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Landscape and Targeting of the Angpt-Tie System in Current Anticancer Therapy

LYu Sun[1-4]* and Boyi Zhang[4]

[1]Key Lab of Stem Cell Biology, Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and Shanghai Jiaotong University School of Medicine, Shanghai, China 200031
[2]Collaborative Innovation Center of Systems Biomedicine, Shanghai Jiaotong University School of Medicine, Shanghai, China 200240
[3]VA Seattle Medical Center, Seattle, WA, USA 98108
[4]University of Washington, Department of Medicine, Seattle, WA, USA 98195

Abstract

Strategies using angiogenesis inhibitors to suppress tumor development via interfering with vascular signaling have been explored for more than a decade. To date, anti-angiogenenic agents such as bevacizumab, sorafenib and sunitinib are incorporated into standard administration to treat many cancer types by targeting the vascular endothelial growth factor A (VEGFA) pathway. However, only mild and transient responses were observed in most cases, raising the topic of drug resistance as one of the major problems that is frequently encountered by clinicians. The angiopoietin (Angpt)-Tie system, previously considered as a second line pathway in tumor angiogenic switch that functionally contributes to angiogenesis and/or lymphangiogenesis, recently attracted substantial interest and is entering the spotlight for meticulous studies in cancer biology. Agents specifically suppressing Angpts are now in clinical trials, and new reports suggest promising anti-cancer activities with a safety profile distinct from those of anti-VEGFA therapeutics. This review provides an updated picture of the Angpt-Tie system, by focusing on the Angpt-Tie2 axis in basic research and clinical investigation, and discusses feasible options to therapeutically target this signaling pathway in the context of precision medicine.

Keywords: Angpt-Tie system; Angiogenesis; Tumor microenvironment; Therapeutic strategy; Precision medicine

Introduction

The family of angiopoietins (Angpts) consists of extracellular proteins that cooperate with VEGFs to regulate vascular and lymphatic vessel growth, by transducing signals via the receptor tyrosine kinase Tie2, and sometimes involving Tie1 [1-4]. Members of the Angpt family, especially Angpt1 and Angpt2, play crucial roles in not only vascular angiogenesis [4-6], lymphangiogenesis [7], inflammation [8,9], but also cancer cell behaviors including invasion and metastasis [10,11]. Biological functions of Angpt family members are unique and highly universal; that is, all proteins bind with similar affinity to the cell surface receptor Tie2, which is expressed by both endothelial cell and cancer cells [1]. Recent studies reported the expression of Angpts in tumor stromal cell lineages such as fibroblasts upon exposure of cancer patients to DNA damaging agents (bleomycin, mitoxantrone and radiation) or anti-cancer targeting drugs including the BRAF kinase target inhibitor PLX4720 [10,12]. The typical phenotype of cellular response to genotoxic insults is modulated by a DNA damage secretory program (DDSP), which refers to a highly conserved stress-responsive program activated by DNA damage repair (DDR) events of eukaryotic, in most cases, mammalian cells [13]. However, an insightful, accurate and complete understanding of regulatory mechanisms and functional implications of DDSP in the course of anti-cancer treatments is still lacking. In this review, we discuss the protein structures, functional properties and pathological implications of Angpts in tumor development. In addition, we summarize recent literature to clarify the in vivo source of Angpts in the tumor microenvironment (TME) under DNA damaging conditions, and explore the possible strategies to effectively target these factors to constrain tumor progression even in therapeutic settings of clinical oncology.

Structural Biology of Angpt1, Angpt2 and Tie2

In human cells, the Angpt-Tie pathway comprises two type I receptor tyrosine kinases (Tie1/Tie2) and three soluble ligands (Angpt1/Angpt2/Angpt4), each molecule well characterized by a variety of studies. Insights into the three-dimensional structure of Angpts help appreciate their spatial profiles, dynamic features and molecular functions upon binding to the transmembrane receptors. In general, Angpts have an amino-terminal superclustering domain (N domain), a central coiled domain (C domain), a linker region and a carboxyl-terminal fibrinogen-related domain (F domain), the latter responsible for binding to the receptor Tie2 [14] (Figure 1). Sharing a high degree of sequence identity, Angpt1 and Angpt2 play indispensable roles in balancing vessel stability and regression during both development-required and tumor-associated angiogenesis (Figure 2). Intriguingly, an Angpt2 chimera containing the Angpt1 loop sequence behaves similarly to Angpt1 as a constitutive Tie2 agonist, efficiently dissociating the inhibitory Tie1/Tie2 complex and eliciting Tie2 clustering and downstream signaling [15].

Crystal structures of the Tie2 ligand-binding region alone and in complex with Angpt2 are reported, data indicating that Tie2 contains three immunoglobulin (Ig) domains which fold together with the three epidermal growth factor (EGF) domains into a compact, arrowhead-shaped structure [16] (Figure 3). Angpt2-Tie2 recognition resembles antibody-antigen association, and all Angpts likely interact with Tie2 in a structurally similar manner. Angpts have evolved unique ways to regulate their Tie2 complex receptor system. When Tie2 is ectopically expressed in fibroblasts, the protein seems to behave like a classic receptor tyrosine kinase that can be activated by simple dimeric ligands.

***Corresponding author:** Yu S, Key Laboratory of Stem Cell Biology, Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and Shanghai Jiaotong University School of Medicine, 320 Yueyang Road, Biological Research Building A, Shanghai 200031, China, Tel: +86 21 54923302; Fax: +86 21 54923302; E-mail: sunyu@sibs.ac.cn

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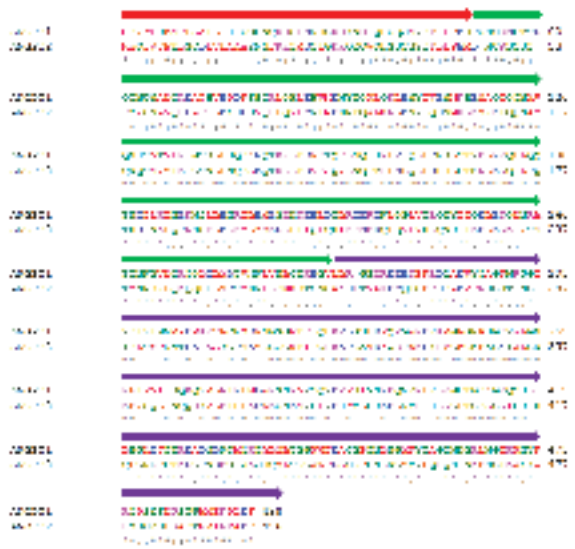

Figure 1: Sequence alignment of the Angpt family members Angpt1 and Angpt2. Structure-based sequence alignment of human Angpt1 and Angpt2 proteins. Secondary structural elements are shown above the alignment and colored individually. Red, N-terminal domain (superclustering domain); green, C domain (central coiled domain); purple, F domain (C-terminal fibrinogen-related domain, responsible for binding to the receptor Tie2).

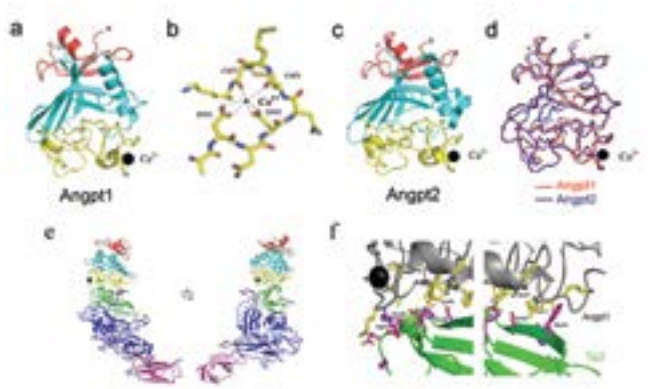

Figure 2: Structure of the Angpt1 receptor binding domain (RBD) and the Angpt1/Tie2 complex. (a) The refined model of Angpt1-RBD with the individual subdomains shown in different colors (A domain, red; B domain, cyan; P domain, yellow). The black sphere represents the bound calcium atom. (b) A close-up view of the Ca^{2+}-binding site. (c) Structure of the previously determined Angpt2-RBD colored as in (a). (d) Structural alignment in coil representation of the Angpt1-RBD (shown in red) and the Angpt2-RBD (shown in blue). (e) Two 90°orientations of the refined model of the Angpt1/Tie2 complex. The Tie2 Ig2 domain is colored green, whereas the remaining domains are colored in blue, with the exception of the FNIII-1 domain, which is colored in magenta; Angpt1 is colored as in (a). The *N*-acetyl-β-D-glucosamine moieties are shown in ball-and-stick format. (f) Close-up view of the Angpt1/Tie2 interface. Angpt1 is shown in gray, whereas Tie2 is in green. Residues at the interface are colored yellow (for Angpt1) or magenta (for Tie2). Color images adapted from Yu et al. with permission from Proc Natl Acad Sci U S A., Copyright 2013.

However, the native Tie2 in endothelial cells seems to be more elaborate, possibly because of the presence of co-receptors and/or co-regulators, thus requiring higher-order multimerization to be activated. Genetic engineering of precise multimers unmasked that Angpt1 tetramers are the minimum size required for receptor activation, whereas dimers and monomers act as antagonists [17]. These structural insights have provided the basis for prospective studies to clarify this fascinating but

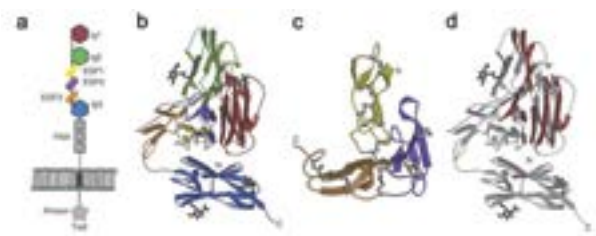

Figure 3: Structure of the Tie2 ligand-binding region.(a) Schematic representation of the Tie2 receptor domain organization. Red, Ig1; blue, Ig2; green, Ig3; yellow, EGF1; purple, EGF2; orange, EGF3; gray, the three fibronectin type III (FNIII) repeats and the cytoplasmic tyrosine kinase catalytic domain. (b) The ligand-binding extracellular region of Tie2, colored as in a. Asparagine-linked carbohydrate groups are shown in ball-and-stick format. (c) The EGF region of Tie2, colored as in a. The 12 disulfide bonds (4 in each EGF repeat) are shown in gray ball-and-stick format. (d) The Tie2 ligand-binding region. Red, the large and highly curved *trans*-domain 11-stranded β -sheet comprised of strands Ig1 (D-E-B-A-)-EGF2(C-D)-Ig2 (A'-G-F-C-C'). Color images adapted from Barton et al. with permission from Nature Structural & Molecular Biology, Copyright 2006.

complex growth factor/receptor system.

Tie1, another member of the Tie receptor family, can also form an interactive complex with native Angpt1 or Angpt4 with similar kinetics but less efficiency as compared with the Cartilage Oligomeric Matrix Protein (COMP)-Angpt1 [18]. In such a case, chimeric structure is formed by replacing the N-terminal portion Angpt1 with the short, pentameric coiled-coil domain of the COMP. Although sharing 55% amino acid sequence and highly similar structure (N-C-F form) with Angpt1, Angpt2 functions either as an agonist or antagonist of Angpt1 in a context-dependent manner [17,19].

Functional Roles of the Angpt-Tie2 System in Tumor Progression

Initiation of Angiogenesis: Both Angpt1 and Angpt2 are actively involved in angiogenesis, but their contributions differ substantially. Due to the multimeric nature of Angpt1 and Angpt2, the Tie2 receptor is likely to function as oligomers, as well [16]. Once bound by Angpt1, Tie2 forms dimers or multimers while being phosphorylated near the carboxyl terminal. Angpt1 is an agonist of Tie2, which is widely expressed by endothelial cells, stromal cells and cancer cells [20,21]. Angpt1-induced Tie2 activation conveys survival signals to endothelial cells and sustains endothelial barrier as quiescent vasculature [22]. Although Angpt1 promotes distinctive vascular remodeling through highly organized angiogenesis and consolidated endothelial cell junctions, it is dispensable for maintaining normal vasculature throughout adulthood [23]. To the contrary, Angpt2 generally acts as an Angpt1 antagonist, enhancing vascular permeability, priming endothelial bed for angiogenesis, promoting cancer cell migration, together causing vessel destabilization [24,25]. In Angpt2-deficient mice, tumor grows slowly in early stage with increased pericyte coverage. Besides, lymphatic and cardiac impairment also occurs upon Angpt2 elimination, indicating that an intact Angpt-Tie2 system is crucial for not only vascularization but also lymphatic and cardiac development [26]. Interestingly, enforced expression of Angpt2 in endothelial cells abolishes Tie2 autophosphorylation, further demonstrating the nature of Angpt2 as a strong Angpt1 inhibitor [27]. Therefore, an appropriate balance of these two Angpts is critical for vascular stability, integrity and overall homeostasis [25].

Vessel Sprouting and Maintenance: Vessel sprouting represents a

complicated and essential step in the course of vascular development. High levels of Angpt2, biologically counteracting Angpt1, interferes with pericyte recruitment to the freshly emerging vessel sprouts while maintaining the dispersed endothelial cells, whose fate (either tip or stalk) is subject to the balance of Notch pathway factors including Delta-like ligand 4 (DLL4) and jagged 1 (JAG1) [28,29]. On the other hand, Angpt1 abrogates the tumor growth factor (TGF) β-mediated suppression of endothelial cell tube formation induced by VEGFA, while Angpt2 reverses such an inhibitory effect caused by Angpt1 [30]. Clearance of Angpt2 can minimize the number of tumor vessel sprouts, potentially due to higher pericyte coverage and fewer loose vessels, indicating the benefit of co-targeting Angpt2 and VEGF on preventing tumor growth [31]. Whether the newly formed unstable endothelial cell tubes become angiogenic sprouts or develop into new vessels mainly depends on the balance of local levels of angiogenic factors, including Angpt1, Angpt2, VEGFA, platelet-derived growth factor (PDGF) B [1].

Angpt1 positively, whereas VEGFA negatively modulates the distribution of vascular endothelial cadherin in adherens junctions, one of the three major forms (the other two are tight junctions and gap junctions, respectively) of associations essential for maintaining blood vessel integrity to prevent extravasation of both immune cells and circulating tumor cells [15]. The Tie2 complex activated by Angpt1 enhances endothelial cell interactions in stable blood vessels, and Angpt1 can cause Tie2 accumulation at the back side of migrating endothelial cells hence stimulating their proliferation, a process independent of tyrosine phosphatase activity but subject to Tie1 suppression upon hypoxic conditions [32]. In addition, Angpt1 promotes the continuous distribution of platelet and endothelial cell adhesion molecule and junctional adhesion molecule A in the vessel walls of tumors [33]. Nevertheless, Angpt2 increases matrix metalloproteinase (MMP) 9 activity while decreasing tight junction protein zonular occludens-1 (ZO-1), an MMP9 substrate that enhances endothelial cell polarity and regulates vascular permeability [34,35]. Together, the data further substantiate that Angpt1 and Angpt2 oppositely recruit mural cells and maintain vessel homeostasis through distinct mechanisms.

Lymphangiogenesis: Although Angpt1 and Angpt2 play distinct roles during angiogenesis, they do function in a similar way in lymphangiogenesis through Tie2 in the lymphatic system [36]. Angpt2 ablation results in poor survival with severe *chylous ascites* and *peripheral lymphoedem*, as is evidenced by the poor muscle coverage of lymphatic vessels found in Angpt2-/- mice [19]. Importantly, overexpressing angiogenic factors, such as COMP-Angpt1, Angpt2, Angpt3 and Angpt4 effectively induces lymphangiogenesis in both cornea and skin of experimental mice, indicating the significance of Angpt signaling in lymphatic vessel formation [6,37]. As upregulation of Angpt2 in the serum of cancer patients is associated with lymph node metastasis, a predictor of poor overall and recurrence-free survival, the involvement of Angpts in lymphangiogenesis potentially enhances cancer cell invasion in the local TME [38,39].

Invasion and metastasis: Cancer invasion is a complicated process, and diverse soluble factors are significantly implicated in this hallmark activity of various malignancies. Tie2 activation in glioma or brain tumor stem cells (BTSCs) results in enhanced invasiveness mainly through upregulating integrin β1 and N-cadherin, whereas neutralizing antibodies against these molecules inhibit the adhesion of Tie2-positive glioma cells to endothelial cells [11]. Increased Angpt2 expression correlates with higher invasive and metastatic potential in multiple cancer types [40,41]. Metastasis remains the most fatal step of tumor development, and the major cause of cancer-associated mortality. Like invasiveness of cancer cells observed in glioma, the molecular mechanism regulating Angpt2-promoted metastasis can be attributed to integrin and integrin-associated kinase pathway [42]. It is recently reported that Ang2 gene silencing can exert an anti-metastasis effect in vitro and in vivo and Ang2 targeted gene therapy has the potential to serve as a novel avenue to treat pancreatic carcinoma [43]. Co-expression of Angpt1 with several other proteins including integrin β3, MMP9 and thrombospondin 1 (THBS1) is observed in cancer stem cells (CSCs) of head and neck squamous cell carcinoma (HNSCC), predicting enhanced possibility of tumor recurrence [44]. These data strongly indicate that Angpts have implications in not only angiogenesis process but also metastatic activities.

Tumor-associated inflammation: Mounting evidence supports the pathological link between inflammation and cancer. In line with the opposing functions of Angpt1 and Angpt2 in regulating angiogenesis, Angpt1 is reported to be an anti-inflammation cytokine while Angpt2 is considered a pro-inflammation factor. The inflammation-suppressive nature of Angpt1 mainly depends on its ability in strengthening the endothelial cells barrier thereby minimizing vascular permeability.

In addition, the nuclear factor-Kappa B (NF-κB)-mediated expression of pro-inflammation molecules, such as intracellular adhesion molecule 1 (ICAM1) and E-selectin, is remarkably blocked upon Tie2 activation by Angpt1 [45,46]. In contrast, Angpt2 exhibits pro-inflammatory effects and induces vessel leakage which is frequently reported in cases of inflammation-associated diseases, including bowel disease, arthritis and sepsis [1]. Angpt2 enhances inflammation by promoting adhesion of tumor necrosis factor (TNF) α-elicited rolling leukocytes to tumor vessels via elevating ICAM1 and vascular cell adhesion molecule 1 (VCAM1) expression, effects that are sharply opposite to those caused by Angpt1 [45]. The Angpt-Tie signaling pathways are essential to maintain a biologically normal homeostasis, involving multiple molecules in a highly complicated network (Figure 4).

Pathological sources of Angpt1 and Angpt2 in the TME under genotoxic conditions

As archived in most literature, Angpts are usually expressed by pericytes and secreted to the extracellular matrix (ECM), whereby contributing to neovascularization in the TME niches. However, recent studies revealed that Angpt1 is upregulated in Kaposi's sarcoma-associated herpesvirus (KSHV)-infected primary effusion lymphoma (PEL) cell lines, with the expression level higher than that in uninfected lymphoma or leukemia cell lines [47]. The reason is, some KSHV-infected PEL cell line-specific DNA-interacting factors, such as Oct 1, binds to the Angpt1 promoter region (-143 to -125) thereby regulating its expression. In addition, breast cancer 1 early onset (BRCA1), complex with transcriptionally interacting protein (CtIP), and zinc finger protein 350 (ZNF350) form a complex to coordinately repress Angpt1 expression via a ZNF350 recognition site in Angpt1 promoter sequence [48]. Consistently, BRCA-deficient mouse breast tumors have accelerated growth, pronounced vascularization, and upregulated Angpt1 [48]. Thus, BRCA1 not only maintains genomic stability as a tumor suppressor, but also directly regulates the expression of angiogenic factors which have the potential to modulate the TME. So far, biological knowledge regarding the regulatory mechanisms of Angpt expression within the TME is limited and much still remains void, thereby deserving continued investigation in future.

New lines of research discovered that certain cancer therapies such as the B-Raf proto-oncogene (BRAF) pathway targeting agent PLX4720, traditional chemotherapies and ionizing radiation can dramatically promote the expression of Angpts by stromal cells and subsequent secretion. For example, Angpt1 is significantly produced and released

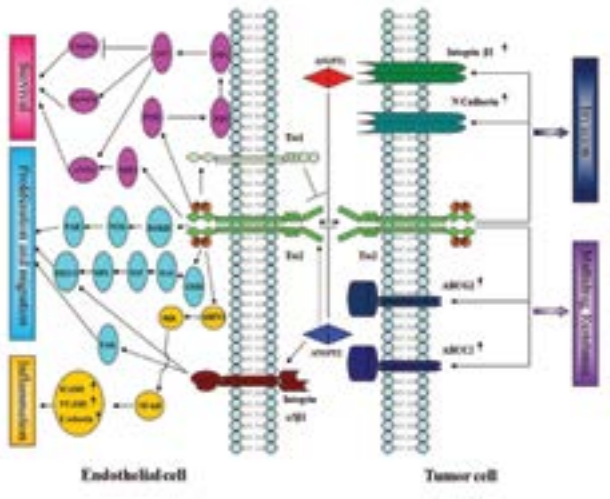

Figure 4: The biological profile of Angpt-Tie signaling axis.Left part, Angpt1 multimers in the extracellular space bind to Tie2 on endothelial cells, causing Tie2 dimerization/multimerization and subsequent autophosphorylation on the membrane. Activated Tie2 binds intracellular molecules, including downstream of tyrosine kinase-related (DOKR), endothelial nitric oxide synthase (eNOS), SH2 domain-containing phosphatase (SHP2), growth factor receptor-bound protein 2 (GRB2) and the PI3K subunit p85. SHP2 and PI3K activate eNOS, thereby increasing NO production [1]. In most cases, Angpt2 functionally antagonizes Angpt1 by increasing vascular permeability and priming the vasculature for angiogenesis; however, it can act as a partial Tie2 agonist under certain conditions (dashed arrow) [66, 67]. At the cell surface, Tie1 can be bound by activated Tie2, and the extracellular domain of Tie1 interferes with the Angpt1-Tie2 interaction thus counteracting Angpt1 signals. Angpt1 is an anti-inflammatory cytokine, whereas Angpt2 exhibits pro-inflammatory activities. Importantly, Angpt1-Tie2 signaling engages A20-binding inhibitor of nuclear factor-κB (NF-κB) activation 2 (ABIN2) and diminishes expression of NF-κB-associated inflammatory proteins such as intercellular adhesion molecule 1 (ICAM1), vascular cell adhesion molecule 1 (VCAM1) and E-selectin [45]. Alternatively, Angpt2 may signal through integrin α5β1, enhancing expression of downstream FAK and ERK1/2 [68]. Right part, in tumor cells, activation of Tie2 upon Angpt1 binding promotes expression of drug pumps or transporters, including ATP-binding cassette, sub-family C (CFTR/MRP), member 2 (ABCC2) and ATP-binding cassette, sub-family G (WHITE), member 2 (ABCG2), each case leading to multidrug resistance (MDR) [69]. Angpt1-Tie2 interaction also results in other changes, such as upregulated N-cadherin, a protein that facilitates epithelial to mesenchymal transition (EMT), and increased integrin β1-dependent adhesion to the extracellular matrix, both activities are associated with invasiveness but can be ameliorated by Angpt2 in the TME [11,70].

Figure 5: Angpt1 expression is induced upon exposure of prostate fibroblasts to genotoxic stress.Bioinformatical analysis revealed gene expression changes post microarray hybridization. The heatmap depicts the relative transcript abundance levels after exposure to hydrogen peroxide (H_2O_2), bleomycin (BLEO), or ionizing radiation (RAD), all agents inducing typical DNA double strand breaks. Note Angpt1 is on the top list of upregulated genes, with Angptl4 showing up as a soluble partner in the same settings. The listed genes have an expression fold change of 3.5 or above, with only secreted factors selected as representative of the DDSP effectors. Microarray image adapted from Sun et al. with permission from Nature Medicine, Copyright 2012.

into the pre-conditioned medium from 18 stroma cell lines at a level detectable by antibody arrays upon treatment with PLX4720, whereas Angpt2 expression decreased at the same time [12]. However, Angpt1 and another angiogenic factor, Angptl4, are remarkably upregulated upon exposure of cells to genotoxicity [10] (Figure 5). Despite the diverse pathological roles of the TME that are increasingly recognized, the regulatory mechanism of stromal cell secretory phenotype remains largely unexplored, particularly in the context of DNA damage generated by therapeutic agents or caused by hypoxia upon malignant expansion in the tumor foci [49].

Several studies have demonstrated that the inflammation-associated transcription factor, NF-κB-mediated signaling is central for the initiation, maintenance and development of the DDSP. The recruitment of Rel A (p65) subunit of NF-κB to the chromatin is necessary for expression of several DDSP factors, including IL-6, IL-8 and WNT16B [10,50-52]. The NF-κB complex can be directly activated by the interaction between ataxia telangiectasia mutated (ATM) and inhibitor of kappaB kinase gamma (IKKγ, or NEMO), which engages NF-κB in damage-stimulated and cell non-autonomous responses by phosphorylating IKK [53-55]. Diverse extrinsic environmental stresses particularly genotoxic treatments provoke a nucleus-to-cytoplasm signaling cascade, while the interior chronic inflammation-stimulated kinase mitogen-activated protein kinase 14 (p38MAPK) also plays a crucial role during DDSP development [56,57]. Data in our lab, intriguingly, indicate that knockdown of NF-κB or several other DDSP-regulating factors, either case, significantly abolishes Angpt1/2 expression in fibroblasts under DNA damage conditions. In spite of these advancements, alternative transcription factors including the family of CCAAT-enhancer binding proteins (c-EBPs) are disclosed to regulate the stress-induced secretory program in synergy with NF-κB [58]. Furthermore, the Poly-ADP-ribose polymerase1 (PARP-1), another important DNA-damage sensor and DDR modulator, is functionally involved in activation of NF-κB in senescent melanoma cells undergoing the senescence-associated secretory program (SASP) [59]. A recent study discovered functional relevance of the mammalian target of rapamycin (mTOR), which regulates the pro-tumorigenic program of SASP or DDSP by promoting translation of interleukin 1A (IL-1A), a key cytokine expressed modestly upon DNA damage but plays a critical role in provoking genome-wide secretion in mammalian cells [60].

Therapeutic strategies to tackle the Angpt-Tie system

Current strategies to target the Angpt-Tie system mainly involves the development of either selective or non-selective traps (for Angpt1 and Angpt2) to systemically deliver Angpts to induce their anticancer

effects, and receptor tyrosine kinase inhibitors (for Tie2) to abolish signaling transduced across the cell membrane (not discussed in this article due to the limited success in trials).

The specific nature of the Angpt-Tie pathways provides unique opportunities to manipulate the TME with the purpose to interrupt pathological processes essential for tumor progression. As Angpt1 promotes vasculature stability, a key aspect of either supportive or suppressive effects on tumor growth, agents designed to target this factor may have therapeutic potential to alter angiogenic switch of multiple malignancies. However, given the anti-inflammatory property of Angpt1, eliminating it may be unfavorable, as is evidenced by a recent study associating the improved disease-free survival (DFS) with enhanced Angpt1 expression in squamous cell cancer patients [33]. Moreover, combined values of circulating Angpt1 and Tie2 concentrations predicted improved progression-free survival (PFS) in bevacizumab-treated ovarian cancer patients in a training set [61]. In contrast, Angpt2, the pro-inflammatory factor that primes vascular bed for enhanced angiogenic response, generates potentially detrimental effects on normal vascularization, thus precluding any attempts for its systemic delivery to treat cancers [1]. According to the serum Angpt2 level, small cell lung cancer (SCLC) patients are divided into high-level group and low-level group, and the patients in the low-level group showed better treatment response during chemotherapy than those in the high-level group, suggesting that Angpt2 might be employed as a prognostic factor for SCLC and for predicting SCLC response to chemotherapy [38].

A significant body of evidence showed that increased Angpt1 signaling through Tie2 helps control pathologies of vascular activation, including sepsis, stroke, diabetic retinopathy and asthma. To the contrary, agents to inhibit Angpt2 action are advocated, and convincing data demonstrated their preclinical and clinical advantages in minimizing tumor-associated angiogenesis [32]. There are at least two Angpt traps in clinical trials, including but not limited to Trebananib (AMG-386) and CVX-060 (PF-4856884). Trebananib is an anti-angiogenic peptibody under investigation in patients with advanced cancer via neutralizing the interaction between angiopoietins (Angpt1/2) and their Tie2 receptors, thus representing a promising drug in terms of both efficacy and toxicity profile [39]. Trebananib provided a clinically meaningful prolongation in PFS in a cohort of patients with recurrent epithelial ovarian cancer, when used in synergy with weekly paclitaxel chemotherapy [62]. A recent clinical IV study examined the effect of trebananib IV combined with the anti-VEGF agents (bevacizumab or motesanib) in advanced solid tumors, and found that Trebananib IV administered at 3 mg kg^{-1} or 10 mg kg^{-1} plus bevacizumab or motesanib is associated with less severe toxicities but better response relative to the conditions where two anti-VEGF agents are combined [40]. In parallel, concomitant Angpt1 and Angpt2 inhibition by Trebananib potentiates their antitumor effects by not only reducing the vessel number but also causing the regression of remaining tumors; consequence of the latter achieved with Trebananib was comparable with bevacizumab, while combination of these two agents exerted a more pronounced effect [63]. Thus, pharmacological blockade of multiple pathways implicated in angiogenesis may result in a more favorable clinical outcomes.

CVX-060 is an Angpt2-targeting agent, composed of two peptides that bind Angpt2 with high affinity and specificity, covalently fused to a scaffold antibody. A predictive model was built to optimize application of this compound in clinics, based on the efficacy of CVX-060 in 13 cell lines and 2 patient-derived xenograft models [64]. The range of CVX-060 efficacy in diverse tissue types was revealed, with a subset of biomarker proteins including Angpt1, EGF and Emmprin defined. The data can be employed to predict tumor growth inhibition by Angpt2 blockade [64]. Chronic treatment with CVX-060 was associated with a significant decrease in inflammatory infiltration, normalization of the hepatic microvasculature and reduction in VCAM-1 vascular expression, indicating that Angpt2 inhibition offers a therapeutic alternative for liver fibrosis and associated neoplasia [65].

Concluding remarks

The Angpt-Tie system is crucial for the angiogenic switch in tumors, and together with VEGFA promotes the initiation of angiogenesis and new vessel maturation. The system is also involved in inflammation, invasion, metastasis and lymphangiogenesis. Significant benefit of combination treatment targeting both the Angpt2 and VEGFA pathways has been demonstrated in preclinical studies. Suppression of the Angpt/Tie2 pathway has gained accumulating interest during recent years as an effective strategy to overcome bevacizumab resistance and toxicities. It is evident that the angiogenic factors, in particular, the Angpt family and certain Angptl proteins including Angptl4 are markedly expressed in resident stromal cells upon exposure to DNA damaging agents, which were originally designed to attack the rapidly proliferating cancer cells. However, as an evolutionarily pleiotropic effect, the DDSP is elicited as a drug-off-target (DOT) but self-defending program when stromal cells are confronted with genotoxicity. However, what information do these damaged cells convey to the highly complicated local tissue that exhibits various extent of cell lineage heterogeneity? The present explanation is that depending on the identity of specific DDSP factors, damaged cells could deliver the dangerous signals to its neighboring cells and arouse the response of individual cell components in the microenvironmental niche. For example, chemoresistance of prostate cancer cells acquired from a TME reprogrammed by the genotoxic treatment is remarkably enhanced by the soluble factor WNT16B generated by damaged fibroblasts. Despite the classical function of Angpt-Tie2 pathway, DDSP-derived Angpts act as extracellular signaling molecules secreted by stomal cells, and can potentially alter the therapeutic outcome. Whether and how Angpt factors confer protective advantage to nearby cancer cells, remains an open but interesting and important topic that needs careful and intensive exploration in the years to come.

Abbreviations

ABIN2: A20-Binding Inhibitor of Nuclear factor-κB (NF-κB) activation 2

Angpt: Angiopoietin

ATM: Ataxia Telangiectasia Mutated

BRAF: B-Raf proto-oncogene

BLEO: Bleomycin

BTSC: Brain Tumor Stem Cell

BRCA1: Breast Cancer 1 Early Onset

CSCs: Cancer Stem Cells

COMP: Cartilage Oligomeric Matrix Protein

c-EBP: CCAAT-Enhancer Binding Protein

CtIP: Complex with transcriptionally Interacting Protein

DLL4: Delta-Like Ligand 4

DFS: Disease-Free Survival

DDR: DNA Damage Repair

DDSP: DNA Damage Secretory Program

DOT: Drug-Off-Target

eNOS: Endothelial Nitric Oxide Synthase

EGF: Epidermal Growth Factor

EMT: Epithelial to Mesenchymal Transition

ECM: Extracellular Matrix

HNSCC: Head and Neck Squamous Cell Carcinoma

H_2O_2: Hydrogen Peroxide

Ig: Immunoglobulin

IKKγ: Inhibitor of Kappab Kinase Gamma

IL-1A: Interleukin 1A

ICAM1: Intracellular Adhesion Molecule 1

RAD: Ionizing Radiation

JAG1: Jagged 1 Protein

KSHV: Kaposi's Sarcoma-Associated Herpesvirus

p38MAPK: Mitogen-Activated Protein Kinase 14

mTOR: Mammalian Target of Rapamycin

MMP: Matrix Metalloproteinase

MDR: Multidrug Resistance

NF-κB: Nuclear Factor-Kappa B

PDGF: Platelet-Derived Growth Factor

PARP-1: Poly-ADP-Ribose Polymerase1

PEL: Primary Effusion Lymphoma

PFS: Progression-Free Survival

RBD: Receptor Binding Domain

SASP: Senescence-Associated Secretory Program

SHP2: SH2 Domain-Containing Phosphatase

SCLC: Small Cell Lung Cancer

THBS1: Thrombospondin 1

TGF: Tumor Growth Factor

TME: Tumor Microenvironment

TNF: Tumor Necrosis Factor

VCAM1: Vascular Cell Adhesion Molecule 1

VEGFA: Vascular Endothelial Growth Factor A

ZNF350: Zinc Finger Protein 350

ZO-1: Zonular Occludens-1

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