THE ANTIANGIOGENIC EFFECTS OF THROMBOSPONDIN-1 AND -2 IN TUMORS

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Abstract: Angiogenesis, the formation of new vessels, is a multistage process and is critical for the growth and proliferation of tumors. The identification of natural modulators of angiogenesis is essential to the understanding of this complex process. All five TSP family members are multimeric and modular heparin-and calcium-binding proteins, but two subfamilies from thrombospondins family (TSP-1 and TSP-2) have anti-angiogenic activity.

INTRODUCTION

The formation of new capillaries from preexisting blood vessels or neoangiogenesis is necessary for a variety of normal physiological processes. It has also been shown to be a crucial event in tumor growth and to precede and facilitate metastatic spread in experimental systems (Zhang L et al.,, 1997).

When a tumor grows, tumor cells stimulate endothelial cell growth, and the newly developed capillaries provide nutrients and oxigen from the systemic circulation into the tumor site (Toi M et al., 2002)

Angiogenesis depends on a balance between positive and negative endothelial regulatory factors produced by the tumour cells themselves or by associated stromal and inflammatory cells (Rice AJ and Quinn CM, 2002)

Two subfamilies from thrombospondins (TSPs) familiy (TSP-1 and -2) have anti-angiogenetic activity.

DISCUSSION

THE THROMBOSPONDINS FAMILY STRUCTURE

The TSPs family consist of five polypeptides called thrombospondins 1-5 (Bornstein P et al.., 1994). All five TSP family members are multimeric and modular heparin-and calcium-binding proteins, but two subfamilies are more important: TSP-1 and -2 which have a similar structure (Lawler J, 2000).

Each subunit of these trimeric molecules is composed of an N-terminal heparin-binding domain, a linker domain enclosing the two cysteine residues implicated in trimerization, a procollagen-homology domain, three properdin-like type I repeats, three epidermal growth factor (EGF)-like type II repeats, seven calcium-binding type III repeats and a globular C-terminal domain. By contrast, TSP-3, -4, and -5 exist as pentamers and contain distinct N-terminal domains, four EGF-like type II repeats, seven type III repeats and a similar C-terminal globular domain (Fraipont F et al., 2001).

TSP-1, the first naturally occurring inhibitor of angiogenesis identified, is present in the extracellular matrix of many normal tissues. TSP-1 is produced by a variety of cells including platelets, megakaryocytes, epithelial, endothelial and stromal cells (Carpizo D et al., 2000).

Unlike TSP-1, TSP-2 is not present in platelets. Kyriakides has recently reported that megakaryocytes contain abundant TSP-2, most of which is probably produced by marrow stromal cells and is taken up by megakaryocytes from the extracellular milieu. It is not completely understood how TSP2 is lost from megakaryocytes during their evolution.

MECHANISMS OF ACTION

TSP-1, a 420 kDa trimeric glycoprotein, was first identified as a thrombin-sensitive protein (TSP) that was released in response to activation of platelets by thrombin, hence its name. TSP-1 binds to the platelet surface in a calcium-dependent manner and interacts with integrins αIIbβ3 and αvβ3, with CD36 and the integrin -associated protein (IAP), and with fibronectin and fibrinogen-bound integrin (Sage EH, 2001). Recently, a sequence in the N-terminal domain was also found to bind integrin α3β1 on tumor cells and aortic endothelial cells (Chandrasekaran L, 2000).

Investigations of the mechanism of action of TSP-1 have implicated CD36, known as a class B scavenger receptor and a collagen binding molecule. The binding of CD36 is mediated by the CSVTCG (cysteine-serine-valine-threonine-cysteine-glycine) motif of the second and third type I repeats of TSP-1 (Greenwalt DE et al, 1992). More recently, studies on the intracellular molecular interactions have determined that TSP-1 induces an apoptotic mechanism that involves CD36, Src-family tyrosine kinase p59fyn, group II caspases and p38 MAPK (p38 mitogen-activated protein kinase) (Jimenez B et al., 2000).

Other proteins which possess type I repeat motifs and affect angiogenesis are : ADAMTS-1 and -8 (two members of the ADAMTS family-a disintegrin and metalloproteinase with TSP motifs) and brain angiogenesis inhibitor (BAI)-1. These proteins inhibit angiogenesis, whereas connective tissue growth factor (CTGF, which contain a single type I repeat, stimulates angiogenesis (Adams J et al., 2000). By contrast, several proteins containing type I repeats have no angiogenic effects. These include properdin, complement system proteins (C6, C7, C8, C9) and several other ADAMTS proteins (Adams J et al., 2000).

HUMAN CANCER AND EXPRESSION OF TSP-1 AND TSP-2

The degree of angiogenesis in breast cancer specimens has been shown to correlate with the rate of metastasis, and the level of angiogenesis in breast cancer was reported to be an independent prognostic factor (Weidner N et al., 1991). Rice et colab (2002) in an original article found that TSP-1 is expressed in the stroma around ductal carcinoma in situ (DCIS) and in the immediately adjacent basement membrane. Also, expression of stromal TSP-1 is lost in DCIS with more aggressive histological features.

Several studies have reported that TSP-1 and TSP-2 expression is higher in carcinomas than in benign tumors or normal tissue. For example in breast tumors, cholangiocarcinomas, colorectal carcinomas, pleural mesothelioma, the stromal expression of TSP-1 was much stronger in tumors than in normal tissue (Pratt DA, 1989, Yoshida Y, 1999; Ohta Y, 1999). In non-small cell lung carcinoma, only TSP-2 expression appeared as a significant prognostic marker (Oshika Y, 1998). Increased TSP -1 expression by tumor cells was found to correlate with reduced metastatic potential in experimental tumors formed from melanoma, lung and breast carcinoma cell lines (Zabrenetzky V, 1994). One possible explanation for the different effects of TSP1 expression in various tumor types relates to its interactions with transforming growth factor β-TGFβ (Fraipont F et al., 2001).

TSP-1 has been found to activate TGF-β1 by releasing it from its latency associated protein (LAP) in vitro as well as in vivo (Crowford SE et al.,1998). Numerous studies have shown that TGF-βs can be detected in tissue specimens from a variety of tumor types. For example, TGF-β1, -2, and-3 specific mRNA can be detected in the majority of primary breast cancer (Maccalum J et al., 1994). In contrast to other tumor types, breast cancer patients with high levels of TGF-β1 appear to have a longer disease-free interval with a significantly better probability of survival (Murray PA et al., 1993). Activation of latent TGF-β is mediated by two sequences present in the type 1 repeats of TSP-1, a sequence (GGWSHW) that binds active TGF-β and a second sequence (RFK) that activates latent TGF-β (Schultz-Cherry S et al., 1995). In cancer formation, the release of active TGF-β can result in upregulation of urokinase plasminogen activator (uPA), uPA-receptor and plasminogen activator inhibitor-1 (PAI-1). These are three components of the proteolytic system which promotes malignant invasiveness (Andreasen PA et al, 2000). For a specific tumor, whether TSP overexpression promotes or inhibits malignancy could be at least partially dependent on the concentration of TGFβ at the tumor site (Fraipont F et al., 2001)

REGULATION OF TSP-1 AND TSP-2 EXPRESSION

In both normal and pathological angiogenesis, hypoxia is the main factor initiating the angiogenic process. Hypoxia up-regulates cyclooxygenase-2 (COX-2), which increases the conversion of prostaglandin E2 (PGE2) from arachidonic acid. It has been shown that PGE2 induces translocation of hypoxia-inducible factor-1a (HIF-1α) from cytosol into the nucleus where it binds to aromatic hydrocarbon nuclear translocator-ARNT/HIF-1α and then induces the expression of vascular endothelial growth factor (VEGF)(Rahman MA and Toi M, 2003)

The regulation of TSP-1 and TSP-2 expression by hypoxia could depend on tissue type, cell transformation, and experimental conditions. In endothelial cells (HUVEC-human umbilical vein endothelial cell), hypoxia was reported to induce TSP-1 gene and protein expression by post-transcriptional stabilization of the TSP-1 mRNA (Phelan MW, 1998). By contrast, hypoxia strongly decreases TSP-1mRNA levels in both p53+/+ and p53-/- human and rodent fibroblast and in several human glioblastoma tumor cell lines (Tenan M, 2000).

Also, several oncogenes such as v-ras, K-ras, src, fos, v-raf induce the upregulation of angiogenic factors like VEGF, insulin –like growth factor-1 (IGF-1) and transforming growth factor –α (TGFα)(Kerbel RS et al., 1993). If oncogenes can indirectly promote angiogenesis by increasing the production of cytokines and proteolitic enzymes (Arbiser JL et al, 1997), the tumor suppressor gene p53 was found to cause degradation of HIF-1α (Ravi R et al, 2000), stimulation of the inhibitor TSP-1 (Dameron KM et al., 1994) and inhibition of VEGF production (Mukhopadhyay D et al, 1995). In von Hippel-Lindau disease, an inherited cancer syndrome characterized by extensively vascularized tumors, VHL tumor suppresor gene product, has been found to play an important role in the activation of HIF-1 (Rahman MA and Toi M, 2003).

Simantov et colab. using immunohistochemical studies of human breast cancer specimens, found that histidine–rich glycoprotein (HRGP) colocalized with TSP-1 in the

matrix tumor, and that this interaction masked the antiangiogenic epitope of TSP-1. HRGP is a plasma protein with an unusually high histidine and proline content that circulates in relatively high concentrations (1,5μM), but has no known function in vivo (Leung L, 1993). In areas where TSP-1 is an important inhibitor of angiogenesis, HRGP could serve as a modulator of TSP-1 activity, promoting angiogenesis. The activity of HRGP may provide a mechanism by which tumors escape or become resistant to the antiangiogenic effects of TSP-1.

CONCLUSIONS

TSP-1 and TSP-2 are considered to be the main physiological inhibitors of angiogenesis which is critical for the growth and proliferation of tumors.

It was shown that TSP-1 production is regulated by the p53 tumor suppressor gene. Mutation of p53 results in the loss of TSP-1 production and a switch to the angiogenic phenotype (Dameron KM, 1994).

Future investigations are expected to reveal the molecular basis of the diverse effects of TSP-1 in different tumor types development and cancer therapies using TSPs might be devised as well. Using TSP-1 and TSP-2 as prognostic markers for cancer patients could improve the precision of clinical predictions (Fraipont F et al., 2001).

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