Seminars in Cell & Developmental Biology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Cell penetrating SERPINA5 (Protein C inhibitor, PCI): More questions than answers

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ARTICLE INFO

Article history:
Received 27 June 2016
Accepted 26 October 2016
Available online xxx

Keywords:
Serpin
Protein C inhibitor
Phospholipid
Cell penetrating peptide
Protease
Spermatogenesis

ABSTRACT

SERPINA5 (protein C inhibitor, plasminogen activator inhibitor-3) is a secreted, extracellular clade A serpin. Its main characteristics are broad protease reactivity and wide tissue distribution (in man). SERPINA5 has originally been described as an inhibitor of activated protein C and independently as an inhibitor of the plasminogen activator urokinase. SERPINA5 binds glycosaminoglycans, phospholipids, and retinoic acid. Glycosaminoglycans and certain phospholipids can modulate its inhibitory activity and specificity. Studies suggest that SERPINA5 may play a role in hemostasis, in male reproduction, in host defense, and as a tumor suppressor. However, its biological role has not yet been defined. So far SERPINA5 deficiency has not been described in man. Mouse models are of limited value, since in mice serpinA5 is almost exclusively expressed in the reproductive tract. Consistently the only obvious phenotype of *serpinA5*-knockout mice is infertility of homozygous males. SERPINA5 can be internalized by cells and translocated to the nucleus. The internalization is dependent on the phospholipid phosphatidylethanolamine and on the intact N-terminus of SERPINA5, which functions as a cell penetrating peptide. Further functional analysis of intracellular SERPINA5 will contribute to our understanding of the biological role of this molecule.

Contents

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http://dx.doi.org/10.1016/j.semcdb.2016.10.007
1084-9521/

Please cite this article in press as: H. Yang, M. Geiger, Cell penetrating SERPINA5 (Protein C inhibitor, PCI): More questions than answers, Semin Cell Dev Biol (2016), http://dx.doi.org/10.1016/j.semcdb.2016.10.007

1. Introduction

Members of the serpin superfamily of proteins are widely distributed in nature. They occur not only in vertebrates, but also in arthropods [1], nematodes [2,3], plants [4], prokaryotes [5–7], and viruses [8]. Most serpins are secreted, extracellular proteins, but there are also intracellular serpins [9]. Serpins are classified into clades and intracellular serpins form clade B (B1-B13)[10]. For some serpins extracellular as well as in intracellular forms have been described [11,12]; and several serpins have intra- as well as extracellular functions [13,14]. Interestingly, a few serpins that are considered as classical extracellular proteins secreted into body fluids have also been detected intracellularly in the nuclear compartment [15–17]. One of these secreted serpins found in the nucleus of certain cells is SERPINA5 (protein C inhibitor, PCI)[16].

In accordance with the serpin nomenclature [10,18] we will use the term SERPINA5/*SERPINA5* for the human protein/gene, and serpinA5/*serpin* for the mouse protein/gene, respectively. In this review we will focus on the interaction of SERPINA5 with non-protein ligands, on its internalization by cells, its nuclear translocation, and on the discussion of possible biological functions. For all other aspects, such as its gene and protein structure, the biochemistry of its interaction with proteases, and its tissue specific expression, we would like to refer to recent reviews [19–24].

2. SERPINA5 (Protein C inhibitor, PCI) is not only an inhibitor of activated protein C in plasma, but a serpin with broad protease reactivity and wide tissue distribution in man

SERPINA5 (M_r = 57,000) is a member of the alpha-1-antitrypsin clade (clade A) of serpins. The gene structure, tissue specific expression, and the characteristics of the SERPINA5 protein have been described in a recent review [19]. Briefly: SERPINA5 has originally been identified as an inhibitor of the anticoagulant protease activated protein C (aPC) and was therefore named protein C inhibitor (PCI) [25,26]. Later it was shown that SERPINA5 has very broad protease reactivity. It inactivates proteases involved in blood coagulation and fibrinolysis [25–27], tissue- [28,29] and plasma kallikreins [30], the sperm protease acrosin [31], hepatocyte growth factor activator [32], and the type II transmembrane serine protease enteropeptidase [33]. As a serpin it inactivates its target proteases in a suicide substrate-like manner by forming stable, enzymatically inactive 1:1 complexes [34]. In addition to serine proteases serpinA5 also inactivates the cysteine protease cathepsin L [35]. Details regarding proteases inhibited by serpinA5 and the respective inhibition rate constants are summarized in [19].

Human SERPINA5 is expressed in many organs and tissues, and the protein is present in most body fluids and secretions [29,36]. SerpinA5 expression has been shown in the liver [37], in the kidney [38], in the skin [39], in the heart [37], and in the male and female reproductive tracts [31,36,40]. The highest concentrations occur in seminal plasma, which contains >4 μM SERPINA5 [36]; human blood plasma contains ~100 nM [36].

3. Non-protein ligands of SERPINA5

SERPINA5 binds heparin [41–44] and other glycosaminoglycans [45,46]. This has been shown not only in purified systems, but also on the surface of cultivated cells [45,46]. SERPINA5 also binds certain phospholipids [47–49]. These phospholipids include phosphatidylserine, oxidized phosphatidylethanolamine, phosphoinositides, and cardiolipin [48,49]. Binding of glycosaminoglycans and phospholipids involves basic amino acids in the H-helix of serpinA5 [48,50,51]. Depending on the target protease binding of glycosaminoglycans and phospholipids can stimulate or suppress the inhibitory activity of serpinA5 [42,47,52,53]. *In vivo* SERPINA5 could bind to glycosaminoglycans on cell surfaces as well as to phospholipids exposed on atherosclerotic plaques [48], on apoptotic and/or activated cells [54], and on microparticles [55]. Binding of SERPINA5 to cell surface glycosaminoglycans could modulate its activity in a heparin-like manner [45]. Binding of SERPINA5 to phospholipids on the surface of apoptotic cells and activated platelets interferes with the phagocytotic removal of these cells [54]. Microparticles containing SERPINA5 are present in normal human plasma. The origin of these microparticles are megakaryocytes and/or platelets. SERPINA5 present on these microparticles seems to be inactive, although it does not seem to be cleaved or complexed [55].

Another non-protein ligand of SERPINA5 is retinoic acid [56]. Two non-inhibitory members of the serpin family, i.e. corticosteroid-binding globulin (CBG) and thyroxine-binding protein (TBG), act as hormone carriers [57–59]. This prompted us several years ago to study binding of different hydrophobic hormones to inhibitory serpins, i.e. to SERPINA5, SERPINC1 (antithrombin), SERPIND1 (heparin cofactor II), and SERPINE1 (PAI-1) [56]. We have shown that serpinA5 bound retinoic acid, but none of the steroid hormones studies (estradiol, progesterone, testosterone, cortisol, or aldosterone). None of the other inhibitory serpins analyzed bound any of the hydrophobic hormones studied. The inhibitory activity of serpinA5 was not affected by retinoid binding, and binding of retinoic acid to serpinA5 was not influenced by the cleavage of SERPINA5 by tissue kallikrein or pancreatic elastase [56]. Binding of ^{3}H-retinoic acid was not only observed with purified SERPINA5, but also with SERPINA5 present in seminal plasma. Huntington and his group have crystalized reactive site cleaved and native SERPINA5 and analyzed its structure. They put special emphasis on its interaction with proteases and non-protein ligands such as heparin and retinoic acid [44,50]. Superposing the structure of the SERPINA5 with that of SERPINA1 (alpha-1-antitrypsin) revealed a two-turn shortening of helix A and a rotation of helix H. This gives rise to a large hydrophobic pocket, which they called helix A gap. They suggested that this helix A gap represents the retinoic acid binding site of SERPINA5. This assumption is supported by data obtained by superimposing the structure of SERPINA5 with that of TBG bound to thyroxine [60]. So far no *in vivo* data supporting role of SERPINA5 as a retinoic acid binding protein are available. It might also be possible that SERPINA5 accommodates *in vivo* (an)other hydrophobic molecule(s) of similar structure and size.

4. Biological functions of SERPINA5

4.1. Role in hemostasis

SERPINA5 has initially been described as an inhibitor of aPC in plasma [25,26] and independently as an inhibitor of the plasminogen activator urokinase (uPA) in urine [61]. Later it was shown that the inhibitor of aPC and the inhibitor of uPA (plasminogen activator inhibitor-3) are immunologically identical proteins [43]. In addition to aPC and urokinase SERPINA5 inactivates several other proteases involved in hemostasis and fibrinolysis [27,30,41,62–65] suggesting a role in the regulation of hemostasis. *In vitro* SERPINA5 can act as a procoagulant as well as an anticoagulant depending on the assay conditions [65]. There are indications that high plasma levels of SERPINA5 represent a mild risk factor for venous thrombosis [66]. Furthermore, significantly elevated plasma concentrations of active SERPINA5 have been described in survivors of myocardial infarction, and seem to represent a risk marker for acute coronary events [67]. SERPINA5 in complex with proteases

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