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A Survey on the Role of Fetuin-A in Breast Cancer: A Review

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A B S T R A C T

Fetuin-A is a major 63-kDa plasma protein synthesized by liver cells and secreted to plasma. It has been suggested to be an extracellular adhesive molecule. In the presence of fetuin-A, breast tumor cells can join and propagate, but the mechanism is not clear. Fetuin-A mediates adhesion by calcium ions, while integrin mediates adhesion by divalent ion manganese and magnesium. Calcium-mediated adhesion is observed in metastatic or more aggressive breast tumor cell line, but not in benign or normal breast epithelial cells. Adhesion and propagation of tumor cells can be only mediated by cellular exosomes carrying histones and fetuin-A. In this article, we review fetuin A and its role in apoptosis and breast cancer.

Keywords: breast cancer, fetuin A, mechanism, calcium ion.

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Introduction

Fetuin-A, $\alpha 2$ -Heremans–Schmid¹ glycoprotein^{1,2}, (AHSG)³⁻⁶ is a major 63-kDa plasma protein⁷ synthesized by liver cells⁸⁻¹¹ and secreted to plasma¹². It is a multifactorial protein^{13,14} with a concentration of about 450–600 $\mu\text{g}/\text{mL}$ in healthy persons¹⁵. Fetuin-A is a cysteine protease inhibitor which is known⁷ as a member of the cystatin superfamily protein with two cystatin domains and a proline and glycine as third domain¹⁶. Due to these properties, it binds to matrix metalloproteinases (MMP), particularly MMP-9, to protect them from degradation¹³. Also, secondary modifications such as N-glycosylation, O-glycosylation, Ser-phosphorylation, and proteolytic process occur in a variety of fetuin-A forms¹⁶. Due to its transforming growth factor alpha (TGF- α) receptor II homology 1 domain (TRH1)¹⁷, fetuin-A is able to compete with epithelial cells for TGF- α ¹⁷. As a circulating calcium-regulatory glycoprotein¹⁸, it can form soluble colloidal complexes with calcium and phosphate, named “calciprotein particles”¹ and is a systemic inhibitor of calcification¹ identified in fetal and newborn calf serum, sheep, pig, goat, mice, rat and humans¹⁹. Fetuin-A has an important role in regulating phagocytosis regulation and innate immunity; low concentrations of serum AHSG are associated with depressed cellular immunity and nonspecific host defense²⁰. Moreover, low serum level of AHSG is a prominent predictor of short-term mortality in liver cirrhosis and liver cancer patients. In addition, AHSG can indirectly influence calcification via regulating energy metabolism or bone metabolism²⁰. Therefore, it seems that fetuin-A can be a strong systemic inhibitor of unwanted ectopic calcification²¹. Several physiological functions have been attributed to fetuin-A, from brain development to bone remodeling and immune function¹⁹, regulation of insulin activity, hepatocyte growth factor activity and in-

hibition of lymphocyte blastic transformation^{15,20}. These diverse functions of fetuins show that as multi-ligand binding proteins, they can interfere with any biochemical pathway by binding and sequestering²⁰. Moreover, AHSG may be involved in tissue regeneration¹⁵. Evidence shows that fetuin mRNA is expressed in a number of developing tissues and organs, including heart, lung, kidney, the nervous system and liver¹⁶.

Fetuin and apoptosis

Apoptosis is a complex network of biochemical and molecular pathways with fine regulatory mechanisms that control the death event in a cell. Apoptosis is a form of cell death that occurs during several pathological situations in multicellular organisms^{22,23}. The mechanism of apoptosis by fetuin is unknown. Fetuin can induce alkaline phosphatase, which is able to induce apoptosis. Studies have shown that cells exposed to high level of alkaline phosphatase die because of apoptosis¹⁶. Furthermore, in rodents, high numbers of apoptotic cells have been found in fibroblasts adjacent to bone and tooth surface where high levels of alkaline phosphatase are expressed. These results confirm that alkaline phosphatase may constitute one mechanism by which fetuin induces apoptosis. Moreover, the activity of fetuin for inducing apoptosis is dependent on zinc. Depletion of zinc ion from fetuin or substitution of zinc ion by barium ion entirely obsoletes the apoptosis-inducing activity of fetuin¹⁶.

Fetuin-A and cell adhesion

Adhesion of cells is a complex process beyond identification of integrins and their ligands in the extracellular matrix. In the past two decades, some components, such as heparan sulfate proteoglycans and exosomes have been considered to play a role in breast cancer, but today, a novel physiological role

has been shown for fetuin-A in these cellular processes²⁴. The role of fetuin-A in cellular adhesion has been discussed, regarding its cooperation with a number of plasma proteins like fibronectin and $\alpha 2$ macroglobulin¹². Therefore, Fetuin-A can act as one of the many adhesion molecules in the extracellular milieu¹². Studies by confocal microscopy show that fetuin-A can produce complexes with histones like H2A and H3 in solution²⁴. The positively charged histone proteins can promote cellular adhesion to surfaces coated with poly-lysine¹². Histones mediate adhesion on the surfaces of exosomes by interaction with cell surface through electrostatic forces. A study by Watson reported a relation between uptake of fetuin-A and secretion of adhesion competent exosomes¹². Kalaby et al. reported that fetuin-A can bind to several cell surface proteins like annexin-II and -VI, and most likely other members of the annexin family which are expressed on the cell surface like lectin with preference for the sialic acid residues on tri-antennary sugar moieties found in fetuin-A¹⁵.

Fetuin A and Breast cancer disease

Breast cancer is the most common cancer in women²⁵⁻²⁸, accounting for approximately one third of all illnesses in women. It affects one of every 8 women in the United States²⁶. Moreover, it is one of the most frequent malignancies among Iranian women²⁶. This disease can be curable at the early stage, when it is identified by tumor markers in blood²⁹, but detection of such biomarkers in early stage of breast cancer is challenging¹². Breast tumor cells can adhere and propagate in the presence of fetuin-A¹², because of its role as extracellular adhesive molecule²¹, although the mechanism is not clear²¹. Fetuin-A mediates adhesion by calcium ions, but integrin mediates adhesion by divalent ion manganese and magnesium¹². Moreover, calcium-mediated adhesion is observed in metastatic or more aggressive breast tumor cell line, but not in benign or normal breast epitheli-

al cells²¹. Sakwe et al. isolated and purified fetuin-A from serum using the Pedersen method and reported that cell adhesive properties are seen in the presence of divalent ions¹³. This adhesion needs sialic acid residues on fetuin-A. Other than sialic acid residue, the overall glycosylation status of fetuin-A may also affect its cell adhesive properties in the presence of calcium²¹. Guillory et al. reported the deficiency in fetuin-A decreases mammary tumors in PyMT transgenic mouse model of breast cancer by more than 60%⁷. Since fetuin-A is an abundant serum protein, it is considered as an important modulator of breast cancer in humans⁷. Watson et al. reported that lack of fetuin-A resulted in failed development of mammary tumors in murine models¹². Fetuin-A also inhibits MAPK activation in breast carcinoma cells. It appears that fetuin-A temporarily binds to most of the extracellular Ca^{2+} with a concomitant reduction in the activation of mitogen activated protein kinase (MAPK) (30). The attachment of tumor cells to fetuin-A is accompanied by Phosphoinositide 3-kinase (PI3 kinase)/Akt activation that is down-regulated in cells that lack annexin-A6, one of the cell surface receptors for fetuin-A. Taken together, these data show the significance of fetuin-A in tumor cell growth mechanisms in vitro and open new research vistas for this protein¹³. Moreover, interaction between breast cancer cells and fetuin-A through annexin- II and VI results in cell spreading and growth¹². It is postulated that annexins can be as cell surface receptors for fetuin-A adhesion¹². Annexins, especially annexin-VI, transport calcium ion from the extracellular milieu into the cytoplasm¹⁹ and some tumor cells with fetuin-A trigger a number of signaling pathways by Ca^{2+} ; this demonstrates the role of extracellular calcium ions as one of the modulators of tumor progression/metastasis pathways in vivo¹⁹. Sakwe et al. compared the expression of annexin-A6 (AnxA6) in breast cancer patients and normal humans and observed a correlation between reduced

expression of AnxA6 in breast cancer carcinoma tissues with enhanced cell proliferation. It seems that decreased expression of AnxA6 contributes to progression of breast carcinoma through promoting the loss of functional cell-cell and/or cell-ECM contacts and anchorage-independent cell proliferation³¹. Therefore, it seems that breast cancer AnxA6 acts as a tumor suppressor and adhesion/motility promoting factor³¹.

Conclusion

Breast tumor cells may adhere and propagate in the presence of fetuin-A, although the mechanism is not clear. Fetuin-A mediates adhesion by calcium ions. Calcium-mediated adhesion is observed in metastatic or more aggressive breast tumor cell line, but not in benign or normal breast epithelial cells. Annexins can act as cell surface receptors for fetuin-A adhesion and interaction between breast cancer cells with fetuin-A through annexin- II and VI, resulting in cell propagation and growth.

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