Growth factors and growth factor receptors in cancer

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Growth factors and growth factor receptors play a major role in growth and development, wound healing and have many physiological functions. Derangement in the function of these molecules plays an important role in cancer. This paper reviews the role of growth factors and their receptors in cancer, particularly, the members of the tyrosine kinase receptor family.

IT is well known that multi-cellular organisms evolved from unicellular ancestors. In the less complex unicellular organisms the key functions of finding food, responding to changes in the environment (external temperature and pH changes) and the stimuli for cell division are mediated by chemical changes within the same cell (Figure 1). However, in a multi-cellular organism the different functions needed to sustain the life of the organism are carried out by different organs. As most of the vital functions require the participation of more than one organ it becomes necessary to develop a system to communicate and co-ordinate events between cells of the same and different organs. The cells in a multi-cellular organism have achieved this by developing a wide array of receptors on their surface to which specific ligands bind and induce specific responses.

Growth factors and growth factor receptors

Growth factors (GF) and growth factor receptors (GFR) play an important physiological role in the normal process of growth and differentiation. In a simplistic model, the binding of the growth factor to its receptor leads to receptor dimerization and cross phosphorylation, activating the receptors. The activated receptors phosphorylate a series of cytoplasmic proteins which in turn sets off a cascade of events leading to the activation of transcription factors in the nucleus, which then leads to increased mRNA synthesis. The translation of the mRNA results in increased protein synthesis finally leading to either growth or differentiation[1].

The peptide and polypeptide growth factors, unlike classical hormones, are produced in a variety of tissues throughout the body and their action is not necessarily restricted to single tissue types. These factors can act by an intracrine, autocrine, juxtacrine, paracrine or endocrine process (Figure 2). Autocrine action is due to the secretion by a cell of growth factors (transforming growth factorα (TGFα) and epidermal growth factor (EGF)) for which it possesses receptors, (epidermal growth factor receptor (EGFR)[2,3]. Some experiments have suggested that this interaction may even occur within a cell, a process called intracrine interaction[4]. Juxtacrine stimulation is when one cell has surface bound growth factors which interact with an adjacent cell containing receptors for the growth factor (TGFα)[5]. Paracrine action is defined as the release by cells, of soluble growth factors which diffuse into the extracellular space and act upon adjacent or closely located cells. In the case of endocrine action, growth factors are carried in the blood stream and may act on distant sites much like a classical hormone.

Aberrations in the growth factor signalling pathways can lead to abnormal growth and development. Loss of function mutations in growth factor receptors can lead to inherited diseases such as insulin-resistant diabetes (insulin receptor)[6] and dwarfism (Achondroplasia) (Fibroblast growth factor receptor 3 (FGFR3 receptor)[7]. Overexpression of growth factors can lead to non-neoplastic disorders like psoriasis (TGFα)[8]. Cancer is now recognized to be the result of a multistep process[9,10]. Among the events that can lead to malignant transformation is the unregulated expression of growth factors or components of their signalling pathways.

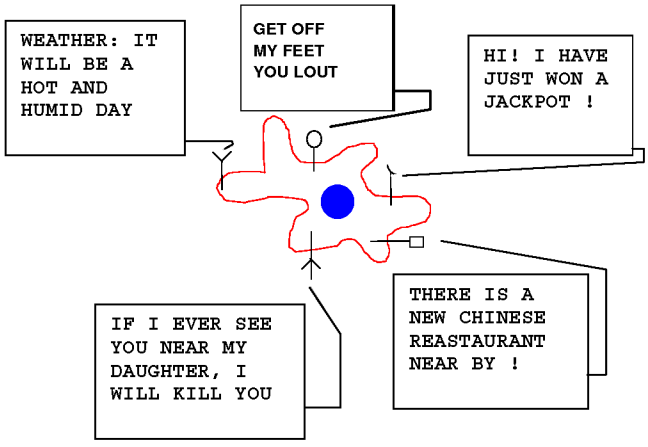

Figure 1.

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Tyrosine kinases

Although a cell may respond to a vast number of growth factors and possess a variety of types of receptors, there are only a few known intracellular second messenger systems through which all these signals can be channelled into the cytoplasm and then into the nucleus. These are the cyclic AMP and the cyclic GMP systems, control of free intracellular calcium levels, usually mediated by the action of inositol 1,4,5-triphosphate, the pathways involving receptor protein tyrosine kinases and the tumour growth factorβ (TGFβ) which utilizes receptor serine/threonine kinases. The activation of tyrosine kinases may be linked to a mechanism for increasing the free intracellular calcium levels (activation of phospholipase Cγ leads to hydrolysis of phosphatidylinositol 4,5-biphosphate to inositol triphosphate which in turn results in the elevation of free calcium levels).

The tyrosine kinases can be classified into receptor tyrosine kinases, cytoplasmic non-receptor tyrosine kinases and membrane associated non-receptor tyrosine kinases[11,12]. These kinases are now thought to phosphorylate other proteins such as PLCγ (ref. 13) and c-Raf[14] on their tyrosine residues leading to their activation.

Receptor tyrosine kinases and their growth factors in cancer

There are as many as 14 types of tyrosine kinase growth factor receptors, as indicated in Table 1. Some of these receptors have been shown to play a critical role in the induction of cancer. The mechanism by which these receptors could contribute to tumourigenesis include overexpression of the receptors or their ligands and mutation of the receptors resulting in abnormal activity even in the absence of the ligand.

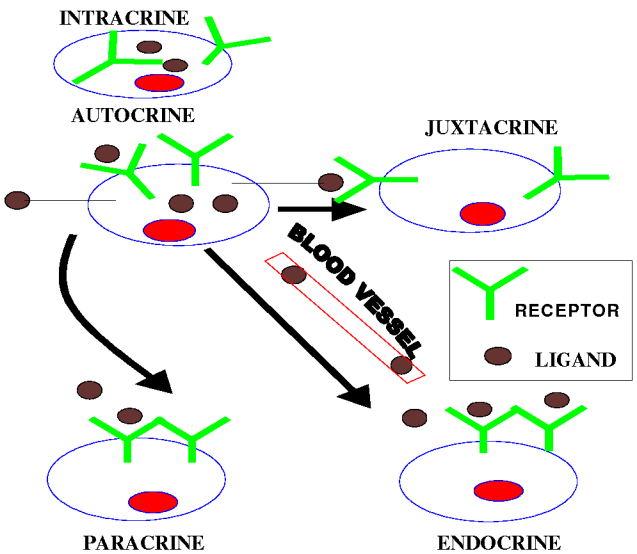

Figure 2. Mechanisms of action of peptide growth factors.

Table 1. Tyrosine kinase membrane receptors and their ligands[59]

Family	Growth factor receptors	Growth factors
1	Epidermal growth factor receptor (EGFR; HER1)	EGF; TGFα, betacellulin, HB-EGF, Amphiregulin
	c-erbB2 (HER2)	None identified
	c-erbB3 (HER3)	Neuregulin
	c-erbB4 (HER4)	Neuregulin
2	Insulin receptor	Insulin
	Insulin-like growth factor receptor 1 (IGF-1R)	Insulin like growth factor 1 and 2 (IGF-I, IGF-II)
	Insulin-like growth factor receptor 2/Mannose–6-phosphate receptor (IGF-II R/M-6-P receptor)	
	Insulin receptor related kinase (IRRK)	
3	Platelet-derived growth factor receptor (PDGFR)	Platelet derived growth factor (PDGF)
	Colony-stimulating factor–1 receptor (CSF-1R) (c-Fms)	Colony Stimulating Factor–1 (CSF-1)
	Steel receptor (c-Kit)	
	Flk2/Flt3	
4	Fibroblast growth factor receptor 1 (Flg/Cek1)	Acidic FGF Basic FGF
	Fibroblast growth factor receptor 2 (Bek/Cek3/K-Sam)	Int-2 Hst/KFGF
	Fibroblast growth factor receptor 3	FGF-5
	Fibroblast growth factor eceptor 4	FGF-6 KGF
5	Nerve growth factor receptor (NGFR) (TrkA)	NGF
	BDNF receptor (TrkB)	BDNF
	NT-3-receptor (TrkC)	
6	Vascular endothelial growth factor receptor 1 (Flt1)	VEGF
	Vascular endothelial growth factor receptor 2/Flk1/KDR	
7	Hepatocyte growth factor receptor (HGF-R/Met)	HGF
8	Eph Eck Eek Cek4/Mek4/HEK Cek5 Elk/Cek6 Cek7 Sek/Cek8 Cek9 Cek10 HEK11	
9	Ror1 Ror2	
10	Ret	
11	Axl	
12	RYK	
13	DDR	
14	Tie	

Signal transduction of growth factor–growth factor receptor interaction

A schematic view of some of the molecules involved in signal transduction is shown in Figure 3. As seen in the figure, following the binding of the growth factor to its receptor, conformational changes occur leading to dimerization and cross-phosphorylation of the receptors. The activated receptor, then phosphorylates molecules like GRB2 and SOS, which in turn activate the GDP bound Ras, to Ras-GTP, which is the active form of Ras. The signal from the activated Ras is then passed on to Raf, MAPKK and MAPK, the latter translocating to the nucleus wherein it activates transcription factors like Jun and Fos. The transcriptional machinery is activated leading to cell division.

Some receptors like the T-cell receptors (TCR) lack a cytoplasmic tyrosine kinase domain. These molecules signal through their association with one of the non-receptor protein tyrosine kinases like JAK.

Cell cycle and growth factors

For the entry of a quiescent cell from the G_0 phase to G_1 phase, competence factors such as PDGF and FGF are needed. Progression through the G_1 phase of cell cycle requires EGF and IGF-1 (progression factors). Baserga[15] has postulated that the primary function of PDGF, FGF and/or EGF is to induce enough IGF-1 and IGF-1 receptor. The growth factor – growth factor receptor signalling act through the G_1 cyclins, particularly the cyclin D family. The induction of cyclin D–cdk4/6 complex leads to phosphorylation of retinoblastoma (Rb) protein, which is bound to E2F. Once phosphorylated, the Rb protein lets go the E2F transcription factor, which is then available for the entry of the cell into the S-phase. IGF-1 has been shown to induce cyclin-D1 expression in human osteosarcoma cell line, MG63[16].

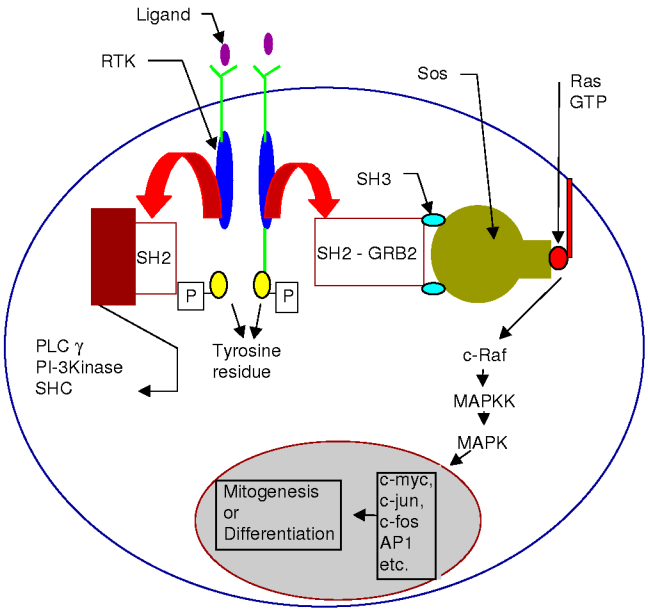

Figure 3. Signalling through tyrosine kinase receptors.

Role of growth factors and their receptors in apoptosis

Apoptosis or programmed cell death is an important physiological phenomenon playing crucial role in growth and development of an organism. It also plays an important protective role in DNA damaged cells which fail to have their DNA damage repaired but attempting to enter the cell cycle. By triggering apoptosis, these abnormal cells are destroyed, thereby preventing tumour induction. Conversely, in the absence or inhibition of apoptosis, these cells survive and cumulate more DNA damage, tend to acquire a more aggressive phenotype.

IGF-1 and PDGF have been shown to inhibit apoptosis in fibroblasts deprived of serum, whereas EGF and FGF do not have any protective effect from cell death[17]. IGF-1 also has been shown to inhibit apoptosis induced by a monoclonal antibody to EGF receptor in a colon cancer cell line. The IGF-1 receptor was also needed for the protective action, as an antibody to the IGF-1 receptor, blocked the inhibition of apoptosis[18].

Role of *EGFR*, c-erbb2, c-erbb3, c-erbb4 and their ligands in cancer

EGFR

Human squamous cell carcinomas have been shown to exhibit a combination of the constitutive secretion of TGFα and overexpression of EGFR, resulting in an autocrine loop-promoting growth[3]. Gastric cancers staining positive for both TGFα and EGFR[19] were found to have a higher bromodeoxyuridine labelling index and poorer prognosis compared to those tumours which were negative for both or one of these molecules. Similar pathways have also been demonstrated in high grade brain tumours[20] and lung carcinomas[21].

Additional evidence for the transforming capabilities of EGF receptor and its ligand TGFα, has come from studies involving transgenic mice. Mice transgenic for TGFα were found to develop breast adenocarcinomas, hepatocellular carcinomas and dramatically accelerated growth of pancreatic tumours[22–24]. The latter two effects were more pronounced in double transgenic expressing TGFα and c-*myc*[23].

Gene amplification of EGFR occurs in about 40% of glioblastoma multiforme[25], 8–20% of head and neck cancers, 8–14% of oesophageal cancers, 3–6% of gastric

cancers and 2% of breast tumours. Overexpression due to altered transcriptional control occurs in a much larger percentage of tumours, for instance, in 25–61% of bladder, 45% of breast, 58% of lung, and 4% of early and 35% of advanced gastric carcinomas. In general, overexpression of EGFR has been found to be associated with poor prognosis. Overexpression of EGFR in breast tumours has generally been associated with an ER (oestrogen negative) phenotype. EGFR positivity has been associated with short disease free (DFS) and overall survival (OS) in node negative patients[26]. In addition, Harris *et al.*[27] have shown that EGFR status predicts response to tamoxifen (more EGFR negative patients' responded to tamoxifen than the EGFR positive patients).

Deletion-mutant EGF receptors have been identified in glioblastomas of the brain[28] and in non-small lung carcinomas[29]. At least three types of mutants have been reported in brain tumours with the type 1 (loss of exons 2–7) being the most common form seen (17%)[28]. These mutant receptors have the potential to act as tumour-specific antigens as none of the normal tissues express them and therefore are good targets for monoclonal antibody directed therapy.

c-erbB2

Breast cancer: High levels of c-erbB2 are found in more than 90% of comedo, large cell, ductal carcinoma *in situ*, and in general low or absent levels of expression are seen with other subtypes such as papillary and cribriform tumours *in situ*[30]. The high levels and high incidence in ductal carcinoma *in situ*, together with the absence of expression in premalignant breast epithelial cells such as atypical ductal hyperplasia, indicate that its expression is an early event in tumourigenesis. In addition, almost all cases of mammary Paget's disease overexpress the protein.

Among the invasive tumours, ductal carcinomas are the types associated with c-erbB2 overexpression whereas the lobular carcinomas are in general negative. In breast carcinomas, the gene is amplified in about 20% of cases, leading to substantial overexpression of the receptor protein[30]. Such elevated expression is generally associated with poor relapse-free and overall survival in node-positive tumours. In node-negative tumours the results have been variable, with some studies showing an association with poor relapse-free and overall survival[31] and others showing no significant effect[32]. One flaw common to some reports is the use of too small a number of cases to detect the predictive power of the effect of overexpression if it is similar to that present in node-positive disease. Most of the larger studies have shown that overexpression of c-erbB2 protein which is inversely related to the presence of oestrogen and progesterone receptors, is associated with inflammatory carcinomas, high tumour grade, lymph node metastasis and poor response to therapy[30].

Ovarian cancer: 20–40% of ovarian carcinomas overexpress c-erbB2 protein with gene amplification seen in 0–30% of cases[32]. Overexpression was associated with poor overall survival[32] or poor relapse free and overall survival[33].

Gastric cancer: About 20% of gastric cancers overexpress c-erbB2 protein either due to amplification and/or increased transcription. Controversy exists regarding the significance of overexpression of c-erbB2, with some studies reporting that it is associated with poor prognosis and others showing that it confers a survival advantage. Additional studies would be needed to clarify this controversy.

Bladder cancers: Widely varying levels of overexpression have been reported in bladder cancer, ranging from 2% to 74%. The widely varying incidence could be due to sampling errors, type of tissue used (frozen sections versus formalin-fixed paraffin-embedded sections), the antibody used and the technique and criteria used to define positivity. Moriyama *et al.*[34] showed that c-erbB2 overexpression in bladder carcinomas was associated with poorly differentiated grade and invasiveness.

Other tumours: c-erbB2 overexpression has been less frequently observed in colorectal, pancreatic, salivary gland and lung carcinomas[30]. Kern *et al.*[35] reported a reduced overall survival in patients with adenocarcinomas of the lungs which overexpressed c-erbB2 protein.

c-erbB3

The c-erbB3 protein overexpression has been reported in gastrointestinal cancers, breast cancers, bladder cancers, cervical cancers and oral cancers[36,37]. Most of the studies have not shown any significant prognostic association with overexpression of c-erbB3. Knowlden *et al.*[38] showed that c-erbB3 and c-erbB4 expression were associated with estrogen receptor (ER) positivity and patients whose ER positive tumours expressed high levels of c-erbB3 were most likely to benefit from endocrine measures.

c-erbB4

Srinivasan *et al.*[39] showed that while 10–20% of adenocarcinomas and astrocytomas overexpress c-erbB4, 40–80% of the adenocarcinomas and 100% of the squamous cell carcinomas showed lack of expression, in their series of cases. Their study also suggested that c-erbB4 was more likely to be associated with better differentiation. In medulloblastomas, a form of brain tumour, expression of c-erbB4 and c-erbB2 was

associated with a aggressive tumour phenotype and a poor prognosis.

Role of IGF-I, IGF-II, IGF-IR and IGF-IIR in cancer

Wilm's tumour

Wilm's tumour is a kidney cancer occurring in children. The Wilm's tumour predisposition gene, WT1, product is a transcriptional factor/regulator. During the normal kidney development, there is an initial high level of expression of IGF-II by the undifferentiated proliferating blastoma but is absent in the epithelial cells of the renal vesicles and the podocyte epithelia. WTI is not expressed during this phase, but occurs later, with declining levels of IGF-II and IGF-I receptor. Down regulation of WT1 expression or deletion or mutation of the *WT1* gene leads to derepression of IGF-II and IGF-I receptor genes, leading to an inappropriate expression of an autocrine/paracrine pathway involving IGF-II and IGF-I receptor, which in turn can lead to mitogenesis[40].

IGF's and their receptors have been found to be expressed in a wide range of tumours, including, Wilm's tumour, liver cancer, lung cancer, breast cancer, etc.

Role of PDGF and PDGF-receptors in cancer

PDGF exists as homo or heterodimer of two polypeptides, the A and B chains. Their receptors (PDGFRα and PDGFRβ) were found to be expressed in microvascular endothelium. It is likely that PDGF might stimulate angiogenesis both by a direct effect on endothelial cells and by inducing angiogenic factors like VEGF[41].

PDGF and PDGFR have been demonstrated in the tumour cells and in the stromal cells, enabling a potential autocrine or paracrine mode of activation of angiogenesis. PDGFR was also found to be expressed on vascular endothelial cells in breast tumour[42].

Role of VEGF and VEGF-receptors in cancer

Vascular endothelial growth factor (VEGF) can bind to VEGFR1/Flt-1or to VEGFR2/Flk-1/KDR These molecules play an important role in angiogenesis during development, wound healing and in the pathogenesis of tumour neovascularization.

Patients with NSCLC or breast cancer with VEGF positive tumours were found to have a poorer prognosis than those tumours which lacked VEGF[43,44]. Bellamy *et al.*[45] have shown that 5/12 human hematopoietic tumour cell lines expressed both VEGF and Flt-1 (VEGFR1) mRNA, indicating a potential autocrine pathway in these tumour cells.

Role of FGF and FGF-receptors in cancer

FGF family of growth factors have diverse actions including their effect on cell proliferation, angiogenesis and neurotrophic effects. In some melanomas and gliomas, FGFs act as autocrine growth factors. Studies have also correlated the presence of the FGFs and their receptors in cancers to more aggressive tumours with a greater tendency to metastasis. FGF2 and FGFR 1 and 2, have been shown to be involved in prostatic cancers[46]. Volm *et al.*[47] using immunohistochemistry have shown that overexpression of FGFR1 in non-small cell lung carcinoma is associated with poorer prognosis. The FGF family of growth factors and their receptors have been shown to be involved in pancreatic cancers[48].

Role of *RET* proto-oncogene in cancer

Patients with multiple endocrine neoplasia 2A (MEN 2A) have missense mutations at the extracellular cysteine-rich domain of c-ret, which leads to constitutive activation of the tyrosine kinase activity or alteration of substrate recognition or both, leading to transformation[49]. MEN 2A syndrome is characterized by bilateral medullary carcinoma of thyroid, pheochromocytoma and hyperparathyroidism.

MEN 2B is associated with a germline point mutation in the c-ret proto-oncogene tyrosine kinase catalytic domain, leading to a methionine to threonine substitution at codon 918 in the kinase domain, which alters the substrate specificity of the protein[50]. MEN 2B is characterized by medullary carcinoma of thyroid, pheochromocytoma, marfanoid body habitus, oral and eye mucosal neuromas and gastrointestinal tract ganglioneuromas. The methionine 918 to threonine mutation seen in MEN 2B is also seen in up to one-third of sporadic medullary carcinoma of thyroid; in a few other sporadic cases, glutamic acid 768 to aspartic acid mutations are seen.

Role of HGF and HGFR/Met in cancer

The hepatocyte growth factor (HGF) is expressed in the stromal cells while its receptor, HGFR/Met, is expressed in a variety of epithelial cells. They are involved in development of organs including lung, kidney, breast particularly with regard to branching morphogenesis and tubulogenesis. They are also necessary for neuronal development, muscle development, hematopoiesis and angiogenesis.

The HGF-HGFR/Met pathway is involved in cancer as well. They promote invasiveness and metastasis of the tumours through rearrangements of the cytoskeleton; by activating cell adhesion molecules and by promoting degradation of extracellular matrix by tumour cells

through induction of synthesis of urokinase-type plasminogen and its receptor. HGF transgenic mice have been shown to develop a wide range of epithelial and mesenchymal cancers like breast cancers, melanoma, fibrosarcoma. In human tumours, HGF-HGFR/Met has been shown to be overexpressed in gastric, liver, colon, lung and thyroid cancers. Overexpression of HGF has been shown to be an independent predictor of poor prognosis in NSCLC. HGF-HGFR/Met also may contribute to B-cell malignancies including large cell lymphomas and myelomas[51].

Hereditary papillary renal carcinoma (HPRC), an autosomal dominant disease with reduced penetrance, is characterized by a predisposition to develop multiple, bilateral papillary renal tumours. Schmidt *et al.*[52] identified missense mutations located in the tyrosine kinase domain of the *Met* gene in the germline of affected members of HPRC families and in a subset of sporadic papillary renal carcinomas. Three mutations in the *Met* gene were located in codons that are homologous to those in *c-kit* and *Ret* proto-oncogenes that are targets of naturally occurring mutations. Their results suggest that missense mutations located in the *Met* proto-oncogene lead to constitutive activation of the Met protein and papillary renal carcinomas.

Targeting growth factors and their receptors

Several strategies aimed at blocking the mitogenic signalling pathway that is activated following ligand–receptor interactions, are being evaluated. These include growth factor antagonists (pentosan polysulphate), monoclonal antibodies, receptor dimerization inhibitors, protein tyrosine kinase inhibitors (genistein, erbstatin, tyrphostins), antisense oligonucleotides and transcriptional inhibitors[53] (Figure 4). Monoclonal antibodies raised against the extracellular domain of the orphan receptors for functional studies can also be used for targeting tumours overexpressing these receptors.

Suramin

This polysulphonated drug inhibits the binding of GF like PDGF, FGF, EGF, TGFα, IGF-1, IGF-2, IL-2 and nerve growth factor to their receptors and can induce disassociation of bound growth factors from their receptors. The drug has shown to have activity against renal cancer, prostate cancer and adrenal cancers.

Monoclonal antibodies to GFR

The introduction of anti-c-erbB2 humanized antibody, trastuzumab, in the treatment of tumours overexpressing c-erbB2, particularly breast cancers is a classic example of taking the advances in the laboratory to the patients' bedside. The antibody has shown a response of around 15% with a median overall survival rate of 13 months in metastatic breast cancer. Combined use of chemotherapy (anthracyclines or taxanes) with the antibody has shown an improved response rate for the combined approach over chemotherapy alone[54].

Similar promising data are available from pre-clinical studies using anti-EGFR antibodies.

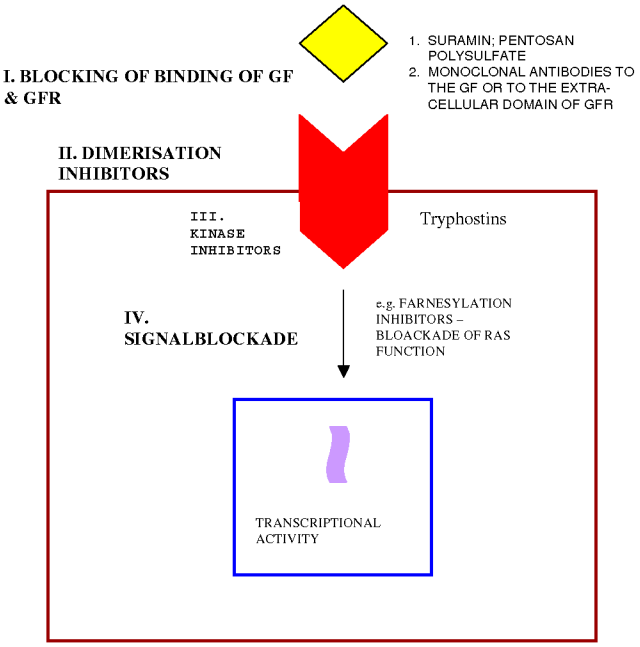

Figure 4. Targeting growth factors and growth factor receptors.

Tyrosine kinase inhibitors

Specific tyrosine kinase inhibitors are being developed, one such example being the 4-anilinoquinazolines which has been developed as an inhibitor of EGFR tyrosine kinase[55]. Tryphostins specific for the EGFR have been shown to inhibit primary glioblastomas[56] and prostate cancers[57]. A c-erbB2 specific tyrosine kinase inhibitor, AG825, was found to sensitize c-erbB2 overexpressing tumour cells to chemotherapy[58].

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