Emerging Roles of Angiopoietin-like 4 in Human Cancer

Ming Jie Tan¹, Ziqiang Teo¹, Ming Keat Sng¹, Pengcheng Zhu¹, and Nguan Soon Tan¹,²

Abstract

Angiopoietin-like 4 (ANGPTL4) is best known for its role as an adipokine involved in the regulation of lipid and glucose metabolism. The characterization of ANGPTL4 as an adipokine is largely due to our limited understanding of the interaction partners of ANGPTL4 and how ANGPTL4 initiates intracellular signaling. Recent findings have revealed a critical role for ANGPTL4 in cancer growth and progression, anoikis resistance, altered redox regulation, angiogenesis, and metastasis. Emerging evidence suggests that ANGPTL4 function may be drastically altered depending on the proteolytic processing and posttranslational modifications of ANGPTL4, which may clarify several conflicting roles of ANGPTL4 in different cancers. Although the N-terminal coiled-coil region of ANGPTL4 has been largely responsible for the endocrine regulatory role in lipid metabolism, insulin sensitivity, and glucose homeostasis, it has now emerged that the COOH-terminal fibrinogen-like domain of ANGPTL4 may be a key regulator in the multifaceted signaling during cancer development. New insights into the mechanistic action of this functional domain have opened a new chapter into the possible clinical application of ANGPTL4 as a promising candidate for clinical intervention in the fight against cancer. This review summarizes our current understanding of ANGPTL4 in cancer and highlights areas that warrant further investigation. A better understanding of the underlying cellular and molecular mechanisms of ANGPTL4 will reveal novel insights into other aspects of tumorigenesis and the potential therapeutic value of ANGPTL4. Mol Cancer Res; 10(6); 677–88. © 2012 AACR.

Introduction

Cancer is a disease in which a group of cells attains the ability to proliferate uncontrollably, invades surrounding tissues, and ultimately spreads to distal tissues and organs through the circulatory system to form metastatic tumors. Based on our current knowledge, the process of cancer development is somewhat perplexing and certainly multifactorial. Genetic instability fueled by DNA damage and errors generated by DNA replication machinery drive the initiation of tumorigenesis (1, 2). Extensive exposure to carcinogens or genotoxic chemicals is a prime factor that causes mutations and increases the risk of cancer. In addition, by-products of normal cellular metabolism, such as reactive oxygen species (ROS), also create a constant threat to DNA integrity (1). Genetic mutations or alterations of critical regulatory genes, such as tumor-suppressor genes and proto-oncogenes, cause uncontrolled proliferation and the deregulation of apoptosis further mediating tumor progression.

Tumors consist of heterogeneous cell types such as cancer-associated fibroblasts, endothelial cells, pericytes, immune inflammatory cells, and proliferating cancerous cells (3). This heterogeneity and interdependency sustain tumor growth and progression. Cancer-associated fibroblasts form the majority of the tumor stromal population and are capable of influencing cancer cell behavior via paracrine regulation. Endothelial cells that form the tumor vasculature act as a transportation route for nutrients not only to support tumor growth, but also to facilitate cancer metastasis. The pericytes provide paracrine support signals to stabilize tumor vascular integrity and its function. Immune inflammatory cells secrete proangiogenic effectors like angiogenic growth factor, VEGF, and proinvasive matrix metalloproteinases to promote tumor progression (3).

Reciprocal cell–cell and cell–matrix communications dictate nearly every aspect of tumor development, and they can drastically affect the efficacy of antitumor therapies. Such communication encourages the tumor cells of many human cancers to acquire several essential capabilities to sustain proliferative signals, evade growth suppressors, resist cell death, enable replicative immortality, induce angiogenesis, activate invasion and metastasis, reprogram their energy metabolism, and evade immune destruction (3). Research to identify critical mediators of these hallmarks will offer novel insights into the network of molecular mechanisms that are central to cancer development.

Ongoing cancer research focusing on understanding the interaction of cancer cells with their microenvironment has intensified over recent years, and a new class of proteins known as matricellular proteins have since been implicated...
Matricellular proteins are a group of nonstructural, extracellular matrix (ECM)–associated glycoproteins secreted by cancer cells and neighboring stromal cells into the extracellular environment (4, 5). This large group of structurally and functionally diverse proteins modulates cell–matrix interactions and cell functions by interacting with membrane receptors, proteases, hormones, and other bioeffector molecules, as well as with structural matrix proteins such as collagen and vitronectin (7, 8). Recent studies have implicated several members of matricellular proteins, such as osteopontin, secreted protein acidic and rich in cysteine, and angiopoietin-like 4 (ANGPTL4) as important factors in tumor progression (4, 9–11). Of particular interest, ANGPTL4 is a novel matricellular protein that was reported to interact with specific ECM proteins and integrins to facilitate cell migration during wound healing (7, 8).

ANGPTL4 is a novel, glycosylated adipokine that belongs to a family of 7 ANGPTL proteins (Fig. 1; refs. 12,13). The 7 proteins, designated angiopoietin-like 1 to 7 (ANGPTL1–7), were identified in systemic circulation and share amino acid sequence similarity with the angiopoietin family (ANG). Despite being structurally similar to ANGs, these ANGPTLs do not bind to the ANG receptors Tie 1 and Tie 2 to mediate their biological functions. The cognate receptors for ANGPTLs remain unknown; thus, ANGPTLs are considered orphan ligands (12, 14). ANGPTL3, 4, and 6 play critical roles in lipid and glucose metabolism (14, 15). Studies have also revealed that ANGPTL2, 3, 5, and 7 regulate hematopoietic stem cell activity (12). Interestingly, ANGPTL2 was recently reported to be a critical factor for carcinogenesis (16). ANGPTL2-associated chronic inflammation was also found to increase cancer susceptibility (16). ANGPTL2 was identified as a causative mediator of chronic inflammation, and this was strongly correlated to the frequency of carcinogenesis in vivo using a chemically induced skin squamous cell carcinoma (SCC) mouse model. Furthermore, tumor-derived ANGPTL2 was shown to enhance metastasis and tumor angiogenesis (16). This finding suggests that other ANGPTL family proteins may have similar roles in cancer development.

Emerging evidence has identified a novel role for ANGPTL4 in cancer progression, although the other ANGPTL members have been mostly characterized as factors involved in angiogenesis and metabolism. The involvement of other ANGPTL family members in cancer remains unclear and warrants further investigation. In this review, we discuss the potentially diverse roles of ANGPTL4 in tumor development and discuss certain discrepancies with ANGPTL4 that require further clarification.

**Functional Domains of ANGPTL4**

ANGPTL4 is characterized by the presence of a highly hydrophobic signal peptide, an N-terminal coiled-coil domain, and a large ANG/fibrinogen-like COOH-terminal domain, which is well conserved in the ANG and ANGPTL families (Fig. 2; ref. 17). The native full-length ANGPTL4 (fANGPTL4) exists in the form of dimeric or tetrameric complexes that can undergo proteolytic processing to generate the N-terminal coiled-coil fragment (nANGPTL4) and the COOH-terminal fibrinogen-like domain (cANGPTL4; ref. 17). Although ANGPTL4s are known to be proteolytically processed, the mechanism of cleavage, the importance of the processing, and the roles of the various ANGPTL4 fragments are only beginning to be elucidated.

The proteolytic cleavage of ANGPTL4 is mediated by proprotein convertases (PC; Fig. 2; ref. 17). Because ANGPTL4 is proteolytically processed in the liver, the hepatocellular carcinoma cell line Huh7 has been used in studies investigating ANGPTL4 cleavage. Using Huh7 cells transfected with expression vectors encoding for the various convertases, Lei and colleagues showed that in vitro several PCs, including furin, PC5/6, paired basic amino acid–cleaving enzyme 4, and PC7, are able to cleave human ANGPTL4 at the -RRXR- consensus cleavage site (17). Although this study showed that ANGPTL4 is cleaved by PCs, the correlation between the expression of PCs and the truncated forms of ANGPTL4 remains unknown. The PCs have been shown to be enzymatically activated after passing through the late Golgi. Therefore, how ANGPTL4 is proteolytically processed remains to be determined (18). Importantly, the expression of these PCs in tumors and whether they are indeed responsible for the in vivo processing of fANGPTL4 is unknown.

The nANGPTL4 is responsible for the oligomeric assembly of ANGPTL4 and binds to lipoprotein lipases (LPL) to inhibit their activities (19). Disulfide bond formation facilitates the oligomeric formation of nANGPTL4 and fANGPTL4, which enhances its inhibitory effects on LPL activity. Mutations that prevent oligomerization severely compromise the capacity of ANGPTL4 to inhibit...
LPL. The fANGPTL4 can also bind to heparin sulfate proteoglycans to participate in the inhibition of endothelial cell migration and tubule formation (20). However, it remains unknown whether fANGPTL4 and nANGPTL4 can directly stimulate intracellular signaling because neither a receptor nor a cell-surface interaction partner has been identified. The cANGPTL4 binds to and activates integrins β1 and β5 to regulate cell migration via the focal adhesion kinase (FAK)/p21-activated kinase (PAK)–signaling cascade (7). Recently, cANGPTL4 was found to activate Src/ERK/PKB signaling pathways that are crucial for effective wound healing. It is also interesting to note that cANGPTL4 has been found to be able to inhibit angiogenesis by suppressing the Raf/MEK/ERK signaling cascade.

Angiopoietin-like 4 in Human Cancer

ANGPTL4 Expression and Its Transcriptional Regulation in Human Cancers

The expression of ANGPTL4 is regulated by the nuclear hormone receptors of the PPAR family, as well as by hypoxia and fasting (15, 22, 23). PPARs act as lipid biosensors and
vascular tight junctions, thereby increasing the permeability of ANGPTL4 to disrupt the integrity of the capillaries in the lung to promote the intravasation into the lung tissue (30). Whether TGF-β can serve as a good therapeutic target still remains unclear because of its dual role in inhibiting and promoting tumor cell growth. However, this ambiguity shows the capacity of the primary tumor microenvironment to promote the progression of cancer via the production of cytokines (29).

Although early expression-profiling studies detected ANGPTL4 in a variety of organs or tissues, such as the skin, intestines, kidneys, adipose tissues, and the liver (14, 15, 22), little is known about the relative expression of the various ANGPTL4 fragments in these tissues. Recently, it was shown that adipocytes express the posttranslationally modified ANGPTL4 (~65 kDa), whereas the liver produces both cANGPTL4 (~47 kDa) and nANGPTL4 (~26 kDa; Fig. 2; refs. 17, 31, 32). The mechanism for such tissue-dependent expression of ANGPTL4 remains unclear.

Although information about the relative expression of the different ANGPTL4 fragments in normal tissues is emerging, much less is known about the expression of various ANGPTL4 fragments in various tumors (Table 1). To date, 4 studies have reported the elevated expression of cANGPTL4 in various tumors. Using an in vitro model in which they overexpressed ANGPTL4 cDNA in B16F0 mouse melanoma cells, Galau and colleagues showed the presence of a small ANGPTL4 fragment whose molecular weight corresponded to the cANGPTL4 fragment (10). Using a monoclonal antibody against cANGPTL4 (mAb11F6C4), Zhu and colleagues showed that cANGPTL4, but not nANGPTL4, is highly expressed in major epithelial tumors such as SCC (11). Similarly, Huang and colleagues detected elevated cANGPTL4 from fine needle aspirates of breast tumors, basal cell carcinomas, melanomas, and in several cancer cell lines derived from breast, lung, and liver cancers (21). Consistent with the above findings, Kim and colleagues showed that colorectal cancer (CRC) cells only secrete cANGPTL4 proteins (25). A high ANGPTL4 expression in oral cancer correlates to an enhanced rate of tumorigenesis and a poor prognosis based on the tumor clinicopathology (Table 2; refs. 33, 34). An elevated ANGPTL4 in CRCs is also correlated with shorter disease-free survival rates (25). Furthermore, the

Table 1. Expression of ANGPTL4 protein in various human cancers

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>Detection technique</th>
<th>Antibody useda</th>
<th>Author (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Various cancers</td>
<td>Immunofluorescence, real-time PCR, and Western blot</td>
<td>nANGPTL4 and cANGPTL4</td>
<td>Zhu et al. (11)</td>
</tr>
<tr>
<td>CRC</td>
<td>Real-time PCR and Western blot</td>
<td>Full-length ANGPTL4</td>
<td>Nakayama et al. (47)</td>
</tr>
<tr>
<td>CRC</td>
<td>Real-time PCR and Western blot</td>
<td>Full-length ANGPTL4 and cANGPTL4</td>
<td>Kim et al. (25)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Real-time PCR and Western blot</td>
<td>Full-length ANGPTL4</td>
<td>Zhang et al. (28)</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>Real-time PCR and Western blot</td>
<td>Full-length ANGPTL4</td>
<td>Nakayama et al. (48)</td>
</tr>
<tr>
<td>Esophageal cancer</td>
<td>Immunohistochemistry</td>
<td>Full-length ANGPTL4</td>
<td>Shibata et al. (49)</td>
</tr>
<tr>
<td>Kaposi sarcoma</td>
<td>Real-time PCR and Western blot</td>
<td>Full-length ANGPTL4</td>
<td>Ma et al. (43)</td>
</tr>
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</table>

aIndicates antibodies raised against full-length, N-terminal, and COOH-terminal ANGPTL4.

are crucial for lipid metabolism and homeostasis (15). Recently, PPARs have been implicated in the development of tumors (24). Studies have shown that ANGPTL4 is one of the PPAR target genes and contains several putative PPAR response element consensus sequences within its promoter (25). Reports have proposed that ANGPTL4 may be regulated by all 3 PPAR isoforms (PPARα, PPARβ/δ, and PPARγ), suggesting that ANGPTL4 may exert tissue-dependent effects governed by the differential expression of the PPAR isoforms (15). Furthermore, despite conflicting results, PPARs are known to be involved in tumorigenesis. This finding further implicates ANGPTL4 in cancer progression, although evidence that PPARs upregulate ANGPTL4 directly in tumor cells is still lacking. Girroir and colleagues suggested that PPARβ/δ modulates ANGPTL4 expression during tumorigenesis in human breast cancer and melanoma cell lines, although Zhu and colleagues reported no apparent correlation between the expression of PPARs and ANGPTL4 in SCC (11, 26). A recent study suggested that prostaglandin E2 (PGE2) may transactivate PPARβ/δ to regulate the expression of ANGPTL4 under hypoxic conditions (25). Because of the aberrant growth of tumor cells and poor vascularization, the tumor microenvironment tends to become hypoxic. Several new studies have reported that hypoxia can induce the expression of ANGPTL4 and that the upregulation of ANGPTL4 is induced by the transcription factor hypoxia-inducible factor-1α (HIF-1α; refs. 11, 25, 27). Indeed, Zhang and colleagues identified a functional HIF-1α–binding site located 1.6 kb upstream of the human ANGPTL4 gene (28). This finding was further confirmed by another independent group using the hepatocellular carcinoma cell lines MHCC-97L and SMMC-7721 (27).

The tumor microenvironment is constantly under chronic inflammatory pressure (29). Recently, the potent regulator of inflammation TGF-β was found to regulate the expression of ANGPTL4 via a Smad3-signaling pathway (30). The upregulation of ANGPTL4 in cancer cells when they extravasate into the circulatory system could explain their inclination toward colonizing lung tissue. The rationale is based on the ability of ANGPTL4 to disrupt the integrity of vascular tight junctions, thereby increasing the permeability of the capillaries in the lung to promote the intravasation into the lung tissue (30).
Table 2. Expression of ANGPTL4 in various human cancers and its implicated roles in tumor growth, metastasis, and angiogenesis

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>Detection technique</th>
<th>Expression in tumor</th>
<th>Disease outcome</th>
<th>Author (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain metastasizing melanoma</td>
<td>Real-time PCR</td>
<td>High expression in metastasized melanoma cells</td>
<td>Expression of ANGPTL4 correlated with metastasis of melanoma</td>
<td>Izraely et al. (46)</td>
</tr>
<tr>
<td>CRC</td>
<td>Real-time PCR and Western blot</td>
<td>High expression in CRC cells under synergistic effect of PGE2 and hypoxia</td>
<td>Increased cancer cell proliferation, survival, motility, and tumor-associated angiogenesis</td>
<td>Kim et al. (25)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Real-time PCR and Western blot</td>
<td>High expression in breast cancer cells under hypoxia</td>
<td>Increased metastasis by promoting extravasation in the lungs</td>
<td>Zhang et al. (28)</td>
</tr>
<tr>
<td>CRC</td>
<td>Real-time PCR and Western blot</td>
<td>High expression in 100% of patients with metastatic tumor</td>
<td>Increased expression facilitates venous invasion and distant metastasis</td>
<td>Nakayama et al. (47)</td>
</tr>
<tr>
<td>Mammary carcinoma</td>
<td>Real-time PCR and bisulfite sequencing</td>
<td>Low expression in 12% of primary carcinoma</td>
<td>Methylation-silenced ANGPTL4 is present in mammary carcinoma</td>
<td>Hattori et al. (55)</td>
</tr>
<tr>
<td>Kaposi sarcoma</td>
<td>Real-time PCR and Western blot</td>
<td>High expression in 92% of oral Kaposi sarcoma</td>
<td>Enhances Kaposi sarcomagenesis</td>
<td>Hu et al. (33)</td>
</tr>
<tr>
<td>Various cancers</td>
<td>Immunofluorescence, real-time PCR and Western blot</td>
<td>Elevated expression in tumors</td>
<td>Tumor-derived ANGPTL4 increases the O$_2$ to H$_2$O$_2$ ratio, conferring anoikis resistance</td>
<td>Zhu et al. (11)</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>DNA microarray, real-time PCR, and immunohistochemistry</td>
<td>Expressed in tumor</td>
<td></td>
<td>Mannelqvist et al. (56)</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>Global transcriptional profiling</td>
<td>High expression in tumors</td>
<td>ANGPTL4 promotes tumorigenesis</td>
<td>Feng et al. (35)</td>
</tr>
<tr>
<td>Esophageal SCC</td>
<td></td>
<td>High expression in metastatic tumor</td>
<td>ANGPTL4 promotes lymphovascular invasion</td>
<td>Shibata et al. (49)</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>Real-time PCR and Western blot</td>
<td>High expression of ANGPTL4 in 36.9% of adenocarcinomas</td>
<td>ANGPTL4 expression supports the progression of human gastric cancer</td>
<td>Nakayama et al. (48)</td>
</tr>
<tr>
<td>Kaposi sarcoma</td>
<td>Real-time PCR and Western blot</td>
<td>Upregulated by vGPCR</td>
<td>ANGPTL4 promotes angiogenesis and vessel permeability</td>
<td>Ma et al. (43)</td>
</tr>
<tr>
<td>Oral tongue SCC</td>
<td>Real-time PCR</td>
<td>High expression in tongue cancer</td>
<td>High expression is predictive of poor prognosis</td>
<td>Wang et al. (34)</td>
</tr>
<tr>
<td>Hepatoma</td>
<td></td>
<td>Increased expression in cancer</td>
<td>ANGPTL4 contributes to anoikis resistance</td>
<td>Zhang et al. (40)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Microarray and real-time PCR</td>
<td>TGF-$\beta$ enhances ANGPTL4 expression</td>
<td>ANGPTL4 facilitates metastasis to lungs</td>
<td>Padua et al. (30)</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>Oligonucleotide microarray</td>
<td>Lowered expression of ANGPTL4 in tumor</td>
<td>Role as a tumor biomarker</td>
<td>Aroa et al. (57)</td>
</tr>
<tr>
<td>General cancer</td>
<td>Transgenic mice, corneal neovascularization, and Miles permeability assays</td>
<td>Low expression in tumor</td>
<td>Mice expressing Arp4 in the skin exhibited remarkable suppression of tumor growth</td>
<td>Ito et al. (37)</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td></td>
<td>Low expression detected in tumor</td>
<td>ANGPTL4 gene is silenced by methylation, contributing to cancer development</td>
<td>Kaneda et al. (58)</td>
</tr>
</tbody>
</table>
downregulation of ANGPTL4 impairs tumor growth and metastasis (11, 35, 36). In contrast, studies have also shown that increased ANGPTL4 expression inhibits melanoma, lung, and colorectal tumor growth, metastasis, and angiogenesis (10, 37). High ANGPTL4 levels in mouse tumors also impaired tumor cell migration and invasiveness, thus inhibiting metastasis (10). The reason for this discrepancy is unclear. These results suggest that the expression and roles of ANGPTL4 may be context and tumor-type dependent. The expression of ANGPTL4 fragments in lymphatic and hematopoietic cancers, such as lymphoma and leukemia, also remains unclear. Despite the increasing emphasis on the differential roles of fANGPTL4, cANGPTL4, and nANGPTL4, most studies have not vigorously addressed the biological significance of this phenomenon in cancer. Emerging studies have also revealed that the posttranslational modification of ANGPTL4 can affect its biological functions, which suggests another level of complexity in understanding the role of ANGPTL4 in cancer. Thus, further investigation is necessary to better understand how tumor microenvironment influences the transcriptional regulation of ANGPTL4 in cancer.

ANGPTL4 and Tumor Growth

Increasing lines of evidence clearly support a tumor-promoting role of inflammation in the development and progression of cancer (38). The tumor microenvironment strongly resembles the wound environment, which contains numerous growth factors and proinflammatory lipid mediators such as prostanooids. However, instead of signaling for the removal of the abnormal cells, these proinflammatory mediators have been found to be coopted by the cancer cells to promote their growth, invasion, and metastasis (38).

COX-2 is an inducible enzyme that converts free arachidonic acid into prostanooids, including prostaglandins and thromboxanes (25). Under normal conditions, COX-2 expression is undetectable in most cells. During inflammation, COX-2 expression is upregulated in activated macrophages to increase the production of prostanooids that modulate the actions of the various immune cells (39). More recently, COX-2 has been shown to be upregulated in various carcinomas and seems to play a fundamental role in tumorigenesis. The expression of COX-2 is elevated in approximately 50% of colorectal adenomas and in up to 85% of adenocarcinomas; it is associated with poor survival among patients with CRC (25). Importantly, the tumor-promoting function of COX-2 is strongly linked to PGE2, which is also the most abundant prostaglandin found in CRCs (25). A recent study showed a synergistic effect between PGE2 and hypoxia in promoting CRC cell proliferation, and the authors ascribe tumor-promoting effects to ANGPTL4 (25). Specifically, hypoxia induced the expression of EP1, a PGE2 receptor, in CRC. The elevated level of PGE2 stimulated EP1 receptor signaling, which enhanced ANGPTL4 expression and cANGPTL4 secretion (Fig. 3). More importantly, the authors of this study went on to show that cANGPTL4, and not fANGPTL4 or nANGPTL4, was responsible for the increased CRC cell proliferation and tumor growth in vitro and in vivo (25). Notably, the study further showed that cANGPTL4 regulates cancer cell proliferation and that STAT1 induction is dependent on an NADPH oxidase-mediated production of superoxide (O2•−), as well as the Src and mitogen-activated protein kinase (MAPK) pathways. This finding is consistent with the findings by Zhu and colleagues who showed that cANGPTL4 stimulates a redox-based mechanism to enhance tumor cell survival via the alteration of the O2•− to H2O2 ratio, leading to the activation of Src and extracellular signal-regulated kinase (ERK; ref. 11).

Given the distinct functions of the different ANGPTL4 fragments, this information provides a focal point for future ANGPTL4 research, highlighting the need to demarcate the different biological functions of cANGPTL4 and nANGPTL4 in cancer progression. More extensive studies will certainly be necessary to elucidate the underlying mechanisms by which ANGPTL4 modulates cancer proliferation. One important discovery will be the identification of the receptors that mediate the downstream effects of ANGPTL4.

ANGPTL4 and Anoikis Resistance

The abnormal growth pattern of tumor cells suggests that tumor cells grow under a myriad of physiologic stresses that should trigger apoptosis and halt cancer progression. However, tumor cells evade apoptosis using multiple dysregulated mechanisms, and anoikis resistance is a hallmark of cancer (3). Given the distinct functions of the different ANGPTL4 fragments, this information provides a focal point for future ANGPTL4 research, highlighting the need to demarcate the different biological functions of cANGPTL4 and nANGPTL4 in cancer progression. More extensive studies will certainly be necessary to elucidate the underlying mechanisms by which ANGPTL4 modulates cancer proliferation. One important discovery will be the identification of the receptors that mediate the downstream effects of ANGPTL4.
suppression of ANGPTL4 via RNA interference or immunosuppression by monoclonal antibody (mAb11F6C4) modulates intracellular ROS generation to attenuate tumor growth that is associated with enhanced apoptosis in vitro and in vivo (11). mAb11F6C4 binds to the human cANGPTL4 protein and has been shown to block the interactions between cANGPTL4 with integrin, VE-Cad, and claudin 5 to attenuate tumor growth and metastasis (11, 21). Similar observations were also recently made in CRC, showing that cANGPTL4-mediated activation of ERK and Src kinase enhanced CRC cell proliferation under hypoxic conditions, further confirming the unique functions of cANGPTL4 in cancer (25). Despite the loss of cell–matrix attachment, the hijacking of integrin-mediated signaling by tumor-derived cANGPTL4 provides an alternative survival mechanism, which is important for cancer cells during

Figure 3. Diverse roles of ANGPTL4 in human cancer. The roles of ANGPTL4 in human cancer are controversial. The fANGPTL4 (depicted by a rectangle and an oval) and cANGPTL4 (depicted by an oval) have been implicated in the various aspects of cancer. The tumor microenvironment is inflammatory and hypoxic in nature. In the event of promoting tumor growth, the expression of ANGPTL4 seems to be upregulated by proinflammatory TGF-β via the SMAD pathway, as well as by COX-2–induced PGE2 signaling in carcinomas. ANGPTL4 subsequently activates NADPH oxidase (NOX) to promote tumor cell proliferation via the Src/ERK/STAT1 pathway. As the aberrant growth of tumor cells progresses, a mechanism to evade apoptosis via anoikis resistance ensures the survival of the tumor cells through ANGPTL4-mediated inhibition of proapoptotic Bcl-2 associated death promoter (BAD; see text). ANGPTL4 is also clearly involved in cancer metastasis. ANGPTL4 was found to increase tumor vascular permeability (see text). In addition, fANGPTL4 was shown to upregulate the expression of VCAM-1 on endothelial cells through an unknown mechanism facilitating the attachment of circulating metastatic cancer cells to endothelial cells, promoting their transendothelial extravasation and metastatic tumor formation. In contrast, antiangiogenic effects of fANGPTL4 have also been reported, whereby it inhibits endothelial cell proliferation, migration, and tube formation via an unknown mechanism. In addition, N-glycosylated cANGPTL4 has been shown to impair bFGF and VEGF-induced angiogenesis by suppressing the Raf/MEK/ERK pathway, suggesting that other posttranslational modifications may also play a role in the modulation of the functions of ANGPTL4. One study has also shown the inhibitory effect that fANGPTL4 has on VEGF-induced vascular permeability; however, the mechanism remains unknown. Despite the establishment of an antiangiogenic effect, whether fANGPTL4 or either of its truncated fragments binds to a receptor to modulate these effects remains to be determined.
metastasis (11). This finding suggests that anticancer strategies focusing on anoikis or redox-based apoptosis in tumors are viable. However, more studies will be required to validate the role of ANGPTL4 in anoikis. Of particular interest would be studies on the effect of ANGPTL4 on other pro-survival pathways during anoikis and whether other ANGPTL4 fragments influence tumor survival.

**ANGPTL4 and Angiogenesis**

The oxygen consumption of rapidly growing tumor cells may outweigh an insufficient oxygen supply, leading to a pathologic condition known as hypoxia (41). This hypoxic environment triggers the onset of pathologic angiogenesis, which forms new blood vessels to supply oxygen and nutrients and remove metabolic waste products supporting tumor survival (41). Angiogenesis is a multistep process that is regulated by angiogenic factors like VEGF, ANG, and, more recently, ANGPTL4 (42). An *in vivo* hypoxic signature includes ANGPTL4 as a robust hypoxia-related gene. Previous studies have indicated that ANGPTL4 mRNA and protein levels become elevated in endothelial cells in response to hypoxia and exert a VEGF-independent proangiogenic effect (42). An increased expression of ANGPTL4 was also observed in the majority of human cancers, with the expression of cANGPTL4 highly correlated to the expression of HIF-1α (see above, “ANGPTL4 Expression and Its Transcriptional Regulation in Human Cancers”). These observations indicate that ANGPTL4 could be a key modulator in tumor angiogenesis in a hypoxic tumor microenvironment.

Recent studies highlighted a prominent role for ANGPTL4 in tumor angiogenesis and vascular permeability. Using a transgenic mouse model expressing a viral G-protein coupled receptor (vGPCR), ANGPTL4 expression was upregulated by vGPCR in Kaposi sarcoma (43). This increase in ANGPTL4 expression enhanced endothelial cell migration and differentiation, both of which are important processes in angiogenesis, resulting in markedly enhanced neovascularization. The inhibition of ANGPTL4 using siRNA prevented the neovascularization process and reduced vascular permeability as well as vGPCR-induced tumorigenesis *in vivo* (43). In subsequent studies, Huang and colleagues revealed that ANGPTL4-knockdown allograft tumors express consistently lower levels of endothelial cell marker CD31 compared with wild-type allograft tumors, which is indicative of impaired angiogenesis, further confirming the proangiogenic role of ANGPTL4 in tumors (21). Although these findings suggest a proangiogenic role for ANGPTL4 in tumors, an antiangiogenic role has also been reported for ANGPTL4. Ito and colleagues found that ANGPTL4 acts as an antiangiogenic factor inhibiting VEGF-induced endothelial cell proliferation, chemotaxis, and tubule formation, which are important processes for vascularization (37). An *in vivo* corneal neovascularization assay revealed that ANGPTL4 strongly impairs VEGF-induced neovascularization and that ANGPTL4 alone does not influence neovascularization. These results seem to suggest that in the context of VEGF-induced angiogenesis, ANGPTL4 exerts antiangiogenic effects. Furthermore, in a study investigating transgenic mice that overexpress ANGPTL4 in the basal epidermis, the authors showed that elevated levels of ANGPTL4 impaired neovascularization, resulting in a decreased amount of invading capillary vessels into the xenograft tumors, effectively limiting tumor growth (37). Interestingly, the same group also reported a proangiogenic effect for ANGPTL4, further highlighting the controversial roles of ANGPTL4 (10, 20, 42).

These findings suggest a role for ANGPTL4 in tumor vascularization, although the underlying mechanism by which ANGPTL4 modulates angiogenesis remains unresolved. The discrepancies between the findings suggest a context- and tissue-dependent effect of ANGPTL4, which is further complicated by posttranslational modification of ANGPTL4 in which N-glycosylated cANGPTL4 was found to suppress the Raf/MAP–ERK kinase (MEK)/ERK signaling cascade in endothelial cells, impairing basic fibroblast growth factor (bFGF) and VEGF-induced angiogenesis (32). To resolve these disagreements, future investigations should focus on understanding the roles of posttranslational modifications on ANGPTL4 and on the mechanisms that govern the pro- and antiangiogenic effect of fANGPTL4 and its truncated fragments.

**ANGPTL4 in Tumor Invasion and Metastasis**

Metastasis is the spread of malignant cells to distant organs and is frequently the final event in tumor progression (3). Metastasis is largely responsible for the majority of cancer deaths, as the metastatic cancer cells are typically highly aggressive and resistant to anticancer drugs. However, metastasis remains the most poorly understood aspect of cancer (3). Metastasis is a complex, multistep process in which tumor cells locally invade the surrounding tissue and then migrate across the vascular endothelium of the lymphatic and vascular systems (invasion). While in circulation, tumor cells must evade immunodetection and resist anoikis before seeding at a distal site. These tumor cells leave the circulation through extravasation to establish a new secondary tumor (44). One of the fundamental mechanisms required during metastasis is the disruption of the vascular integrity, which facilitates the transmigration of the tumor cells across the endothelium (44).

Early studies have suggested that fANGPTL4 inhibits VEGF-induced angiogenesis and vascular permeability (37). In a different study, ANGPTL4 was shown to inhibit vascular leakiness, and this impaired the intravasation and extravasation of tumor cells *in vivo* (10). Notably, only a single form of ANGPTL4 was detected in sera *in vivo*. Because the molecular weight of the ANGPTL4 fragment size was not provided in the study, it was not possible to know whether the detected protein band corresponded to fANGPTL4, nANGPTL4, or cANGPTL4. Galaup and colleagues went further by using the overexpression of ANGPTL4 in melanoma to show that the expression of ANGPTL4 impairs the migration and invasion of melanoma
cells (10). Interestingly, fANGPTL4 in the presence of a smaller ANGPTL4 fragment, whose molecular weight corresponds to the cANGPTL4, was found to be produced by the melanoma cells (10). However, it is unclear which ANGPTL4 fragment was actually responsible for the inhibition of cell migration and invasion.

However, recent studies have shown that ANGPTL4 is highly expressed in metastatic cancers, suggesting that it may play a role in the metastatic processes. Furthermore, these studies have pinpointed ANGPTL4 as a critical mediator in the transmigration process (11, 21, 45). ANGPTL4 enhances vascular permeability and promotes the metastasis of breast tumor cells to the lung and the metastasis of melanoma cells to the brain (21, 28, 30, 46). TGF-β upregulates ANGPTL4 in breast tumor cells and disrupts the endothelial cell–cell junctions, leading to enhanced vascular leakiness and transendothelial migration of the tumor cells. Furthermore, clinical studies have correlated ANGPTL4 expression with venous and lymphatic invasion in human gastric and CRCs, which further emphasizes the role of ANGPTL4 in tumor metastasis (47–49). ANGPTL4 was found to promote transendothelial migration and metastasis of hepatocellular carcinoma cells through the upregulation of vascular cell adhesion molecule 1 (VCAM-1) on endothelial cells and the activation of the VCAM-1/integrin β1–signaling axis (27). In this mechanism, the increased VCAM-1 expression on endothelial cells facilitates the attachment of the metastatic cancer cells in circulation and the subsequent transendothelial extravasation to establish a metastatic tumor. ANGPTL4 also disrupts endothelial cell–endothelial cell interactions, leading to enhanced vascular leakiness, and promotes the metastasis of cancer cells to the lung (21). Another study pinpointed the vascular disruptive effect of ANGPTL4 to the cANGPTL4 fragment. In this study, the authors showed that cANGPTL4 interacts with integrin α5β1 to activate the Rac/PAK-signaling axis, weakening the endothelial cell–endothelial cell contacts and facilitating cANGPTL4 interaction with VE-Cad and claudin-5 (Fig. 3; ref. 21). This interaction results in the internalization and declustering of the endothelial cell tight junction and adherens junction proteins, leading to the disruption of the vascular barrier. Clearly, more investigations are needed to clarify the prometastatic role of ANGPTL4 and its underlying mechanism of action. Taken together, these results suggest that ANGPTL4 may have both homotypic and heterotypic influences in the tumor microenvironment (Fig. 3).

The role of ANGPTL4 in vascular permeability and metastasis is controversial. The apparent discrepancy that was observed in previous studies seems to derive from the use of different experimental approaches and models in each study. Additionally, the functions of ANGPTL4 may be affected by the tumor microenvironment, suggesting a context- and tissue-specific activity. Presently, the lack of structural information on ANGPTL4 limits our ability to fully explain this phenomenon. Unfortunately, numerous studies that have attempted to decipher the role of ANGPTL4 in cancer have failed to address the posttranslational modifications and proteolytic processing of ANGPTL4, which clearly have a significant influence on the functions of ANGPTL4 (10, 30, 37, 47–49). For example, the expression of either the nANGPTL4 or the cANGPTL4 truncation in transgenic mice remains unknown (43). The differential processing of ANGPTL4 has a major effect on the different biological functions of ANGPTL4 under various contexts and may have contributed to the observed discrepancies (11, 21, 50). Indeed, only cANGPTL4 can interact with integrin and stimulates intracellular signaling to promote tumor cell survival (7, 8, 11). In addition, it seems that the processing of ANGPTL4 in humans is tissue dependent, as different organs such as the liver and kidney have been found to use different forms of ANGPTL4 (50). One recent finding has implicated the glycosylated form of ANGPTL4 in augmenting the leakiness of the kidney glomerular epithelium, suggesting that this type of posttranslational modification of ANGPTL4 may direct its function in this case (50). Further in vitro and in vivo experiments are necessary to clarify the role of ANGPTL4 in metastasis.

Clinical Relevance of ANGPTL4

The endocrine regulatory role of ANGPTL4 in lipid metabolism, insulin sensitivity, and glucose homeostasis has been extensively studied (14, 15, 22). As a critical mediator in energy homeostasis, it is not surprising that ANGPTL4 has been suggested as a potential therapeutic target for metabolic and cardiovascular diseases. Antibodies against nANGPTL4 could potentially lower the levels of triglycerides and cholesterol in patients with heart disease (http://www.lexpharma.com/news/2007-07-02-160145.html). The targeted silencing of ANGPTL4 offers another potential therapeutic strategy in the treatment of atherosclerosis, dyslipidemia, and diabetic wounds (8, 14, 51, 52). These reports emphasize the clinical relevance of ANGPTL4 as an attractive therapeutic candidate.

ANGPTL4 as an antigrowth and antimitotic target remains debatable because the multifaceted roles of ANGPTL4 in tumorigenesis need further clarification. However, several research groups have shown promising results suggesting that ANGPTL4 could be a potential interventional therapeutic target in the battle against cancer (11, 21, 25, 30). On one hand, it was shown that the immunosuppression of cANGPTL4 by a neutralizing antibody results in tumor regression, which was attributed to reduced cell proliferation and enhanced redox-based cell apoptosis of the regressed tumor (11). The suppression of cANGPTL4 by either a neutralizing antibody or RNA interference also reduces vascular disruption and, thus, metastasis (21). In addition, the inhibition of cANGPTL4 may confer chemosensitivity to cancer cells (25). On the other hand, an increased level of ANGPTL4 has been found to inhibit tumor angiogenesis, vascular permeability, and invasiveness of cancer cells (10, 20, 37, 42). Although discrepant in their results, these studies have used different cancer cell models, suggesting that potential inhibitory therapy may have a restricted therapeutic range or may be restricted to certain cancer types.
ANGPTL4 could also be a potential diagnostic biomarker in cancer. ANGPTL4 expression was observed to be elevated in numerous cancers at both the mRNA and protein level (Table 2). Given the implications that ANGPTL4 may have on cancer progression, a recent report suggested that ANGPTL4 mRNA could be used as a diagnostic marker to distinguish primary and metastatic clear cell renal cell carcinoma (53). Several other studies have also delineated the ANGPTL4 expression level in primary and invasive tumors, showing that high ANGPTL4 mRNA and protein expression in human tumor biopsies often correlates with a poor prognosis and a poor disease outcome (25, 33, 34). As a secreted protein, ANGPTL4 protein levels could also be detected in the serum (23). Zhu and colleagues have also shown that increased cANGPTL4 protein levels could be detected in the serum of tumor-bearing mice (11). cANGPTL4 was also found to be secreted by hypoxic cancer cells, lending further credence to the potential use of ANGPTL4 as a biomarker (25).

**Conclusion and Future Directions**

Recent work has suggested that ANGPTL4 has increasingly important roles in cancer development and progression. Therefore, many researchers have postulated that diverse and sometimes conflicting roles for ANGPTL4 exist in cancer. The collective data show that ANGPTL4 exerts a proangiogenic effect by promoting vascularization under hypoxic conditions in tumors (42). Moreover, ANGPTL4 promotes invasion and metastasis by enabling vascular permeability and conferring anoikis resistance to the tumor cells (11, 21, 28, 46). However, antitumorigenic roles of ANGPTL4 have also been established, particularly in the inhibition of tumor angiogenesis and metastasis (10, 37). The role of ANGPTL4 in human cancer could certainly be context dependent or tissue dependent. These seemingly controversial results underscore the need to address the function of full-length ANGPTL4 and its truncated fragments in cancer and the posttranslational modifications that may clarify the context-dependent roles of the different fragments of ANGPTL4 in cancer.

The role of ANGPTL4 in systemic lipid and glucose metabolism has been established recently (15). However, it would be interesting to examine the impact that ANGPTL4 may have on tumor cell metabolism. Evidence has shown that AKT/PKB can regulate glucose consumption and glycolysis during the metabolic reprogramming that occurs in cancer cells. Coupled with the ability of ANGPTL4 to regulate the function of AKT/PKB in conferring tumor cell resistance against anoikis, it remains to be observed if ANGPTL4 plays a role in the alteration of cancer cell metabolism as well (11, 54). Another area that remains to be elucidated is the biological function and the inherent signaling pathway of the different fragments of ANGPTL4 (Fig. 2). It is interesting to note that a "receptor" for the full-length and the N-terminal ANGPTL4 has yet to be identified (see above, "Functional Domains of ANGPTL4"). The cANGPTL4 has been reported to bind to several interacting partners, resulting in the activation of different pathways in a context- and tissue-dependent manner (11, 21, 25). However, it is unknown whether the COOH-terminal domain of the ANGPTL4 activates similar signaling pathways and biological functions.

New and promising findings have already earmarked ANGPTL4 as a diagnostic biomarker in various cancer types. Despite the reported conflicting roles of ANGPTL4, several exciting findings offer a novel perspective promoting the therapeutic use of cANGPTL4 antibody as a primary clinical drug. Clearly, the diverse roles of ANGPTL4 in cancer remain to be discovered. However, given the complex nature of cancer development and the many proteins involved, the likelihood of curing cancer by targeting a single protein is unlikely to achieve the desired outcome. Indeed, multimodal therapy is a new, yet extremely important, advance in cancer treatment. By building on the current mechanistic insights of ANGPTL4 function, elucidating the diversified roles of ANGPTL4 in human cancer promises to be a challenging yet provocative endeavor.

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No potential conflicts of interest were disclosed.

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