Cancer is a disease involving abnormal cell growth and increased propensity of cells to invade other parts of the body. Cancer is the second most common cause of death; the most common types of cancer being lung, colorectal and breast cancer [1]. The great majority of cancers are due to environmental factors (95%) and the remaining are caused by genetic factors (5%) [2]. Treatments like chemotherapy, radiation and surgical interventions are conventional and burdening cancer therapies. In most cancer cells specific signalling pathways are overactivated by overexpressions, autocrine loops, alternative activation [3]. The targeted inhibition of these signalling pathways provides more specific and less burdening treatments.

Malignant cells are characterized by irregular size and shape, large nucleus, disorganized arrangement and scarce cytoplasm [4]. Besides changes in their morphology, cancer cells are also altered in their physiology, for example they proliferate autonomously, they develop a resistance against growth inhibitors. Furthermore, apoptosis is suppressed, which leads to an unlimited proliferation. In addition the malignant cells are able to express growth factors that induce enhanced angiogenesis. These cells have the ability to anchorage independent growth and can invade in other tissues and metastasize [3]. The alterations of the properties of cells during tumorigenesis are caused by mutations in specific genes controlling various signalling pathways. Some of these important pathways are connected to G-protein coupled receptors and receptor tyrosine kinases (RTK). Both receptors are membrane bound proteins, which have intra- and extracellular domains. A ligand binds to the extracellular domain and transactivates the intracellular domains, which then activates an intracellular signal cascade. In case of G-protein coupled receptors they are activated by conformation changes and in case of RTKs, they are activated by phosphorylation, which is also coupled to changes in their conformation. Some example of pathways, which play a key role in carcinogenesis are the VEGF-, CXCR4-, MET- and Eph -
pathways. C-Met and CXCR4 are important for proliferation and migration, while VEGF and Eph are important ligands for angiogenesis.

Ephrin receptors (Eph) are membrane bound receptor tyrosine kinases (RTKs) that play a crucial role in migration, repulsion and adhesion in normal development, but also in cancer, for example non-small cell lung cancer and breast cancer. They can be divided into two groups, EphA and EphB, according to their respective ligand. Ephrin ligands (ephrin) are also membrane bound proteins. Therefore their interaction requires direct cell-cell interaction [5]. EphrinB has a transmembrane and an intercellular domain. The signalling is mediated either by phosphorylation of tyrosine residues or by the PDZ domain. The signalling occurs bidirectionally into Eph-expressing cells (forward signaling) or into ephrin-expressing cells (reverse signaling) [6].

This type of signalling plays an important role in axon path finding. Since axonal growth cones and capillary tip cells have filopodial extensions in common, they could both use similar attractive and repulsive signals. Tip cells are regulated by gradients of VEGF-A. Because EphrinB ligands have a well-defined function as repulsive molecules for axon guidance Sawamiphak et al. investigated whether ephrin-B2 would play a role in tip cell guidance and function and possibly controls VEGFR2 internalization and activation [7]. They proposed a model of ephrin-B2 function at tip cell filopodia. Here, Ephrin-B2 is activated by its receptor EphB4 and functionally cooperates with VEGF to stimulate VEGFR2 through PDZ interactions. This induces VEGFR2 internalization, activation and downstream signalling, resulting in tip cell filopodial extension and vascular sprouting. An orthotopic glioma tumor model revealed that ephrin-B2 reverse signaling, via PDZ interaction, controls VEGFR2 function, which regulates tip cell filopodial dynamics during tumor angiogenesis. They observed smaller tumors and less vascularization. Sawamiphak et al. concluded that blocking ephrin-B2 reverse signalling could be an alternative or combinatorial anti-angiogenic treatment to interfere with VEGFR2 function in tumor angiogenesis [7].

While the described pathway is an example for the impact of ephrin-B2 reverse signalling, cancer development is also affected by forward signaling. This signalling is reported when the ligand EphrinB stimulates its receptor EphB. Unlike other receptor tyrosine kinases, described in this review, EphB does not promote tumor progression, but suppresses it in forward signalling.
A correlation between kinase activation of EphB3 and non-small-cell-lung-cancer (NSCLC) cell migration was examined by Li et al [8]. It was shown that a low ephrin-ligand expression leads to the lowest life-expectation within the observation of NSCLC-patients.

Analysis of EphB3 overexpressing cells with Co-immunoprecipitation (Co-IP) and boyden chamber migration assays elucidate a signalling pathway activated by EphB3: After activation of the intracellular EphB3 kinase domain, RACK1 (receptor for activated C-kinase 1) is recruited and mediates the assembly of PP2A/RACK1/Akt signalling complex. Within this complex PP2A dephosphorylates Akt, which triggers an inhibitory signal to cell migration.

Analysis of the phosphorylation level of Akt in NSCLC-tissues showed similar results and further indicated that this model can be applied to therapeutic treatment. Ephrin-B2-Fc or antibodies to the extracellular domain of EphB3 are proposed for lung cancer therapy.

The suppression due to EphB forward signalling also occurs in breast cancer. Noren et al., (2006) investigated that there is a widespread expression of EphB4 receptors but not of the ephrinB2 ligand in breast cancer cells [9]. In in vivo xenograft models they could feature that there is tumor growth suppression after treatment with soluble ephrinB2-Fc. This tumor suppressing effect had been examined with in vitro invasion and migration assays, as well. The investigation of the pathway involved revealed the transactivation of the adaptor protein Crk targeting the non-receptor tyrosine kinase Abl. Activated Abl has multiple roles in cell growth, cell stress, apoptosis and remodeling of the actin cytoskeleton [10]. In these experiments, Abl was observed to reduce proliferation, migration and invasion and to cause an increase of apoptotic cells. Here, inhibition of Abl by treatment with Gleevec resulted in opposite effects, e.g. enhanced tumor growth. The outcome of Noren et al.’s results could be used to design new strategies for breast cancer therapies, for example by inhibiting tumor growth by stimulating the EphB4 receptor with ephrinB2-Fc [9].

The activities of the Eph system in cancer are complex and paradoxical in their effects, since dependent on forward or reverse signaling of this receptor-ligand system a tumor suppressing or activating function is reported [11].

Besides Eph, there is another ligand for angiogenesis like VEGF (vascular endothelial growth factor). VEGF is a growth factor produced by endothelial cells that stimulates vasculogenesis and angiogenesis. Angiogenesis, the formation of blood vessel from existing ones, is required for tumor
growth and metastasis. Tumor cells induce angiogenesis in order get oxygen and nutrient supply. An overexpression of VEGF can contribute to cancers like colitis-associated cancer. To investigate the correlation between VEGFR-2 signaling and cell senescence, Foersch et al., 2015 quantified a decrease of tumor development in the colon of VEGFR-2ΔIEC mice compared to control mice with colitis. They investigated that VEGFR-2 plays an important role in tumorigenesis due to the fact that there is an interaction between VEGFR-2, PI3K, AKT and P21. Moreover, they showed that VEGFR-2 signalling bypasses the protective effect of P21 by inactivating it. The Inhibition of VEGFR-2 leads to senescence and an anti-tumor immune response by CD8⁺ T-cells [12].

In lung cancer it was shown that also the expression of the receptor for VEGF, namely VEGFR-2 is increased in 20% of patients and a high VEGFR-2 gene copy number was shown to be associated with chemoresistance and shorter survival. Chatterjee et al. identified a VEGF/VEGFR2 signalling loop that promotes blood supply through angiogenesis. The inhibition of VEGFR2 caused an increase of ERK, which in turn activates an ERK dependent proliferative pathway. It was therefore proposed, that non-small-lung-cancer cells reduce proliferation in case of nutrient shortage in a VEGF/VEGFR2-dependent manner. The feed-forward loop of VEGF/VEGFR2 is proposed to interact with MAPK signaling through ERK. A dual therapeutical approach was suggested in which both the VEGF/VEGFR2 and ERK pathways are inhibited.

Targeted therapy against VEGF is already used in the clinics. One of the drug used is Bevacizumab a humanized monoclonal antibody against VEGF [13, 14]. In glioblastoma multiforme (GBM), a rapid and invasive tumor growth in brain parenchyma, VEGF has high expression levels. GBM is one of the most vascularized and edematous tumors [15, 16]. The survival rate without a therapy lasts only a few months and with a treatment with Bevacizumab up to one year [17]. Bevacizumab binds to VEGF, which leads to an anti-angiogenesis effect and stops tumor growth. But the beneficial effects of Bevacizumab are transient, so that GBM uses an alternative pathway to sustain tumor growth [18]. By activating the alternative MET –pathway with the ligand HGF (hepatocyte growth factor) by phosphorylation the tumor is able to enhance proliferation. In case of inhibition of VEGF by Bevacizumab MET is therefore able to be phosphorylated, so that the tumor regrowth is enhanced. This means that in the presence of VEGF, MET is inhibited. To inhibit MET-activation VEGF directly competes with HGF for MET binding. Another way to block MET- signaling VEGF antagonizes MET activity by signaling through VEGF receptors (VEGFR2) on tumor cells. This demonstrate the connection between VEGF and HGF. They revealed that VEGF enhanced the
recruitment of the non-receptor protein tyrosine Phosphatase PTP1B, which can create a MET/VEGFR2 heterocomplex to facilitate MET dephosphorylation [19]. Combines VEGF and MET inhibition might also be useful in other cancer types. In other types of cancers, previous approaches revealed an increased aggressiveness of tumors treated with anti-VEGF antibodies like Bevacizumab [20–22].

In pancreatic tumors of the Rip-Tag2 mice, the increased aggressiveness is reflected by an increased invasiveness of tumors and metastasis, which appear hand in hand with an increased c-Met expression and activation [23, 24]. Rip-tag2-mice have been treated with anti-VEGF-antibodies or sunitinib, in combination or not with the c-Met inhibitor PF-04217903. The additional appliance of this c-Met inhibitor showed a drastic decrease in aggressiveness in both cases (antibody & sunitinib treatment). Due to these results, it seems recommendable to add the c-Met-inhibition on tumor treatments by VEGF-inhibition. In fact previous work already showed an advanced tumor-treatment by multi-targeted tyrosine kinases, which target VEGF and c-Met [25].

The c-Met proto-oncogene [26] influences multiple biological functions involved in normal development and cancer progression. This includes proliferation, survival, invasion and motility. c-Met is a receptor tyrosine kinase (RTK) which is activated by its ligand hepatocyte growth factor (HGF) [27]. The cell surface receptor is expressed in epithelial cells of many organs. Binding of HGF to c-MET results in homodimerisation and phosphorylation which results in the activation of several signalling cascades, for example the MAPK cascade, through recruitment of effectors [28]. In many types of cancer, an abnormal activity of c-Met expression is observed [29]. Consistently, high expression of HGF has been observed in the reactive stroma of primary tumors [30]. This indicates the establishment of paracrine positive feedback loops which promotes proliferation and angiogenesis. Therefore, inhibition of c-Met is a promising therapeutical approach in cancer treatment. Such a distinct feed forward loop was found in adrenocortical carcinoma by Phan et al. (2015). They identified HGF and c-Met as important components in the development of this rare malignant form of cancer with a low survival rate of patients and limited response to chemo- and radiotherapeutical approaches. A highly elevated expression of HGF and c-Met in adrenocortical carcinoma was demonstrated. Treatment with cisplatin and mitotane led to increased c-Met expression. A similar result was obtained with radiation treatment. Besides this, cell survival increased with the serum HGF-concentration. Treatment of adrenocortical carcinoma xenograft models with the tyrosine kinase inhibitor Cabozantinib caused interference with tumor growth.
Phan et al. could therefore show that the inhibition of the c-Met autocrine loop could be a possible therapeutical approach in adrenocortical carcinoma. Alongside this mechanism, c-Met/HGF signalling in cancer is also relevant for invasion and metastasis of tumor cells.

The Met tyrosine kinase receptor is often involved in promoting tumor growth and metastasis by enhancing motility, survival and proliferation of cancer cells and stimulating angiogenesis [31, 32]. During metastasis and stem cell motility membrane blebbing is characteristic and often observed.

Membrane blebs are bubble-like fluid filled protrusions, which enhances cell mobility and amoeboid migration. Membrane blebbing is characteristic for cells undergoing apoptosis [33, 34]. Due to this the research group around Laser-Azogui investigated whether there is a correlation between the Met receptor activity and blebbing. They observed membrane blebbing in high Met expressing breast cancer cells whereas in low expressing breast cancer cell lines no blebbing could be observed. To confirm membrane blebbing is Met-induced they transfected cell lines with constructs encoding for overexpressed fluorescence-tagged Met (FP-Met) and encoding for a dominant-negative fluorescent-tagged Met (DN-FP-Met), respectively. They could show that an overexpression of Met leads to constitutive receptor activation even without its ligand stimulation. Moreover, it could be observed that this constitutive activation leads to enhanced membrane blebbing. In contrast, DN-FP-Met-expressing cells do not show any membrane blebbing even after ligand treatment.

To exclude that in this case membrane blebbing is not related to apoptosis they performed TUNEL assays revealing that, in fact, Met overexpression is the reason for blebbing. Besides, membrane blebbing Met overexpressing cells showed a rounded cell shape enhancing migration ability and invasion. Taken together, Laser-Azogui et al., 2013 results may give a better understanding of cellular and molecular mechanisms in controlling metastatic development, which could be used for future therapy approaches by blocking Met-induced blebbing [35].

Another receptor to influence cancer signaling is the CXCR4 chemokine transmembrane receptor of the G protein-coupled receptor family. It promotes cellular adhesion, chemotaxis via ERK and other pathways by stimulation with its ligand CXCL12. Besides its function in signaling pathways it plays a crucial role in stem cell mobilization and migration and immune reactions [36]. The chemokine ligand CXCL12 activates leukocytes and is important for the formation of large blood vessels. Both are also known to be overexpressed in cancer cells and are associated with cancer proliferation,
metastasis and cell invasion [37]. It was shown that CXCR4 is highly expressed on the surface of T-ALL (T-cell acute lymphoblastic leukemia) cells and therefore the impact of its knockdown was further investigated. Ablation of CXCR4 causes a reduced number of T-ALL cells in all tissues and this leads to the survival of the infected mice. Further in vivo knockdown experiments revealed that CXCR4 plays a key role in T-ALL localization and cell survival [38].

Based on the knowledge that CXCL12 is also highly expressed in the bone marrow and T-ALL cells associate with bone marrow as well, Pitt et al. checked for interaction between CXCL12 components with T-ALL cells. It has been shown immotile and stable association between T-ALL cells with CXCL12 through immunofluorescence staining in mouse bone marrow cells. This is mainly the case in vascular endothelial CXCL12 producing cells. It was demonstrated that an ablation of vascular endothelial cells leads to a reduced number of T-ALL cells. All in all, CXCL12 produced in vascular endothelial cells, as a niche, and CXCR4 play a key role in T-ALL progression. The interruption of the CXCL12 signalling by small molecules could be an important point of application in therapy.

One of these small molecules could be AMD3100 as a promising therapeutic target for the pharmacological inhibition of CXCR4. Pharmacological inhibition with AMD3100 was already demonstrated in MPNST (malignant peripheral nerve sheath tumors) and resulted in arrested proliferation and tumor growth In MPNS tumors receptor activity rely on the presence of CXCL12. CXCL12 is secreted by MPNST cells themselves and triggers specific downstream signalling pathways. CXCR4 impacts the expression of the cell-cycle regulatory gene cyclin D1 via the AKT/GSK-3β cascade. The CXCR4 receptor activates the PI3K/AKT signalling pathway. This results in the inactivation by phosphorylation at Ser9 of GSK-3 by AKT. That leads to an accumulation of β-catenin and activation of the transcription of cyclin D1. In non-deficient cells GSK-3β destabilizes β-catenin by phosphorylation at Ser33/37 and therefore inhibits the elevated expression of cyclin D1 in the cells and consequently proliferation of cells [39].

In a pathway that also participates in tumor proliferation around the CXC receptor 4, IQGAP1 is involved. IQGAP1 (IG motif-containing GTPase-activating protein 1) is a scaffold protein that regulates the actin and microtubule networks. Furthermore, it regulates ERK and gene expression in response to signaling by cell surface receptors [40]. IQGAP1 is required for CXCL12-dependent CXCR4 signal transduction, traffic and recycling of CXCR4 and is also a critical regulator of the
receptor expression. Inhibiting the IQGAP1 function could also be useful for further cancer therapy studies because of its function in cell migration pathways [41].

Based on the examples of these pathways it is reported that targeting the inhibition of specific factors, which leads to an overexpression in cancer, could be a possible alternative therapy for cancer patients. By inhibiting overexpressed pathways this therapy approach could able to be more specific than in conventional therapy methods like chemotherapies and radiation. To explore secondary effects of these specific inhibitors the cellular signal cascades have to be figured out more in detail.
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