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Invited critical review

Annexin A5 as a potential marker in tumors

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ABSTRACT

Annexin A5 (Anxa5) promotes pancreatic adenocarcinoma, sarcoma, tumorigenesis and progression of breast cancer and prostate cancer stem cells. It is involved with metastasis, invasion and development of squamous cell carcinoma, and facilitates nodal progression of bladder cancer and angiogenesis and progression of glioma. Anxa5 de-regulation is associated with drug resistance in nasopharyngeal carcinoma and gastric cancer. Although Anxa5 protein up-regulation promotes cervical cancer progression, it is markedly suppressed in cervical carcinoma cells. Anxa5 is negatively correlated with thyroid cancer malignancy. In this review, we explore the mechanisms of Anxa5 action in tumors. Anxa5 could be a predictive biomarker for tumor development, metastasis and invasion, and be of diagnostic, prognostic and therapeutic significance in cancer.

Contents

1. Introduction

1.1. Annexins: structural determinants to functions

As Ca^{2+}-regulated phospholipid- and membrane-binding proteins, annexins are classified into families A (vertebrates), B (invertebrates), C (fungi and some groups of unicellular eukaryotes), D (plants), and E

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(protists) [1]. Annexins are predominantly cytosolic soluble proteins that can reversibly bind to negatively charged phospholipids in a Ca^{2+}-mediated manner. 12 annexins common to vertebrates are named as annexins A1–A11 and A13 [1,2].

Annexins share a COOH-terminal core domain composed of four homologous tandem annexin repeats, each is about 70-amino-acid long. The annexin repeats share a sequence identity of 25–35% between each other [3] and of 45–55% [4] among different annexins. Each annexin repeat is a right-handed super-helix structure formed by five α-helices. Four annexin repeats pack into a compact and slightly curved disk with convex surface for harboring the calcium- and membrane-binding sites. The concave side facing cytoplasm is available for NH_2- and COOH-termini. NH_2-termini of annexins are varied from 11 to 196 residues. Annexin NH_2-terminus is probably located on the concave side of core region on the opposite of membrane-binding surface. It is the site for protein–protein interaction and annexin–membrane association. The unique NH_2-terminal structure determines the biochemical properties and functions of annexins [1,5–10]. Annexins functionalize in the processes of endo- and exocytosis, anti-inflammation, anticoagulation, signal transduction, ion channel formation, cell proliferation, division and apoptosis, tumor development, invasion, metastasis and drug resistance [1,5,6,10–14].

1.2. Properties of Anxa5

Anxa5, also named as placental anticoagulant protein I, thromboplastin inhibitor V, endonexin II, calphobindin I and lipocortin V, was first described functionally as a vascular anticoagulant in 1985 [7,15]. It is a non-glycosylated single chain protein composed of 319 amino acid residues with a molecular mass of ~35.7 kDa [16,17].

Anxa5 was the first annexin characterized for three-dimensional structure in 1990 [18]. It contains annexin repeats I to IV. Asp-226 of Anxa5 participates as a molecular switch in a Ca^{2+}- and pH-dependent conformational manner. In Ca^{2+}-bound conformation, the repeat III of Anxa5 exposes its Trp-187 to bind phospholipids [9,19]. The inter-domain interactions within Anxa5 make two tightly associated modules formed by repeats I/IV and repeats II/III. The interaction between I and IV is mediated in a non-covalent manner by NH_2-terminal tail. Repeats II and III are covalently linked via a short helical turn [17]. Conserved arginine residues present in the endonexin fold of each homology segment are crucial for tertiary structural stability of Anxa5 [19]. It can bind to membrane that has a higher phosphatidylserine (PS) content or at higher Ca^{2+} concentrations [9,20]. Anxa5 is monomeric, but once bound to membrane, three Anxa5 monomers spontaneously form a trimer. The trimers assemble in a two-dimensional lattice covering the PS exposing surface through trimer–trimer interactions [16,17,21,22]. The self-assembly of intracellular Anxa5 into two-dimensional crystal lattice is involved in stabilizing plasma-membrane structures, in changing membrane curvature and cell shape and in promoting cell membrane repair during anti-inflammatory, profibrinolytic and anti-thrombotic activities [23]. Intracellular Anxa5 exhibits calcium channel activity in plasma membrane and interacts with actin in platelets, where it represents a key regulator of the coagulation process [23]. Anxa5 is generally considered a cytosolic protein due to a lack of a 5′-leader sequence in its mRNA. However, release of Anxa5 into the extracellular space has been proposed to occur via unconventional secretory pathways [17]. Extracellular Anxa5 can bind to the outer leaflet of PS-externalized plasma membrane of activated platelets, dying cells and viable leukocytes for playing important roles in hemostasis, apoptosis and phagocytosis [17]. The two-dimensional lattice formed by extracellular Anxa5 causes the invagination of membrane followed by endocytosis and intracellular trafficking of endocytic vesicle [17]. Additionally, extracellular Anxa5 plays an essential role in docking hepatitis B virus and influenza viruses [23].

Anxa5 deregulations were observed as causative phenomena in a range of physiological and pathological processes. We summarized the associations of Anxa5 with tumor progression, invasion, metastasis, drug resistance and tumor treatments as schemed in Fig. 1 and Table 1. The relevant study progresses and corresponding action mechanisms of Anxa5 in tumors were also explored.

2. Anxa5 and tumors

2.1. Anxa5 and hepatocarcinoma

Hepatocarcinoma is characterized with high recurrence, high metastasis and poor prognosis. Hepatocarcinoma could infiltrate to portal vein by developing into macroscopic tumor thrombus, which is one of the most conspicuous growth patterns of hepatocarcinoma for most patients with middle and late primary hepatocarcinoma [24–26]. 2D DIGE–MALDI-TOF-MS analysis of five pairs of matched primary tumor and tumor thrombus samples from patients indicated that Anxa5 was up-regulated by 134% in tumor thrombus samples [27]. Anxa5 might be a potential biomarker for portal vein tumor thrombus (PVTT) forming (Fig. 1). Although it cannot stand for the full view of the differential proteome between primary tumor and PVTT, it's conducive not only to expound PVTT forming mechanism, but also to make corresponding antibodies to block and prevent PVTT forming come true.

Anxa5 was associated with hepatocarcinoma lymphatic metastasis, as shown in Fig. 1. About half of tumor metastases are via lymphatic system, resulting in poor prognosis. Comparative proteomics analysis of two well-established mouse hepatocarcinoma cell lines, Hca-F with 75% lymph node metastatic potential and Hca-P with 25% lymph node metastatic potential, from our group using 2D DIGE–HPLC–ESI-MS/MS approach indicated that Anxa5 was increased by 216% in Hca-F compared to Hca-P [28–30]. Hereby, we up-regulated Anxa5 by transfection of Anxa5 eukaryotic expression plasmid in Hca-P to confirm if Anxa5 could influence the proliferation, migration and invasion capacity of Hca-P, so that we could gain some experimental basis, which might open up the possibility for delving into lymphatic metastasis. The study of Anxa5 in lymphatic metastasis might provide new diagnostic and therapeutic insights into early lymphatic metastasis of tumors.

2.2. The association of Anxa5 with breast cancer

Breast cancer is the commonest cancer with an estimated lifetime risk of 1:8. Despite the impressive advances in clinical treatments, breast cancer is still a major health problem for women [31].

Anxa5 was involved in carcinogenesis of breast cancer (Fig. 1). As one of the receptor tyrosine kinase (RTK)-activated signal transduction pathways, Ras/Raf/MEK/ERK pathway is essential for cell metabolism, growth, differentiation and oncogenes. The activation of GTP binding protein Ras by extracellular stimuli results in a cascade of phosphorylation reactions involving Raf, mitogen-activated protein kinases (MAPKs; extracellular signal-regulated kinases, ERK1/2) and mitogen-activated protein kinase/extracellular signal-regulated kinase kinases (MEK1/2) in mitogenic signaling. Shc, an adaptor protein, relays RTK-induced signals through Ras/Raf/MEK/ERK. Its association with Grb2 is an early signaling event preceding Ras activation [32]. Being tyrosine-phosphorylated by active RTK, Shc recruits Grb2 to activate Ras by catalyzing Ras-GTP into Ras-GDP [32]. ELISA analysis revealed that Anxa5 level was 10.48 times higher in MCF-7 breast cancer cells following the transfection of expression plasmid pcDNA3.1(−)-Anxa5 [31]. Anxa5 up-regulation suppressed Ras activation by decreasing the association of Shc with Grb2 [32]. Interestingly, Shc is likely a substrate for protein kinase C (PKC) in RTK–Ras/Raf/MEK/ERK signaling pathway [33,34]. The serine phosphorylation of Shc induced by PKCs facilitated its association with Grb2. Anxa5 overexpression might suppress Shc through inhibiting PKCs. Following the up-regulation of Anxa5, the levels of Raf-1, MEK1/2 and ERK1/2 phosphorylation were found significantly suppressed [32]. However, it is unclear whether Anxa5 overexpression directly inhibits these phosphorylations. Anxa5 dysexpression may