Annexin A2 (ANXA2) In Colorectal Cancer

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Abstract

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death. The prognosis of CRC usually depends on staging. However, a complex interaction of pathological features, clinical factors and molecular markers help in guiding prognosis and therapy. Identification of new markers and underlying molecular pathway of cancer development and progression will improve treatment response in cancer. Annexin A2 (ANXA2) belongs to a family of calcium-dependent phospholipid and membrane binding proteins called annexins. High ANXA2 expression has been detected in various tumors as CRC, gastric cancer, hepatocellular carcinoma, and ovarian cancers. Annexin A2 is essential in repair of injury in the normal colorectal epithelial cell. ANXA2 expression is increased in CRC compared to normal colonic tissue, that is why it is supposed that it has a role in colon cancer malignant transformation. The association of high ANXA2 expression with poor tumor pathologic features indicates its role as poor prognostic factor as it is involved in tumor proliferation, adhesion, angiogenesis, progression and invasion. In this review , we summarize structure, subcellular localization , mode of action and roles of ANXA2 ,as well as therapeutic techniques targeting ANXA2.

Keywords: Colorectal cancer; Annexin 2; immunohistochemical expression

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Introduction

Colorectal cancer (CRC) is the third most common cancer (10.2%) and the second leading cause of cancer-related death (9.2%) in both sexes worldwide ***(1).*** GLOBOCAN 2020 estimated that the new cancer cases number reached 19.3 million globally, and about 10 million people died from cancer ***(2).*** It is a disease of older adults that commonly occurs after the age of 50 years ***(3).*** In Egypt CRC incidence rates below the age of 40 is 38%, compared with 2%–8% in the U.S. and the European Union in the same age group ***(4).*** The World Health Organization (WHO) (5th edition) classified tumors of the colon and rectum into benign and malignant tumors. Benign epithelial tumors and precursors include Serrated dysplasia, adenomatous polyp and glandular intraepithelial neoplasia, while malignant epithelial tumors involve adenocarcinoma and its variants , neuroendocrine tumor, and Mixed neuroendocrine - nonneuroendocrine neoplasm (MiNEN) ***(5)***.

Annexin A2 (ANXA2) is a calcium regulated phospholipid-binding protein that is expressed in various cell types, such as macrophages, monocytes, dendritic cells, endothelial cells, epithelial cells, bone marrow cells, neuron and tumor cell ***(6).*** It is encoded by annexin A2 gene, located on chromosome 15 (15q21) ***(7).*** The name came from the Greek word “annex”, meaning to attach or bridge as they can link membranes together or to other structures ***(8).***

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Currently, 13 members of the annexin family have been identified. The ANXA2 is most widely studied in human biology and disease ***(9)***.Annexin A2 has two characteristic criteria. The first criterion is the ability to bind negatively- charged phospholipid in a calcium-dependent manner. The second is containing an Annexin repeat ***(10).*** Annexin repeat is a structure of 70 to 80 homologous amino acids repeats, packed in an α-helix disc ***(11).*** All annexins are characterized by a conserved C-terminal core domain which comprises 70 amino acids segments that are repeated four or eight times and harbors Ca2+dependent binding sites for acidic phospholipids ***(12).*** The variable N-terminal domain interacts with different host proteins and undergoes posttranslational modifications giving each annexin molecule unique properties ***(13).***

Annexin A2 is involved in several cytoskeleton-membrane interactions and intracellular processes; such as membrane domain organization, membrane fusion, and vesicle aggregation, which are involved in exocytosis, endocytosis, and phagocytosis ***(14).*** Also, it participates in fibrinolysis, inflammation and immune reaction regulation and tissue repair so, its dysfunction has been implicated in multiple human diseases ***(15).***

Abnormal ANXA2 expression has been linked to multiple malignancies, such as gastric carcinoma, esophageal cancer, colorectal cancer, prostate cancer, breast cancer and pancreatic cancer ***(16).*** It serves as a potent target of therapy and multiple therapeutic strategies targeting ANXA2 have been tested and showed favorable anti-tumor efficacy both in vitro and vivo ***(17).***

Subcellular localization of ANXA2

ANXA2 has diverse subcellular localization, it can be present in the cell membrane, cytoplasm, or even in the nucleus ***(17).*** ANXA2 exists in two forms: a monomer and a heterotetrameric complex where two molecules of ANXA2 are bound to a S100A10 homodimer via a site containing the first 12 N-terminal amino acids of ANXA2 ***(18).*** The monomer exists in the cell cytoplasm, nucleus and on early endosomes, while the heterotetramer is located on cell membranes ***(19).***

Mode of action of ANXA2

Annexins bind negatively charged phospholipids in the presence of calcium. It interacts with S100 protein in a Ca2+-dependent and Ca2+-independent manner producing anexin-S100 complexes that possess biological activities ***(20).*** ANXA2 in the cytoplasm and on the surface of membranes acts as mediator of membrane-related processes, involving exocytosis, endocytosis and membrane trafficking ***(19).*** ANXA2 monomers in nucleus stimulate DNA synthesis, cell division and proliferation by promoting transition into the G1-S phase ***(6).***

Role of ANXA2 in colorectal tissue repair

The intestinal epithelium is critical barrier between microbiota and mucosal immune cells .The initial response to epithelial injury includes hemostasis, which limits blood loss and seals damaged tissue ***(21).*** The intestinal epithelial cells rapidly migrate to reseal wounds. It is observed that a membrane-associated, actin-binding protein, ANXA2, is up regulated in these migrating cells and promoting wound closure ***(22).***

Role of ANXA2 in cell cycle regulation, cell survival and proliferation

ANXA2 transcription and translation progressively increase as the mitotic cells advances through cell cycle, reaching their maximum level during transition from G1 to S phases ***(9).*** It regulates DNA synthesis, replication and the cell cycles. ANXA2 activates both NF-κB and β-catenin signaling pathways thus causing cell proliferation in vivo ***(17).***

Nuclear ANXA2 disrupts coilin, causing it to localize to centromeres, triggering chromosomal instability (CIN) as well as increasing DNA replication. According to some reports, chromosomal instability

promotes cellular resistance to chemotherapy and hastens the growth of tumors ***(23).***

Role of ANXA2 in neoangiogenesis

The ANXA2/S100A10 complex, residing on the endothelial cell surface, converts plasminogen into plasmin which activate matrix metalloproteases (MMP) to proteolyze the basement membrane components, liberating endothelial cells and allowing their migration, so ANXA2 may be involved in pathological angiogenesis ***(8).***

Role of ANXA2 in cell invasion and metastasis

ANXA2 may be important for progastrins and gastrins effect, mediating growth factors effect on colon cancer cells ***(10).*** Researchers proved a significant increase in ANXA2 expression in colonic adenocarcinoma tissue compared to adjacent normal colonic tissue tissue ***(24, 25, 26).*** Its overexpression is associated with CRC invasion and TGF-ß induced epithelial mesenchymal transition via Src/ANXA2/STAT3 ***(27).*** TGF-β signaling inhibits epithelial growth in normal tissues, but it promotes tumor cell progression, which is known as TGFβ paradox. Consensus molecular subtype of CRC (CMS4) has mesenchymal features, with a high stromal content and activation of TGF-β suggesting its role in promotion the tumor-stromal interaction to induce a malignant CRC phenotype and poor prognosis ***(28).***

ANXA2 is a receptor for plasminogen (PLG), tissue plasminogen activator (tPA).On the cancer cell surface, it converts PLG into plasmin ***(9).*** Plasmin is an important regulator of ECM degradation, fibrin polymers lysis, tumor migration, invasion and angiogenesis ***(17***).

ANXA2 mediates its role in EMT by signal transducer and activator of transcription 3 (STAT3) through Src/ANXA2/STAT3 axis. ANXA2 promotes STAT3 phosphorylation and translocation to the nucleus mediating transcription factor Slug expression. As a result, E-cadherin gene transcription is inhibited, and vimentin and matrix metalloprotinase (MMP) 2&9 expression are upregulated ***(27).*** MMPs are a family of zinc-dependent endopeptidases MMP-9 plays a role in tumor invasion, metastasis and angiogenesis and to mediate tumor microenvironment ***(29).*** Plasmin cleaves the tissue inhibitor of metalloproteinases on pro-matrix metalloproteases (MMPs), and then the pro-MMPs become the active forms of MMPs. MMPs (e.g., MMP-2 and MMP-9) degrade fibronectin and the ECM, accelerating invasion and metastasis ***(30).***

ANXA2 co-localizes with epithelial cell adhesion molecule (EpCAM) at the plasma membrane, EpCAM supports ANXA2 to function as a co-receptor for the tPA that convert the plasminogen to the active plasmin leading to degradation of the extracellular matrix helping invasion and metastasis ***(31).***

ANXA2 expression enhances cancer progression via cytoskeleton structural rearrangements as it enhances tubulin polymerization that is is essential to maintain the motility in cancers with high-metastatic potential. ANXA2 can be activated by insulin receptor activation relevant to the actin accumulation and subsequent cell detachment ***(32).***

Cancer Stem Cell formation

ANXA2 expression in cancer cells is associated with acquirement of stem cell-like properties, giving them the ability for self-renewal and differentiation. CSCs promote invasion, metastasis, suppress chemotoxicity and radiotoxicity .ANXA2 can be a novel marker to detect circulating CSCs. Akt protein upregulates stemness-related transcription factors (Oct4, Sox2, and Nanog) and is involved in CSC maintenance in different cancer types. Experimental silencing of ANXA2 inactivates Akt, with subsequent suppression of the protein levels of stemness-related transcription factors (Oct4, Sox2, and Nanog) ***(33).***

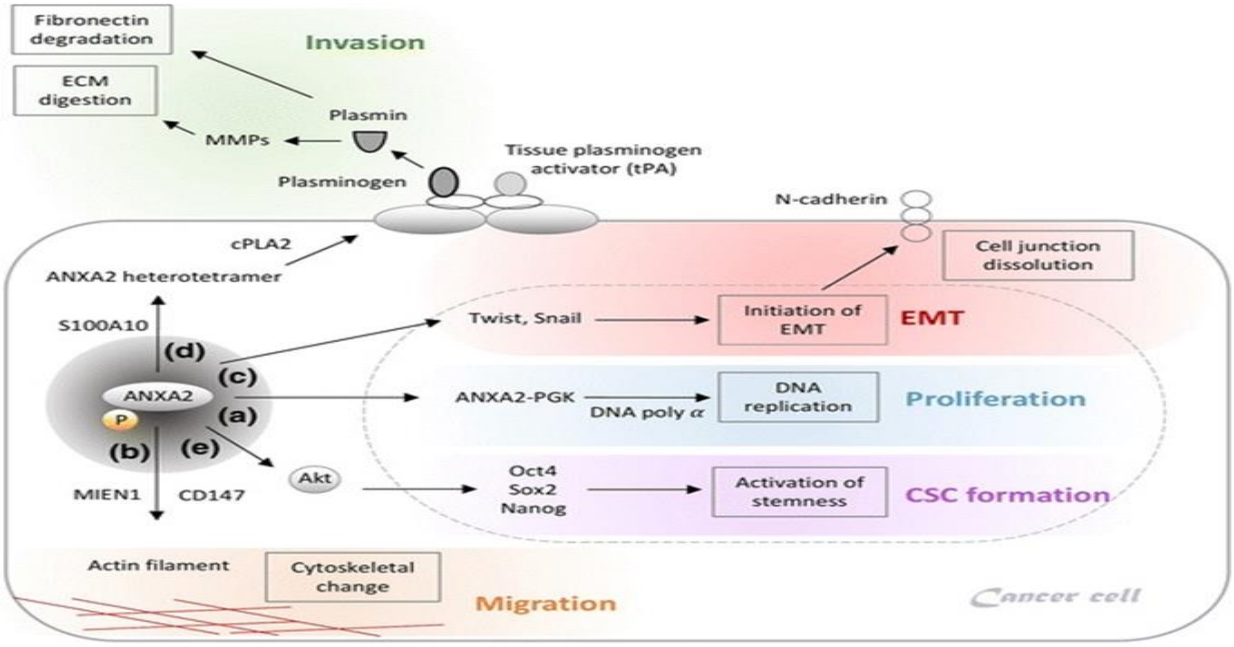

Figure (1) Annexin A2 (ANXA2) in cancer progression. **a** The ANXA2-3-phosphoglycerate kinase (ANXA2-PGK) complex serves as a primer recognition protein to initiate DNA replication with the support from DNA polymerase alpha, which contributes to cell proliferation. **b** MIEN1 phosphorylates ANXA2 and supports ANXA2's binding to actin filaments to modulate cytoskeletal change, thus resulting in cell migration. **c** ANXA2 initiates the endothelial-mesenchymal transition (EMT) via the Twist/Snail pathway. After initiation of the EMT, cells changed to a mesenchymal-like morphology, and cell junctions dissolved. **d** The ANXA2 heterotetramer complex links to the plasminogen and tissue plasminogen activator (tPA). After plasminogen is cleaved into plasmin, plasmin activates pro-matrix metalloproteases (MMPs) to become MMPs. MMPs digest the extracellular matrix and fibronectin, thus resulting acceleration of invasion. **e** ANXA2 increases stemness-related transcription factors (Oct4, Sox2, and Nanog) through the Akt signaling pathway, which activates cancer stem cell formation***(Chen et al., 2018).***

Role of ANXA2 in cell apoptosis

ANXA2 is a ligand of C1q that binds to apoptotic cells mediating recognition by phagocytes. It suppresses expression of p53 and its downstream genes, p21, Growth arrest and DNA-damage-inducible protein (GADD45) and BAX, resulting in promoting apoptosis ***(17).***

Role of ANXA2 in immune escape

The immune system identifies and acts against tumor by cytotoxic T lymphocytes and natural killer (NK) cells ***(34).***CRC tissue showed a higher proportion of Tregs and a lower proportion of CD4+ and CD8+ cells suggesting that tumor progression and metastasis was affected by the immunosuppressive systems produced by Tregs ***(35).*** ANXA2 is involved in tumor immune escape by upregulation of the number of Treg cells and the expression of checkpoint molecules as well as the downregulation of the number of activated NK cells and DCs ***(36).***

Effects of ANXA2 on patient prognosis

In addition to its role in tumorigenesis and cancer progression, ANXA2 is strongly expressed in poorly differentiated tumors, late stage and lymph node positivity. Moreover, patients with strong ANXA2 expression showed lower overall survivals compared with those with weak-expression which indicates its important role as a poor prognostic factor in CRC ***(24, 25, 26)***.

ANXA2 as therapeutic target

Colorectal cancer binding peptide (CBP12), an ANXA2-targetting peptide motif, represents a promising hope for targeted therapies, especially that it has specifically affinity to colorectal cancer cells ***(17).***

ANXA2-targeted prospective treatment beginning in 2013, various research teams started to form treatment that targets ANXA2. One of Teams made use of an anti-ANXA2 antibody to lessen tumor development and metastasis in a mouse model of ovarian cancer ***(37).*** After a year, Mandip Singh and his coworkers used RNA targeting ANXA2 and inserted short hairpin (sh)RNA targeting ANXA2 (shANXA2) into a liposomal cationic ligand-guide (CLG) to create a CLG-ANXA2 molecule. The goal of the CLG-ANXA2 was to detect cancer cells and CSCs in a mouse model of lung cancer. Once tumor cells have taken up CLG-ANXA2, shANXA2 reduced the level of ANXA2 messenger (m) RNA and prevented its expression. The CLG-shANXA2 group inhibited tumor growth as it is reduced from 72% to 75% in comparison to the control ($p < 0.001$) ***(38).***

Targeting ANXA2 raises the possibility of being able to overcome the low therapeutic efficacy of cancers with high ANXA2 expression ***(33).***

Conclusions

ANXA2 is highly expressed in CRC compared to adjacent normal tissues. Through its signaling and interactions with other pathways, it plays a role in cancer initiation, proliferation, progression, invasion and metastasis predicting a poor prognosis. ANXA2 can be used as target for therapy.

Conflicts of Interest

The authors declared no conflict of interest.

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