Uniparental Disomy in Cancer – A New Tool in Molecular Cancer

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Article Contents

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The cancer genome project has attempted to provide the complete landscape of existing mutations in tumours, but sequencing the whole genome for all tumour types is a challenging goal. Recently a novel form of abnormality in various cancers, acquired uniparental disomy (aUPD), has been revealed. aUPD regions may pinpoint the mutated genes for next generation sequencing. Therefore, identifying the aUPD regions can help to identify novel candidate genes for mutation analysis instead of randomly sequencing the genome, may help to distinguish driver genes from passenger, lead to the discovery of novel therapeutic targets and provide important prognostic information, which may thus lead to important clinical applications.

Introduction

Cancer results from the accumulation of all types of genetic and genomic changes, including deoxyribonucleic acid (DNA) copy number changes, copy number neutral changes, sequence alterations and global or promoter methylation of genes. Numerous aberrations have been reported in various cancers, but it is unknown which genes 'drive' ('drivers') the cell to clonal selection, owing to the challenges of selecting candidate genes in extremely large regions of DNA copy number changes and distinguishing the 'driver' genes from the 'passengers', which tag along because of physical proximity to the drivers. Integrating genomic and genetic data may improve our understanding of cell transformation and help us identify the driver genes.

A novel form of abnormality, acquired uniparental disomy (aUPD, also known as copy number neutral change), has been revealed in various sporadic cancers as a somatic event. UPD was first identified in constitutional DNA by Engel (1980) in hereditary disease and occurs through meiotic error as a germline event (now called constitutional UPD). Recently, it was shown that UPD can also occur through mitotic error as a somatic event (called acquired UPD, aUPD) (Fitzgibbon *et al*., 2005). a UPD can pinpoint the homozygous mutated or methylated genes; therefore, aUPD can be used as a new tool in molecular cancer for discovering novel genes that are candidates for mutation in the aUPD regions.

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Genetic and Genomic Changes

Numeric and structural aberrations of cells are hallmarks of cancer. Numeric aberrations involve the loss or gain of one or more chromosomes, which is called *aneuploidy*, or the addition of one or more complete haploid complements, which is called *polyploidy*. Aneuploidy is a condition in which a cell has an incorrect number of chromosomes; for example, a cell might have three copies of a particular chromosome, a condition called trisomy (i.e. the cell is trisomic), or it might have only one copy of a particular chromosome, which is called monosomy (i.e. the cell is monosomic). Numeric alterations can be detected by using karyotyping, fluorescence *in situ* hybridisation (FISH), comparative genomic hybridisation (CGH), CGH array and single nucleotide polymorphism (SNP)- and oligonucleotide-based arrays (**Table 1**).

Structural aberrations can be either balanced or unbalanced rearrangements. Balanced rearrangements are chromosomal abnormalities that give rise to structurally altered chromosomes without the gain or loss of genetic material: reciprocal translocations, inversions and insertions. Unbalanced rearrangements are chromosomal abnormalities that give rise to structurally altered chromosomes with the gain or loss of genetic material: unbalanced

Table 1 Methods for detecting structural, numerical alterations and UPD in the human genome

Methods	Resolution	UPD detection	Dividing cells needed	Translocation	Inversion	DNA copy number changes
Genome-wide scans						
Metaphase cytogenetics	Low, dependent on mitotic index ($\sim$5 Mb)	No	Yes	Yes	Yes	Yes
SKY (M-FISH)	1–2 Mb	No	Yes	Yes	No	Yes
CGH	2–10 Mb	No	No	Yes[a]	No	Yes
aCGH (BAC array)	1 Mb	No	No	Yes[a]	No	Yes
Oligonucleotide array						
Affymetrix (SNP-based, SNP and MIP arrays)	0.68–210 kb	Yes	No	No	No	Yes
Illumina (SNP-based)	1.5–4.9 kb	Yes	No	No	No	Yes
NimbleGen (oligonucleotide probe)	713 bp–40 kb	No	No	No	No	Yes
Agilent (oligonucleotide probe)	2.1–43 kb	No	No	No	No	Yes
Targeted scans						
Interphase FISH	50 kb–2 Mb	No	No	Yes	Yes	Yes
Fiber FISH	1–500 kb	No	No	Yes	No	Yes
MSI	Low	Yes	No	No	No	Yes (deletion)

Notes: CGH, comparative genomic hybridisation; M-FISH; multiplex-fluorescence *in situ* hybridisation; MIP, molecular inversion probe and SKY, spectral karyotyping.
[a]CGH/aCGH can detect unbalanced translocation, but not reciprocal translocation.

translocations, isochromosomes, ring chromosomes, deletions and amplifications. Methods to identify all of these chromosomal abnormalities are summarised in **Table 1**. *Translocations* occur in three different types: reciprocal, Robertsonian and unbalanced. A *reciprocal translocation* involves breakage of at least two chromosomes and an exchange of the fragments between nonhomologous chromosomes. In this case, the chromosomal number does not change from the normal 46. A *Robertsonian translocation*, or centric fusion, results from the breakage of two acrocentric chromosomes at or close to their centromeres; the short arms are lost and the long arms become fused. Because the short arms of these acrocentric chromosomes contain genes for only ribosomal ribonucleic acid (RNA), for which there are multiple copies on the various other acrocentric chromosomes, the loss of the short arms has no clinical importance. In this case, however, the total chromosomal number is reduced to 45. Both reciprocal and Robertsonian translocations can be detected by using the conventional cytogenetic techniques of chromosome banding (metaphase spread), interphase FISH, and multiplex-FISH (M-FISH)/spectral karyotyping (SKY). An *unbalanced translocation* is an unequal exchange of chromosomal material that results in either extra or missing genes. In cancer, the most frequently seen translocations are reciprocal translocations (e.g. t(9;22)(q34;q11), which results in *BCR–ABL* (breakpoint cluster region–c-abl oncogene, receptor tyrosine kinase) gene fusion) and unbalanced translocations (e.g. der(17)t(X;17)(p11;q25), which results in alveolar soft part sarcoma chromosome region, candidate 1 *(ASPSCR1)*–transcription factor E3 *(TFE3)* gene fusion). These aberrations can be detectable by karyotyping, SKY, FISH and microarrays (bacterial artificial chromosome (BAC), oligonucleotide- or SNP-based arrays).

An *inversion* is a two-break rearrangement in which a segment of a chromosome is reversed end to end (i.e. inverted). Inversions can be either *paracentric*, which involve just one chromosomal arm, or *pericentric*, which includes the centromere and breaks in both arms. Inversions can be detected by karyotyping.

An *insertion* is the addition of DNA material, any size between one base pair to one chromosome, incorrectly inserted into another one. An insertion can be detected by karyotyping (depending on the size and type of the inserted segment), FISH (via a chromosome painting probe) and SKY. If the inserted material involves translocations between two chromosomes that exchange material, then the karyotype is considered balanced.

An *isochromosome* results when one arm of a chromosome is lost and the remaining arm is duplicated by transverse rather than longitudinal division of the centromere.

A *ring* chromosome is formed when a break occurs on both arms of a chromosome, leaving two 'sticky' ends on the more central portion that then connect to form a ring. The distal fragments of both chromosomes are lost. Isochromosomes and ring chromosomes can be detected by karyotyping, SKY and FISH.

A *deletion* involves the loss of one part of a chromosome, which results in monosomy for that segment of the chromosome.

Finally, an *amplification* involves the creation of multiple copies of a chromosomal region. This can occur as double minutes (many small diploid copies of small part of a chromosome) or homogeneously staining regions.

Other hallmarks of cancer are mutations, methylation and copy number neutral changes. *Mutations* can range from single-base substitutions, to insertions and deletions of single or multiple bases, to loss or gain of entire chromosomes. There are also silent, missense, nonsense, splice-site, promoter, frameshift and dynamic mutations as well as partial or whole-gene deletions or duplications. *Methylation* can occur either as promoter methylation, which causes gene silencing or global methylation.

Copy number neutral changes, also called UPD, can occur either in the whole chromosome or segmentally. This type of alteration does not cause any DNA copy number changes. Genome-wide UPD can be detected only by using SNP-based microarray such as Affymetrix (Santa Clara, CA) and Illumina (San Diego, CA) and proper analytical tool such as allele-specific copy number (AsCNAR) (Yamamoto *et al*., 2007) and segmental UPD in a certain chromosome by microsatellite analysis (**Table 1**). UPD is not detectable with conventional cytogenetic methods, BAC probe-based array such as PerkinElmer Life and Analytical Sciences (Waltham, MA), oligonucleotide probe-based comparative hybridisation array (aCGH) such as NimbleGene (Roche NimbleGen, Inc. Madison, WI) and Agilent (Foster City, CA) microarrays. **See also**: Genetic Variation: Polymorphisms and Mutations; Microarrays and Single Nucleotide Polymorphism (SNP) Genotyping; Polymorphisms: Origins and Maintenance; Single Nucleotide Polymorphisms (SNPs): Identification and Scoring

Mechanism(s) Underlying aUPD

Each cell carries two copies of each somatic chromosome – one inherited from the mother and the other from the father (**Figure 1a**). In addition, each cell has two copies of the sex chromosomes, either two copies of the X-chromosome, in females (one from the mother, called maternal and the other from the father, called paternal), or, in males, one X-chromosome (maternal) and one Y-chromosome (paternal). UPD occurs when an individual inherits both homologous chromosomes from the same parent either maternal UPD (both inherited from the mother) or paternal UPD (both inherited from the father) (**Figure 1d** and **e**). Therefore, UPD can occur in somatic chromosomes as well as in X-chromosome, and it can occur in both sporadic and hereditary cancers. aUPD can occur either on the whole chromosome or can be segmental. The mechanism underlying whole-chromosome aUPD can be loss of one chromatid through anaphase lag, followed by duplication of the remaining allele. It is unknown whether whole-chromosome aUPD can also occur through a nondisjunction error. If that is the case, trisomy would result for the specific chromosome in one daughter cell, and this specific chromosome would be inherited from both parents but with two copies of one of the chromatids from one parent and one copy from the other. In this case, the daughter cell would not display aUPD (**Figure 1c**). However, if one chromatid is lost (**Figure 1b**), called monosomy, and then the remaining allele is duplicated or triplicated, disomy or trisomy results for that chromosome; but that type of trisomy differs from the trisomy that arises from a nondisjunction error, because that certain chromosome inherited is only from the one parent (**Figure 1d** and **e**).

On the contrary, a mechanism underlying segmental aUPD is mitotic recombination, a term that describes the recombination of homologous chromosomes at the G_2/S phase of the cell cycle that is followed by chromosome segregation and cell division. The role of mitotic recombination in tumourigenesis was first defined in retinoblastoma (Cavenee *et al*., 1983), in which the mutant *RB1* (retinoblastoma 1) gene was duplicated. This alteration results in replacement of the deleted DNA, so the aUPD region eludes discovery by conventional techniques. Such detection difficulties were insurmountable until the development of genome-wide high-density SNP arrays and analytical tools capable of detecting aUPD. Nonetheless, we are still in the beginning stages of understanding the mechanism underlying aUPD.

aUPD has now been detected in all types of cancer, including solid tumours and haematologic malignancies, with the highest frequency of aUPD observed in ovarian cancer and the lowest frequency observed in leukaemia (our unpublished observations). It is well known that one of the functions of the *BRCA1* (breast cancer 1, early onset) and *BRCA2* genes is in DNA replication and double-strand break repair and control of homologous recombination (Scully *et al*., 1997). Therefore, a mutation in *BRCA1* may cause or increase somatic mitotic recombination and aUPD in cancer. The mutations in the genes that involve a DNA double-strand break repair and homologous recombination (e.g. *BRCA1*, *BRCA2*, *RecQ* (DNA helicase Q), *RAD50*, *RAD51*, *XRCC2* (X-ray repair complementing defective repair in chinese hamster cells 2), *XRCC3*, *XRCC4* and *LIG4* (ligase IV, DNA, ATP-dependent)) may also be involved in the mechanisms of UPD. Therefore, dysfunction of DNA double-strand break repair and homologous recombination pathways through the mutation may be a general phenomenon in many cases of cancer.

It is also possible that the genes responsible for somatic recombination are different from those responsible for meiotic recombination. Recently, it was shown that *JAK2* (Janus kinase 2) mutation stimulates homologous recombination and genetic instability (Plo *et al*., 2008). If that is the case, then the following questions arise about the mechanism: (a) Does a mutated gene (heterozygous) on one chromosome lead to mitotic recombination and results

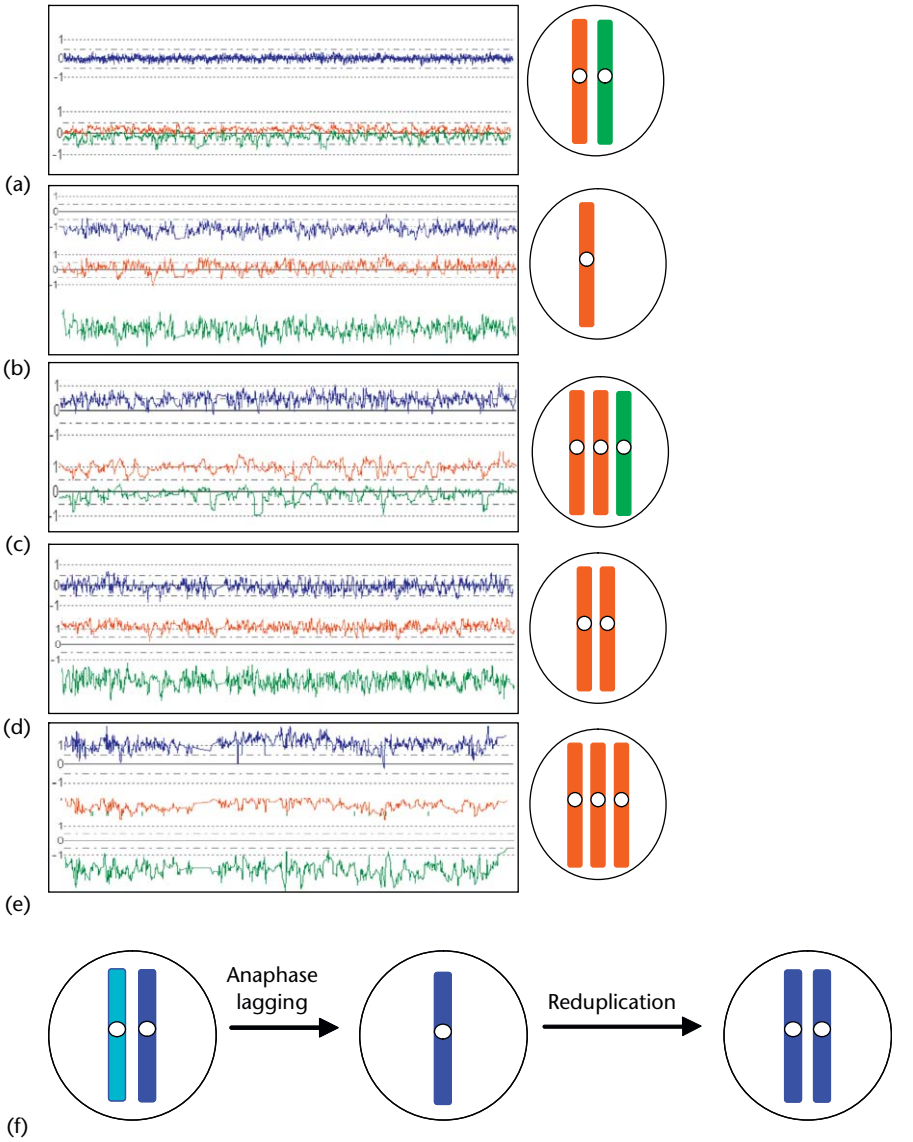

Figure 1 Illustration depicts the normal chromosome, numeric (monosomy and trisomy) and structural (losses and gains) chromosomal rearrangements, and uniparental disomy (UPD) identified by single-nucleotide polymorphism (SNP) array analysis (on the left panel) and illustration of homologous chromosomes in somatic cells (i.e. *red chromosome* represents maternal and *blue chromosome* represents paternal one or vice versa) (on the right panel). In the upper area of each panel, the *blue line* represents the average copy number signal intensity of the SNPs on the array. In the lower panel, the *green and red lines* show the relative signal intensity for individual homologous identified by using the AsCNAR software. (a) Depicts normal chromosome with no gains or losses, (b) loss of one copy and (c) trisomy, the gain of one copy resulting from a nondisjunction error in mitotic division. In this scenario, the cell harbours two copies of this particular chromosome from one parent, and the third homologous chromosome is from the other parent. Therefore, this change is not called uniparental disomy. aUPD, in which one copy is lost and the remaining is duplicated ((d) and (f)), or triplicate (e); therefore, all of them come from the same parent for this specific chromosome. Mechanism underlying UPD (f).

aUPD region (homozygous) in the same location on that same chromosome? or (b) Does a mutated gene on one chromosome cause mitotic recombination, leading to a homozygous mutation in a different location on a different chromosome? It also would be interesting to observe whether patients with polymorphisms in those genes that function in mitotic recombination who develop cancer are more likely to have UPD than are patients without such polymorphisms.

Other questions also remain unanswered: Does the frequency of aUPD depend on mutation(s) of the genes that function in homologous recombination? Do the mutations in multiple genes imply that a synergistic effect is involved in the frequency of aUPD? Do the frequencies of aUPD depend on tissue or cell type? Does each type of tumour harbour mutations in different genes that function in DNA double-strand break repair or control of homologous recombination?

No matter what mechanism or mechanisms are at play, aUPD creates stretches of homozygous regions that lead to homozygous mutation or deletion, loss or gain of imprinting and homozygous methylation of promoter region of genes. Mutations in those regions may not just be inactivating mutations in tumour-suppressor genes, they may also be activating mutations in proto-oncogenes. In this article, we focused on homozygous mutations of the genes in the UPD regions. There have not been any reported mutations in the miRNA in UPD regions yet, but UPD at chromosome 13 results in homozygous deletions of 13q14 in chronic lymphoblastic leukaemia that has been mapped as miRNA 15a/miRNA-16-1 (Lehmann *et al*., 2008). **See also**: Mutations in Human Genetic Disease; Oncogenes; Tumor Suppressor Genes

Mutations in aUPD Regions

Accumulating data provide strong evidence that aUPD is a common feature of the cancer genome and is associated not only with loss-of-function mutations of tumour-suppressor genes, but also with gain-of-function mutations of proto-oncogenes. Determination of aUPD in various cancers which led to the identification of homozygous mutations in *c-CBL* (Cas-Br-M (murine) ecotropic retroviral transforming sequence), *FLT-3* (FMS-related tyrosine kinase 3), *TET2* (ten-eleven-translocation gene) and *A20* (A20-binding inhibitor of NFκB (nuclear factor-κ-B) activation 2) paved the way for further studies (Frohling *et al*., 2007; Kato *et al*., 2009; Langemeijer *et al*., 2009; Sanada *et al*., 2009). Mutated genes that have been found in UPD regions are summarised in **Table 2**. For example, mutations were found in *JAK2* V617F at aUPD 9p in myeloproliferative neoplasia (MPN) (Baxter *et al*., 2005). These data indicate that the JAK/STAT pathway is affected in MPNs. This finding was followed shortly by the discovery of an *NF-1* mutation that resulted in aUPD at 17q in juvenile myelomonocytic leukaemia (JMML) (Flotho *et al*., 2007). Other discoveries followed as well: *FLT3-ITD* (FLT3-internal tandem duplication) or *FLT3-TKD* (FLT3-tyrosine kinase domain) homozygous mutations resulted in aUPD at 13q, and *CEBPA* (CCAAT/enhancer-binding protein alpha) (Wouters *et al*., 2007) or *AML1/RUNX1* mutations resulted in aUPD at 21q and *WT1* (Wilms tumour 1) mutation resulted in aUPD at 11p in acute myeloid leukaemia (AML) (Fitzgibbon *et al*., 2005). Accumulated data have demonstrated an association between homozygous mutations and aUPD in various cancers, mostly leukaemias: homozygous *c-MPL* (myeloproliferative leukaemia virus oncogene) mutation in aUPD at 1p (Gupta *et al*., 2008), *c-KIT* (v-kit Hardy–Zuckerman 4 feline sarcoma viral) in aUPD at 4q (Raghavan *et al*., 2008), *PTPN11* (protein tyrosine standard phosphatase nonreceptor 11) in aUPD at 12q in acute lymphoblastic leukaemia (ALL) (Karow *et al*., 2007), in *MLL* and *c-CBL* in aUPD at 11q in AML, in atypical *BCR–ABL* negative chronic myeloid leukaemia (aCML) and in a subset of MPNs (Dunbar *et al*., 2008; Grand *et al*., 2009; Raghavan *et al*., 2008; Sanada *et al*., 2009; Serrano *et al*., 2008). Mutations were identified in *A20* in aUPD at 6q23.3 in B-cell lymphoma (Kato *et al*., 2009), in *TET2* in aUPD at 4q24 in myelodysplastic syndrome (MDS) (Jankowska *et al*., 2009; Langemeijer *et al*., 2009) and in *MPL* in aUPD at 1p in refractory anaemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T) (Szpurka *et al*., 2008).

Table 2 Homozygouse mutations identified in aUPD regions

Gene	Mutation	Tumour-suppressor gene/oncogene	Chromosomal location	Tumour type
JAK2	Activating	Oncogene	9p24.1	MPN, AML
FLT3	Activating	Oncogene	13q12.2	AML
NRAS	Activating	Oncogene	1p13.3	CMML
c-MPL	Activating	Oncogene	1p34.2	CMML, RARS-T
MLL	Activating	Oncogene	11q23.3	AML
c-KIT	Activating	Oncogene	4q12	AML
AML/RUNX1	Inactivating	Tumour suppressor	21q22.12	AML
c-CBL	Inactivating	Tumour suppressor	11q23.3	sAML, aCML, CMML, RAEB
A20 (*TNFAIP3*)	Inactivating	Tumour suppressor	6q23.3	B-cell lymphoma
CEBPA	Inactivating	Tumour suppressor	19q13.11	AML
WT1	Inactivating	Tumour suppressor	11p13	AML
NF-1	Inactivating	Tumour suppressor	17q11.2	JMML
TET2	Inactivating	Tumour suppressor	4q24	MDS

Notes: aCML, atypic chronic myleogenous leukaemia; AML, acute myeloid leukaemia; CMML, chronic myelomonocytic leukaemia; JMML, juvenile myelomonocytic leukaemia; MDS, myelodysplastic syndromes; MPN, myeloproliferative neoplasia; RARS-T, refractory anemia with ringed sideroblasts and thrombocytosis; RAEB, refractory anemia with excess blasts; sAML, secondary acute myeloid leukaemia.

Function(s) of those mutated genes in aUPD regions

JAK2 encodes a cytoplasmic tyrosine kinase protein involved in various signalling pathways. *JAK2* plays a key role in signal transduction initiated by multiple growth factor receptors and cytokines. In contrast, the acquired *JAK2* V617F mutation has an inhibitory effect on the kinase domain JH1. And the JAK2 mutant enzyme has enhanced kinase activity and leads to hyperactivation of the downstream signalling pathways: STATs, PI3K (phosphatidylinositol 3 kinase)/AKT and mitogen-activated protein kinase (MAPK) (Verma *et al*., 2003). This mutation leads to constitutive tyrosine phosphorylation activity, which causes polycytemia vera (James *et al*., 2005).

MPL belongs to the haematopoietin receptor superfamily. This gene encodes the receptor for thrombopoietin, a haematopoietic growth factor that regulates the production of multipotent haematopoietic progenitor cells and platelets. Mutation of *MPL* results in the constitutive activation of JAK–STAT signalling and cytokine-dependent proliferation of haematopoietic cells. In addition, *MPL* mutation leads to activation of G_1/S transition in murine Ba/F3 pro-B cells (Chaligne *et al*., 2008).

CEBPA belongs to the CCAAT/enhancer-binding protein family, which is involved in the balance between cell proliferation and terminal differentiation. This gene encodes a transcription factor that plays a crucial role during differentiation. *CEBPA* is also a master regulator of haematopoietic differentiation of multipotent myeloid progenitor cells into mature neutrophils via multiple ways: downregulation of c-MYC (v-myc myelocytomatosis viral oncogene homolog) expression involving differentiation; upregulation of the expression of granulocytic lineage-specific genes and interaction with other key genes or proteins involved in myeloid development. The partners for these protein–protein interactions include p21, CDK2, CDK4 and E2F. CEBPA protein inhibits cell proliferation through p21 by inhibiting CDK2 and CDK4.

FLT3 encodes a membrane-bound receptor tyrosine kinase (RTK). *FLT3* mutations consist of two major types: *FLT-ITD*, which has an ITD in exons 14 and 15, and *FLT-TKD*, which has a missense mutation in exon 20, within the activation loop of the TKD. *FLT* mutations lead to phosphorylation of the receptor in the absence of FLT3 ligand and subsequent activation of the downstream signalling pathways, such as PI3K/AKT, Ras/MAPK and JAK2/STAT5, and they enhance proliferation and survival of multipotent progenitors (Frohling *et al*., 2007). The downstream cellular responses to *FLT-ITD* and *FLT3-TKD* mutations are substantially different in the activation of STAT5, but is the same in PI3K/AKT and MAPK (Bagrintseva *et al*., 2004). FLT-ITD also induces constitutive phosphorylation of STAT5, AKT and ERK1/2 and causes MPN in various murine models (Lee *et al*., 2005). From the clinical perspective, *FLT3* mutations have prognostic relevance because constitutively activated *FLT3* is an important therapeutic target. Moreover, *FLT*-ITD mutations are associated with an increased risk of relapse and short survival (Yanada *et al*., 2005). Currently, four selective FLT3 inhibitors are available: PKC412 (midostaurin), CEP-701 (lestaurtinib), MLN518 (tandutinib) and SU11248 (sunitinib); they are well tolerated and have shown moderate activity in cases of relapsed or refractory AML with activating *FLT3* mutations (Knapper, 2007).

c-CBL encodes an E3 ubiquitin ligase and is involved in degradation of activated RTKs and other tyrosine kinases, including Src kinases (Thien and Langdon, 2005). c-CBL protein is expressed in various cell types. E3 ubiquitin ligase is an enzyme that attaches an ubiquitin molecule to growth-factor receptors and other cellular proteins. Ubiquitination of these growth-factor receptors triggers their internalisation and degradation, thereby reducing the signalling cascades that promote cellular proliferation. In contrast, an inactivating mutation of *c-CBL* ubiquitin ligases, which are important regulators of RTK signalling found in MPNs, introduces amino acid substitutions that disable ubiquitin ligase activity (Sanada *et al*., 2009). Sanada *et al*. reported that mutations in *c-CBL* contribute in a complex way to the development of cancer. Consistent with previously reported findings, Sanada *et al*. found that mice lacking the *CBL* gene produced increased numbers of immature blood cells. In addition, *CBL* inactivation promoted the development of leukaemia in mice engineered to express the pro-leukaemic *BCR–ABL* gene. Mutant c-CBL proteins inhibit ubiquitination of growth factor receptors in blood-cell lines, even in cells that retain a normal copy of the *CBL* gene, and this inhibition is associated with prolonged receptor activation and an enhanced proliferative response to cytokine growth factors (Sanada *et al*., 2009). Sanada's group also found that expression of the mutant c-CBL proteins is associated with aberrant phosphorylation of STAT5.

A20 (also known as TNFAIP3 (tumor necrosis factor, alpha-induced protein 3)) is a negative regulator of NFκB through a variety of cell receptors and viral proteins (Kato *et al*., 2009). *A20* suppresses cell proliferation, enhances apoptosis and accumulates of IκBβ and IκBε and downregulates NFκB activity. Mutated *A20* has the opposite effects on cell growth and proliferation, apoptosis, accumulation of IκBβ and IκBε and regulation on NFκB activity. In addition, mock-transduced and *A20* absent mice generated B-lineage lymphomas (Kato *et al*., 2009).

c-KIT encodes a transmembrane glycoprotein, which is a member of the type III RTK family, and whose ligand is a stem cell factor. Activation of downstream signalling pathways with different mutations of *c-KIT* (ligand independent) are involved in proliferation, differentiation and survival, in particularly, of haematopoietic stem cells. *c-KIT* mutations appear to have a role in prediction of outcome as well as therapeutic implications because an activating mutation of *c-KIT* may be a target for tyrosine inhibitors (Renneville *et al*., 2008).

AML1 (also known as *CBFA2* and *RUNX1*) encodes for one of the two subunits that form the human core-binding factor (CBF). AML1 is a transcription factor that regulates

the expression of macrophage colony-stimulating factor (M-CSF) receptor, *IL-3*, myeloperoxidase and *TCRβ* genes that are specific to leukaemogenesis (Lutterbach and Hiebert, 2000). Thus, *AML1* mutations are involved in the development of leukaemia. A monoallelic mutation was found in familial platelet disorder, which causes haploinsufficiency (Song *et al*., 1999); biallelic mutations also were found in AML (FAB M0 type) (Roumier *et al*., 2006). Even though a monoallelic mutation is sufficient to lead to AML, biallelic mutation is selected in some circumstances.

WT1 encodes a zinc-finger DNA-binding protein. Depending on the cellular context and interaction with other genes or proteins, *WT1* can be involved in transcriptional activation or repression. Also, *WT1* can function as either a tumour-suppressor gene or an oncogene, depending on tissue type and/or expression of different isoform, post-trascriptional modifications, and interaction with other proteins. The role of WT1 in haematopoiesis and its contribution to leukaemogenesis are also still not clear, although it has been suggested that WT1 protein could promote stem cell proliferation and induce a differentiation block of haematopoietic cells (Keilholz *et al*., 2005). *WT1* mutations were first described in Wilms tumours. Most mutations associated with AML are in the zinc-finger domain, suggesting that they would cause loss of function, and most of mutations are heterozygous, although a few homozygous mutations associated with aUPD have been described in AML (Fitzgibbon *et al*., 2005).

PTPN11 encodes a cytoplasmic protein tyrosine phosphatase called SHP-2. SHP-2 participates in signal transduction downstream of growth factors, cytokines, hormones and cell-adhesion molecules. Its role in transducing signals is mediated partly through the RAS/MAPK cascade (Tartaglia *et al*., 2004). *PTPN11* mutations contribute to leukaemia by deregulating the RAS pathway (Loh *et al*., 2004).

TET2 belongs to the *tet* oncogene family member 2 and catalyses the conversion of 5-methylcytosine in DNA to 5-hydroxymethylcytosine, which indicates a potential role of TET proteins in epigenetic regulation (Tahiliani *et al*., 2009). *TET2* may be a tumour-suppressor gene, and a*TET2* biallelic mutation is likely to be important in the pathogenesis of MPN (Langemeijer *et al*., 2009).

Interestingly, these homozygous mutated genes either contribute to cell proliferation by alteration of tyrosine kinase (e.g. *JAK2*, *FLT3* and *c-KIT*) or components that signal upstream (e.g. *MPL* and *c-CBL*) or downstream (e.g. *NRAS*) of tyrosine kinase or tyrosine phosphatase (e.g. *PTPN11*) pathways or involved in myeloid differentiation (e.g. *AML1* and *CEBPA*) and/or involved in regulation of the cell cycle and apoptosis (e.g. *NPM1*). It is well accepted that deregulation of tyrosine kinase signalling by activating mutations or gene fusions results as critical drivers of excess cell proliferation (De Keersmaecker and Cools, 2006). Therefore, we conclude that identifying the presence of aUPD could be useful as a tool for identifying novel mutated 'driver' genes.

It appears that even monoallelic mutations in some genes (*c-CBL*, *AML*/*RUNX1*, *WT1*) are haploinsufficient for leukaemogenesis. Even though monoallelic mutations are haploinsufficient for leukaemogenesis, biallelic mutations compared to monoallelic mutations may result in more severe phenotypic effects and a selective growth advantage; therefore, cells undergo selection of biallelic mutation.

Clinical significance of an aUPD region or mutations in such aUPD regions

From a clinical perspective, it appears that the presence of either an aUPD region or a mutation in a certain region correlates with disease outcome. For example, the presence of aUPD at chromosome 1p36 correlates with shorter overall survival lymphoma and at chromosome 16p correlates with shorter progression-free survival and is predictive of transformation. UPD is also noted frequently at the time of diagnosis of follicular lymphoma (O'Shea *et al*., 2009). Further, patients with glioblastoma multiforme who had an aUPD region at chromosome 13q or 17p had significantly shortened survival time (Yin *et al*., 2009).

Grand *et al*. (2009) found that patients with *CBL* mutations had shorter (although not statistically significant) overall and progression-free survival than patients without such mutations. In addition, they identified an oncogenic function of a P417A mutation, which abrogates CBL ubiquitin ligase activity, that conferred a proliferative advantage to 32D cells overexpressing of wild-type FLT3, and homozygous mutations of *CBL* with aUPD have a selective advantage over those that are heterozygous (Grand *et al*., 2009).

Functional studies have shown that *JAK2* V617F mutations are capable of inducing myeloproliferative phenotypes in transgenic mice (Xing *et al*., 2008); for example, thrombocythaemia was induced in low expressing animals and polycythaemia was present in animals with high transgenic expression. Additionally, an oncogenic function of *MPL* W515L/K mutations has been proven in murine Ba/F3 cell lines (Chaligne *et al*., 2008).

aUPD is predictive of poor overall and event-free survival in AML (Tiu *et al*., 2009), and aUPD at 7q is associated with poor overall survival (Gondek *et al*., 2008) in patients with MDS/MPNs.

Concluding Remark

Taken together, the accumulating data about aUPD are indicating the importance of using genome-wide approaches to identify genetic and therapeutic targets in human cancers. Identifying aUPD has paved the way to discover homozygous mutated genes, which will provide greater insight into the mechanisms of tumourigenesis, help to distinguish driver from passenger genes, lead to the discovery of novel therapeutic targets and provide important prognostic information.

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Further Reading

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