Review

Protein Kinases, Their Function and Implication in Cancer and Other Diseases

(protein kinase / cancer therapy / protein phosphorylation)

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Abstract. Protein phosphorylation is known to play an important role in various cellular processes such as cell division, metabolism, survival and apoptosis. It is driven by specific enzymes, tyrosine and serine-threonine protein kinases. Human protein kinases constitute a complicated system with intricate internal and external interactions. The complexity and sophistication of the system implies its vulnerability. Alterations in functions of these enzymes may launch series of pathological changes within the cell and as a result cause diseases. Protein kinases have been shown to be involved in various pathological processes, first of all malignancies. Deregulation of different protein kinases has been found in chronic myelogenous leukaemia, gastrointestinal stromal tumours, various other sarcomas and cancers as well as non-malignant disorders. Therefore, they are regarded as important effectors in human pathology and represent prospective therapeutic targets.

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Abbreviations: Abl – Abelson tyrosine kinase; AML – acute myeloid leukaemia, CDK – cyclin-dependent kinase; CML – chronic myelogenous leukaemia; EGFR – epidermal growth factor receptor; ERK – extracellular signal-regulated kinase; Flt-3 – FMS-like tyrosine kinase-3; HER–1, 2 – human epidermal growth factor 1, 2; Hsp90 – heat-shock protein 90; IL – interleukin, JNKs – c-Jun N-terminal kinases; MAP – mitogen-activated protein; MAPK – mitogen-activated protein kinase; M-CSF – macrophage colony-stimulating factor; MKK – MAP kinase kinase; PDGF – platelet-derived growth factor; PDGFR – platelet-derived growth factor receptor; PDK-1 – phosphoinositide-dependent kinase-1; PI3K – phosphatidylinositol 3-kinase; PK – protein kinase; PKB – protein kinase B; PKC – protein kinase C; RPTK – receptor protein tyrosine kinase; STAT – signal transducer and activator of transcription family, TKs – tyrosine kinases.

Introduction

It has been shown that protein phosphorylation regulates various aspects of cellular function such as division, metabolism, movement, survival and apoptosis. Hence, any disruption of the phosphorylation will alter cell function and may cause disease (Cohen, 2002). Phases of the cell cycle are driven by a great number of specific proteins including protein kinases (PKs). These enzymes undergo timely destruction or inactivation after they have performed their function. This enables the cell to properly perform its functions and proceed through the cycle (Blagden and de Bono, 2005). Manning et al. (2002) have defined the human „kinome space“, consisting of 518 protein kinase genes and 106 protein kinase pseudogenes. This study has provided important foundations for understanding kinase signalling pathways and their role in various pathological processes.

A number of diseases are caused or accompanied by deregulation of the phosphorylation. Alterations in 218 genes of the kinome are supposed to be associated with human diseases. Thus, PKs are regarded as a promising therapeutic target in malignant diseases, viral infections and in other disorders implicating PKs (Manning et al., 2002).

Protein kinases

Series of phosphorylation events are indispensable for the greater part of cellular processes. These events are catalysed by enzymes known as kinases.

Function. Kinases transfer phosphoryl groups onto target proteins, altering their activity as a result. This process is called phosphorylation and is reversed by the action of phosphatases, which remove phosphoryl moieties from target proteins (Blagden and de Bono, 2005). Phosphorylation of the target proteins leads to the activation of signal-transduction pathways, which play an important role in a great number of biological processes (Cheetham, 2004, Kondapalli et al., 2005). It is an essential mechanism by which intracellular and extra-

cellular signals are transmitted throughout the cell and to the nucleus. Thus, PKs play a crucial role in intracellular signalling pathways that regulate cell growth, differentiation, development, functions, and death.

Classification. There are different classifications of PKs. The two most conventional of them are based on substrate specificity and amino acid sequences of the catalytic domains.

The first one implies that kinases are classified by the amino acids they phosphorylate. The two main classes of kinases are tyrosine kinases (TKs), which phosphorylate tyrosine, and serine-threonine kinases, which phosphorylate serine or threonine. Both have a common catalytic core, which contains a glycine-rich N-terminal ATP-binding pocket and a central conserved aspartic acid residue required for its catalytic activity (Knighton et al., 1991).

The tyrosine kinases in turn are divided into two large classes: receptor and non-receptor TKs. Receptor TKs are transmembrane proteins with a ligand-binding extracellular domain and a catalytic intracellular kinase domain, whereas non-receptor TKs lack transmembrane domains and are found in the cytosol, the nucleus, and the inner surface of the plasma membrane. The enzymatic activities of both types of TKs are under tight control, so that non-proliferating cells have very low levels of tyrosyl phosphorylated proteins (Krause and Van Etten, 2005).

Of the two classes of kinases, tyrosine kinases were the first identified class and have been well described.

About 90 TK genes and 43 TK-like genes have been discoverd (Madhusudan and Ganesan, 2004). Of these 90 tyrosine kinases, 58 are receptor type, divided into 20 subfamilies according to similar structural organization and sequence similarities within the kinase domain (Dewji, 2004). The 32 non-receptor tyrosine kinases can be sorted in 10 subfamilies. Their products regulate cellular proliferation, survival, differentiation, functions, and motility (Krause and Van Etten, 2005). The most recently discovered and investigated receptor TKs belong to the type III family. The members of receptor tyrosine kinases type III family include platelet-derived growth factor receptors (PDGFR α and β), colony-stimulating factor receptor (CSF-1R, c-fms), FMS-like tyrosine kinase-3 (Flt-3), and stem cell or steel factor receptor (c-kit) (Yarden et al., 1986). The type III family of receptor TKs is implicated in several highly malignant human cancers (Hubbard, 2004).

The second classification rests on sequence comparisons of the catalytic domains. The human kinome (Manning et al., 2002) is divided into seven main families: AGC family comprises protein kinases A, G and C; CAMK family contain Ca^{2+}/CAM-dependent PK; CK1 family – casein kinase 1; CMGC family contains CDK, MAPK, GSK3, CLKs; STE family – homologues of yeast sterile 7, 11, 20 kinases; TKs – tyrosine kinases, TKL – tyrosine kinase-like PKs.

Mode of action. Kinases can act as receptors on the cell membrane or as intracellular signal mediators. Activation of a cell surface transmembrane receptor kinase by its ligand or other stimulus launches a chain of intracellular kinase interactions that leads to changes in gene transcription and cell response. Uncontrolled kinase activity as a result of loss of inhibitory mediators or activating mutations leads to subsequent cell proliferation and is common in human cancers (Kondapalli et al., 2005).

The process of ligand binding and activation of the catalytic domain is well known in receptor TKs. Normally ligands bind to the external region of a PK receptor, resulting in activation of the intracellular kinase domain, autophosphorylation and activation of a down-stream effect or pathway (Fehm et al., 2004). Receptor protein kinases can exist in two distinct conformations: active and inactive, also called closed and open, respectively. The difference between the two conformations consists in the position of the so-called activation loop. In its open conformation, the activation loop turns away from centre of the molecule, allowing productive substrate binding. In its closed conformation, the activation loop occludes the mouth of the kinase, resulting in an inactive state (Deininger, 2004). In the absence of the ligand, receptor PKs are unphosphorylated and monomeric, i.e. the conformation of their kinase domains is inactive. Besides, the cytoplasmic juxtamembrane region takes part in the enzyme inhibition by interacting with the kinase domain. Receptor PKs become activated when the ligand binds to the extracellular domain, resulting in receptor oligomerization, disruption of the auto-inhibitory juxtamembrane interaction, and autophosphorylation of a regulatory tyrosine within the activation loop of the kinase. This rearranges critical amino acid residues increasing the catalytic activity of the enzyme with consequent autophosphorylation, which generates binding sites for signalling proteins, recruiting them to the membrane and activating multiple signalling pathways (Schlessinger, 2000).

Activation of transmembrane tyrosine kinase receptors is connected with various intracellular signalling events, such as SH2- and SH3-mediated protein-protein interactions and activation of signalling enzymes such as phospholipase C, protein kinase C (PKC), mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3-kinase (PI3K) (Buchdunger et al., 2000).

While receptor PKs have an auto-inhibitory juxtamembrane domain, the non-receptor PKs, typified by c-Abl, are maintained in an inactive state by cellular inhibitor proteins and lipids and through intramolecular auto-inhibition. Non-receptor PKs are activated by diverse intracellular signals through dissociation of inhibitors, by recruitment to transmembrane receptors (causing oligomerization and autophosphorylation), and through transphosphorylation by other kinases.

Non-receptor PK signalling is terminated in part through the action of tyrosine phosphatases that hydrolyse tyrosyl phosphates and by the induction of inhibitory molecules (Krause and Van Etten, 2005).

Protein kinases are known to regulate the cell cycle. A number of kinases are required for cell cycle progression. Among them are cyclin-dependent kinases (CDKs), Aurora, Plk-1, Bub1, BubR1, Mps1, Nek kinases and the checkpoint kinases Chk1 and Chk2. They play various roles in the cell cycle. Some of them are potential cancer targets since they have been shown to have a cancer-related activity.

Blagden and de Bono (2005) distinguished a group of so-called cell cycle protein kinases. According to these authors the cell cycle kinases are PKs that, by cooperating with other protein families, allow systematic and coordinated cell cycle progression. They are usually non-membrane serine-threonine kinases (see below), while CDKs are supposed to be the largest and most important family of them.

Protein kinases are also involved in cardiac Na^+-Ca^{2+} exchange (Harvey, 2004). Ion channels have been identified as frequent targets of TK activity in many different cell types, including cardiac myocytes (Davis et al., 2001). Some of the discovered signalling pathways that are important in acute functional responses in the heart comprise serine-threonine kinases, e.g. protein kinase A and protein kinase C. Also, TKs have been shown to play an important role in cardiac function. Some lines of evidence show that modulation of TK activity can also produce acute functional responses, including changes in both electrical and mechanical activity. It has been found that activation of receptors linked to TK-dependent signalling pathways leads to functional changes. As has been found by means of pharmacologic inhibition of TK and tyrosine phosphatase activity, basal tyrosine phosphorylation plays an important role in affecting functional responses (Harvey, 2004).

Liew et al. reported that the phytoestrogen genistein can enhance cardiac myocyte contraction by inhibition of TK activity. This effect is supposed to result from the capacity of the compound to inhibit Na^+-Ca^{2+} exchange activity and attenuate extrusion of cytosolic Ca^{2+} (Liew et al., 2003).

Hence, the importance of PKs in the cell is undeniable. As it has already been mentioned, these enzymes comprise a large family, with more than 500 being encoded by the human genome (Kondapalli et al., 2005) and in each cell, approximately 200–300 PKs are present (Traxler et al., 2001).

Structure. Crystal structures of PKs may provide deeper insight into the activation and inactivation mechanisms of the enzymes. Crystal structures of many kinases have been determined recently. Among the first were phosphoinositide-dependent kinase-1 (PDK-1), Aurora-A, interleukin-2 TK, c-kit (C-terminal receptor TK encoded by the kit oncogene), and Flt-3 (Cheetham, 2004).

The kinase domains of all TKs have a bilobar structure, with an N-terminal lobe that binds ATP and magnesium, a C-terminal lobe containing an activation loop, and a cleft between the lobes to which polypeptide substrates bind (Krause and Van Etten, 2005). Thus, the main parts of the catalytic unit of PKs are two structural subdomains, called the N and C lobes. N lobes consist of β-sheets and C lobes of α-helices. The adenosine moiety of ATP binds in a primarily hydrophobic cleft formed by the intersection of these two lobes within the catalytic domain. It is attached to the pocket by van der Waals contacts and H-bond interactions to the flexible hinge segment that connects the N and C subdomains (Kesari et al., 2005).

Although different kinases have a similar general structure of the ATP-binding site, there are certain differences between them, which enable drugs to specifically target one subclass without affecting the others. Small molecule inhibitors competitively occupy the ATP-binding pocket, often mimicking the hydrogen bonds made by the adenine fragment of ATP (Johnson et al., 2002). This is why it is necessary to know the structure of the ATP-binding site.

Five regions are distinguished in the ATP-binding site: adenine region, sugar region, hydrophobic pocket, hydrophobic channel and phosphate binding region (Engh and Bossemeyer, 2002).

The adenine region is a hydrophobic region that forms the two key hydrogen bonds formed by the interaction of the N-1 and N-6 amino groups of the adenine ring with the NH and carbonyl groups of the adenine anchoring hinge region of the PK. Many PK inhibitors use at least one of these hydrogen bonds. Some of the backbone carbonyl residues in the hinge region can also serve as hydrogen bond acceptors for inhibitor binding.

The sugar region is hydrophilic in most of the PKs, except for the EGFR (Traxler et al., 1996).

The hydrophobic pocket (or selectivity pocket) is not used by ATP, but is exploited by most of the kinase inhibitors; this plays an important role in the inhibitor selectivity.

The hydrophobic channel on the N-terminal lobe is induced by the ordered αC helix. As it is not used by ATP, it can be exploited to gain binding affinity.

In the phosphate-binding region the triphosphate group of ATP is constrained by a glycine-rich loop and is bound by an array of basic amino acid residues, which are involved in the catalytic process (Engh and Bossemeyer, 2002).

Kinases are most similar to each other when the kinase domain is in an active state, but are different in an inactive conformation (Blagden and de Bono, 2005).

The receptor TKs are characterized by five Ig-like domains in the extracellular domain and a cytoplasmic region containing a hydrophilic catalytic kinase insert domain (Heldin, 1995), a single transmembrane helix and an auto-inhibitory juxtamembrane domain (for

Table 1. Protein kinases and their functions

PK	Alternative names	Involved in	References
c-kit	CD117	– proliferation and differentiation of stem cells – migration of germ cells – development of normal haematopoiesis – survival, expansion and maturation of NK – maintenance of normal haematopoiesis, melanogenesis, gametogenesis – growth and differentiation of mast cells – growth and differentiation of interstitial cells of Cajal	Colucci and Di Santo, 2000 Manning et al., 2002 Savage and Antman, 2002 Kitamura et al., 2003
PDGF	MDGF GSM FDGF ODGF MDF OBIF T47Dfactor GDGF	– mesenchymal cell migration and proliferation – correct development of kidneys, cardiovascular system, brain, lungs and connective tissue in embryogenesis – inflammation – angiogenesis – wound healing – blood vessel maintenance – indirect regulation of endothelial cell functions	Zhang et al., 2003 Saharinen and Alitalo, 2003. Jones and Cross, 2004
CDKs		– cell cycle and cell division regulation	Banchio et al., 2004 Madhusudan and Ganesan, 2004 Knight and Shokat, 2005 Schang, 2005
MAPKs	ERKs	– transduction of intracellular signals in a wide range of different biological responses differing from cell to cell	Johnson et al., 2002 Johnson et al., 2005
EGFR		– cell survival – angiogenesis – cell proliferation – cell differentiation – inhibition of programmed cell death – cell motility	Barlesi et al., 2005 Ready, 2005
JAKs		– signal transduction from cytokine receptors	Leonard and O'Shea, 1998
ROCK	RhoGTP binding protein	– regulation of cell functions, including actin – cytoskeletal reorganization and cellular motility	Leung et al., 1996 Amano et al., 2003
PKC		– signal transduction in mediating the effects of many extracellular stimuli including growth factors, hormones and drugs	Jenny et al., 2005
Plk		– multiple roles in meiotic and mitotic cell division – regulation of many aspects of mitosis necessary for successful cytokinesis	Conn et al. 2000 Blagden and de Bono, 2005
Aurora		– cytokinesis – regulation during mitotic cell cycle progression	Katayama et al. 2003
VEGFR1/ VEGFR2/ VEGFR3	flt1/flk1/flt4	– positive regulation of angiogenesis	Mendoza et al. 2005
Bub1, BubR1, Mps1		– spindle assembly checkpoint during mitosis	Lew and Burke, 2003
Chk		– DNA damage checkpoint	Chen and Sanchez, 2004 Tort et al., 2005
Flt-3	CD135 Flk-2 STK-1 Ly72	– long-term expansion and differentiation of human pro-B-cells – growth and maintenance of various types of progenitor/precursor cells	Krause and Van Etten, 2005
ret		– a role in proliferation of neural crest cells is suggested – ret can compensate for the defect of kit during embryogenesis and postnatal life	Ogilvie et al., 2006
PKB/Akt		– a critical component of the intracellular signalling pathway that exerts the effects of growth and survival factors and that mediates the response to insulin and inflammatory agents	Brazil and Hemmings, 2001
Src family		– control of many functions, including cell adhesion, growth, movement and differentiation	Warmuth et al., 2003
PDK-1		– activation of protein kinase B – cell growth – cell proliferation – protection from pro-apoptotic stimuli – stimulation of neo-angiogenesis	Mora et al., 2004

details see c-kit). In normal cells, the auto-inhibitory juxtamembrane domain tightly regulates the activity of receptor TKs (Hubbard, 2004).

Deregulation. Different protein kinases are deregulated and over-expressed in human cancers and are thus attractive targets for selective pharmacologic inhibitors. One of the most extensively studied is the Bcr-Abl TK of chronic myelogenous leukaemia (CML) (Deininger et al., 2000).

A frequent mechanism of TK activation in haematologic cancers is the fusion of a receptor or non-receptor TK with a partner protein, usually as a consequence of balanced chromosomal translocation. The partner protein causes constitutive oligomerization of the TK in the absence of ligand binding or physiologic activating signals, thereby promoting autophosphorylation and activation (Krause and Van Etten, 2005). A second important mechanism of TK deregulation is a mutation disrupting auto-regulation of a kinase. Mutations in the Flt-3 receptor in acute myeloid leukaemia (AML) render this TK active in the absence of the ligand (Krause and Van Etten, 2005). In another instance, small deletions and point mutations in the kinase domain of epidermal growth factor receptor (EGFR) in a subset of non-small-cell lung cancers increase the sensitivity of the receptor to its ligand (Lynch et al., 2004) and alter receptor signalling (Sordella et al., 2004). A third mechanism of TK deregulation is increased or aberrant expression of a receptor TK, its ligand, or both. Examples include over-expression of the receptor TK ERBB2 (HER-2/neu) in breast cancer and over-expression of a mutant form of platelet-derived growth factor (PDGF), a receptor TK ligand, in dermatofibrosarcoma protuberans with t(11; 17) (Krause and Van Etten, 2005). Lastly, increased TK activity can result from a decrease in factors that limit TK activity, such as impaired tyrosine phosphatase activity or decreased expression of TK inhibitor proteins (Krause and Van Etten, 2005). Aberrant TK activation can increase the survival, proliferation, and cytotoxic drug resistance of malignant cells, and in tumours it can increase angiogenesis, invasiveness, and metastatic potential (Krause and Van Etten, 2005).

Many TKs have been shown to be amplified, up-regulated or mutated in cancers. As a result, some TKs (e.g. HER-2 in breast cancer) are both prognostic markers and therapeutic targets (Fehm et al., 2004).

c-kit

The kit receptor tyrosine kinase represents a transmembrane receptor that is expressed in a variety of different tissues. It mediates pleiotropic biological effects through its ligand, stem cell factor. Initially, the *kit* gene was identified as a viral oncogene in the Hardy-Zuckerman IV feline sarcoma virus (HZ4-FeSV) and associated with its transforming activity (Holyoak, 2001).

Kit is encoded by the c-*kit* gene, at the White spotting (W) locus, chromosome 5, in mice (Sommer et al., 2003). In humans, the c-*kit* gene maps to the long arm of chromosome 4 (4q11-q12), which is in close proximity to the PDGF-α receptor (d'Auriol et al., 1998). The c-*kit* gene product is expressed in haematopoietic progenitor cells, mast cells, germ cells, epithelial breast cells and interstitial cells of Cajal (the gastrointestinal "pacemaker" cells), and some human tumours (Kitamura et al., 2003); a critical role of c-kit for survival, expansion, and maturation of natural killer (NK) cells has been proved (Colucci and Di Santo, 2000). Studies with mice with inactivating mutations of c-kit or its ligand have also demonstrated that the c-*kit* gene product is essential for maintenance of normal melanogenesis and gametogenesis (Savage and Antman, 2002).

Kit is structurally similar to the receptors for macrophage colony-stimulating factor (M-CSF), PDGFR-α and β (Kitamura et al., 2003), and Flt-3 (Rosnet et al., 1991). On this basis it belongs to the type III receptor tyrosine kinase family (Villalba et al., 2001).

Functionally the c-kit belongs to growth factor receptors with intrinsic tyrosine kinase activity, which also include receptors such as c-Ret, the receptor for glial cell-derived neurotrophic factor (GDNF) and the platelet-derived growth factor (PDGF) receptors (Sattler and Salgia, 2004).

The type III receptor TKs are characterized by an extracellular (EC) domain made up of five immunoglobulin-like repeats, a short juxtamembrane region, and a TK domain that is split into two domains (TK-I and TK-II) by an insert sequence of variable length separating the ATP-binding and phospho-transferase regions (Duensing et al., 2001). The structure and amino acid sequence of kit are well preserved in humans, mice and rats (Kitamura et al., 2003). The three amino-terminal Ig-like motifs determine the specificity of c-kit for ligand binding to stem cell factor (SCF), followed by an Ig-like domain that facilitates dimerization of the receptor. The function of the membrane proximal Ig-like domain is not fully understood (Sattler and Salgia, 2004). The functional part of the c-kit protein is localized on the cell surface, and allows transduction of downstream signalling to the nucleus via phosphorylation of tyrosine residues in the signalling proteins (Nakatani et al., 2005). The c-kit protein is also known as CD117 (Verweij, 2004).

Nakatani et al. revealed that tyrosine phosphorylated c-kit was involved in the cluster formation at the cell membrane, while dephosphorylated c-kit molecules did not show cell-surface clustering (Nakatani et al., 2005).

The ligand for c-kit (KitL) has been identified as Steel factor or SCF. It is the only known specific ligand of the kit receptor. KitL is encoded at the Steel (Sl) locus, chromosome 10, in mice (Sommer et al., 2003). Steel factor has been originally described to support

growth and survival of immature haematopoietic cells of multiple lineages. The kitL is synthesized as a transmembrane protein and a soluble form is produced by a proteolytic cleavage. *In vivo*, the membrane form only is important for normal haematopoiesis, but *in vitro*, both the membrane-bound and the soluble form are biologically active (Sattler and Salgia, 2004).

KitL binding to the receptor leads to receptor dimerization, activation of kinase activity and autophosphorylation of kit. The activated tyrosine kinase induces rapid, but transient, tyrosine phosphorylation of several cellular proteins, including kit itself. Subsequently, kit activates several signalling cascades, leading to cell proliferation, cell survival, and other cellular responses (Sattler and Salgia, 2004).

The phosphatidylinositol-3-kinase (PI3K)/Akt system is one of the major pathways of the c-kit. PI3K is composed of an 85-kDa regulatory subunit and a 110-kDa catalytic subunit. The 85-kDa regulatory subunit is associated with activated kit through an SH2 domain, and then is phosphorylated on tyrosine. The 110-kDa catalytic subunit of the activated PI3K produces phophatidylinositol-3, 4-bisphosphate, which in turn is used for phosphorylation of Akt, a serine-threonine kinase. The activated Akt plays an important role in inhibition of apoptosis of cells whose survival depends on kit signalling.

Another major signalling pathway is the Ras/MAP kinase cascade (Linnekin, 1999). The activated kit recruits SH2-containing proteins such as Grb2, Shc and SHP2. Grb2 may bind kit either directly or indirectly through interaction with Shc or SHP2. Grb2 is constitutively associated with Sos, a guanine nucleotide exchange factor, therefore the recruitment of Grb2 to the activated kit leads to co-localization of Sos and Ras with subsequent activation of Ras. This promotes the interaction of Ras with Raf serine-threonine kinase and then the activation of MEK (a MAP kinase kinase). MEK phosphorylates ERK (a MAPK system member) and ERK phosphorylates a number of substrates including $pp90^{rsk}$ (Linnekin, 1999), leading to cell proliferation.

Both Ras/Raf/MAPK and PI3-K/AKT cascades are supposed to be regulated by the binding of Grb2 and PI3-K to the phosphorylated forms of kit tyrosine residues 703 and 721, respectively (Thommes et al., 1999).

Pathways with JAK/STAT (namely STAT1, STAT3, and STAT5) or with Src family (lyn) members may also be involved in kit signalling (Linnekin, 1999), leading to proliferation.

Nakatani et al. (2005) showed by means of immunoprecipitation analysis that tyrosine-phosphorylated c-kit could biochemically interact with Hsp90, which strongly bounds to SCF-stimulated c-kit. This interaction was shown to occur at the plasma membrane.

Not only KitL can activate c-kit. Kit is known to be involved in tight interaction with other cell proteins. kit has thus been shown to be up-regulated and activated by Bcr-Abl (Hallek et al., 1996). This activation plays a role in haematologic malignancies (Wong et al., 2004).

Mutations in the murine locus for SCF (SL, steel) ligand and its receptor c-kit (W, white spotting) result in defects of melanogenesis, gametogenesis, or haematopoiesis (Geissler et al., 1998).

Various types of loss-of-function mutants have been reported at the W locus. Among them, double heterozygous mice of W/W^v genotype are most frequently used. The W mutant allele encodes a truncated kit without any transmembrane domain; as a result, the extracellular domain is not expressed on the cell surface. The W^v mutant allele is a point mutation at the TK-I domain, resulting in a remarkable decrease in the TK activity (Nocka et al., 1990). W/W^v mice show several abnormalities as a result of the loss of kit function. They have anaemia because of hypo-production of red blood cells, are infertile due to depletion of germ cells in both males and females, and have distinctive coloration due to lack of melanocytes (white-colour coat) (Russell, 1979). Lack of the kit function also leads to depletion of mast cells (Kitamura et al., 1978) and interstitial cells of Cajal (ICCs) (Maeda et al., 1992). In addition, Shimada et al. (1980) found in such mice spontaneous development of forestomach papillomas and antral ulcers. Yokoyama et al. (1982) confirmed these findings in W/W^v and Sl/Sl^d mutant mice. Bile reflux from duodenum to stomach appeared to be the cause of the stomach lesions in mice.

Ws/Ws rats have the phenotype similar to those of W/W^v mice. However, severe anaemia present in suckling Ws/Ws rats ameliorates within growth, and only slight anaemia can be found in adult animals. Besides, male and female Ws/Ws rats are fertile in spite of the decreased weights of gonads. Most humans heterozygous for kit mutations do not have a significant haematopoietic disorder, but do have mild defects in hair or skin pigmentation (de Mestie and des Guetz, 2005).

Although SCF and kit are thought to play a central role in the proliferation and differentiation of stem cells, SCF itself does not promote colony formation *in vitro*, because it functions in concert with other growth factors such as GM-CSF, G-CSF, IL-3, IL-6, IL-7, and Epo to stimulate formation of both differentiated progenitor cells and more primitive multi-lineage progenitor cells of the myeloid and erythroid lineages (McNiece and Briddell, 1995).

The frequency of gain-of-function mutations in c-kit is high. These mutations lead to the permanent activation of c-kit signalling in the absence of binding of SCF resulting in uncontrolled cell proliferation and resistance to apoptosis (Verweij, 2004). The TK activity of c-kit is likely to be critical for transformation in activating mutants of c-kit as well as in paracrine/autocrine mechanisms (Sattler and Salgia, 2004). During ligand-activated signalling, c-kit action is equalized by phosphatases, therefore it provokes only a transient increase

in tyrosine phosphorylation of cellular proteins. In cells with oncogenic forms of c-kit, there is a chronic increase in tyrosine phosphorylation of cellular proteins (Sattler and Salgia, 2004).

Both sporadic mutations of c-kit and autocrine/paracrine activation mechanisms of the SCF/kit pathway are implicated in a variety of malignancies, leading to tumour growth and apoptosis reduction. For example, c-kit is frequently mutated and activated in gastrointestinal stromal tumours (GISTs) and there is ligand-mediated activation of kit exemplified by some lung cancers. It has been shown to be implicated in pathogenesis of Merkel cell carcinoma and Kaposi's sarcoma, germ cell tumours (Tian et al., 1999), mast cell tumours, melanoma, breast cancer testicular malignancies, gynaecological cancers and neuroblastoma (Buchdunger et al., 2000). SCF and c-kit have also been linked to several haematological neoplastic disorders.

A pivotal role of c-kit has been proved in GISTs. These tumours originate from the multiplication of the cells of Cajal, which normally regulate intestinal motility and express the c-*kit* gene on their surface (de Mestie and des Guetz, 2005). The mechanism of constitutive c-kit phosphorylation in the majority of the GISTs involves a gain of function mutations in exon 11 (cytoplasmic juxtamembrane domain), and other mutations in exon 9 (extracellular membrane domain), exon 13 (first part of the split TK domain) and exon 17 (phosphotransferase domain) (Nakatani et al., 2005). Duensing et al. (2001) characterized oncogenic kit signalling mechanisms in GISTs and detected target signaling pathways, which might be of potential relevance to therapeutic interventions in GISTs. The authors also proved the effects of SCF-mediated kit activation in GIST cell cultures and investigated their dependence on the oncogenic kit signal. The results of Nakatani et al. (2005) suggested that interaction of c-kit with Hsp90 is necessary and sufficient for activation of c-kit in GIST-T1 cells.

c-kit is activated by point mutations in many cases of systemic mastocytosis or mast-cell leukaemia and less frequently in AML. The most common c-kit mutation involves D816 in the activation loop of the kinase domain. This mutant is not inhibited by imatinib. Besides, the normal c-kit receptor is expressed on most AML blasts and may be over-expressed and activated in some patients (Krause and Van Etten, 2005).

Sommer and collaborators (2003) produced a mouse model of the constitutive activation of kit in oncogenesis by a knock-in method importing a kit exon 11-activating mutation into the mouse genome. The authors introduced a mutation, $kit^{V558\Delta/+}$, found in a case of human familial GIST syndrome into the mouse germ line to produce a mouse model for GIST and kit mutation-associated haematopoietic diseases. $kit^{V558\Delta/+}$ mice reproduced the human conditions associated with this mutation consisting of myenteric plexus hyperplasia, GIST, and mastocytosis.

So-called Asp-816 mutations of c-kit have also been found in various types of mast cell neoplasms of adults, such as cutaneous mastocytosis and systemic mastocytosis. Besides, an appreciable number of testicular seminomas show the gain-of-function mutations of kit.

Therefore, kit targeting is an undoubtedly topical therapeutic strategy, and inhibition of this receptor with small molecule drugs, e.g. Gleevec (imatinib mesylate, STI571 has shown a dramatic efficacy in GIST) (Sattler and Salgia, 2004). Nevertheless, the sole expression of c-kit in itself in a tumour does not imply that the tumour will respond to a c-kit inhibitor. The tumour must be dependent on the activity of c-kit for the drug to produce an anti-tumour effect (Savage and Antman, 2002).

PDGFR

Platelet-derived growth factor receptors α and β (PDGFRs α and β) are widely expressed in various tissues. However, their expression in normal tissues is minimal or undetectable. Normally, expression of PDGFR can be found in the fibroblasts and the smooth muscle cells in lungs and airways (Zhang et al., 2003).

Structurally PDGFR belongs to the type III receptor TK family (Villalba et al., 2001). Like other members of the family it has an extracellular domain made up of five immunoglobulin-like repeats, and a TK domain (see c-kit).

PDGFRs α and β and their ligands, platelet-derived growth factors A and B (PDGFs) are important for mesenchymal cell migration and proliferation. In embryogenesis the PDGFR signalling is essential for the correct development of kidneys, cardiovascular system, brain, lungs and connective tissue. In adults, PDGFR participates in wound healing, inflammation and angiogenesis (Jones and Cross, 2004). PDGFR-β takes part in angiogenesis and blood vessel maintenance. PDGF-B has been shown to be an essential factor regulating pericyte, and thus, indirectly, endothelial cell function. Targeted disruption of PDGF-B or PDGFR-β in mice results in loss of microvascular pericytes, which normally form part of the capillary wall. The mutant mouse embryos develop abnormal vasculature with an irregular vessel diameter, increased leakiness, and capillary microaneurysms (Saharinen and Alitalo, 2003).

Signal transduction from activated PDGFR is partially mediated by the Ras/Raf/MAPK pathway as well as signalling through the PI3K/Akt pathway (de Bono et al., 2003). Both pathways lead to stimulation of the cell proliferation, while PI3K/Akt signalling inhibits apoptosis as well. The cell migration regulation is performed by signalling through PLC-γ and PKC.

The results of Plattner et al. (2004) show that specific stimulation of both PDGFRs results in activation of the endogenous c-Abl and Arg tyrosine kinases. The authors revealed that Arg is activated by PDGF and that the mechanism of activation involves activity of the

enzymes Src and PLC-γ1, which are recruited to PDGFR after growth factor stimulation. Thus, these data show the tight interactions between PDGFRs, Abl family kinases and PLC-γ1 that make up a signalling complex in which they undergo reciprocal phosphorylation and activation (see Bcr-Abl).

Normally PDGFRs are activated by PDGF. In pathological conditions, their ligand-independent activation is possible. PDGFR is thus activated by cryptic interstitial chromosome 4 deletions that generate a FIP1L1-PDGFR fusion TK in some patients with hyper-eosinophilic syndrome or systemic mastocytosis, whereas in some patients with chronic myelomonocytic leukaemia, fusion of PDGFR with one of several partner proteins has been detected (Krause and Van Etten, 2005).

Tumour cell-derived PDGF seems to play a role as a paracrine growth factor in tumorigenesis. PDGF is a potent mitogen and chemoattractant for both fibroblasts and endothelial cells, therefore PDGF may contribute to tumour development by stimulating the growth of a supporting connective tissue stroma and endothelial cell growth (Buchdunger et al., 2000).

Over-expression of PDGFR has been reported in multiple malignancies, such as myeloproliferative disorders, gliomas, carcinomas, melanomas (Barnhill et al., 1996), sarcomas, pancreatic and colonic cancers, breast, bone and ovarian tumours (Buchdunger et al., 2000). Aberrant PDGF receptors appear to deregulate the growth in these tumours. Moreover, PDGF has been implicated as an important factor involved in the vascular response to injury in cardiovascular diseases such as atherosclerosis (Ross, 1995), restenosis (Pauletto et al., 1994), and transplant arteriosclerosis (Gordon, 1992). There is evidence that over-expression and activation of PDGFRs may be an important factor contributing to the transformed phenotype of malignant gliomas (Kilic et al., 2000). The PDGF A and B ligands are expressed in most glioma cell lines. Several studies have revealed that the majority of gliomas co-express both PDGF and PDGFRs, suggesting an autocrine mechanism of growth stimulation in this type of malignancy (Hermanson et al., 1992).

In many cases it is not quite clear whether the expression of PDGFR is the real factor driving the tumour growth. PDGFR-α activating mutations have been described in GIST as a factor of responsiveness beside kit mutations. Gain-of-function mutations of PDGF receptor α have been identified in about one-third of such GISTs without kit mutations (Corless et al., 2005). According to the studies of Heinrich et al. (2003), PDGFR-α and kit mutations in GISTs are mutually exclusive.

A mutant form of PDGF is over-expressed in dermatofibrosarcoma protuberans with t(11; 17) (Krause and Van Etten, 2005), and low-grade astrocytomas show over-expression of PDGF and PDGFR (Kesari et al., 2005).

PDGFR also plays a role in lung cancer. Zhang et al. (2003) have shown convincingly that lung cancer cells A549 express PDGFR-α.

The findings presented by Bergers and collaborators (2003) suggest that pericytes of tumour blood vessels and their signalling mechanisms via the PDGFR-β are functionally important for the maintenance of tumour blood vessels. Their study suggests that the inhibition of PDGFR-β kinase activity is sufficient to inhibit tumour growth even if the tumour cells themselves do not express PDGFR-β. Instead, PDGFR-β-positive cells aligning the tumour blood vessels may be the main target of the TK inhibitors, e.g. imatinib mesylate.

The tumour must be dependent on the activity of PDGFR to respond to a corresponding TK inhibitor. The sole expression of PDGFR does not imply a therapeutic effectiveness of PDGFR inhibition (Savage and Antman, 2002).

Bcr-Abl

C-Abl. The name of the c-*abl* gene and protein originates from the Abelson virus, which contains the viral oncogene v-*abl* and causes leukaemia in mice (Holyoak, 2001). The mammalian Abelson family of non-receptor tyrosine kinases is comprised of c-Abl encoded by the *abl1* gene and Arg (*abl*-related gene) encoded by the *abl2* gene (Pendergast, 2002). Both genes in human are located on chromosome 9.

Abl has structural homology with the Src family of kinases, having tyrosine kinase (SH1), SH2 and SH3 domains (Holyoak, 2001). The N-terminus is homologous to c-Src and other Src-family members, but c-Abl has a large unique C-terminal half (Van Etten, 1999). The functional domains of c-Abl have been well investigated. SH2 and SH3 domains are involved in protein-protein interactions that are required for assembly of signalling proteins. The tyrosine kinase domain responsible for the phosphorylation of target proteins is flanked on the amino-terminal end (NH2 terminus) by the SH2 and SH3 domains (Holyoak, 2001).

Abl TK activity is strictly regulated under physiologic conditions. c-Abl is maintained in an auto-inhibited state by intra-molecular interactions involving the SH3 domain and a proline residue located in the linker region connecting the SH2 and TK (SH1) domains. Activation of c-Abl may occur through binding or phosphorylation events that disrupt the intra-molecular inhibitory interactions (Hantschel et al., 2003). Additional interactions that orient the SH3 and SH2 domains against the kinase domain play a regulatory role as well (Pendergast, 2002, Hantschel et al., 2003). However, the role of SH3 domain here seems to be critical. A deletion or positional alteration of this domain activates the kinase (Van Etten, 1999).

Several proteins binding to the SH3 domain have been identified that are supposed to control Abl activity (Cicchetti et al., 1992). The nuclear pool of c-Abl in quiescent cells in G1 phase of the cell cycle is bound with a complex involving the retinoblastoma protein (Rb), which inhibits the activity of Abl (Holyoak, 2001). In this complex, the C-terminal pocket of Rb binds to the ATP-binding lobe of the Abl kinase domain. Phosphorylation of Rb by cyclin-dependent kinases 4 and 6 between G1 and S results in release of c-Abl and activation of Abl kinase activity during S phase (Van Etten, 1999).

Several functions have been attributed to Abl. The Abl protein is involved in the regulation of the cell cycle, in the cellular response to genotoxic stress, and in transmission of cellular environment information through integrin signalling. The Abl protein proved to serve a complex role as a cellular integrator of various extracellular and intracellular signals that influences decisions in regard to cell cycle and apoptosis (Deininger et al., 2000). Several observations also suggest that mammalian c-Abl may also play a role in neuronal development (Van Etten, 1999).

The role of c-Abl depends on its location in the cell. The subcellular location of c-Abl was first determined in murine fibroblasts. It proved to be prevailingly nuclear, but with a significant fraction in the cytoplasm that was associated mostly with filamentous actin and the cell membrane. Such allocation of c-Abl has been confirmed in other cell types. However, in some tissues, such as haematopoietic cells and neurons, the cytoplasmic fraction prevails over the nuclear one (Van Etten, 1999).

c-Abl can move within the cell, transducing signals in response to physiological stimuli. It is presumed to have different functions in different cellular compartments. Localization of c-Abl to these different compartments is supposed to be a regulated process controlled by specific signals (Van Etten, 1999).

When over-expressed, c-Abl induces cell arrest, suggesting its probable role as a negative regulator of cell growth (Holyoak, 2001). Nevertheless, disruption of the c-*abl* gene in mice does not lead to uncontrolled cell growth. Mice homozygous for a null mutation in c-*abl* show undersized phenotype, lymphopoenia, shortened survival, have abnormal eyes, frequent rectal prolapse, and defective spermatogenesis. Some animals also have splenic and thymic atrophy, with a 10–30 fold decrease in the number of mature B and T lymphocytes. Many of the mutants die in perinatal period (Schang, 2005).

There is evidence that in S phase, c-Abl can participate in phosphorylation of the C-terminal domain of RNA polymerase II, which probably stimulates the transcription of S-phase genes. This suggests that c-Abl may also have a growth-promoting action during S phase (Van Etten, 1999).

In comparison with nuclear Abl, less is known about the function of its cytoplasmic fraction. A large portion of Abl in the cytoplasm is associated with the F-actin cytoskeleton through the C-terminal actin-binding domain. Abl is supposed to locally influence the cytoskeleton, while the kinase activity is dependent on cytoskeletal signals (Van Etten, 1999).

In addition, the data of Plattner et al. (2004) demonstrate that both c-Abl and Arg are regulated by phosphatidylinositol-4,5-bisphosphate, a lipid shown to modulate the function of a number of proteins involved in the regulation of the actin cytoskeleton such as profilin, vinculin, gelsolin, the Arp 2/3 complex, and the Wiskott-Aldrich syndrome protein *in vivo* and *in vitro*. The authors suggest that the binding of phosphatidylinositol-4,5-bisphosphate to the SH1 domains of c-Abl and Arg results in the inhibition of their TK activities. Besides, it has been proved that the Abl family kinases are part of a signalling complex involving PDGFRs as well as phospholipase C-γ1 (PLC-γ1), an enzyme that plays a crucial role in intracellular signal transduction pathways. Moreover, PDGFRs and Abl kinases undergo reciprocal phosphorylation (Plattner et al., 2004).

Bcr. The breakpoint cluster region protein (*bcr*) gene on chromosome 22 in human (in mouse – chromosome 12) includes 23 exons. The gene expression gives rise to two mRNAs, both of which are translated into a 160-kDa soluble oligomeric multidomain protein. Like Abl, Bcr is expressed ubiquitously. Nevertheless, its functions remain largely unclear (Deininger et al., 2000). In quiescent cells, the Bcr protein is localized in the cytoplasm, during mitosis it may be found in perichromosomal location, suggesting a possible role in cell cycle regulation. In Bcr knock-out mice haemopoiesis appears to be intact. The only abnormality in these mice seems to be an increased neutrophil respiratory burst resulting in an increased susceptibility to septic shock. A role of Bcr in signal transduction is a point at issue and its true biological relevance remains to be determined (Deininger et al., 2000).

Bcr-Abl. The *bcr-abl* gene usually arises as a result of breakpoints in introns 1 or 2 of the *abl* gene and in the MBCR region of the *bcr* gene, either between exons 13 and 14 or 14 and 15. These breakpoints produce *bcr-abl* fusion genes. A reverse rearrangement and the *abl-bcr* gene may also arise by the t(9; 22) translocation. However, no Abl-Bcr protein has been identified and the role of *abl-bcr* in pathogenesis of CML is unclear (Holyoak, 2001).

In contrast to Abl, Bcr-Abl is a deregulated, constitutively active enzyme. It has been found exclusively in the cytoplasm of cells, in complex with a number of cytoskeletal proteins. Two features underlie the ability of Bcr-Abl to induce leukaemia: increased tyrosine kinase activity and growth factor independence. As a result of the increased activity, Bcr-Abl activates several signal transduction pathways and promotes cell growth and differentiation (Holyoak, 2001).

Three major mechanisms of the malignant transformation have been described in cells carrying Bcr-Abl: altered adhesion to stroma cells and extracellular matrix, lastingly active mitogenic signalling, and reduced apoptosis (Deininger et al., 2000).

CML progenitor cells exhibit decreased adhesion to bone marrow stroma cells and extracellular matrix. Adhesion to stroma is known to negatively regulate cell proliferation, and CML cells escape this regulation because of their altered adhesion properties.

The artificial expression of Bcr-Abl in cytokine-dependent immortalized murine cell lines may induce growth factor-independent proliferation. Different pathways of mitogenic activation are supposed in CML cells: Ras and the MAP kinase, Jak-Stat, PI3 kinase and Myc pathways (Deininger et al., 2000). Under certain conditions, the increased proliferation has been associated with autocrine production of interleukin 3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Lugo and Witte, 1989). In primary CML cells or Bcr-Abl-transfected cells lines, many biological and biochemical changes have been detected that are similar to those which occur in normal cells treated with high concentrations of IL-3 (Holyoak, 2001).

The last mechanism: expression of Bcr-Abl in factor-dependent murine and human cell lines prevents apoptosis after growth-factor withdrawal, an effect that is critically dependent on TK activity (Daley and Baltimore, 1992)

Tight interactions between Abl and kit have also been proved. Bcr-Abl has been shown to up-regulate and activate c-kit (Hallek et al., 1996), which may play a considerable role in haematologic cancers. It has been shown that the sole Bcr-Abl inhibition is insufficient to eliminate all leukaemic cell populations. Wong et al. produced a mutant allele of *bcr-abl* (T315A) that can be inhibited by the small molecule 4-amino-1-tert-butyl-3-(1-naphthyl)pyrazolo[3,4-D]pyrimidine. Using this compound, they demonstrated that the suppression of Bcr-Abl activity was insufficient to eliminate both Bcr-Abl and kit expressing immature murine myeloid leukaemic cells. In contrast, imatinib mesylate, which is known to inhibit both PKs, effectively eliminated Bcr-Abl- and kit-expressing leukaemic cells (Wong et al., 2004).

CDKs

Cyclin-dependent kinases are a large family of serine-threonine protein kinases that usually phosphorylate a serine or threonine followed by proline. They are only active in complexes with regulatory subunits called cyclins (Schang, 2005). The human genome encodes 14 putative CDKs and 34 putative cyclins (Liu and Kipreos, 2000).

Like other kinases, CDKs phosphorylate target proteins. They are, in turn, activated through phosphorylation by other kinases and also through their association with cyclins (Blagden and de Bono, 2005). Hence, active CDKs are constituted by a catalytic subunit (the CDK itself) and a regulatory subunit (cyclin) (Schang, 2004).

Different CDKs are active at different times in the cell cycle (sequential activation and inactivation of cyclin-dependent kinases), which enables it to pass from one phase to another (Banchio et al., 2004). CDKs crucial for a particular step of the cell cycle undergo timely destruction after they have performed their function. This may occur either directly or through destruction of their partner cyclin, by the 26S proteasome. Proteins fated to destruction are ubiquitinated, i.e. numerous ubiquitin molecules covalently attach to them. This process is catalysed by the anaphase-promoting complex (Blagden and de Bono, 2005).

The levels of cyclins vary depending on the cell cycle phase. When cyclin levels rise, they associate with CDKs, forming stable active heterodimeric complexes. When cyclin levels fall, CDKs lose catalytic activity and become unable to phosphorylate their substrates (Blagden and de Bono, 2005). Each cyclin binds to a certain set of CDKs, and the resulting cyclin-CDK complexes typically display the highest kinase activity during a certain period in the cell cycle.

Not only periodic accumulation of cyclins regulates CDK activities. While the cyclin-CDK complexes regulate distinct downstream effector proteins by means of phosphorylation (Banchio et al., 2004), these complexes become activated themselves by phosphorylation at specific sites on the CDKs by CDK7/cyclin H, also referred to as CDK-activating kinase (CAK) (Kaldis et al., 1998).

A variety of other mechanisms are also important, such as degradation, nuclear localization, phosphorylation of CDKs (Banchio et al., 2004), and association with CDK endogenous inhibitors p15, p16, p21, p27 and p57 (Blagden and de Bono, 2005). Besides, there exist corresponding cell cycle inhibitory proteins (endogenous cyclin-dependent kinase inhibitors) that serve as negative regulators of the cell cycle and prevent the cell from entering the next phase of the cell cycle (Knight and Shokat, 2005).

Other regulatory mechanisms may be relevant, e.g. it has been shown that CDK1 is phosphorylated and maintained in the inactive state by the kinases Wee1 and Myt1, and dephosphorylated and activated by the phosphatase Cdc25 (Blagden and de Bono, 2005), an important regulator of normal cell division and the cell response to DNA damage.

Cyclin-dependent kinases are also indirectly inhibited by the kinases Chk1 and Chk2, which modulate the activity of the phosphatase Cdc25 mentioned above.

When activated, the CDKs drive the cell from one phase of the cell cycle to the next (G1 to S or G2 to M). If DNA damage occurs, the CDKs get inhibited and the cell undergoes cell-cycle arrest. This gives the cell an

opportunity to repair its own damaged DNA before it resumes the cell proliferation (Knight and Shokat, 2005).

CDKs 1, 2, 3, 4, 6 and 7 are involved in regulation of the cell cycle (Schang, 2005).

CDK1 in complex with cyclin B allows entry into mitosis (G2-M transition). In complex with cyclin A it is required for G1-S passage (Blagden and de Bono, 2005). It is fundamental for cell division in all eukaryotes studied so far.

CDK2 is activated by cyclins E and A and allows progression into and through S phase, resulting in the phosphorylation of retinoblastoma susceptibility protein (Rb), activation of the E2F family of transcription factors, and indirect promotion of DNA synthesis (Madhusudan and Ganesan, 2004). It phosphorylates the transcription factor SP1 serving for recognition of glycine-rich sequences present in many promoters, and thus takes part in the regulation of the S phase of the cell cycle (Banchio et al., 2004).

CDK3 in association with cyclin C is supposed to participate in the regulation of the G1-S transition and Rb-dependent G0/G1 transition (Ren and Rollins, 2004).

CDK4 and 6 are activated by the cyclin D and allow progression through G1, in response to growth factor stimulation (Sausville, 2002).

CDK7, 8 and 9 are involved in the regulation of transcription (Schang, 2005). These kinases phosphorylate RNA polymerase II (Sausville, 2002). Of these CDKs involved in transcription, CDK7 is activated by cyclin H and Mat3 and activates transcription initiation, CDK8 is activated by cyclin C and inhibits transcription initiation, and CDK9 is activated by cyclin T and activates transcription elongation (Schang, 2004).

CDKs 5 and 11 are involved in neuronal functions. CDK2, 5, 6 and 9 are also involved in cell differentiation and CDK1, 2, 4, 5, 6, and 11 also participate in apoptosis. CDKs likely participate in other cellular functions (Schang, 2005).

Deregulation of CDKs is supposed to underlie many pathological processes. CDK2 has been demonstrated to be over-expressed in colorectal cancer and lung cancer and CDK4 has been shown to be amplified in various types of sarcomas, and more recently in lung cancers. Over-expression of cyclins D and E or CDKs 4 and 6, as well as loss of endogenous CDK inhibitors (INK4 and KIP1), leads to loss of Rb function, negatively regulated by CDK activity. Inactivation of Rb protein, which is known to be an inhibitor of cell cycle progression and to control entry into G1, in turn leads to carcinogenesis (Blagden and de Bono, 2005).

A great role of CDKs in viral infection has been proved. Only few human pathogenic viruses encode their own protein kinases. Nevertheless, viral proteins must often be phosphorylated. Viruses therefore use cellular protein kinases to regulate many functions. This is exemplified by DNA viruses, which replicate in the nucleus and demand cellular CDKs for the replication (Schang, 2004).

Schang at al. (2004) studied CDK expression in neuronal and non-neuronal cells infected by Herpes simplex virus. The authors concluded that neuronal levels of CDK2 were among the factors that determined the outcome of HSV infections of neurons. In non-neuronal cells, CDK1, 2, or 7 were required for HSV replication.

MAPK and the three-kinase phospho-relay system

MAPKs are expressed ubiquitously. However, they regulate very specific biological responses, distinct in different cell types (Johnson et al., 2005). These kinases are activated by dual phosphorylation of threonine and tyrosine residues in the activation segment.

The MAPK superfamily belongs to serine-threonine kinases, it comprises MAPKs, MAPK kinases (MKKs) and MAPK kinase kinases (MKKKs), which constitute the three-kinase phospho-relay system. At least 11 MAPKs, seven MKKs and 20 MKKKs are known by now. MKKKs phosphorylate and activate MKKs, which in turn phosphorylate and activate MAPKs. In mammalian cells, five MAPK families are regulated by MKKK-MKK-MAPK phospho-relay systems: ERK1/2, JNK1/2/3, p38a/b/g/d, ERK5, and ERK7. There are also splice variants of several of the MAPKs, which further contribute to the complexity of the system (Johnson et al., 2005).

The MAPK family participates in signal transduction providing cellular response to various stimuli, including cytokines, antigens, toxins, pharmacological drugs, stress insults and cell-cell interactions. The MAP kinases play integral roles in the transduction of intracellular signals and have been extensively studied as drug targets for diseases, including inflammation and cancers (Kesari et al., 2005). It is the diversity of members of the three-kinase phospho-relay system and their protein-protein interactions that allow the integration of specific MAPK pathways in the cellular response (Johnson et al., 2005).

The subfamily of c-Jun N-terminal kinases (JNKs1, 2 and 3) belongs to the MAPK family. The JNKs are essential signal transmitters and regulators of physiological and pathological processes. They are known to be involved in several diseases including diabetes, atherosclerosis, stroke, Parkinson's and Alzheimer's diseases. JNKs are clinically relevant mediators of inflammatory diseases such as rheumatoid arthritis and Crohn's disease, acute ischemic damage following myocardial infarction and stroke (Waetzig and Herdegen, 2005). JNK1 is an important mediator of insulin resistance associated with obesity (Hirosumi et al., 2002), but it is also indispensable for the intact cytoarchitecture of the brain (Chang et al., 2003). JNK2 is recruited by apoptotic stimuli (Coffey et al., 2002),

but it is also important for the coordinated differentiation of activated immune cells (Yang et al., 1998) and for brain development (Kuan et al., 1999).

Other pharmaceutically relevant proteins of the MAPK family include p38, ERK1, ERK2 (Kesari et al., 2005).

EGFR

Epidermal growth factor receptor (EGFR) represents a receptor TK superfamily of four receptors (c-ERBB1 or EGFR, c-ERBB2 or HER2, c-ERBB3 and c-ERBB4) and is a member of the type I TK family (Raizer et al., 2005). All four members of the EGF superfamily are close homologues and share common structural features, such as an extracellular ligand-binding domain and a cytoplasmic TK domain (Traxler et al., 2001).

The EGFR transmembrane glycoproteins have three domains. There are the ligand-binding extracellular domain, a lipophilic transmembrane domain, and a signal-transducing tyrosine TK domain located within the cell (except for c-ERBB3). The EGFR kinase family is structurally unique in that it has a cysteine residue in the ATP-binding pocket (Kesari et al., 2005).

EGFR has several ligands, while the most important natural ligands of EGFR are EGF and transforming growth factor-α (TGF-α) (Raizer et al., 2005).

The autophosphorylation of EGFR, which follows after homo- or hetero-dimerization, leads to intracellular responses affecting cell survival, angiogenesis, cell proliferation, cell differentiation (Barlesi et al, 2005), and inhibition of programmed cell death (Ready, 2005).

The 3-D structure for the EGFR kinase domain showed that the activation loop took on an open conformation similar to other phosphorylated RTKs such as the insulin receptor kinase (Stamos et al., 2002).

Signal transduction from activated EGFR is mediated in part by the Ras/Raf/MAPK pathway (de Bono et al., 2003). Binding of a ligand induces receptor dimerization, followed by internalization of the receptor-ligand complex that subsequently leads to the activation of the TK signal transduction. The EGFR TK activates ERK1/2 using a Ras-dependent mechanism. c-Raf1 and B-Raf are both activated by the Ras-GTP complex and both activate the ERK1/2 pathway. Ras-dependent c-Raf1 and B-Raf activation of the ERK1/2 pathway is involved in regulating cell proliferation and survival (Morrison, 2004).

Signal transduction from activated EGFR is mediated by signalling through the PI3K/Akt pathway as well (de Bono et al., 2003).

Expression of EGFR has been related with various cellular processes involved in carcinogenesis such as cell proliferation, inhibition of apoptosis, angiogenesis, cell motility, and metastasis. Over-expression of EGFR has been associated with progression of disease, poor response to therapy, chemotherapy resistance (Vallbohmer and Lenz, 2005), and finally with poor prognosis (Mendoza et al., 2005).

EGFR is over-expressed, mutated, or both in many solid tumours (Dewji, 2004). Expression of EGFR has been found in several tumour types, including 77% of cases of colorectal cancer (Vallbohmer and Lenz, 2005), 40-80% of non-small-cell lung cancer (NSCLC) cells (Ready, 2005), and 40-50% glioblastoma multiforme (Raizer et al., 2005). Small deletions and point mutations in the kinase domain of EGFR in a subset of non-small-cell lung cancers increase the sensitivity of the receptor to its ligand and alter receptor signalling (Lynch et al., 2004).

There are different mechanisms of activation of EGFR in malignancies. Approximately 40% of the tumours with EGFR amplification have gene rearrangements resulting in a constitutively active mutant (EGFR vIII), e.g. in glioblastomas. Primary glioblastomas, which arise *de novo*, often have loss of phosphatase and tensin homologue deleted on chromosome 10, along with amplification and over-expression of EGFR (Kesari et al., 2005).

In non-small-cell lung cancer, EGFR mutations do not cause constitutive activation, rather, they enhance the responsiveness of the receptor to EGF ligand (Sordella et al., 2004). In any case, all these mechanisms in malignancies lead to increased cell proliferation, angiogenesis, and inhibit programmed cell death (Ready, 2005).

Laboratory studies have shown that anti-EGFR agents are able to inhibit this EGFR signalling and additionally potentiate the effectiveness of traditional anti-cancer therapy. Thus, the EGFR is an attractive therapeutic target (Mendoza et al., 2005).

Some malignant cells may exhibit EGFR inhibitor resistance. For example, some recurrent non-small lung cancers have a common secondary mutation in the EGFR kinase domain, providing drug resistance, but in other cases the mechanism underlying acquired resistance is unknown. Kwak and collaborators (2005) detected this mutation (T790M) in only a small proportion of tumour cells. In the other drug-resistant cells they found dependence on EGFR and ERBB2 signalling for their viability. These cells did not show secondary EGFR mutations, but displayed increased internalization of ligand-activated EGFR, consistent with altered receptor trafficking.

JAKs

The Janus kinases (JAKs) are non-receptor tyrosine kinases that are the most proximal elements in a signal transduction cascade transmitting signals from the cell surface to the nucleus after cytokine binding (Leonard and O'Shea, 1998).

Four JAKs (JAK1, JAK2, JAK3 and Tyk2) have been described. Whereas three JAKs (JAK1, JAK2,

Tyk2) are used by a variety of cytokine receptors and are largely expressed in haematopoietic cells, JAK3 is used only by cytokines that contain a glycine in their receptor and is expressed at high levels only in NK cells and thymocytes (Leonard and O'Shea, 1998). This tyrosine kinase is crucial for mediating signals from the common γ-chain of cytokine receptors and its expression is limited to cells that actively participate in the immune response to allografts (Borie et al., 2004).

Furthermore, JAK3 is required to maintain T-cell function, because the loss of JAK3 protein in peripheral T cells leads to the JAK3K/K phenotype with a notable decrease in peripheral CD8+ T cells, whose available pool appears anergic (Thomis and Berg, 1997). JAK3 is the protein TK the deficiency of which results in severe combined immune deficiency (SCID) (Leonard, 1996). Although B cells are present in normal or even elevated numbers in T-B+NK- SCID, they are poorly functional (Borie et al., 2004).

The JAKs are activated within the JAK/STAT pathway following ligand binding to a receptor and initiate signalling by phosphorylating cytokine receptors that *per se* have no kinase activity. This phosphorylation creates docking sites on the receptors for molecules known as STATs (signal transducers and activators of transcription), which are subsequently phosphorylated. It is a precondition for STAT dimerization, transport to the nucleus, DNA binding and subsequent transcriptional activity (Borie et al., 2004).

Non-haematopoietic expression of JAK3 has also been reported, but its functional significance is not yet clear (Borie et al., 2004).

JAKs have been shown to be implicated in pathological processes. Several authors reported a clonal somatic mutation in the pseudo-kinase domain of the JAK2 protein, substituting phenylalanine at position 617 with valine (V617F). This mutation causes constitutive activation of the JAK/STAT pathway in polycythemia vera patients (Kralovics et al., 2005). V617F has been found in the majority of such patients. It has also been described in some patients with essential thrombocytosis and idiopathic myelofibrosis (Krause and Van Etten, 2005).

ROCKs (Rho kinases)

ROCK is a serine-threonine kinase that formerly was identified as a RhoGTP-binding protein. Two isoforms encoded by two different genes of ROCK have been described: ROCKI (also known as ROK-β) and ROCKII (which is also known as ROK-α).

The two ROCK isoforms have similar protein sequences but different tissue distribution, which indicates distinct functions of each isoform *in vivo*. While ROCKII is preferentially expressed in the brain (mainly observed in the pyramidal neurons of the hippocampus, cerebral cortex and in the Purkinje cells of the cerebellum), ROCKI is mostly found in non-neuronal tissues, such as heart, lung, and skeletal muscles (Mueller et al., 2005).

Activation of ROCK by GTP-bound Rho or by the special lipid mediators leads to phosphorylation of a number of target proteins. One of the main substrates of ROCK is myosin light chain (MLC). In fact, ROCKs were initially characterized according to their roles in cytoskeletal rearrangements through their effects on MLC (Leung et al., 1996). Phosphorylation of MLC results in stimulation of myosin-actin interactions. Furthermore, ROCK can indirectly regulate the amount of phosphorylated MLC by inactivating MLC phosphatase (MLCP) (Kimura et al., 1996). Observations indicate that the inactivation of MLCP contributes to the elevation of MLC phosphorylation more than the direct phosphorylation of MLC itself.

Other downstream targets of ROCKs include the Ser/Thr LIM kinases 1 and 2 (LIMK1 and LIMK2) (Sumi et al., 2001). Phosphorylation of LIMKs by ROCKs inhibits cofilin-mediated actin-filament disassembly, which leads to an increase in the number of actin filaments. In addition to LIMK, ROCK phosphorylates the ezrin/radixin/moesin (ERM) protein complex and other proteins involved in cytoskeletal regulation.

Further substrates of ROCK are intermediate filament proteins such as vimentin, glial fibrillary acidic protein (GFAP), neurofilaments, as well as microtubule-associated protein 2 (MAP2) and tau (Amano et al., 2003). In addition, ROCKII phosphorylates collapsin response mediator protein 2 (CRMP2) (Arimura et al., 2000), a neuronal protein playing an important role in axon guidance during development of the nervous system. ROCKII therefore seems to be an integration point for signalling pathways regulating actin-myosin contractility (Mueller et al., 2005).

ROCKI and ROCKII knock-out mice have different phenotypes, but have also some common features. While ROCKI–/– mice cannot close their eyelids and have omphalocele, ROCKII loss results in prenatal growth retardation and the defect in the placenta-embryo interaction resulting in increased foetal death. If the perinatal period is survived, ROCKII knock-out mice develop without deviations and are fertile. They seem to have normal anatomy and physiology (Thumkeo et al., 2003).

ROCKs have been found to be activated in atherosclerosis, restenosis, hypertension, pulmonary hypertension, and cardiac hypertrophy (Loirand et al., 2006). Besides, they were shown to contribute to glioma and NSCLC pathogenesis (Rattan et al., 2005).

PKC

The protein kinase C (PKC) family of serine-threonine protein kinases participates in signal-transduction

pathways mediating the effects of many extracellular stimuli including growth factors, hormones, and drugs and promotes lipid hydrolysis. PKC is an important signalling molecule in the VEGF pathway. It is therefore regarded as an attractive therapeutic target (Jenny et al., 2005).

At least 11 related protein kinases belong to the PKC family. They can be divided into three subgroups based on their structural and biochemical properties. The novel PKC subgroup including PKCθ, PKCδ, PKCε, PKCη, and PKCμ represents a particular practical interest (Villalba et al., 2001).

Of the novel subgroup, protein kinase C θ is the most investigated. PKCθ is a serine-threonine kinase that has been found to be up-regulated in GISTs (Griffin et al., 2003). PKCθ is also the PKC family member that is selectively expressed in the interstitial cell of Cajal lineage (Poole et al., 2004). In T lymphocytes, PKCθ is a key signalling molecule in T-cell receptor activation pathways, serving as a positive regulator of cell survival (Villalba et al., 2001).

PKCθ inhibition results in p53-independent cell cycle arrest in various cell types. These findings suggest that the protein kinase expression may be relevant diagnostically and therapeutically in GISTs (Villalba and Altman, 2002). Duensing at al. reported that PKCθ protein is largely expressed, constitutively phosphorylated and enzymatically active in GISTs, which does not depend on kit or PDGFR expression and mutational status. By contrast, PKCθ protein expression is not detectable, or is very low, in tumours that represent histopathological mimics of GISTs. These findings demonstrated that PKCθ was a diagnostic marker and provided therapeutic promise for GIST. PKCθ may therefore serve, along with kit and PDGFRA, as a therapeutic target (Corless et al., 2005). Some lines of evidence indicate that kit and PDGFR signalling may be relevant for PKCθ activation in GISTs (Duensing et al., 2004).

Thus, PKCs represent an attractive target for therapy. Jenny et al. (2005) described a model in which atypical PKC isotypes play a fundamental role in balancing cell proliferation and programmed cell death, cellular architecture and cell polarity.

VEGFR

The vascular endothelial growth factor (VEGF) is the only mitogen that specifically acts on endothelial cells through interactions with their cell surface receptors, VEGFR1, 2, 3 (Flt1, Flk-1, Flt4, respectively) (Mendoza et al., 2005), located on vascular endothelium. VEGFRs have an intracellular TK activity. Together these interactions constitute a well-characterized positive regulatory pathway for angiogenesis.

Solid tumours need vessels supplying them with blood for their growth beyond a certain size. They establish a blood supply by inducing formation of new vessels branching from the existing host capillaries. VEGF is known to play a key role in this angiogenic process. It has been shown to be secreted by tumour cells and macrophages.

Inhibition of VEGF-induced angiogenic signals is expected to target the tumour-associated vessels selectively. Anti-angiogenic therapy through inhibition of the VEGF-mediated effects might be safe and well tolerated in cancer patients (Breier and Risau, 1996). VEGF is a major target for anti-angiogenic therapy because its over-expression has been associated with vascularity, poor prognosis, and aggressive disease in most malignancies (Mendoza et al., 2005). Studies of the signalling pathways of VEGF receptors, integrins, and cadherins have provided new anti-angiogenic strategies for inhibition of tumour growth and inhibition of VEGF signals that stimulate lymphangiogenesis. This approach seems to be effective in inhibition of lymphatic metastasis in mice (Alitalo and Carmeliet, 2002).

Polo-like kinases

Polo-like kinases belong to the family of serine-threonine kinases. Humans have four Plk members: Plk1, Plk2, Plk3, and Plk4 (McInnes et al., 2005). All Polo-like kinases comprise an N-terminal Ser/Thr kinase catalytic domain and a C-terminal region that contains a serine-threonine-binding domain known as the Polo-box domain (Lowery et al., 2005). Their activity is controlled by phosphorylation and they are inactivated by ubiquitination (Blagden and de Bono, 2005).

Plk-1 is the most investigated member of the family. Many studies have focused on Plk-1. Most functions attributed to the Plk family have been studied on it. PLK-1 is known to play crucial roles in meiotic and mitotic cell division. Less is known about the activities of Plk-2, 3 and 4, although there is likely to be considerable functional overlap between the three members of the polo-like kinases. Most recent studies suggest that normally Plk-2 and Plk-4 are required for centriole duplication at the G1-S stage of the cell cycle (Habedanck et al., 2005), and that Plk-3 is required for cytokinesis (Conn et al., 2000).

Plk-1 up-regulation has been detected in many cancers, especially those with high mitotic activity such as head and neck cancer, ovarian cancer, endometrial cancer, non-small-cell lung cancer, glioma, breast cancer, melanoma, and colorectal cancer. In many cases Plk-1 expression levels seem to correlate with tumour aggressiveness and prognosis. Cultured cells can be malignantly transformed by Plk-1 over-expression and induce tumours in a mouse model. Plk-1 inhibition can stop cancer cell growth. There are indications that Plk-3 may also be an important factor in cancer development. It has been demonstrated to be down-regulated in rat azoxymethane-induced colon carcinogenesis specimens (Blagden and de Bono, 2005).

Aurora

The Aurora kinases were first described in the fruit fly. Later homologues have been identified in organisms throughout evolution. There are three members in humans, named Aurora A, B and C. The members of the Aurora family are serine-threonine kinases. The Aurora family is believed to control important cellular events (Katayama et al., 2003), such as progression through the mitotic phase of the cell cycle, checkpoint activation, glucose metabolism, cell survival, and protein translation (Cheetham, 2004).

Aurora A is present on centrosomes/spindle poles. It is required for centrosome separation and spindle formation at the onset of mitosis. Aurora B is a chromosomal passenger protein required for chromosome condensation, chromosome segregation, and cytokinesis. The role of Aurora C is not quite clear, but it has been shown to be present on centrosomes from anaphase until cytokinesis (Blagden and de Bono, 2005).

Auroras are suspected to play a part in various pathological processes. Over-expression of both Aurora A and B has been demonstrated in breast and colon cancers, while gene amplification of Aurora A has been observed in bladder, gastric and colorectal cancers (Bischoff et al., 1998). Nevertheless, Aurora A is in the main focus of attention of the three Auroras. Some studies have linked its over-expression to malignant transformation of cell lines (Zhou et al., 1998), it is now suspected to support transformed cell growth rather than to directly cause it (Tatsuka et al., 2005).

The Auroras are currently under intense investigation as therapeutic targets.

Other protein kinases

Src. The Src protein was the first TK discovered. Src family kinases (SFKs) are important regulators of ligand-induced cellular responses including proliferation, survival, adhesion and migration. SFKs are expressed ubiquitously, but different members of the family persist in different cell types. These enzymes belong to non-receptor TKs. Structurally SFKs have SH1 (kinase), SH2, SH3 domains and resemble Abl. The downstream signalling mechanisms of Src include the activation of STAT3, PDK1 and Akt, which shows their oncogenic potential and the importance of Src family kinases (Warmuth et al., 2003).

Src inhibition is attractive because Src activation may play a role in the development and progression of many tumours. Src kinase modulates signal transduction through multiple oncogenic pathways including PDGFR, VEGFR and others (Lombardo et al., 2004).

Flt-3. FMS-like tyrosine kinase-3 (Flt-3 also known as foetal liver kinase-2 (Flk-2)) is the transmembrane TK, a member of the type III receptor TK subfamily. The Flt-3 is expressed predominantly on primitive haematopoietic progenitors, CD34+ cells lacking the lineage-specific markers. Flt-3 signalling is known to be important in the early stages of haematopoiesis. Expression of Flt-3 differs within the stem cell pool. It has been found on cells with the long- and short-term marrow-reconstituting activity. Mice with targeted disruption of Flt-3 have specific deficiency in B lymphoid development as well as deficiency in T-cell and myeloid reconstitution by mutant stem cells (Mackarehtschian et al., 1995).

Flt-3 is expressed on blasts in most cases of acute myeloid leukaemia. It is activated by duplications within the juxtamembrane domain or point mutations at D835. The inhibition of Flt-3 leads to growth arrest and induces apoptosis in haematopoietic cell lines expressing activated Flt-3. It has therapeutic efficacy in murine models of Flt3-induced leukaemia (Krause and Van Etten, 2005).

BRAF. BRAF is a member of the RAS/RAF/MEK /ERK /MAP kinase pathway, playing an important role in intracellular signal transduction. It has been shown to play a significant part in development of melanoma (Kondapalli et al., 2005). BRAF mutation V600E has been detected in many cases of papillary thyroid carcinoma. However, correlation between BRAF mutation and aggressive clinical behaviour has not been found (Moretti et al., 2006).

PDK-1. Phosphoinositide-dependent protein kinase-1 is an activator of protein kinase B (PKB), which in turn plays a role in the phosphorylation of various downstream effectors such as NF-κB, mTOR, Forkhead, Bad, GSK-3 and MDM-2. PDK-1 is also a key effector in pathways that are triggered by the stimulation of RPTKs such as IGF-1R, HER2/Neu, VEGFR, PDGFR, and PI3K. Thus, PDK-1 has oblique effects on cell growth, proliferation, protection from pro-apoptotic stimuli and stimulation of neo-angiogenesis. It is highly expressed in some tumour types (Mora et al., 2004). All this makes PDK-1 a highly attractive therapeutic target (Cheetham, 2004).

Bub1, BubR1, Mps1. Bub1, BubR1, and Mps1 are serine-threonine kinases involved in the spindle assembly checkpoint, which prevents anaphase onset until chromosomes have correct alignment and tension along the metaphase plate. The kinases Bub1, BubR1, and Mps1 are recruited, along with other proteins, to the kinetochores (Lew and Burke, 2003). The substrates for each of these kinases are still unknown. Mutations to Bub1 and Bub1b (encoding Bub1 and BubR1, respectively) have been identified in certain colorectal cancer cell lines. However, the checkpoint has been shown to be intact in these cell lines (Tighe et al., 2001) and the clinical relevance of these findings is still unclear. Mps1 is required for spindle checkpoint regulation, as well as centrosome duplication, normal mitotic progression, and cytokinesis (Fisk et al., 2004). It is not yet known whether expression levels of Mps1 are altered in cancer cells (Blagden and de Bono, 2005).

Ret. Activating point mutations and fusions of the receptor TK Ret occur in different endocrine neoplasia, e.g. radiation-associated thyroid cancer or hereditary medullary thyroid cancers, which have been shown to have an autosomal dominant pattern of inheritance and to be caused by activating point mutations in the *ret* proto-oncogene (Ogilvie and Kebebew, 2006). Besides, the *ret* proto-oncogene is the major gene involved in the pathogenesis of Hirschsprung disease, a complex genetic disease characterized by lack of ganglia along variable lengths of the gut (Lantieri et al., 2006).

ITK. Inducible T-cell kinase is a member of the Tec family of non-receptor tyrosine kinases and plays an important role in T-cell signalling. ITK is expressed in T-cells, NK cells and mast cells, and appears to be essential for T-cell activation. It plays a role in signalling downstream of the T-cell receptor (Cheetham, 2004). Selective inhibition of ITK may be an important way of modulating many diseases that involve inappropriate activation of the immune system (Uckun and Mao, 2004).

Nek. The human Nek (NIMA-related kinases) family of serine-threonine kinases has evolved from a common ancestor gene with never in mitosis gene A (NIMA). This means that these genes are orthologous. Eleven members belong to the human Nek family. The function of Nek is not yet quite clear. It is believed to be required for mitotic progression, possibly by controlling G2-M transition (Blagden and de Bono, 2005). Only separate members have been investigated. For example, human Nek2 is a centrosome-associated protein kinase. It is required for centriole splitting during the cell cycle. Nek6, Nek7 and Nek9 also seem to regulate mitosis. Nek8 has been found to be overexpressed in multiple breast tumour samples, indicating a role of this kinase in breast cancer progression (Bowers and Boylan, 2004). Nek8 has also been linked with polycystic kidney disease alone with Nek1.

Checkpoint kinases. There are two checkpoint kinases, Chk1 and Chk2. Both Chk1 and Chk2 are effector kinases, activated upon phosphorylation by ATM and ATR, the DNA damage response serine-threonine kinases. If DNA is damaged, Chk1 and 2 mediate a G2/M cell cycle arrest by phosphorylating Cdc25. After the phosphorylation, Cdc25 is not able to activate the mitotic entry kinase, Cdk1 (Chen and Sanchez, 2004). Chk1 and 2 are activated by different forms of DNA damage. It is unclear whether or not their functions overlap. By arresting the cell at G2/M, time is allowed for DNA repair to take place. After that the cell cycle can resume. Chk1 protein and mRNA levels have shown to be down-regulated in aggressive non-Hodgkin's lymphoma (Tort et al., 2005).

Rhe. A new fusion kinase has been discovered by Griffin et al. (2003). The authors described a gene rearrangement in the eosinophilic cell line EOL-1 that resulted in the expression of a yet unknown fusion protein. It was constituted by an N-terminal region encoded by a gene of unknown function and a C-terminal region coming from the intracellular domain of PDGFR-α. Griffin et al. proposed the name Rhe for this new kinase originating from „rearranged in hypereosinophilia".

Other fusion PKs. Fusion of the fibroblast growth factor receptor 1 (FGFR1) TK with one of several partners, such as ZNF198, BCR (Roumiantsev et al., 2004), TIF1 (Belloni et al., 2005) and others, occurs in the 8p11 myeloproliferative syndrome, also known as stem cell leukaemia-lymphoma syndrome (SCLL).

Translocations involving the anaplastic lymphoma kinase (*ALK*) gene have been found in anaplastic large-cell lymphoma (ALCL) and generate fusion of the ALK receptor TK with several partners. In up to 50 % of patients with ALCL, NPM-ALK fusion protein has been detected (Gu et al., 2004).

Fusion of JAK2 with Bcr has been discovered in cases of acute leukaemia and atypical CML (Krause and Van Etten, 2005).

The receptor TKs Ros, ALK, NTRK1, and NTRK3 are activated through the generation of fusion proteins in a number of carcinomas and sarcomas. ALK and NTRK3 fusion proteins are also found in haematologic cancers. The type of disorder depends on the tissue of expression, e.g. TEL-NTRK3 fusion triggers malignant transformation in congenital fibrosarcoma, but it induces haematologic cancers when expressed in bone marrow (Krause and Van Etten, 2005).

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