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Review

Long pentraxin 3: A novel multifaceted player in cancer

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ABSTRACT

Since its discovery in 1992, long pentraxin 3 (PTX3) has been characterized as soluble patter recognition receptor, a key player of the innate immunity arm with non-redundant functions in pathogen recognition and inflammatory responses. As a component of the extra-cellular matrix milieu, PTX3 has been implicated also in wound healing/tissue remodeling, cardiovascular diseases, fertility, and infectious diseases. Consequently, PTX3 levels in biological fluids have been proposed as a fluid-phase biomarker in different pathological conditions.

In the last decade, experimental evidences have shown that PTX3 may exert a significant impact also on different aspects of cancer biology, including tumor onset, angiogenesis, metastatic dissemination and immune-modulation. However, it remains unclear whether PTX3 acts as a good cop or bad cop in cancer. In this review, we will summarize and discuss the scientific literature data focusing on the role of PTX3 in experimental and human tumors, including its putative translational implications.

1. Introduction

Tumor growth is the result of complex and still not fully understood interactions among cellular and non-cellular players. Tumor cells, tumor associated fibroblasts, endothelial cells, and infiltrating immune cells (macrophages, lymphocytes, mastocytes and other myeloid cells) [1,2] establish autocrine and paracrine loops of interaction and cross-talk that deeply affect and sometimes reprogram tumor fate. These cells also contribute to the deposition of extracellular matrix (ECM) components that actively participate in the modulation of tumor microenvironment. Long pentraxin 3 (PTX3) is present in body fluids and as an ECM-associated component produced by different cell types. Originally, PTX3 was characterized as a pattern recognition receptor, member of the innate immunity with relevant functions in inflammatory responses and pathogen recognition [3]. Then, different studies demonstrated the pleiotropic roles exerted by PTX3 in different settings such as wound healing/tissue remodeling, cardiovascular diseases, fertility, and infectious diseases. PTX3 expression has been associated with inflammation and proposed as a biomarker for the aforementioned pathological conditions and efforts have been undertaken in order to understand the biological role and function of PTX3 in these contexts.

In the last decade, it has become evident that PTX3 is involved also in different aspects of cancer progression, including tumor onset, angiogenesis, metastatic dissemination and cancer immune-modulation. Since both tumor and stromal cells produce and are affected by PTX3, it is not trivial to clarify the impact of PTX3 in these different aspects of tumor biology. Moreover, since inflammation is for better or for worse part of the tumor “life cycle” [4], it is difficult to assess whether PTX3 may exert anti- or pro-tumor effects.

The different and sometime contradictory information about PTX3 function in cancer appears to be dependent upon context and cannot be easily generalized. In this review, we will summarize the most significant data focusing on PTX3 in experimental and human tumors, offering relevant information for the discussion about the role of PTX3 in cancer and its possible translational implications.

2. The long pentraxin 3

2.1. PTX3 gene and expression

Discovered in 1992 [5], PTX3 (also named TSG-14) is the prototypic member of the long-pentraxin subfamily [6] that includes also guinea pig apexin, rat, human, and murine neuronal pentraxins 1 (NP1 or NPTX1) and 2 (NP2, also named Narp or NPTX2), and the putative integral membrane pentraxin NRP [7]. Long pentraxins have been found in *Xenopus* [8], *Drosophila melanogaster* [9], and zebrafish (*Danio rerio*) [7]. In recent times, a new long pentraxin, named PTX4 and characterized by an unrelated N-terminal domain coupled to a C-terminal pentraxin domain, has been identified. *In silico* and transcript expression analyses show that the *PTX4* gene is conserved from mammals to lower vertebrates and has a unique pattern of mRNA expression,

Abbreviationss: CRP, C-reactive protein; EC, endothelial cells; ECM, extracellular matrix; PTX3, long pentraxin 3; SAP, serum amyloid P component

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distinct from that of the other members of the family [10].

The human *PTX3* gene has been originally identified using a differential screening of cDNA libraries created from human umbilical vein endothelial cells stimulated by IL-1β [5] and from fibroblasts stimulated by TGF-α and TNF-α [11]. Unlike the short pentraxin C-reactive protein (*CRP*) and serum amyloid P component (*SAP*) genes that map on chromosome 1, the human *PTX3* gene is located on chromosome 3q25.

The complete nucleotide sequence of the *PTX3* gene consists of a 5′-UTR (Untraslated Terminal Region) of 68 bp, an open reading frame of 1143 bp, a polyadenylation signal (at position 1802) and a 3′-UTR containing two consensus sequences for mRNA instability [5]. The *PTX3* gene comprises three exons, the first two exons (nucleotides 1–197 and 198–599) encode for the signal peptide and the N-terminal domain of the PTX3 protein, whereas the third exon (nucleotides 600 up to the 3′-terminus) encodes for the C-terminal domain and exactly matches the second exon of the short-pentraxin *CRP* and *SAP* genes [12]. Thus, the PTX3 gene represents the fusion between regions encoding a specific N-terminal polypeptide with non-redundant functions and a C-terminal polypeptide homologous to short pentraxins.

PTX3 is synthesized locally at the inflammatory site by several cell types (myeloid cells, vascular/lymphatic endothelial cells, mesenchymal and epithelial cells) upon exposure to different inflammatory signals (Fig. 1). Indeed, the enhancer/proximal promoters of both human and murine PTX3 genes are characterized by numerous potential binding sites for transcription factors, including Pu1, AP-1, NF-κB, SP1, and NF-IL-6 sites [13,14].

Accordingly, inflammatory cytokines (IL-1β, TNFα), microbial components (LPS, lipoarabinomannans), TLR agonists, thrombin and the anti-inflammatory cytokine IL-10 induce a rapid and transient PTX3 expression (maximal levels being measured 4–6 h after stimulation) in different myeloid cells [15–17]. A peculiar PTX3 production pipeline occurs in neutrophils, where PTX3 is stored in secondary granules to be quickly released after activation or it localizes in neutrophil extracellular traps [18].

Different signaling pathways regulate PTX3 production, including IL-1R and TLRs/MyD88/NF-κB signaling in immune and stromal cells [19], TNF-α/JNK axis in mesenchymal and epithelial cells [20] and the lysosphingolipid receptor-PI3K/Akt axis activated by high-density lipoproteins in endothelial cells [21]. PTX3 expression is negatively regulated by IFNγ in human monocytes [22] and by glucocorticoids (GCs) that interfere with NF-κB and AP-1 pathways in myeloid dendritic cells [23] (Fig. 1).

Epigenetic regulation of *PTX3* expression by hypermethylation has been observed in some types of cancers [*e.g.* leiomyosarcoma, esophageal squamous cell carcinoma, and colorectal cancer (CRC)] [24–27]. A recent study has clarified that, under inflammatory conditions, *PTX3* expression levels result by the control of two enhancers playing different roles in gene transcription: enhancer-1 increases the response to pro-inflammatory transcription factors whereas enhancer-2 tunes *PTX3* transcription by recruiting different components of the pre-initiation complex [27].

2.2. PTX3 protein structure

PTX3 primary sequence is highly conserved among animal species (human and murine PTX3 sharing 92% of conserved amino acid residues), suggesting a strong evolutionary pressure to maintain its structure-function relationship [7]. Indeed, as described below, PTX3 protein has a broad ligand-binding spectrum that is probably due to its structural complexity.

Human PTX3 is a glycoprotein composed of multiple protomer subunits of 381 amino acids held together by a disulphide bond network. Each protomer is composed of a signal peptide (residues 1–17), an N-terminal domain (residues 18–178) and a C-terminal domain (residues 179–381) [8,28] (Fig. 2).

The C-terminal 203 amino acid domain corresponds to the “pentraxin-like” domain highly homologous among the various members of the pentraxin family (57% of conserved amino acids with short pentraxins) and its structure has been modeled on the similarity to CRP and SAP [29]. The structural model of PTX3 C-terminus shows a hydrophobic core composed by two anti-parallel β-sheets organised as a typical β-jelly roll. A single α-helix, spanning amino acid residues 344–351, is located on the protein surface, whereas Cys^{210} and Cys^{271} are located on opposite sides of the two anti-parallel β-sheets. These cysteine residues establish a disulphide bond in CRP and SAP and appear as covalently linked in the C-terminal model of PTX3. The proximity to Cys^{179} and Cys^{357} residues suggests that they are reciprocally engaged in another disulphide bond potentially linking the N-terminal end of PTX3 to the tail of the C-terminus domain [30].

In the C-terminal domain of PTX3, Asn^{220} is the only site of N-glycosylation identified and is located on an exposed loop of the protein monomer [29]. Notably, PTX3 glycosylation (mainly constituted by fucosylated and sialylated biantennary sugars) modifies the protein binding capacity to various ligands, including the complement fraction C1q [29], and contributes to the complex fine-tuning of the biological activity of this long pentraxin.

The N-terminal region of PTX3 is unrelated to any known protein domain and no crystallographic data are available for this portion of long pentraxins. A secondary structure prediction for PTX3 N-terminal domain includes three α-helices (amino acids 78–97, 109–135, and 144–170), which participate in the formation of coiled-coil assemblies; moreover, the occurrence of short loops between α-helices suggests an up-down topological distribution [31].

PTX3 N-terminus contains three cysteine residues in position 47, 49, and 103 that are present also in the N-terminal coiled-coil α-helices of the human long pentraxins NP1 and NP2 [5]. Initial studies about the quaternary structure of human PTX3 had suggested a complex multidomain organization with eight protomer subunits held together by both covalent and non-covalent interactions [28]. Then the final quaternary structure has been resolved by mass spectrometry and site-directed mutagenesis followed by low-resolution modeling based on electron microscopy and small-angle X-ray scattering (SAXS) data [32]. These studies revealed that $Cys^{47,\ 49,\ 103}$ residues in the N-terminal region form three inter-chain disulphide bonds holding four protein subunits in a tetrameric structure (Fig. 2). Then, two tetramers are linked together by $Cys^{317,\ 318}$ residues in the C-terminal region to form the final octameric structure [33] where the eight subunits fold into an elongated structure with one large and one small domain interconnected by a stalk region [32].

2.3. PTX3 interactome

The physiological functions ascribed to pentraxins involve recognition and binding to different ligands, including microbial moieties, complement components, and extracellular matrix (ECM) proteins.

The complex building-block structure of PTX3 reflects the heterogeneous spectrum of ligands and functions exerted by this soluble pattern recognition receptor in different contexts (Fig. 2).

As a free protein in body fluids, PTX3 binds a number of selected bacteria, fungi, and viruses: a specific binding has been observed to conidia of *Aspergillus fumigatus* [34], *Paracoccidoides brasiliensis*, and zymosan [35], selected gram-positive and gram-negative bacteria [36], and some viral strains, including human and murine cytomegalovirus and influenza virus type A (IVA) [37,38].

The first characterization of the protein revealed the capacity of PTX3 to interact with the complement system [33]. PTX3 binds the first component of the classical complement cascade C1q [39,40], but no other components of the complement system such as C3 and C4. PTX3/C1q interaction requires multimer formation, involves the C-terminal domain of PTX3 and the globular head region of C1q [35,39]. At