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Thrombospondin-based antiangiogenic therapy

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Abstract

Thrombospondins (TSPs) are a family of extracellular matrix proteins that regulate tissue genesis and remodeling. TSP-1 plays a pivotal role in the regulation of both physiological and pathological angiogenesis. The inhibitory effects of TSP-1 on angiogenesis have been established in numerous experimental models. Among other TSP members, TSP-2 has equivalent domain structure as TSP-1 and shares most functions of TSP-1. The mechanisms by which TSP-1 and -2 inhibit angiogenesis can be broadly characterized as direct effects on vascular endothelial cells and indirect effects on the various angiogenic regulators. The fact that TSP-1 and -2 are potent endogenous angiogenic inhibitors has prompted studies to explore their therapeutic applications, and detailed understanding of the mechanisms of action of TSP-1 and -2 has facilitated the design of therapeutic strategies to optimize these activities. The therapeutic effects can be achieved by up-regulation of endogenous TSPs, or by the delivery of recombinant proteins or synthetic peptides that contain sequences from the angiogenic domain of TSP-1. In this article, we review the progress in thrombospondin-based antiangiogenic therapy and discuss the perspectives on the significant challenges that remain.

Keywords: Thrombospondin; Angiogenesis; Antiangiogenic therapy; Endothelial cell

Contents

Introduction

Thrombospondins (TSPs) are a family of five multidomain, calcium-binding extracellular glycoproteins that are synthesized, secreted, and incorporated into the extracellular matrix of a wide variety of normal and transformed cells of both mesenchymal and

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epithelial origin (Adams and Lawler, 2004). Thrombospondin-1 (TSP-1) was the first identified and therefore the prototypic member of the family and has been studied most intensively. TSP-1 is the first protein to be shown to play a critical role as a naturally occurring inhibitor of angiogenesis. Of the members of TSP family, TSP-2 has equivalent domain structure as TSP-1 and is also a potent inhibitor of angiogenesis.

The expression of TSP-1 in adult tissue is limited for the most part to sites of tissue remodeling. At these sites, TSP-1 acts in the pericellular space to regulate cellular phenotype and extracellular matrix structure. Virtually every domain of TSP-1 has a receptor on the cell surface. The specific repertoire of receptors that a given cell expresses may determine its response to TSP-1. Whereas TSP-1 promotes the migration of vascular smooth muscle cells, it is a potent inhibitor of endothelial cell migration. TSP-1 modulates extracellular matrix structure by binding to matrix proteins such as fibronectin and collagen and by modulating the activity of extracellular proteinase such as matrix metalloproteinases (MMPs) and plasmin. The ability to regulate cellular phenotype and extracellular matrix structure enables TSP-1 and -2 to be key regulators of the tissue remodeling that is associated with angiogenesis, development, wound healing, and synaptogenesis. They are also involved in the pathological tissue remodeling that is associated with atherosclerosis, neoplasia and tumor angiogenesis.

The TSPs can be divided into structural domains that reflect exon shuffling during evolution (Fig. 1) (Chen et al., 2000). TSP-1 and -2 have an N-terminal domain of approximately 200 amino acids that contains a high-affinity-binding site for heparin and heparan sulfate proteoglycans. This domain also mediates the uptake and clearance of the TSPs through a low-density lipoprotein receptor-related protein (LRP)-dependent mechanism. Three copies of the thrombospondin type 1 repeat (TSR) and three copies of the epidermal growth factor (EGF), or type 2, repeat are found in the middle of TSP-1 and -2. The TSRs are found in approximately 100 proteins in the human genome (Tucker, 2004). The TSRs have been shown to inhibit tumor angiogenesis and growth (Lawler and Detmar, 2004). Furthermore, a therapeutic agent, designated ABT-510, is based on an 8-amino-acid sequence within the second TSR (see below) (Haviv et al., 2005). The TSRs of TSP-1 bind to β1 integrins, CD36 and transforming growth factor (TGF)-β. Whereas TSP-1 and -2 probably have similar functions, they differ in their ability to activate TGFβ. Activation of TGFβ requires the sequence RFK between the first and second TSR of TSP-1 (Young and Murphy-Ullrich, 2004b). Since TSP-2 lacks this sequence, it is not able to activate TGFβ. The C-terminal portion of TSP-1 and -2 (including the sequence from the last type 2 repeat to the C-terminal of the protein) is composed of a series of contiguous calcium-binding sites that are wrapped around a β sandwich structure that is formed by the last 200 amino acids of the proteins (Carlson et al., 2005). This C-terminal domain is highly conserved in all five members of the thrombospondin gene family and thus has been designated the thrombospondin signature domain. This domain appears to bind 30 calcium ions, suggesting that the TSPs are involved in calcium homeostasis within the cell; however, this function of the thrombospondins has not been explored in detail.

Mechanisms of inhibition of angiogenesis by TSP-1 and -2

Numerous *in vitro* and *in vivo* approaches have been used to identify multiple mechanisms by which TSP-1 and -2 inhibit

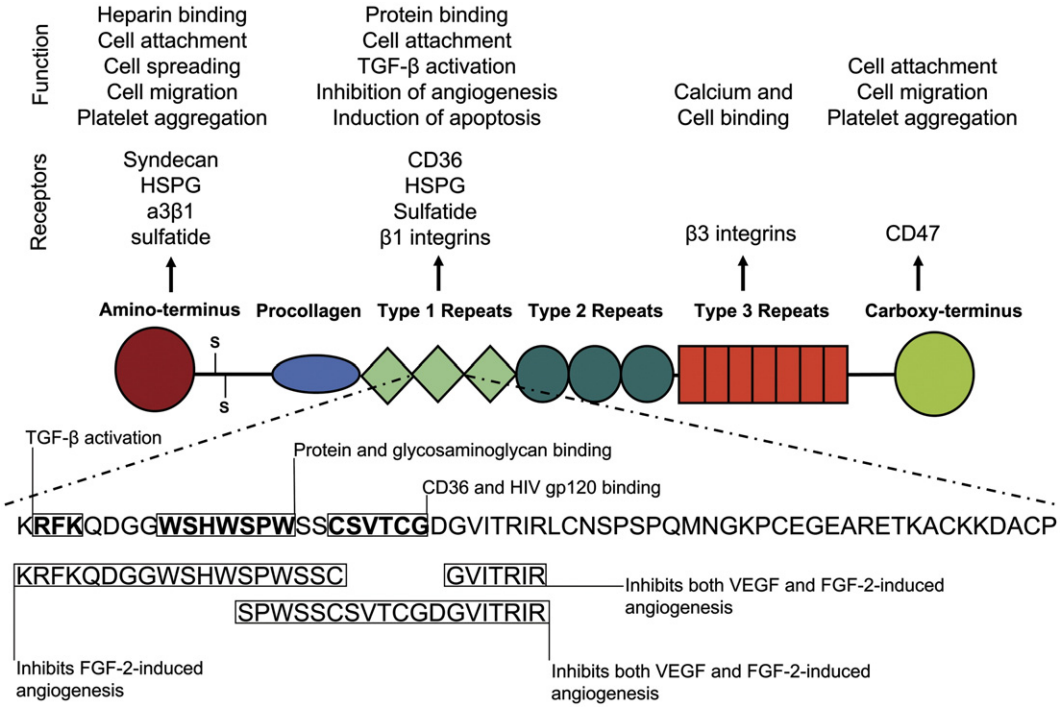

Fig. 1. The structure of TSP-1 and the TSRs. TSP-1 comprises multiple functional domains and each domain has different receptors. The amino acid sequence of the second type 1 repeat (TSR+RFK) is shown, and the active amino acid sequences and the anti-angiogenic peptides are indicated.