Receptor tyrosine kinase signalling as a target for cancer intervention strategies

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Abstract

In multicellular organisms, communication between individual cells is essential for the regulation and coordination of complex cellular processes such as growth, differentiation, migration and apoptosis. The plethora of signal transduction networks mediating these biological processes is regulated in part by polypeptide growth factors that can generate signals by activating cell surface receptors either in paracrine or autocrine manner. The primary mediators of such physiological cell responses are receptor tyrosine kinases (RTKs) that couple ligand binding to downstream signalling cascades and gene transcription. Investigations over the past 18 years have revealed that RTKs are not only key regulators of normal cellular processes but are also critically involved in the development and progression of human cancers. Therefore, signalling pathways controlled by tyrosine kinases offer unique opportunities for pharmacological intervention. The aim of this review is to give a broad overview of RTK signalling involved in tumorigenesis and the possibility of target-selective intervention for anti-cancer therapy.

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Introduction

On the basis of their structural characteristics, receptor tyrosine kinases (RTKs) can be divided into 20 subfamilies which share a homologous domain that specifies the catalytic tyrosine kinase function (van der Geer et al. 1994). All RTKs consist of a single transmembrane domain that separates the intracellular tyrosine kinase region from the extracellular portion (Ullrich & Schlessinger 1990). The latter exhibit a variety of conserved elements including immunoglobulin (Ig)-like or epidermal growth factor (EGF)-like domains, fibronectin type III repeats or cysteine-rich regions that are characteristic for each subfamily of RTKs.

The catalytic domain that displays the highest level of conservation includes the ATP-binding site that catalyses receptor autophosphorylation and tyrosine phosphorylation of RTK substrates (Yarden & Ullrich 1988). Ligand binding to the extracellular domain leads to conformational changes that induce and stabilize receptor dimerization leading to increased kinase activity and autophosphorylation of tyrosine residues (Greenfield et al. 1989, Ullrich & Schlessinger 1990, Heldin 1995). Phosphorylation of distinct tyrosine residues of the activated receptor creates binding sites for Src homology 2 (SH2)- and phosphotyrosine binding (PTB) domain-containing proteins. Molecules recruited via these binding motifs are either enzymes that are tyrosine phosphorylated and activated, such as Src and phospholipase Cγ, or adaptor molecules that link RTK activation to downstream signalling pathways (Pawson 1995). One important adaptor protein complex is the SHC–Grb2–Sos complex that couples RTKs via Ras to the extracellular regulated kinase (ERK)/mitogen-activated protein (MAP) kinase pathway which is crucial for RTK-induced cell proliferation (Dhanasekaran & Reddy 1998) (Fig. 1).

Deregulation of RTKs

Since the first connection between a viral oncogene, a mutated RTK and human cancer was made in 1984 (Ullrich et al. 1984), it is well known that aberrant signalling by RTKs is critically involved in human cancer and other hyper-proliferative diseases. Constitutive activation of RTKs, which has been shown to be important for malignant transformation and tumour proliferation, can occur by several mechanisms (Kolibaba & Druker 1997). In most cases gene amplification, overexpression or mutations are responsible for the acquired transforming potential of oncogenic RTKs. Somatic and germline mutations, which are associated with distinct inherited and spontaneous human cancer syndromes, have been observed in at least ten different RTK families (Robertson et al. 2000). These alterations include deletion or...
mutation within the extracellular region or alterations of the catalytic domain, especially of the ATP-binding motif, which all result in a constitutive active RTK. In addition, mutations within the transmembrane domain have been shown to lead to ligand-independent kinase activation as reported for the RTK HER2/neu.

Activation of autocrine growth factor loops is another mechanism of aberrant RTK signalling and has been frequently described for the epidermal growth factor receptor (EGFR) and insulin-like growth factor-I receptor (IGF-IR) family (Derynck et al. 1987, Kaleko et al. 1990). This potent mechanism of activation occurs when a RTK is aberrantly expressed or overexpressed in the presence of its cognate ligand, or when overexpression of the ligand occurs in the presence of its associated receptor. In many solid tumours it has been shown that elevated levels of both growth factor receptor and its ligand are expressed concomitantly (Salomon et al. 1995).

**Intervention strategies**

Increasing knowledge of the structure and activation mechanism of RTKs and the signalling pathways controlled by tyrosine kinases provided the possibility for the
development of target-specific drugs and new anti-cancer therapies (Plowman et al. 1994). Approaches towards the prevention or interception of deregulated RTK signalling include the development of selective components that target either the extracellular ligand-binding domain or the intracellular tyrosine kinase or substrate binding region (Fig. 1).

The most successful strategy to selectively kill tumour cells is the use of monoclonal antibodies (mAbs) that are directed against the extracellular domain of RTKs which are critically involved in cancer and are expressed at the surface of tumour cells (Fan & Mendelsohn 1998). In the past years, recombinant antibody technology has made an enormous progress in the design, selection and production of new engineered antibodies and it is possible to generate humanized antibodies, human–mouse chimeric or bispecific antibodies for targeted cancer therapy (Farah et al. 1998, Hudson 1999). Mechanistically, anti-RTK mAbs might work by blocking the ligand–receptor interaction and therefore inhibiting ligand-induced RTK signalling and increasing RTK downregulation and internalization. In addition, by binding of mAbs to certain epitopes on the cancer cells they induce immune-mediated responses such as opsonization and complement-mediated lysis and trigger antibody-dependent cellular cytotoxicity by macrophages or natural killer cells. In recent years, it became evident that mAbs control tumour growth by altering the intracellular signalling pattern inside the targeted tumour cell, leading to growth inhibition and/or apoptosis (Cragg et al. 1999). In contrast, bispecific antibodies can bridge selected surface molecules on a target cell with receptors on an effector cell triggering cytotoxic responses against the target cell that is thus killed (Segal et al. 1999). Despite the toxicity that has been seen in clinical trials of bispecific antibodies, advances in antibody engineering, characterization of tumour antigens and immunology might help to produce rationally designed bispecific antibodies for anti-cancer therapy.

Another promising approach to inhibit aberrant RTK signalling are small molecule drugs that selectively interfere with the intrinsic tyrosine kinase activity and thereby block receptor autophosphorylation and activation of downstream signal transducers (Levitzki 1999). The tyrphostins, which belong to the quinazolines, are one important group of such inhibitors that compete with ATP for the ATP binding site at the receptor’s tyrosine kinase domain and some members have been shown to specifically inhibit the EGFR (Levitzki & Gazit 1995). But also potent and selective inhibitors of receptors involved in neovascularization have been developed and are now undergoing clinical evaluation. Using the advantages of structure-based drug design, crystallographic structure information, combinatorial chemistry and high-throughput screening new structural classes of tyrosine kinase inhibitors (TKIs) with increased potency and selectivity, higher in vitro and in vivo efficacy and decreased toxicity have emerged (Klohs et al. 1997, Al-Obeidi & Lam 2000).

Recombinant immunotoxins provide another possibility of target-selective drug design. They are composed of a bacterial or plant toxin either fused or chemically conjugated to a specific ligand such as the variable domains of the heavy and light chains of mAbs or to a growth factor (Kreitman 1999). Immunotoxins either contain the bacterial toxins Pseudomonas exotoxin A or diphtheria toxin or the plant toxins ricin A or clavin. These recombinant molecules can selectively kill their target cells when internalized after binding to specific cell surface receptors (Frankel et al. 2000).

The use of antisense oligonucleotides represents another strategy to inhibit the activation of RTKs. Antisense oligonucleotides are short pieces of synthetic DNA or RNA that are designed to interact with the mRNA to block the transcription and thus the expression of specific-target proteins (Marcusson et al. 1999). These compounds interact with the mRNA by Watson–Crick base-pairing and are therefore highly specific for the target protein. On the other hand bioavailability may be poor since oligonucleotides can be degraded upon internalization by cellular endo- and exonucleases. Nevertheless, several preclinical and clinical studies suggest that antisense therapy might be therapeutically useful for the treatment of solid tumours (Pawlak et al. 2000).

**Epidermal growth factor receptor (EGFR) family**

The epidermal growth factor receptor (EGFR) family of RTKs comprises four members: the archetypal EGFR/ErbB1, which was the first RTK to be molecularly cloned (Ullrich et al. 1984), HER2/ErbB2, HER3/ErbB3 and HER4/ErbB4. The four receptors share two extracellular cysteine-rich domains and an intracellular portion with a long C-terminal tail carrying most of the autophosphorylation sites. Receptors of the EGFR family are frequently co-expressed in various combinations and depending on the activating ligand they can form various homo- or heterodimers generating a complex signal transduction network (Alroy & Yarden 1997, Riese & Stern 1998). EGFR family members are activated by a large group of EGF-related growth, which all contain a conserved EGF-like domain and are synthesized as transmembrane precursor proteins (Heldin 1996). These include EGF, transforming growth factor-α (TGFα), epiregulin, betacellulin, heparin-binding EGF-like growth factor, amphiregulin and the large family of alternatively-spliced neuregulins (Burden & Yarden 1997). These growth factors bind with different specificities and...
affinities to EGFR, HER3 and HER4, while no ligand for HER2 has yet been identified.

HER2

The RTK HER2 was originally identified as both the transforming gene in a chemically transformed rat neuroblastoma cell line (Schechter et al. 1984) and as an EGFR-related cDNA clone (Coussens et al. 1985), and has been most frequently implicated in human neoplasias and cancer (King et al. 1985, Slamon et al. 1987, Hynes & Stern 1994).

Despite the fact that no ligand is known to bind with high affinity to HER2, it is well known that HER2 acts as a co-receptor for the other EGFR family members. In particular, either the EGFR ligands or the neuregulins that bind HER3 or HER4 are able to induce heterodimer formation between a high-affinity ligand binding receptor and HER2. These heteromolecular interactions are of pathophysiological relevance, because such receptor combinations show strong mitogenic signalling and tumorigenicity. Signalling pathways involving Ras, Src, PI3-kinase and the MAP kinases ERK and JNK have been shown to be activated by HER2-expressing cell lines and seem to be important for tumour management (Zwick et al. 2000).

HER2 gene mutation and high level overexpression are other potent mechanisms resulting in HER2 activation. A single point mutation at position 664 within the transmembrane region of HER2, encoding a change from the hydrophobic amino acid valine to the negatively charged glutamic acid residue, renders the receptor constitutively active (Bargmann et al. 1986, Weiner et al. 1989). Although the mutation is not observed in human tumours, a polymorphism at codon 655, which results in a valine to isoleucine substitution, has been identified in the transmembrane coding region of the HER2 gene (Papewalis et al. 1991). Performing a large-scale population-based, case-control study in China, an association of the genetic HER2 polymorphism and an increased risk of breast cancer, particularly among younger women, was recently identified (Xie et al. 2000).

Overexpression and/or gene amplification of HER2 was found in various types of human cancer and especially in human breast carcinomas where HER2 gene amplification has been identified with a frequency of 30% (Slamon et al. 1987, 1989). Subsequently, it became evident that aberrantly elevated levels of HER2, either with or without gene amplification, correlated with a more aggressive progression of disease and a reduced patient survival time (Paik et al. 1990).

The mechanism underlying the oncogenic potential of an overexpressed HER2 may relate to the increased availability for heterodimer formation and thus for high level autophosphorylation and constitutive signalling (Lonardo et al. 1990, Brennan et al. 2000). It was shown that overexpression of HER2 led to a prolonged and enhanced cellular signalling through the MAP kinase pathway (Karunagaran et al. 1996, Harari & Yarden 2000).

The role of HER2 in tumour growth

Recently it became evident that the transforming ability of HER2 is linked to cell survival and the ability of HER2 to directly affect components of the cell cycle machinery. Progression through the cell cycle is critically dependent on active complex formation of cyclin-dependent kinases (CDKs) with cyclin D1, which is a key regulator of G1/S phase transition of the cell cycle. Interestingly, cyclin D1 has been identified as a downstream target of HER2/neu in transgenic mice or HER2-overexpressing cell lines, which is necessary for HER2/neu-induced transformation (Lee et al. 2000). It was shown that cyclin D1 expression is upregulated by activated HER2/neu and that blocking HER2 specifically inhibits the growth of tumour cells by arresting the cells in the G1 phase of the cell cycle. Concomitantly, accumulation of p27Kip1, a CDK inhibitor, and inactivation of cyclin E–CDK2 complexes were seen (Lane et al. 2000). Moreover, the level of e-Myc and D-cylins, proteins that are involved in the sequestration of p27Kip1, decreased in the absence of functional HER2 (Neve et al. 2000). These results suggest that HER2 induces the potentiation of cyclin E–CDK2 activity via regulation of p27Kip1 sequestration. As a consequence, HER2 overexpression deregulates the G1/S phase transition and results in aberrant proliferation and tumour formation. Very recently it was demonstrated that activation of the PI3-kinase/Akt pathway by HER2-overexpressing cells promotes cell survival by phosphorylating the CDK inhibitor p21Cip1 (Zhou et al. 2000). Phosphorylated p21Cip1 localizes to the cytoplasm where it can form a complex with apoptosis-signal-regulating kinase 1 (ASK1) conferring its anti-apoptotic effect. At the same time the HER2-induced cytoplasmic localization of p21Cip1 suppresses its growth-inhibiting activity in the nucleus and consequently leads to HER2-mediated cell growth.

HER2 as a prognostic and predictive indicator

The relatively low expression level of HER2 in normal epithelial cells is significantly enhanced in several types of human cancers, including breast, ovarian, gastric, lung, bladder and kidney carcinomas (Hynes & Stern 1994). Especially in breast cancer a significant correlation between HER2 overexpression and reduced survival of breast cancer patients exists (Slamon et al. 1987). Moreover, clinical as well as laboratory data revealed that overexpression of HER2 increases the metastatic potential of human breast and lung cancer cells and correlates with the number of lymph node metastases in node-positive breast cancer patients (Yu &
Hence, HER2 expression represents a pivotal biological marker that can help to determine more accurately the prognosis for individual patients (Cooke et al. 2001).

In recent years, it became evident that HER2 is also a predictive marker for responses to various therapeutic agents used in cancer therapy. It has been reported that HER2 overexpression negatively influences the response rate of breast tumours to chemotherapeutic agents (Yu & Hung 2000). In addition, elevated levels of a processed HER2 extracellular domain in metastatic breast cancer patients seem to reduce the efficacy of certain chemotherapy combinations (Colomer et al. 2000). Of most important clinical relevance is the fact that HER2 overexpression correlates with a lack of response to anti-oestrogen hormonal therapy (Houston et al. 1999) and confers resistance to tamoxifen, an anti-oestrogen that is administered as endocrine therapy in breast cancer patients.

**Herceptin – the first RTK-specific anti-oncogene drug**

Several approaches towards the selective prevention and interception of HER2-relevant signalling are possible (Bange et al. 2001). When it was demonstrated that MAb 4D5, a mAb directed against the extracellular domain of HER2, could specifically inhibit the proliferation of HER2-overexpressing tumour cell lines and prevent tumour growth in nude mice, an excellent weapon towards the suppression of HER2-relevant signalling was established (Hudziak in nude mice, an excellent weapon towards the suppression of HER2-overexpressing tumour cell lines and prevent tumour growth could specifically inhibit the proliferation of HER2-mAb directed against the extracellular domain of HER2, (www.endocrinology.org 165). The EGFR is frequently overexpressed in non-small cell lung, bladder, cervical, ovarian, kidney, and pancreatic cancer and occurs with very high incidence in squamous cell carcinomas of the head and neck (Salomon et al. 1995, Grandis et al. 1996, Fontanini et al. 1998). The predominant mechanism leading to EGFR overexpression is EGFR gene amplification, with more than 15 copies per cell reported in certain tumours (Velu 1990). In general, elevated levels of EGFR expression are associated with late stage of disease progression and often correlate with high metastatic rate and increased rate of tumour proliferation (Pavelic et al. 1999). In addition, tumorigenic changes in EGFR activity can also occur via mutations that activate the receptor in the absence of ligand binding. A number of EGFR deletions have been identified in human cancer and most of these mutations alter

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<tr>
<th>RTK</th>
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FDA, Food and Drug Administration; MAb, monoclonal antibody; RTK, receptor tyrosine kinase; TKI, tyrosine kinase inhibitor.

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the extracellular ligand binding domain of the receptor and result in a truncated EGFR with constitutively active kinase function (Tang et al. 2000).

A very potent mechanism of constitutive EGFR activation in a variety of human cancers is autocrine stimulation via growth factor loops. The most prominent ligand, which is involved in autocrine growth receptor activation, is TGFα (Seth et al. 1999, Hsieh et al. 2000). Coexpression of TGFα and EGFR is frequently observed in glioblastomas and squamous cell carcinomas of the head and neck where it is correlated with poor prognosis (Grandis et al. 1999). Moreover, it was recently shown that the G protein-coupled receptor (GPCR)-induced cleavage of EGFR-like growth factors leads to EGFR transactivation and EGFR-related signalling in cancer cells (Gschwind et al. 2001). Mechanistically, GPCR stimulation activates a metallopeptase that cleaves a transmembrane EGFR-like ligand precursor and allows the released growth factor to transactivate the EGFR (Prenzel et al. 2001). Moreover, Prenzel et al. demonstrated that the metallopeptase inhibitor batimastat blocks bombesin-induced transactivation of the EGFR in PC3 human prostate cancer cells and significantly reduces the high constitutive level of EGFR tyrosine phosphorylation (Prenzel et al. 1999). This finding suggests that GPCR–EGFR cross-communication plays a prominent role in the development and progression of human cancer.

**EGFR as a target for anti-cancer therapies**

Since aberrant EGFR signalling is implicated in many cancers and seems to correlate with poor prognosis, it is an excellent target for anti-cancer therapy (Huang & Harari 1999, Hong & Ullrich 2000). Currently, the monoclonal antibody-based treatments using cetuximab (C225) represent the most successful anti-EGFR therapies (Table 1). C225 is directed against the extracellular domain of the EGFR and inhibits receptor activation and downstream signalling by blocking ligand binding to the EGFR and inducing receptor internalization. The growth inhibiting and anti-tumour effect of C225 was shown in a variety of human cancer cells including pancreatic, renal and breast carcinomas (Prewett et al. 1998, Overholser et al. 2000). Therefore, it is now undergoing a number of clinical trials either alone or in combination with chemotherapeutic treatments such as cisplatin, paclitaxel or gemcitabine (Baselga et al. 2000, Bruns et al. 2000, Inoue et al. 2000).

Inhibition of the tyrosine kinase activity by TKIs represents another promising approach towards the intervention of EGFR signalling in cancer cells. The most advanced of these compounds are ATP analogues of the quinazoline and pyridopyrimidine family, which interact with the conserved ATP-binding site in the kinase domain of the receptor and thus block EGFR activation (Levitzki 1992, Noonberg & Benz 2000). The quinazoline ZD-1839 (Iressa) shows a significant anti-tumour effect on human breast and colon cancer cells that is synergistically enhanced in combination with various cytotoxic agents (Ciardiello et al. 2000) and is currently being evaluated in phase II clinical trials.

**Insulin growth factor receptor (IGFR) family**

Structurally quite different from the EGFR family are the insulin receptor (IR) and the insulin-like growth factor (IGF) receptor (IGF-IR). Both receptors consist of two extracellular α subunits, which are responsible for ligand binding, and two membrane-spanning β subunits bearing the tyrosine kinase domain and the autophosphorylation sites (Yarden & Ullrich 1988). The ligands for the two receptors include insulin, IGF-I and IGF-II. While insulin is mostly a metabolic hormone, IGF-I and IGF-II are crucial for normal development and carcinogenesis. Interestingly, in the circulation IGFs are found to be complexed to a number of different IGF-binding proteins (IGFBPs), which serve as transport vehicles for these ligands and modify the stability and the proliferative effect of the growth factors (Jones & Clemmons 1995).

In the last 10 years it has been demonstrated that IGF-IR and its ligands are involved in the pathogenesis of a variety of human tumours, particular in breast and prostate cancer (Perks & Holly 2000, Surmacz 2000). In primary breast tumours, IGF-I and IGF-II are predominantly expressed in stromal fibroblasts surrounding the normal and malignant breast epithelium (Gebauer et al. 1998, Rasmussen & Cullen 1998), whereas the IGF-IR is overexpressed in breast cancer cells and shows enhanced tyrosine kinase activity (Resnik et al. 1998). These findings indicate that IGFs are able to stimulate breast carcinoma growth in a paracrine manner. Moreover, it has been demonstrated that high plasma IGF-I levels correlate with an elevated prostate cancer risk and several prostate cancer cell lines were shown to be responsive to IGF-I (Chan et al. 1998). On the basis of these results several approaches have been initiated to develop new small molecule inhibitors (Blum et al. 2000) or other strategies to interfere with the pathogenic IGF-IR signalling (Brodt et al. 2000) (Table 1).

**Vascular endothelial growth factor receptor (VEGFR) family**

The vascular endothelial growth factor (VEGF) is one of the main inducers of endothelial cell proliferation and permeability of blood vessels. The two RTKs that bind VEGFs, VEGFR-1 and VEGFR-2, are expressed on endothelial cells during embryonic development and are the key regulators for angiogenesis, a process which leads to the formation of new blood vessels developing from pre-existing...
ones. Blood vessel formation is usually quiescent in the adult organism except for wound repair and the female menstrual cycle. However, expansion of solid tumours beyond a diameter of 1–2 mm requires de novo formation of a vascular network that provides the growing tumour with oxygen and essential nutrients. Many studies have provided evidence for the role of the VEGF–VEGFR ligand–receptor system in tumour vascularization and metastasis. Mechanistically, the tumour secretes VEGF that in turn activates VEGFR-2 and induces proliferation of stromal endothelial cells and sprouting of new blood vessels. The critical role of VEGF-2 in the above-mentioned process was demonstrated by delivering of a retrovirus encoding a dominant negative VEGFR-2 into nude mice which prevented glioblastoma xenograft growth (Millauer et al. 1994). These findings have opened a new avenue of cancer therapies and several strategies have been developed for targeting the VEGFR pathway (Table 1).

Inhibition of angiogenesis has several advantages compared with other attempts in cancer therapy. This includes the fact that endothelial cells are easily accessible from the blood stream and are not likely to develop resistance to cancer therapy, since endothelial cells are genetically stable compared with tumour cells (Boehm et al. 1997). Therefore, over 20 anti-angiogenic drugs, which prevent endothelial cell proliferation directly by blocking activators of angiogenesis like the VEGF and fibroblast growth factor (FGF) receptors, are currently undergoing evaluation in phase I, II or III clinical trials (Table 1). For example the small molecule inhibitor SU5416, which specifically inactivates VEGFR-2 (Fong et al. 1999), is the most advanced in clinical trials for the treatment of a variety of solid tumours (National Institutes of Health). The drug was well tolerated in phase I studies and stable disease was demonstrated in a variety of tumours like Kaposi’s sarcoma, non-small cell lung cancer, colorectal cancer and breast cancer (Mendel et al. 2000, Fong et al. 1999). Another synthetic RTK inhibitor currently being evaluated is the tyrosine kinase inhibitor SU6668 that targets not only the VEGFR but also the fibroblast growth factor receptor (FGFR) and the platelet-derived growth factor receptor (PDGFR) (Laird et al. 2000). Both receptors are additionally involved in endothelial cell growth and angiogenesis. Other active VEGF-2 inhibitors that are now undergoing clinical trials are the TKIs PTK787, ZK22584 and a mAb that targets the VEGFR-2 ligand.

**Fibroblast growth factors receptor (FGFR) family**

The fibroblast growth factors (FGFs) represent the largest family of growth factor ligands. To date more than 20 distinct members have been identified. The FGFs and their designated receptors (FGFRs) appear to play critical roles not only in normal development but also in tumour formation and progression. Two classes of FGFRs were discovered in the past. The first class comprise the four high affinity FGFRs, whereas the second class is defined by low affinity FGF binding sites. Considerable evidence indicates that those low affinity binding sites represent heparan sulphate proteoglycan molecules (HSPG) located on the cell surface. These HSPG receptors may support the fine tuning of cell responses to the FGFs present and also regulate their availability and their transport within a tissue.

The first members of the large FGF family to be identified were the FGF-1 (aFGF) and FGF-2 (bFGF). They were purified on the basis of their mitogenic activity towards fibroblasts. Both ligands are potent mitogens for a variety of cells of mesodermal, ectodermal and endodermal origin (Basilico & Moscatelli 1992). In addition, they play a role as positive regulators for endothelial cell growth and angiogenesis (Folkman & Shing 1992).

The family of the high affinity FGFRs comprises four members: FGFR1 (Ig), FGFR2 (bek), FGFR3 and FGFR4. Ligand binding to FGFRs induces dimerization and phosphorylation of the cytoplasmic tyrosine residues, but full activation is only achieved in the presence of heparin (Spivak-Kroizman et al. 1994). A common explanation is that heparin is able to bind a number of monovalent FGFs allowing the formation of receptor oligomers, which bind to the clustered FGFs.

**FGFs and FGFRs in cancer development**

The fact that a remarkable number of FGFs were identified as genes isolated from tumours due to their ability to induce proliferation or transformation of fibroblasts indicates their functional role in tumorigenesis. Indeed, a number of studies point out that changes in the expression patterns of FGFs may contribute to growth deregulation of human tumour cells (Eguchi et al. 1992, Kornmann et al. 1997). Furthermore, it has been demonstrated that expression of FGF-1, FGF-2 or FGF-8b in fibroblasts leads to transformation and tumour formation in nude mice. In addition, strategies to interfere with growth factor action, such as dominant negative FGFRs or anti-sense oligonucleotides, result in impaired tumour growth in nude mice (Becker et al. 1992, Li et al. 1994, Wang & Becker 1997, Auguste et al. 2001). These data indicate that endogenous FGFs are autocrine mediators of neoplastic cell growth.

However, changes at the level of FGFRs, such as point mutations, elevated expression or different splicing, result in dis-regulated FGFR signalling and have been identified in a variety of human tumours. For example, FGF overexpression was observed in tumours arising from numerous tissues, including breast, prostate, melanoma, thyroid and salivary gland (Myoken et al. 1996, Shingu et al. 1998, Giri et al. 1999). Somatic mutations in the FGFR-3,
similar to the activating mutations in skeletal dysplasias, have been identified in bladder cancer and in multiple myeloma (Chesi et al. 1997, Capellen et al. 1999).

Since FGFR signalling shows a physiological profile of action that includes mitogenic and angiogenic activity and is frequently altered in human tumours, this system might be a useful target for anti-cancer therapy. Suramin, a compound that complexes heparin-binding growth factors, such as FGF-2, has been shown to inhibit cell migration and FGF-2-mediated induction of urokinase-plasminogen activator (Takano et al. 1994). However, efficacy in clinical trials is often limited by toxicity. Targeting the FGFRs was first achieved by using a toxin chimera between FGF-2 and the potent ribosome-inactivating protein saporin and shows anti-tumour activity towards melanoma cells expressing high FGFR levels (Beitz et al. 1992).

Another possibility to block FGFR signalling is represented by the use of small molecule inhibitors such as SU6668, which interferes with FGFR tyrosine kinase activity. SU6668 has been shown to induce striking regression of large established human tumour xenografts and investigations are ongoing in phase I clinical trials (Laird et al. 2000) (Table 1).

Hepatocyte growth factor receptor (HGFR)

The hepatocyte growth factor receptor (HGFR), which is encoded by the proto-oncogene met (Naldini et al. 1991), was identified as a regulator of a variety of morphogenic processes like cell migration, cell scattering and invasion of extracellular matrices (Birchmeier & Gherardi 1998). HGFR is a disulphide-linked heterodimer with a glycosylated extracellular α-chain and a β-chain, which consists of the transmembrane and cytoplasmic tyrosine kinase domain (Giordano et al. 1989). Hepatocyte growth factor (HGF) or scatter factor (SF), the corresponding ligand, is expressed in mesenchymal-derived cells where it is supposed to act in a paracrine manner on epithelial cells in close proximity and has been described as a growth modulator for hepatocytes, melanocytes and keratinocytes in vitro (Kan et al. 1991, Sonnenberg et al. 1993). Since HGF/SF is an important motility factor it might influence the migration of tumour cells and may facilitate invasive growth and metastasis. For example, HGF overexpression was demonstrated in a variety of human tumours, such as thyroid and colorectal carcinomas and seems to have prognostic significance for non-small lung and breast cancer (Di Renzo et al. 1995, Siegfried et al. 1997). HGFR mutations have been identified in patients with inherited predisposition to develop multiple papillary renal cell carcinomas (HPRCC) (Schmidt et al. 1997). Most of these mutations lie adjacent to the kinase domain, leading to enhanced enzymatic activity, transformation of fibroblasts and invasive growth as shown by in vitro experiments (Jeffers et al. 1998). Somatic mutations in the HGFR have been demonstrated in childhood hepatocellular (Park et al. 1999) as well as head and neck squamous cell carcinomas (Di Renzo et al. 2000).

Since HGF/SF is synthesized as a single chain precursor polypeptide devoid of biological activity and can be activated through a critical step involving proteolytic cleavage, this initial step of HGF/SF activation provides a possible point of interference by potential inhibitors. In the past few years numerous inhibitors and antagonists have been identified (Parr & Jiang 2001). These factors have demonstrated a possible role in minimizing the action of HGF/SF on cancer cells, and may be of therapeutic value in the future.

RET receptor tyrosine kinase

Multiple endocrine neoplasia type 2 (MEN2) is a dominant autosomal inherited cancer syndrome which exists in three different subtypes and is characterized by the development of medullary thyroid carcinoma. The gene responsible for MEN2 was identified as the Rearranged during Transformation (RET) RTK. The RET proto-oncogene encodes a protein that is characterized by a cadherin-like and a cysteine-rich domain in the extracellular part of the receptor. RET is expressed during embryogenesis in the peripheral nervous system and in the urogenital system and is involved in the development of the neural crest and the kidney (Edery et al. 1997).

MEN2 cancer syndromes are caused by dominant activating germline mutations in the RET proto-oncogene. For example, mutations at five different cysteine residues have been found in the extracellular domain, which result in an unpaired cysteine and intermolecular disulphide bonding, leading to constitutively activated receptors (Chappuis-Flament et al. 1998).

Interestingly, in 50% of patients with Hirschsprung disease (HSCR), a disorder characterized by the absence of enteric ganglia, gene deletions or point mutations that are predicted to inactivate the RET receptor have been identified (Pasini et al. 1996, Eng & Mulligan 1997). Therefore, the RET proto-oncogene is a unique example of either dominant gain-of-function or dominant loss-of-function mutations in the same gene associated with severe human disorders.

Platelet-derived growth factor receptor (PDGFR) family

The two RTKs platelet-derived growth factor receptor (PDGFR) and Kit are members of the PDGFR family. These proteins are characterized by an extracellular domain with five Ig-like domains and an intracellular tyrosine kinase domain split by 100 amino acids. Two genes encoding PDGFR-α and PDGFR-β have been identified and both receptors are activated by a ligand dimer consisting of
PDGF-A and/or PDGF-B. This in turn leads to receptor dimerization with three possible configurations: αα, ββ, αβ (Heldin et al. 1998). Coexpression of PDGFR and its ligands has been identified in glioblastoma and other human astrocytotic brain tumours, whereas normal brain tissue does not express these proteins (Hermanson et al. 1992). These findings suggest an autocrine loop that stimulates the uncontrolled growth of human brain tumours. However, the PDGF receptor–ligand system may exert its main function in tumour progression via the stimulation of neovascularization of solid tumours (Plate et al. 1994, Dunn et al. 2000).

The RTK Kit is predominantly expressed in mast cells, melanocytes and bone marrow (Wang et al. 1989), whereas its cognate ligand, stem cell factor (SCF), is found in stromal cells, fibroblasts and endothelial cells (Heinrich et al. 1993). The involvement of Kit in human malignancy is paradoxical. On the one hand functional expression of Kit has a positive effect on the growth of small lung cancer cells (Krystal et al. 1996) and mutations in Kit have been identified in patients with familial and sporadic gastrointestinal tumours (Moskaluk et al. 1999). On the other hand, loss of Kit is associated with progression of melanoma and thyroid carcinoma (Natali et al. 1992, 1995), suggesting a tumour suppressor function in this particular tumour type.

Conclusion

Over the past decade tremendous progress has been made in the elucidation of cellular signalling mechanisms and the establishment of molecular pathways activated by RTKs and their ligands in normal and malignant cells. Empirical observations and experimental studies led to an emerging consensus that several classes of RTKs and polypeptide growth factors play an important role in cancer management. Therefore, the potential of RTKs and their relevant signalling as selective anti-cancer targets for therapeutic intervention has been recognized. As a consequence, a variety of successful target-specific drugs such as mAbs and RTK inhibitors have been developed and are currently evaluated in clinical trials. Moreover, the growing field of rational drug design and computational prediction, in combination with advanced molecular understanding of cell signalling and genomic profiling, may make possible the development of an entire new generation of target-specific and molecularly based drugs and this promises a new era of anti-cancer therapy.

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