

# Signal transduction pathways in cancer cells; novel targets for therapeutic intervention

## Review Article

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**Key Words:** Human neoplasms, signal transduction, oncogenes, transcription factors, kinases

**Received: 26 August, 2000; Accepted: 26 August, 2000**

## Summary

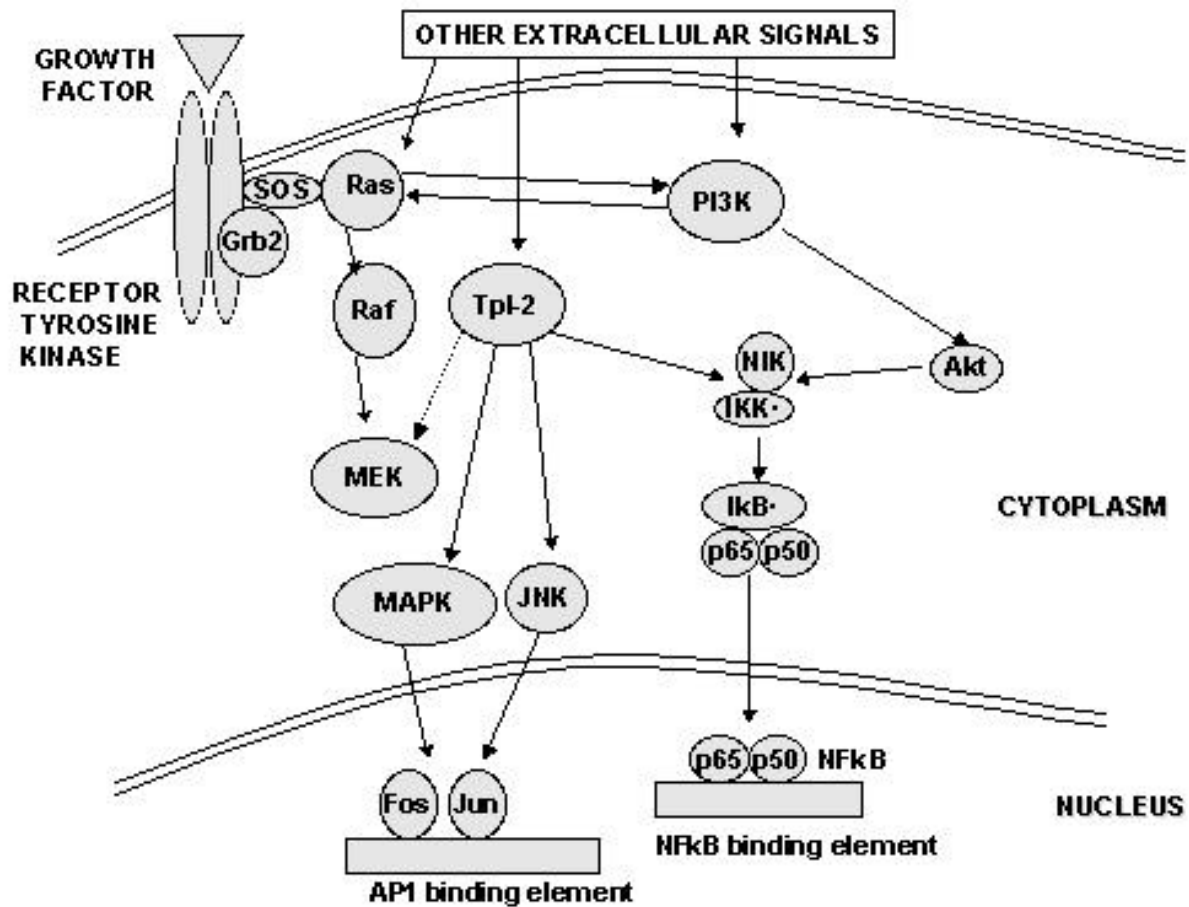
**Oncogenic proteins participate in signal transduction cascades that induce cell transformation. Understanding the molecular events that take place during oncogenesis is necessary to find novel, more effective therapeutic interventions. Signal transduction in cancer cells involves signaling from the extracellular environment, through the membrane, into the cytoplasm and towards the nucleus where transcription is initiated to generate proteins that will eventually contribute to the oncogenic phenotype. Alterations in such signaling cascades via mutations, gene amplifications or deletions frequently occur in human neoplasms. The result of such alterations has an impact in the cell cycle control, the cell morphology and the regulation of apoptosis. An overview on the signaling molecules altered in human tumors and their potential role as therapeutic targets is presented.**

## I. Introduction

Cancer originates in the genetic material of the tumor cell. Alterations that occur in the genetic material deregulate the cellular functions and lead to uncontrolled proliferation and alterations in the cell morphology. To find effective therapeutic interventions for cancer we need to understand the events that take place during cell transformation. The first step will be the identification of the genes that are altered in the tumor cell. Such genes are defined as oncogenes, and are usually either overexpressed or mutated in a way that they cannot be regulated as they used to leading to the oncogenic phenotype. A second category are the onco-suppressor genes, genes that normally function as brakes in the cell cycle or repair damaged DNA and when their function is lost the cell loses control of its division rate or acquires mutations that lead to faster proliferation (Fearon, 1997; Hanahan and Weinberg, 2000). Following the identification of these genes we need to elucidate the role of the proteins encoded by these genes in the cellular environment. In other words we need to understand the function of these proteins in the

normal cell and in the tumor cell. By understanding the mechanism through which these proteins induce the tumor we can interfere with therapeutic agents that will be able either to specifically inhibit the function of these genes and therefore eliminate these cells or perturb their proliferation and lead them to extinction (Denhardt, 1996).

Oncogenic proteins participate in signal transduction pathways that play central role in the transmission of a signal from the extracellular environment, through the cell membrane, into the cytoplasm and to the nucleus where transcription is initiated to generate proteins that will eventually contribute to the oncogenic phenotype. The function of these proteins is vital for cell and tissue homeostasis and they control processes such as cell division, differentiation and apoptosis. All these molecules are potential targets for anti cancer drug design since inhibition or activation of their function will lead to elimination of the tumor cells.



**Figure 1.** Growth factors and other extracellular signals initiate cascades that lead to activation of transcription factors and gene expression

## II. Growth factors and transmembrane receptors

Growth factors normally play a role in controlling the proliferation and metabolic activation of certain cells. They act by binding on specific receptors of the cell membrane, which, in turn, transmit the signal into the cytoplasm. They are frequently found overexpressed in a variety of tumors. The result is that the respective receptors are stimulated at a higher rate and, therefore, the signal that is transmitted is constant. Often tumors are found to secrete growth factors such as epidermal growth factor (EGF), colony stimulating growth factor 1 (CSF1), insulin growth factor I (IGF-I) and platelet-derived growth factor (PDGF) (Kolibaba and Druker, 1997). These factors bind to their receptors and initiate growth and proliferative signals. This mechanism establishes an autocrine loop that leads to tumor growth.

Alternatively, receptors can be mutated in a way that they transmit the signal without ligand binding. For instance tyrosine kinase receptors dimerize or oligomerize following ligand binding. The dimerization and the conformational changes that are induced by ligand binding

bring the cytoplasmic tails in such proximity to trigger autophosphorylation. Autophosphorylation in most cases activates a cascade of phosphorylation events that include phosphorylation of intracellular signaling molecules and recruitment of SH2 (src homology 2) domain-containing proteins that bind to specific tyrosine phosphorylated residues (Cooper and Howell, 1993; Rodrigues and Park, 1994). In various tumors tyrosine kinase receptors can be constitutively activated by mutations that render them active independent of ligand binding. Such mutations were found on NEU/c-erbB-2 (Bargmann and Weinberg, 1988; Weiner et al, 1989). Mutation of the transmembrane domain was also found in other viral oncogenes such as v-ROS, which obtains very broad substrate specificity (Zong et al, 1993).

Alternatively, tyrosine kinases can become oncogenic by mutations that make them active independent of ligand binding or dimerization. Non-receptor tyrosine kinases are also activated by mutations that affect their negative regulation such as the mutation on tyrosine 527 of Src that leads to deregulation of its activation (Sawyers and Denny, 1994).

There are several tyrosine kinases that are activated in tumors via mutations. Most of these mutations result from chromosomal translocations that give rise to hybrid gene products. A major example is the BCR-ABL that is a mutant protein caused by the reciprocal translocation between chromosomes 9 and 22, the Philadelphia chromosome, that juxtaposes sequences of the breakpoint cluster region BCR on chromosome 22 with the c-ABL kinase on chromosome 9 (Groffen et al, 1984; Heisterkamp et al, 1985). This translocation is present on 95% of chronic myelogenous leukemias, which account for 20% of the adult leukemias. The BCR-ABL fusion gene in CMLs produces a protein in which the first exon of c-ABL has been replaced by BCR sequences encoding 927 or 902 aminoacids (Shtivelman et al, 1985; Ben-Neriah et al, 1986). In other cases 185 kd BCR portion is fused with exons 2-11 of the c-ABL protein (Hermans et al, 1987). The BCR-ABL chimeric protein exhibits tyrosine kinase activity several fold higher than that of the c-ABL. This kinase can transform fibroblasts and is considered highly oncogenic (Daley et al, 1987; Lugo et al, 1990). The pathways that this protein uses to cause transformation are not clearly defined. It is known that it binds and activates GRB-2 (Pendergast et al, 1993) which, in turn, activates the Ras pathway, a key pathway for triggering MAPK activation and cell proliferation. Other cases of fusion proteins is this of TEL-ABL, present in acute lymphoblastic leukemia (ALL), in acute lymphoblastic leukemia (AML) and in chronic myeloblastic leukemia (CML) with a reciprocal t (9; 12) translocation which links the Ets-like transcription factor TEL with the ABL tyrosine kinase (Golub et al, 1996; Golub et al, 1996; Papadopoulos et al, 1995). TEL has also been found fused to the PDGF receptor (TEL-PDGFR) in chronic myelomonocytic leukemias (CMML) through an acquired translocation in hematopoietic cells, t(5;12)(q33;p13) (Berkowicz et al, 1991; Lerza et al, 1992; Golub et al, 1994).

Receptors molecules such as the cytokine receptors can contribute to the oncogenic phenotype by transducing signals from cytokines often expressed by tumor cells, such as the TGF in breast tumors (Chakravarthy et al, 1999). Antigen receptors also play a significant role in the tumor formation either by giving the tumor cell the ability to escape the immune system surveillance or by rendering hematopoietic cells sensitive to proliferation signals.

Interfering with the mutated receptor kinases may contribute to inhibition of the signal they transmit and subsequent elimination of the tumor cell. Chemical inhibitors are currently in trial such as tyrosine kinase inhibitors and growth factors are being used in therapeutic strategies in order to induce tumor cells to differentiate into a non-proliferating type of cell or cause apoptosis. Gene therapy strategies can, therefore, be used to produce such substances locally and thus eliminating side effects.

### **III. Cytoplasmic molecules**

The signal initiated by the growth factors at the cell surface is then transmitted into the cytoplasm and transduced by a cascade of events that includes phosphorylation, farnesylation, ubiquitination and other changes that alter molecules in order to promote or inhibit their activity or interaction with other molecules. Kinases and phosphatases play an important role in the transduction of oncogenic signals.

The MAPKinase and the PI3Kinase cascades play a central role during cell activation and proliferation. Several oncogenes are known to act on these pathways and several molecules that participate on these cascades when deregulated they become oncogenic. Ras, a well-studied family of oncogenes, structurally altered in about 25% of all human tumors, functions on activating the MAPK cascade (Kinzler and Vogelstein, 1996; Spandidos and Anderson, 1990; Zachos and Spandidos, 1997). Raf1, a serine threonine kinase that is activated by Ras, is also activated in some myeloid leukemias (Okuda et al, 1994; Schmidt et al, 1994).

Serine threonine kinases are another important group of oncogenes. This family of oncogenes includes the Akt family (Akt1, Akt2, Akt3). Akt2 was activated in pancreatic adenocarcinomas, small cell lung cancer, and ovarian cancers (Cheng et al, 1992; Bellacosa et al, 1995; Ruggeri et al, 1998). Akt3 has also been found activated in estrogen receptor deficient breast cancers and androgen independent prostate cancers (Nakatani et al, 1999). The Tpl-2/Cot oncogene is activated in breast (Sourvinos et al, 1999), thyroid and colon tumors (Ohara et al, 1995).

The Tpl-2 oncogene activates the MAPKinase (Mitogen Activated Protein Kinase) and the SAPKinase (Stress Activated Protein Kinase) pathways (Patriotis et al, 1994; Salmeron et al, 1996). Activation of these two pathways leads to the activation of transcription factors such as AP1 and NFAT (Ballester et al, 1997; Tsatsanis et al, 1998). Tpl-2 also activates the transcription factor NFkB, a major transcription factor, by activating the kinase that phosphorylates and induces degradation of the NFkB inhibitor IkB (Tsatsanis et al, 1998; Belich et al, 1999; Lin et al, 1999). Activation of these factors induces transcription of several genes that contribute to the tumor phenotype.

The Akt proto-oncogene (Bellacosa et al, 1991) is activated by PDGF receptor via activation of the PI3Kinase, a kinase that phosphorylates lipids (Franke et al, 1995; Chan et al, 1999). Activation of Akt inhibits apoptosis by inhibiting BAD, a pro-apoptotic, Bcl-2-binding protein (Franke et al, 1997; Khwaja, 1999; Wang et al, 1999). Akt is also involved in inducing cell cycle progression possibly by activating transcription factors such as NFkB (Ozes et al, 1999; Romashkova and Makarov, 1999). Akt kinase is known to induce phosphorylation of IkB via NIKinase and IKK (Ozes et al, 1999). It is also a transducer of growth factor signals such as PDGF, G-CSF, IL-2, hepatocyte growth factor,

IGF and other mitogenic signals. Most of these signals lead to phosphorylation of Akt which results in signals that lead to inhibition of apoptosis (Ahmed et al, 1997; Kennedy et al, 1997; Chan et al, 1999). When a combination of oncogenes is activated a particular phenotype is favored. For example, in breast tumor cells Akt phosphorylates Raf at a highly conserved serine residue in its regulatory domain *in vivo*. This phosphorylation of Raf by Akt inhibited activation of the Raf-MEK-ERK signaling pathway and shifted the cellular response from cell cycle arrest to proliferation (Zimmermann and Moelling, 1999). Such interactions can occur and determine the levels of crosstalk and fine regulation of different signaling pathways, the MAPK and the PI-3Kinase.

Raf, Akt and Tpl-2 are kinases that contribute to the oncogenic phenotype through divergent mechanisms. On the one hand they can induce transcription of genes that are normally not expressed in these cells and on the other hand they can directly interfere with cell cycle machinery and promote progression through the cell cycle. Alternatively, they can inhibit programmed cell death and, therefore, allow the survival of a cell that carries other defects and would otherwise apoptose. Interference with the function of these kinases via chemotherapeutic agents requires caution since the effect could be the opposite from the expected. On the contrary such molecules can be used in gene therapy approaches by introducing mutated forms that will favor a particular function.

#### **IV. Transcription factors**

Transmission of the signals from the cytoplasm will lead to the activation of transcription factors. Transcription factors are activated by several mechanisms during tumorigenesis and contribute to tumor formation. Phosphorylation and ubiquitination are mechanisms that control and regulate the activation of transcription factors. This is the case for NFkB where a sequence of phosphorylation events leads to degradation of its inhibitory molecule, I $\kappa$ B, and its subsequent translocation into the nucleus. I $\kappa$ B is ubiquitinated and recognized by the proteasome complex where its degradation takes place. In the case of NFAT, dephosphorylation by calcineurin in the cytoplasm leads to its nuclear translocation and a nuclear kinase, GSK3 phosphorylates NFAT and pushes it to translocate into the cytoplasm (Beals et al, 1997).

In tumor cells a transcription factor can be mutated and activated independent of extracellular or cytoplasmic signals. Expression of the transcription factors Ets-1 and Ets-2 is induced during cell proliferation but it has also been directly linked to a complex chromosomal translocation, t (6;18;21), in acute non lymphoblastic leukemias. Ets-2 is overexpressed during hepatic regeneration and hepatocellular carcinomas (Dittmer and

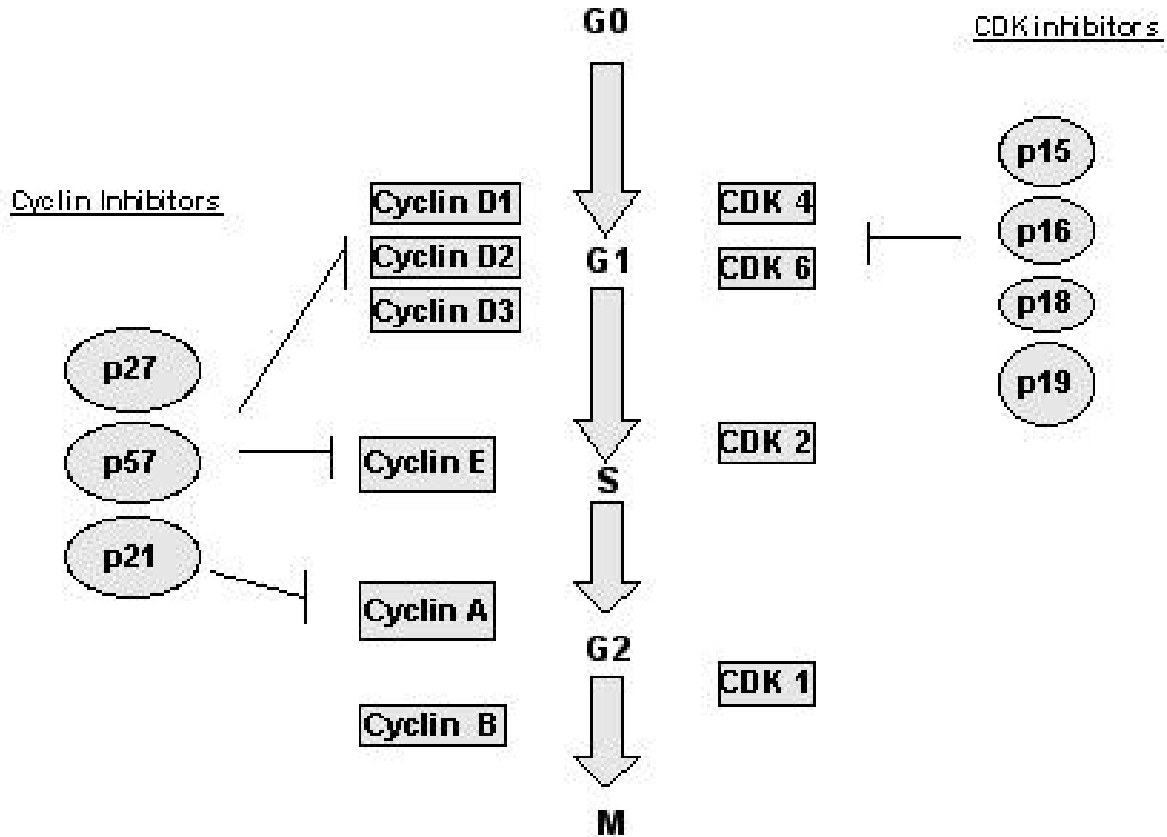
Nordheim, 1998). NFkB is a transcription factor that regulates expression of several genes and was activated in a series of tumors such as breast tumors, pancreatic adenocarcinomas, lung cancers and acute T cell leukemias (Bukowski et al, 1998; Sovak et al, 1997; Wang et al, 1999). In this case we do not know whether it is the immediate effect or the consequence of other oncogenes that have been activated and lead to NFkB induction. Regardless, inhibition of its activity in tumors may be beneficial for the elimination of the neoplastic cells.

C-myc is a transcription factor implicated in a variety of human tumors. When overexpressed it dimerizes with Max, a complex that elicits growth signals, while the Mad-Max complex promotes differentiation signals (Bouchard et al, 1998; Brandt-Rauf and Pincus, 1998; Schmidt, 1999). Overexpression of c-myc has been involved in a series of human tumors including colon, stomach, cervix, breast and hematological neoplasms (Spandidos et al, 1991; Agnantis et al, 1992; Porter et al, 1994; Nesbit et al, 1999).

#### **V. Cell cycle control proteins**

Deregulation of the cell cycle control is crucial for the development of a cancer cell since it has to proliferate at a faster than the normal rate. This effect can be direct, involving mutations of the cell cycle control proteins or indirect when an oncogenic protein targets the cell cycle regulators.

Oncogenic processes exert their greatest effect by targeting particular regulators of the G1 to S phase progression. Control of the G1 to S progression is a crucial checkpoint for the cell fate. Deregulation of the checkpoint proteins can contribute to uncontrolled proliferation (Sherr, 1996). Progression from the G1 to S phase occurs when cyclins respond to growth factor signals. Thus, such signals can be initiated by different growth stimuli that transmit the signal to the cytoplasm where cyclins are bound to cyclin dependent kinases and control the restriction point. Release of the cyclin dependent kinases from the complex pinpoints the passage from G1 to S phase. Cyclins D1, D2 and D3 control that stage. They are bound to the cyclin dependent kinases CDK4 and CDK6 which, when released, phosphorylate the retinoblastoma protein Rb (Morgan, 1995). Phosphorylation of Rb is a critical point in the cell cycle progression since it appears to be necessary for the transcriptional initiation of several genes. Hyperphosphorylated form of Rb is present past the G1 to S restriction point and all through the cell cycle until cell division (Zhu et al, 1996). The cyclin/CDK complex is inhibited by a family of proteins that include p15, p16, p18 and p19, frequently mutated in human melanomas, gliomas and leukemias, that specifically interact with CDK4 and CDK6 and therefore block the function of D type cyclins (Nobori et al, 1994; Zhang et al, 1994). On



**Figure 2.** Progression of a cell through the cell cycle is tightly controlled by inhibitors of the CDKs or inhibitors of the cyclins

the other hand the p21, p27, and p57 family of cyclin inhibitors are capable of interacting with cyclins type D, E and A exhibiting a broader spectrum of inhibition (el-Deiry et al, 1993; Toyoshima and Hunter, 1994; Lee et al, 1995).

Several types of tumors carry mutations on genes that control the cell cycle. Inactivation of the Rb gene is a primary event in retinoblastomas (Knudson, 1971), but overall the gene is targeted more often in adult cancers, particularly small-cell carcinomas of the lung (Sumitomo et al, 1999). Similarly, inherited loss of INK4a gene that encodes p16 confers susceptibility to melanoma (Palmero and Peters, 1996). Cyclin D1 is also overexpressed in many human cancers as a result of gene amplification or translocations targeting the D1 locus on human chromosome 11q13 (Masciullo et al, 1997). The gene encoding its catalytic partner CDK4, located on chromosome 12q13 is also amplified in sarcomas and gliomas (Nobori et al, 1994) although several other potential oncogenes including MDM2, the p53 antagonist, map on the same region (Hall and Peters, 1996).

Although cell cycle transition depends on the underlying CDK cycle, superimposed checkpoint controls help ensure that certain processes are completed before others begin. The role of such mechanisms is to act as a brake on the cell cycle in the face of stress and damage and allowing repair to take place. The best-studied checkpoint regulator is the p53 gene and is most frequently mutated in human cancer (Baker et al, 1989; Nigro et al, 1989). Even though p53 is a short-lived protein, it stabilizes and accumulates when the cell undergoes damage (Ko and Prives, 1996). The precise signal transduction pathway that activates p53 has not been elucidated but is likely to include genes like ATM (mutated in *ataxia telangiectasia*) (Enoch and Norbury, 1995). The p53 protein acts as a transcription factor and cancer related mutations cluster in its binding domain (Ko and Prives, 1996). Targeting the cell cycle control proteins is a possible approach to eliminate tumor growth.

## VI. Apoptosis related proteins

Programmed cell death occurs when a cell has suffered DNA damage that cannot be repaired, is under environmental stress or receives extracellular apoptotic signals. Stress-induced apoptosis is regulated by a mechanism that involves cytochrome c release from the mitochondria and subsequent activation of several proteolytic molecules termed caspases that lead to degradation of cellular components, DNA cleavage ('laddering') and death (Green, 1998). Receptor-mediated apoptosis, such as Fas or the TNF- receptors, initiate signals that lead to caspase 8 activation, cytochrome c release from the cytoplasm, activation of caspase 9 and the APAF complex and subsequent cleavage and activation of caspase 3, caspase 6 or caspase 7 (Alnemri, 1999; Qin et al, 1999). Caspases also translocate into the nucleus triggering their pro-apoptotic effects (Alnemri, 1999).

In cancer cells an anti-apoptotic mechanism is often activated to rescue the transformed cell from programmed cell death. The most common mechanism is activation of the bcl-2 family of proteins (Bcl-2, Bcl-xL, Bcl-W) that are able to inhibit cytochrome c release from the mitochondria and rescue the cell from apoptosis. Inactivation of the pro-apoptotic molecules Bax, Bak, Bid or Bim also contributes to rescuing the cell from apoptosis. Activation of oncogenic kinases such as Akt-1 protects cells from apoptosis by inhibiting the pro-apoptotic molecule Bad (Khwaja, 1999). Several anti-apoptotic signals such as growth factors (PDGF, EGF etc) lead to the activation of signaling pathways including the PI3Kinase or MAPK pathways that can also be activated by oncogenic kinases like Akt and Tpl-2. Thus, activation of these oncogenic kinases rescues the cell from the apoptotic signals and promotes survival.

Activation of the apoptotic mechanism is, therefore, a key stage where therapeutic agents could interfere. Gene therapy approaches could be used by introducing pro-apoptotic molecules into tumor cells, whereas pharmacological inhibitors of anti apoptotic molecules such as the bcl-2 family of proteins may be a therapeutic approach in tumors where these molecules are activated. Several conventional chemotherapeutic agents induce apoptosis in tumor cells and drug resistant tumors exhibit activated anti-apoptotic mechanism. The fact that pro-apoptotic and anti-apoptotic molecules are important in maintaining the homeostasis in all tissues may be a potential drawback for such pharmacological treatments.

## VII. Conclusions

Human tumors are a result of accumulation of two or more mutations in a cell. These mutations alter the protein profile of the cell and lead to faster proliferation and transformation. The mutant or the overexpressed proteins can be targeted with therapeutic agents to inhibit their action and kill the tumor cells. Gene therapy approaches

can be used, introducing mutant proteins that compete or inhibit the transforming ones. In the case of a deleted protein a gene therapy approach is beneficial to re-introduce the gene that is deleted. Thus, understanding of the signal transduction pathways altered in tumor cells is important for detecting novel targets for cancer therapy.

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