The mechanisms that regulate the localization and overexpression of VEGF receptor-2 are promising therapeutic targets in cancer biology

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The vascular endothelial growth factor (VEGF) family has been proposed to be the most important signaling protein family in vessel formation and maturation. VEGF receptor-2 (VEGFR-2) plays an abundant role in the most common forms of cancer. The localization of VEGFR-2 expression is important in cancer pathogenesis; however, so far, little attention has been paid to this phenomenon. Induced cytoplasmic VEGFR-2 transition from the nucleus is associated with poor prognostic cancer stages. Current VEGFR-2-targeted therapy approaches are effective in inhibiting or arresting tumor growth. Moreover, VEGFR-2-targeted therapy was demonstrated to restore the abnormal vasculature in tumors, enhancing their susceptibility toward conventional therapy. Most effects can be found when VEGFR-2-targeted therapy inhibits not only the induced angiogenesis but also the cancer cells that sometimes overexpress VEGFR-2. Nevertheless, we still have little knowledge about the mechanisms that regulate VEGFR-2 expression and how its localization is exactly involved in cancer prognosis. Further research and evaluation of VEGFR-2 regulation and its nuclear transition is necessary to develop more accurate therapeutic strategies to improve the patients' quality of life and their survival. *Anti-Cancer Drugs* 23:347–354 © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2012, **23:**347–354

Keywords: angiogenesis, cancer therapy, cancer prognosis, kinase insert domain receptor, vascular endothelial growth factor receptor-2

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Received 12 October 2011 **Revised form accepted** 5 December 2011

Introduction

Vascular endothelial growth factor (VEGF) signaling is important for the human system. VEGF is necessary for the formation of blood vessels and their extension. The activated VEGF signaling pathway induces angiogenesis, vasculogenesis, and lymphangiogenesis. VEGF induces proliferation and migration of endothelial cells to form new vessels by binding to VEGF receptors on lymphatic and vascular endothelial cells [1,2]. These innocent and 'normal' human mechanisms become a patient's worst enemy when he or she is suffering from cancer. VEGF plays a turbulent role in human cancers by enhancing the blood vessel formation, which provides the tumor with all the nutrients that are important for growth and eventually results in tumor progression [3]. Cancer cell-induced angiogenesis is a prominent target in current treatment strategies.

The human VEGF family consists of VEGF-A, VEGF-B, VEGF-C, and VEGF-D. They can bind to one or more VEGF receptors: VEGFR-1, VEGFR-2, and/or VEGFR-3. Most of the VEGF family members can bind to VEGFR-2. Signaling through VEGFR-2 (i.e. kinase insert domain receptor) can increase angiogenesis [3,4]. The activated form of VEGFR-2 can induce intrinsic downstream signaling, which is important in cancer pathogenesis (Fig. 1). VEGFR-2 can activate downstream PI3-kinase to increase cell survival. The receptor can also activate the MEK/Erk pathway to enhance cell proliferation. These signal transduction routes are frequently overactivated signaling pathways that regulate and induce cancer development [5].

The purpose of this study was to gain more insight into the importance of VEGFR-2 signaling in cancer pathogenesis and to evaluate its role in a broader perspective. Analyzing current literature and investigating promising important aspects of VEGFR-2 signaling may reveal possible new targets for cancer therapy approaches.

Vascular endothelial growth factor receptor-2 overexpression in solid tumors

VEGFR-2 signaling has an important role in various cancers. VEGFR-2 overexpression in vascular endothelial cells mediates tumor formation. In some cases, the cancer cells also (over)express VEGFR-2 on their cell membrane, with prognostic significance [4]. Urothelial tumors demonstrate VEGFR-2 expression in 50% of patients with bladder cancer [6]. Increased VEGFR-2 expression correlates with the stage of disease and the invasiveness of the phenotype. Hepatocellular carcinomas also show a correlation between high VEGFR-2 expression and prognostic significance [7]. High VEGFR-2 expression is significantly related to a large tumor diameter, poorly

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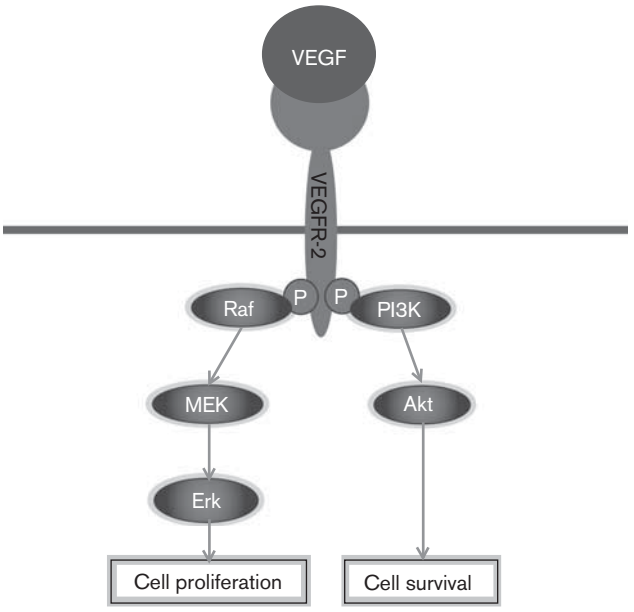
Fig. 1



VEGF/VEGFR-2 signaling pathway. VEGF binding to VEGFR-2 phosphorylates the membrane receptor and activates intrinsic downstream signaling that can induce the Raf/MEK/Erk pathway and the PI3-kinase/Akt pathway. These induced signaling pathways regulate cell proliferation and survival of VEGFR-2-expressing endothelial cells and cancer cells. VEGF, vascular endothelial growth factor; VEGFR-2, vascular endothelial growth factor receptor.

differentiated tumors, and short survival. Normal ovarian epithelial cells display little or no VEGFR-2 expression, whereas ovarian cancer cells show VEGFR-2 expression in 85% of patient samples [8]. VEGFR-2 overexpression is also observed in malignant pleural mesotheliomas (MPMs) [9]. Both the epithelioid as well as the sarcomatoid MPMs demonstrate higher levels of VEGFR-2 compared with normal lung tissue. VEGFR-2 expression is strong in epithelioid MPMs and moderate in sarcomatoid MPMs. Furthermore, VEGFR-2 has been proven to be important in the pathogenesis of colorectal cancer [10]. Expression of the activated form of VEGFR-2 in colorectal cancer cells is associated with tumor size and poor histological differentiation. Phosphorylated (activated) VEGFR-2 (pVEGFR-2) expression was mainly centered at the invading tumor edge.

The occurrence of cancer metastasis also provides some evidence of involvement of VEGFR-2 overexpression. Patients with metastatic medullary thyroid carcinomas are indicated with a poor prognosis. The overexpression and activation of VEGFR-2 is related to occurrence of metastasis in patients with medullary thyroid carcinoma [11]. The development of metastasis in pediatric patients similarly associates with VEGFR-2 expression levels. The occurrence of metastasis in pediatric patients with sarcomas, central nervous system tumors, and lymphomas correlates with high levels of circulating VEGFR-2-positive bone marrow-derived progenitor cells [12].

To date, only a few publications have focused on VEGFR-2 gene aberrations and its role in cancer progression. VEGFR-2 copy number gains are detected in 32% of non-small-cell lung cancer tumors [13]. The abnormally high VEGFR-2 copy number state is significantly associated with higher VEGFR-2 protein expression levels, a higher microvessel density, and an increased risk of death. Gene expression analysis of glioblastomas demonstrated that alterations in the VEGFR-2 gene are present in 3.3% of glioblastomas without prognostic relevance to the overall survival rates [14]. Although VEGFR-2 has proven to be important in various cancers, there are also forms of cancer in which VEGFR-2 plays no evident pathogenic role. Gastric cancer patients with VEGFR-2 expression in tumor tissue showed similar survival rates compared with patients who did not express VEGFR-2 [15].

Nonhematological tumors that arise in the spleen, the lungs, and the genitourinary and gastrointestinal system frequently demonstrate VEGFR-2 overexpression. The level of VEGFR-2 overexpression in tumors is sometimes associated with the aggressiveness of tumor cells and their capacity to metastasize. The role of VEGFR-2-related single-nucleotide polymorphisms, gene mutations, and copy number gain analysis in the pathogenesis of cancer requires attention and might reveal promising mechanisms responsible for VEGFR-2 overexpression.

VEGFR-2 overexpression in hematological malignancies

Hematological malignancies are rare malignant disorders that account for less than 10% of deaths from malignancies. Nevertheless, VEGFR-2 overexpression is also involved in hematological malignancies. The bone marrow of patients with multiple myeloma demonstrates induced levels of pVEGFR-2 in 36.8% of cases [16]. Leukemia is known to demonstrate increased angiogenesis in the bone marrow [17]. This enhanced angiogenesis is associated with the patient's outcome. Bone marrow biopsies from acute myeloid leukemia patients show significantly higher VEGFR-2 expression levels compared with normal bone marrow controls [18].

VEGFR-2 overexpression plays an important role in lymphomas. The neoplastic lymphocytes in B-cell non-Hodgkin's lymphomas show a significant upregulation of pVEGFR-2 [19]. A significantly higher expression of pVEGFR-2 was observed in diffuse large B-cell lymphomas (DLBCL) compared with follicular lymphomas (FLs). Moreover, diffuse VEGFR-2 expression in FLs is associated with a higher risk of aggressive histological transformation into DLBCL [20]. In addition, VEGFR-2 is shown to predict poor overall survival and progression-free survival for patients with DLBCL who have been treated with immunochemotherapy [21].

Similar to solid tumors, hematological cancers demonstrate frequent VEGFR-2 overexpression. VEGFR-2 overexpression

might also contribute to aggressive transformation of cancer cells in hematological malignancies.

Significance of VEGFR-2 overexpression in cancer

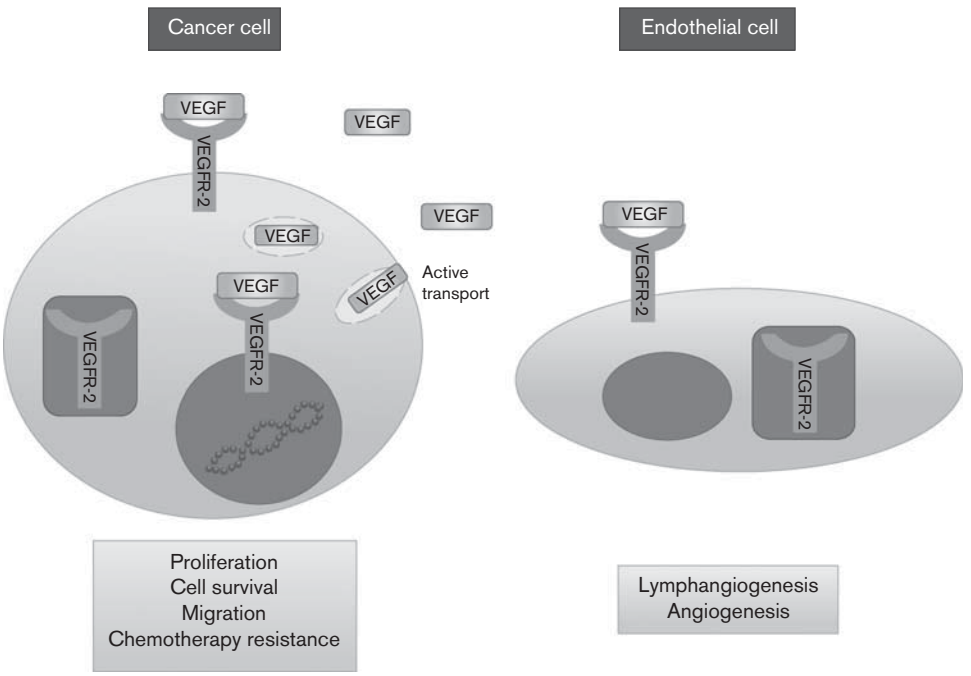
The relevance of VEGFR-2 overexpression in cancer cell biology is not similar across all cancers. In contrast, a basal process that is common in all cancers is the induced vessel formation mediated through paracrine signaling pathways. In addition, cancer cells that overexpress VEGFR-2 are reported to stimulate themselves through autocrine signaling mechanisms. The autocrine VEGF/VEGFR-2 signaling pathway enables the cancer cells to produce VEGF that they can bind to in order to activate their own VEGFR-2. This will result in intrinsic downstream signaling to regulate cancer cell proliferation, cell survival, and chemotherapy resistance (Fig. 2). This activated autocrine signaling pathway is found in breast cancer, acute myeloid leukemia, ovarian cancer, glioblastoma, and so on [22–26]. The VEGFR-2-targeted therapy in these cancers might work synergistically in reducing the angiogenesis parallel to blocking the proliferative and survival advantages of these autocrine signaling cancer cells. VEGFR-2 is an important protein in many cancers and has shown to be significant in cancer prognosis and pathogenesis. Nevertheless, little research has been conducted on VEGFR-2 gene aberrations and differences in VEGFR-2 expression patterns in these cancers. Unraveling these VEGFR-2 features provides more insight into the role of VEGFR-2 in cancer.

VEGFR-2 localization involvement in cancer pathogenesis

Does the localization of VEGFR-2 indicate anything about its role in cancer pathogenesis? VEGFR-2 consists of an extracellular domain (ligand-binding domain), a transmembrane domain, and an intracellular domain (catalytic domain). VEGFR-2 expression can be detected on the cell membrane, the nucleus, or, when VEGFR-2 is internalized, in the cytoplasm within the endosomes. Moreover, the internalized VEGFR-2 retains its signaling activity [1,24].

VEGFR-2 plays a role in the proliferation and migration of breast cancer cells through its tyrosine kinase activity. VEGFR-2 is localized within the cytoplasm in breast cancer cells [27]. In addition, only the invasive lobular breast carcinomas demonstrate nuclear VEGFR-2 staining, which is not found in invasive ductal carcinomas. The VEGFR-2 that migrated to the nucleus might not retain

Fig. 2



VEGF/VEGFR-2 autocrine and paracrine signaling. VEGFR-2 localization is important for its function. Extracellular binding of VEGF ligands to VEGFR-2 on cancer cells as well as on endothelial cells induces phosphorylation of the receptor, which results in the intracellular activation of signal transduction cascades. Internalization of VEGFR-2 allows endosomal transition to either recycle or degrade the receptor. Intrinsic VEGF/VEGFR-2 activation at the nucleus has been found in cancer cells and has been shown to induce signal transduction cascades as well. In cancer cells the intrinsic activation leads to increased proliferation, migration, cell survival, and sometimes chemotherapy resistance. In endothelial cells the intrinsic activation results in enhanced proliferation and migration, which leads to lymphangiogenesis and angiogenesis. VEGF, vascular endothelial growth factor; VEGFR-2, vascular endothelial growth factor receptor.

its proliferative and promigratory activity. Lobular breast carcinoma patients do have slightly better survival rates compared with ductal breast carcinoma patients [28].

In the colorectum, we can observe different stages of neoplasms: hyperplastic polyps, serrated adenoma, and adenocarcinoma of the colorectum [29]. These neoplasms show VEGFR-2 expression in 17% of hyperplastic polyp patients, in all of the serrated adenomas, and in all adenocarcinomas. VEGFR-2 could be detected at the membrane and in the cytoplasm of serrated adenomas and adenocarcinomas. Differences in vessel counts were not observed between groups, which might indicate that the VEGFR-2 pathway has tumor growth and progression effects that are angiogenesis independent. In this study, they did not focus on differences between cytoplasmic and membrane staining patterns in relation to cancer progression. This distinction is unfortunately absent in most studies.

VEGFR-2 immunohistochemical analysis revealed that there are different VEGFR-2 expression patterns in melanocytic tumors of the skin [30]. The vast majority (90%) of melanomas presented VEGFR-2 expression. In-situ and microinvasive melanomas showed a nuclear membrane-like VEGFR-2 expression pattern, whereas invasive melanomas displayed a combined cytoplasmic and nuclear VEGFR-2 expression pattern. The additional cytoplasmic localization, besides the nuclear expression, is associated with progression toward invasive melanoma.

In the lymph nodes of B-cell non-Hodgkin's lymphoma patients, the expression pattern of pVEGFR-2 was compared with those in normal lymph nodes. The expression of pVEGFR-2 in normal lymph nodes is very low and demonstrated cytoplasmic localization in 26% of the nodes [14]. FLs are slowly growing indolent lymphomas with a median survival of around 10 years. DLBCLs are more aggressive lymphomas that demonstrate poor survival rates. The activated VEGFR-2 expression pattern seems very different between groups. Overall, the pVEGFR-2 expression was detectable in 82% of DLBCL patients and in 74% of FL patients. Moreover, the lymph nodes of DLBCL patients showed increased cytoplasmic pVEGFR-2 localization in comparison with the lymph nodes of FL patients. DLBCL patients demonstrated increased numbers of lymph nodes with very high cytoplasmic pVEGFR-2 expression.

In all these studies, we can observe that induced VEGFR-2 localization in the cytoplasm is more pronounced in poor prognostic patients. VEGFR-2 localization in the cytoplasm is important for autocrine intrinsic cell signaling mechanisms that regulate cell proliferation, migration, and survival functions. The significance of VEGFR-2 localization and trafficking in cancer pathogenesis needs to be taken into account for future research and for the development of new therapeutic approaches.

VEGFR-2 regulation in cancer

There is little knowledge about the mechanisms that regulate VEGFR-2 expression. One of the regulators described in epithelial ovarian carcinoma cells is glucose [25]. Ovarian cancer cells use an autocrine VEGF/VEGFR-2 loop. Glucose deprivation seems to reduce the VEGFR-2 protein synthesis and glycosylation through degradation mechanisms. Glucose levels in the blood are dependent on a patient's diet. This can be taken into consideration during patient treatment periods. Leptin is a small nonglycosylated protein that is also dependent on the energy balance of its microenvironment. Leptin was described to increase VEGFR-2 expression levels in endometrial cancer cells *in vitro* and in breast cancer cells *in vitro* and *in vivo* [22].

Hypoxic areas can be frequently found within tumors. Moreover, the hypoxic areas in tumors are a common cause of treatment failure for chemotherapy and radiotherapy. The leaky abnormal vessels in tumors result in inadequate oxygen supply, which leads to the formation of hypoxic areas [3]. Moreover, these hypoxic conditions reduce the cancer cell sensitivity toward chemotherapeutics. Hypoxia is a local environmental condition that regulates VEGFR-2 expression. Hypoxia induces VEGFR-2 expression in endothelial cells, sarcomas, cancer stem cells, and so on [31–34].

Another compound that has been proposed to regulate VEGFR-2 expression is EMMPRIN [35]. EMMPRIN is an extracellular matrix metalloproteinase inducer that is expressed on the surface of tumor cells [36]. EMMPRIN was found to upregulate VEGFR-2 expression in primary melanoma cells. These upregulated VEGFR-2 levels by EMMPRIN were demonstrated to mediate melanoma cell migration, proliferation, and apoptosis. The upregulation of VEGFR-2 by EMMPRIN was induced through increasing levels of hypoxia inducible factor 2α.

Recently, it was shown that the *hSulf-1* gene regulates VEGFR-2 expression as well [37]. HSulf-1 expression is often downmodulated in cancer cells [38]. Downregulation of hSulf-1 upregulates the activation of VEGFR-2. The *hSulf-1* gene exhibits antiangiogenic activity in tumors. Moreover, it was demonstrated that *hSulf-1* gene and protein expression is decreased by hypoxia through hypoxia inducible factor 1α in breast cancer cells [39].

The most important mechanism that regulates VEGFR-2 expression in cancer cells seems to be the microenvironment. Glucose and hypoxia are environmental conditions that upregulate the VEGFR-2 expression. These stress-induced conditions enhance the cell survival mechanisms in cancer cells, in which VEGFR-2 signaling plays an important role. The microenvironment is known to be responsible for influencing the fate of cancer cells and reversing the cancerous properties of metastatic cancer cells [40]. The influence of the microenvironment on

Table 1 Overview of VEGFR-2-targeted therapeutics tested in clinical trials for the treatment of cancer

Therapeutic	Targets	Forms of cancer
1. IMC1121b, i.e. ramucirumab	VEGFR-2	Leukemia, breast cancer, liver cancer, nonsmall lung cancer, ovarian cancer, renal cancer
2. XL184, i.e. cabozantinib	VEGFR-2, HGFR, RET, GDNFR	Breast cancer, lung cancer, glioblastoma, nonsmall lung cancer, medullary thyroid cancer
3. ZD6474, i.e. vandetanib	VEGFR-2, EGFR	Breast cancer, lung cancer, head and neck cancer, glioblastoma, transitional cell carcinoma, colorectal cancer
4. PTK787/ZK222584, i.e. vatalanib	VEGFRs, PDGFRs, SCFR, M-CSFR	Leukemia, breast cancer, multiple myeloma, colorectal cancer, pancreatic cancer/adenocarcinoma, hemangioblastoma, hepatocellular carcinoma, glioblastoma, lung cancer, gastrointestinal stromal tumors, neuroendocriene tumors, mesothelioma
5. SU5416, i.e. semaxanib	VEGFR-2, SCFR, FLT-3	Prostate cancer, colorectal cancer, breast cancer, kidney cancer, ovarian cancer, head and neck cancers, cervical cancer, central nervous system tumors, sarcomas, leukemia, melanoma, myeloma

EGFR, epidermal growth factor receptor; FLT-3, fms-like tyrosine kinase receptor-3; GDNFR, glial cell-derived neutrophic factor receptor; HGFR, hepatocyte growth factor receptor; M-CSFR, macrophage stimulating factor receptor; PDGFR, platelet-derived growth factor receptor; RET, rearranged during transfection; SCFR, stem cell factor receptor; VEGFR-2, vascular endothelial growth factor receptor.

cancer cell behavior has become an important aspect of cancer research.

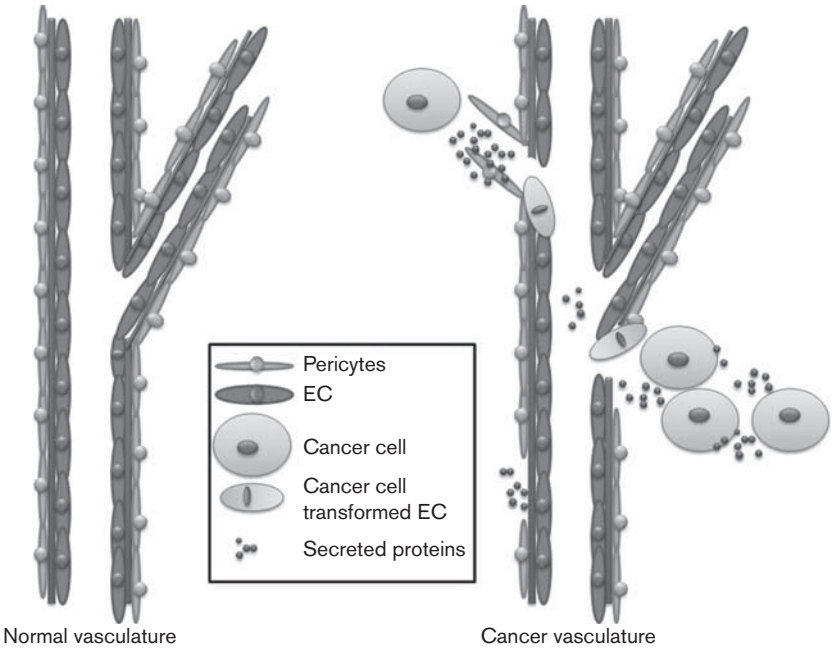
VEGFR-2-targeted cancer therapy

VEGFR-2 is a prominent player in vascular endothelial cell signaling for the enhancement of tumor angiogenesis and cancer development. VEGFR-2 is expressed on endothelial tip cells that guide the formation of angiogenic sprouts [41]. This implicates the need of VEGFR-2 at the early stage of disease to accomplish full growth of vascular networks. In contrast to the enhanced vessel formation, the appearance of VEGFR-2 overexpression in cancer cells is a frequent observation. This indicates a dual function for VEGFR-2-targeted therapy. VEGFR-2-targeted therapy seems to be an effective adjuvant therapy approach along with conventional therapy in many cancers. Normal tissue demonstrates low expression of VEGFR-2, which indicates that VEGFR-2-targeted therapy is an attractive therapeutic approach. There is a range of VEGFR-2-targeted therapeutics reported to reduce tumor growth, cancer cell proliferation, and cancer cell survival [42–45]. Table 1 displays VEGFR-2-targeted therapeutics, their selectivity, and in which type of cancers they have been investigated, according to the US National Institute of Health clinical trials [46–67]. IMC1121b is the only anti-VEGFR-2 monoclonal antibody that is used in clinical trials. XL184, ZD6474, PTK, and SU5416 are all multitargeted tyrosine kinase inhibitors that focus on VEGFR-2 as one of its targets. Multitargeted kinase inhibitors block many important membrane proteins and are described to be quite successful in the treatment of several cancers [68]. YN968D1 is a very new multitargeted kinase inhibitor, which demonstrated promising results in a preliminary patient study [69]. VEGFR-2-targeted therapeutics demonstrate promising results by arresting tumor growth and sometimes by reducing the tumor size by changing the vasculature to a more normalized situation. The normal/healthy-appearing vascular networks demonstrate smooth regular vessels that, in the case of cancer progression, develop into highly vascular networks of irregular structure that in most cases show some leakiness [70]. Figure 3 represents how the normal vasculature is changed in cancer. An important role of VEGFR-2-targeted therapy is to normalize the vasculature so that conventional therapy can reach its destination more accurately and thereby reduce tumor growth. Anti-VEGFR-2-targeted therapy is effective in reducing the number of vessels and in restoring their normal structure *in vitro* and *in vivo* [9,45]. Blocking or reducing angiogenesis relies on the inhibition of vascular endothelial cells [71]. One of the VEGFR-2-targeted therapeutics, SU5416, causes endosomal accumulation of VEGFR-2 with subsequent VEGFR-2 degradation in endothelial cells. The induction of VEGFR-2 degradation diminishes its tyrosine kinase activity, making the cells more vulnerable to chemotherapy.

VEGF has been shown to protect cancer cells from proceeding to apoptosis/cell death upon chemotherapy exposure. When cancer cells express VEGFR-2 and use this receptor to induce autocrine VEGF signaling, VEGFR-targeted therapy will immediately affect the cancer cells as well. In leukemia, we can see that upon VEGFR-2 inhibition the phosphorylation of VEGFR-2 is downregulated, resulting in apoptosis of leukemic cells. Furthermore, VEGFR-2 antibody treatment (IMC1121b) was shown to induce overall survival in a leukemic mouse model [52]. In ovarian cancer, in-vitro analysis demonstrated that VEGFR-2 antibody treatment (IMC1121b) reduced ovarian cancer cell migration and invasion by 70% [8]. Moreover, IMC1121b treatment significantly reduced in-vivo tumor growth. This reduced tumor growth is correlated to an increase in tumor cell apoptosis, a decrease in tumor cell proliferation, and a lower microvessel density. These results clearly demonstrate how the cancer cells are vulnerable to anti-VEGFR-2-targeted therapy and are then more susceptible to conventional therapy when they overexpress VEGFR-2.

VEGFR-2-targeted therapy may hold antiangiogenic properties only when cancer cells do not overexpress VEGFR-2, whereas in others it might inhibit the autocrine VEGFR-2 cancer cell signaling as well. Overall,

Fig. 3



Normal vessel structure versus cancer vasculature. The normal vasculature changes upon cancer progression. Increased angiogenesis is found in almost all cancers. The increase in angiogenesis is associated with an increased number of vessels and vascular sprouting and also demonstrates that the structure of the vessels has changed; larger vessels, irregular vessels, and leaky vessels. The cancer cells secrete proteins that stimulate endothelial cells (EC) to proliferate and migrate to induce the vessel formation from preexisting vessels. Cancer cells have been described to transform into endothelial-like cells, supporting the aberrant vessel formation.

the best results in clinical trials are obtained when anti-VEGFR-2-targeted therapy is combined with chemotherapy and/or radiation therapy.

Conclusion

VEGFR-2 overexpression plays a significant role in cancer cell biology. VEGFR-2 overexpression in cancer cells is a frequent observation in the most common forms of cancer, whereas normal tissue displays only marginal expression of VEGFR-2. Moreover, the level of VEGFR-2 overexpression is often related to the stage of disease and the patient's prognosis. The location of VEGFR-2 expression was shown to be relevant to the patient's prognosis as well. VEGFR-2 expression in cancer cells that resides within the cytoplasm is shown to be more pronounced in cancer patients with poor prognosis. The role of VEGFR-2 localization in cancer pathogenesis is an important feature that needs further evaluation, identification, and characterization for better understanding of the biological consequence of VEGFR-2 trafficking in cancer cells. The regulation of VEGFR-2 expression is dependent on its microenvironment. Glucose and hypoxia are important microenvironmental conditions that upregulate the expression of VEGFR-2 in cancer cells.

Induced angiogenesis is a common pathological condition in cancer patients and a prominent target in current therapeutic approaches. VEGFR-2-targeted therapy targets the induced angiogenesis as well as the cancer cells that overexpress VEGFR-2. Normal tissue is only slightly affected by VEGFR-2-targeted therapy. This therapy is most effective in combination with conventional therapy. Overall, VEGFR-2 is important for cancer progression through regulation of optimal microenvironment cancer cell conditions. It is very important to unravel the mechanisms that regulate VEGFR-2 expression, localization, and trafficking to provide substantial information necessary to improve VEGFR-2-targeted cancer therapeutic strategies in the future.

Acknowledgements

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

Conflicts of interest

There are no conflicts of interest.

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