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The role of oncogenic kinases in human cancer (Review)

CHRISTOS TSATSANIS and DEMETRIOS A. SPANDIDOS

Medical School, University of Crete, 71409 Heraklion, Crete, Greece

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Abstract. Tumorigenesis in humans is a multistep process, which reflects genetic alterations that lead to cell transformation and malignancy. Cellular genes that are altered are normally involved in maintaining cell homeostasis by participating in signaling pathways tightly regulated to maintain the functional integrity of the cell. When these genes are altered they escape from the regulatory control and transmit signals that lead to the progressive conversion of normal cells into cancer cells. Oncogenic signals involve activation of kinases, which can be either a primary event when they are directly mutated in a tumor cell or a secondary event as recipients and mediators of oncogenic signals. Transmembrane (e.g. EGFR, PDGFR) or cytoplasmic (Src, Abl) tyrosine kinases are found mutated in a variety of human tumors. Cytoplasmic serine threonine kinases (Raf, Akt, Tpl-2) are also mutated or activated in several types of human malignancies. Kinases transduce signals that lead to cell proliferation or inhibition of programmed cell death by activating transcription factors (e.g. AP1, NFκB, Myc), inhibiting pro-apoptotic molecules (e.g. Bad, Bax), or they participate in deregulating the cell cycle control. Thus, kinases play a central role in oncogenesis rendering them putative targets for anti-cancer drug design.

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Correspondence to: Dr Christos Tsatsanis, Medical School, University of Crete, 71409 Heraklion, Crete, Greece

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1. Introduction

Cancer arises when one or more cells lose their ability to control cell division and they begin to proliferate in an uncontrolled fashion. The origin of cancer lies in the genetic material of the cell and is a result of an accumulation of mutations that promote clonal selection of cells with an aggressive phenotype. This phenotype is underlined by the faster proliferation rate 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. Since cancer originates in the genetic material of the affected cell, the primary step is the identification of the genes that are altered in the tumor cell. These genes are defined as oncogenes, genes that are usually either overexpressed or mutated so that they cannot be regulated as they used to, and oncosuppressor 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 (1,2). The second step is the understanding of 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 elucidating 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 the genes involved eliminating the cells, or perturb their proliferation and lead them to extinction (3).

All oncogenic proteins participate in cellular functions that involve transduction of signals from the extracellular environment, through the membrane, into the cytoplasm towards the nucleus, where transcription is initiated to generate proteins that will eventually contribute to the oncogenic phenotype. Study of the signal transduction in cancer can be therefore divided into the following areas: a) signaling from growth factors and cytokines via transmembrane receptors, b) role of the cytoplasmic signaling molecules in cancer c) regulation of transcription factors in cancer. These signaling events have effects a) on the regulation of the cell cycle and b) the regulation of apoptosis.

Cellular signaling involves phosphorylation events that occur through interactions of kinases that are localized on the cell membrane, in the cytosol or in the nucleus. Kinases that are often deregulated in human cancers are able to initiate or alter signals that eventually lead to cell proliferation and transformation. Such kinases transfer phosphates in tyrosine residues or serine and threonine residues of other kinases or

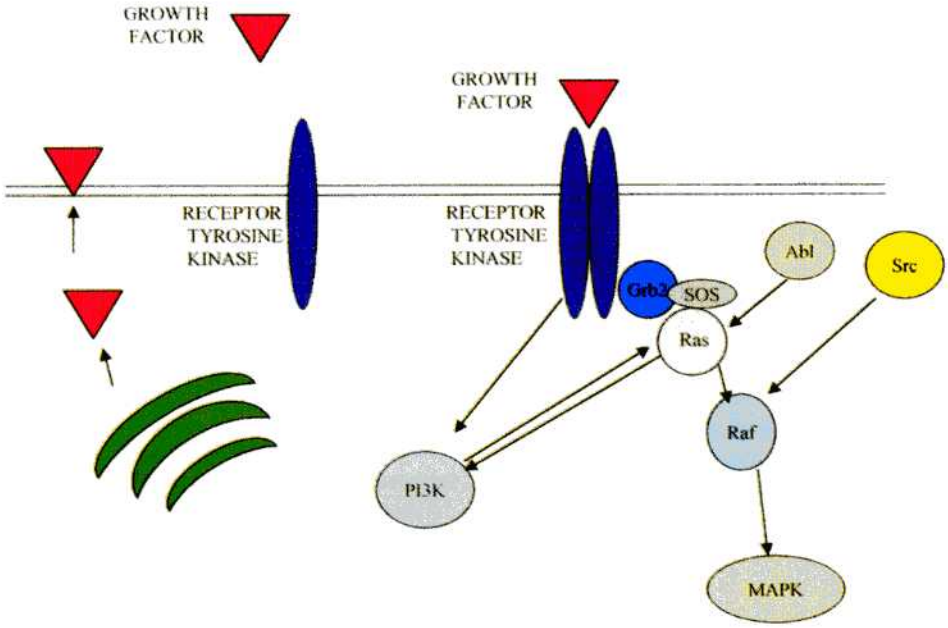

Figure 1. Signaling via transmembrane receptors. Receptor tyrosine kinases are bound to their ligands, dimerize and transmit signals that lead to proliferation and differentiation.

other proteins that participate in various signaling pathways in the cell.

2. Signaling via transmembrane receptors

Transmembrane receptors often contain domains that exhibit catalytic properties. The tyrosine kinase receptors are a major family of transmembrane receptors. Tyrosine kinases act to transfer phosphate from ATP to tyrosine residues on specific cellular proteins. Phosphorylation of these proteins alters their catalytic properties or their association with other molecules and therefore initiates biochemical signals that lead to gene transcription or morphological changes. Tyrosine kinases can be either transmembrane or cytoplasmic. The first oncogene identified was the tyrosine kinase v-*src* which was encoded by the Rous sarcoma virus in chicken. The cellular homologue c-*src* is a non-receptor kinase tightly regulated in contrast with its oncogenic viral counterpart (4,5).

Tyrosine kinase receptors can contribute to the oncogenic phenotype by different mechanisms:

a) Secretion of particular growth factors may be deregulated and as a result the receptor will be triggered at a higher than the normal level. 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) (6). These factors bind to their receptors and initiate growth and proliferative signals. This mechanism establishes an autocrine loop that leads to tumor growth (Fig. 1).

b) Tyrosine kinase receptors often 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 as 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 (7,8). 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-*erb*B-2 (9,10). Mutation of the transmembrane domain was also found in other viral oncogenes such as v-ROS, which obtains a very broad substrate specificity (11).

c) 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 (12).

d) Several tyrosine kinases are activated in tumors via mutations. 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 (13,14). 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 amino acids (15,16). In other cases 185 kDa BCR portion is fused with exons 2-11 of the c-ABL protein (17). 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 (18,19). The pathways through which this protein causes transformation are not clearly defined. It is known that it binds and activates GRB-2 (20) which in turn activates the Ras pathway, a key pathway for triggering MAPK activation and cell proliferation. Other

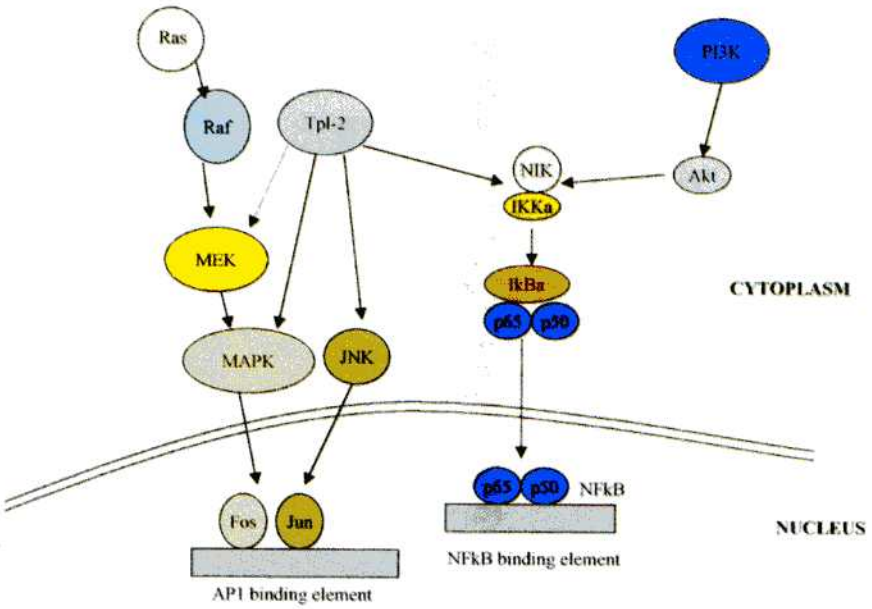

Figure 2. Signaling via cytoplasmic proteins. Activation of transcription factors. Oncogenic serine threonine kinases transduce signals to activate the MAPK, JNK and NFκB pathways which in turn promote gene expression.

known fusion proteins are TEL-ABL, present in acute lymphoblastic leukemia (ALL), in acute myeloid 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 (21-23). 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) (24-26).

Other receptors involved in the signaling in tumor cells are the cytokine and growth factor receptors that transduce signals from cytokines and growth factors often expressed by tumor cells, such as the TGFß in breast tumors (27). Antigen receptors also play a significant role in 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.

3. Signaling via cytoplasmic proteins

The signal that is initiated at the transmembrane receptors is being transduced through cytoplasmic proteins via the cytoplasm into the nucleus. The cytoplasmic signaling molecules can be protein kinases, phosphatases or other proteins such as adaptor molecules. Most transmembrane proteins are associated with intracellular tyrosine or serine threonine kinases, which, in turn activate signaling cascades towards the nucleus.

Activation of the MAPKinase and the PI3Kinase cascades are critical events during cell activation and proliferation. Several oncogenes are known to act on these pathways and several molecules that participate on these cascades when deregulated become oncogenic. *Ras*, a well-studied family of oncogenes, structurally altered in about 25% of all human tumors, functions on activating the MAPK cascade (28-30). Raf1, a serine threonine kinase that is activated by Ras, is also found to be activated in myeloid leukemias (31,32).

Cytoplasmic oncogenes can be serine threonine kinases. In this family of oncogenes the most important ones are the Akt family (Akt1, Akt2, Akt3). Akt2 was found to be activated in pancreatic adenocarcinomas, small cell lung cancer, and ovarian cancers (33-35). Akt3 was also found activated in estrogen receptor deficient breast cancers and androgen independent prostate cancers (36). The Tpl-2/Cot oncogene is activated in breast (37), thyroid and colon tumors (38).

The Tpl-2 oncogene activates the MAPKinase (mitogen activated protein kinase) and the SAPKinase (stress activated protein kinase) pathways (39,40). Activation of these two pathways leads to the activation of transcription factors such as AP1 and NFAT (41,42). Tpl-2 also activates the transcription factor NFκB, by activating the kinase that phosphorylates and induces degradation of the NFκB inhibitor IkBα (43-45). Activation of these factors induces transcription of several genes that contribute to the tumor phenotype (Fig. 2).

The Akt proto-oncogene (46) is activated by PDGF receptor via activation of the PI3Kinase, a kinase that phosphorylates lipids (47,48). The lipids bind to the PH domain of Akt and induce its serine threonine kinase activity (49). Activation of Akt inhibits apoptosis by inhibiting BAD, a pro-apoptotic, Bcl-2-binding protein (50-52). Akt is also involved in inducing cell cycle progression possibly by activating transcription factors such as NFκB (53,54). Akt kinase is known to induce phosphorylation of IkBα via NIKinase and IKKα (53). 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 (48,55,56). The pleiotropic effect of Akt and other oncogenic molecules is often regulated by other oncogenic molecules. Thus, when a combination of such oncogenes is activated a particular phenotype is favored. For example, in breast tumor cells Akt phosphorylates Raf at a highly conserved serine

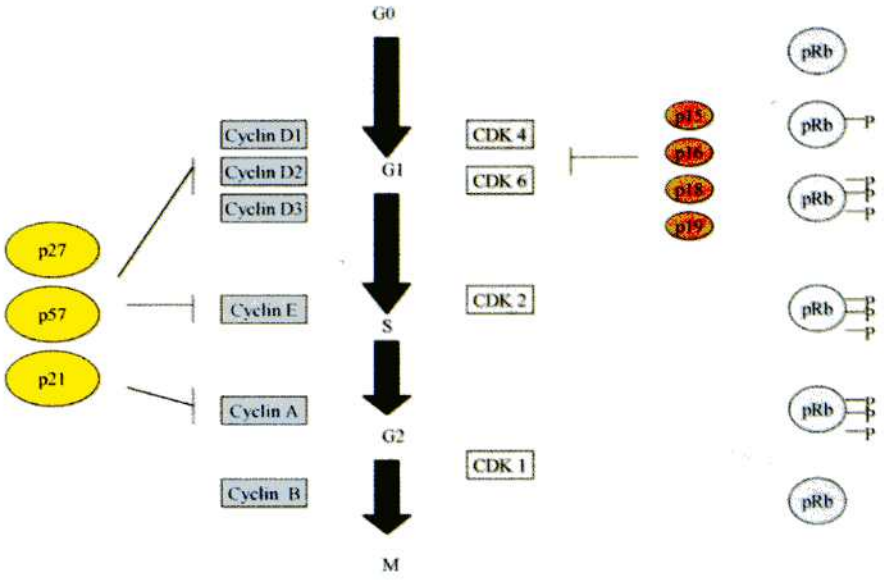

Figure 3. Cell cycle control. Progression through the cell cycle involves different regulatory mechanisms that require phosphorylation of cyclins by the CDKs and subsequent change in the phosphorylation status of the Rb protein.

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 (57). Such interactions can occur and determine the levels of crosstalk and fine regulation of different signaling pathways, the MAPK and the PI-3Kinase.

The examples of Raf, Akt and Tpl-2 indicate that an oncogenic serine threonine kinase can contribute to tumor formation through pleiotropic effects. 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 enter apoptosis.

4. Signaling to the nucleus

Cytoplasmic oncogenes often lead to the activation of transcription factors. The transcription factors themselves, though, can be activated by several mechanisms during tumorigenesis and contribute to tumor formation. Such signals are often regulated via phosphorylation of transcription factors either in the cytoplasm or in the nucleus. In the case of NFκB a sequence of phosphorylation events leads to degradation of its inhibitory molecule, IkBα and its subsequent translocation into the nucleus. In other cases, such as NFAT, dephosphorylation by calcineurin leads to its nuclear translocation and a nuclear kinase, GSK3 phosphorylates NFAT which translocates into the cytoplasm (58).

In some cases a transcription factor is 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 in hepatocellular carcinomas (59). In other cases activation of the signaling pathways previously mentioned lead to cell differentiation and proliferation. These events require different genes to be expressed. NFκB is a major transcription factor found to be activated in breast tumors, pancreatic adenocarcinomas, lung cancers and acute T cell leukemias (60-62). Another transcription factor involved in various human tumors is c-*myc*. When overexpressed in human tumors it dimerizes with Max, a complex that elicits growth signals, while the Mad-Max complex promotes differentiation signals (63-65). Overexpression of c-*myc* has been shown to be involved in human tumors including colon, stomach, cervix, breast and haematological neoplasms (66-69).

5. Cell cycle progression

A tumor cell is characterized by short and uncontrolled proliferation. All oncogenic events lead to deregulation of the cell machinery that controls the cell cycle. In other cases the cell cycle components have been affected and the cell loses the ability to control its proliferation. Whether the effect is direct, involving mutation of genes that regulate the cell cycle, or indirect, the result is a shorter proliferation time.

The life cycle of a cell is divided in the following stages: G0 where the cell has just emerged from mitosis and is growing to reach its mature stage; G1, the most prolonged stage where the cell does not divide and functions as part of the tissue where it belongs; S stage where the cell enters the mitotic stage and duplicates its DNA and finally the M stage where the cell enters mitosis.

Most oncogenic processes exert their greatest effect by targeting particular regulators of the G1 to S phase progression. When a cell exits from the G1 phase to enter the S phase it is

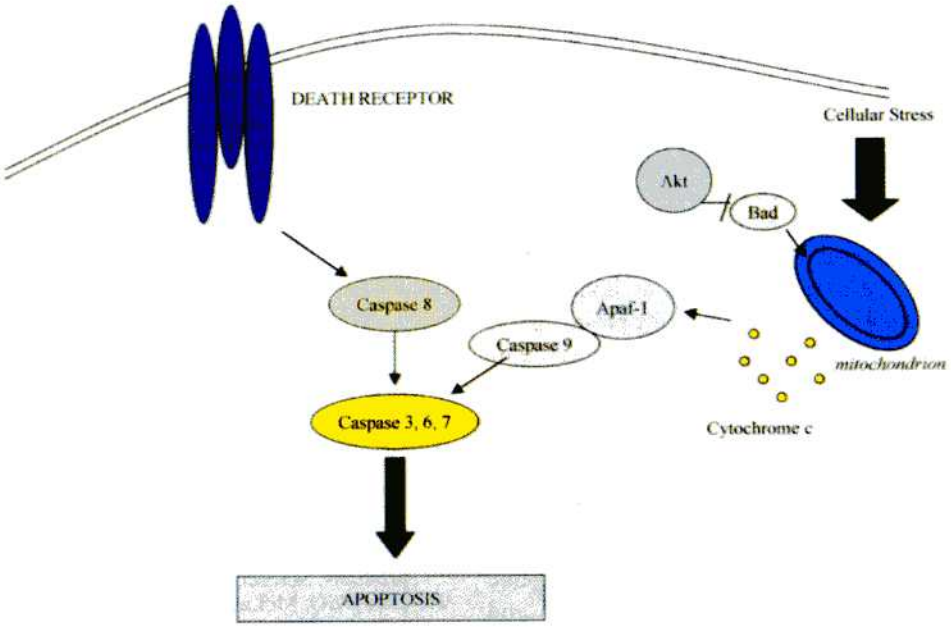

Figure 4. Regulation of apoptosis. Apoptosis can be triggered either by sensors of cellular stress or through ligation of the death receptors with their ligands. Activation of the apoptotic mechanism involves proteolytic cleavage and activation of the caspase family of proteases.

bound to divide. Control of the G1 to S progression is, therefore, a crucial checkpoint for the cell fate. Deregulation of the checkpoint proteins can contribute to uncontrolled proliferation (70). 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 are known to be involved in controlling this stage. They are bound to the cyclin dependent kinases CDK4 and CDK6 which, when released, phosphorylate the retinoblastoma protein Rb (71). Phosphorylation of Rb seems to be a critical point in the cell cycle progression since it appears to be necessary for the transcriptional initiation of several genes. Hyper-phosphorylated form of Rb is present past the G1 to S restriction point and all through the cell cycle until cell division (72). 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 (73,74). On 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 (75-77) (Fig. 3).

Mutations in genes that regulate the cell cycle have been detected in several types of tumors. Inactivation of the Rb gene is a primary event in retinoblastomas (78), but overall the gene is targeted more often in adult cancers, particularly small-cell carcinomas of the lung (79). Similarly, inherited loss of INK4a gene that encodes p16 confers susceptibility to melanoma (80). 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 (81). The gene encoding its catalytic partner CDK4, located on chromosome 12q13 is also amplified in sarcomas and gliomas (73) although several other potential oncogenes including MDM2, the p53 antagonist, map on the same region (82).

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 brake 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 (83,84). Even though p53 is a short-lived protein, it stabilizes and accumulates when the cell undergoes damage (85). The precise signal transduction pathway that activates p53 has not been elucidated but is likely to include genes such as ATM (mutated in ataxia telangiectasia) (86). The p53 protein acts as a transcription factor and cancer-related mutations cluster in its binding domain (85).

6. Regulation of apoptosis

Cytoplasmic or nuclear kinases transduce mitogenic signals that lead to cell proliferation and transformation. Activation of such molecules may also inhibit cells to undergo programmed cell death. Cells that suffer from DNA damage, environmental stress, or lose their ability to maintain homeostasis due to mutations, are destined to die. 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 (87). Ligation of the Fas or the TNF-α receptors with their ligands initiate signals that lead to caspase 8 activation, cyto-

chrome 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 (88,89) (Fig. 4). Caspases also translocate into the nucleus triggering their pro-apoptotic effects (89).

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 (50). 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 such as Akt and Tpl-2. Thus, activation of these oncogenic kinases rescues the cell from the apoptotic signals and promotes survival.

7. Concluding remarks

The paths that a cell can take to become malignant are variable. Each different cancer type and tissue requires activation of a different set of oncogenes in order to promote transformation. These oncogenes initiate or transduce signals that lead to deregulation of gene expression and uncontrolled proliferation. Kinases are key molecules in all signaling cascades since they deliver phosphates, the 'currency' of most signaling pathways. Different kinases are being activated in different human tumors, either by being directly mutated or due to other events that induce signals of the pathway(s) they participate in. Involvement of kinases in vital signaling events renders them valuable targets for therapeutic intervention in human cancers.

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