_2C_{(7-11)}$ $CX_2CX_{A5}CX_2CX_{(5-9)}$ $HX_{(1-3)}$ H (X_A: acidic residues). Using differential display approach with basic fibroblast growth factor (b-FGF) inducible genes in gastric cancer cells, it was observed that FIGC upregulation in response to bFGF in gastric cancer. This suggests that FIGC might be implicated in gastric carcinogenesis through dysregulation of growth modulator.

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Gene Section
Review

METAP2 (methionyl aminopeptidase 2)

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DOI: 10.4267/2042/44778

Identity

Other names: MAP2; MNPEP; P67EIF2; p67; p67eIF2

HGNC (Hugo): METAP2

Location: 12q22

DNA/RNA

Description

The gene spans 41237 bp on strand plus; 11 exons; coding sequence: 1437 nucleotides.

Pseudogene

No known pseudogenes.

Protein

Description

Methionine Aminopeptidase 2. E.C. 3.4.11.18. Also known as methionyl aminopeptidase and peptidase M. Catalyzes release of N-terminal amino acids, preferentially methionine, from peptides and arylamides. Methionine aminopeptidases (MetAPs) are the enzymes responsible for the removal of methionine from the amino-terminus of newly synthesized proteins (Jackson and Hunter, 1970; Solbiati et al., 1999). The removal of methionine is essential for further amino terminal modifications (e.g., acetylation by N-alpha-acetyltransferase and myristoylation of glycine by N-myristoyltransferase, NMT) and for protein stability (Selvakumar et al., 2006; Selvakumar et al., 2007; Lowther et al., 2000; Bradshaw et al., 1998).

Expression

Ubiquitous expression. MetAP2 protein is highly expressed in all tissues.

Localisation

Cytoplasm.

Function

This protein function both by protecting the alpha subunit of eukaryotic initiation factor 2 from inhibitory phosphorylation and by removing the amino-terminal methionine residue from nascent protein (Jackson and Hunter, 1970; Solbiati et al., 1999). Increased expression of this gene is associated with various forms of cancer and the anti-cancer drugs fumagillin and ovalicin inhibit the protein by irreversibly binding to its active site (Selvakumar et al., 2006). This gene is a member of the methionyl aminopeptidase family and encodes a protein that binds to cobalt or manganese ions.

Homology

The human MetAP2 has DNA homology with Pan troglodytes (99.7%), Canis lupus familiaris (95%), Bos taurus (95.3%), Mus musculus (89.2%), Rattus norvegicus (89.2%), Gallus gallus (80.8%), Danio rerio (73.5%) and Arabidopsis thaliana (63.8%).

The human MetAP2 has protein homology with Pan troglodytes (100%), Canis lupus familiaris (98.1%), Bos taurus (96.6%), Mus musculus (95%), Rattus norvegicus (94.1%), Gallus gallus (87.6%), Danio rerio (82.9%), and Arabidopsis thaliana (66.2%).

Mutations

No mutations have been reported for MetAP2 that cause congenital anomalies.

Implicated in

Mesothelioma

Disease

Various reports suggested that MetAP2 plays an important role in the growth of different types of tumors. Malignant mesothelioma cells expressed higher MetAP2 mRNA levels compared to normal mesothelioma cells (Catalano et al., 2001). Transfection of mesothelioma cells with a MetAP2 anti-sense oligonucleotide revealed a time-dependent inhibition of cell survival and induced nucleosome formation. MetAP2 is a main regulator of the proliferative and apoptotic pathways in mesothelioma cells and MetAP2 inhibition may represent a potential target for therapeutic intervention in human mesothelioma (Catalano et al., 2001).

Lymphomas

Disease

A high level of MetAP2 was reported in malignant lymphomas exclusively in B-cell lymphoma subtypes (Kanno et al., 2002).

Colorectal adenocarcinoma

Disease

It has been reported that a high expression of MetAP2 in colorectal adenocarcinoma patients (Selvakumar et al., 2004a). Since myristoylation reaction is catalyzed by NMT, we reported that a cross-talk among the MetAP2, and NMT in HT29 cells (Selvakumar et al., 2004b). The expression of pp60c-src, MetAP2, and NMT was dependent on the cell density (Selvakumar et al., 2004b).

Esophageal squamous carcinoma

Disease

Microarray gene expression analysis of human esophageal squamous cell carcinomas revealed that MetAP2 was down-regulated when irradiated (Bo et al., 2004).

Hepatoma

Disease

Anti-sense of MetAP2 also induces apoptosis in rat hepatoma cells (Datta and Datta, 1999). A recent study suggested that fumagillin effectively inhibits both liver tumor growth and metastasis in rats in vivo (Sheen et al., 2005).

Neuroblastoma

Note

The angiogenesis inhibitor TNP470, O-(chloro-acetyl-carbamoyl) fumagillol, a synthetic analogue of fumagillin, suppressed the expression of MetAP2 in human neuroblastoma and thus, MetAP2 may be an important molecular target for human neuroblastomas (Morowitz et al., 2005). The intracellular enzyme MetAP2 became such a candidate target enzyme due to its inactivation by the widely investigated anticancer agent TNP470 (Abe et al., 1994; Adams et al., 2004; Griffith et al., 1997; Hu et al., 2006; Hu et al., 2007; Sin et al., 1997). Previously, inhibition of MetAP2 by TNP470 has been shown to activate p53 for cell-cycle arrest. In fact, the primary mouse embryonic fibroblasts were demonstrated to be sensitive to TNP470 and other MetAP2-specific inhibitors in a p53-dependent fashion. Several MetAP2 inhibitors were studied based on the inhibition of MetAP activity (Griffith et al., 1998; Antoine et al., 1994; Kusaka et al., 1994; Wang et al., 2000; Wang et al., 2003; Yeh et al., 2000; Zhang et al., 2000; Kim et al., 2004; Towbin et al., 2003).

Various cancer

Note

MetAP2 inhibitors

It has been reported that MetAP2 could function as an oncogene (Tucker et al., 2008). Furthermore, various Src family tyrosine kinases, ADP ribosylation factors and eukaryotic transcription elongation factor-2 were substrates of MetAP2 which plays a significant role in the progression of metastasis (Tucker et al., 2008). A derivative of the natural product fumagillin, TNP470 has been shown to be safe and effective in the treatment of solid tumors in several animal studies and preclinical trials. TNP470 entered human clinical trials for the treatment of AIDS-related Kaposi's sarcoma, metastatic breast cancer, androgen-independent prostate cancer, pediatric solid tumors, lymphomas, acute leukemia, advanced squamous cell cancer of the cervix, and metastatic renal carcinoma (Dezube et al., 1998; Kruger and Figg, 2000; Kudelka et al., 1997). Several MetAP2 inhibitors were studied based on the inhibition of MetAP activity (Griffith et al., 1998; Antoine et al., 1994; Kusaka et al., 1994; Wang et al., 2000; Yeh et al., 2000; Zhang et al., 2000; Kim et al., 2004). Previously, inhibition of MetAP2 by TNP470 has been shown to activate p53 for cell-cycle arrest (Yeh et al., 2000; Zhang et al., 2000).

The Src family kinases have been shown to play pivotal roles in cell-cycle progression, making them potential candidates to mediate the cell-cycle effects of MetAP inhibitors. MetAP2 plays a critical role in the proliferation of endothelial cells and certain tumor cells and thus serves as a promising target for anti-angiogenesis and anti-cancer drugs (Bo et al., 2004). The inhibition of MetAP2 expression in mesothelioma cells leads to cell death and that such apoptosis is avoided in cases where there is overexpression of Bcl-2 (Catalano et al., 2001). The upregulation of Bcl-2 in colorectal cancer is well established by various investigators (Rajala et al., 2000; Yu et al., 2003; Valassiadou et al., 1997).

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Gene Section
Review

MUC5AC (mucin 5AC, oligomeric mucus/gel-forming)

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Identity

Other names: LeB; MUC5; mucin 5AC; TBM

HGNC (Hugo): MUC5AC

Location: 11p15.5

DNA/RNA

Description

MUC5AC gene approximately extends 150 kb-long on the chromosome 11 in the region p15.5. The central region has sequences repeated in tandem (TR) with a consensus motif composed of 24 bp. The variable number of TR (VNTR) polymorphism is low compared with MUC2 and MUC6. The MUC5AC alleles present small differences in length, but the tandem repeat sequence is highly polymorphic and differs in length by 0.5-1 kb.

Transcription

To date, there is a discrepancy regarding the total number of exons present in MUC5AC gene.

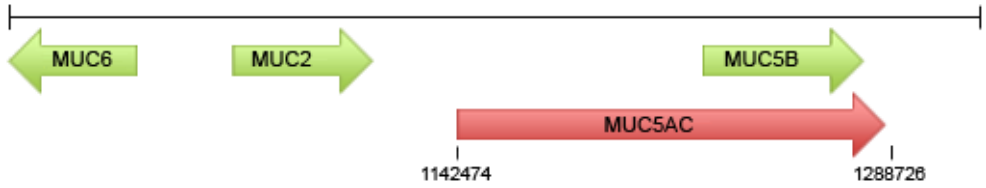
The full size 5' UTR of MUC5AC has not been yet determined, but it is estimated that the mRNA length is approximately 17.5 kb.

The 4 kb fragment upstream is essential for the cell-specific expression of MUC5AC. It contains a TATA box at -29/-23 and potential transcription factor binding sites are described for NFkappaB, Sp-1, GRE and AP-2. One CACCC box able to bind SP1 and initiate transcription has been identified.

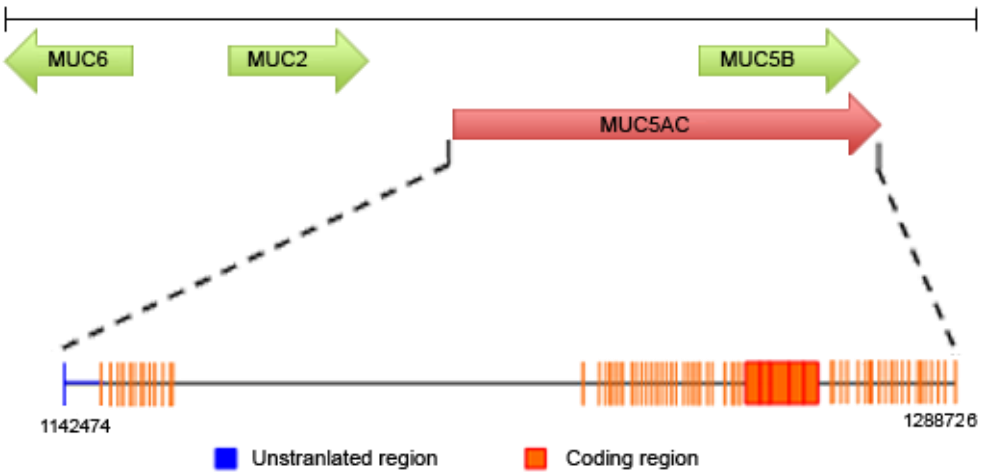
At present no splice variant forms have been reported.

The MUC5AC promoter has lower number of CpG dinucleotides compared to the other mucin genes located at 11p15, and no silencing of this gene could be explained by methylation.

Several factors have been shown to induce the transcription of MUC5AC such as cytokines, inflammatory mediators, growth factors, some bacterial exproducts and toxic agents like tobacco smoke and pollutants. Furthermore, it is reported that glucocorticoids downregulate MUC5AC expression.



Location of MUC5AC gene.



Genomic organization of MUC5AC gene (not to scale).

Protein

Note

MUC5AC is a secreted, gel-forming mucin with a high molecular weight (approximately 641 kDa). Up to 80% of the total weight is due to the large number of O-glycosilated chains attached to Thr and Ser residues in the TR sequence.

Description

MUC5AC is a polymeric mucin with a N-terminal region, a central region, and a C-terminal region.

At the N-terminal region, D1, D2, D' and D3 cysteine-rich domains (Cys) similar to von Willebrand factor (vWF) are present, and are responsible for the disulfide-mediated polymer formation.

At the central region, coded by a single large exon, nine Cys domains are located: Cys1 to Cys5 are interspersed by domains rich in Ser, Thr and Pro (STP) with no repetitive sequences, whereas Cys5 to Cys9 domains are interspersed by four TR domains. The consensus repetitive sequence most frequent is TTSTTSAP containing a high number of potential O-glycosilation sites. The C-terminal region has the cysteine-rich vWF-like domains D4, B, C and CK. The CK domain mediates the formation of disulfide-linked dimmers by an autocatalytic process. Towards the C-terminus, contains an autocatalytic protein-cleavage site at the motif GDPH.

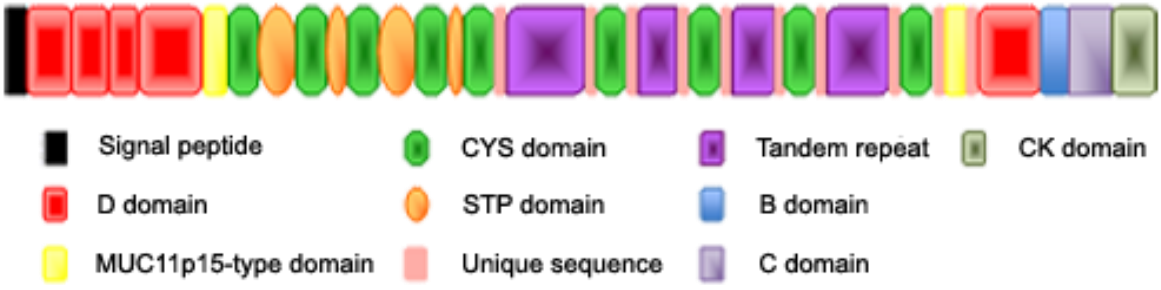
Expression

MUC5AC was initially isolated from a human tracheobronchial cDNA library, and it is highly expressed in the goblet cells of the respiratory epithelium. MUC5AC is also highly detected in the superficial gastric epithelium, and it is also expressed in pancreas, endocervix and gallbladder.

Under pathological conditions, MUC5AC expression can be altered, as it is reported below. The changes associated with neoplastic transformation and inflammatory diseases, can be induced by the activation of signaling pathways in response to several factors such as inflammatory cytokines, growth factors, and bacterial products.

Function

MUC5AC is a gel-forming mucin and it is a major constituent of the mucus lining mainly the respiratory tract and the stomach. In the surface of the normal respiratory epithelium, MUC5AC is one of the major contributors to the rheological properties of the mucus that has a critical role in the defense against pathogenic and environmental challenges. In the gastric mucosa, MUC5AC and MUC6 are the main components of the protective layer over the surface, and act as a selective diffusion barrier for HCl. MUC5AC also protect the gastric epithelium from Helicobacter pylori, and the glycan structures on MUC5AC, Le^b and sialyl Le^x, act as ligands for the bacterium competing with the ligands located on the epithelial cell surface.



Schematic representation of MUC5AC peptide structure (not to scale).

Homology

Several orthologues of MUC5AC have been identified in Mus musculus, Rattus norvegicus, Canis lupus familiaris, Equus caballus and Pan troglodytes. The chicken, horse and mouse Muc5AC have a similar domain structure. Murine N-terminal and C-terminal regions showed striking similarities with human MUC5AC, whereas the TSP domains are specific for species. Furthermore, MUC5AC tissue-specific expression is conserved in murine and equine organisms.

Implicated in

Gastric cancer

Disease

Gastric cancer remains the second leading cause of cancer related deaths and the fourth most common cancer in the world, although its incidence is gradually decreasing.

Prognosis

Gastric neoplastic transformation is associated with a decreased expression of MUC5AC. MUC5AC is used as a marker of gastric phenotype in stomach tumours, and its expression is associated with antral carcinomas. MUC5AC expression have been also related to tumour stage: it is expressed in early carcinomas while advanced gastric cancers present reduced levels of MUC5AC.

Colon cancer

Disease

Colorectal cancer is one of the commonest cancers and the third leading cause of cancer death. However, its incidence has decreased due to a most effective intervention and life-style changes in the western countries.

Prognosis

MUC5AC has been detected in precancerous lesions as well as in colon cancer, and this ectopic expression may represent a nonspecific repair function of the colon cells to compensate for damage to barrier function.

Endometrial adenocarcinoma

Disease

Endometrial adenocarcinoma is the most common malignant neoplasm of the female genital tract in developed countries, and it occurs predominantly after menopause.

Prognosis

Increased levels of MUC5AC have been found in endometrial adenocarcinoma compared to normal endometrium and endometrial hyperplasia, suggesting a potential role for MUC5AC as a marker of endometrial neoplastic transformation.

Pancreatic cancer

Disease

Pancreas cancer is a very aggressive tumor with a 5-year survival of less than 5%, and approximately 85% of them correspond to ductal adenocarcinomas.

Prognosis

The ectopic expression of MUC5AC in pancreas ductal adenocarcinomas is an early event, already detected in the PanIN1A (pancreatic intraepithelial neoplasia 1A) stage. The MUC5AC expression is maintained to reach 85% of the pancreatic tumors.

Biliary tract cancer

Disease

Biliary tract carcinomas are uncommon tumors that includes cholangiocarcinomas and gallbladder carcinomas. These tumors has a poor prognosis: more than 80% of the patients are unresectable with a 6-9 month survival, and this rate is increased to 5-year after surgery.

Prognosis

MUC5AC is detected at very low levels in biliary tract carcinomas and its expression do not correlate with the clinical stage of the tumor. However, the detection of MUC5AC in sera from biliary tract carcinoma patients, associated to the MUC4 expression in the tumor, have been suggested as a highly specific markers for this neoplasia.

Airways pathologies: asthma, cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD) and nasal polyps (NP) in upper airways

Disease

Asthma has grown, particularly among children, in prevalence and it is characterized by an airflow obstruction caused by inflammation-induced changes in airway smooth muscle contraction and by mucus hypersecretion.

CF is characterized by impaired mucociliary clearance, leading to chronic airflow obstruction and to recurrent infections.

COPD is the fourth leading cause of death in the U.S. and Europe. Submucosal gland hypertrophy and airway surface metaplasia are the hallmarks of COPD.

NP is an inflammatory disease whose aetiology is still unknown and affects 2-4% of general population.

Prognosis

MUC5AC levels have been found to be increased in asthma, CF and COPD that alter the transport properties of the mucus gel and provide a favourable environment for pathogens. In NP a decrease of MUC5AC levels are detected.

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Gene Section
Review

NNMT (nicotinamide N-methyltransferase)

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Identity

Other names: EC 2.1.1.1

HGNC (Hugo): NNMT

Location: 11q23.2

DNA/RNA

Description

The human NNMT gene is approximately 16,5 kb in length, consists of 3 exons and 2 introns, and is mapped to chromosome 11q23.1. The first intron is 1,240 bp in length, while the second is approximately 14 kb long. The sequence of the 5'-untranslated region (UTR) of the NNMT cDNA is present in exon 1, while the sequence of the cDNA 3'-UTR is present in exon 3 (Aksoy et al., 1995). The initiation of transcription for the human NNMT gene occurs at or near a nucleotide located -108 bp upstream from the translation initiation codon and approximately 30 nucleotides 3'-downstream from an atypical TATA box element (TCTAAA) (Aksoy et al., 1995). The 3'-UTR ends with a poly(A) tract, and the polyadenylation signal ATTAAA is located 19 nucleotides upstream from the poly(A) region (Aksoy et al., 1994).

A strong promoter is located within the initial 700 bp of 5'-flanking sequence of the human NNMT gene (Yan et al., 1999).

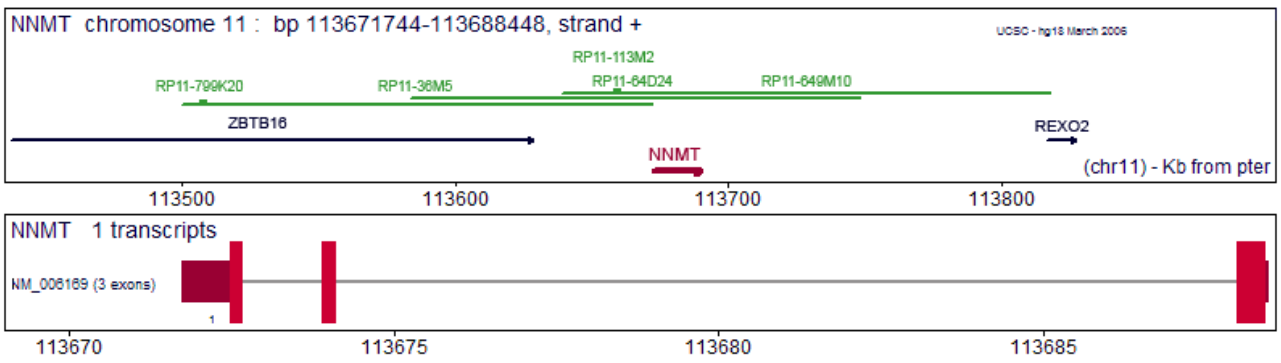
Transcription

Transcription of the human NNMT gene produces a full-length mRNA transcript of 1,579 bp.

It was recently shown that NNMT expression in some thyroid cancer cells may be regulated by hepatocyte nuclear factor beta (HNF-1beta).

HNF-1beta is able to function as a transcription activator of NNMT promoter, binding to specific sites in the basal promoter region (between nucleotides -148 and -162 relative to the translation initiation codon) (Xu et al., 2005).

In BHP 18-21 papillar thyroid cancer cells, the histone deacetylase inhibitor depsipeptide reduces NNMT mRNA level through down-regulation of transcription activator HNF-1beta (Xu et al., 2006).



Structure of human Nicotinamide N-methyltransferase (NNMT) gene and transcript. NNMT gene is encoded on 3 exons which span 16,704 bp at chromosome 11 (nucleotides 113,671,745-113,688,448). Exons are depicted as red boxes separated by intron sequences (solid lines). Brown boxes contain the untranslated sequences (5'-UTR and 3'-UTR).

Enhanced NNMT expression has been also correlated to activation of STAT3 in Hep-G2 liver cancer cells stimulated with IL-6 and in colorectal cancer tissues (Tomida et al., 2008).

Pseudogene

No pseudogene of NNMT was reported in human.

Protein

Note

The NNMT gene encodes a full-length monomeric protein of 264 amino acids with a predicted molecular weight of 29.6.

Description

Nicotinamide N-methyltransferase (NNMT, EC 2.1.1.1) is an S-adenosyl-L-methionine (Ado-Met) - dependent enzyme that catalyzes the methylation of nicotinamide and other pyridines to form pyridinium ions (Rini et al., 1990).

NNMT was first identified by cDNA cloning from the liver and the protein is predicted to be present in the cytosol (Aksoy et al., 1995).

A radiochemical microassay was developed by Rini et al. (1990) to study selected characteristics of NNMT activity in human liver preparations.

These studies suggested that human hepatic NNMT is a cytoplasmic enzyme with a pH optimum of approximately 7.4. Apparent K_m values for its two substrates, nicotinamide and S-adenosyl-L-methionine, are 347 and 1.76 µmol/l, respectively. The enzyme activity is inhibited by the reaction products, N^1-methylnicotinamide and S-adenosyl-L-homocysteine, while its activity is not affected by inhibitors of other methyltransferases. Basal enzyme activities, detected in human liver biopsy samples, show large individual variations with a bimodal frequency distribution.

Aksoy et al. (1994) set out to clone and express a cDNA for human liver NNMT to study molecular mechanisms involved in the regulation of individual differences of NNMT activity in humans. The cloning strategy involved purification of human liver NNMT, leading to partial amino acid sequence, followed by direct PCR-based cloning with the use of the rapid amplification of cDNA ends (RACE). The combined use of these techniques resulted in the isolation of a human liver NNMT cDNA that was 969 bp long, with a 792-bp open reading frame that encoded a 264-amino acid protein with a calculated molecular mass of 29,600 Daltons. Transient expression of the protein encoded by this cDNA demonstrated that it catalyzed the methylation of nicotinamide and had biochemical characteristics similar to, or identical with, those of human liver NNMT.

Recently, 2-DE experiments revealed that NNMT exists in multiple spots in gastric tissues and the presence of multiple NNMT spots is highly specific to cancer tissues of stomach. This suggests that NNMT could receive a post-translational modification in cancer-specific manner, but the mechanism by which NNMT is modified is still unknown (Lim et al., 2006).

Expression

NNMT is predominantly expressed in the liver, while a lower expression has been detected in the kidney, lung, skeletal muscle, placenta, heart, and brain. The N-methylation of nicotinamide is known to be altered in some diseases including Parkinson's disease (Green et al., 1991), hepatic cirrhosis (Cuomo et al., 1994), COPD (chronic obstructive pulmonary disease) (Debigarè et al., 2008), atherosclerosis (Mateuszuk et al., 2009), etc. The abnormal expression of NNMT has been identified in several kinds of tumors, such as glioblastoma (Markert et al., 2001), stomach adenocarcinoma (Jang et al., 2004; Lim et al., 2006), papillary thyroid cancers (Xu et al., 2003; Xu et al., 2005), renal carcinoma (Yao et al., 2005; Sartini et al., 2006), oral squamous carcinoma (Sartini et al., 2007), colorectal cancer (Roessler et al., 2005), hepatocellular carcinoma (Kim et al., 2009), bladder cancer (Wu et al., 2008), lung cancer (Tomida et al., 2009) and pancreatic cancer (Rogers et al., 2006).

Localisation

NNMT is a cytosolic enzyme.

Function

NNMT is an important cytosolic methyltransferase, belonging to Phase II Metabolizing Enzymes. The enzyme catalyzes the N-methylation of nicotinamide, pyridines and other structural analogs, playing a crucial role in the biotransformation and detoxification of many xenobiotic compounds. In fact, the metabolism of drugs, toxic chemicals, hormones, and micronutriens is an important topic in the fields of pharmacology and endocrinology, and it is often implicated in many diseases and pathophysiological processes, such as cancer and resistance to chemotherapy (Szakàcs et al., 2004). N-methylation is one method by which drugs and other xenobiotic compounds are metabolized by the liver and the enzyme NNMT is responsible for this activity which uses S-adenosyl-L-methionine as the methyl donor. The NNMT reaction yields two products: S-adenosyl-L-homocysteine and N^1-methylnicotinamide. S-adenosyl-L-homocysteine is converted into homocysteine by S-adenosyl-L-homocysteine hydrolase. N^1-methylnicotinamide is mostly excreted into urine and partly further converted via catalysis by aldehyde oxidase to N^1-methyl-2-pyridone-5-carboxiamide and N^1-methyl-4-pyridone-5-carboxiamide, which are also excreted into urine. N-methylation has been proposed as a metabolic pathway for nicotinamide excretion, and NNMT is the only enzyme known to utilize nicotinamide as methyl acceptor substrate. Therefore, NNMT could participate in the regulation of nicotinamide intracellular levels, modulating its excretion after N-methylation.

Nicotinamide, the amide of nicotinic acid, is the precursor of the coenzyme beta-nicotinamide adenine dinucleotide (NAD), an essential cofactor for several oxidoreductases, which participates in a wide range of biological processes, including energy supply, cellular resistance to stress or injury, and longevity (Williams et al., 2005). In addition, several enzymes, which use NAD as substrate can be inhibited by nicotinamide. Because of this type of product inhibition, the salvage and/or elimination of nicotinamide are crucial steps in NAD metabolism and the enzyme NNMT could be involved in controlling these cellular events. NNMT activity may also play a role in regulating biological processes related to N^1-methylnicotinamide. It has recently become apparent that it possesses anti-inflammatory (Bryniarski et al., 2008), anti-thrombotic (Chlopicki et al., 2007), vasoprotective (Bartus et al., 2008), and gastroprotective (Brzozowski et al., 2008) properties. NNMT was characterized by Cantoni in 1951 (Cantoni et al., 1951) and it is highly expressed in liver where its activity displays a 5-fold variation among individuals and has a bimodal frequency distribution. This observation raises the possibility that this enzyme activity may be regulated by a genetic polymorphysm. Such a polymorphism could have functional implications for individual differences in the metabolism and therapeutic effect of drugs (Aksoy et al., 1994) and in the formation of potentially toxic pyridine metabolites. Moreover, heightened NNMT activity was reported in many kinds of tumours. The up-regulation of this enzyme suggests a possible role of NNMT in cancer growth, migration, and metastasis (Sartini et al., 2007; Wu et al., 2008). However, the biological significance of alterations in NNMT activity in various pathological conditions remains largely unknown.

Homology

NNMT belongs to the NNMT/PNMT/TEMT family. The amino acid sequence of the protein encoded by human liver NNMT cDNA is 52% identical to that of mouse thioether S-methyltransferase (TEMT) and 37, 39, 38 and 39% identical to those of human, rat, mouse, and bovine phenylethanolamine N-methyltransferase (PNMT), respectively (Aksoy et al., 1994).

Human NNMT shows a very high level of identity to other non-human NNMTs: Sus scrofa (88%), Rattus norvegicus (87%) and Mus musculus (85%).

Mutations

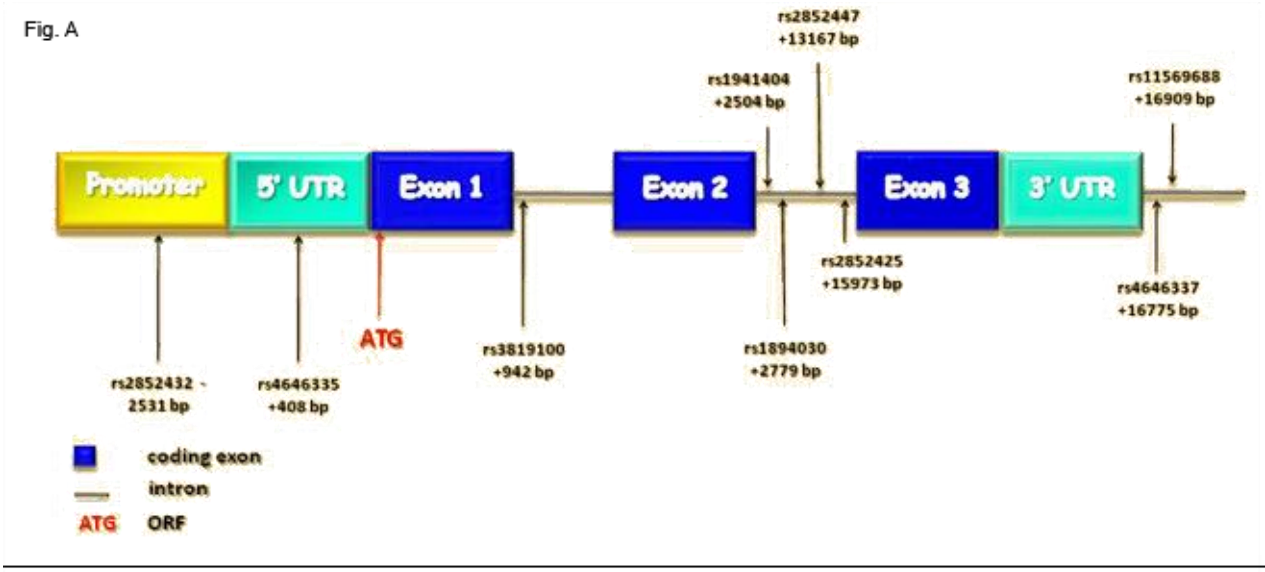
In humans NNMT is highly polymorphic. About a hundred of polymorphisms, most of which are SNPs, have been identified. The figure A below shows the positions of investigated NNMT polymorphisms, taking as a reference the start site of transcription in 5'UTR region.

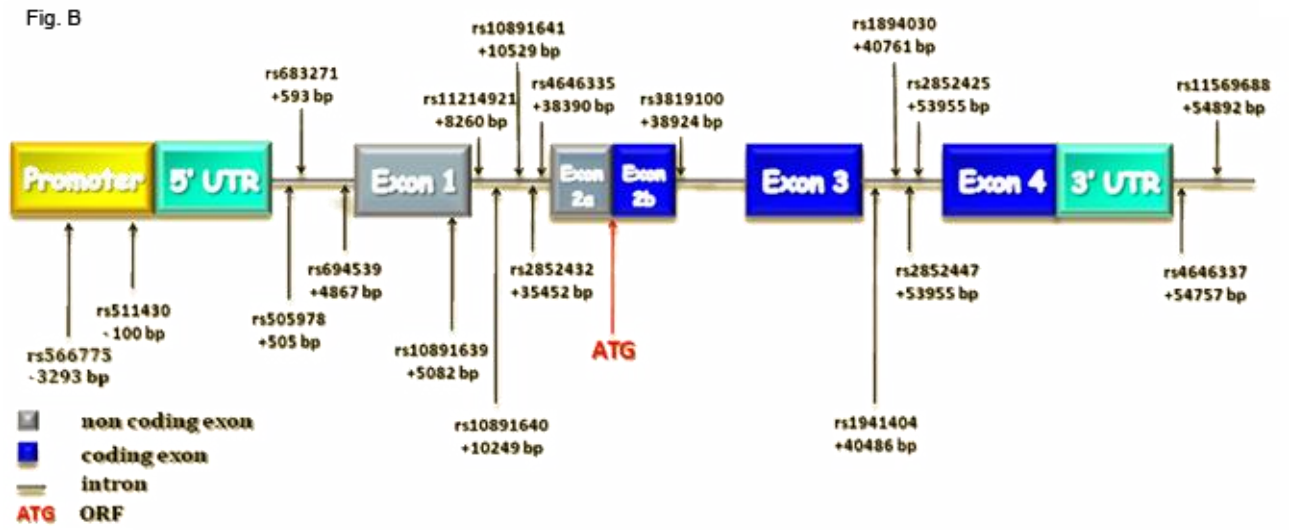
An alternative NNMT gene organization is available at UCSC web site. This sequence, depicted in the figure B below, displays another putative 5'UTR region located at 38,732 bp upstream of the open reading frame.

NNMT is one of at least 39 SAM-dependent methyltransferases and is involved in different metabolic pathways such as folate and homocysteine ones. Several independent studies have investigated some NNMT polymorphisms that could reflect differences in catalytic activity or in transcriptional efficiency of gene; however data available in literature are contrasting because some archived SNPs are very rare substitutions or limited to some ethnic group. Yan (Yan et al., 1999) and Smith (Smith et al., 1998) detected no association between SNPs (either insertion/deletion events within exons or into 5' flanking region) and NNMT activity variation in healthy population. Saito (Saito et al., 2001) suggested that some SNPs in the NNMT 5' flanking region may influence its transcriptional efficiency. Several studies about association between NNMT genetic variations and alteration of cellular pathways are present in literature.

Homocysteine (Hcy) pathway. In humans, the only source of Hcy is the demethylation of methionine, through several methyl transferase activities, such as NNMT. Hyperhomocysteinemia is a condition characterized by high plasma level of Hcy and it is implicated in several diseases, as Alzheimer and other clinical status such as atherosclerosis, ischemic strokes (Furie et al., 2006) and osteoporosis. The causes of hyperhomocysteinemia are both genetic and environmental (e.g.: life-style, sex, age), but genetic basis are still poorly understood. Different studies have investigated an association of NNMT polymorphisms with hyperhomocysteinemia. Souto (Souto et al., 2005) carried out the GAIT (Genetic Analysis of Idiopathic Thrombophilia) Project in a Spanish population, where 10 SNPs of NNMT gene were investigated. The results of this study suggested a strong correlation between plasma Hcy level and a specific haplotype. Because these genetic variants are in non-coding regions, they could influence the regulation of transcription but evidence on the functionality of the NNMT polymorphisms is still conflicting.

A consistent study was carried out by Ling Zhang (Zhang et al., 2007) in about three hundred healthy japanese workers. Authors focused on a specific NNMT polymorphism (rs694539) localized in the first intron. The results confirmed that SNPs in non-coding regions affected the regulation of transcription, but they weren't the main determinant





of the plasma Hcy levels, because other factors were involved: age, sex, plasma folate levels and the associations with MTHFR polymorphisms.
The association between NNMT polymorphisms and hyperhomocysteinemia has been investigated by Bathum (Bathum et al., 2007). Six hundred and three danish adult twin pairs were included in the study. Experimental results suggested that MTHFR C677T is the only SNP responsible for the disease progression, leaving only minor influence to other genetic variations.

Implicated in

Thyroid cancer

Note

Gene expression profiles obtained by DNA microarray showed NNMT overexpression in papillary thyroid carcinoma cells, but not in primary goiter cell O4 and in other cancer cell lines (follicular, medullary, and anaplastic). The results were validated using RT-PCR and Northern Blot analysis. High levels of NNMT enzyme activity were detected in eight of ten papillary lines, and in three of six of the follicular cell lines tested, while in the anaplastic and medullary cancer cell lines, as well as in primary thyroid cultures, and normal thyroid tissue enzyme activity was low or undetectable. Immunohistochemical staining of human papillary carcinoma specimens for NNMT showed positive and strong staining in 94% of the specimens, but not in the normal follicular cells (Xu et al., 2003). Even though the molecular mechanism leading to NNMT over-expression is at present unknown, the hepatocyte nuclear factor-1beta (HNF-1beta), expressed in many papillary cancer cell lines, seems to be involved in the activation of NNMT transcription (Xu et al., 2005). Moreover, the repression of NNMT observed in BHP 18-21 papillar thyroid cancer cells treated with depsipeptide, a histone deacetylase inhibitor, is at the transcription level through downregulation of transcription activator HNF-1beta (Xu et al., 2005).

Gastric cancer

Note

The differential proteome profile of gastric cancer obtained through a series of 2-DE experiments combined with peptide mass finger printing analysis by MALDI-TOF mass spectrometry showed overexpression of NNMT in tumour tissues compared to the adjacent normal mucosa. Moreover, Western Blot revealed that NNMT exists as a single spot in gastric tissue, while four to five spots (with different pI values and similar MW) were detected in most gastric tumour tissues. The pattern of multiple NNMT spots is highly specific to tumour tissue and might lead to hypothesize that NNMT in gastric cancer carries a post-translational modification, possibly phosporylation (Jang et al., 2005; Lim et al., 2006).

Colorectal cancer

Note

Roessler et al. (2005) found that NNMT is upregulated in malignant tissues compared with normal colonic epithelium and they suggested that NNMT serum levels could be useful as a biomarker in the early detection of patients with colorectal cancer. In addition, immunostaining of NNMT and phospho-Stat3 in colon cancer tissues showed that enhanced expression of NNMT is correlated with activation of Stat3 (Tomida et al., 2008).

Renal cell carcinoma

Note

Elevated levels of NNMT mRNA were first detected in clear cell renal cell carcinoma (RCC) by Yao et al. (2005), who examined the gene expression profiles of several normal kidneys and several cancerous specimens, although adjacent normal tissue was not available. NNMT expression was further investigated in paired tissue samples from cancerous and non-cancerous parts of the kidneys of patients with clear cell /chromophobe renal cell carcinoma and with oncocytoma. The authors observed significant NNMT overexpression in 100% of ccRCCs tested, with 41-fold higher mean expression in cancerous tissue than in adjacent non-cancerous tissue. In keeping with NNMT mRNA level results, in ccRCC NNMT protein was found upregulated, and a marked increase in its enzymatic activity was detected (Sartini et al., 2006). The increase in the level of NNMT correlated inversely with tumour size, thus suggesting that NNMT activity may be significant in an early stage of malignant transformation.

Prognosis

NNMT mRNA levels did not correlate with survival (Yao et al., 2005).

Oral cancer

Note

Compared with normal mucosa, favorable oral squamous cell carcinomas (OSCCs) (N0) exhibited significantly increased expression of NNMT, while no marked enzyme expression alterations between tumour and adjacent normal mucosa were detected in most of the unfavorable OSCCs (N+). The low NNMT expression detected in subjects with metastasis supports the hypothesis that NNMT plays a role in tumour expansion, and tumours which downregulate this enzyme may be able to evade immunosurveillance and grow.

Prognosis

NNMT mRNA levels appear to be inversely related to pT and pathological staging, suggesting the possibility of this enzyme as a prognostic factor (Sartini et al., 2007). Kaplan-Meier analysis shows an improved overall survival rate for patients bearing tumours with higher NNMT expression levels than patients with tumours with lower NNMT expression, although without reaching statistical significance (Emanuelli et al., 2009, in press).

Insulinoma and pancreatic cancer

Note

NNMT was found to be downregulated in human insulinoma, when compared with normal islets preparations. Its underexpression was associated with reduced TGFbeta1 mRNA levels, being NNMT a target gene of this cytokine, which acts via the activation of Smad proteins (Nabokikh et al., 2007). Conversely, NNMT seems to be overexpressed in the malignant pancreatic ductal carcinoma. Gene expression alterations were explored by profiling the RNA isolated from pancreatic juice of patients with pancreatic cancer and patients with non-neoplastic disease (Rogers et al., 2006).

Lung cancer

Note

Patients with non-small cell lung cancer (NSCLC) exhibit increased NNMT serum levels compared to patients with chronic obstructive pulmonary disease (COPD) and healthy donors (Tomida et al., 2009). ROC curves were employed to evaluate the sensitivity and specificity of NNMT serum levels measurement for the detection of lung cancer. The results obtained seem to indicate that NNMT is slightly better than the currently available lung cancer biomarker CEA, although both the sensitivity and specificity displayed appear relatively low. No significant correlation between NNMT and CEA serum levels were found. Therefore, the measurement of serum levels of both markers could contribute to improve sensitivity for detection of NSCLC.

Liver cancer

Note

A large number of Hepatocellular carcinoma (HCC) specimens were analyzed by real-time reverse

transcription PCR. NNMT mRNA level appeared markedly reduced in tumour samples compared to the surrounding healthy tissue. Moreover, NNMT expression was significantly associated with tumour stage.

Prognosis

NNMT mRNA levels appear to be inversely related to overall survival time as well as to disease-free survival time, suggesting the posssibility of this enzyme as a prognostic factor (Kim et al., 2009). It has been found that nuclear factor-interleukin 6 and STAT3 induce NNMT promoter activity in the transformed Hep-G2 cells (Tomida et al., 2008). Therefore, the prognostic power of NNMT mRNA level determination could be improved by the simultaneous measurement of related regulatory molecules (Kim et al., 2009).

Bladder cancer

Note

NNMT expression in the radioresistant bladder carcinoma cell line MGH-UI has been reported to be higher than that observed in its radiosensitive subclone S40b (Kassem et al., 2002). The NNMT possible involvement in determining radioresistance might be related to its catalytic activity, which could lead to a decrease of intracellular levels of nicotinamide, compound known as a radiosensitizer able to enhance the damage produced by radiation treatments.

Prognosis

Recently, transcriptional profiling of several bladder cancer cell lines and human bladder cancers identifies NNMT as gene involved in cancer migration, while being associated with tumour stage in patients. Moreover, NNMT silencing appears to decrease cell proliferation, making the enzyme a promising target for chemotherapy (Wu et al., 2009).

Acute lymphoblastic leukemia (ALL)

Note

Folate pathway. Folate metabolism is essential for cellular functioning because it provides methyl donors for some important biochemical reactions such as methylation of homocysteine. Genetic variations of some folate related genes have been associated with low folate levels, influencing the risk of cancer.

de Jonge (de Jonge et al., 2009) investigated the association between folate pathway polymorphisms and susceptibility to lymphoid leukemia in 245 pediatric ALL patients. Authors demonstrated that specific polymorphisms of MTHFR (C677T), RFC1 (G80A) and NNMT (IVS C-151T) and their association are related to ALL risk. Specifically, NNMT IVS -151TT and NNMT IVS -151 CT+TT/ RFC1 80AA subjects showed a 2,2 and 4,2-fold increased ALL risk, respectively, while NNMT IVS -151CC/ MTHFR 677CT+TT patients exhibited a 2-fold reduction in ALL risk. Authors suggested that the mechanism of this increased risk is related to a reduction of cellular folate uptake and change in methylation status.

Chronic obstructive pulmonary disease (COPD)

Note

Chronic obstructive pulmonary disease (COPD) is an inflammatory disorder characterized by progressive bronchial obstruction and often associated with peripheral muscle wasting. Microarray analysis performed on vastus lateralis muscle tissue revealed that NNMT was up-regulated (5.8-fold than normal subjects) in patients with COPD and muscle atrophy. Real-Time PCR analysis confirmed an higher expression of NNMT in patients with COPD. Statistical analysis revealed a positive correlation between NNMT deltaC_t and FEV_1 (forced expiratory volume in 1 s). This correlation suggests that the airway obstruction, through NNMT up-regulation, could lead to an alteration of energy metabolism in these patients (Debigarè et al., 2008).

Parkinson's disease

Note

NNMT has been recently demonstrated to be present in the brain with a regional distribution, being in relatively high concentration in spinal cord and cortex and present in lower concentration in substantia nigra. In Parkinson's Disease (PD) brain, NNMT is present in increased concentrations. This enhanced NNMT activity seems to be responsible for the production of toxic N-methylpiridinium compounds that have been advanced as possible neurotoxins underlying nigrostriatal degeneration. In fact the enzyme displays a wide substrate specificity that enables it to convert a large range of substrates to their corresponding pyridinium ions, involved in Complex I poisoning, which leads to diminished ATP production. This reduced ATP synthesis may be also related to NNMT upregulation, in that high enzyme levels lower the amount of nicotinamide available for NADH synthesis (Williams and Ramsden, 2005; Williams et al., 2005).

Abdominal aortic aneurysm (AAA)

Note

NNMT and its genetic variants are candidate risk factors for AAA.

Giusti and co-workers, using a multiplex PCR oligonucleotide extension approach (Giusti et al., 2008b), investigated the correlation between some genetic variants of fifteen genes involved in the methionine metabolism (including NNMT) and AAA (Giusti et al., 2008a) in 423 subjects affected from AAA. They demonstrated that only seven genes, including NNMT, have at least one specific haplotype that represents a probable risk factor for AAA. They found also that the influence of the single gene in this pathology is independent from the role in homocysteine metabolism.

Spina bifida

Note

Moderate hyperhomocysteinemia is a risk factor for Neural Tube Defects (NTDs).

Several SNPs of NNMT gene have been analysed in 252 cases (infants with spina bifida) and 335 controls (non malformed infants) by Lu (Lu et al., 2008). Findings showed no association between any single genetic variation and NTDs. Only a specific haplotype was significantly associated with decreased risk for spina bifida in non Hispanic Whites.

Congenital heart defects (CHDs)

Note

CHDs seem to be multifactorial phenomenon due to polymorphisms of NNMT gene, maternal nutrition and medicine use in the peri-conception period.

van Driel (van Driel et al., 2008) investigated the SNP rs694539, probably involved in the regulation of NNMT transcription (Souto et al., 2005). In the analyses, he included two hundred and ninety-two cases and three hundred and sixteen control families. No association between NNMT polymorphism and risk of CHDs was detected. On the other hand, children with the combination of heterozygous or mutant genotype for rs694539, peri-conception medicine use and low dietary nicotinamide intake showed eight-fold increased risk for CHDs.

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Atlas of Genetics and Cytogenetics in Oncology and Haematology

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Gene Section
Mini Review

RBBP7 (retinoblastoma binding protein 7)

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Identity

Other names: RBAP46; RbAp46; RBBP-7; MGC138867; MGC138868

HGNC (Hugo): RBBP7

Location: Xp22.2

Note: RBBP7 is located at contig NP_002884.1 of GenBank. The retinoblastoma binding protein 7 gene symbol for human is RBBP7 whereas the symbol for the same gene for rat and mice is Rbbp7. RBBP7 was one of the two most abundant proteins from HeLa cell lysates that were specifically retained by an RB1 affinity column (Qian et al., 1993). Qian and Lee (1995) isolated cDNAs encoding RBBP7 by screening a HeLa cell cDNA expression library with monoclonal antibodies against RBBP7, which they called as RbAp46. Southern blot analysis indicated that the human genome contains a single copy of the RBBP7 gene.

DNA/RNA

Description

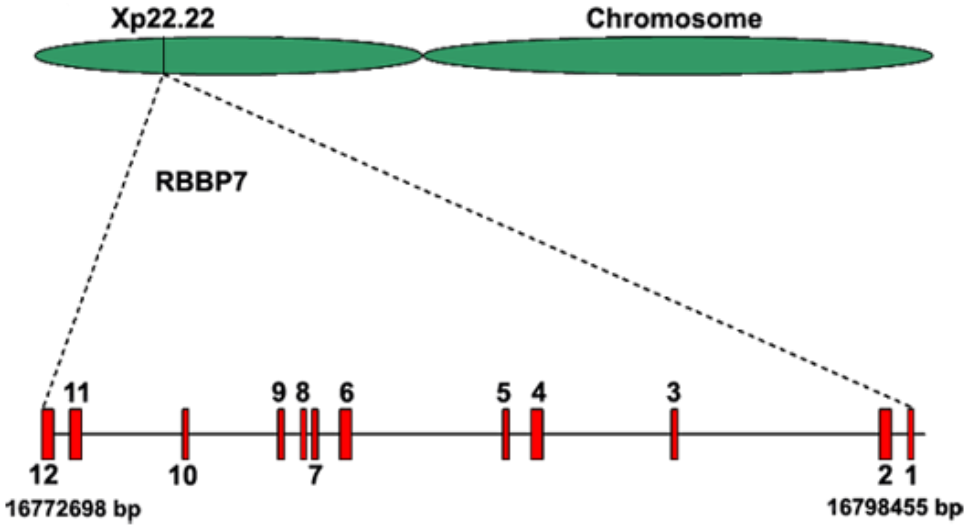
DNA size 27.75 kb; mRNA size 2021 bp; 12 exons.

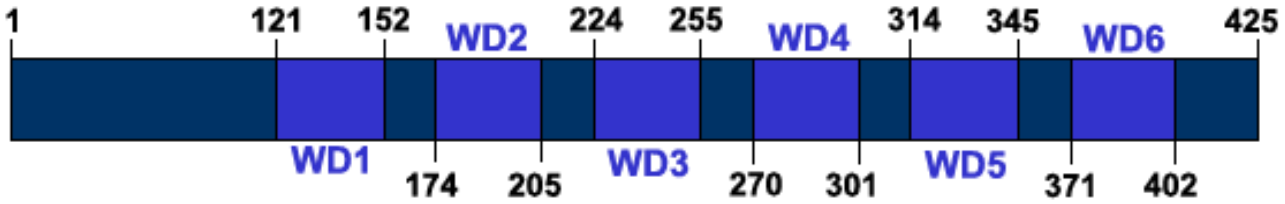
Protein

Description

425 amino acids; 47.82 kDa protein.

Post translational modifications: Phosphorylation enhances DNA binding. Phosphorylation occurs at position 95, 99, 354, 413 (Serine) and 416 (Threonine). Acetylation brings in a negative charge, acting to neutralise the positive charge on the histones and decreases the interaction of the N termini of histones with the negatively charged phosphate groups of DNA. As a consequence, the condensed chromatin is transformed into a more relaxed structure which is associated with greater levels of gene transcription.





The acetylation sites are: at 2 (Alanine), and 119 (Lysine).
Isoform: The following isoforms have been identified:
- RBBP7.iApr07
- hPA25320.1 (469 aa)
- hPA25320.2 (425 aa)
- hPA25320.3 (410 aa)
- hPA25320.7 (420 aa)

Expression

It is widely expressed.

Localisation

Nucleus.

Function

This protein is an ubiquitously expressed nuclear protein and it belongs to a highly conserved subfamily of WD-repeat proteins. It is found among several proteins that bind directly to retinoblastoma protein, which regulates cell proliferation. The encoded protein is found in many histone deacetylase complexes, including mSin3 co-repressor complex. It is also present in protein complexes involved in chromatin assembly, which include the type B histone acetyltransferase (HAT) complex, which is required for chromatin assembly following DNA replication; the core histone deacetylase (HDAC) complex, which promotes histone deacetylation and consequent transcriptional repression; the nucleosome remodeling and histone deacetylase complex (the NuRD complex), which promotes transcriptional repression by histone deacetylation and nucleosome remodelling. This protein can interact with BRCA1 tumor-suppressor gene and may have a role in the regulation of cell proliferation and differentiation.

Homology

The percent identity below represents identity of RBBP7 over an aligned region in UniGene.
- M. musculus : 100 (percentage identity)
- C. lupus familiaris : 100
- B. taurus : 100
- R. norvegicus : 100
- G.gallus : 96.2
- D. rerio : 94.4

Mutations

Note
Two types of mutation have been detected in the RBBP7 gene. A827G is a silent mutation and the other one is a missense type of mutation that changes N276S.

Implicated in

Breast cancer

Note
RBBP7 (also known as RbAp46) overexpression has shown to inhibit the tumorigenicity of neoplastigenic breast epithelial cells (Li et al., 2003). RBBP7 activates stress-induced apoptosis, the JNK-dependent apoptotic cell death, possibly through upregulation of GADD45 (Growth arrest- and DNA damage-inducible 45). GADD45 binds and activates MAPKKK MTK1/MEK4, the upstream regulator of JNK, triggering JNK-dependent apoptosis. Thus, overexpression of RBBP7 facilitates stress-induced apoptosis and suppresses tumorigenicity of neoplastigenic breast epithelial cells.

Leukemia

Note
Expression level of RBBP7 in initial acute leukemia has been found to be significantly higher than in chronic myelogenous leukemia. The Wilms tumor suppressor gene (WT1) expression level was also correlated with RBBP7 expression. WT1 encodes a zinc finger transcription factor that regulates transcription of its downstream gene. RBBP7 is a downstream effector of WT1 gene, and acts in a similar manner as WT1 does. It has been seen that high expression of RBBP7 suppresses the tumorigenicity of neoplastic breast epithelial cells but its overexpression possibly may induce leukemia. This phenomenon suggests that the regulatory pathway for RbAp46 gene expression in acute leukemia may be different from that in solid tumor.

Human embryonic kidney (HEK) 293 cell tumorigenesis

Note
High levels of RbAp46 suppress the tumorigenicity of adenovirus-transformed human embryonic kidney 293 cells. High level of RbAp46 resulted in G2/M cell population and augmented apoptosis in serum starved cells. It is possible that overexpression of RbAp46 may interfere with normal cell cycle and/or enhance apoptotic cell death which inhibits the transformation of tumor cells.

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Gene Section
Review

SLC5A5 (solute carrier family 5 (sodium iodide symporter), member 5)

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Identity

Other names: NIS

HGNC (Hugo): SLC5A5

Location: 19p13.11

Local order: Telomeric to CCDC124, centromeric to JAK3.

DNA/RNA

Note

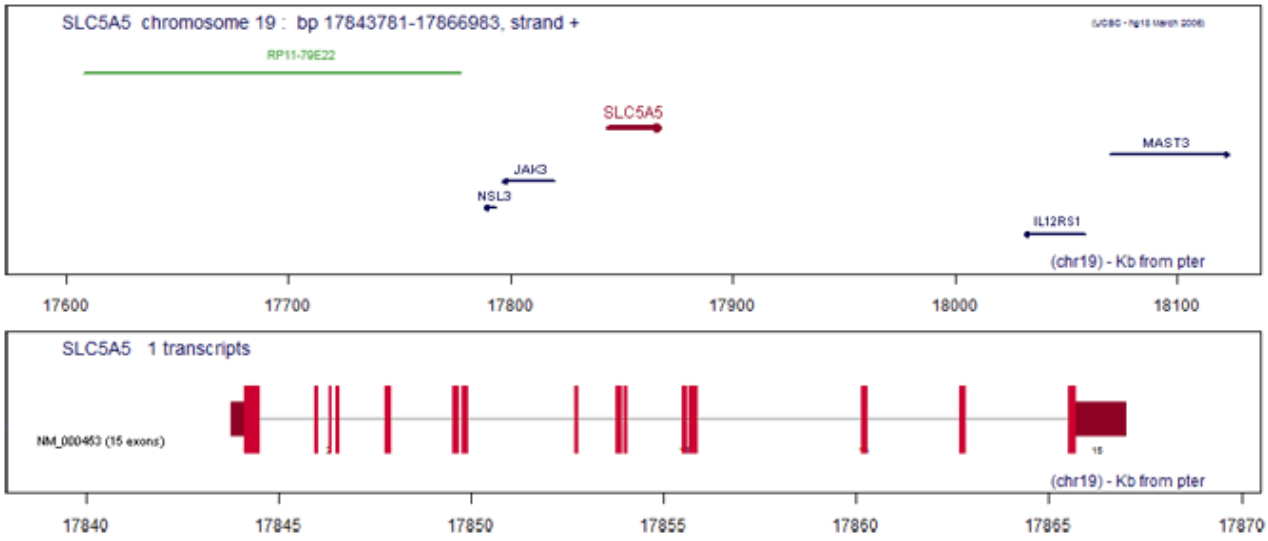
The SLC5A5 gene was first sequenced in 1996 from rat and subsequently human thyroid (Dai et al., 1996; Smanik et al., 1996) and the exon-intron organization characterized in 1997 (Smanik et al., 1997).

Description

15 exons, spanning 23202 bp.

Transcription

Transcription starts at -375 relative to the ATG site. The minimal promoter is localized to a region of 144 bp that includes a 90-bp stretch (-478 and -389 bp) with 73% identity to the rat NIS proximal promoter and containing a TATA- and a GC-box. The region between -596 and -415 is essential for full promoter activity in human thyroid cells. A human NIS gene 5' far-upstream enhancer (hNUE) (-9847 to -8968) confers thyroid-specific and TSH-cAMP responsive transcription and contains an essential Pax-8 binding site and a cAMP response element (CRE)-like sequence activated by a CRE



modulator (CREM) (Taki et al., 2002; Fenton et al., 2008).
RNA: 3576 bases, open reading frame: 1929 bp. No splice variants are reported.

Pseudogene

No pseudogenes have been identified.

Protein

Note
The protein encoded by the SLC5A5 gene is more commonly referred to in the scientific literature as the Sodium Iodide Symporter or NIS.

Description

NIS is a glycoprotein with 643 aa and predicted molecular weight 69k Da. It is composed of 13 transmembrane domains, an extracellular N-terminal, a cytosolic C-terminal and three N-linked glycosylation sites at positions 225, 485 and 497. NIS is phosphorylated in vivo, mostly at the level of serines.

Expression

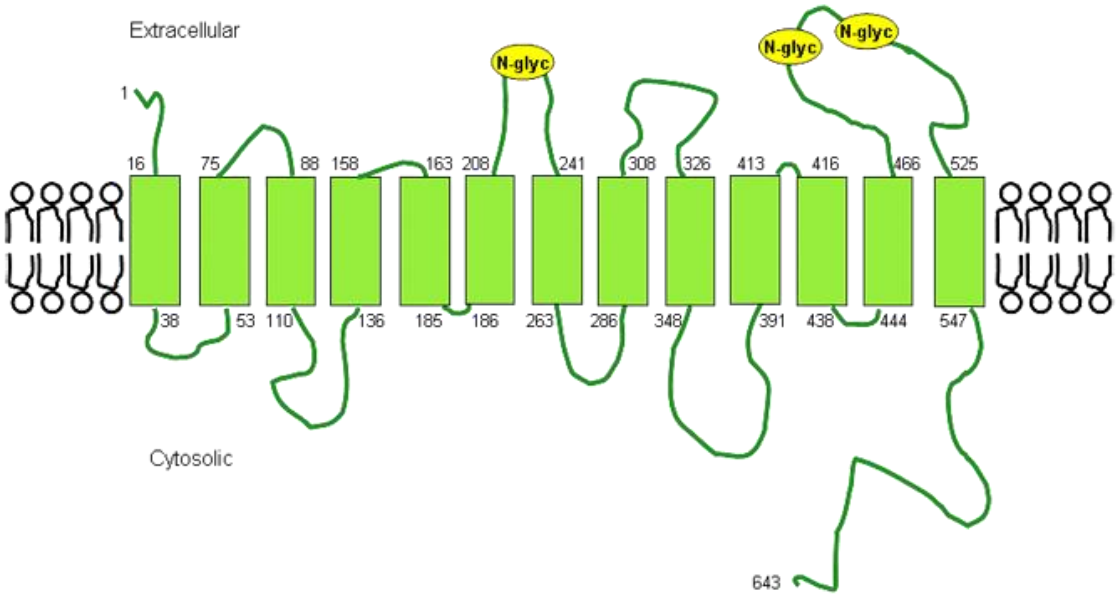
NIS is highly expressed and is active in the thyroid, stomach, salivary glands and lactating mammary gland. Low levels of NIS have also been detected by immunohistochemistry and/or RT-PCR in other extrathyroidal tissues (small intestine, colon, rectum, pancreas, kidney, bile duct, lung, lacrimal gland, heart, placenta, testis, ovaries, prostate gland, adrenal gland, thymus and pituitary gland), but it is not clear to what extent it is active in these tissues.

Localisation

Cell membrane. NIS is located on the basolateral membrane of thyroid follicular cells, lactating mammary gland alveolar cells, salivary gland ductal epithelial cells and gastric mucin-secreting cells. In contrast, NIS is located on the apical membrane of placental trophoblasts and enterocytes. In the kidney, NIS has a diffuse cytoplasmic distribution in distal tubular cells, but is more prominent in the basolateral aspect of proximal tubular cells.

Function

NIS mediates the transport of iodide (I^-) into cells; it cotransports Na^+ and I^- on a 2:1 basis, using the inwardly directed Na^+ concentration gradient generated by the Na^+-K^+ ATPase to concentrate I^- to 30-50 times the extracellular concentration.
The major function of NIS is to concentrate I^- in the thyroid for the synthesis of thyroid hormones triiodothyronine (T3) and tetraiodothyronine (T4). Iodine, a trace element obtained with the diet, is organified into the thyroid hormone precursor thyroglobulin by thyroid peroxidase in the presence of hydrogen peroxide. Thyroidal NIS is regulated by thyroid stimulating hormone (TSH) under control of the hypothalamic-pituitary axis. Low circulating levels of T3 and T4 stimulate the release of thyrotropin-releasing hormone (TRH) from the hypothalamus, which in turn stimulates the secretion of TSH from the anterior pituitary gland. TSH increases NIS expression resulting in enhanced I^- uptake and thyroid hormone synthesis. In contrast, high levels of circulating T3 and T4 inhibit TSH production through a negative feedback loop reducing iodide uptake and thyroid hormone production. TSH regulates NIS transcription via a cAMP-dependent pathway requiring binding of transcription factors Pax-8 and CREM to the hNUE enhancer element. TSH also regulates NIS trafficking, promoting NIS targeting to the plasma membrane.



The diagram has been drawn following UniProtKB/Swiss-Prot database prediction and maintaining approximate length proportions among extracellular and intracellular segments. Transmembrane segments are represented by green rectangles, N-glycosylation sites in yellow.

Mammary gland NIS drives the secretion of I^- into milk in fulfillment of the newborn's dietary requirement for iodine and is induced by lactogenic hormones (prolactin, oxytocin).
Placental NIS may provide the fetus with the necessary I^- to synthesize thyroid hormones.
NIS function in other tissues is unclear. I^- secretion may play a role in mucosal host defense through the formation of reactive metabolites of iodine with antimicrobial activity. A role for NIS in the transport of thiocyanate and nitrate across mucosal barriers has also been proposed, again resulting in the formation of antimicrobial molecules.

Homology

NIS belongs to the SLC superfamily of solute carriers. The SLC5 family has 12 members to date (SLC5A1-SLC5A12) and includes Na^+-coupled cotransporters that rely on the Na^+ electrochemical gradient to drive solute transport into cells. NIS (SLC5A) has the highest homology with SLC5A12 (48% identity) and SLC5A8 (46% identity), both of which are thought to be sodium/monocarboxylate transporters and SLC5A6 (42% identity), a sodium/multivitamin transporter.

Mutations

Germinal

Germinal NIS mutations cause Iodide Transport Defect (ITD), a rare form of dyshormogenic congenital hypothyroidism with autosomal recessive inheritance (OMIM 274400). Twelve loss-of-function mutations have been reported to date: V59E, G93R, R124H, ΔM143-Q323, Q267E, C272X, T354P, G395R, ΔA439-P443, frame-shift 515X, Y531X, G543E.

Mutations reduce thyroidal iodide uptake as a result of impaired NIS expression, maturation, trafficking or transport activity.

Somatic

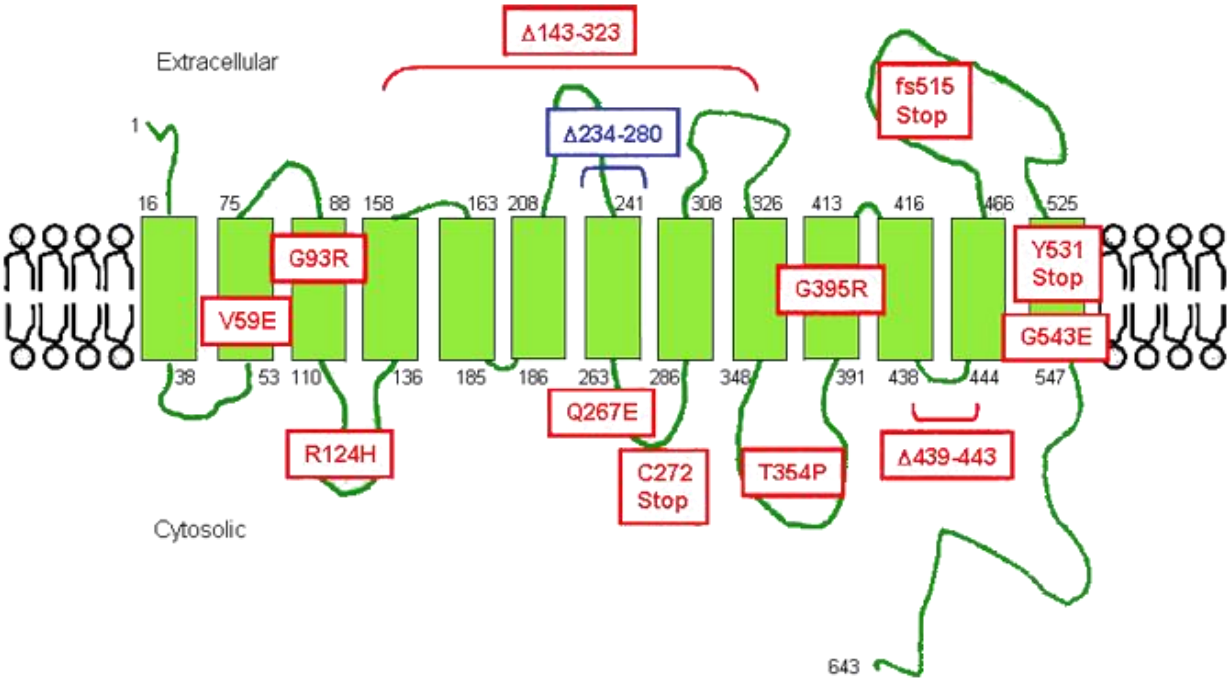
A loss-of-function deletion of exon 6 was identified in a single case of follicular thyroid adenoma (Liang et al., 2005). No other somatic mutations have been reported in association with cancer.

Implicated in

Thyroid cancer

Disease

NIS-mediated uptake of radionuclides has long been exploited in diagnostic scintigraphic imaging (^{123}I, ^{131}I, $^{99m}TcO_4^-$) and radiotherapy (^{131}I) of thyroid carcinoma of follicular cell origin. Compared to other cancers, the prevalence of thyroid cancer is relatively low and its prognosis after surgery and radioiodine therapy is mostly favorable. However, radioiodine uptake is frequently decreased in differentiated thyroid carcinoma (papillary and follicular) and is completely absent in 20% of differentiated carcinomas and most anaplastic thyroid carcinomas. Furthermore, the recurrence rate of thyroid cancer is high (10-30% for papillary thyroid carcinoma) and only one third of patients with distant metastases respond to ^{131}I therapy with complete remission.
NIS expression in thyroid cancer is controversial with reports of under-expression as well as over-expression (Arturi et al., 1998; Saito et al., 1998; Venkataraman et al., 1999; Lazar et al., 1999; Castro et al., 2001; Dohan et al., 2001; Ward et al., 2003; Trouttet-Masson et al., 2004).



Localisation of NIS mutations identified in iodide transport defect (ITD) (in red) and thyroid follicular adenoma (in blue).

Low NIS expression identifies aggressive thyroid tumors and correlates with reduced radioiodine uptake and tumor dedifferentiation. Loss of NIS expression may be associated with hypermethylation of the NIS gene promoter, or may be secondary to reduced expression of nuclear transcription factors. When over-expressed, NIS is mostly intracellular suggesting defective targeting of the protein to the plasma membrane in these cases. Hypofunctioning thyroid tumors express low levels of non-glycosylated NIS suggesting that protein maturation may also be impaired.

Several pharmacological approaches are being tested for their ability to promote cellular re-differentiation, increase endogenous NIS expression and restore iodide transport in thyroid carcinoma cell lines and in patients. Agents include retinoic acid, demethylating agents, histone deacetylase inhibitors and reverse transcriptase inhibitors (Schmutzler et al., 1997; Venkataraman et al., 1999; Zarnegar et al., 2002; Fortunati et al., 2004; Landriscina et al., 2005). The effectiveness of these agents, however, is variable and their clinical utility has yet to be proven.

Oncogenesis

Although no somatic NIS mutations have been identified in thyroid carcinoma, alterations in other genes or gene products may be associated with NIS impairment.

BRAF: Papillary thyroid carcinomas (PTC) harboring the BRAF V600E mutation have reduced NIS expression and impaired targeting to the plasma membrane, which correlates with reduced radioiodine uptake and high risk of recurrence (Riesco-Eizagirre et al., 2006). BRAF V600E-positive PTC also have reduced expression of other thyroid-specific genes such as thyroperoxidase and thyroglobulin, suggesting that impaired NIS expression may be part of an early dedifferentiation process present at the molecular level in BRAF V600E-mutated PTC (Durante et al., 2007; Romei et al., 2008).

RET/PTC: Expression of RET/PTC rearrangements reduces radioiodide uptake and NIS expression in thyroid cells in vitro and transgenic mice (Cho et al., 1999; Knauf et al., 2003). No change in NIS expression, however, was detected in papillary thyroid carcinoma with RET/PTC rearrangements (Romei et al., 2008).

PTTG: Differentiated thyroid cancer over-expresses pituitary tumor transforming gene (PTTG), a proto-oncogene involved in the control of sister chromatid separation. PTTG overexpression correlates with reduced radioiodine uptake and is a prognostic factor for persistent disease (Saez et al., 2006).

PTTG downregulates NIS expression and I^- uptake in vitro, possibly by repressing the binding of transcriptional regulators to the hNUE upstream enhancer (Boelaert et al., 2007).

Breast cancer

Disease

NIS is up-regulated in breast cancer and attention has recently focused on the potential application of radioiodine in the diagnosis and therapy of breast cancer. Several studies have detected NIS immunohistochemically in 30-90% of primary and metastatic breast carcinomas, with variable degrees of intracellular and plasma membrane staining (Tazebay et al, 2000; Wapnir et al, 2003; Wapnir et al., 2004; Beyer et al., 2008; Renier et al., 2009). Estimates of NIS expression in breast cancer, however, may be overestimated due to non-specific binding of some anti-NIS antibodies resulting in a diffuse intracellular staining. One study failed to detect significant NIS immunostaining in 30 cases of primary breast cancer (Peyrottes et al., 2009). In vivo scintigraphic imaging detected ^{123}I or $^{99m}TcO_4$ uptake in up to 25% of NIS-expressing breast tumors, suggesting that the expression of functional NIS in breast cancer is low (Moon et al., 2001; Wapnir et al., 2004). Current research is aimed at identifying strategies that increase the expression and membrane targeting of NIS in breast cancer, in order to improve the efficiency of NIS-mediated radionuclide uptake.

Cholangiocarcinoma (CCA)

Disease

NIS is up-regulated in CCA and is localized to the plasma membrane and/or cytoplasm of bile duct epithelial cholangiocytes. In the diethylnitrosamine rat model of liver cancer, NIS is expressed at the preneoplastic stages of liver carcinogenesis and enables tumor suppression after ^{131}I radiotherapy (Liu et al., 2007). Radioiodide therapy may therefore represent a novel strategy for the treatment of CCA.

Gastric cancer

Disease

NIS expression, normally present in the gastric mucosa, is markedly decreased or absent in gastric cancer (Altorjay et al., 2007) and distinguishes malignant from benign gastric lesions (Farnedi et al., 2009).

Various carcinomas

Note

Targeted NIS gene therapy is being evaluated as a potential diagnostic and therapeutic option for various cancers, enabling tumor cells to accumulate NIS-transported radionuclides. Preclinical studies demonstrate NIS expression, radioiodide uptake and tumor cell death in vitro and in vivo following targeted adenoviral NIS gene transfer to tumor cells. A phase I clinical trial is ongoing to study the efficacy and safety of NIS gene therapy and radioactive iodine for the treatment of prostate cancer (NCT00788307, www.clinicaltrials.gov).

Disease

Carcinomas of the prostate, cervix, breast, head and neck, lung, liver, thyroid, colon, ovaries and pancreas; myeloma; glioma.

Thyroid adenoma

Disease

Benign nonfunctioning thyroid adenomas are characterized by reduced radioiodine uptake due to reduced NIS expression or defective targeting of NIS to the plasma membrane (Tonacchera et al., 2002). A loss-of-function deletion of exon 6 of the NIS gene was identified in a single case of follicular thyroid adenoma (Liang et al., 2005). Hyperfunctioning toxic adenomas harbor activating mutations of the TSH receptor and are characterized by increased NIS expression with correct plasma membrane localization (Lazar et al., 1999).

Congenital hypothyroidism

Disease

Germinal NIS mutations causing iodide transport defect (ITD) are a rare cause of dyshormogenic congenital hypothyroidism (OMIM 274400). To date, 12 mutations have been reported (V59E, G93R, R124H, ΔM143-Q323, Q267E, C272X, T354P, G395R, ΔA439-P443, frame-shift 515X, Y531X, G543E) leading to reduced or absent thyroidal radioiodine uptake, low iodide saliva: plasma ratios and a variable degree of hypothyroidism and goiter.

Prognosis

Goitre, severe neuro-developmental impairment and infertility if not treated. Hypothyroidism treated with T_4-replacement therapy and I^- supplementation.

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Leukaemia Section

Short Communication

1q triplication in hematologic malignancies

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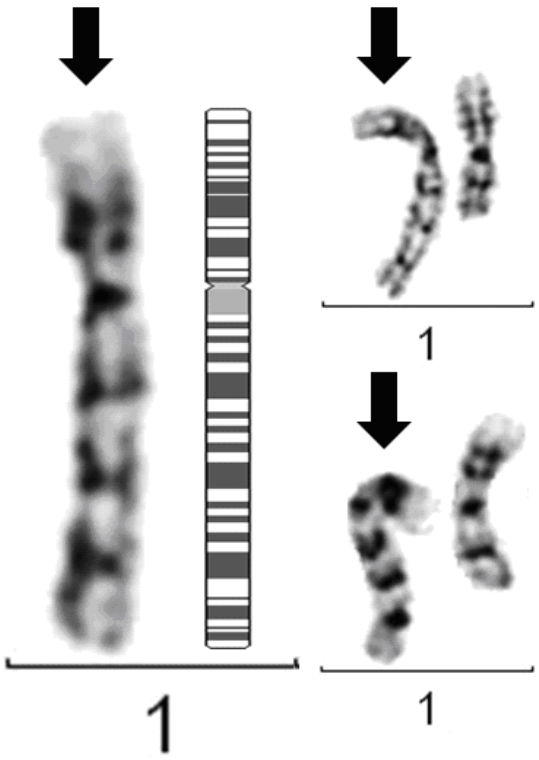

Giemsa-banding partial karyograms of 1q triplication and its representative ideogram. The arrows indicate a trp(1)(q21q32) chromosome.

Clinics and pathology

Disease

Acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), Burkitt lymphomas or non-Burkitt type lymphomas, acute lymphoblastic leukemia (ALL), multiple myeloma (MM), myeloproliferative neoplasm (MPN) and Fanconi anemia.

Note

29 cases have been reported in the literature.

Phenotype/cell stem origin

Rare secondary karyotypic event in various hematologic malignancies; AML/MDS (8 cases), Lymphoma (9 cases), ALL (6 cases), MM (3 cases), MPN (2 cases), Fanconi anemia without other hematologic malignancies (1 case).

Epidemiology

Male predominance (71%), patients ranged in age from 14 to 69 (median 41.5 years).

Prognosis

Most of 1q triplication cases did not provide detailed information for the patients' survival. Although partial duplication/triplication of 1q or trisomy 1 was reported to be correlated with a poor outcome, further studies are needed for the evaluation of prognosis in such patients.

Cytogenetics

Cytogenetics morphological

1q triplication is a distinct secondary chromosomal abnormality. Most repeated region (tandem triplication) of 1q is q21-q32 (33% of total cases).

Additional anomalies

Most cases showed a complex karyotype except three solitary abnormalities of trp(1)(q) cases.

Genes involved and proteins

Note

The gene involved in trp(1)(q) is unknown. However, it was suggested that the most common region of duplication, 1q23-q24, harbors genes associated with tumor cell invasiveness.

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Leukaemia Section

Mini Review

Peripheral T-cell lymphoma not otherwise specified (PTCL-NOS)

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Clinics and pathology

Disease

Peripheral T-cell lymphomas not otherwise specified (PTCL-NOS) include a heterogeneous group of diseases involving lymph nodes and extra nodal sites deriving from the clonal expansion of mature T-lymphocytes bearing clonally rearranged TCR genes.

Phenotype/cell stem origin

The cell of origin is an activated mature CD4+ lymphocyte.
The phenotype is usually CD4+/CD8-, TCRβ+ whereas the expression of CD7 and CD5 may be low. Occasionally, CD30 may be positive.

Epidemiology

There is geographic variation in the incidence of T-cell lymphoma. PTCL-NOS accounts for approximately 4-7% of all non Hodgkin's lymphomas and for 30-70% of all mature T-cell lymphomas.

Clinics

The disease runs an aggressive clinical course.

Pathology

The proliferation effaces the lymph node architecture, with paracortical or diffuse growth pattern. The cells are medium-to-large sized, with irregular nucleus, distinct nucleoli. Mitotic figures may be numerous.

Treatment

Anthracycline-based regimes such as CHOP yields

unsatisfactory results with lower CR rates than in B-cell diffuse large cell lymphomas and high relapse rate.

Intensive regimens such as hyperCVAD with or without autologus bone marrow transplantation may be effective in this type of lymphoma, though the superiority of this approach over conventional treatment has not been definitely proven.

Prognosis

Reported failure free survival rates ranged between 12 and 45% (Armitage, 2006).

Cytogenetics

Cytogenetics molecular

Complex karyotypes are reported in 70-90% of the cases (Rizvi et al., 2006).
Recurrent chromosome gains were described to involve 7q, 8q, 17q and 22q, whereas recurrent regions of loss of chromosome material were represented at 4q, 5q, 6q, 9p, 10q, 12q and 13q (Pileri et al., 2008).
In a recent study, frequent gains involved 7q22q31 (33%), 1q (24%), 3p (20%), 5p (20%) and 8q24qter (22%). Losses occurred at 6q22q24 (26%) and 10p13pter (26%).
Complex karyotypes were predictive of an inferior outcome, but no association was noted between specific aberrations and survival (Nelson et al., 2008).
Array comparative genomic hybridization (CGH) for high-resolution analysis of PTCL-NOS identified a region with high copy number gain at 14q32.2, and a region with homozygous loss at 9p21.3. Gains of 7p and 7q and loss of 9p21.3 showed a significant association with poor prognosis (Nakagawa et al., 2009).
p53 protein overexpression and mutation of p53 may be found in 30% of the cases and may correlate

significantly with treatment failure and worse overall and disease-free survival (Pescarmona et al., 2001).
Recurrent copy number gain may also involve chromosomes 8, 9 and 19. Other genomic imbalances may include overexpression of CARMA1 at 7p22 and of MYCBP2 at 13q22, both genes being localized within regions of frequent copy number gain.
LOH was found at 2q34 (Fujiwara et al., 2008).

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Atlas of Genetics and Cytogenetics in Oncology and Haematology

OPEN ACCESS JOURNAL AT INIST-CNRS

Leukaemia Section

Mini Review

t(11;14)(q23;q32)

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Clinics and pathology

Disease

Treatment related leukemia (treatment related acute myeloid leukemia, t-AML)

Epidemiology

The involvement of MLL in 11q23 and KIAA0284 in 14q32 was shown in only 2 cases (Burmeister et al., 2008; De Braekeleer et al., 2009). These 2 cases were treatment related leukemia cases (t-AML for: treatment related acute myeloid leukemia). These t-AML cases occurred in a 45-year-old male patient (a M1 case) and in a 65-year-old female patient, 2 years after an urothelial carcinoma and 5 years after a ductal mammary carcinoma respectively. In 1 other case of t(11;14)(q23;q32), a myelodysplastic syndrome case, the involvement of MLL was excluded, and IGH in 14q32 was rearranged (Yujiri et al., 2009). Finally, in 2 other cases of t(11;14)(q23;q32), no molecular studies were available (Kaneko et al., 1982; Hanson et al., 1993). The two latter cases were a biphenotypic leukemia (BAL) case and an acute lymphoblastic leukemia (ALL) in a 33-year-old male patient.

Prognosis

One of the t-AML cases died 2 months after diagnosis, while the other one was lost to follow-up 5 years after diagnosis of the t-AML. The ALL case died 7 months after diagnosis.

Cytogenetics S

Cytogenetics morphological

The t(11;14)(q23;q32) was the sole anomaly in the 2 cases were MLL and KIAA0284 involvements were ascertained. The BAL case showed a complex karyotype with -7 and i(17q), the ALL case also had a complex karyotype, with +12.

Genes involved and proteins

MLL

Location
11q23

DNA/RNA
36 exons, multiple transcripts 13-15 kb.

Protein
3969 amino acids; 431 kDa; contains two DNA binding motifs (a AT hook and a CXXC domain), a DNA methyl transferase motif, a bromodomain. MLL is cleaved by taspase 1 into 2 proteins before entering the nucleus, called MLL-N and MLL-C. The FYRN and a FRYC domains of native MLL associate MLL-N and MLL-C in a stable complex; they form a multiprotein complex with transcription factor TFIID. MLL is a transcriptional regulatory factor. MLL can be associated with more than 30 proteins, including the core components of the SWI/SNF chromatin remodeling complex and the transcription complex TFIID. MLL binds promotors of HOX genes through acetylation and methylation of histones. MLL is a major regulator of hematopoesis and embryonic development.

KIAA0284

Location
14q32.33

Protein
KIAA0284 presents an amino acids similarity of 30% with CEP170. CEP170 is a forkhead-associated

domain protein which associates with centrosomes during interphase and with spindle microtubules during mitosis (Guarguaglini et al., 2005).

Result of the chromosomal anomaly

Hybrid gene

Description

5' MLL - 3' KIAA0284

Transcript

The breakpoint was located in intron 9 of MLL. The breakpoint in KIAA0284 was located in intron 2 in one case, and intron 4 in the other case.

Fusion protein

Description

N-term MLL - C-term KIAA0284

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Leukaemia Section
Short Communication

t(3;9)(q27;p24)

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Clinics and pathology

Disease

Non Hodgkin lymphoma (NHL).

Epidemiology

Only one case to date, a gastric lymphoma (Chen et al., 2006).

Prognosis

No data.

Genes involved and proteins

BCL6

Location: 3q27

Protein

706 amino acids; composed of a NH2-term BTB/POZ domain (amino acids 1-130 (32-99 according to Swiss-Prot)) which mediates homodimerization and protein-protein interactions with other corepressors (including HDAC1 and NCOR2/SMRT) to constitute a large repressing complex, another transcription repression domain (191-386), PEST sequences (300-417) with a KKYK motif (375-379), and six zinc finger at the C-term (518-541, 546-568, 574-596, 602-624, 630-652, 658-681), responsible for sequence specific DNA binding.
Transcription repressor; recognizes the consensus sequence: TTCCT(A/C)GAA (Albagli-Curiel, 2003).

DMRT1

Location: 9p24

Protein

373 amino acids; contains a DM (Doublesex/Mab-3) domain (amino acids 72-118) and a Proline/Serine-rich region (aa 322-347) according to SwissProt; binds DNA. Transcription factor; sex determination gene of major role in the platypus and in birds; expressed at a higher level in the embryonic testis than in the embryonic ovary in vertebrates. Postnatally, DMRT1 is only expressed in undifferentiated spermatogonia and in Sertoli cells, and silenced in granulosa cells by repression of DMRT1 promoter by FOXL2 (Lei et al., 2009). Required for testicular development in vertebrates.

Result of the chromosomal anomaly

Hybrid gene

Description

5' DMRT1 - 3' BCL6.

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Solid Tumour Section

Short Communication

t(1;22)(q23;q12) in myoepithelioma

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Clinics and pathology

Disease

Myoepitheliomas are tumours which can occur in the salivary glands, in soft tissues and other organs, with a variable but generally low to intermediate aggressiveness (myoepithelial carcinoma for the most adverse histology).

Epidemiology

One case to date, a 59-year-old female patient with a 10 years long history. The patient was well 7 months after treatment of a myoepithelioma with an uncertain degree of malignancy (Brandal et al., 2008).

Cytogenetics

Cytogenetics Morphological

The t(1;22)(q23;q12) was the sole anomaly.

Genes involved and proteins

PBX1

Location: 1q23

Protein

Homeobox protein (homeodomain in amino acids 233-295). Binds the sequence 5'-ATCAATCAA-3'. Transcription factor.

EWSR1

Location: 22q12

Protein

From N-term to C-term: a transactivation domain (TAD) containing multiple degenerate hexapeptide repeats, 3 arginine/glycine rich domains (RGG regions), a RNA recognition motif, and a RanBP2 type Zinc finger. Role in transcriptional regulation for specific genes and in mRNA splicing.

Result of the chromosomal anomaly

Hybrid Gene

Description

5' EWSR1 - 3' FLI1. EWSR1 exon 7 is fused in frame to PBX1 exon 5.

Fusion Protein

Description

Fusion of the N terminal transactivation domain of EWSR1 to the homeobox (DNA binding domain) of PBX1.

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Cancer Prone Disease Section

Mini Review

Familial tylosis

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Identity

Alias: Howell-Evans syndromes; Tylosis oesophageal cancer; Focal non epidermolytic palmoplantar keratoderma (NEPPK) with carcinoma of the oesophagus

Note: Synonyms include: (a) Tylosis oesophageal cancer or (b) Focal Non Epidermolytic Palmoplantar Keratoderma (NEPPK) with carcinoma of the oesophagus (Howel-Evans et al., 1958; Stevens et al., 1996).

Inheritance: Is a rare autosomal dominant condition with full penetrance of skin phenotype by puberty. No race prevalence has been noted (Howel-Evans et al., 1958).

Clinics

Phenotype and clinics

Tylosis is divided into to types: Type A with late onset of NEPPK between age of 5 to 15 years and Type B with early onset around the first year of age (Maillefer and Greydanus, 1999; Nagai et al., 2000).

It usually involves the pressure areas mainly sole of feet and later mild involvement of palms (more obvious in manual workers). It can also affect frictional areas like elbows and knees. It regresses completely on bed rest (Howel-Evans et al., 1958; Stevens et al., 1996).

The affected skin has a thickened epidermis which sheds horny large flakes, usually in autumn, to leave a red tender surface which quickly get covered with another thick layer of epidermis (Howel-Evans et al., 1958).

Oral leukokeratosis (which are white "spongy" plaques) and follicular hyperkeratosis (which are pinkish-to-tan papules) on the body and flexure areas, are often seen in patients with tylosis and it could be a possible indication for developing oesophageal cancer (Tyldesly and Osborne-Hughes, 1973; Tyldesly, 1974).

See example of Tylosis on DermAtlas.

Neoplastic risk

Malignancy Risk: Type A has a higher risk of developing squamous oesophageal carcinoma up to 95% by age of 65 years, while Type B runs a benign course (Howel-Evans et al., 1958; Ellis et al., 1994; Stevens et al., 1996).

These malignancies are predominantly in the distal esophagus whereas acquired squamous cell carcinomas are mostly mid-thoracic in location (Howel-Evans et al., 1958; Maillefer and Greydanus, 1999). Increase risk has been noted with history of smoking (Stevens et al., 1996).

Histological findings: Thickening of the all skin layers especially epidermis, hypertrophy of sweat glands and their ducts which often occluded by hyperplastic epithelium (Howel-Evans et al., 1958).

Treatment

Monitoring: Annual endoscopic surveillance with biopsies taken should be offered to affected individuals in view of risk of oesophageal cancer (Robertson et al., 2008).

Prognosis

Prognosis of squamous cell cancer of oesophagus: In general is poor with 5 year survival of 75% in Stage 0 (intraepithelial cancer) to <5% in stage IV (Distant metastasis). Overall survival is about 20-25% (Mayer, 2001).

Cytogenetics

Note

The tylosis oesophageal cancer gene (TOC) is localized to a small region on band 17q25, a region frequently deleted in persons with sporadic squamous cell oesophageal tumours (Kelsell et al., 1996; Risk et al., 2002).

This region contains 5'end of uncharacterized (FM8) gene, which is likely non coding RNA, a promoter of another gene and the whole cytoglobin gene (Langan et al., 2004).

So far studies has failed to identify TOC specific mutations in any of the 3 genes above (Langan et al., 2004).

However recent studies of the gene expression in the 42.5 kb TOC minimal region has shown down regulation of cytoglobin gene expression by 70% in tylotic patients which might contribute to TOC phenotype. This reduction exceeds the expected 50% effect from autosomal dominant conditions therefore rules out a simple haplo-insufficiency as a mechanism of the disease, instead a novel trans-allele interaction (ie the mutated allele causing suppression of the normal allele) has been suggested (McRonald et al., 2006).

Genes involved and proteins

TOC

Location

17q25

Note

TOC gene or tylosis with oesophageal cancer gene.

DNA/RNA

Note: Abnormality in this area has been noted in breast and ovarian cancer (Nagai et al., 2000; Harada et al., 2001).

Description: 42.5kb.

No mutations have been identified in the gene.

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Cancer Prone Disease Section

Mini Review

Hereditary diffuse gastric cancer (HDGC)

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Identity

Alias: Signet ring carcinoma or isolated cell type carcinoma.

Inheritance: Autosomal dominant with high penetrance (about 80%), average age of onset is in the 4th decade of life but it could be as early as the teens to the seventies. Germline mutations in CDH1 gene have been associated with this condition (Gayther et al., 1998; Guilford et al., 1998).

Clinics

Note

Criteria for diagnosis (Brooks-Wilson et al., 2004):

- Two or more cases of gastric cancer in a family, with at least one diffuse gastric cancer diagnosed before age 50 years.
- Three or more cases of gastric cancer in a family, diagnosed at any age, with at least one documented case of diffuse gastric cancer.
- An individual diagnosed with diffuse gastric cancer before 45 years of age.
- An individual diagnosed with both diffuse gastric cancer and lobular breast cancer (no other criteria met).
- One family member diagnosed with diffuse gastric cancer and another with lobular breast cancer (no other criteria met).
- One family member diagnosed with diffuse gastric cancer and another with signet ring colon cancer (no other criteria met).

Phenotype and clinics

HDGC forms less than 3% of all gastric cancers (Stone et al., 1999). It often affects younger people in contrast to the other types of gastric cancer. It consists of scattered clusters of poorly differentiated cells involving a large area of the stomach without a macroscopically recognisable margin or formation of a mass or ulcer (linitis plastica).

There is no known association between genotypic and phenotypic character of the disease (Kaurah and Huntsman, 2006).

Malignant risk: Four fifths of female carriers with CDH1 gene mutations are estimated to develop HDGC by age of 80 years with an additional 40% risk for lobular breast cancer, adding up to 90% for both cancers, while two thirds of males are expected to develop gastric cancer by the same age (Paul et al., 2001).

Treatment

Aim of the management is: (1) Curative treatment through early detection and resection of the tumour completely, but unfortunately gastric cancer especially HDGC are usually incurable at presentation. (2) Identifying Germline mutation in CDH1 can provide help and support for family members who are unaffected but carrier of the genetic mutations by developing a plan to reduce the risk of cancer (Brooks-Wilson et al., 2004), through either (a) prophylactic gastrectomy which may be life saving as cancer cells have been detected in all resected stomach specimens in asymptomatic carriers (Huntsman et al., 2001), but with high morbidity and mortality (22-30% and 4-5% respectively (Kelsen et al., 2008)), or through (b) extensive biannual chromo endoscopic surveillance which has its limitation in detecting submucosal lesions in a normal looking mucosa, therefore the best preventive approach is yet to be established (Cisco et al., 2008).

In view of increase risk of colorectal cancer by 2-3 times and lobular breast cancer in females, surveillance colonoscopy every 3-5 years and regular MRI check of

the breast may be required (Cisco et al., 2008; Porter et al., 2002).

Prognosis

Overall survival in gastric cancer is poor with 28% at 5 years and 20% at 10 years. However if the cancer is detected at early stages (i.e. confined to mucosa and submucosa), >90% will be alive at 5 years compare to 10-20% in advanced gastric cancer even when potentially curative surgery has been carried out (Kelsen et al., 2008; Leung et al., 2009).

Genes involved and proteins

CDH1

Location

16q22.1

DNA/RNA

Description: The gene consists of 16 exons and a 65-kb-long intron 2 that span around 100 kb (Berx et al., 1995).

Protein

Description: E cadherin is a transmembrane calcium dependant glycoprotein (728 AA) with cytoplasmic domain which binds to actin cytoskeleton via catenins (catenin alpha, catenin beta and catenin gamma), single transmembrane domain, and extracellular domains which adhere to neighbouring cells and form a tight homophilic bond which is an important part in cell-cell adhesions, tissue architecture, cell differentiations and proliferations (Conacci-Sorrell et al., 2002; Roy and Berx, 2008).

Function: CDH1 gene encodes for Cadherin protein which plays an important role in maintaining normal cell physiology like differentiation, growth, motility and tissue architecture through tight cell-cell adhesions (Conacci-Sorrell et al., 2002; Robertson and Jankowski, 2008).

Loss of cell adhesions have been noted in cancers for a long time. CDH1 suppression has been associated with poorly differentiated, aggressive, metastatic cancers. Mutation in E-cadherin is also associated with breast, colorectal cancers, thyroid, endometrial, ovarian, head and neck, skin, prostate, bladder cancer and other tumours (Birchmeier, 1995).

Mutations

Germinal: Germline mutations in CDH1 have been associated with HDGC. First mutations were described by Guilford et al. in three Maori families in New Zealand in 1998 (Guilford et al., 1998). Nowadays more than 50 different types of mutations have been described and new ones are emerging (Robertson and Jankowski, 2008). Types of mutations described are mainly truncating and missense mutations.

Up to 50% of families meeting the criteria above, which was set by the International Gastric cancer Linkage Consortium (IGCLC) in 2004, will have mutations in CDH1 (Brooks-Wilson et al., 2004).

Promoter methylation of the wild type allele in the mutated CDH1 is associated with loss of gene expression and might work as a "second genetic hit" predisposing to cancer and explain the absence of loss of heterozygosity in this condition (Grady et al., 2000).

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Deep Insight Section

Detection of minimal residual disease in acute lymphoblastic leukemia

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I. Introduction

In patients with acute lymphoblastic leukemia (ALL), the degree of treatment response guides clinical decisions, and information about this response is essential for selecting the optimal clinical management approach. Unfortunately, determining whether residual leukemia is present during treatment by traditional methods, i.e. the morphologic examination of cells in bone marrow smears, is typically a subjective and imprecise endeavor owing to the fact that the morphology of ALL cells is very similar to that of normal bone marrow cell subpopulations, such as immature B cells and activated mature lymphocytes. Hence, the remission status of patients with ALL often raises doubt in the mind of pathologists and clinicians; this uncertainty can lead to overtreatment (and excessive toxicities) or undertreatment (and increased risk of relapse). The advent of methods for detecting minimal residual disease (MRD) has revealed that many patients considered to be in "remission" by morphologic analysis still have substantial amounts of residual leukemia (Campana, 2008a). Because of the strong correlation between MRD levels and treatment outcome, MRD testing is increasingly being incorporated in clinical trials.

II. A brief review of methods for MRD detection

Polymerase chain reaction

Two main types of molecular targets can be used to identify leukemic cells. One is represented by clonally rearranged antigen-receptor genes, i.e,

immunoglobulin (IG) and T-cell receptor (TCR) genes. The junctional regions of the rearranged genes are unique to the leukemic clone. Typically, the unique gene signature is identified at diagnosis in each case using PCR primers matched to the V and J regions of various IG and TCR genes. If a rearrangement is found, the PCR product is further analyzed to ensure its clonal origin by using heteroduplex analysis (van der Velden et al., 2007). The junctional regions of the IG/TCR gene rearrangements are then sequenced to design specific oligonucleotides which are then applied to monitor MRD (van der Velden et al., 2007). Investigators have developed methods to detect clonal IG/TCR gene rearrangements without the need for patient-specific oligonucleotides. These efforts have relied on high-resolution electrophoresis, such as radioactive fingerprinting or fluorescent gene scanning, but this approach has a considerably lower sensitivity, usually not better than 0.1%, and date interpretation may be difficult (Delabesse et al., 2000; Knechtli et al., 1998).

Because the majority of B-lineage ALL cases have IG (Beishuizen et al., 1993) and cross-lineage TCR gene rearrangements (Szczepanski et al., 1999a), MRD monitoring by using these genes as targets is feasible in > 90% of cases of B-lineage ALL. Likewise, TCR genes are rearranged in most cases of T-lineage ALL and cross-lineage IG gene rearrangements occur in approximately 20% of T-ALL (Szczepanski et al., 2000; Kneba et al., 1995). In sum, the method can be used to monitor MRD in most cases of childhood and adult ALL (van der Velden et al., 2003; van der Velden et al., 2007; Bruggemann et al., 2006; Flohr et al., 2008).

Detection of MRD by PCR using IG/TCR gene rearrangements is most frequently performed by using "real-time" quantitative PCR (RQ-PCR) (van der Velden et al., 2003) and less commonly by limiting

dilution (Neale et al., 1999). Because rearranged IG and TCR genes are present in one copy per cells, very precise estimates of the MRD levels can be achieved. IG and TCR genes may be affected by continuing or secondary rearrangements (Szczepanski et al., 1999b), resulting in subclones with distinct clonal IG/TCR gene rearrangements, and minor clones at diagnosis may become predominant at relapse (Szczepanski et al., 2002; van der Velden et al., 2004). These possibilities have prompted the recommendation of targeting two or more different rearrangements during MRD studies (van der Velden et al., 2007). Multiple targets are identifiable in the majority of ALL cases although in approximately 30% of cases it is not possible to identify multiple targets that allow detection of MRD with a high sensitivity (e.g., 0.01%) (Pongers-Willemse et al., 1999; Flohr et al., 2008).

The second type of gene target for MRD monitoring by PCR is represented by gene fusions, such as BCR-ABL1, MLL-AFF1, TCF3-PBX1, and ETV6-RUNX1, and their resulting aberrant mRNA transcripts (van Dongen et al., 1999; Gabert et al., 2003). Recurrent fusions are identified in less than half of patients with newly diagnosed ALL (Gabert et al., 2003), thus limiting the applicability of this approach. However, with the systematic use of novel whole-genome screening technologies (Mullighan et al., 2007; Mullighan et al., 2009), it is very likely that additional genetic targets will enrich the available array of gene targets for MRD studies.

One potential advantage of using fusion transcripts to monitor MRD is that it might be possible to detect pre-leukemic cells (Hong et al., 2008). If so, the clinical significance of such finding needs to be investigated. A clear disadvantage of using fusion transcripts as targets is an accurate estimate of the number of leukemic cells present in the sample is difficult. This is because that ratio between amount of PCR product and target cell number is uncertain, there may be interpatient variability in the number of transcripts per leukemic cell within the same genetic subtype of ALL, and this number could be altered by chemotherapy (Gabert et al., 2003).

Flow cytometry

Immunophenotypes characteristic of leukemic cells can be used to distinguish ALL from normal cells by flow cytometry (Campana, 2008). There are three main categories of leukemia-associate immunophenotypes. One is characterized by the expression of fusion proteins derived from fusion transcripts, such as BCR-ABL1, ETV6-RUNX1, or TCF3-PBX1. However, suitable antibodies for reliable flow cytometric analysis of these proteins are lacking. A second group is represented by the immunophenotype of T-lineage ALL cells, which is normally expressed only by a subset of thymocytes and it is not expressed by cells outside the thymus. Immature T-cell phenotypes can be effectively used to monitor MRD in T-lineage ALL (Coustan-Smith et al., 2002a), and also to detect disease dissemination in T-cell lymphoblastic lymphoma (Coustan-Smith et al., 2009a). The third group of leukemia-associated immunophenotype is constituted by multiple marker combinations that are found in B-lineage ALL cells but are normally not expressed during lympho-hematopoiesis. The use of these immunophenotypes, named "asynchronous" or "aberrant" (Hurwitz et al., 1988; Lucio et al., 1999; Campana and Coustan-Smith, 1999; Ciudad et al., 1998), requires a particularly good knowledge of the immunophenotypes expressed by normal hematopoietic cells, in both normal and recovering bone marrow.

Leukemia-associated immunophenotypes that are suitable for MRD studies and afford a sensitivity of at least 0.01% can be identified in nearly all patients with ALL (Coustan-Smith et al., 2002b; Campana and Coustan-Smith, 1999). Results obtained by flow cytometry are very similar to those obtained by PCR amplification of IG/TCR genes, if MRD is present at a $\geq$ 0.01% level (Neale et al., 1999; Neale et al., 2004; Kerst et al., 2005).

Current methods for MRD testing by flow cytometry typically require the use of extensive antibody panels and considerable interpretative expertise. We developed a simplified flow cytometric MRD test that can detect residual B-lineage ALL cells (which express CD19 plus CD10 and/or CD34) on day 15-26 of treatment with a minimum panel of antibodies (Coustan-Smith et al., 2006). The rationale for this strategy is that normal immature CD19+ cells, or those expressing CD10 and/or CD34, are consistently undetectable in bone marrow samples collected from children with T-lineage ALL after 2 weeks of remission induction chemotherapy, because of their high sensitivity to glucocorticoids and other antileukemic drugs. We therefore reasoned that any cell with this immunophenotype detected in patients with B-lineage ALL on day 19 of induction treatment would likely be residual leukemic cells. Indeed, our findings indicate that the results of the simplified test correlate very well with those of more complex flow cytometric assays or PCR amplification of IGH/TCR genes. It should be stressed that this test cannot be used beyond this early treatment interval because of the high risk of false-positive results in recovering marrow samples.

III. Results of correlative studies with treatment outcome

Studies in pediatric ALL

The clinical significance of MRD testing during the initial phases of treatment was definitively demonstrated by 3 prospective studies published in 1998 by the EORTC (Cave et al., 1998), St Jude (Coustan-Smith et al., 1998) and BFM groups (van Dongen et al., 1998). The results these studies consolidated those of many other previous reports of smaller series, and have been confirmed by several

subsequent studies (reviewed in Campana, 2009). MRD testing is also clinically informative for patients with specific ALL subtypes (Coustan-Smith et al., 2000; Biondi et al., 2000; Attarbaschi et al., 2008; van der Velden et al., 2009), patients with relapsed ALL who achieve a second remission (Eckert et al., 2001; Coustan-Smith et al., 2004; Paganin et al., 2008), patients with extramedullary relapse (Hagedorn et al., 2007) and patients undergoing allogeneic hematopoietic stem cell transplantation (Knechtli et al., 1998; van der Velden et al., 2001; Bader et al., 2002; Uzunel et al., 2001; Krejci et al., 2003; Goulden et al., 2003).

Levels of MRD are directly proportional to the risk of subsequent relapse. Thus, MRD ≥ 1% at the end of remission induction therapy predicted an extremely high rate of relapse in St Jude studies (Coustan-Smith et al., 2000), while MRD ≥ 0.1% on both day 33 and day 78 of treatment had a very high risk of relapse in the I-BFM Study Group studies (van Dongen et al., 1998; Flohr et al., 2008). The threshold level commonly used to define MRD positivity is 0.01% of bone marrow mononuclear cells. Patients with ≥ 0.01% MRD at any time point during treatment had a higher risk of relapse in earlier St Jude studies (Coustan-Smith et al., 1998; Coustan-Smith et al., 2000; Coustan-Smith et al., 2002b), as had those with ≥ 0.01% MRD on day 29 of treatment in studies of the Children's Oncology Group (Borowitz et al., 2008). In other studies, however, a threshold of 0.1% appeared to be more informative (Cave et al., 1998; Dworzak et al., 2002; Zhou et al., 2007).

In addition to providing a parameter to identify patients at a higher risk of relapse, MRD can also identify patients with excellent early treatment response and undetectable (< 0.01%) MRD after 2-3 weeks of therapy. We found that 183 of 402 (45.5%) B-lineage ALL patients were MRD < 0.01% on day 19 of treatment (Campana, 2008b), a feature that is associated with excellent prognosis overall (Panzer-Grumayer et al., 2000; Coustan-Smith et al., 2002b).

The prevalence of MRD differs among different genetic subtypes of childhood ALL (Pui et al., 2001; Borowitz et al., 2003). Thus, MRD is generally more prevalent among patients with BCR-ABL1 ALL and less prevalent among those with ETV6-RUNX1, hyperdiploid (> 50 chromosomes) and TCF3-PBX1 ALL (Campana, 2008c). More recently, it has been shown that patients with B-lineage ALL and mutations or deletions of the Ikaros (lIKZF1) gene had a higher prevalence of MRD during remission induction therapy than those without this abnormality (Mullighan et al., 2009). In addition, among patients with T-lineage ALL, MRD-positive findings were strikingly more frequent and levels higher in the subgroup of patients with early thymic precursor (ETP)-ALL (Coustan-Smith et al., 2009b).

MRD studies have now been included in clinical trials to guide therapy. Thus, the AIEOP-BFM group uses MRD to classify patients with newly diagnosed ALL into three risk groups: standard risk (MRD negative on days 33 and 78), intermediate risk (any MRD positivity on days 33 and 78 but < 0.1% on day 78) and high risk (MRD ≥ 0.1% on day 78) (Flohr et al., 2008). In the AIEOP-BFM ALL 2000 trial, of the 3341 diagnostic samples examined, 88 (3%) lacked suitable gene rearrangements targets for PCR analysis, and an additional 217 (7%) had a target but not sufficient to reach a sensitivity of 0.01% (Flohr et al., 2008). At least two sensitive gene rearrangement targets could be identified in 71% of patients. Adequate data for MRD-based stratification were obtained in 2594 (78%) of the 3341 patients (78%).

In the St Jude Total XV trial for children with newly diagnosed ALL, our laboratory monitored MRD by using flow cytometric detection of aberrant immunophenotypes and/or PCR amplification of antigen-receptor genes (Pui et al., 2009). Overall, 482 of 492 patients (98%) were monitored by flow cytometry and 403 of 492 (82%) by PCR (applied only to patients with B-lineage ALL). As previously shown (Neale et al., 1999; Neale et al., 2004; Kerst et al., 2005), both methods yielded virtually identical results above the threshold level of 0.01%. The two methods in combination could be applied to study 491 of 492 patients (99.8%) (Pui et al., 2009). The single patient with no available immunophenotypic or antigen-receptor gene rearrangements had a MLL-AF9 fusion transcript and was monitored by RQ-PCR using that marker. In our current Total XVI trial, patients with MRD ≥ 1% on day 15 receive intensified remission induction therapy; further intensification is reserved for patients with ≥ 5% leukemic cells. By contrast, patients with MRD < 0.01% on day 15 receive less intensive reinduction therapy and lower cumulative doses of anthracyclin. Patients with standard-risk ALL who have MRD of ≥ 0.01% on day 42 are reclassified as high-risk; patients with MRD ≥ 1% are eligible for transplant in first remission. Because in patients with T-lineage ALL MRD levels in peripheral blood are similar to those in bone marrow (Coustan-Smith et al., 2002a; van der Velden et al., 2002), it is our current practice to use blood instead of marrow to monitor MRD after day 42 in these patients.

Studies in adult ALL

Several studies have also demonstrated the prognostic importance of MRD in adult ALL patients (Mortuza et al., 2002; Bruggemann et al., 2006; Raff et al., 2007; Holowiecki et al., 2008; Bassan et al., 2009). Bruggeman et al. (Bruggemann et al., 2006) studied MRD in 196 standard-risk patients using PCR amplification of antigen-receptor genes and segregated three groups: 10% of patients had < 0.01% MRD on days 11 and 24 of treatment and 23% had persistent MRD ≥ 0.01% until week 16. The 3-year relapse rates were 0% and 94%; for the remaining patients, the relapse rate was 47%. The same group subsequently studied post-consolidation samples from 105 patients

who were in hematologic remission, had completed the first-year chemotherapy, and were MRD-negative before enrolling in the study. MRD was detected in 28 patients, 17 of whom relapsed. By contrast, 77 patients remained MRD-negative and only 5 relapsed (Raff et al., 2007). Using IG/TCR gene rearrangements or fusion transcripts as targets, Bassan et al. (Bassan et al., 2009) measured MRD at the end of consolidation. Five-year overall disease-free survival estimates were 72% among 58 MRD negative patients and 14% among the 54 patients with positive MRD. In a study using flow cytometry, Holowiecki et al. (Holowiecki et al., 2008) measured MRD in 116 patients with Philadelphia-negative ALL and found that MRD $\geq$ 0.1% after remission induction therapy was an independent predictor for relapse. Together, the results of these studies provide convincing evidence of the clinical significance of MRD in adult ALL, although the strengths of the correlations with outcome depend on the subgroup of patients studied and the type of treatment.

Monitoring of MRD in adult patients with Philadelphia-positive ALL receiving transplant and/or imatinib therapy has been shown to predict treatment outcome (Radich et al., 1997; Wassmann et al., 2005; Pane et al., 2005). It has been shown that MRD detected before initiation of conditioning is a significant predictor of failure post-transplant (Sanchez et al., 2002; Spinelli et al., 2007).

Areas for further research

Measuring MRD provides unprecedented insights into the kinetics of treatment response in patients with acute leukemia which not only have prognostic ramifications but can also provide novel endpoints for correlative studies with cellular and biologic features. For example, the correlation between MRD and gene expression of leukemic lymphoblasts at diagnosis revealed genes associated with treatment response (Cario et al., 2005; Flotho et al., 2006; Flotho et al., 2007), while correlations with gene polymorphisms has pointed to drug-metabolizing molecules which may have a direct impact on leukemia response to treatment (Rocha et al., 2005; Yang et al., 2009). These areas are clearly worthy of further research, which may lead to the identification of new prognostic factors and provide clues about targets for molecular therapies.

Although MRD can be studied in virtually all patients with ALL using molecular and/or flow cytometric methods, MRD assays require considerable expertise and can be performed well only in specialized centers. Simplification of the methodologies to widen the applicability of MRD testing should be an objective for future research. At the same time, increasingly sophisticated methodologies provide new opportunities for investigation. To this end, the availability of reliable flow cytometers that can detect 6 or more fluorochromes together with the a wide array of commercial antibodies open the possibility to investigate the biologic features of the leukemic cells that contribute to MRD in extraordinary detail. In turn, such studies should help unearthing some of the biologic roots of drug resistance in ALL and ultimately lead to more effective and less toxic treatment.

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Deep Insight Section

RLN2 and its role in cancer

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Abstract

There is clear evidence that relaxin (RLN2 9p24) is involved in tumorigenesis. Relaxin, and a family of related peptides, has significant actions on connective tissue, cell growth and death and vascularization. Originally identified and named for its action on relaxing the ligaments of the pelvic girdle, over the last thirty years a picture has emerged that relaxin is involved in a number of critical tissue and cellular functions which are important attributes of cancer development and growth. This review provides an overview of the relaxin superfamily and focuses attention on evidence that relaxin is involved in different aspects of tumorigenesis.

I. Introduction

In 1926, F.L. Hisaw reported that injection of serum from pregnant guinea pigs or rabbits into virgin guinea pigs resulted in relaxation of the pubic ligament (Hisaw, 1926) and, shortly after in 1930, was able to develop an aqueous extract of this relaxative agent (Fevold et al., 1930). The hormone was named "relaxin" - it was one of earlier peptide hormones to be discovered and its method of discovery and its name have left an indelible impression that it is a hormone of pregnancy. But almost one hundred years on, the hormone is now known as one of a family of related peptides with putative and accepted roles in a variety of tissues and organs throughout the body and across many animal species from paramecium to humans.

Despite its relatively early discovery, relaxin research was hampered by technological barriers - primarily the lack of an ability to isolate pure extracts of relaxin. However, in 1974 techniques were developed to isolate and produce large quantities of purified hormone spawning a renewed interest in relaxin research (Sherwood and O'Byrne, 1974). Relaxin was isolated from a number of species and purified forms were used to determine its primary structure, develop a radioimmunoassay, identify actions in a number of tissues, and develop monoclonal antibodies and knock-out mice to elucidate its action (Bathgate et al., 2006a). But almost all the reports focused on its role in the female (Sherwood, 1994). Although there were reports of its presence in males or in non-reproductive tissue, the predominant focus of relaxin research was in its role as a hormone of pregnancy.

The first substantive observation that relaxin might have actions outside of the reproductive system was published by Summerlee and co-workers in 1984 who showed that relaxin affected the release of other peptide hormones from the brain. Since this discovery, many other actions of relaxin have been identified in tissues ranging from the heart and vascular system (Han et al., 1994), kidney (Novak et al., 2001), and neoplastic tissue (Silvertown et al., 2003). It is now clear that relaxin acts on a multiplicity of tissues in males and females (Bathgate et al., 2006a).

The advent of molecular techniques paved the way to cloning the first relaxin gene: cloning the rat (Hudson et al., 1981) and porcine (Haley et al., 1982) relaxin genes confirmed previous work that relaxin is structurally similar to insulin and is synthesized as a prohormone with three distinct regions or chains designed A, B and C. The A and B chains, with a characteristic signature of disulphide bridges cementing the tertiary structure, form the mature hormone but as relaxin was cloned from different species a remarkable lack of sequence homology between species was confirmed. Two human relaxin genes were cloned - RLN1 (Hudson et al., 1983) and RLN2 (Hudson et al., 1984). We now know that the second of these genes RLN2 is the gene encoding the relaxin peptide

produced in the corpus luteum and released in the circulation in women. It is the ortholog of circulating relaxins in other species and is known as H2 relaxin and has more recently been named systematically as RLX2 (Bathgate et al., 2006b).

The availability of recombinant H2 relaxin and the availability of genome databases rapidly led to the discovery that there were five novel genes with high homology to relaxin: four of these were named insulin-like peptides (INSL) - designated 3-6 (Adham et al., 1993; Chassin et al., 1996; Conklin et al., 1999; Hsu, 1999; Kasik et al., 2000; Lok et al., 2000). The insulin-like peptides do not share the relaxin-binding motif and are unable to mimic the actions of relaxin. Interestingly, in 2002 Bathgate and co-workers reported on a new relaxin gene with almost exclusive expression in the brain; termed RLN3 this discovery also provided researchers with new avenues for study with respect to the central actions of relaxin (Bathgate et al., 2002). Further studies investigating the sequence of RLN3 provide evidence that this peptide is indeed the ancestral form of all relaxins, insulin-like peptides, and insulin itself leading researchers to classify this group of peptides as a family of hormones (Hsu, 2003; Wilkinson et al., 2005; Bathgate et al., 2006b).

Concurrent with the rapid expansion in our knowledge of relaxin genes, there has been a substantial growth in our knowledge of the potentially physiological actions of relaxin; indeed there may be instances where relaxin has pathological actions (e.g. cancer). The hormone acts on a variety of tissues including connective tissue (Unemori and Amento, 1990), blood vessels (Bani, 1997) and neurons (Geddes and Summerlee, 1995) and on a number of organs including the brain (Geddes and Summerlee, 1995), heart (Han et al., 1994), and on the male and female productive reproductive tracts (Sherwood, 2004). And most recently has been implicated in tumour biology (Silvertown et al., 2003a) with a number of putative roles including modulation of tumour growth, neovascularization, migration and tumour progression (Silvertown et al., 2003a; ; Silvertown et al., 2006, Silvertown et al.,2007). The purpose of the current review is to focus on the potential role of relaxin in facilitating and supporting tumour development and metastasis and spread but before highlighting some of the key actions of relaxin in cancer, it is important to highlight one other fascinating feature of this unique, pleiomorphic hormone - the nature of its receptors.

Once again, the story of the discovery of "the"
relaxin receptor is remarkable - remarkable for three reasons: (1) it took almost eighty years from the discovery of the hormone to the first receptor was
identified (Hsu et al., 2002); (2) despite the structural similarities and in some cases sequence homology with insulin, relaxin appears to use a completely different family of receptors (Hsu et al., 2002; Kumagi et al., 2002; Liu et al., 2003a, Liu et al., 2003b; Liu et al., 2005) from insulin; and (3) there are several receptors and specific ligand-receptor pairings and even some specific peptide and species specific interactions between ligands and receptors (Bathgate et al., 2006b) that may complicate our understanding of the way these hormones may bring about their effects at the cellular level.

With all these complexities, it is important to understand and situate the biology of RLN2 9p24 within the framework of the family of peptides and to appreciate that the observations about the potential role of relaxin in cancer biology in one species may not necessarily be extrapolated to another species. There have been a number of critically important reviews of the actions of relaxin published over the years which provide a more detailed account of the history, chemistry and biology of relaxin (Sherwood, 1994; Schwabe and Büllesbach, 1994; Goldsmith et al., 1995; Bani, 1997; Ivell and Einspanier, 2002; Bathgate et al., 2003; Dschietzig and Stangl, 2003; Samuel et al., 2003; Silvertown et al., 2003b) and conference proceedings from meetings in 2000 (Tregear et al., 2001), 2004 (Sherwood et al., 2005) and 2008 (Bryant-Greenwood et al., 2009a). However, the current review is focused on the role of relaxin in cancer. It therefore outlines the isolation and cloning of relaxin and the relationship between the relaxin family of genes using RLN2 as the principal reference point. We then provide information on identification of the binding sites and receptors for relaxin and the actions of relaxin, primarily in non-reproductive tissues, that might underlie roles of relaxin in cancer biology. Finally, we review the evidence that supports the contention that relaxin has a role in the development and maintenance of cancer and in metastasis. We conclude with some remarks about the opportunities and challenges for further work in this field.

II. Isolation and purification of relaxin

The initial work isolating and purifying relaxin was published by Fevold et al., (1930) who reported that relaxin was probably a peptide as it was soluble in water, amphoteric and could be readily digested by trypsin (Fevold et al., 1930; Fevold et al., 1932). However, the early studies were limited by the lack of techniques for isolating and purifying proteins and by the lack of an ability to determine the purity of a substance. A significant break-through was achieved by Sherwood and O'Byrne (1974) who described a procedure for isolating the peptide in high yields from pig ovaries in kilogram quantities. For the first time it was possible to sequence the hormone and show the similarity between relaxin and insulin (Figure 1).

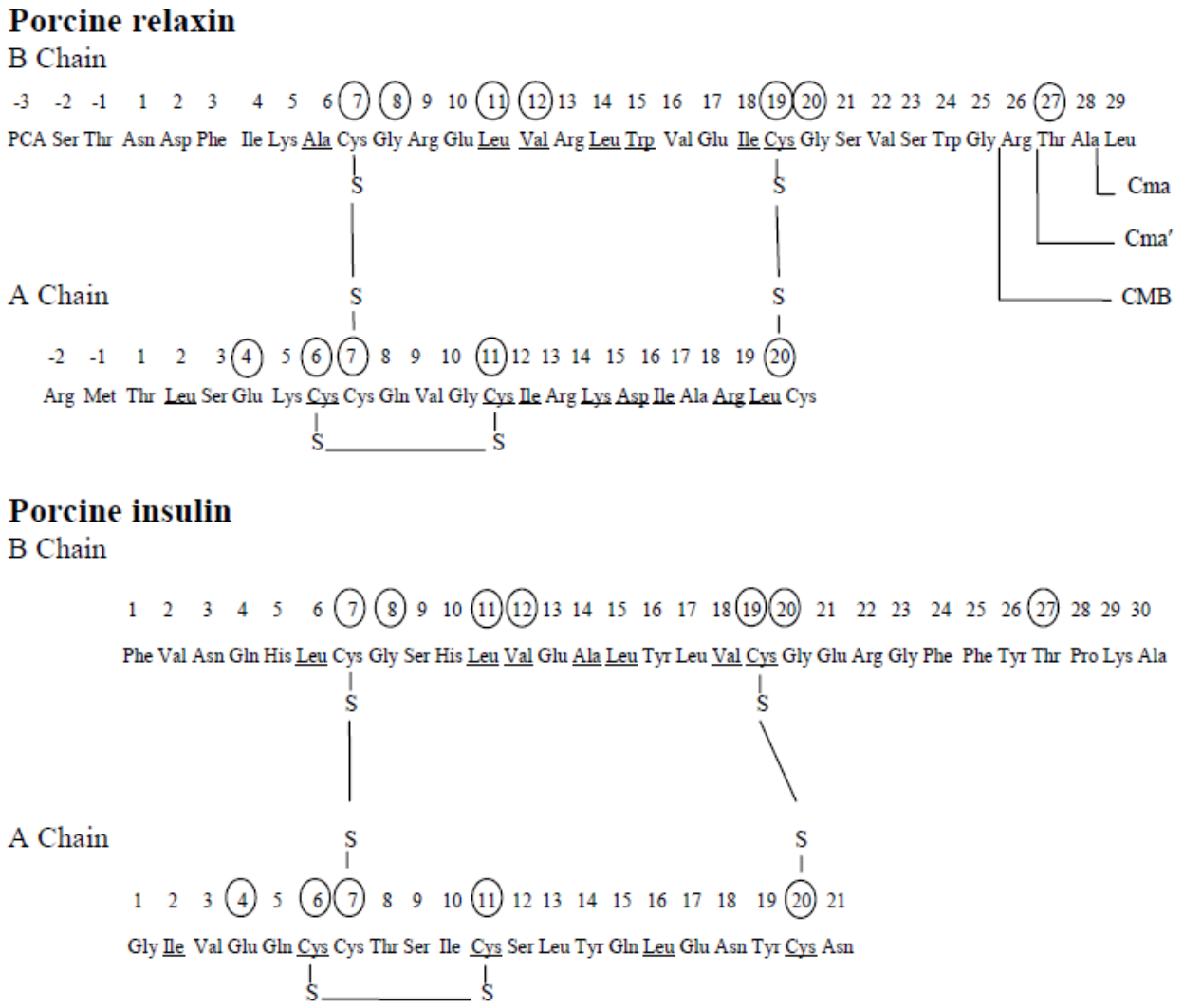

Figure 1: The structure of porcine relaxin (equivalent to H2 relaxin) and porcine insulin to illustrate the similarities and differences between the two peptides. The residues are numbered according to the insulin sequence to facilitate comparison. There are minor differences between three forms of porcine relaxin reported (CMB, Cma and Cma') which are shown on the B Chain of porcine relaxin. There are minor differences in the lengths of the B Chain between residues 25 and 26, 26 and 27 and 28 and 29 respectively. The amino acids which are identical in the two hormones are circled and those which contribute to the hydrophobic core of insulin and the comparable positions in relaxin are underlined.

Much of the work done on the structure of relaxin has been focused on the isolation and purification in three species - the pig (Sherwood and O'Byrne, 1974); the rat (Sherwood, 1979) and the horse (Stewart and Papkoff, 1986). The comparisons between these three types of relaxin underscore that despite the overall framework of two peptide chains held together in a characteristic tertiary conformation with an approximate molecular weight of roughly 6000 Da, there is considerable heterogeneity. Despite the notion that sequence homology is not highly conserved between species, three invariant structural characteristics are highly conserved: (1) the overall two-chain structure designated A and B; (2) the location of the disulfide bridges yielding the tertiary structure of the peptide; and (3) because the tertiary structure is highly conserved, the distinctive binding motif (R-x-x-x-R-x-x-I/V) is exposed and confers biological activity of the peptide.

Isolation of human relaxin did not occur until the late 1980s and early nineties because the hormone is present in lower concentrations in human tissues and initial attempts to isolate the hormone were confounded by lack of purity of the isolate but eventually sufficient hormone was extracted and purified for amino acid sequence analysis from human relaxin corpora lutea (Winslow et al., 1989) and later Winslow et al., (1992) were also able to extract relaxin from seminal plasma and show that the luteal and seminal relaxin were identical.

The heterogeneity of relaxin between species is remarkable with differences in lengths of the chains - particularly the B chain and considerable differences and differences within the chains. In some animals, the B chain is particularly long, for example, the domestic

dog (Canis familiaris) (Stewart et al., 1992) and the skate (Raja erinacea) has the longest B chain (Büllesbach et al., 1987): in some species not only is hormone different but its biological activity is considerably different - for example, shark relaxin shows very poor bioactivity in the mouse interpubic ligament bioassay (Büllesbach et al., 1986, Reinig et al., 1981); whilst in some species there is an astonishing conservation of amino acid sequence - for example, there is virtually no difference between porcine (Sus scrofa) relaxin and relaxin obtained from a mike whale (Blaenoptera acutorostrata) (Schwabe et al., 1989) or the porpoise (Phocaena phcaena) (Woods et al., 1991).

III. Cloning of relaxin

Work began on the cloning of relaxin genes with the activities of Niall and colleagues (Hudson et al., 1981; Haley et al., 1982) who determined the complete amino acid sequences of porcine (Haley et al., 1982) and rat (Hudson et al., 1981) preprorelaxin by cloning of relaxin cDNA. They confirmed that relaxin is synthesized as one single chain peptide with a signal tail connected to the B chain, a connecting peptide and the A chain in that order. Since then, first porcine (Haley et al., 1987) and then rat (Soloff et al., 2003) relaxin genes were cloned from genomic libraries. The sequences are identical in both circumstances with the potential of a single allelic variation in the porcine sequence (Haley et al., 1987) and the structure conforms to the gene structure for all relaxin genes.

There has now been analysis of the genomic DNA from humans, primates, pigs, rats and mice and their general structure is similar. There is a consistent view that an intron interrupts the coding region at the Glu in position 46 of the C peptide (Hudson et al., 1983; Haley et al., 1987; Crawford et al., 1989; Evans et al., 1993; Soloff et al., 2003) and the position of this intron matches that of one of the two introns found in insulin genes (Bell et al., 1980). Although there is no evidence of the second intron seen in insulin (Bell et al., 1980).

IV. Relaxin-family of related peptides

The first hint that there might be other members of the relaxin family came in the early 1990s when two groups independently identified a new cDNA clone that was differentially expressed in porcine (Adham et al., 1993) and mouse (Pusch et al., 1996) testis.

The newly identified clone encoded for a protein that was structurally similar to insulin and relaxin. In both cases the cDNA was highly expressed in the Leydig cells and initially known as Leydig cell insulin-like peptide (Adham et al., 1993) and relaxin-like factor (Büllesbach and Schwabe, 1995) which provides some confusion in the early literature. Subsequently, the gene for this peptide was cloned from human, porcine, mouse and rat (Burkhardt et al., 1994; Koskimies et al., 1997; Zimmerman et al., 1997; Spiess et al., 1999) and showed to be a single-copy gene similar to the relaxin gene - two exons and a single intron in the middle of the coding for the C-peptide - remarkably similar to relaxin. (See Figure 2). The name of this new member of the relaxin family was rationalized to insulin-like peptides and the peptide produce from this particular gene designated INSL3 because it was the third insulin-like gene to be discovered.

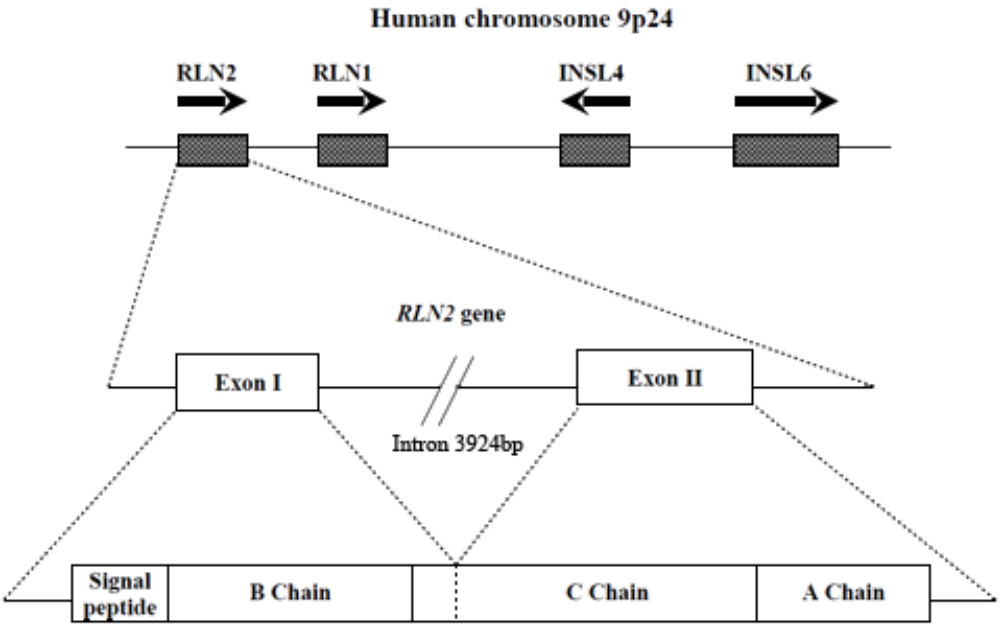

Figure 2: Schematic representation of the transcription of the human RLN2 gene. Adapted from Bathgate et al. 2006a (with permission). The gene is located with the RLN1, INSL4 and INSL6 genes on chromosome 9 at 9p24. The RLN2 gene consists of two exons and is transcribed to give preprorelaxin-2 mRNA. Exon I encodes for the signal peptide, the B Chain and part of the C Chain, and Exon II encodes for the remainder of the C Chain and the A chain of H2 relaxin. The arrows on the diagrams indicate the orientation of the genes. Although insulin and H2 relaxin are similar, there is no report that the insulin gene posses an intron.

Although the gene for INSL3 was discovered in the early 1990s, it was not until 2002 that the structure of INSL3 synthesized in vivo was identified (Büllesbach and Schwabe, 2002). Comparing the predicted and the actual sequence of the peptide revealed another surprise about these relaxin-like peptides: the A chain of bovine INSL3 was exactly as predicted but the B chain is longer by 8-9 amino acids - paradoxically the longer, naturally-produced INLS3 is less bioactive than an artificially synthesized version with a shorter B chain (Büllesbach and Schwabe, 2002). This implies that there may be mechanisms for processing relaxins once it is released or at the target tissue. Although this observation has only been specifically verified for INSL3, there are reports that transfected cells in vitro and in vivo with a cDNA for prorelaxin (H2 relaxin) will produce a peptide that appears to be prorelaxin which is as biologically active as relaxin. Such a possibility raises more questions about the possibility to there could be local control at the site of action for relaxin and members of the relaxin family of peptides that might be critical in cancer or in mitigation of the effects of relaxin in cancer.

V. The Evolving Story of the Relaxin Family of Peptides

In total, six human relaxin-like genes have been discovered. These are shown in Table I along with their specific chromosomal location. The key facet that links these genes and their products is the greater similar to relaxin (H2) than to either insulin or the insulin-like growth factors although there are clearly similarities across these three groups (Hsu, 2003).

Phylogenetic analysis has revealed that there is a common ancestor (Hsu, 2003) and this is most likely to be the third relaxin gene that was identified most recently (RLN3) and is located predominantly in the brain (Bathgate et al., 2002).

The similarities and differences among the relaxins, insulin and insulin-like growth factors are highlighted by their clustering on different chromosomes. These are illustrated in Figure 3. The focus of the remainder of the review will concentrate on RLN2 which is located on chromosome 9p24 closely associated with RLN1, INSL4 and INSL6 on the same chromosome.

VI. Binding sites and receptors for relaxin

Relaxin binding sites were identified in reproductive and non-reproductive tissue before the discovery of the relaxin receptor. The principal challenge was labeling pure hormone in a way that the labeled relaxin retained its biological activity. The early studies used two different techniques to label porcine relaxin: (1) iodination of tyrosine residues added to the N terminus producing a polytyrosyl-relaxin (Sherwood et al., 1975) or (2) incorporation of a ^{125}I group directly into the N terminus of porcine relaxin (McMurtry et al., 1978). Both methods produced labeled hormone that was biologically active and binding sites were demonstrated in reproductive tissues such as uterus (McMurtry et al., 1978; Mercado-Simmen et al., 1980; Mercado-Simmen et al., 1982; Weiss and Bryant-Greenwood, 1982) and placental membranes (Koay et al., 1986). Binding sites were also reported in fibroblasts in human skin (McMurtry et al., 1980). Although neither approach yielded completely pure iodinated forms, binding of the radioactive labeled hormone could not be displaced by insulin, IGF-1 or IGF-2.

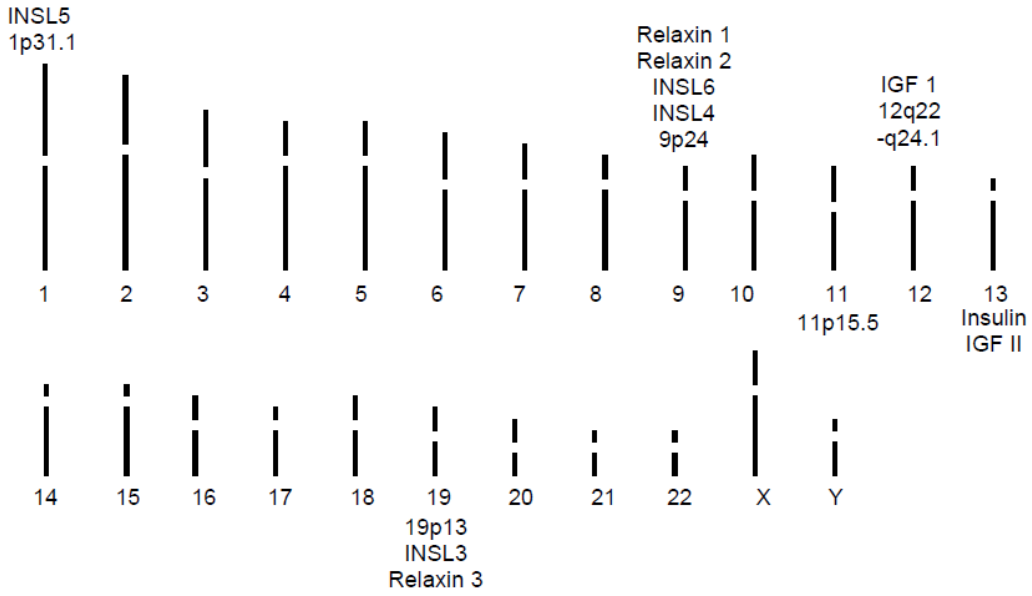

Figure 3: Schema showing the human chromosomal locations of the 10 members of the relaxin and insulin-like family of peptides genes. The relaxin peptide family genes are in different locations from the insulin and IGF-1 genes. The human RLN1 and RLN2 genes map in a tight cluster with INSL4 and INSL6 genes on chromosome 9 at 9p24. The RLN3 gene is located on chromosome 19 at 19p13.3 in close proximity to INSL3 at 19p13.2. In contrast, the INSL5 gene is located in chromosome 1 at 1p31.1 and is not closely associated with the other relaxin-like genes.

Peptide name	Abbreviations	Gene name
Insulin	INS	INS
Insulin-like growth factor-1	IGF-1	IGF1
Insulin-like growth factor-2	IGF-2	IGF2
Relaxin-1	RLX1 (human H1)	RLN1
Relaxin-2	RLX2 (human H2)	RLN2
Relaxin-3	RLX3 or INSL7	RLN3
Insulin-like peptide 3 Leydig-insulin-like peptide Relaxin-like factor	INSL3 Ley-I-L RLF	INSL3
Placentin Early placental insulin-like factor	INSL4 EPIL	INSL4
Insulin-like peptide 5 Relaxin-insulin-like factor 2	INSL5 RIF2	INSL5
Insulin-like peptide 6 Relaxin-insulin-like factor 1	INSL6 RIF1	INSL6

Table I
Members of the relaxin and insulin-like peptide family of genes.

More recently, relaxin binding studies were expanded using a ^{32}P (Osheroff et al., 1990) or ^{33}P (Tan et al., 1989) labeled relaxin. Specific binding with the ^{32}P labeled relaxin was confirmed in the uterus and cervix but also extended to the brain (Osheroff et al., 1990; Osheroff and Phillips, 1991; Osheroff and Ho, 1993). Interestingly, latter studies demonstrated binding in the rat heart atrium (Osheroff et al., 1992) and rat atrial cardiomyocytes (Osheroff and King, 1995).

In 1990, Büllesbach and Schwabe reported that the relaxin molecule could be biotinylated yet preserve its biological activity. Binding sites have subsequently been confirmed in the rat cervix, mammary gland and nipple (Kuenzi and Sherwood, 1995), cervix, mammary gland, nipple, small intestine, skin, ovary and testis of pigs (Min and Sherwood, 1998); the reproductive tract and breast tissue of women (Kohsaka et al., 1998); and prostate (Hornsby et al., 2001).

As a result of the chemical structure of members of the relaxin and insulin families of peptides and the evidence for the coevolution of the two peptide families and probably their receptors, it was assumed that receptors for relaxin and the INSLs 3-7 would be related to the known insulin receptors with tyrosine kinase activity. Indeed some of the initial work suggested that stimulation with relaxin resulted in tyrosine phosphorylation (Büllesbach and Schwabe, 2000). But the relaxin receptor remained illusive and attempts to purify the receptors were confounded by high levels of non-specific binding of tracer and apparently low levels of binding sites in target tissues.

At the turn of the twenty first century, there was a significant break through in relaxin receptor biology. Investigating the phenotypes of mice deficient in INSL3 (Nef and Parada, 1999; Zimmerman et al., 1999) two groups of researchers reported that bilateral cryptorcidism was a consistent feature of the INSL3 knock-out mouse and it was suggested that a leucine-rich glycoprotein receptor might be the receptor for relaxin (Hsu et al., 2002). It was shown that porcine relaxin stimulates both LGR7 and LGR8 receptors and results in increased cAMP (Hsu et al., 2002). Subsequent work has shown that LGR7 transcripts are located in a number of reproductive and non-reproductive tissues throughout the body. Although there is some evidence that relaxin activates both LGR7 and LGR8 there are clearly species differences in both the ability of relaxin to bind to LGR8 and the sensitivity of that binding (Bathgate et al., 2006b).

The complexity of the receptor-ligand story for relaxin was further compounded by the discovery that RLX3 has a relatively low affinity for LGR7 (Bathgate et al., 2002; Sudo et al., 2003). It now appears as if RLX3, which is located specifically within the brain, is a ligand to two orphan receptors GPCR135 (also known as somatostatin and angiotensin-like peptide receptor [SALPR]) and GPCR142 (Liu et al., 2003a; Liu et al., 2003b). There close links between the sites of concentration of these GPCR receptors and binding sites for relaxin and for relaxin-3 message (Osheroff and Phillips, 1991; Bathgate et al., 2006b) but low levels of GPCR142 message have also been reported in a variety of non-neural tissues throughout the body (Liu et al., 2003b).

Identification of receptors for relaxin created the possibility of confirming the intricate signaling cascade in normal and neoplastic tissues (Hsu et al., 2002; Kumagi et al., 2002; Sudo et al., 2003).

VII. Signaling pathways

Relaxin enacts its many physiological actions through a number of distinct signaling pathways that ultimately upregulate cAMP (Braddon, 1978; Sanborn et al., 1980; Sanborn and Sherwood, 1981; Hsu et al., 1985). Interaction of relaxin and its cognate GPCR stimulates cAMP production in a bi-phasic manner through G_S to enhance the activity of adenylate cyclase (Halls et al., 2006). Relaxin has also been reported to act through Gbetagamma thereby activating PI3K and further increasing cAMP production (Nguyen et al., 2003; Nguyen and Dessauer 2005). Downstream signaling of PI3K has also indicated that relaxin stimulates PKCzeta to stimulate cAMP (Nguyen and Dessauer, 2005). PKA has also been implicated in the signaling cascade initiated by relaxin. Inhibition of PKA has been reported to reduce contractility of heart cells (inotropy) (Han et al., 1994) and also has been demonstrated to be involved in affecting contractility of the myometrium by modulating potassium channels (Meera et al., 1995). Taken together it is clear that relaxin stimulates profound changes in cAMP levels in many cell types and tissues in order to bring about diverse physiological actions.

Relaxin has also been demonstrated to affect expression of NOS expression both acutely and chronically (Nistri and Bani, 2003). Modulation of NOS expression has been reported in endothelial cells (Failli et al., 2001) and vascular smooth muscle cells (Bani et al., 1998). It appears that two NOS isoforms are implicated: NOSII (iNOS) is likely affected by chronic administration of relaxin (Quattrone et al., 2004) while shorter term NO production is likely through NOSIII (eNOS) (Willcox et al., 2009).

The intracellular signaling pathways affected by relaxin have a number of implications in cancer and may explain the invasive, growth promoting, and angiogenic phenotypes promoted by relaxin in tumours. Relaxin has been reported to increase cAMP levels in a number of tumour cell lines including MCF-7 breast cancer cells (Bigazzi et al., 1992), PC-3 prostate cancer cells (Silvertown et al., 2007), and MDA-MB-231 human breast cancer cells (Radestock et al., 2008). Liu and colleagues (2008) also reported an involvement of the PI3K/PKB (Akt) pathway in a LNCaP prostate cancer cell model. Taken together these studies indicate that congruent to physiological actions, relaxin retains a diverse signaling profile and an ability to activate multiple signaling pathways in order to promote tumour growth and invasion characteristics. Whether or not these pathways are working in parallel or converge remains to be elucidated and requires further study in order to further understand relaxin's action in these cancers and develop potential therapeutic targets to treat this disease.

Relaxin has also been reported to increase NO production through increased iNOS activity in MCF-7 breast cancer cells (Bani et al., 1995). In spite of this observation, whether or not this is a positive effect of relaxin remains to be determined. It is possible that this phenotype contributes to the inhibition of tumour cell growth by the inhibition of DNA synthesis and mitochondrial respiration (Silvertown et al., 2003) however conversely increased NO may also induce cellular resistance to apoptotic events thereby contributing to cellular growth of the tumour. However other studies investigating the effect of NO on tumour development clearly report on the increased tumour cell migration (Jadeski et al., 2003) and tumour cell growth and angiogenesis (Jadeski et al., 2000). Furthermore, relaxin-induced expression of NO may affect the blood supply of the tumour contributing to the increased blood supply required by tumours to promote their own growth. In a number of vascular beds, relaxin has been noted to increase NO and therefore induce vasodilation in tissues ranging from the heart (Fisher et al., 2002) to skeletal muscle (Willcox et al., 2009). Given that NO is a potent vasodilator and has been reported to increase blood flow (Di Bellow et al., 1995) and angiogenesis in mammary cancer (Jadeski et al., 2000) the fact that relaxin-induced NO signaling may play a role in the development of tumours presents opportunities for further and intruiging studies.

VIII. Biological actions of relaxin that might underlie a role in cancer biology

A number of actions of relaxin at the tissue and cellular level are also important components of tumour growth, development, and metastasis and present the possibility that relaxin is involved the progression of cancer. Its action modulating connective tissue, inducing angiogenesis and affecting cell growth and apoptosis are critical in tumorigenesis and metastasis.

Evidence that relaxin affects tumour growth and development

Relaxin, acting in concert with estrogen and progesterone plays a critical role in mammary gland development (Min and Sherwood, 1996; Winn et al., 1994). In the mouse, the hormone induces mammary growth and differentiation (Bani and Bigazzi, 1984). Conversely, mammary development is retarded and nipple development impaired in the relaxin-deficient mouse (Zhao et al., 1999). Although lactational changes do occur in the mammary tissue in the knock-out mice, the young are unable to suck milk and starve to death which confirms the essential role that relaxin plays in remodeling connective and epithelial tissue and development of the nipples. Similarly, both H1 and H2 relaxin are present in human breast and have been linked to normal development. They have also been implicated in neoplastic growth of the breast (Tashima et al., 1994; Mazoujian and Bryant-Greenwood, 1990; Bryant-Greenwood et al., 1994). Moveover, Tashima et al., (1994) reported the presence of relaxin (H2) transcripts were identified in 100% of neoplastic mammary tissue (benign and malignant) with relatively

low proportions in non-neoplastic tissue. LGR7 receptors are present in both malignant human breast cancer tissues and in human mammary tumour cell lines (Silvertown et al., 2003a) suggesting that the neoplastic tissue is not only producing relaxin but is also a target for the hormone. The possible extracellular roles of relaxin in tumour growth, development and metastasis are discussed later in the review. Low concentrations of relaxin over short periods of time appear to promote the growth of breast adenocarcinoma cells in vitro (Sacchi et al., 1994; Bani et al., 1999) and Binder et al., (2004) reported that there are elevated circulating levels of relaxin in women with breast cancer - particularly those with metastatic disease. Relaxin stimulates invasiveness and migration of breast tissue, thyroid, and endometrial carcinoma cells in vitro and is accompanied by up-regulation of matrix metalloproteinase activity and expression of vascular endothelial growth factors (VEGF) (Binder et al., 2002; Kamat et al., 2006; Hombach-Klonisch et al., 2006). Prorelaxin 2 (the precursor of relaxin) also stimulates the invasiveness of canine mammary carcinoma cells (Silvertown et al., 2003b).

Similar to reports of the presence and action of relaxin in normal development of human breast tissue, relaxin is present in prostatic tissue (Ivell et al., 1989; Sokol et al., 1989; Hansell et al., 1991) and has been implicated in development and maturation of prostatic tissue in rats (Hornsby et al., 2001; Feng et al., 2007). The prostate gland undergoes a number of structural changes during life and prostatic hypertrophy and tumour are condition of men over 45 years of age (Carter and Coffey, 1990) with similar age-related changes reported in other species (Gann et al., 1996). Much of the work on the etiology of both prostatic hyperplasia and carcinoma and adenocarcinoma has focused on the role of steroid hormones (Montie and Pienta, 1994; Barret-Connor et al., 1990; Normura et al., 1988) but the findings are not entirely consistent and there is a persistent view that peptides may also be involved in the disease. There is a clear progression of the disease from hypertrophy to cancer which is characterized by an unresponsive switch to a differentiated state and uncontrollable proliferation of cells (Hanahan and Weinberg, 2000) reported in both men and male dogs (Nomura et al., 1988). The hyperplastic state is associated with a change in the connective tissue framework of the gland and a marked angiogenesis (Lissbrant et al., 1997): changes which are further exaggerated in the neoplastic state - both of these changes are hallmarks of the action of relaxin (Bathgate et al., 2006a; Bathgate et al., 2006b). Gunnerson et al. (1995) reported that the human prostate adenocarcinoma cell line LNCaP. FGC expresses high levels of relaxin transcripts which implies a link with prostatic cancer. Lentiviral-mediated delivery of relaxin into PC-3 prostate cancer cells increases growth of prostate tumour xenografts (Silvertown et al., 2006) and it has been shown that relaxin is a direct downstream target of R273H p53 mutation in prostate carcinoma cells (Vinall et al., 2006). Moreover, relaxin expression appears to be up-regulated by androgen withdrawal both in vivo and in vitro (Thompson et al., 2006). Finally, Feng and colleagues (Feng et al., 2007) reported that there is a strong correlation between significantly higher levels of relaxin message and message for its receptor LGR7 in recurrent prostate cancer samples from human patients and congruent with reports in breast tissue, relaxin stimulates cell proliferation, invasiveness and adhesion in vitro (Feng et al., 2007). Interfering with the production of relaxin and its receptor in vitro on prostate adenocarcinoma cells decreased cell invasiveness and growth and increased cell death in vitro (Feng et al., 2007). Finally, experiments conducted by Feng and colleagues, (2007) in vivo using a transgenic mouse with overexpression of RLN1 demonstrated a shorter survival time for mice with excess relaxin in the presence of prostate adenocarcinoma compared with controls. Further evidence that relaxin modulated tumour growth and progression was provided by Silvertown et al., (2007) when this group reported that an analog of relaxin which appears to be a relaxin anatgonist impairs prostate tumour growth in vivo both reducing the growth of a prostate cell line xenograft and reducing the incidence of metastasis. This was the first study to indicate the possible use of a relaxin antagonist to both investigate the progression and course of tumourigenesis as well as it suggest a possible therapeutic agent for use in the treatment of prostate cancer.

Relaxin-like peptides and INSL3 have been associated with a number of other tumours (Klonisch et al., 2005) including malignancies in the gastrointestinal tract (Stemmermann et al., 1994) thyroid gland (Homach-Klonisch et al., 2006), colorectum (Alfonso et al., 2005), and the male and female reproductive tracts (Silvertown et al., 2003a) in addition to the report above on relaxin and tumour development in breast and prostate. Although the data are not as fulsome for these other cancers, common themes emerge: there are higher levels of expression of transcripts for relaxin and its receptor in malignant cell forms, and in some cases correlations reported between increased relaxin expression, circulating levels of hormone, tendency to malignancy and incidence of metastasis (Homach-Klonisch et al., 2006). Studies in vitro suggest that relaxin promotes proliferation, invasion and metastasis of tumour cells. There is some evidence that levels of circulating hormone can be linked to survival times. Taken together, evidence is accumulating to suggest that relaxin signaling plays a significant role in tumour development and progression.

Relaxin and cell growth

Relaxin affects cancer cell differentiation and growth. Relaxin induced a transient growth followed by a

reduction in growth of mammary tumours induced by estrogen and radiation in rats (Segaloff, 1983). Human breast cancer MCF-7 cells show marked proliferation and differentiation to relatively low levels of relaxin. However at higher doses relaxin seems to suppress proliferation although differentiation is still observed both in coculture (Bani et al., 1994) and in an in vivo preparation in nude mice (Bani et al., 1999). This raises intriguing questions about the possible role of relaxin in cancer suppression that need to be answered but at the same time Zhang and colleagues demonstrated that relaxin caused cellular proliferation by increasing MAPK and MEK protein expression in a variety of cells including normal human endometrial stromal cells, THP-1 myelomonocytic leukemia cells, and coronary and pulmonary artery smooth muscle cells (Zhang et al., 2002). Insulin, IGF-1 and platelet derived growth factor (PDGF) activate proliferative, apoptotic and metabolic signals via both MAPK and P13-Kinase/Akt. Although relaxin appears to stimulate P13-Kinase in blood vessels (Willcox et al., 2009) it appears that its action in human endometrial stromal cells stimulates the transcription factor CREB but does not involve Akt or Jun N-terminal kinase (JNK) (Zhang et al., 2002).

One of the principal intracellular pathways activated by relaxin is the nitric oxide (NO) cascade (see previous section). Activation of NO results in cytoskeletal and organellular changes and, depending on conditions be involved in antiapoptosis or cytostasis (Rivoltini et al., 2002): suppression of NO synthesis in human melanoma results in induction of the intrinsic apoptosis pathway. Cell survival is thereby promoted against chemotherapeutic drugs, mediating hypoxia induced drug resistance in human and murine tumours and assisting neoplastic cells to avoid immune destruction. Nitric oxide also induces a cytostatic state by inhibiting DNA synthesis, mitochondrial respiration and cytochroms P-450 activity (Bani et al., 1995; Bani et al., 1998; Bogdan, 2001). There is either spontaneous or induced expression of NO-synthase (iNOS) in both mouse mammary and melanoma cell lines (Lala and Orucevic, 1998; Xie and Fidler, 1998; Li et al., 1991). This results in increased NO which inhibits DNA synthesis and this is inversely correlated with metastasis. Bani and colleagues (Bani et al., 1995) reported that MCF-7 cells incubated with porcine relaxin showed an increased expression of two isoforms of NOS. They reported a dose dependent, bi-phasic increase in Ca^{2+}/calmodulin dependent NOS (cNOS) and a graduate increase in iNOS activity. This implies that relaxin may indirectly attenuate tumour growth by activating the NO pathway to inhibit DNA synthesis that results in cytostasis and/or relaxin may facilitate tumorigenesis by assisting cells to avoid apoptosis.

Relaxin has been shown to activate protein kinase A (PKA) in a number of cells including the human tumour cell lines MCF-7 and THP-1 (Parsell et al., 1996; Fei et al., 1990; Hsu et al., 2000; Failli et al., 2002) and evidence in most cells confirms that the PKA pathway not PKC mediates the actions of the LGR7 and 8 receptors (Hsu et al., 2000; Hsu et al., 2002; Willcox et al., 2009) but there is one exception. It appears as if the action of relaxin in cardiac myocytes is mediated through PKC (Shaw et al., 2009). Through a complex cascade (Xi et al., 1994): increased PKAc activity results in enhanced phosphorylation of the NFkappaB p65 subunit and an increase in transcriptional activity of NFkappaB. This change in transcription has been suggested to promote tumour growth (Zhong et al., 1997).

Relaxin and cell invasion

Remodeling of connective tissue is a hallmark action of relaxin (Bathgate et al., 2006a) and the hormone has been implicated in anti-fibrotic action (Casten and Boucek, 1958). Relaxin acts directly on transforming growth factor-beta-stimulated human dermal fibroblasts (Unemori and Amento, 1990), lung fibroblasts (Unemori et al., 1996) and cardiac fibroblasts (Samuel et al., 2004) to promote both a decrease in type I and type II collagen synthesis and an increase in MMP expression and activation (Samuel et al., 2004). As a result, relaxin has actually been used in a number of animal models to alleviate fibrosis where it has been used to remodel the extracellular matrix including in the skin (Kibblewhite et al., 1992; Unemori et al., 1993), lung (Unemori et al., 1996); liver (Williams et al., 2001), liver (Bennett et al., 2003; Bennett et al., 2007; Bennett et al., 2009) and kidney (Garber et al., 2001; Garber et al., 2003). However, apart from the original report of clinical trials with porcine relaxin in humans by Casten and Boucek, (1958), a more rigorous clinical trial with genetically engineered relaxin was not successful in demonstrating an effective anti-fibrotic therapeutic action for relaxin in the human disease scleroderma (Seibold et al., 2000; Khanna et al., 2009). Nevertheless, relaxin has been reported to improve wound healing (Casten et al., 1960) although the prime site of action may not be on the connective tissue but on blood supply (see later) and in serving as a cardioprotective agent to experimentally produced ischemia (Masini et al., 1997; Bani et al., 1998).

It has been strongly suggested that loosening connective tissue may assist in tumour migration as a result of the actions of relaxin, mediated through the matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMP) (Silvertown et al., 2003a). In tumour biology, MMP/TIMP has been implicated in degradation of the extracellular matrix to facilitate cell migration, alteration in the cellular environment that fosters cell migration, and the activation of tissue specific molecules that modulate TIMPs (Vu and Werb, 2000). MMPs are also involved in angiogenesis, invasion and metastasis (Duffy et al., 2000; Hiraoka et al., 1998) and they affect tumour suppressing growth factor (TGF-beta) (Yu and Stamenkovic, 2000),

heparin-binding epidermal growth factor (HBEGF) (Pierce et al., 2001; Prenzel, 1999) various binding proteins (Fowlkes et al., 1994a; Fowlkes et al., 1994b); and proteases (Polette and Birembaut, 1998; Ugwu et al., 1998). These data clearly lead to the conclusion that by activating MMPs and TIMP, relaxin could support and enhance tumour invasion. However, at the same time there is evidence that MMPs can induce programmed cell death in anchorage-dependent cells and might defy tumour progression (Li et al., 1999: Will et al., 2000).

In a similar vein, the effects of relaxin on the MMP/TIMP system appear to be cell-type dependent: relaxin is reported to stimulate MMPs in cervical fibroblasts (Palejwala et al., 2001) but reduces pro-MMP-1 in endometrial cells (Palejwala et al., 2002). There is certainly evidence that relaxin can stimulate MMP release: Binder et al., (2002) showed that relaxin upregulated the expression of mRNA of MMP-2, -9 and -14 in MCF-7 and SK-BR3 cell lines and increased cellular migration; and Silvertown et al., (Silvertown et al., 2001; Silvertown et al., 2003a) showed that human relaxin could stimulate the migration of L6 cells and the movement of canine mammary tumour cells (CF33.Mt) respectively, through a porous membrane. Again, suggesting perhaps that the response is cell-type specific, Silvertown and colleagues, (2003a) reported that human relaxin resulted in a decreased migration of the human mammary cancer cell line MDA-MB-435.

Binder and colleagues, (2001) reported that patients with active metastatic breast cancer have elevated circulating levels of relaxin in the serum. In an interesting study on the incidence of breast cancer in the early nineties, Lambe et al., (1994) had postulated that one pregnancy increased the risk of breast cancer but multiple pregnancies decreased the risk which was confounding. Silvertown and colleagues (2003a) suggested that perhaps the short- and long-term risks of breast cancer and pregnancy might be related to the differential action of relaxin but this remains to be explored.

Relaxin and angiogenesis

Tumour growth depends on blood supply and there is critical point in the growth phase when a switch towards an angiogenic phenotype is absolutely critical (Ellis et al., 1996; Hanahan and Folkman, 1996; Tonini et al., 2003; Kerbel, 2008). The modelling and remodeling of vascular supply depends on a balance of proangiogenic and antiangiogenic factors that are produced by neoplastic tissue or induced in the surrounding cells (Tonini et al., 2003). Proangiogenic factors include vascular endothelial growth factor (VEGF), angiopoietins and ephrins, and a variety of other molecules and transcriptional factors. A number of these have been implicated as a possible product of relaxin stimulation. Reports and claims that relaxin stimulates these angiogenic substances are provided in Table II. In contrast, although there is a wide cadre of potential antiangiogenic factors known, only a limited number have been reported to be stimulated by relaxin.

Originally identified as a single compound, it is now known that VEGF is one of the most potent angiogenic cytokines and comprises a family of related molecules VEGF A-D and placental growth factor (Ogawa, 1998; Meyer et al., 1999; Neufeld et al., 1999; Ferrar, 2002; Hicklin and Ellis, 2005; Kerbel, 2008). The critical importance of VEGF to the integrity of the vascular system is supported by knockout studies: disruption of one VEGF allele in mice results in lethal abnormalities and removal of both alleles results in a virtually complete absence of vasculature in embryos (Cameliet et al., 1996; Ferrara et al., 1996; Carmeliet, 2000). All the members of the VEGF family have overlapping abilities to interact with the different receptors expressed primarily in the vascular endothelium (Eriksson and Alitalo, 1999). The vital importance of angiogenesis in tumour growth and development and the major role of VEGF has led to a great deal of basic and clinical research directed towards the VEGF family and the receptor tyrosine kinases that mediate their proangiogenic effects (Ferrara, 2002; Hicklin and Ellis, 2005). Relaxin has been shown to upregulate VEGF in stromal and glandular epithelial cells of the endometrium in wound healing (Palejwala et al., 2002; Unemori et al., 1999; Unemori et al., 2000), and in the myelomoncytic leukemia cell line THP-1 (Parsell et al., 1996). The THP-1 cells also exhibit relaxin receptors (Unemori et al., 1999; Unemori et al., 2000) which implies there may be some autocrine function of relaxin that may be related to angiogenesis in tumour development (Silvertown et al., 2003a; Kerbel, 2008). But again, the action of relaxin may be dependent on cell type and by inference on tumour cell type - Zhang and colleagues (2002) report that human endometrial stromal cells incubated with relaxin showed a reduced level of VEGF transcription.

The major mediator of tumour angiogenesis appears to be VEGF-A (Kerbel, 2008) which acts preferentially through the VEGF receptor 2. This is highly expressed by endothelial cells engaged in angiogenesis and by circulating bone marrow-derived endothelial precursor cells (Shibuya and Claesson-Welsh, 2006). There is also a VEGF receptor 1 which has a ten-fold higher affinity with VEGF-A but its signal transducing properties are extremely weak (Shibuya and Claesson-Welsh, 2006). Consequently, the role of VEGF receptor-1 remains unknown (Kerbel, 2008).

Known Angiogenic Factor	Relaxin stimulates production
Adenosine	Chen et al. 1988
Angiogenin	Unemori et al. 1999
Angiopoetin-1 (Ang-1)	Hewitson and Samuel 2009
Collagen	Unemori et al. 1993
Epidermal growth factor	Steinetz et al. 2009
Ephrins	Davison et al. 2004
Fibroblast growth factors (a and b)	Taylor and Clark 1992
Fibronectin	McDonald et al. 2003
Follistatin	Petraglia et al. 1994
Granulocyte colony-stimulating factor	Moore et al. 2007
Heparin	Masini et al. 1994
Interleukin 8 (IL-8)	Bryant-Greenwood et al. (2009a)
Leptin	Steinetz et al. (2009)
Midkine	Sacchi et al. (1994)
Nicotinamide	Berne 2002
Proliferin	Conrad et al. 2004

Table II
Known angiogenic factors which have been linked with or claimed to be linked with relaxin.

Most types of human cells have been shown to express high levels of VEGF and it appears as if hypoxia, which is a characteristic of solid tumours (Semenza, 2003) is important for inducing VEGF release. There are no data to date that indicate the hypoxia results in relaxin release but this is an intriguing possibility, especially as the appears to be conductance phenomena among branches of the microcirculation which might explain both an increased blood flow (Willcox et al., 2010) and angiogenic effect of relaxin.

It is assumed that VEGF has paracrine effects as tumour cells produce VEGF but lack cell-surface receptors for VEGF whereas endothelial cells express the receptors but produce relatively little VEGF. It has been suggested that VEGF originates from host cells in the body such as platelets and muscle cells (Kut et al., 2007) and tumour-associated tumour cells (Fukumura et al., 1998; Liang et al., 2006).

As mentioned earlier, relaxin upregulates NO through NOS in both vascular cells (Willcox et al., 2009) and neoplastic cells (Parsell et al., 1996; Fei et al., 1990; Hsu et al., 2000; Failli et al., 2002; Davel et al., 2002). Furthermore, tumour-associated angiogenic activity in vivo has been linked with increased levels of iNOS (Jadeski and Lala, 1999) and endothelial cells NOS (eNOS) (Jadeski et al., 2000) and inhibition of NOS with N-nitro-L-arginine methyl esther (L-NAME) results in a marked reduction in angiogenesis (Jadeski and Lala, 1999; Jadeski et al., 2000). Relaxin has been shown to increase microvascular arterial diameter in vitro (Bani et al., 1998) and in vivo (Willcox et al., 2009; Willcox et al., 2010). Arteriolar dilation decreases leukocyte-endothelial adhesive properties and increases vascular permeability (Fukumura and Jain, 1998). Bearing in mind that microvessel density, in both mammary and prostate tumours, is positively correlated with tumour cell survival and negatively correlated with longevity of the patient (Lissbrandt et al., 1997), Silvertown and colleagues suggested that high circulating and/or local levels of relaxin might upregulate VEGF and NO to increase blood flow to the region and stimulate an active angiogenesis to support tumour growth (Silvertown et al., 2006).

Other possible pathways for relaxin-involvement in angiogenesis

There is a body of literature supporting a pivotal new signaling pathways in angiogenesis related to tumorigenesis: notch delta-like ligand (DII) (Sainson and Harris, 2007; Noguera-Troise et al., 2006; Lobov et al., 2007; Ridgway et al., 2006; Gale et al., 2004). Notch cell-surface receptors are expressed by various cell types and generally involved in cell differentiation, proliferation and apoptosis. These receptors interact with transmembrane ligands on adjacent cells and may be involved in vital angiogenic activity which implies a possible role in vascular growth in tumorigenesis (Gale et al., 2004; Carmeliet et al., 1996; Ferrara et al., 1996). Although there are no reports to date of the possible role for relaxin in stimulating pathways that might interact with the Notch cell-surface receptors, this remains a possibility that deserves further investigation. Finally, it is known that a number of cell types can be mobilized from bone marrow that may be important in new blood vessel formation (Betolini et al., 2006).

These include various monocytic and myeloid cells that express endothelial cells markers such as VE-Cadherin, VEGF-1 and VEGF-2 (Okazaki et al., 2006; Conejo-Carcia et al., 2005; Grunewald et al., 2006). As relaxin has been reported to upregulate VEGF and bFGF in the myelomonocytic leukemia THP-1 cells (Parsell et al., 1996) this raises the interesting spectre that relaxin could also affect the responses of circulating bone-marrow derived cells in promoting angiogenesis.

IX. The next steps

The evidence that RLN2 9p24 and other members of the relaxin superfamily of peptides are involved in tumorigenesis is now unequivocal. There are data suggesting that relaxin is upregulated in tumour tissue, that receptors are present and that the hormone appears to be involved in the growth, vascularization and spread of cancer. There is a picture emerging of the signaling events induced by relaxin. Under specific conditions, relaxin appears to facilitate growth, limit apoptosis, induced angiogenesis and facilitate connective tissue remodeling that would support local and metastatic spread. This raises the spectre that inhibitors of inhibitors of relaxin could be part of the arsenal of weapons to be used in the fight against cancer. Recently, Silvertown and colleagues (Silvertown et al., 2006) showed that transfecting tumour xenografts implanted in mice with a modified relaxin cDNA not only reduced tumour size and vascularization but also reduced the incidence of metastasis raising the exciting possibility that anti-relaxin agents might suppress tumour development.

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