J Biomed Sci (2008) 15:675–685
DOI 10.1007/s11373-008-9264-9

REVIEW

Connective tissue growth factor (CTGF) and cancer progression

Chia-Yu Chu · Cheng-Chi Chang · Ekambaranellore Prakash · Min-Liang Kuo

Received: 30 November 2007 / Accepted: 6 June 2008 / Published online: 13 July 2008

Abstract Connective tissue growth factor (CTGF) is a member of the CCN family of secreted, matrix-associated proteins encoded by immediate early genes that play various roles in angiogenesis and tumor growth. CCN family proteins share uniform modular structure which mediates various cellular functions such as regulation of cell division, chemotaxis, apoptosis, adhesion, motility, angiogenesis, neoplastic transformation, and ion transport. Recently, CTGF expression has been shown to be associated with tumor development and progression. There is growing body of evidence that CTGF may regulate cancer cell migration, invasion, angiogenesis, and anoikis. In this review, we will highlight the influence of CTGF expression on the biological behavior and progression of various cancer cells, as well as its regulation on various types of protein signals and their mechanisms.

Keywords Angiogenesis · Anoikis · Cancer · Connective tissue growth factor · CTGF · Invasion · Migration

C.-Y. Chu
Department of Dermatology, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei 100, Taiwan, ROC

C.-Y. Chu · C.-C. Chang · E. Prakash · M.-L. Kuo
Angiogenesis Research Center, National Taiwan University, Taipei 100, Taiwan, ROC

C.-C. Chang · E. Prakash · M.-L. Kuo (✉)
Laboratory of Molecular and Cellular Toxicology, Institute of Toxicology, College of Medicine and Angiogenesis Research Center, National Taiwan University, No.1 Sec.1 Jen-Ai Rd., Taipei 100, Taiwan, ROC
e-mail: kuominliang@ntu.edu.tw; toxkml@gmail.com

Introduction

Connective tissue growth factor (CTGF) was initially discovered in 1991 as a secreted protein in the conditioned media of cultured human umbilical vascular endothelial cells [1]. CTGF is a member of the CCN family of secreted, matrix-associated proteins encoded by immediate early genes that play various roles in angiogenesis and tumor growth [2–9]. The name CCN stands for **C**yr61 (cysteine-rich 61), **C**tgf (connective tissue growth factor), and **N**ov (nephroblastoma overexpressed) [6]. The CCN family now comprises six members including Cyr61 (CCN1), CTGF (CCN2), Nov (CCN3), Wisp-1/elm1 (CCN4), Wisp-2/rCop1 (CCN5), and Wisp-3 (CCN6). These proteins share uniform modular structure which mediates various cellular functions such as regulation of cell division, chemotaxis, apoptosis, adhesion, motility, angiogenesis, neoplastic transformation, and ion transport [2–9]. Recently, CTGF expression has been shown to be associated with tumor development and progression [10–17]. For example, the level of CTGF expression is positively correlated with bone metastasis in breast cancer [10], glioblastoma growth [11], poor prognosis in esophageal adenocarcinoma [12], aggressive behavior of pancreatic cancer cells [13], and invasive melanoma [14]. In contrast, CTGF expression was negatively correlated with proliferation activity and tumor grade of chondrosarcomas [15], and overexpression of CTGF has been shown to suppress the tumor growth of oral squamous cell carcinoma (SCC) cells transplanted into mice [16]. Accordingly, there is growing body of evidence that CTGF may regulate cancer cell migration, invasion, angiogenesis, and anoikis. In this review, we will highlight the influence of CTGF expression on the biological behavior and progression of various cancer cells, as well as its regulation on various types of protein signals and their mechanisms.

The biological functions of CTGF

The basic structure of CTGF, as in other CCN family proteins, consists of up to four modules that resemble the functional domains previously identified in major regulatory proteins (Fig. 1). These four modules share identity with insulin-like growth factor binding proteins (IGFBP), the von Willebrand factor (VW), thrombospondin-1 (TSP1), and a cysteine knot motif [3]. There are also a few biologically active isoforms, generated by either post-translational processing or alternate splicing. A carboxy-truncated CTGF protein, lacking the CT module, was also isolated in high concentration from the primary human osteoblasts [5]. The absence of CT module does not necessarily abrogate its ability to promote adhesion of osteoblasts. Nevertheless, this module is responsible for determining the ability of CTGF to induce dose-dependent adhesion to various other cell types [18, 19]. The existence of two biologically active truncated isoforms of CTGF in normal uterine secretory fluids provided the functional clue for the importance of post-translational processing of CTGF [2].

CTGF, a 36–38 kD cysteine-rich peptide containing 349 amino acids, is predominantly identified in endothelial cells, fibroblasts, cartilaginous cells, smooth muscle cells, and some cancer cell lines [20]. After synthesis, CTGF is secreted through the Golgi apparatus [21], which requires its N-terminal 37 amino acid signal sequence. Given that CTGF is secreted, most of the studies regarding CTGF function have focused on the effect of exogenously added recombinant CTGF in cell cultures [22]. Although CTGF is glycosylated, the glycosylational modification is neither a prerequisite for secretion [21] nor it has yet been attributed to any of the reported functions of CTGF.

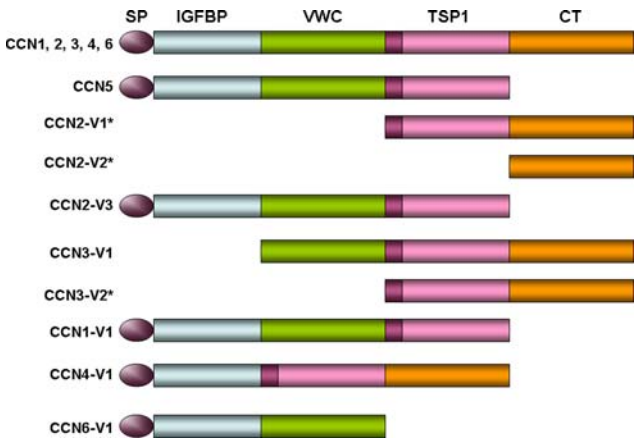

Fig. 1 Structure of full-length and truncated CTGF, in comparison with other members of CCN family proteins. IGFBP: insulin-like growth factor-binding protein; VWC: von Willebrand type C motif; TSP1: thrombospondin type 1 motif; CT: carboxyl-terminal cysteine knots module. *: These isoforms are believed to originate from post-translational processing. They are present in biological fluids and cell-culture medium

One of the main functions of CTGF is to promote cell adhesion through a unique integrin and heparin sulfate proteoglycan dependent mechanism [23]. The carboxyl-terminal 10 kDa of CTGF, which binds heparin, has been shown to be sufficient to promote cell adhesion [18]. This fragment also promotes fibroblast proliferation [24]. The integrins through which CTGF promotes adhesion vary depending on the cell type; for example, CTGF promotes adhesion to human foreskin fibroblasts through integrin $\alpha 6\beta 1$, to human platelets through integrin $\alpha \mathrm{II}\beta 3$, to endothelial cells through integrin $\alpha v\beta 3$, and to blood monocytes through integrin $\alpha \mathrm{M}\beta 2$ [22].

High levels of CTGF expression has been detected in many fibrotic lesions, indicating its role in promoting fibrosis. CTGF exhibits mitogenic and chemotactic effects on fibroblasts [1] and is also reported to enhance the mRNA expression of $\alpha 1$(I) collagen, fibronectin, and $\alpha 5$ integrin in fibroblasts [25]. The finding that TGF-β increases CTGF synthesis and that TGF-β and CTGF share many functions in common, is consistent with the hypothesis that CTGF is a downstream mediator of TGF-β [20]. In endothelial cells, CTGF mediates several functions such as proliferation, migration, differentiation, and survival, leading to enhanced angiogenesis [26, 27]. It also induces chondrocyte proliferation and differentiation [28, 29].

Expression of CTGF in different cancers

CTGF is believed to be a multifunctional signaling modulator involved in a wide variety of biologic or pathologic processes, such as angiogenesis, osteogenesis, renal disease, skin disorders, and tumor development [2–6, 17]. There are at least 21 different human tumors or cancers that have been found to have CTGF expression (Table 1), signifying its influence on the biology and progression of cancer [10–16, 30–62]. Of particular interest is the fact that CTGF is found to be expressed in human tumor cells or surrounding stromal cells, including acute lymphoblastic leukemia (ALL) [30, 31], breast cancer cells [32, 34–39], cervical cancer [41], chondrosarcoma [15], cutaneous fibrohistiocytic and vascular tumors [42], esophageal cancer [12], gastric cancer [43], glioblastoma and gliomas [11, 44], hepatocellular carcinomas [45], laryngeal squamous cell carcinoma (SCC) [46], non-small-cell lung cancer [47–49], melanoma [14, 62], myofibroblastic tumors [51], oral SCC [16], ovarian cancer [52], pancreatic cancer [13, 53–56], prostate cancer [57], rhabdomyosarcoma [58], and Wilms tumor [59–61]. In glioblastoma, CTGF is strongly stained in tumor cells and proliferating endothelial cells, strongly emphasizing a role for CTGF in angiogenesis [11]. Furthermore, the level of CTGF

Table 1 Summary of the expression of CTGF in cancer cells

Cancers	Cells expressing CTGF	Effects of CTGF expression	References
1. Acute lymphoblastic leukemia (ALL)	1. Leukemic cells 2. B cell lineage of ALL	1. Worsening of overall survival	[30, 31]
2. Breast cancer	1. Breast cancer cell 2. Fibrous stroma cells	1. Promote osteolytic bone metastasis 2. Promote angiogenesis 3. Induce apoptosis (MCF-7 cells) 4. Enhance breast cancer cell motility	[10, 32–40]
3. Cervical cancer	NM	Cancer progression	[41]
4. Chondrosarcoma	Chondrosarcoma cells	Negative correlation with proliferation, tumor grade and prognosis	[15]
5. Cutaneous fibrohistiocytic tumors (dermatofibroma, dermatofibrosarcoma protuberans, malignant fibrous histiocytoma)	Tumor cells	Loss of CTGF expression when benign fibrohistiocytic tumors achieve malignant potency	[42]
6. Cutaneous vascular tumors (angiolipoma, angioleiomyoma)	Tumor cells	Loss of CTGF expression when benign vascular tumors achieve malignant potency	[42]
7. Esophageal cancer	1. Adenocarcinoma cells 2. Surrounding stroma cells	1. Tumor progression and poor survival in adenocarcinoma cells 2. Longer survival in SCC	[12]
8. Fibrosarcoma	Fibrosarcoma cell	Enhanced angiogenesis	[36]
9. Gastric cancer	Cancer cells	1. Increased lymph node metastasis 2. Poor survival	[43]
10. Glioblastoma and glioma	1. Glioblastoma and glioma tumor cells 2. Proliferating endothelial cells in glioblastoma	1. Stimulate angiogenesis 2. Positive correlation between CTGF mRNA levels and tumor grade, gender and pathology	[11, 44]
11. Hepatocellular carcinoma (HCC)	NM	1. CTGF expression was higher in HCC tissue compared to those of control 2. Positively correlated with venous invasion and tumor grade	[45]
12. Laryngeal squamous cell carcinoma (SCC)	Tumor cells	1. Negatively correlated with metastasis and clinical staging	[46]
13. Lung adenocarcinoma	Tumor cells	1. Inhibit invasion and metastasis 2. Suppression of cell proliferation and signaling transduction 3. Negatively correlated with patient survival and metastasis 4. Inhibit angiogenesis	[47–50]
14. Melanoma	Tumor stroma	Correlated with invasive histological type	[14]
15. Myofibroblastic tumors[a]	1. Tumor cells 2. Endothelial cells	Involved in the pathogenesis of myofibroblastic tumors	[51]
16. Oral SCC	Tumor cells	1. Attenuated cell growth 2. Less potent tumorigenicity	[16]
17. Ovarian cancer	Tumor cells	Inhibit growth of ovarian cancer cells	[52]
18. Pancreatic cancer	1. Pancreatic stellate cells 2. Cultured pancreatic cancer cells 3.Cancer-associated fibroblasts	1. Increased proliferation and invasiveness of PANC-1 cells 2. Enhanced pancreatic tumor growth	[13, 53–56]
19. Prostate cancer	Stromal cells	CTGF-expressing stroma induced significant increases in microvessel density and xenograft growth	[57]

Table 1 continued

Cancers	Cells expressing CTGF	Effects of CTGF expression	References
20. Rhabdomyosarcoma	Tumor cells	CTGF emerges as a survival and differentiation factor	[58]
21. Wilms tumor	Tumor cells	1. The expression of CTGF was suppressed by WT1 2. May be related to tumor progression	[59–61]

[a] Including angiofibroma, malignant fibrous histiocytoma, infantile myofibromatosis, and malignant hemangiopericytoma; NM: not mentioned

expression is positively correlated with bone metastasis in breast cancer [10, 39], progression of cervical cancer [41], poor prognosis in esophageal adenocarcinoma [12], aggressive behavior of pancreatic cancer cells [13], invasiveness of melanoma [14], lymph node metastasis and poor survival of gastric cancer [43], progression of gliomas [44], recurrence and metastasis of hepatocellular carcinoma [45], increased proliferation, anchorage-independent growth and invasiveness of pancreatic cancer cells [54, 55] (Fig. 2). Based on these studies, CTGF seems to be a positive regulator of tumor development and progression.

In contrast to the above reports on CTGF as tumor inducer, ectopic overexpression of CTGF in oral squamous cell carcinoma cells induces attenuated cell growth and decreases tumorigenicity in an animal model [16]. CTGF has also been found to induce apoptosis in the human breast cancer cell line MCF-7 [33] while the expression of CTGF was negatively correlated with proliferation activity and tumor grade of chondrosarcomas [15], and also negatively correlated with lymph node metastasis and clinical staging of laryngeal SCC [46]. CTGF has been found to suppress cell proliferation and behave as a tumor suppressor in non-small-cell lung cancer [47–49]. In ovarian cancers, epigenetic silencing by hypermethylation of the CTGF promoter leads to a loss of CTGF function, which may be a causative factor in the carcinogenesis of ovarian cancer [52]. In addition, exogenous restoration of CTGF expression or treatment with recombinant CTGF inhibited the growth of ovarian cancer cells lacking its expression, whereas knockdown of endogenous CTGF accelerated growth of ovarian cancer cells expressing *CTGF* gene [52]. Collectively, these results suggest that the role of CTGF in different types of cancer may vary considerably, depending on the tissue involved. The question as how the action of CTGF protein is determined in a cell or tissue context, is interesting and deserves further investigation [63].

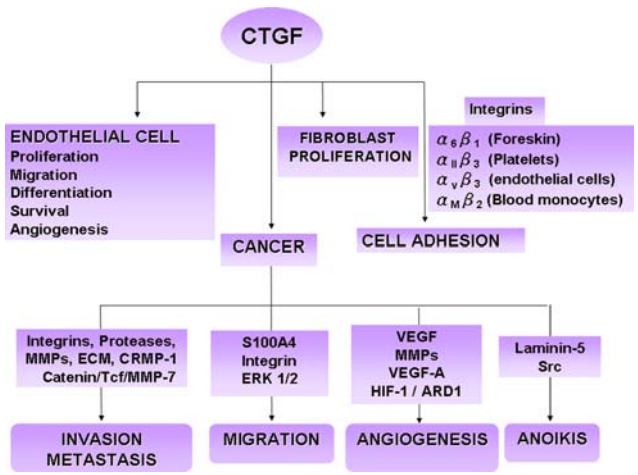

Fig. 2 The roles of CTGF in various aspects of cancer progression

Roles of CTGF on cancer cell invasion and metastasis

Cancer cell invasion is the most intricate step in the cascade of events leading to metastasis, which occurs through biological activities and interaction of cancer cells with the surrounding environment. The invasion process is categorized into three steps: binding of extracellular matrix (ECM) with tumor cell; protease production; tumor cell motility via matrix degradation, and after completing these steps tumor cells enter into the blood stream and lymph system to initiate distant metastasis [64–68]. Several factors including integrins, protease, metalloproteinases (MMPs) and ECM components have been correlated with cancer cell invasion and metastasis [69]. The MMPs degrade basement membrane and stroma, facilitating the invasion of the cancer cells into the adjacent tissues [70].

Metastasis is the dissemination of cancer cells from primary tumor to distant site, which is a major prognostic factor governing cancer patient mortality. The metastatic process involves detachment of tumor cells from primary tumor mass, micro-invasion of tumor cells into stromal tissue, intravasation of tumor cells into blood vessels and extravasation of tumor cell growth in secondary site [71, 72]. Metastatic competence requires a complex set of cellular functions that are associated with a cadre of molecular and cellular changes [73, 74]. To enable metastasis, tumor cells must coordinate the expression of metastasis promoter genes and/or decrease the expression of metastasis suppressor genes. Metastasis suppressor genes are operationally defined as genes that encode proteins which could suppress the formation of overt metastases but exert no measurable effect on in vitro or

in vivo proliferation [73, 74]. As a dynamic process, metastasis requires cells to sequentially invade local tissues and disseminate from the primary tumor, lodge in and extravasate from the microvasculature at a secondary site, and finally form microscopic colonies giving rise to clinical metastases [75]. Proteins encoded by metastasis suppressor genes can block any of the steps in this process, the net result being suppression of overt metastases [76].

Recently, we found that CTGF appears to be a suppressor of lung tumor invasion and metastasis [47]. Our studies directly demonstrated that overexpression of CTGF not only suppressed the ability of lung adenocarcinoma cells to invade Matrigel in vitro, but also strongly inhibited tumor metastasis in an animal model. Decreased CTGF expression in tumor tissues was associated with advanced tumor stage, lymph node metastasis, early postoperative relapse, and reduced patient survival rates. We have also demonstrated that the level of CTGF protein was significantly higher in normal lung type I and II epithelial cells than in the majority of metastatic adenocarcinoma specimens, suggesting that the level of CTGF protein decreases during metastasis. Furthermore, we showed that a metastasis suppressor gene, collapsin response mediator protein 1 (CRMP-1), was functionally involved in the CTGF-mediated invasion and metastasis inhibition of human lung adenocarcinoma. CTGF-mediated increase in CRMP-1 expression was abolished by treatment with antibodies that specifically block the function of integrins $\alpha v\beta 3$ and $\alpha v\beta 5$. In addition, antisense CRMP-1 oligonucleotides essentially abolished the CTGF-mediated inhibition of cell invasion. These data indicate that CRMP-1 acts downstream of CTGF and is regulated by an integrin-related signaling pathway.

Integrins are important receptors for CCN proteins, and receptor activation may produce a variety of effects. CTGF protein can bind directly to integrins $\alpha v\beta 3$ and $\alpha IIb\beta 3$ [27]. Interaction of CTGF with integrin $\alpha v\beta 3$ promotes endothelial cell adhesion, migration, and survival and also induces angiogenesis in vivo [27]. CTGF also stimulates human skin fibroblast migration and proliferation through integrin $\alpha 6\beta 1$. In contrast, we show that both $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins were required for the inhibition of CTGF-mediated invasion [47].

In addition, we also found that colorectal cancer patients with decreased CTGF expression pronounced strong lymph node metastasis, with low recurrence time, and a short survival period [63]. Particularly, CTGF seemed to be an independent prognostic factor that will allow the successful differentiation of a high-risk population from the group of patients with stage II and stage III disease. At a mechanistic level, overexpression of CTGF in human colorectal cancer cells results in a decrease in the invasive ability. Consistent with these reports, reduced CTGF expression significantly enhanced hepatic metastasis of colorectal cancer cells in a mouse model. Interestingly, CTGF transfection strongly reduced β-catenin/Tcf signaling and the level of its downstream gene target MMP-7 in human colorectal cancer cells [63]. The accumulating evidence indicates that the signaling/oncogenic activity of β-catenin/Tcf can be trans-repressed by the activation of nuclear receptors such as the retinoic acid receptor, the vitamin D receptor, and the androgen receptor. Our preliminary data from microarray analysis show that retinoic acid receptor mRNA was up-regulated in CTGF transfectants. It is therefore possible that CTGF expression up-regulates nuclear receptor genes and inhibits β-catenin/Tcf activity by interacting with them [63]. We suggest that CTGF inhibits colorectal cancer invasion and metastasis perhaps through inhibition of the β-catenin/Tcf/MMP-7 pathway. Furthermore, the ability of CTGF to inhibit metastasis and invasion suggests that this growth factor may be a potential candidate of therapeutic importance for patients with colorectal cancer [63].

Roles of CTGF on cancer cell migration

Migratory ability of a cancer cell is a major prerequisite for the successful execution of metastatic process, including movement within the connective tissue of primary site, entry of cancer cells into circulation by intravasation, and invasion of the target organ by extravasation; therefore the migration ability is positively correlated with tumor metastasis [40]. In order to invade, a tumor cell must undergo major changes in shape. Cellular motility depends on localized actin polymerization at the leading edge of the cells [77], and the polymerization and depolymerization of actin filaments must be under dynamic control. Simultaneously, paxillin and vinculin interact at focal contacts of the actin stress fibers, providing a link to the extracellular matrix (e.g. fibronectin and vitronectin). These cytoskeletal changes enable the invading cell to pass through the stromal cells, extracellular matrix and endothelial cell layer. Integrins, paxillin, selectins, transmembrane receptor tyrosine kinases, phospholipids, focal adhesion kinases (FAKs), GTPases and the S100 calcium binding protein A4 (S100A4) calcium binding protein have been described as being involved in regulating the organization of the actin cytoskeleton [78–80].

Previous large scale microarray analysis revealed that CTGF is crucial for the formation of osteolytic bone metastasis in beast cancer [10, 81]. Recently, we further demonstrated that the molecular mechanism by which CTGF-confers cellular metastatic ability of breast cancer is mediated by S100A4 upregulation by integrin $\alpha v\beta 3$ and/or ERK1/2 [40]. This is based on the following evidence. First, overexpression of CTGF appreciably increases the

migratory ability of MCF-7 cells. Conversely, knockdown of CTGF abolishes the migratory ability of MDA231 cells. Second, CTGF expression leads to morphological alterations and formation of F-actin and focal adhesions. Third, ERK1/2 activation is essential for the CTGF-mediated migratory effects. Fourth, blockade of the CTGF–integrin-$\alpha v\beta 3$ axis attenuates CTGF-induced ERK1/2 activation and subsequent cellular migration. Fifth, the prometastatic *S100A4* gene is regulated by the signaling cascades of CTGF–integrin-$\alpha v\beta 3$–ERK1/2 and contributes to the metastatic ability. Finally, CTGF expression levels are correlated with S100A4 expression levels in primary human breast tumors [40].

Roles of CTGF on cancer cell angiogenesis

Angiogenesis, the formation of new blood vessels, is a highly coordinated process involving several molecules and is vital for tumor growth and metastasis [82–85]. Proteins mediating angiogenesis are widely known as angiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and other growth factors. Among them, VEGF is the major regulator of tumor-associated angiogenesis [86]. It has previously been reported that VEGF-A protein expression is strongly associated with many types of cancer progression and with clinical manifestation [87, 88]. Inhibition of VEGF reduces angiogenesis and tumor growth in vivo [88]. Conversely, VEGF overexpression is associated with increased microvessel density, tumor metastasis, and poor prognosis [89–95].

Hypoxia-inducible factor (HIF)-1 α is a transcription factor that regulates the supply of blood to tissues through its effects on VEGF expression [96]. HIF-1 α activity is induced by hypoxia in almost all cell types; however, under normoxic conditions, the protein is quite sensitive to ubiquitin-dependent degradation. HIF-1 α protein degradation is reduced under hypoxic conditions by posttranslational modification and binding to the von Hippel-Lindau (pVHL) tumor suppressor protein [97]. HIF-1 α binds to pVHL only after it is hydroxylated by HIF-prolyl hydroxylase (PHD1) and acetylated by arrest-defective 1 protein (ARD1) acetyltransferase [98–101]. HIF-1 α protein levels also increase in response to growth factor stimulation by mechanisms that differ from those that occur under hypoxic conditions. These oxygen-independent mechanisms are not clearly understood, but they are thought to involve growth factors, cytokines, and other signaling molecules that stimulate synthesis of HIF-1 α or decrease its degradation [102–107].

One possible mechanism involves the activity of connective tissue growth factor (CTGF), an extracellular matrix—associated signaling molecule that binds directly with moieties in the pericellular environment. CTGF has recently been identified as a regulator of angiogenesis and has many functions in normal and pathologic processes [1, 6, 11, 17, 26, 27, 35–37, 57, 108–110]. Although CTGF was originally purified from medium conditioned by human umbilical vein endothelial cells [111], it can be produced by and acts on many cell types, including fibroblasts, smooth muscle, endothelial, neural, and cancer cells [28, 29, 47, 112–115]. CTGF can influence several functions of endothelial cells, including in vitro proliferation and tube formation [26], cell adhesion and migration [27], and induction of angiogenesis in vivo [26, 27, 108]. However, it has also been reported that CTGF inhibits angiogenesis in vitro through an interaction with VEGF [111]. Angiogenic activity of VEGF was restored after the CTGF–VEGF complex was digested by matrix metalloproteinase 3 or −7 [112]. Therefore, CTGF may play an inhibitory role through VEGF-induced angiogenesis during embryonic development, tissue maintenance, and the pathological processes of various diseases. Although CTGF itself is an angiogenesis factor, its interaction with other molecules may alter its function.

One previous study revealed that stromal expression of CTGF promotes angiogenesis of prostate cancer cells [57]. To evaluate the role of stromal-expressed CTGF in tumor progression, either engineered mouse prostate stromal fibroblasts expressing CTGF or 3T3 fibroblasts engineered with mifepristone-regulated CTGF were combined with LNCaP human prostate cancer cells in the "differential reactive stroma (DRS)" xenograft tumor model. The results showed that the expression of CTGF in tumor-reactive stroma induced significant increases in microvessel density and xenograft tumor growth [57].

In breast cancer cells, CTGF has been found to be abundantly present in MDA-MB-231 cells, and its gene expression and protein secretion are up-regulated by hypoxia [35]. In vivo experiments also confirmed that the xenograft formation and neovascularization activity of this breast cancer cell line could be suppressed by neutralizing CTGF-specific polyclonal antibody [36]. In addition, hypoxia-induced release of CTGF has been found to initiate the invasive angiogenesis cascade by modulating the balance of extracellular matrix synthesis and degradation via matrix metalloproteinases (MMPs) secreted by endothelial cells in response to CTGF [37].

In contrast, CTGF may inhibit tumor angiogenesis by regulating the expression of VEGF-A. Recently, we have demonstrated that CTGF expression can inhibit tumor growth in primary or metastatic sites by reducing VEGF-A gene expression and its subsequent angiogenic effects in tumor cells [50]. We also observed that the effect of CTGF on VEGF-A gene expression was mediated by accelerating HIF-1α protein degradation through ARD1-dependent

acetylation. Most importantly, we have provided functional evidence that CTGF acts as an angiogenesis suppressor, inhibiting tumor growth and metastasis in mouse models of human lung adenocarcinoma [50].

Roles of CTGF on cancer cell anoikis

Anoikis is a form of apoptosis induced by loss of cell anchorage [116], a remarkably complex process involving several adhesion molecules that interact with an array of different structural components referred to as the extracellular matrix (cell–matrix anchorage) and neighboring homotypic or heterotypic cells (cell–cell anchorage). The process of 'loss of cell anchorage' therefore embraces the dissolution of potentially many different kinds of cell–cell/cell–matrix interactions. Several means of cell anchorage provide survival signals to the cell and therefore the term 'anoikis' describes rather the final outcome of what results once these different means of cell adhesion are disturbed than standing for a distinct and single molecular mechanism leading to apoptosis [116].

Anoikis resistance or anchorage-independent survival and growth are certain hallmarks of tumor cells. This property of tumor cells suggests altered activities in tumor cells that compensates for the cell survival signals lost by disrupting cell–matrix interactions. However, the exact mechanisms that are responsible for anoikis resistance of naturally occurring human cancer cells have not been clearly understood [117]. Recently, several studies found that the anoikis resistance of lung adenocarcinoma may be related to the expression of laminin-5 or Src [117–119]. However, we also found CTGF expression in lung adenocarcinoma cell lines may induce their sensitivity to anoikis and may lead to inhibited metastasis potential (unpublished results), indicating that CTGF may also play a role in regulating anoikis of cancer cells.

Conclusion

CTGF is a multifunctional signaling modulator involved in a wide variety of biologic or pathologic processes, such as angiogenesis, osteogenesis, renal disease, skin disorders, and tumor development. Although it was originally isolated from the conditioned media of cultured human umbilical vascular endothelial cells, it could also be detected in endothelial cells, fibroblasts, cartilaginous cells, smooth muscle cells, and some cancer cells. Recently, CTGF expression has been found to regulate cancer cell migration, invasion, angiogenesis, and anoikis. Although CTGF expression seems to be associated with progression of many kinds of cancers, its expression may have tumor suppressive effects in lung adenocarcinoma cells, colorectal cancer cells and oral squamous cell carcinoma cells. Therefore, the role of CTGF in different types of cancer may vary considerably, depending on the tissue involved. Furthermore, the expression of some important cancer progression-related molecules, such as CRMP-1, HIF-1α, β-catenin/Tcf/MMP-7, and S100A4, has been found to be regulated by CTGF. Understanding the detailed mechanisms involved in CTGF-mediated regulation will extend us further insight on the progression and metastasis of human cancers. Moreover, deciphering tumor suppressive effects of CTGF may have future important therapeutic applications.

References

1. Bradham DM, Igarashi A, Potter RL, Grotendorst GR (1991) Connective tissue growth factor: a cysteine-rich mitogen secreted by human vascular endothelial cells is related to the SRC-induced immediate early gene product CEF-10. J Cell Biol 114:1285–1294
2. Brigstock DR (1999) The connective tissue growth factor/cysteine-rich 61/nephroblastoma overexpressed (CCN) family. Endocr Rev 20:189–206
3. Lau LF, Lam SC (1999) The CCN family of angiogenic regulators: the integrin connection. Exp Cell Res 248:44–57
4. Perbal B (2001) The CCN family of genes: a brief history. Mol Pathol 54:103–104
5. Perbal B (2001) NOV (nephroblastoma overexpressed) and the CCN family of genes: structural and functional issues. Mol Pathol 54:57–79
6. Bork P (1993) The modular architecture of a new family of growth regulators related to connective tissue growth factor. FEBS Lett 327:125–130
7. Ristimaki A, Honkanen N, Jankala H, Sipponen P, Harkonen M (1997) Expression of cyclooxygenase-2 in human gastric carcinoma. Cancer Res 57:1276–1280
8. Thun MJ, Namboodiri MM, Calle EE, Flanders WD, Heath CW Jr (1993) Aspirin use and risk of fatal cancer. Cancer Res 53:1322–1327
9. Uefuji K, Ichikura T, Mochizuki H (2001) Expression of cyclooxygenase-2 in human gastric adenomas and adenocarcinomas. J Surg Oncol 76:26–30
10. Kang Y, Siegel PM, Shu W, Drobnjak M, Kakonen SM, Cordon-Cardo C, Guise TA, Massague J (2003) A multigenic program mediating breast cancer metastasis to bone. Cancer Cell 3:537–549
11. Pan LH, Beppu T, Kurose A, Yamauchi K, Sugawara A, Suzuki M, Ogawa A, Sawai T (2002) Neoplastic cells and proliferating endothelial cells express connective tissue growth factor (CTGF) in glioblastoma. Neurol Res 24:677–683
12. Koliopanos A, Friess H, di Mola FF, Tang WH, Kubulus D, Brigstock D, Zimmermann A, Buchler MW (2002) Connective tissue growth factor gene expression alters tumor progression in esophageal cancer. World J Surg 26:420–427
13. Wenger C, Ellenrieder V, Alber B, Lacher U, Menke A, Hameister H, Wilda M, Iwamura T, Beger HG, Adler G, Gress TM (1999) Expression and differential regulation of connective tissue growth factor in pancreatic cancer cells. Oncogene 18:1073–1080
14. Kubo M, Kikuchi K, Nashiro K, Kakinuma T, Hayashi N, Nanko H, Tamaki K (1998) Expression of fibrogenic cytokines

in desmoplastic malignant melanoma. Br J Dermatol 139: 192–197
15. Shakunaga T, Ozaki T, Ohara N, Asaumi K, Doi T, Nishida K, Kawai A, Nakanishi T, Takigawa M, Inoue H (2000) Expression of connective tissue growth factor in cartilaginous tumors. Cancer 89:1466–1473
16. Moritani NH, Kubota S, Nishida T, Kawaki H, Kondo S, Sugahara T, Takigawa M (2003) Suppressive effect of overexpressed connective tissue growth factor on tumor cell growth in a human oral squamous cell carcinoma-derived cell line. Cancer Lett 192:205–214
17. Planque N, Perbal B (2003) A structural approach to the role of CCN (CYR61/CTGF/NOV) proteins in tumourigenesis. Cancer Cell Int 3:15
18. Ball DK, Rachfal AW, Kemper SA, Brigstock D (2003) The heparinbinging 10 kDa fragment of connective tissue growth factor (CTGF) containing module 4 alone stimulates cell adhesion. J Endocrinol 176:R1–R7
19. Takigawa M (2003) CTGF/Hcs24 as a multifunctional growth factor for fibroblasts, chondrocytes and vascular endothelial cells. Drug News Perspect 16:11–21
20. Ihn H (2002) Pathogenesis of fibrosis: role of TGF-β and CTGF. Curr Opin Rheumatol 14:681–695
21. Chen Y, Segarini P, Raoufi F, Bradham D, Leask A (2001) Connective tissue growth factor is secreted through the Golgi and is degraded in the endosome. Exp Cell Res 271:109–117
22. Leask A, Abraham DJ (2003) The role of connective tissue growth factor, a multifunctional matricellular protein, in fibroblast biology. Biochem Cell Biol 81:355–363
23. Chen CC, Chen N, Lau LF (2001) The angiogenic factors Cyr61 and connective tissue growth factor induce adhesive signaling in primary human skin fibroblasts. J Biol Chem 276:10443–10452
24. Brigstock DR, Steffen CL, Kim GY, Vegunta RK, Diehl JR, Harding PA (1997) Purification and characterization of novel heparin-binding growth factors in uterine secretory fluids. Identification as heparin-regulated Mr 10,000 forms of connective tissue growth factor. J Biol Chem 272:20275–20282
25. Frazier K, Williams S, Kothapalli D, Klapper H, Grotendorst GR (1996) Stimulation of fibroblast cell growth, matrix production, and granulation tissue formation by connective tissue growth factor. J Invest Dermatol 107:404–411
26. Shimo T, Nakanishi T, Nishida T, Asano M, Kanyama M, Kuboki T, Tamatani T, Tezuka K, Takemura M, Matsumura T, Takigawa M (1999) Connective tissue growth factor induces the proliferation, migration, and tube formation of vascular endothelial cells in vitro and angiogenesis in vivo. J Biochem 126:137–145 (Tokyo)
27. Babic AM, Chen CC, Lau LF (1999) Fisp12/mouse connective tissue growth factor mediates endothelial cell adhesion and migration through integrin alphavbeta3, promotes endothelial cell survival, and induces angiogenesis in vivo. Mol Cell Biol 19:2958–2966
28. Nakanishi T, Nishida Y, Shimo T, Kobayashi K, Kubo T, Tamatani T (2000) Effects of CTGF/Hcs24, a product of a hypertropic chondrocyte-specific gene, on the proliferation and differentiation of chondrocytes in culture. Endocrinology 141:264–273
29. Yosimichi G, Nakanishi T, Nishida T, Hattori T, Takano-Yamamoto T, Takigawa M (2001) CTGF/Hcs24 induces chondrocyte differentiation through a p38 mitogen-activated protein kinase (p38MAPK), and proliferation through a p44/42 MAPK/extracellular-signal regulated kinase (ERK). Eur J Biochem 268:6058–6065
30. Vorwerk P, Wex H, Hohmann B, Oh Y, Rosenfeld RG, Mittler U (2000) CTGF (IGFBP-rP2) is specifically expressed in malignant lymphoblasts of patients with acute lymphoblastic leukaemia (ALL). Br J Cancer 83:756–760
31. Sala-Torra O, Gundacker HM, Stirewalt DL, Ladne PA, Pogosova-Agadjanyan EL, Slovak ML, Willman CL, Heimfeld S, Boldt DH, Radich JP (2007) Connective tissue growth factor (CTGF) expression and outcome in adult patients with acute lymphoblastic leukemia. Blood 109:3080–3083
32. Frazier K, Grotendorst G (1997) Expression of connective tissue growth factor mRNA in the fibrous stroma of mammary tumors. Int J Biochem Cell Biol 29:153–161
33. Hishikawa K, Oemar BS, Tanner FC, Nakaki T, Luscher TF, Fujii T (1999) Connective tissue growth factor induces apoptosis in human breast cancer cell line MCF-7. J Biol Chem 274:37461–37466
34. Xie D, Nakachi K, Wang H, Elashoff R, Koeffler HP (2001) Elevated levels of connective tissue growth factor, WISP–1, and CYR61 in primary breast cancers associated with more advanced features. Cancer Res 61:8917–8923
35. Shimo T, Kubota S, Kondo S, Nakanishi T, Sasaki A, Mese H, Matsumura T, Takigawa M (2001) Connective tissue growth factor as a major angiogenic agent that is induced by hypoxia in a human breast cancer cell line. Cancer Lett 174:57–64
36. Shimo T, Nakanishi T, Nishida T, Asano M, Sasaki A, Kanyama M, Kuboki T, Matsumura T, Takigawa M (2001) Involvement of CTGF, a hypertrophic chondrocyte-specific gene product, in tumor angiogenesis. Oncology 61:315–322
37. Kondo S, Kubota S, Shimo T, Nishida T, Yosimichi G, Eguchi T, Sugahara T, Takigawa M (2002) Connective tissue growth factor increased by hypoxia may initiate angiogenesis in collaboration with matrix metalloproteinases. Carcinogenesis 23:769–776
38. Jiang WG, Watkins G, Fodstad O, Douglas-Jones A, Mokbel K, Mansel RE (2004) Differential expression of the CCN family members Cyr61, CTGF and Nov in human breast cancer. Endocr Relat Cancer 11:781–791
39. Shimo T, Kubota S, Yoshioka N, Ibaragi S, Isowa S, Eguchi T, Sasaki A, Takigawa M (2006) Pathogenic role of connective tissue growth factor (CTGF/CCN2) in osteolytic metastasis of breast cancer. J Bone Miner Res 21:1045–1059
40. Chen PS, Wang MY, Wu SN, Su JL, Hong CC, Chuang SE, Chen MW, Hua KT, Wu YL, Cha ST, Babu MS, Chen CN, Lee PH, Chang KJ, Kuo ML (2007) CTGF enhances the motility of breast cancer cells via an integrin-$\alpha v\beta 3$-ERK1/2-dependent S100A4-upregulated pathway. J Cell Sci 120:2053–2065
41. Wong YF, Cheung TH, Tsao GS, Lo KW, Yim SF, Wang VW, Heung MM, Chan SC, Chan LK, Ho TW, Wong KW, Li C, Guo Y, Chung TK, Smith DI (2006) Genome-wide gene expression profiling of cervical cancer in Hong Kong women by oligonucleotide microarray. Int J Cancer 118:2461–2469
42. Igarashi A, Hayashi N, Nashiro K, Takehara K (1998) Differential expression of connective tissue growth factor gene in cutaneous fibrohistiocytic and vascular tumors. J Cutan Pathol 25:143–148
43. Liu L, Li Z, Feng G, You W, Li J (2007) Expression of connective tissue growth factor is in agreement with the expression of VEGF, VEGF-C, -D and associated with shorter survival in gastric cancer. Pathol Int 57:712–718
44. Xie D, Yin D, Wang HJ, Liu GT, Elashoff R, Black K, Koeffler HP (2004) Levels of expression of CYR61 and CTGF are prognostic for tumor progression and survival of individuals with gliomas. Clin Cancer Res 10:2072–2081
45. Zeng ZJ, Yang LY, Ding X, Wang W (2004) Expressions of cysteine-rich61, connective tissue growth factor and Nov genes in hepatocellular carcinoma and their clinical significance. World J Gastroenterol 10:3414–3418

46. Li Y, Lu Y, Ceng Y, Yang X (2007) Expression of connective tissue growth factor (CTGF), osteopontin (OPN) and clinical significances in the laryngeal squamous cell carcinoma tissues. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 21:121–123
47. Chang CC, Shih JY, Jeng YM, Su JL, Lin BZ, Chen ST, Chau YP, Yang PC, Kuo ML (2004) Connective tissue growth factor and it's role in lung adenocarcinoma invasion and metastasis. J Natl Cancer Inst 96:364–375
48. Chien W, Yin D, Gui D, Mori A, Frank JM, Said J, Kusuanco D, Marchevsky A, McKenna R, Koeffler HP (2006) Suppression of cell proliferation and signaling transduction by connective tissue growth factor in non-small cell lung cancer cells. Mol Cancer Res 4:591–598
49. Chen PP, Li WJ, Wang Y, Zhao S, Li DY, Feng LY, Shi XL, Koeffler HP, Tong XJ, Xie D (2007) Expression of Cyr61, CTGF, and WISP-1 correlates with clinical features of lung cancer. PLoS ONE 2:e534
50. Chang CC, Lin MT, Lin BR, Jeng YM, Chen ST, Chu CY, Chen RJ, Chang KJ, Yang PC, Kuo ML (2006) Effects of connective tissue factor on hypoxia-inducible factor 1 degradation and tumor angiogenesis. J Natl Cancer Inst 98:984–995
51. Kasaragod AB, Lucia MS, Cabirac G, Grotendorst GR, Stenmark KR (2001) Connective tissue growth factor expression in pediatric myofibroblastic tumors. Pediatr Dev Pathol 4:37–45
52. Kikuchi R, Tsuda H, Kanai Y, Kasamatsu T, Sengoku K, Hirohashi S, Inazawa J, Imoto I (2007) Promoter hypermethylation contributes to frequent inactivation of a putative conditional tumor suppressor gene connective tissue growth factor in ovarian cancer. Cancer Res 67:7095–7105
53. Hartel M, Di Mola FF, Gardini A, Zimmermann A, Di Sebastiano P, Guweidhi A, Innocenti P, Giese T, Giese N, Buchler MW, Friess H (2004) Desmoplastic reaction influences pancreatic cancer growth behavior. World J Surg 28:818–825
54. Aikawa T, Gunn J, Spong SM, Klaus SJ, Korc M (2006) Connective tissue growth factor-specific antibody attenuates tumor growth, metastasis, and angiogenesis in an orthotopic mouse model of pancreatic cancer. Mol Cancer Ther 5:1108–1116
55. Dornhofer N, Spong S, Bennewith K, Salim A, Klaus S, Kambham N, Wong C, Kaper F, Sutphin P, Nacamuli R, Hockel M, Le Q, Longaker M, Yang G, Koong A, Giaccia A (2006) Connective tissue growth factor-specific monoclonal antibody therapy inhibits pancreatic tumor growth and metastasis. Cancer Res 66:5816–5827
56. Kwon S, Munroe X, Crawley SC, Lee HY, Spong S, Bradham D, Gum JR Jr, Sleisenger MH, Kim YS (2007) Expression of connective tissue growth factor in pancreatic cancer cell lines. Int J Oncol 31:693–703
57. Yang F, Tuxhorn JA, Ressler SJ, McAlhany SJ, Dang TD, Rowley DR (2005) Stromal expression of connective tissue growth factor promotes angiogenesis and prostate cancer tumorigenesis. Cancer Res 65:8887–8895
58. Croci S, Landuzzi L, Astolfi A, Nicoletti G, Rosolen A, Sartori F, Follo MY, Oliver N, De Giovanni C, Nanni P, Lollini PL (2004) Inhibition of connective tissue growth factor (CTGF/CCN2) expression decreases the survival and myogenic differentiation of human rhabdomyosarcoma cells. Cancer Res 64:1730–1736
59. Stanhope-Baker P, Williams BR (2000) Identification of connective tissue growth factor as a target of WT1 transcriptional regulation. J Biol Chem 275:38139–38150
60. Zirn B, Samans B, Spangenberg C, Graf N, Eilers M, Gessler M (2005) All-trans retinoic acid treatment of Wilms tumor cells reverses expression of genes associated with high risk and relapse in vivo. Oncogene 24:5246–5251
61. Zirn B, Hartmann O, Samans B, Krause M, Wittmann S, Mertens F, Graf N, Eilers M, Gessler M (2006) Expression profiling of Wilms tumors reveals new candidate genes for different clinical parameters. Int J Cancer 118:1954–1962
62. Olbryt M, Jarzab M, Jazowiecka-Rakus J, Simek K, Szala S, Sochanik A (2006) Gene expression profile of B 16(F10) murine melanoma cells exposed to hypoxic conditions in vitro. Gene Expr 13:191–203
63. Lin BR, Chang CC, Che TF, Chen ST, Chen RJ, Yang CY, Jeng YM, Liang JT, Lee PH, Chang KJ, Chau YP, Kuo ML (2005) Connective tissue growth factor inhibits metastasis and acts as an independent prognostic marker in colorectal cancer. Gastroenterology 128:9–23
64. Liotta LA, Rao CN, Wewer UM (1986) Biochemical interactions of tumor cells with the basement membrane. Annu Rev Biochem 5:1037–1057
65. Kramer RH, Bensch KG, Wong J (1986) Invasion of reconstituted basement membrane matrix by metastatic human tumor cells. Cancer Res 46:1980–1986
66. Terranova VP, Hujanen ES, Loeb DM, Martin GR, Thornberg L, Glushko V (1986) Use of a reconstituted basement membrane to measure cell invasiveness and select for highly invasive tumor cells. Proc Natl Acad Sci USA 83:465–469
67. Albini AY, Iwamoto HK, Kleinman GR, Aaronson A, Kozlowski JM, McEwan RN (1987) A rapid in vitro assay for quantitating the invasive potential of high and low metastatic variants. Cancer Lett 38:137–147
68. Hendrix MJC, Seftor EA, Seftor REB, Fidler IJ (1987) A simple quantitative assay for studying the invasive potential of high and low metastatic variants. Cancer Lett 38:137–147
69. Stracke ML, Liotta LA (1953) Molecular mechanisms of tumor cell metastasis. In: Mendelson T, Howley P, Israel MA, Liotta LA (eds) The molecular basis of cancer. W. B. Saunders, Philadelphia, pp 233–250
70. Visse R, Nagase H (2003) Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function and biochemistry. Circ Res 92:827–839
71. Sleeman JP (2000) The lymph node as a bridgehead in the metastatic dissemination of tumors. Recent Results Cancer Res 157:55–81
72. Pepper MS (2001) Lymphogiogenesis and tumor metastasis: myth or reality? Clin Cancer Res 7:462–468
73. Vander Griend DJ, Berger JC, Rinker-Schaeffer CW (2004) Suppression of metastasis—a new function for known proteins. J Natl Cancer Inst 96:344–345
74. Yoshida BA, Sokoloff MM, Welch DR, Rinker-Schaeffer CW (2000) Metastasis suppressor genes: a review and perspective on an emerging field. J Natl Cancer Inst 92:1717–1730
75. Fidler IJ (1991) Cancer metastasis. Br Med Bull 47:157–177
76. Vander Griend DJ, Rinker-Schaeffer CW (2004) A new look at an old problem: the survival and organ-specific growth of metastases. Science's STKE 2004:pe3
77. Kirfel G, Rigort A, Borm B, Herzog V (2004) Cell migration: mechanisms of rear detachment and the formation of migration tracks. Eur J Cell Biol 83:717–724
78. Kim EJ, Helfman DM (2003) Characterization of the metastasis-associated protein, S100A4 Roles of calcium binding and dimerization in cellular localization and interaction with myosin. J Biol Chem 278:30063–30073
79. Turner CE (2000) Paxillin interactions. J Cell Sci 23:4139–4140
80. Brunton VG, MacPherson IR, Frame MC (2004) Cell adhesion receptors, tyrosine kinases and actin modulators: a complex three-way circuitry. Biochim Biophys Acta 1692:121–144
81. Minn AJ, Kang Y, Serganova I, Gupta GP, Giri DD, Doubrovin M, Ponomarev V, Gerald WL, Blasberg R, Massague J (2005) Distinct organ-specific metastatic potential of individual breast cancer cells and primary tumors. J Clin Invest 115:44–55

82. Carmeliet P, Jain RK (2000) Angiogenesis in cancer and other diseases. Nature 407:249–257
83. Carmeliet P (2000) Mechanisms of angiogenesis and arteriogenesis. Nat Med 6:389–395
84. Bergers G, Benjamin LE (2003) Angiogenesis: tumorigenesis and the angiogenic switch. Nat Rev Cancer 3:401–410
85. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100:57–70
86. Fukumura D, Xavier R, Sugiura T (1998) Tumor induction of VEGF promoter activity in stromal cells. Cell 94:715–725
87. Yuan A, Yu CJ, Shun CT, Luh KT, Kuo SH, Lee YC, Yang PC (2005) Total cyclooxygenase-2 mRNA levels correlate with vascular endothelial growth factor mRNA levels, tumor angiogenesis and prognosis in non-small cell lung cancer patients. Int J Cancer 115:545–555
88. Kerbel RS (2000) Tumor angiogenesis: past, present and the near future. Carcinogenesis 21:505–515
89. Ferrara N (2002) Role of vascular endothelial growth factor in physiologic and pathologic angiogenesis: therapeutic implications. Semin Oncol 29:10–14
90. Forsythe J, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD, Semenza GL (1996) Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol Cell Biol 16:4604–4613
91. Semenza GL (2002) HIF-1 and tumor progression: pathophysiology and therapeutics. Trends Mol Med 8:S62–S67
92. Yamamoto S, Yasui W, Kitadai Y, Yokozaki H, Haruma K, Kajiyama G, Tahara E (1998) Expression of vascular endothelial growth factor in human gastric carcinomas. Pathol Int 48:499–506
93. Saito H, Tsujitani S, Kondo A, Ikeguchi M, Maeta M, Kaibara N (1999) Expression of vascular endothelial growth factor correlates with hematogenous recurrence in gastric carcinoma. Surgery 125:195–201
94. Kido S, Kitadai Y, Hattori N, Haruma K, Kido T, Ohta M, Tanaka S, Yoshihara M, Sumii K, Ohmoto Y, Chayama K (2001) Interleukin 8 and vascular endothelial growth factor—prognostic factors in human gastric carcinomas? Eur J Cancer 37:1482–1487
95. Cascinu S, Staccioli MP, Gasparini G, Giordani P, Catalano V, Ghiselli R, Rossi C, Baldelli AM, Graziano F, Saba V, Muretto P, Catalano G (2000) Expression of vascular endothelial growth factor can predict event-free survival in stage II colon cancer. Clin Cancer Res 6:2803–2807
96. Berra E, Pagès G, Pouysségur J (2000) MAP kinases and hypoxia in the control of VEGF expression. Cancer Metastasis Rev 19:139–145
97. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER, Ratcliffe PJ (1999) The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 399:271–275
98. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, Kriegsheim AV, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW, Ratcliffe PJ (2001) Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. Science 292:468–472
99. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS, Kaelin WG Jr (2001) HIF-1 alpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. Science 292:464–468
100. Masson N, Willam C, Maxwell PH, Pugh CW, Ratcliffe PJ (2001) Independent function of two destruction domains in hypoxia-inducible factor-alpha chains activated by prolyl hydroxylation. EMBO J 20:5197–5206
101. Semenza GL (2003) Targeting HIF-1 for cancer therapy. Nat Rev Cancer 3:721–732
102. Fukuda R, Hirota K, Fan F, Jung YD, Ellis LM, Semenza GL (2002) Insulin-like growth factor 1 induces hypoxia-inducible factor 1-mediated vascular endothelial growth factor expression, which is dependent on MAP-kinase and phosphatidylinositol 3-kinase signaling in colon cancer cells. J Biol Chem 277:38205–38211
103. Fukuda R, Kelly B, Semenza GL (2003) Vascular endothelial growth factor gene expression in colon cancer cells exposed to prostaglandin E2 is mediated by hypoxia-inducible factor 1. Cancer Res 63:2330–2334
104. Hellwig-Bürgel T, Stiehl DP, Jelkmann W (2003) Hypoxia-inducible factor-1: more than a hypoxia-inducible transcription factor. In: Lahiri S, Semenza G, Prabhakar MR (eds) Oxygen sensing: responses and adaptation to hypoxia (Lung biology in health and disease). Marcel Dekker, New York, pp 95–108
105. Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL (2001) HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1 (HIF-1) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. Mol Cell Biol 21:3995–4004
106. Zhong H, Chiles K, Feldser D, Laughner E, Hanrahan C, Georgescu MM, Simons JW, Semenza GL (2000) Modulation of hypoxia-inducible factor-1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. Cancer Res 60:1541–1545
107. Zundel W, Schindler C, Haas-Kogan D, Koong A, Kaper F, Chen E, Gottschalk AR, Ryan HE, Johnson RS, Jefferson AB, Stokoe D, Giaccia AJ (2000) Loss of PTEN facilitates HIF-1-mediated gene expression. Genes Dev 14:391–396
108. Latinkic BV, O'Brien TP, Lau LF (1991) Promoter function and structure of the growth factor-inducible immediate early gene cyr61. Nucleic Acids Res 19:3261–3267
109. Grotendorst GR (1997) Connective tissue growth factor: a mediator of TGF-beta action on fibroblasts. Cytokine Growth Factor Rev 8:171–179
110. Moussad EE, Brigstock DR (2000) Connective tissue growth factor: what's in a name? Mol Genet Metab 71:276–292
111. Inoki I, Shiomi T, Hashimoto G, Enomoto H, Nakamura H, Makino K, Ikeda E, Takata S, Kobayashi K, Okada Y (2002) Connective tissue growth factor binds vascular endothelial growth factor (VEGF) and inhibits VEGF-induced angiogenesis. FASEB J 16:219–221
112. Hashimoto G, Inoki I, Fujii Y, Aoki T, Ikeda E, Okada Y (2002) Matrix metalloproteinases cleave connective tissue growth factor and reactivate angiogenic activity of vascular endothelial growth factor 165. J Biol Chem 277:36288–36295
113. Passaniti A, Taylor RM, Pili R, Guo Y, Long PV, Haney JA, Pauly RR, Grant DS, Martin GR (1992) A simple, quantitative method for assessing angiogenesis and antiangiogenic agents using reconstituted basement membrane, heparin, and fibroblast growth factor. Lab Invest 67:519–528
114. Jeong JW, Bae MK, Ahn MY, Kim SH, Sohn TK, Bae MH, Yoo MA, Song EJ, Lee KJ, Kim KW (2002) Regulation and destabilization of HIF-1alpha by ARD1-mediated acetylation. Cell 111:709–720
115. Nishida T, Kubota S, Nakanishi T, Kuboki T, Yosimichi G, Kondo S (2002) CTGF/Hcs24, a hypertrophic chondrocyte-specific gene product, stimulates proliferation and differentiation, but not hypertrophy of cultured articular chondrocytes. J Cell Physiol 192:55–63
116. Grossmann J (2002) Molecular mechanisms of detachment-induced apoptosis–Anoikis. Apoptosis 7:247–260

117. Wei L, Yang Y, Zhang X, Yu Q (2004) Altered regulation of Src upon cell detachment protects human lung adenocarcinoma cells from anoikis. Oncogene 23:9052–9061
118. Wei L, Yang Y, Yu Q (2001) Tyrosine kinase-dependent, phosphatidylinositol 3'-kinase, and mitogen-activated protein kinase-independent signaling pathways prevent lung adenocarcinoma cells from anoikis. Cancer Res 61:2439–2444
119. Kodama K, Ishii G, Miyamoto S, Goya M, Zhang S, Sangai T, Yoshikawa T, Hasebe T, Hitomi Y, Izumi K, Ochiai A (2005) Laminin 5 expression protects against anoikis at aerogenous spread and lepidic growth of human lung adenocarcinoma. Int J Cancer 116:876–884