

Deregulation of Nuclear Factor Kappa B in Human Cervical Cancer

**D. Karunagaran
Department of Biotechnology
Indian Institute of Technology Madras
Chennai – 600 036**

Avinashilingam University, Coimbatore 15-12-2006

Six characteristics of metastatic (malignant) tumor cells

Benign tumor cells
are not metastatic.

Self-sufficiency in
growth signals

Necessary for continued
proliferation.

Sustained
angiogenesis

Blood supply
necessary for
the tumor to grow
and spread.

Intravasation and
extravasation.

Insensitivity to
antigrowth signals

Necessary for
continued
proliferation.

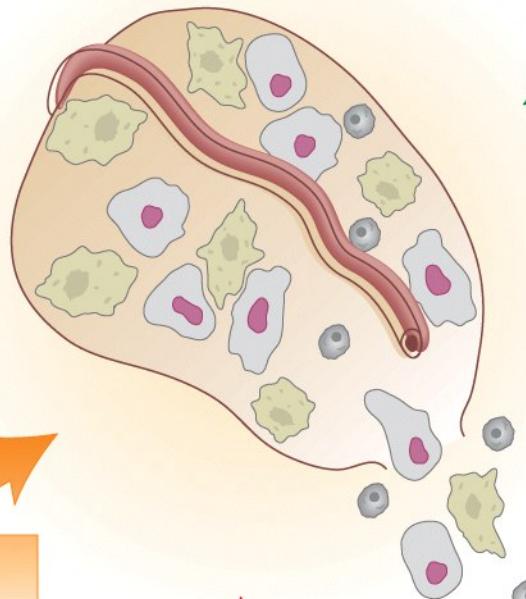
Tissue invasion
and metastasis

Requires protease activity
to penetrate basement
membranes and the
extracellular matrix.

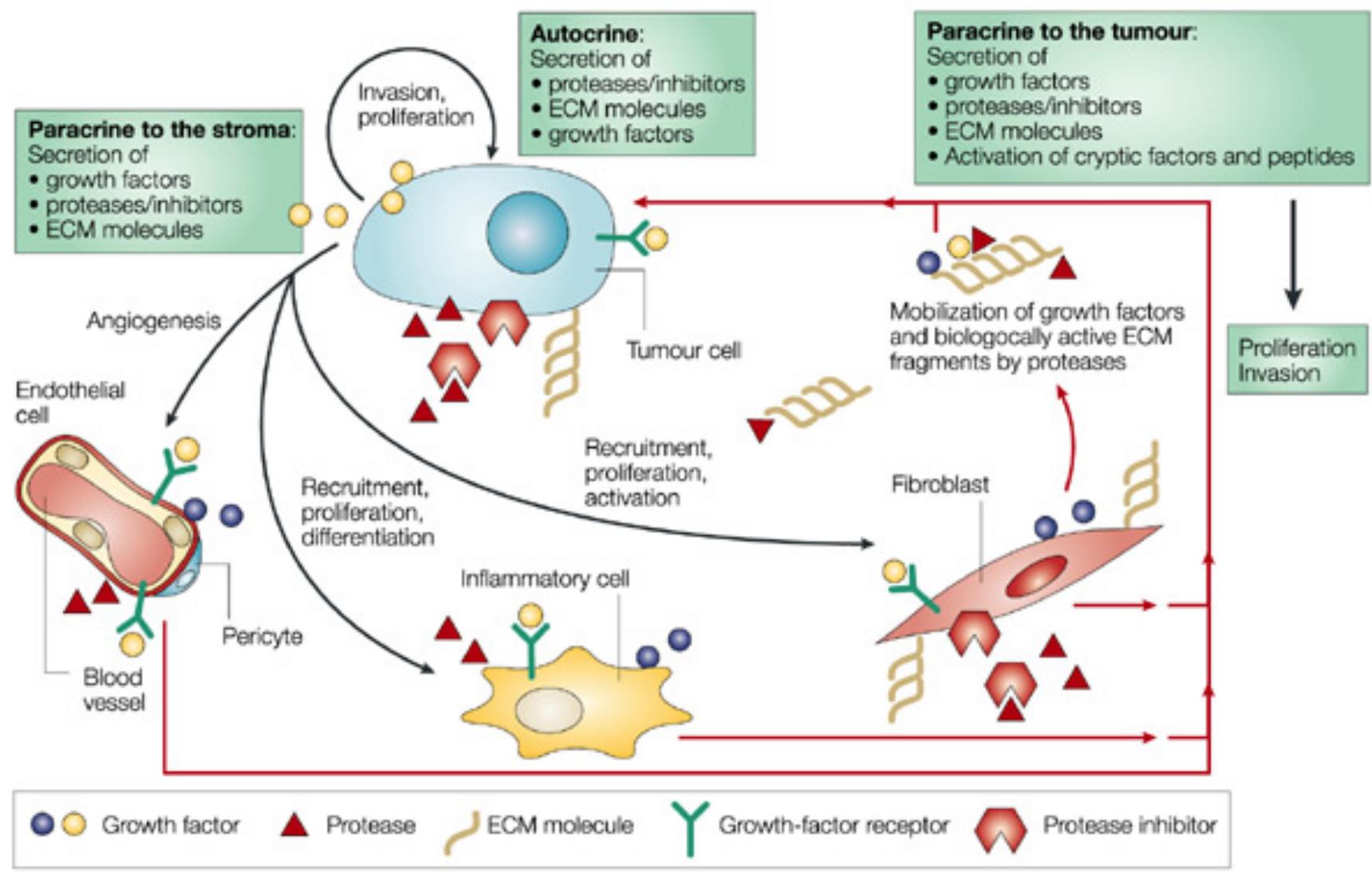
Limitless replicative
potential

Evasion of
apoptosis

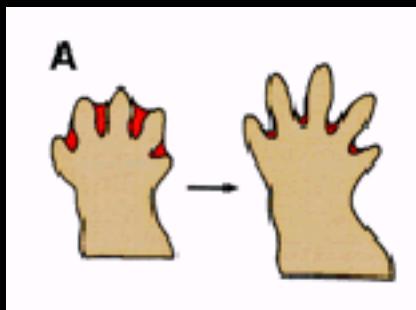
Otherwise the
tumor cells will die,
Otherwise the
tumor will not grow.



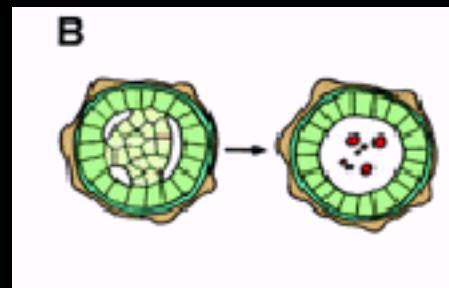
Tumor cells interact with surrounding stroma



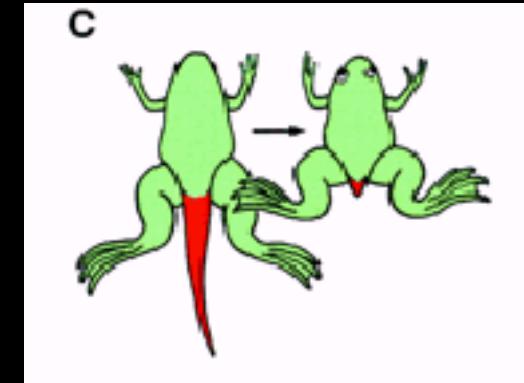
Forming digits



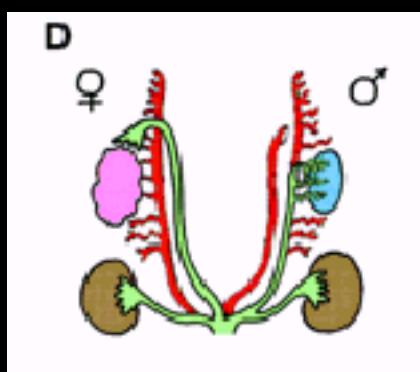
Forming lumen



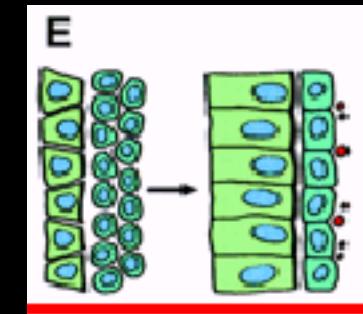
Losing tail



Mullerian or wolffian

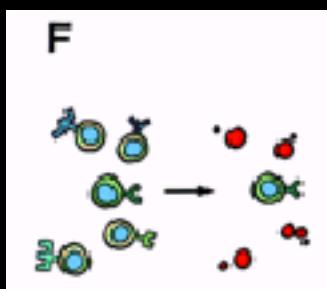


Adjusting cell number

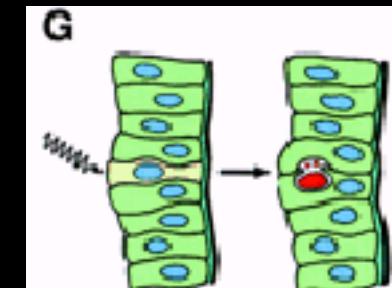


**Different roles for
Apoptosis**

Eliminating infected cells



Eliminating injured cells



Research News by Marcia Barinaga

Three separate research groups report in this issue of *Science* (pages 782, 784, and 787) that a cellular protein called nuclear factor kappa B (NF-kappa B) blocks the programmed form of cell suicide known as apoptosis in several cell types. This finding has led to the suggestion that blocking NF-kappa B in tumor cells might make them more vulnerable to drugs or radiation.

An Essential Role for NF-κB in Preventing TNF- α Induced Cell Death

Amer A. Beg and David Baltimore *Science* 1 November 1996: 782-784.

TNF- and Cancer Therapy-Induced Apoptosis: Potentiation by Inhibition of NF-κB

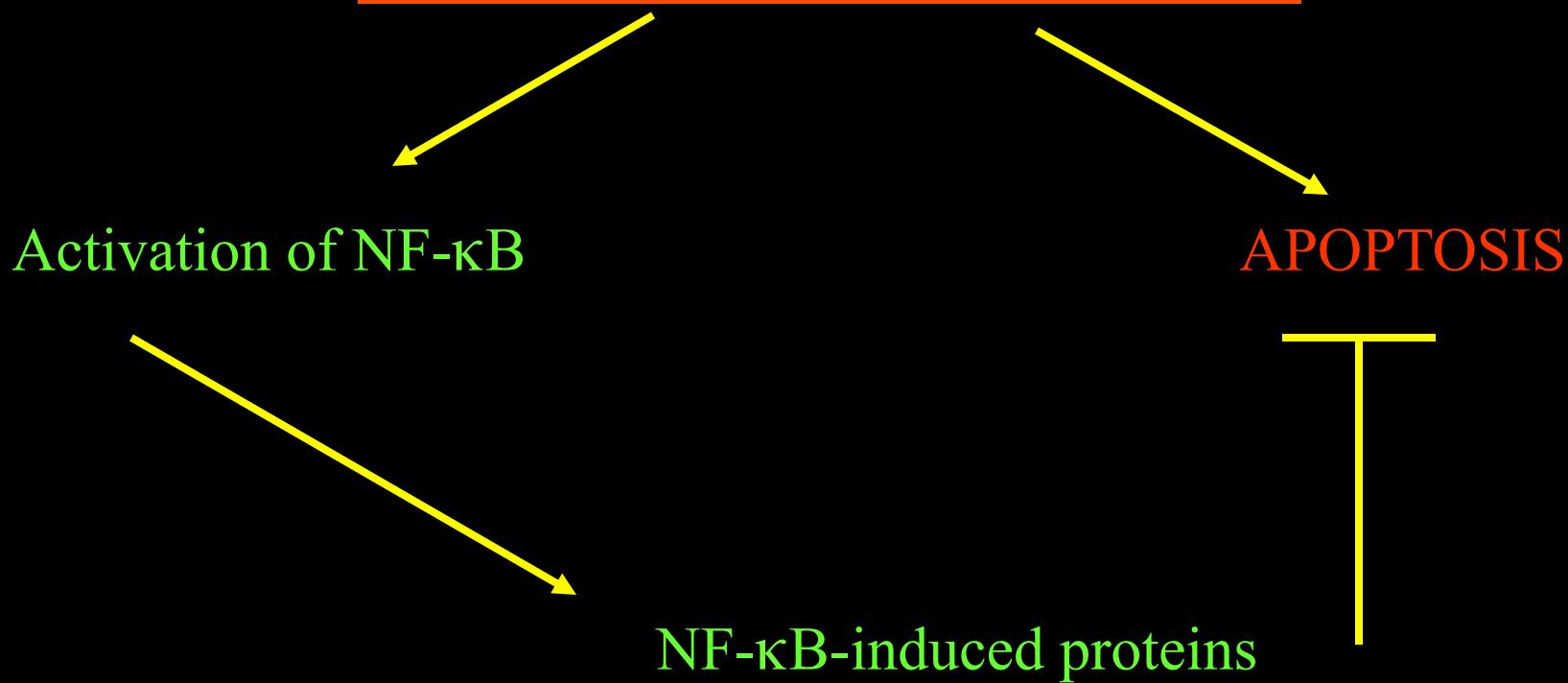
Cun-Yu Wang, Marty W. Mayo, and Albert S. Baldwin, Jr. *Science* 1 November 1996: 784-787.

Suppression of TNF- α Induced Apoptosis by NF-κB

Daniel J. Van Antwerp, Seamus J. Martin, Tal Kafri, Douglas R. Green, and Inder M. Verma *Science* 1 November 1996: 787-789.

NF-κB PREVENTS APOPTOSIS

Tumor Necrosis Factor
Radiation
Chemotherapeutic compounds



NUCLEAR FACTOR-KAPPA B (NF-κB)

- A family of closely related dimeric transcription factors
- Bind a common sequence of immunoglobulin gene called κB site
- Held inactive in the cytoplasm by Inhibitory proteins (IκBs)
- Activated mainly by degradation of Inhibitory proteins (IκBs)
- Translocate into the nucleus
- Bind to specific gene promoter
- Promote transcription

Members of NF-κB in mammals

Two classes based on their synthesis and transactivation

Class I

Rel A / p65

Rel B

c-Rel

Synthesized in mature forms

REL Homology Domain (RHD) with NLS

Transcription- modulating domains

Class II

P50 (p105/NF-κB1)

P52 (p100/NF-κB2)

Synthesized as large precursors

RHD and Ankyrin repeats

Ubiquitin dependent proteolytic cleavage required for processing into DNA binding p50 & p52

Inhibitor of κB (IκB) protein family

All members have 6-7 ankyrin repeats that block NLS on the RHD of NF-κB members & retain them in cytoplasm

IκB α

Contain an amino terminal regulatory domain within which are two conserved serines, phosphorylation of which targets ubiquitin mediated degradation

IκB β

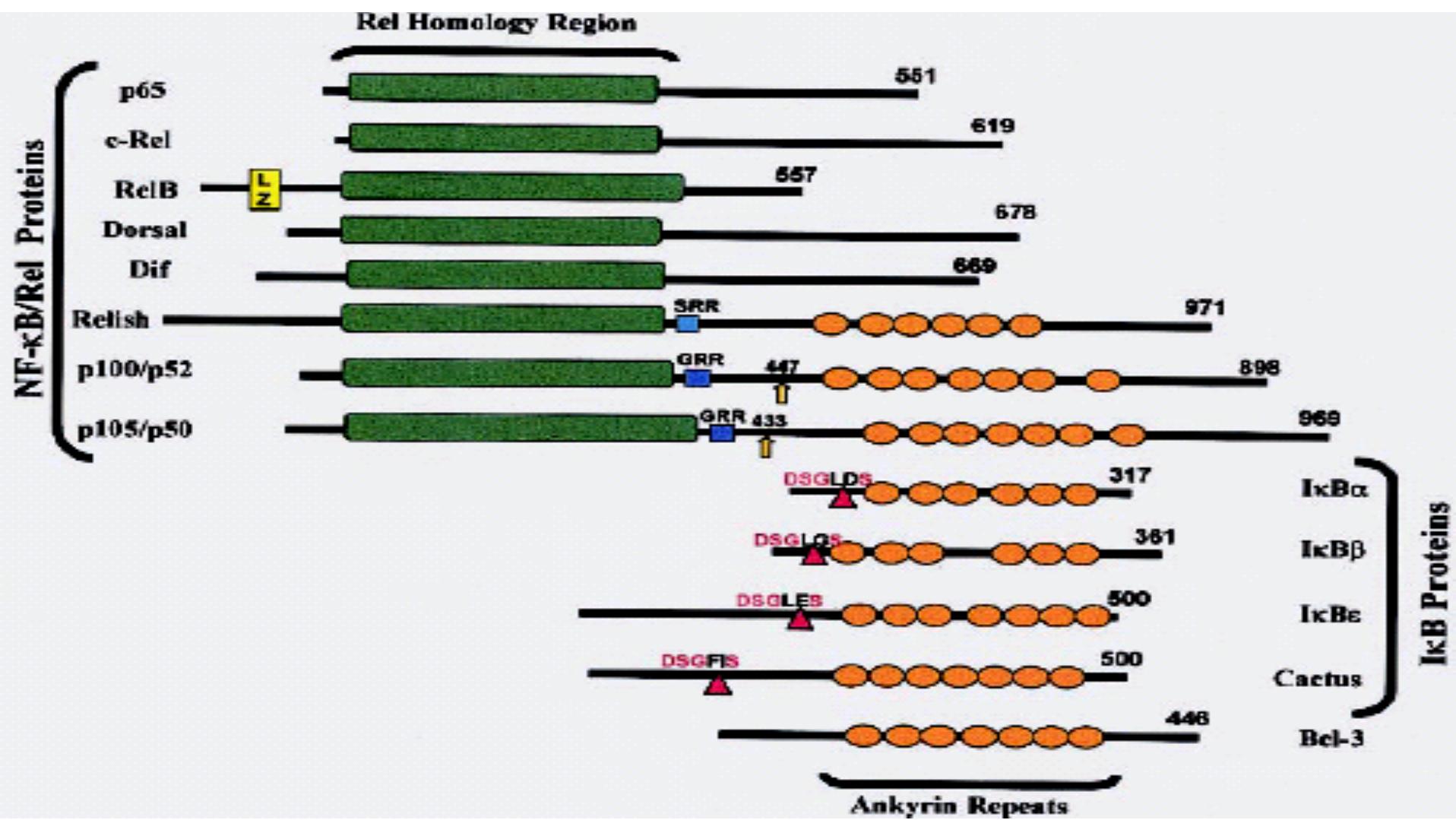
Lysine residue which are targets of polyubiquitination are also present in the amino terminal regulatory domain

IκB γ

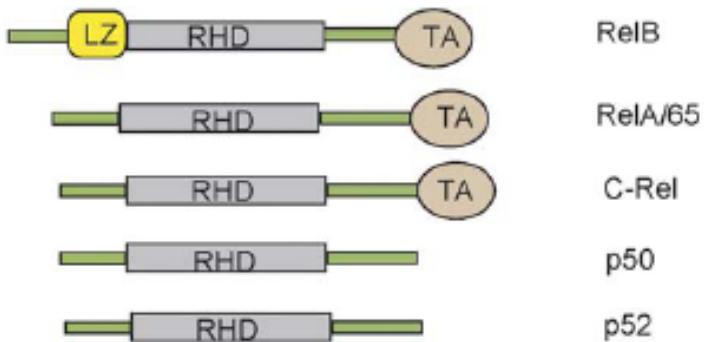
Bcl3

Transcriptional co-activator for p50 and p52

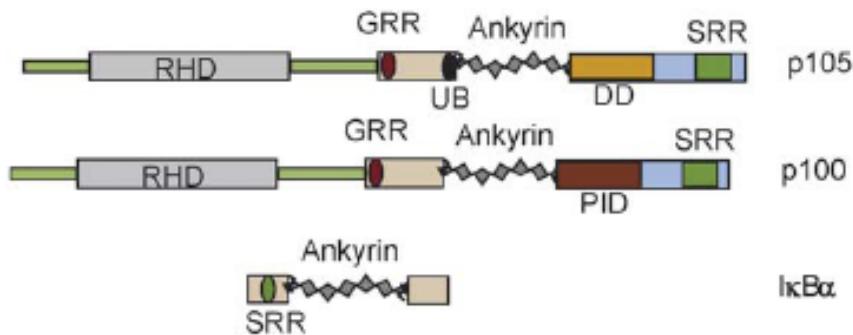
Nuclear Factor-kappa B Family



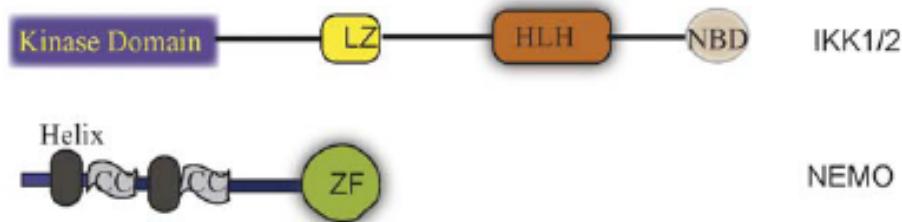
(A) DNA binding subunits



(B) $\text{I}\kappa\text{B}\alpha$ and $\text{I}\kappa\text{B}$ like proteins



(C) IKK complex proteins



Activators of NF-κB

Bacteria or their products

Helicobacter pylori, *Mycobacterium tuberculosis*,
Lipopolysaccharide (LPS)

Viruses or their products

HIV-1: Tax, gp-160, Influenza virus, HTLV-1: Tax 1, Double-stranded RNA viruses, HCV: core protein

Cytokines

Interleukins (1, 2, 12, 15, 17, 18), TNF-α, Lymphotoxin, CD-29L, CD-30L, CD-40L, 4-1BBL, OX-40L, RANKL

Growth factors

Serum, **EGF, Insulin, PDGF, TGF-β, NGF**

Stress conditions

UV and ionizing radiation, Hyperosmotic shock, Shear stress, H₂O₂, Pervanadate

Chemotherapeutic compounds

Cisplatin, Daunorubicin, Etoposide, Tamoxifen, Taxol

Chemical agents

Phorbol ester, N-methyl-D-aspartate, Okadaic acid, Calcium ionophore

Targets of NF-κB

Cytokines/chemokines - **TNF-α, interleukins (1, 2, 6, 8, 11, 12), interferon-γ, IP-10**

Growth factors - G-CSF, GM-CSF, **VEGF**

Cell cycle regulators - **CyclinD1, p21WAF**

Apoptosis regulatory proteins - **Bcl-2 homologs (A1, Bcl-X_L, Nrl-3), Fas, FasL, IAPs, IEX-1L**

Stress response proteins - Angiotensin II, **COX-2, MnSOD, iNOS**

Transcription factors - c-Myc, JunB, **c-Rel, NF-κB p100, NF-κB p105, IκB-α, p53**

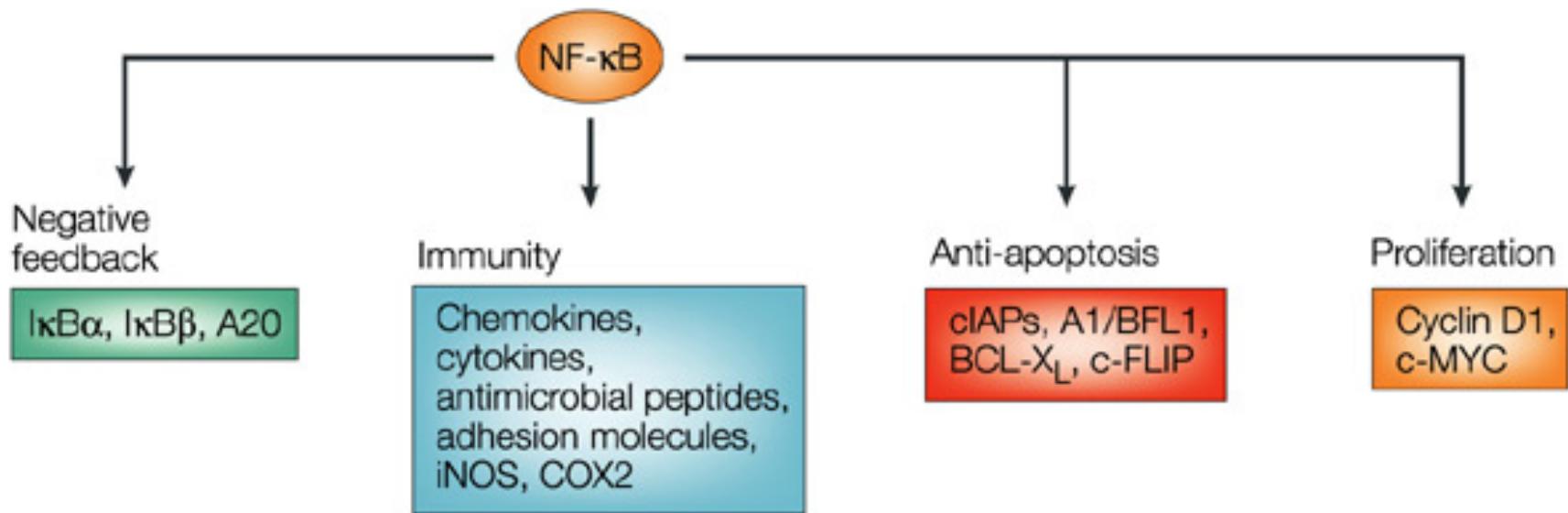
Viruses - HIV-1, CMV, Adenovirus, SV-40

Cell adhesion proteins - E-Selectin, **ICAM-1, VCAM-1**

Acute phase proteins - Angiotensinogen, C-4b binding protein, complement factors B and urokinase type plasminogen activator

Immunoreceptors - CD-23, CD-48, T-cell receptor b-chain

Genes Regulated by NF-κB



Nature Reviews | Cancer

Nat.Rev.Cancer 2:301-310, 2002

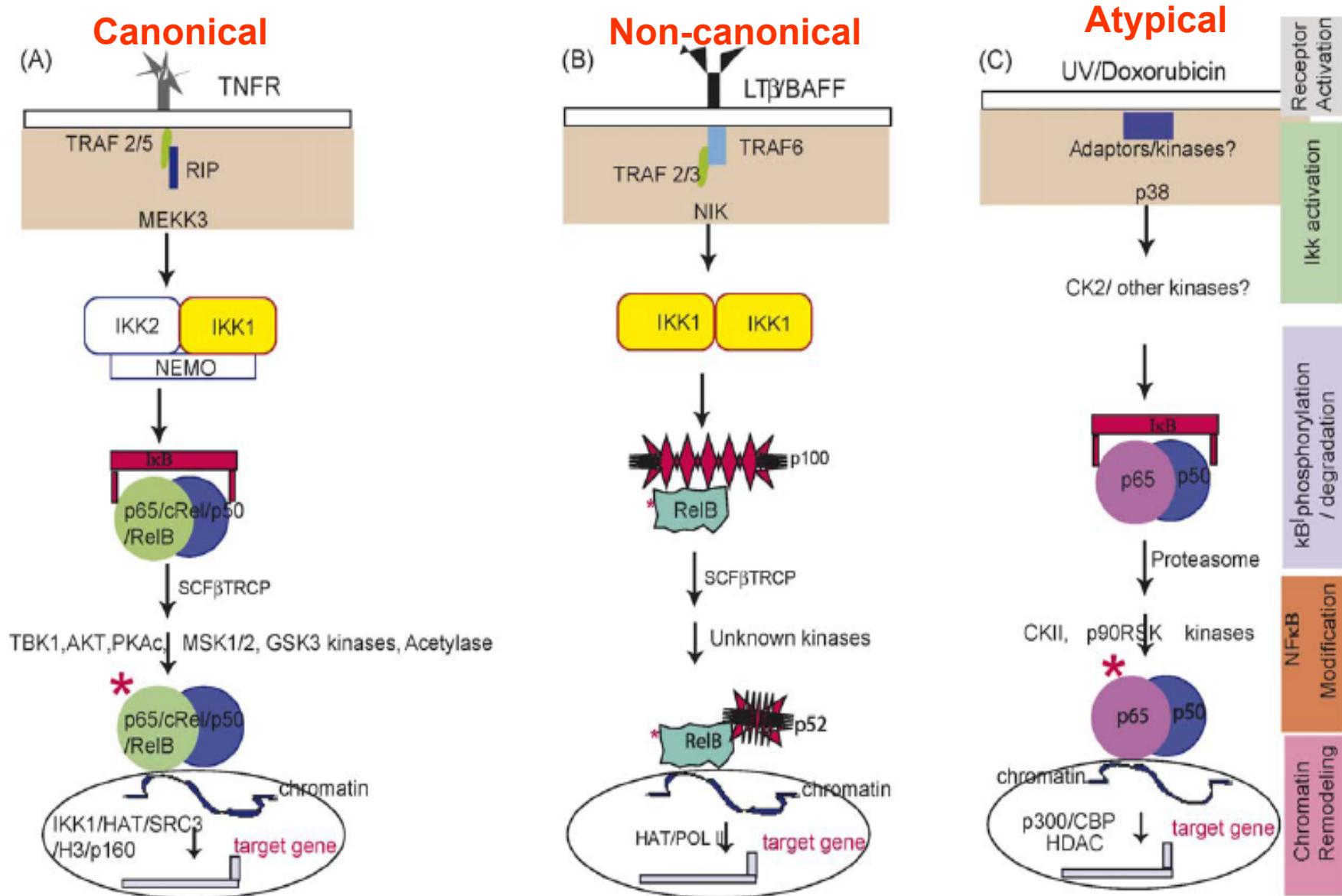
Table 1
Natural and synthetic inhibitors of NF κ B pathway

| Biological Inhibitors | Synthetic inhibitors | Molecular target |
|--|--|--|
| Poxvirus decoy receptors | Anti TNF (Remicade), Soluble TNF-receptor (Enbrel) | TNF receptor |
| Adenoviral E3-10.4K/E3-14.5 complex | IL-1R α (Kineret) | IL1 receptor |
| | TIRAP peptide | TLR4; TIRAP interaction |
| <i>Yersinia</i> YopJ protein, Adenoviral E1A and E3-10.4/14.5, curcumin, EGCG from green tea. | Antioxidants, non steroid anti inflammatory agents (NSAID) like aspirin, Thalidomide, Small molecule IKK inhibitors such as quinazoline analogues (SPC839), β -carboline derivatives (PS-1145), Ureido-thiophenecarboxamide derivatives, diarylpyridine derivatives, | IKK activation and function |
| African Swine fever virus encoded truncated I κ B protein, Vaccinia virus K1L gene product, Plant products such as sanguinarine and emodin. | Proteasome inhibitors like PS-341 (Bortezomib), antioxidants like vitamin C/E and MnSOD, Phosphopeptides that compete for SCF β ^{TRCP} | I κ B phosphorylation and degradation |
| <i>Porphyromonas gingivalis</i> , <i>Human cytomegalovirus</i> encoded proteins and Caffeic acid phenethyl ester from honey bee hives | Glucocorticoids, SN-50 peptide, decoy oligodeoxynucleotides (ODN), p65 phosphorylation domain peptides, antisense and siRNA to various NF κ B subunits | NF κ B translocation, DNA binding, chromatin remodeling and transcription |

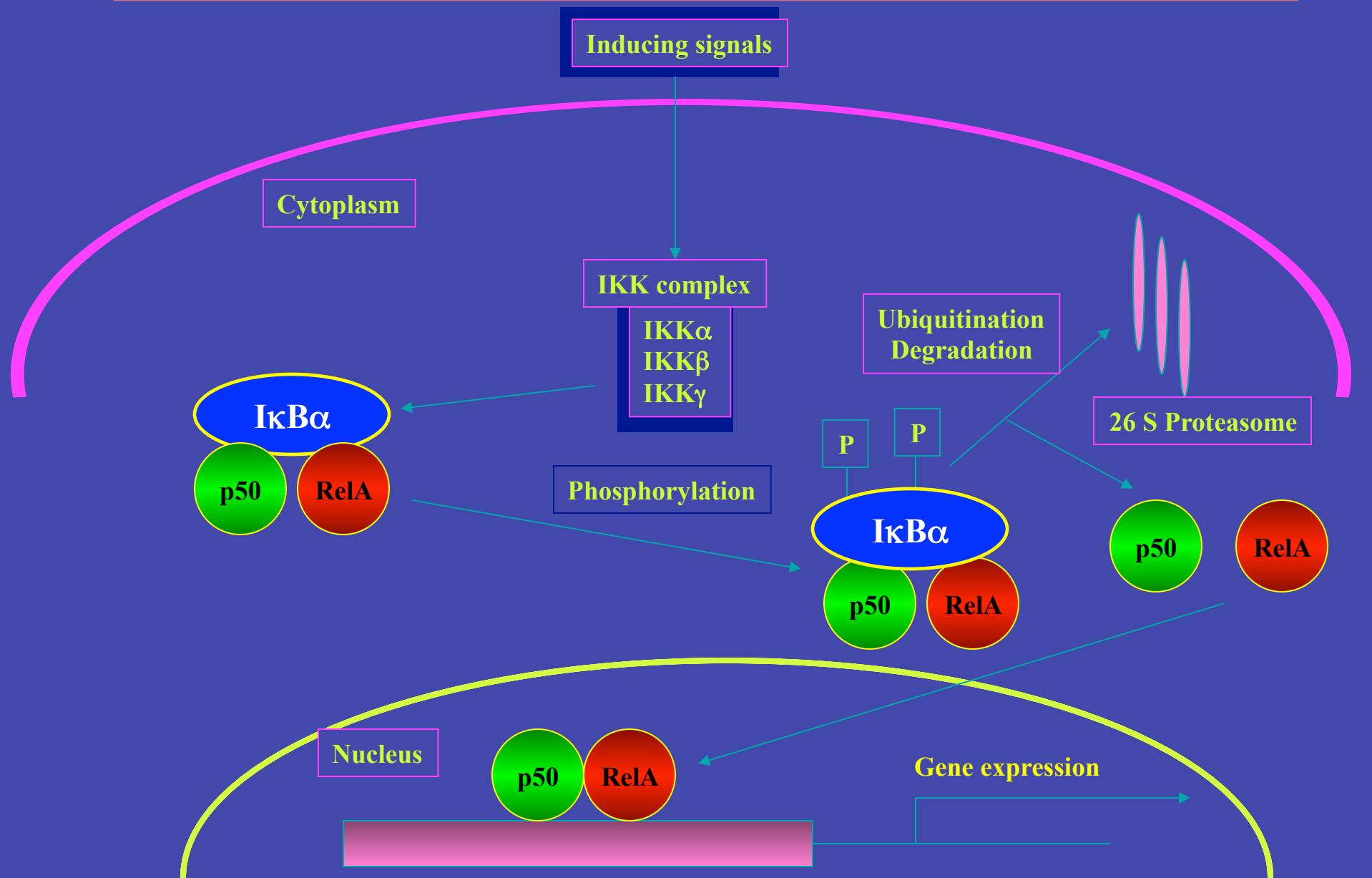
Three Pathways of NF-κB Signaling

1650

V. Tergaonkar / The International Journal of Biochemistry & Cell Biology 38 (2006) 1647–1653



NF-κB Signaling Pathway



NF-κB and CANCER

| Class of tumor | Type of cancer | Mechanism |
|----------------------|--|---|
| Solid tumors | Breast carcinoma | Defective IκB-α activity |
| | Colon carcinoma | Defective IκB-α activity |
| | Ovarian carcinoma | Defective IκB-α activity |
| | Pancreatic carcinoma | Defective IκB-α activity |
| | Thyroid carcinoma | Defective IκB-α activity |
| | Bladder carcinoma | Defective IκB-α activity |
| | Prostate carcinoma | Defective IκB-α activity |
| | Melanoma | Defective IκB-α activity |
| | Oncogenic Ras-induced transformation | Increased RelA transcriptional activity |
| Hematopoietic tumors | Hodgkin's disease | IκB-α mutation |
| | Hodgkin's disease | IκB-ε mutation |
| | Hodgkin's disease | Constitutive IKK activity |
| | Childhood acute-lymphoblastic leukemia | Constitutive IKK activity |
| | Acute lymphoblastic leukemia | Bcr-Abl kinase:Ras pathway |
| | Chronic myelogenous leukemia | Bcr-Abl kinase:Ras pathway |
| | Adult T-cell leukemia | Bcr-Abl kinase: Ras pathway |

Cervical Cancer

Second most common cancer Worldwide

Most common cancer among Indian women

Global Incidence

4.7×10^5 new cases

2.3×10^5 cancer deaths

Incidence in India

1.25×10^5 new cases

0.705×10^5 cancer deaths

26% of global incidence

Molecular alterations in Cervical Cancer

HPV infection is necessary but not sufficient

Although HPV infection is the major risk factor for cervical cancer, molecular alterations of tumor suppressor genes and / or oncogenes associated with or without HPV infection are necessary for the progression of cervical cancer

Classification

Three histological types of epithelial tumors in cervix

Squamous Cell Carcinoma (SCC) of ecto cervix

60-80% incidence

HPV- 16 risk factor

Adenocarcinoma (ADC) of endo cervix

Less than 20%

HPV- 18 risk factor

Less common epithelial tumors

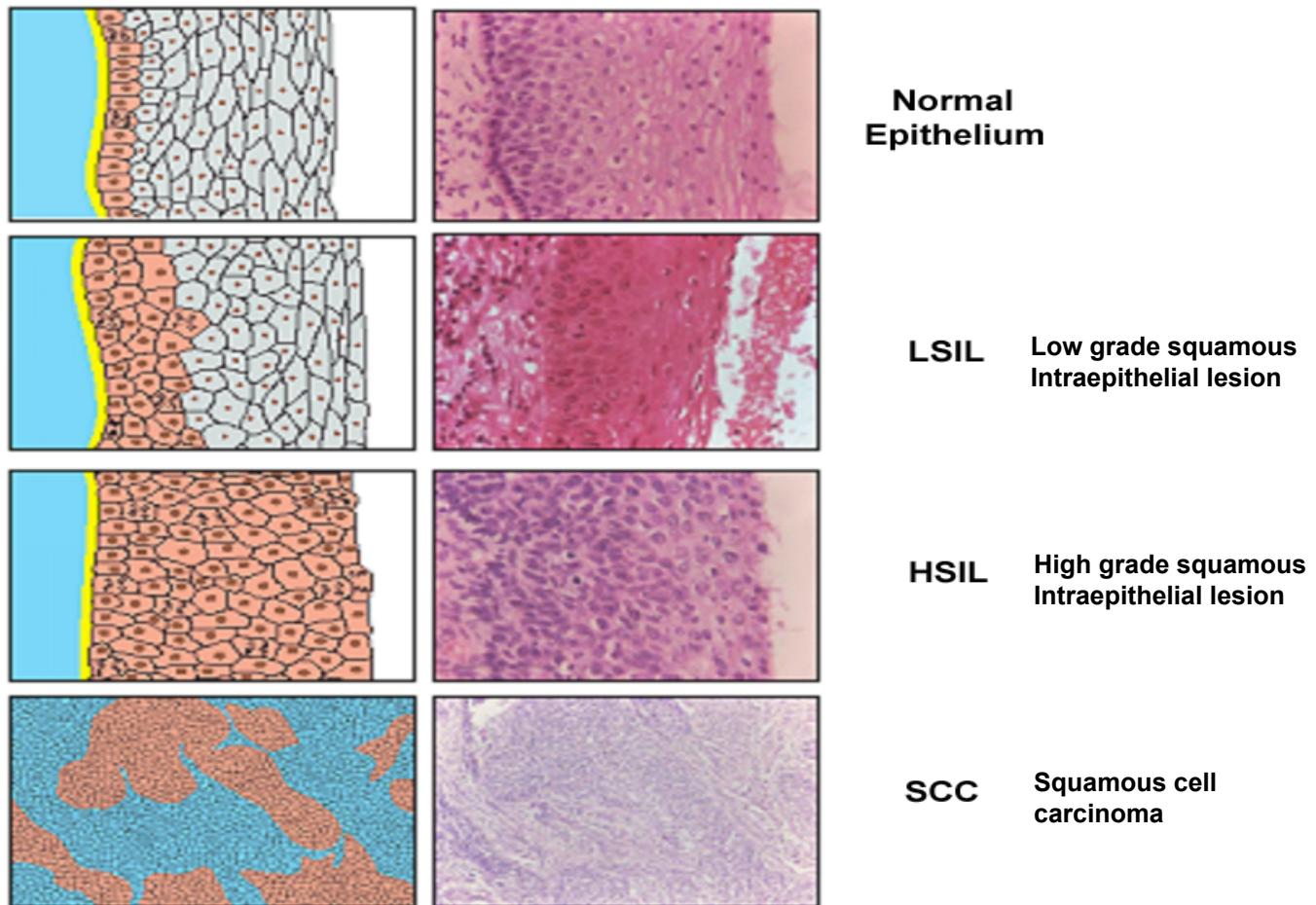


Fig. 1.5. Histological grades indicating ectocervical cancer progression

HPV and NF-κB Activation

| | | |
|--|--|---|
| Functional components of NF-κB pathway are (p50,P65,c-Rel,IκB-α) upregulated | Normal Keratinocytes-KN Transformed Keratinocytes –E6,E7,E6&E7 HPV+ cervical carcinoma derived keratinocyte cell lines- CaSki,SiHa HPV- cervical carcinoma derived keratinocyte cell line – HT3 | Virology. 2002 Jul;298(2):271-85 |
| Papillomavirus type 16 oncogenes upregulate proliferation-associated NF-kappaB-responsive genes. | cervical keratinocytes | J Virol. 2001 May;75(9):4283-96 |
| Differential production of cytokines and activation of NF-kappaB in HPV-transformed keratinocytes | Normal Keratinocytes-KN Transformed Keratinocytes –E6,E7,E6&E7 | Virology. 2002 Jul;298(2):271-85 |
| Human papillomavirus type 16 E6 activates NF-kappaB, induces cIAP-2 expression, and protects against apoptosis in a PDZ binding motif-dependent manner | primary human airway epithelial cells (AECs) | J Virol. 2006 Jun;80(11):5301-7 |

HPV and NF-κB Inhibition

| | | |
|--|---|--|
| High levels of p105 (NFKB1) and p100 (NFKB2) proteins in HPV16-transformed keratinocytes | Normal Keratinocytes-KN Transformed Keratinocytes –E6,E7,E6&E7 | Virology. 2005 Jan 20;331(2):357-66 |
| The E6 protein of human papillomavirus type 16 binds to and inhibits co-activation by CBP and p300 | Soas-2,Cos-1 and U2OS cell lines | EMBO J. 1999 Sep 15;18(18):5061-72 |
| The human papillomavirus oncoprotein E7 attenuates NF-kappa B activation by targeting the Ikappa B kinase complex. | U2OS and H1299 cell lines | J Biol Chem. 2002 Jul 12;277(28):25576-82 |
| Down regulation of the interleukin-8 promoter by human papillomavirus type 16 E6 and E7 through effects on CREB binding protein/p300 and P/CAF | primary keratinocytes from human neonatal foreskins (HFKs) | J Virol. 2002 Sep 76(17): 8710-8721 |

Increased nuclear translocation of p50 and RelA, and loss of I κ B- α immunoreactivity during human cervical cancer progression

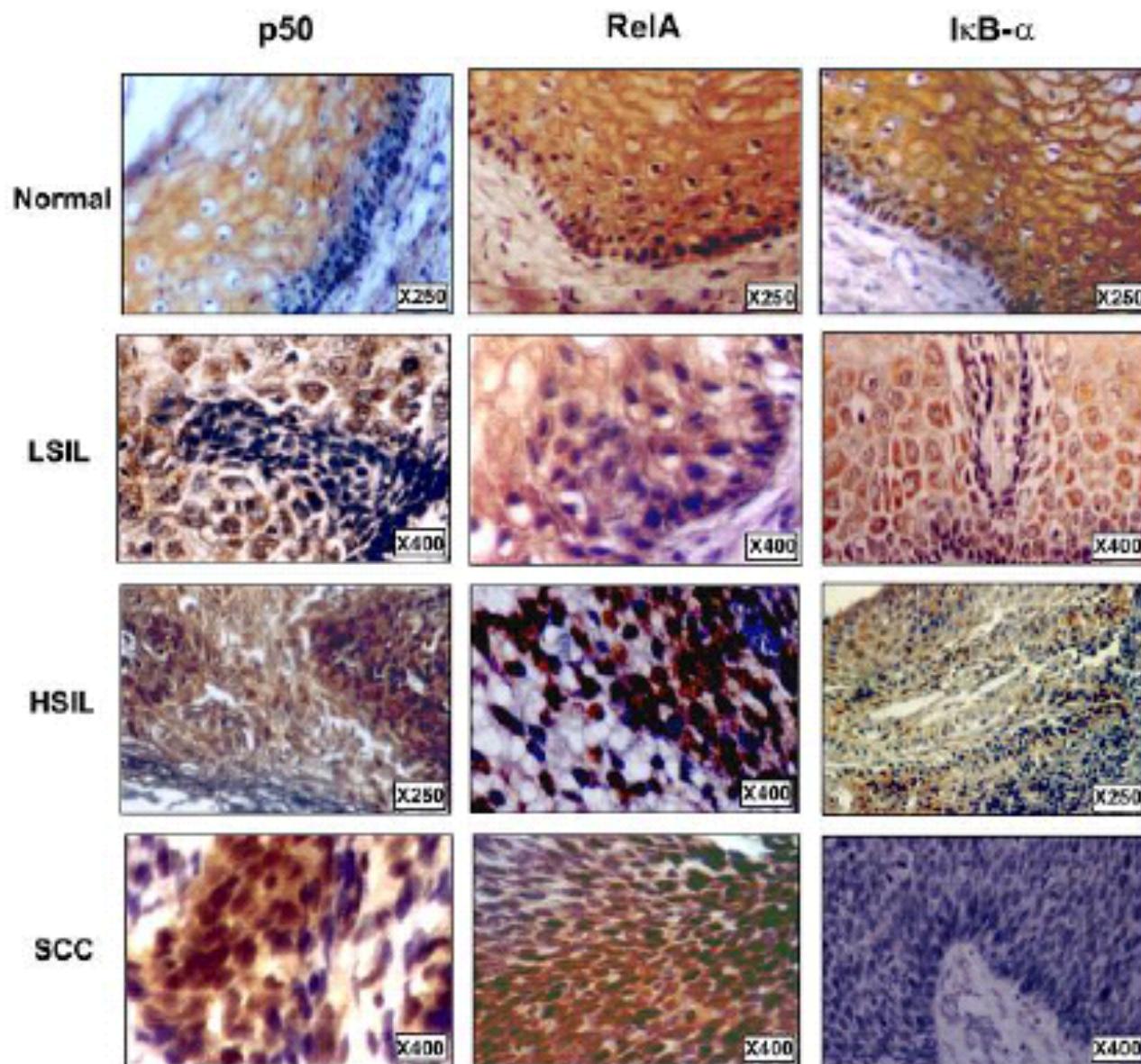


Table 1 Analysis of immunohistochemical data on the expression of p50, RelA, and I κ B- α

| <i>Histology</i> | <i>p50</i> | <i>RelA</i> | <i>IκB-α</i> |
|--------------------|------------|-------------|--|
| Normal | +++ (12) | +++ (15) | +++ (16) |
| <i>n</i> =18 | ++ (6) | ++ (3) | ++ (2) |
| LSIL | +++ (14) | +++ (20) | +++ (12) |
| <i>n</i> =23 | ++ (9) | ++ (3) | ++ (11) |
| HSIL | | | ++ (7) |
| <i>n</i> =27 | 65% | 71% | +(16) |
| WDSCC <i>n</i> =9 | 64% | 75% | +(3) |
| MDSCC <i>n</i> =7 | 69% | 76% | +(2) |
| PDSCC <i>n</i> =22 | 81% | 92% | - |

n=total number of samples analyzed. Intensity scoring in cytosol: intense: +++; moderate: ++; mild: +; negative: -. Nuclear positivity is shown in percentage. Figures in parentheses indicate the number of samples found positive.

*N*74

*C*20

*C*31

Nuc

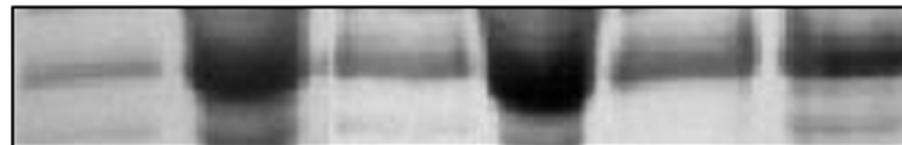
Cyt

Cyt

Nuc

Cyt

Nuc



p50



RelA

*C*31

*C*20

*N*21

*C*20

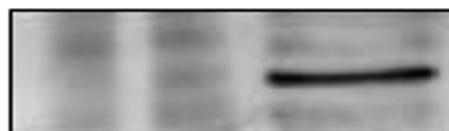
*C*23

*N*75

*N*74

*C*72

*C*19



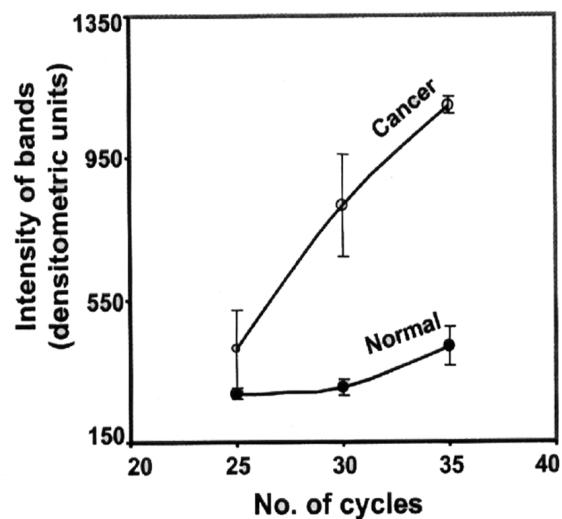
I κ B- α



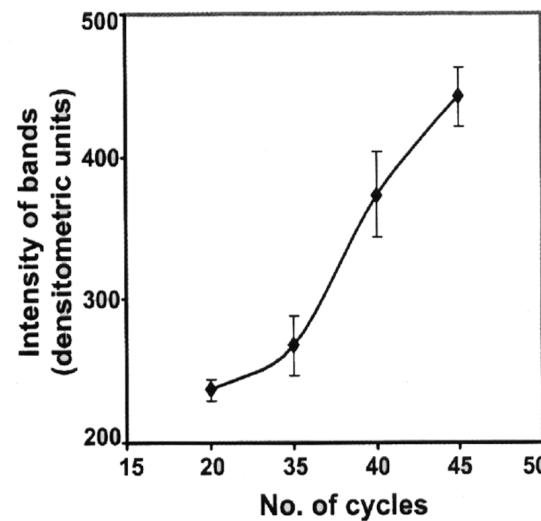
β -actin

Loss of I_KB- α is not due to defective transcription

Expression of I_KB- α

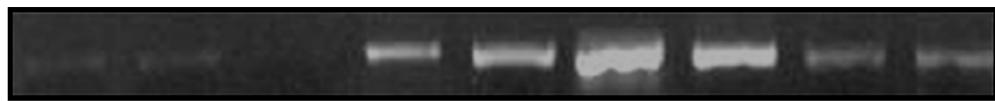


Expression of β 2-microglobulin

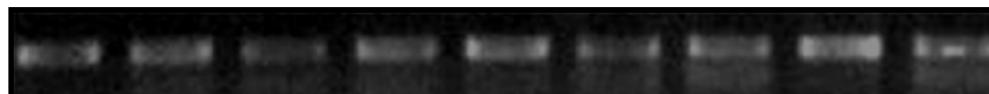


C

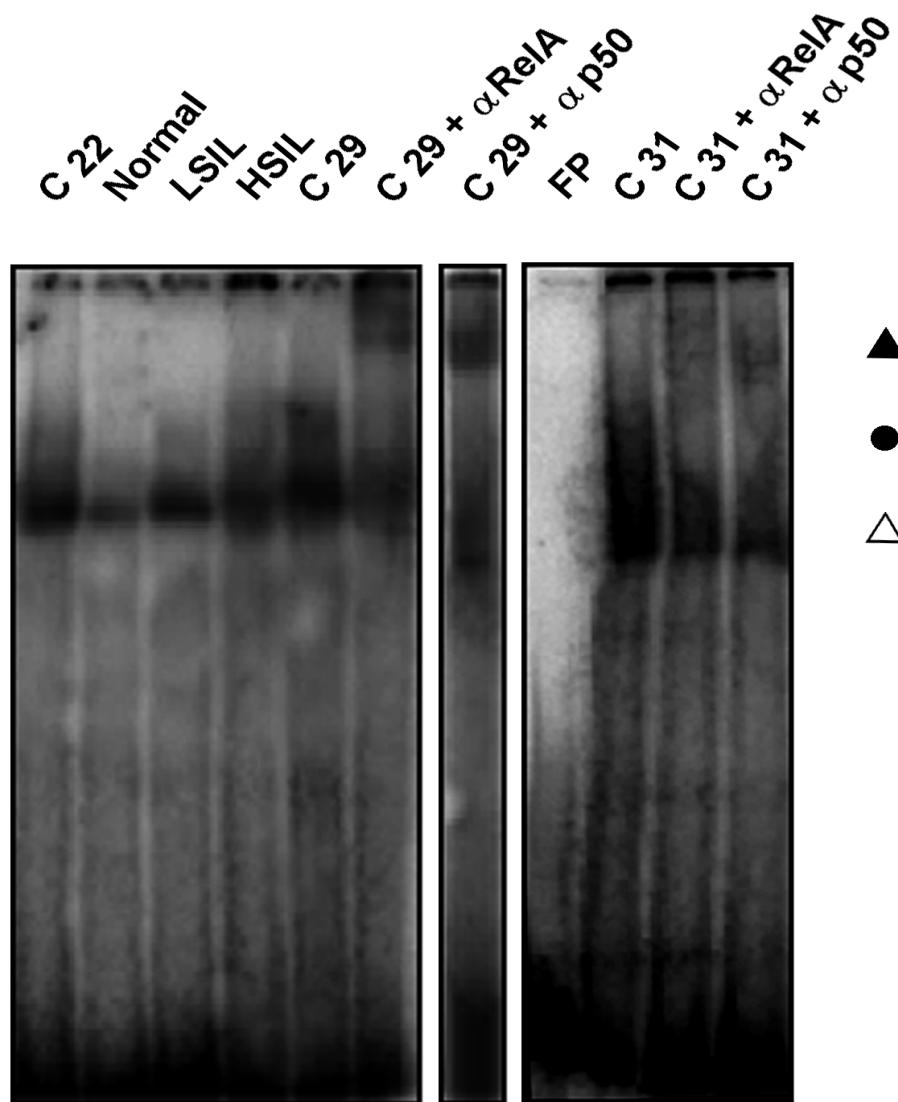
N⁶⁵ N⁶⁶ N²⁴ C⁶¹ C²⁰ C²² C²³ C²⁹ C⁶³



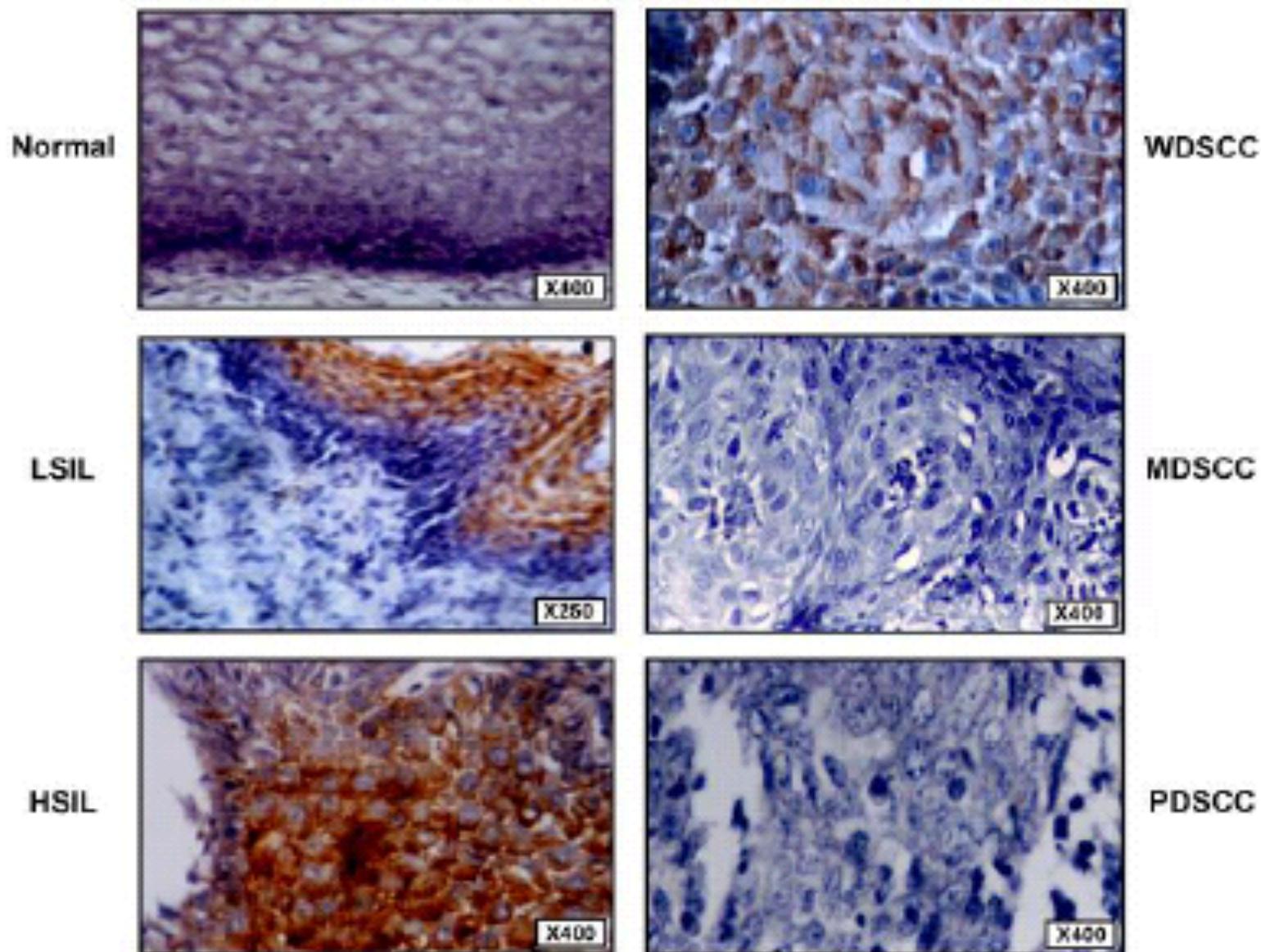
β 2-microglobulin



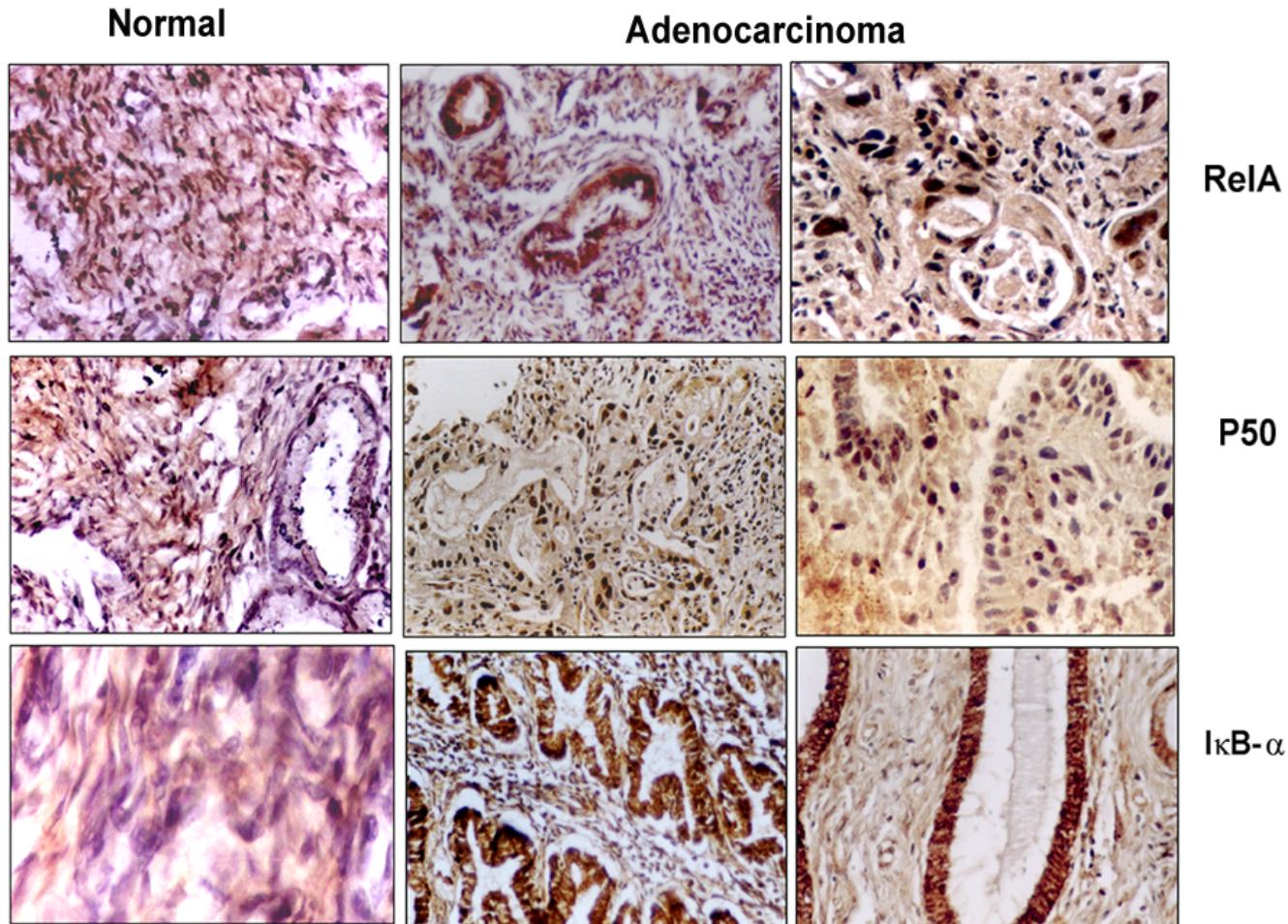
Constitutive NF-κB DNA binding activity during cervical cancer progression



Phosphorylation of I κ B- α occurs in the squamous intraepithelial lesions but not in the advanced stages of squamous cell carcinoma



Nuclear translocation of p50 and RelA, and I κ B- α immunoreactivity in uterine adenocarcinomas.



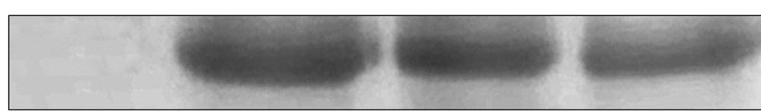
RelA and p50 expression in endocervix

Normal

Cancer



RelA



p50

Nuclear



RelA



p50

Cytosol

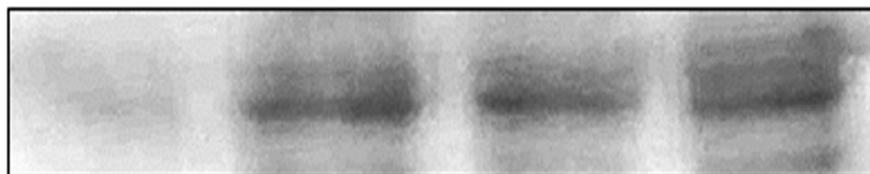


β-actin

$I\kappa B-\alpha$ expression in endocervix

Normal

Cancer



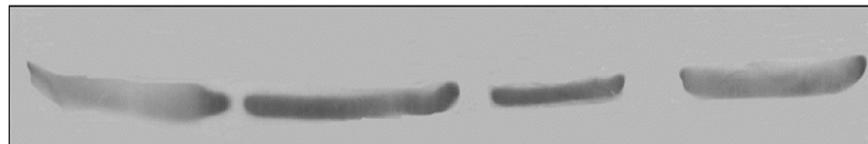
$I\kappa B-\alpha$

Nuclear



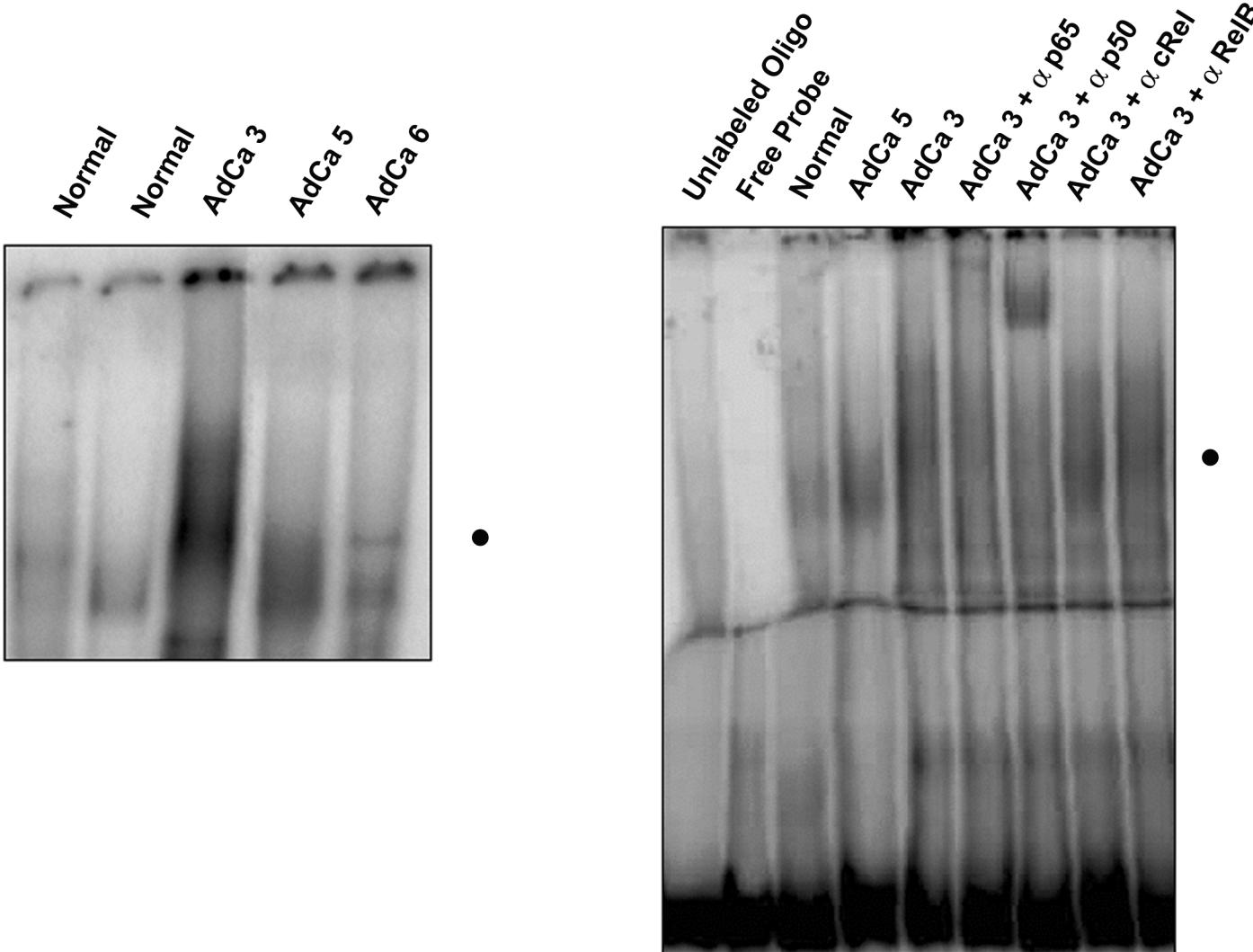
$I\kappa B-\alpha$

Cytosol



β -actin

Constitutive NF- κ B DNA binding activity in cervical adenocarcinomas



NF-κB is constitutively activated in high-grade squamous intraepithelial lesions and squamous cell carcinomas of the human uterine cervix

Asha Nair¹, Manickam Venkatraman¹, Tessy T Maliekal¹, Balaraman Nair² and Devarajan Karunagaran*,¹

¹*Division of Cancer Biology, Rajiv Gandhi Center for Biotechnology, Thiruvananthapuram, Kerala 695 014, India;* ²*Department of Pathology, Doctors Diagnostic and Research Center, Thiruvananthapuram, Kerala 695 011, India*

Publications on NF-kappa B

- 1. Asha Nair, Manickam Venkatraman, Tessy T. Maliekal, Balaraman Nair, and Devarajan Karunagaran (2003) Nuclear Factor-κB is Constitutively Activated in High grade Squamous Intraepithelial Lesions and Squamous Cell Carcinomas of the Human Uterine Cervix. *Oncogene* 22, 50-58.**
- 2. Ruby John Anto, Manickam Venkatraman, and Devarajan Karunagaran (2003) Inhibition of NF-κB sensitizes A431 Cells to EGF-induced apoptosis whereas its activation by ectopic expression of RelA confers resistance *J. Biol. Chem.* 278, 25490-25498**
- 3. Manickam Venkatraman, Ruby John Anto, Asha Nair, Merina Varghese and Devarajan Karunagaran (2005) Biological and chemical inhibitors of NF-κB sensitize SiHa cells to cisplatin-induced apoptosis. *Mol. Carcinogenesis* 44, 51-59**
- 4. Karunagaran, D., and Bharat Aggarwal (2003) Transcription factors as targets for drug development “In Pathomechanisms- New trends in drug research: Molecular mechanisms and new trends in drug development” Ed. Gyorgy Keri and Istvan Toth, Taylor and Francis, London and New York**

A trigger for cervical cancer progression

Thiruvananandapuram Why do cervical lesions in some women turn into cancer but regress in others? Scientists at the Rajiv Gandhi Centre for Biotechnology in Thiruvananandapuram reckon they are well on the way to finding out.

Their findings could help the search for a drug and a diagnostic tool for cervical cancer, which causes about 250,000 deaths worldwide each year, 80% of them in developing countries.

Before a normal cervical epithelial cell turns cancerous it exhibits increasing dysplasia — that is, its development becomes increasingly abnormal. Only about a third of women who reach a stage known as severe dysplasia progress to cervical cancer over a period of 10–15 years, whereas most cases of low-grade dysplasia regress spontaneously, according to Devarajan Karunagaran, who led the research.

Karunagaran and co-workers detected the spontaneous activation of a transcription factor called NF- κ B in cells that went on to become cancerous.

NF- κ B belongs to a family of transcription factors that are known to regulate the expression of genes important for tumour initiation and the promotion and progression of other types of cancer. "Many cancer cells protect themselves against ionizing radiation or chemotherapeutic compounds by activating NF- κ B, which helps in cell survival," Karunagaran told *NewsIndia*.

"We have demonstrated for the first time that the transcription factor NF- κ B is spontaneously activated during human cervical cancer progression," report the Thiruvananandapuram researchers¹.

They arrived at this conclusion after immunohistochemical analysis of 106 cervical tissue specimens of different histological grades. In normal cervical tissue and tissue with mild lesions, NF- κ B was found to be localized mainly in the cytosol (outside the cell nucleus), whereas in severe lesions it was translocated into the nucleus.

The researchers say their results suggest that the nuclear expression of

NF- κ B might be an indicator of malignant transformation. This means that a test to detect the proteins p50 and RelA, which together make up classical NF- κ B, in cervical tissues would be useful for diagnosing whether a cervical lesion is likely to become cancerous or regress.

Further studies using cervical cancer cell lines may help to identify the mechanisms by which NF- κ B is activated, and shed more light on the potential use of members of the NF- κ B family as specific targets for the treatment of human cervical cancer, the team report.

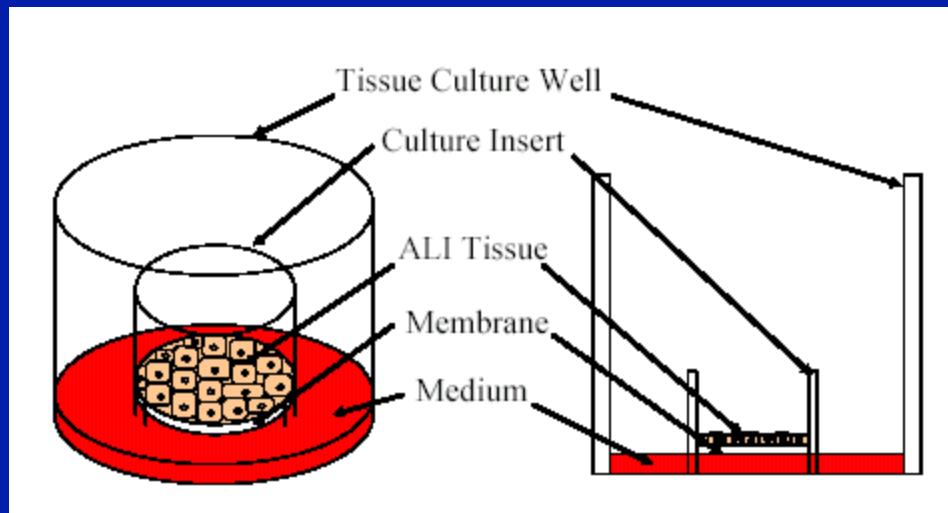
The mechanism of constitutive NF- κ B activation in cervical cancer remains unclear. Little is known about how constitutive nuclear NF- κ B activity contributes to the malignancy of these cells, Karunagaran says.

1. Nair, A., Venkatraman, M., Maliekal, T., Nair, B. & Karunagaran, D. *Oncogene* 22, 50–58 (2003).

e-mail: dkarunagaran@hotmail.com

Methodology for proving the hypothesis that secretion of cytokines may maintain an autocrine feedback loop that maintains constitutive NF- κ B activity

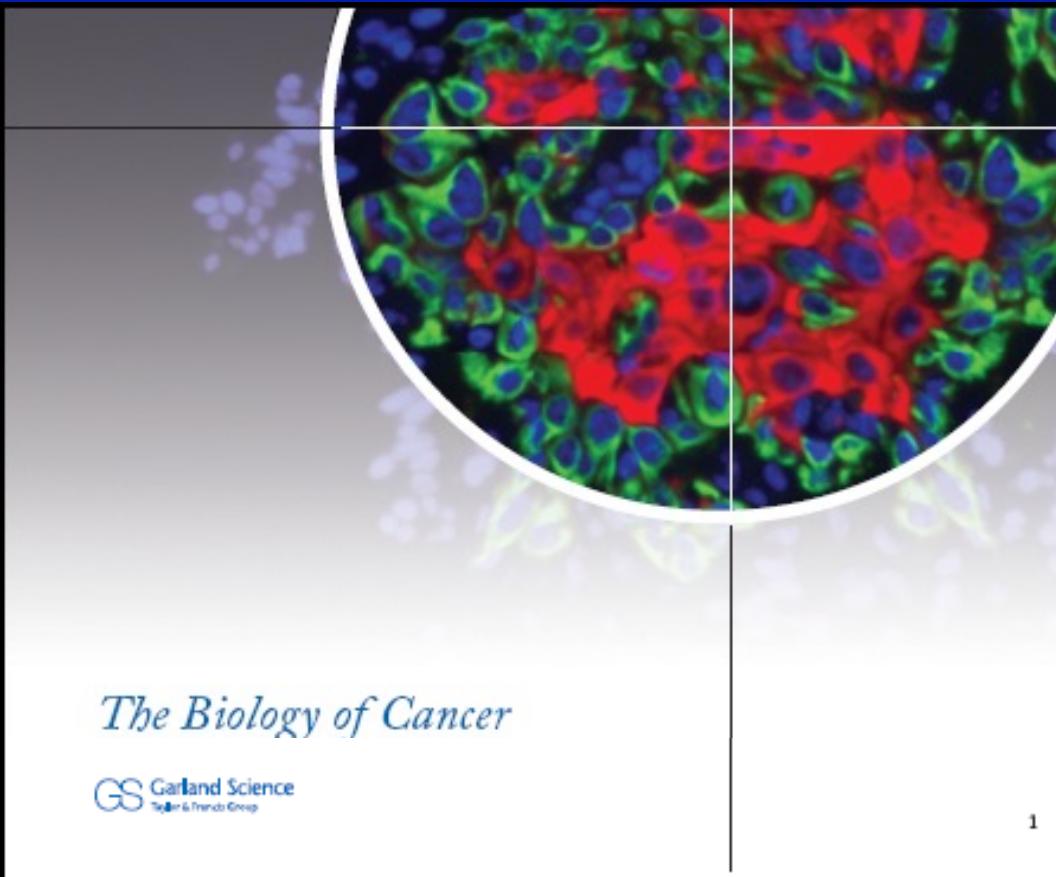
Organotypic raft culture



Provides *in vivo* like physical and biochemical properties

Freedom for controlled experimentation compared to *in vivo* models

Avoids disadvantages of cell line work since the changes can be observed in a 3D environment



The Biology of Cancer

GS Garland Science
Taylor & Francis Group

1

Thank You