Tumor Angiogenesis in Breast Cancer: Clinical Implications

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Abstract

Angiogenesis, the formation of new blood vessels from pre-existing ones represents a critical aspect of tumor progression and metastasis. Because inhibition of angiogenesis represents a major approach to cancer treatment, the development of inhibitors of angiogenesis is a major challenge. Therefore, inhibition of angiogenesis is an attractive strategy for treatment of cancer. Currently, the most promising approach has been the use of bevacizumab, a humanized monoclonal antibody directed against the most potent pro-angiogenic factor, vascular endothelial growth factor (VEGF). In metastatic breast cancer its use has shown enhanced response rates and prolonged progression-free survival, unfortunately without overall survival benefit. Small molecular inhibitors of VEGF tyrosine kinase activity or inhibitors of mammalian target of rapamycin (mTOR) in breast cancer has to be defined. Several unanswered questions remain, such as choice of drug(s), optimal duration of therapy and patient selection criteria, thus there is an urgent need for a more rational use of anti-angiogenic based treatment with greater insight into predictive factors for toxicities, therapy efficacy, and clinical benefit.

Keywords: Angiogenesis; Inhibitors; Breast cancer

1. The Tumour Angiogenic Process

The ability of solid tumours to sustain growth beyond a few millimetres (2-3 mm) in size is dependent on their capacity to acquire nutrients and oxygen and to dispose of metabolic waste products and carbon dioxide through the formation of new blood vessels (Kerbel, 2008) (Carmeliet, 2001). This process, termed angiogenesis is considered one of the essential hallmarks underlying cancer development and metastasis (Hanahan & Weinberg, 2000). Classically, there are 2 distinct types of angiogenesis that have been described: the sprouting process, which involves branching of new blood vessels from pre-existing blood vessels and the non-sprouting angiogenesis, which involves the splitting of a lumen of an existing vessel (Fox & Harris). Recently, other mechanisms of tumour vascularization have been discovered including the recruitment of endothelial progenitor
There is an elevated number of signalling pathways involved in tumour angiogenesis which represent an unbalanced expression of angiogenic factors and inhibitors within the tumour (Table 1) (Weis & Cheresh, 2011) and they play into the multiple steps of the angiogenic process.

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<tr>
<th>Pro-angiogenic Factors</th>
<th>Anti-angiogenic Factors</th>
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<tr>
<td><strong>Growth Factors and growth factor receptors</strong></td>
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<td>Angiogenin</td>
<td>Angiostatin,</td>
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<td>Epidermal growth factor (acid and basic)</td>
<td>Endostatin,</td>
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<td>Fibroblast Growth Factor (FGF) and FGF</td>
<td>Vasostatin</td>
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<td>Granulocyte colony-stimulating factor</td>
<td>Chemokines/Chemokine receptors</td>
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<td>Hepatocyte growth factor</td>
<td>Vascular Endothelial Growth Factor inhibitor</td>
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<td>Insulin Growth Factor (IGF) and IGFR</td>
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<td>Tumor necrosis factor α</td>
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<td>Vascular Endothelial Growth Factors: VEGF-A, B, C, D</td>
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<td>Scatter factor</td>
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<td>MIG (monokine induced by interferon γ),</td>
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<td>Tumour Necrosis Factor- α,</td>
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<td>Fragment of Platelet Factor-4,</td>
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The majority of anti-angiogenesis treatments are currently tailored toward the sprouting biology of angiogenesis. Numerous agents that are currently in clinical practice and other undergoing clinical development aim to interfere with signals promoting angiogenesis and therefore angiogenesis is currently one of the most promising avenues of investigation in the study of tumour biology. Based on the complexity of the tumour angiogenetic process, the optimal treatment strategies would target multiple steps of the angiogenic process with widespread applicability, low potential toxicity, and possibly a synergistic effect combined with classical cytotoxic therapy and radiotherapy.

In most cases anti-angiogenic therapy is combined with other types of treatments against cancer.
such as radiation and chemotherapy. This combination is believed to give better results compared to only one type of treatment. However, the chemotherapy, needs to be delivered directly to the target (tumour) via blood flow. At the same time the patient is treated with agents to inhibit tumour vasculature growth by decreasing oxygen and nutrients uptake. It seems a paradox but a new concept in anti-angiogenic therapy came into view in a paper by Jain (Jain, 2001): he proposed the hypothesis that by trying to normalize the tumour vasculature will positively help different anti-cancer treatments. Developing a normal vasculature we might be able improve the delivery system to get to the tumour cells and in this way we also achieve a sufficient concentration of drugs delivered and taken up by the tumour as also showed in clinical setting (Jain, 2005), (Willett et al, 2004).

However, while highly specific inhibition can be advantageous in targeting only the specific signalling transduction cascade, it can also mean modest outcome in the clinical setting especially when complicated processes such as angiogenesis, known to have signalling redundancy, are involved. Therefore, other strategies for anti-VEGF treatment potentially include using tyrosine kinase inhibitors (TKIs), soluble receptors, anti-sense oligonucleotides, and RNA interferences.

2. The Anti-Angiogenic Strategies

Fig 1. Schematic cartoon demonstrating the angiogeneic inhibitors and their targets. ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3’ kinase; PKB, protein kinase B

There are two main classes of such approved drugs: the monoclonal antibodies, e.g. bevacizumab and oral small molecule as Tirosyne Kinase Inhibitor (TKI)s, e.g. sunitinib (Sutent®), sorafenib (Nexavar®) or everolimus (Afinitor®) (Figure 1). Whereas bevacizumab binds circulating and local VEGF and hence neutralizes its biologic activity, the TKIs inhibit the intracellular catalytic function of VEGF receptors expressed by vascular endothelial cells, particularly VEGFR-2, the major
signaling receptor for VEGF-mediated (tumour) angiogenesis. The TKIs are not totally specific for VEGF receptors; invariably they also antagonize the function of other kinase domain receptor tyrosine kinases similar in structure to VEGF receptors, e.g. platelet-derived growth factor (PDGF) receptors, c-kit, Flt-3, and Raf is also targeted in the case of sorafenib. It is worthwhile mentioning that also chemotherapy could have anti-angiogenic activity, particularly metronomic therapy [e.g. cyclophosphamide or metotrextate or capecitabine](Kerbel, 2011; Penel et al, 2012).

**Conventional therapy/regimes with anti-angiogenic properties**

Regarding conventional strategies, studies have suggested that several classes of chemotherapeutic drugs have anti-angiogenic activity *in vitro* or *in vivo*, including several agents that are routinely used in clinical treatment of breast cancer (Miller et al, 2001) as cyclophosphamide, paclitaxel, doxorubicin, tamoxifen or aromatase inhibitors (Bottini et al, 2006; Browder et al, 2000). Paclitaxel, a microtubule inhibitor that is an active agent in the treatment of many different cancers, was shown to possess anti-angiogenic properties independent of its anti-proliferative action in *in vivo* models (Klauber et al, 1997). The weekly regimen as used routinely with paclitaxel could be viewed as ‘metronomic-like’ (see below) and thus may be a factor in the results that were obtained compared to regimens such as once-every-3-week docetaxel plus bevacizumab, i.e., the AVADO trial (Pivot et al, 2011). Among the various approaches to inhibiting angiogenesis, metronomic therapy is now broadly used in treatment cancer. The “metronomic therapy” approach refers to the frequent, even daily, administration of chemotherapy in dose below the maximum tolerated dose, for long period of time, with no prolonged drug-free breaks (Kerbel & Kamen, 2004). Administering chemotherapy in this investigational fashion is thought to cause anti-tumour effects by a variety of mechanisms which include inhibition of angiogenesis (Browder et al, 2000), stimulation of the immune system, targeting HIF-1 expression as well as possibly direct tumour cell targeting (Pasquier et al, 2010).

The phase II trials based on this approach involved a daily oral metronomic methotrexate plus cyclophosphamide (Colleoni et al, 2002) or this chemotherapy drug combined with a targeted agent such as trastuzumab (Orlando et al, 2006) or a selective estrogen receptor antagonists or aromatase inhibitors, e.g. fulvestrant or letrozole (Aurilio et al, 2012; Bottini et al, 2006), or an EGFR antagonist such as erlotinib or bevacizumab (Montagna et al, 2012). However, in order to validate the clinical data obtained so far, there are three other randomized phase III trials, either in the adjuvant or metastatic setting, currently underway evaluating oral metronomic chemotherapy regimens alone or in combination with a targeted agent such as bevacizumab (e.g NCT01589159; NCT00496665; NCT01924078; NCT00925652; NCT01112826; NCT01067989). There are also a number of phase II clinical trials currently underway evaluating the combination of a small molecule anti-angiogenic TKI plus metronomic chemotherapy, including for the treatment of breast cancer ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)).

COX-2 inhibitors suppress growth factor induced angiogenesis in endothelial cells, suggesting that endothelial derived COX-2 is essential in directly regulating angiogenesis (Jones et al, 1999). Emerging data suggest that celecoxbib may cause a time-dependent reduction in circulating angiogenic markers. Considering these properties the combination of anti-angiogenic chemotherapy with a COX-2 inhibitor warrants clinical evaluation (Gasparini et al, 2003).
It is already known that a part specific activity on bone resorption by suppressing the osteoclast activity, pamidronate and zoledronic acid, potent biphosphonates clinically used, could also have a direct anti-tumour activity such as inhibition of tumour cell adhesion, invasion and viability, and anti-proliferative and pro-apoptotic effect, maybe due to presence of nitrogen molecule (Wood et al, 2002). Also in a recent meta-analysis, clodronate, a non-nitrogenous bisphosphonate, have been shown to interfere with the metastatic cascade and may improve the overall survival, bone metastasis-free survival and non-bone metastasis-free (mainly visceral metastases) survival in patients with early breast cancer (Zhu et al, 2013). Validated evidence suggests that bisphosphonates have anti-angiogenic effects (Wood et al, 2002; Foroni et al, 2014). In clinical trials with zoledronate there was a significant reduction of VEGF and PDGF serum levels induced by a single administration in a window of opportunity trial (Santini et al, 2003).

Old and New drugs targeting angiogenesis

Regarding the anti-angiogenic drugs, bevacizumab has been studied in a number of solid tumours and has been approved by the FDA for the treatment of advanced colorectal cancer, non–small cell lung cancer (NSCLC), advanced renal cell cancer, and recurrent glioblastoma multiforme. Bevacizumab is a humanized monoclonal antibody that binds to VEGF-A, preventing it from binding to receptors and activating signaling cascades that lead to angiogenesis. Initial proof of the concept that targeting VEGF-A could inhibit the growth of tumours (despite its having no effect on the growth rate of the tumour cells in vitro) was demonstrated in a mouse model in 1993 using a monoclonal antibody against VEGF-A-121 leading to the clinical development of bevacizumab. The first combination trial in advanced breast cancer was based on capecitabine alone or capecitabine in combination with bevacizumab. Unfortunately, despite the significant ORR in favour to the combination, the primary endpoint of improved Progression Free Survival (PFS) was not achieved (Miller et al, 2007). The higher response rate suggested a potential benefit with bevacizumab, and any improvements in survival may have been masked by the fact that the patients enrolled had been heavily pre-treated. The E2100 based on newly metastatic HER2-negative cancers to receive either paclitaxel or paclitaxel in combination with bevacizumab a significantly higher response rate and improvement in PFS but not the Overall Survival (OS) led to the accelerated approval of bevacizumab in this setting (Miller et al, 2007). The subsequent clinical trials of bevacizumab in metastatic breast cancer have failed to support the encouraging data from E2100. The AVADO trial was a three-arm trial comparing docetaxel as a single agent with docetaxel and bevacizumab at two dose levels: 7.5 mg/kg and 15 mg/kg. A 1-month improvement was observed in PFS favouring the bevacizumab arms, but no difference was observed in OS. The median OS in the control arm was 31.9 months compared with 30.8 months with low-dose bevacizumab and 30.2 months with high-dose bevacizumab. Patients receiving bevacizumab experienced more treatment disruptions (Miles et al, 2010).

The RIBBON-1 trial randomized patients to receive or not receive bevacizumab. The data were analyzed in two distinct chemotherapy groups: “anthracycline-based” or “taxane-based” chemotherapy with or without bevacizumab. A significant improvement in both response rate and PFS was reported when bevacizumab was added to each chemotherapy regimen. However, OS was not improved (Robert et al, 2011).

Bevacizumab was studied in the second-line setting in RIBBON-2. Women with HER2-negative
disease that progressed after first-line therapy were randomly assigned to receive chemotherapy according to the choice of the treating oncologist with or without bevacizumab. With subsequent disease progression, bevacizumab could be added to the third-line regimen. PFS favored the bevacizumab-treated patients but, again, no improvement in OS was observed (Brufsky et al., 2011).

In the neoadjuvant setting, the GeparQuinto trial showed the pathological complete response rates (pCR) were higher for those assigned to bevacizumab: 18.4% vs 14.9% for those receiving chemotherapy alone (P = .04) (von Minckwitz et al., 2012). The same results were obtained into The National Surgical Adjuvant Breast and Bowel Project (NSABP) B40 trial that showed a significant increase in pCR rate which was observed for those women assigned to bevacizumab (34.5% vs 28.2%; P = .02). Consistently higher response rates with bevacizumab added to chemotherapy are reported in all stages of breast cancer. However, clinically meaningful endpoints of improved OS in advanced disease have not been seen. Despite a clear signal of increased efficacy, we have yet to identify a population of women who clearly benefit from bevacizumab.

Based on these data, two additional FDA reviews by the Oncology Drug Advisory Committee ultimately led to the removal of the breast cancer indication for bevacizumab.

The VEGF-Trap as Aflibercept is a soluble fusion protein of some of the human extracellular domains of VEGFR-1 and VEGFR-2 and the Fc portion of human immunoglobulin (Ig) G. Aflibercept binds to both VEGF-A and Placental Growth Factor (PIGF) with an high affinity and essentially renders the VEGF-A and PIGF ligands unable to bind and activate cell receptors (Holash et al., 2002). Aflibercept inhibited tumour growth in xenograft models and blocked all tumour-associated angiogenesis. Aflibercept showed in phase 2 trials on ovarian cancer 41% of patients with stable disease (Holash et al., 2002). In contrast, in metastatic breast cancer it demonstrated a response rate of 5% and the PFS rate at 6 months was only 10% (Holash et al., 2002).

The Receptor Tyrosine Kinase (RTK) inhibitors are particularly useful in treating cancer because of their dual block: oncprotein signal transduction and the downstream angiogenic processes. They also often target more than one type of receptor and affect both ECs and cancer cells because the receptors are expressed on both types of cell (Young & Reed, 2012).

Sunitinib (SU11248) is an orally available compound that inhibits the VEGFR, PDGFR, Flt-3,c-kit and RET (Gan et al., 2009). It plays an important role in metastatic Renal Cell Carcinoma (RCC) or in gastrointestinal stromal tumours (GIST) and it showed to improve the disease progression (Demetri et al., 2006). Unfortunately, it failed to improve the Overall Response rate (ORR) and Progression Free Survival (PFS) in metastatic breast cancer when compared to bevacizumab (Yang et al., 2012). Also Sorafenib (BAY 43-9006) is an oral inhibitor of the intracellular Raf kinase (B-Raf, C-Raf), but it targets also the VEGFR (VEGFR-2 and VEGFR-3), PDGFR, and c-kit (Wilhelm et al., 2008), even it is active in different clinical setting in metastatic breast cancer data from trial named SOLTI-0721 based on sorafenib and capecitabine combination showed an advantage in PS but it was unfortunately accompanied by significantly increased toxicity. The confirmatory phase III trial evaluating this regimen (called RESILIENCE) (Rugo, 2012) involving a lower dose of sorafenib (Baselga et al., 2012) is ongoing.

Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) signaling contributes to many cell processes, including angiogenesis, cell proliferation, survival, and motility, and is initiated by RTK
activation (Engelman, 2009). Up-regulation of the PI3K pathway can increase angiogenesis through multiple pathways, including increasing the levels of Hypoxia Inducible Factor (HIF)-1alpha under normoxic conditions (Jiang et al., 2001; Laughner et al., 2001). Initial evidence that PI3K and AKT were involved in the regulation of angiogenesis in vivo was obtained when constitutively active PI3K and AKT were shown to induce angiogenesis and increase levels of VEGF (Jiang et al., 2001). Inhibitors of the PI3K pathway have been found to decrease tumour angiogenesis and demonstrate HIF inhibition, including rapamycin analogues as temsirolimus (CCI-779) and everolimus (RAD001) (Land & Tee, 2007) (Hudson et al., 2002) (Guba et al., 2002). The blocked angiogenesis is believed to be due at least partially to the inhibition of HIF-1alpha caused by the inhibition of mTOR (Land & Tee, 2007) (Hudson et al., 2002) (Guba et al., 2002).

Clinical trials of temsirolimus and everolimus as single agents demonstrated improved survival in patients with advanced RCC, in metastatic breast cancer and neuroendocrine cancer, leading to FDA approval for this indication.

3. Resistance to the Anti-Angiogenic Treatments

Various mechanisms are thought to underlie the resistance to VEGF blockade observed in some patients with cancer. Understanding the molecular bases of these cancer type-dependent resistance mechanisms against VEGF blockade offers opportunities to improve anti-angiogenic treatment.

Hyoxia induced by anti-angiogenic therapy

VEGF inhibition induced vessel regression leading to hypoxia in tumour tissues; these circumstances favour the induction of a high rate of tumour cell death. However, some cells are hypoxia-tolerant, survive in poorly oxygenated niches and elicit tumour adaptation to anti-angiogenesis. Some reports suggest that the resultant selection of tumour cells renders tumours even more invasive and metastatic (Lu & Kang, 2010). However, contradictory results from preclinical and clinical studies indicate that the concept of cancer aggravation by VEGF blockade is still unproven. Randomized, placebo-controlled phase III studies in 4205 cancer patients did not support a decreased time to disease progression, increased mortality or altered disease progression pattern after cessation of bevacizumab therapy (Smith et al., 2011). In line with this, novel strategies are warranted where tumour cell migration or glycolysis are targeted when such resistance mechanisms develop. Additional targeting of the Hypoxia Inducible Factor-1 (HIF-1) alpha or the Carbonic Anhydrase (CA9), induced by HIF and protecting against acid pH has show synergistic effects in xenograft experiments. Disrupting the pH homeostasis by targeting carbonic anhydrases in hypoxic tumour cells has been proposed as a tumour-selective approach. Drug development efforts have identified a range of compounds with varying selectivity for CAIX. Treatment of mice with CAIX-positive mammary tumours with sulfonamides resulted in significant inhibition of tumour growth and metastasis formation in both spontaneous and experimental models of metastasis. Therefore, it represents an important clinical opportunity for window studies to select appropriate combinations for randomised trials. Up to now, indisulam (chloro-indolyl sulfonamide) is currently in Phase II clinical trials treating various tumour types as renal clear cell carcinoma and metastatic breast cancer (Jones & Harris, 2012).
Cytokine and growth factor up-regulation by therapy

A number of preclinical studies have shown that interfering with the VEGF pathway using antibodies can result in the up-regulation of higher levels of the pro-angiogenic molecule such as the basic fibroblast growth factor (bFGF) (Casanovas et al, 2005), interleukin-8, stromal derived factor-1 (SDF-1), various inflammatory cytokines, etc (Huang et al, 2010; Shojaei et al, 2010). The induction or up regulation of such growth factors may be the consequence of the original anti-angiogenic treatment causing an increase in tumour hypoxia and hence also up-regulating the transcription factor HIF-1, which can act as a master switch to up-regulate several compensatory pro-angiogenic growth factors (Rapisarda & Melillo, 2012; Semenza, 2003). Consequently, targeting HIF-1 or HIF-2 regulated specific molecular mediators of angiogenesis represents a possible strategy to prolong the tumour inhibition caused by an anti-angiogenic drug treatment (Rapisarda & Melillo, 2012).

Microenvironment

Tumour cells and other components of the tumour microenvironment, such as myeloid cells and stromal cells, produce a multitude of pro-angiogenic growth factors, exposing endothelial cells to the essential growth factors needed (Weis & Cheresh, 2011). Inhibition of a single cytokine is therefore in many cases not enough to block the angiogenesis process. Furthermore, pro-angiogenic growth factors can be secreted by various cell populations within a malignant tumour. This is exemplified by VEGF, which is secreted by malignant cells, endothelial cells and fibroblasts within the tumour, indicating that several cell compartments need to be targeted simultaneously to stop VEGF production. Accordingly, if VEGFR-2 is blocked on the endothelium, VEGF can still be produced by tumour cells and cancer-associated fibroblasts to stimulate angiogenesis via VEGFR-1 and VEGFR-3 (Nyberg et al, 2008). Thus, the tumour microenvironment can promote drug resistance in a passive way, by preventing penetration of drugs into the tumour or in an active way by secreting protective cytokines or changing gene transcription within the tumour cells to override the cytotoxic effects of anti-cancer agents (Meads et al, 2008).

Tumour-associated Endothelial Mutation (TEM) biology

A major controversy in the field of angiogenesis inhibition is whether tumour-associated endothelial (TE) cells harbor mutations that will contribute to drug resistance. At present, data in support of such a phenomenon are limited (Engelman, 2009). Therefore, endothelial genetic alterations are probably not the basis for drug resistance, and they should retain a partial sensitivity to anti-VEGF therapy. This is supported by a recent clinical study showing that bevacizumab had a significant therapeutic effect when added to chemotherapy in second-line treatment, beyond progression on bevacizumab in the first-line setting (Bennouna et al, 2012). This could imply that the drug resistance develops against the chemotherapeutic agent(s) which bevacizumab is combined with, and that a therapeutic benefit from anti-VEGF treatment can still be derived if another type of chemotherapy is added in the second-line setting.

Another mechanism relates to vascular phenotype as the presence of subtypes of tumour-associated blood vessels that are refractory to VEGF deprivation. Dvorak's group has described the extensive heterogeneity of the tumour vasculature, there being six major types of tumour-
associated blood vessels, which can be broadly classified into ‘early’ versus ‘late’ vessels. Late vessels appear to be insensitive to VEGF inhibition (Sitohy et al., 2011), in contrast to the ‘early’ vessels. However such ‘resistant’ vessels may be sensitive to anti-angiogenic attack using other types of anti-angiogenic drugs or vascular targeting therapeutic strategies. Thus determining the heterogeneity and composition of blood vessels in human breast cancer may help clinically the treatment decision making.

**Epithelial-to-Mesenchymal Transition (EMT)**

If the neovascularization process is halted, cancer cells can grow along pre-existing blood vessels to obtain adequate oxygen and nutrient supply, so-called vascular co-option (Rubenstein et al., 2000). Another avoidance mechanism has been observed in malignant brain and pancreatic tumours where angiogenesis inhibition switches the cancer from solid tumour growth into diffuse infiltrative growth to obviate the need for neovascularization (Keunen et al., 2011). This invasive phenotype seems related to epithelial-to-mesenchymal transition (Foroni et al., 2012). A crucial mechanism by which carcinoma cells enhance their invasive capacity is the dissolution of intercellular adhesions and the acquisition of a more motile mesenchymal phenotype as part of an epithelial-to-mesenchymal transition (EMT). Up-regulated expression of these EMT-activating transcription factors such as ZEB, Snail or Twist families promotes tumor invasiveness in cell lines and xenograft mice models and has been associated with poor clinical prognosis in human cancers. Evidence accumulated in the last few years indicates that EMT related-factors also regulate an expanding set of cancer cell capabilities beyond tumor invasion. Thus, EMT related-factors have been shown to cooperate in oncogenic transformation, regulate cancer cell stemness, override safeguard programs against cancer like apoptosis and senescence, determine resistance to chemotherapy and promote tumor angiogenesis(Sanchez-Tillo et al., 2012).

**4. Conclusions**

Angiogenesis plays a critical role in the local growth and metastasis of many different solid tumours. Development of drugs targeting angiogenesis is in progress. However, up to now, the efficiency of the angiogenesis inhibitors clinically used has so far not resulted in drugs which completely and permanently interrupt neo-vascularization and although, clinical benefits where obtained in treatment of various cancers such as renal cancer or colon cancer, concern by oncologists arose at the evidence of the clinical observation of resistance to anti-angiogenic therapy and subsequent tumour re-growth during or after treatment. Results of ongoing trials and maturation of the published trials will hopefully lead to more precise knowledge, and thus more cost-effective use of recent developments. Identification of predictive biomarkers and improvement of our understanding of molecular mechanisms is fundamental in daily clinical practice. In the future, tailored treatments based on dynamic assessment of response should result in individualised patient therapy and improved progression free and survival free outcomes, as well as being more cost effective and importantly reduce unnecessary toxicity in patients and promote more a more cost-effective treatment.
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