Review

FETUIN-A – ALPHA2-HEREMANS-SCHMID GLYCOPROTEIN: FROM STRUCTURE TO A NOVEL MARKER OF CHRONIC DISEASES PART 1. FETUIN-A AS A CALCIUM CHAPERONE AND INFLAMMATORY MARKER

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Summary

Fetuin-A is a major plasma glycoprotein released mainly by the liver. Its functions include inhibition of the activity of insulin receptor, regulation of response to inflammation, inhibition of calcified matrix metabolism and ectopic mineralization, etc. Three major functional domains of fetuin-A have been identified: one similar to the Ca-binding domains, one inhibiting cysteine protease, and a domain with high affinity to insulin receptor. The fetuin-A molecule may be considered as a highly pleomorphic protein with an important impact in a variety of clinically expressed metabolic and pathological processes. It could be used as a marker in clinical practice in the future.

Key words: fetuin-A, inflammation, cystatin, calcium chaperone, hepatokine

Introduction

Fetuin-A is a major plasma glycoprotein, which belongs to the cystatin superfamily of protease inhibitors, and is released mainly by the liver. It performs important functions, including those of inhibition of the insulin receptor activity, and inhibition of bone mineralization regulation of calcified matrix metabolism. It has also been recognized as a multifunctional plasma protein and a key participant in various processes like cellular protein metabolism, attenuation of acute inflammatory response, neutrophil and platelet degranulation, lymphocyte stimulation, binding of fatty acids, thyroid hormones and calcium ions, etc.

Fetuin-A was discovered as an important calf serum protein during fetal life by K. O. Pedersen in 1944 [1]. Its homologue in humans was isolated later and was named α2–Heremans-Schmid glycoprotein (AHSG)/ fetuin-AHSG, after J. F. Heremans (1960) and Schmid et Burgi (1961) [2, 3]. The half-life of fetuin-A is 1-2 days [4]. Structurally, a closely related protein – fetuin-B, was detected by searching expression databases of human, mouse, and rat genes [5]. The A and B fetuins were found to be similar in terms of function and tissue distribution [6].

Structure and function

Fetuin-A is a highly expressed plasma protein consisting of two disulfide bond-linked polypeptide chains, both of which are produced by cleavage of a pre-protein, synthesized from a single messenger RNA [7]. The mature form is produced from pre-protein after proteolytic processing [8, 9], and posttranslational modifications: glycosylation [10], phosphorylation [11, 12] and sulfation [13]. These posttranslational modifications probably regulate the expression of fetuin-A, its biological stability and activity [14]. Phosphorylation is essential for fetuin-A interaction with the insulin receptor [15].

Fetuin-A is a glycoprotein containing approximately 23.6% of carbohydrates. The major part is that of three N-linked branched heteropolysaccharides of similar composition [16]. Apart from N-linked glycans, the protein also has four sites of O-linked glycosylation [17].

The Kratky plot indicates that fetuin-A is mostly a well-ordered globule with a small disordered part [18]. Based on the sequence similarity, it has been demonstrated that fetuin-A consists of two cystatin domains D1 and D2

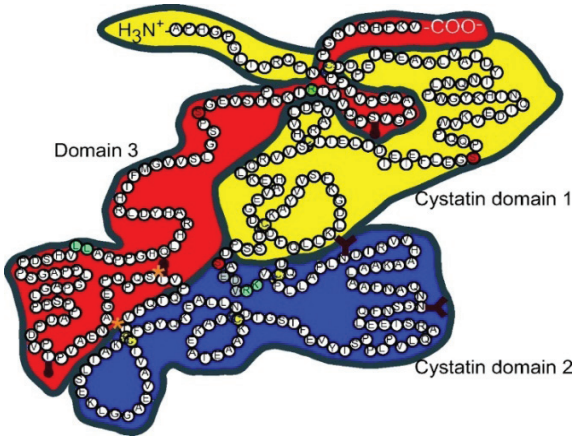

Figure 1. Human fetuin-A/Fetuin-AHSG. The domains are presented in yellow – cystatin-like domain D1, in blue – cystatin-like domain D2 and in red a C terminal domain 3. The disulfide bridges (C-C in yellow; the sites of Serine-phosphorylation (S in dark red), the sites sensitive to protease cleavage (dibasic tryptic cleavage site R-K; chymotryptic cleavage site L-L; furin-sensitive cleavage site R-T; in turquoise), and N-glycosylation sites Asn-linked complex, O-glycosylation sites Ser/Thr are presented as violet-blue symbols [19]

(positions 8-118 and 132-230, respectively), followed by a 100-residue C-terminal portion (residues 240-320), as shown on Figure 1 [19].

Its molecular weight is 39.2 kDa, calculated on the basis of 367 amino acids. Due to the high glycan mass, the molecular weight determined by Static light scattering and Small-Angle X-Ray Scattering was found to be higher, approximately 49.4kDa [20]. However, on electrophoresis it runs with alpha 2 globulins with a molecular weight around 55 kDa.

Human blood abounds in fetuin-A and fetuin-B. By structural homology, these proteins belong to the fetuin family and are members of the cystatin superfamily [21]. Cystatins are cysteine protease inhibitors, which usually possess a domain of conserved residues necessary for their protease inhibition capacity and four conserved cysteins, which form disulphide bonds [22]. The cystatin superfamily includes the cystatins themselves, as well as kininogens, histidine-rich glycoprotein, the intracellular stefins, and the fetuin family [23].

Physiological role

During fetal development, fetuin-A is abundantly synthesized by multiple tissues: liver, placenta, and choroid plexus. The fetuin-A fraction in fetal calf serum is larger than that of albumin. According to human and animal studies, the highest serum concentration of fetuin-A is presented during fetal life [24], accumulating in tissues with high turnover, such as the brain [25], and the immune and hematopoietic systems [26] of the developing organism [27]. In adults it is secreted predominantly by the liver (>95%) [28], and by monocyte/macrophages [29].

Human fetuin-A function

Fetuin-A inhibits insulin receptor tyrosine kinase, thus impeding autophosphorylation of the insulin receptor, leading to insulin resistance and related morbidities:

- Fetuin-A inhibits calcification process in soft tissue, and acts as a potent inhibitor of ectopic mineralization [30];
- It is a negative acute-phase protein with normal circulating levels in adults (300-600 μg/mL), which decrease significantly

(30-50%) during injury and/or infection [31];

- The level of fetuin-A in plasma correlates reciprocally with inflammatory cytokines, activation biomarkers and chemokines in patients with Type 2 Diabetes Mellitus (T2DM); fetuin-A levels are related to T2DM, metabolic syndrome, obesity and cardiovascular disease (CVD);
- Fetuin-A is a broad-range protease modulator [32]. It is an endogenous proteolytic regulator of meprin activity [33];
- Fetuin-A is a carrier protein, like albumin;
- Fetuin-A blocks transforming growth factor beta 1 (TGF-β) binding to cell surface receptors through binding to TGF-β. Fetuin-A is an important growth and development factor [34];
- It enhances the entry of cationic inhibitors into macrophages and modulates innate immunity [35].

Fetuin-A – calcium chaperone

Fetuin-A is highly associated with the regulation of calcium metabolism. Fetuin-A/AHSG is an important inhibitor of systemic calcification. Its low serum level has been associated with soft-tissue calcifications, as well as stiffening and calcification of arteries.

Fetuin-A is involved in the regulation of calcium metabolism, and remodelling of bones, and prevention of undesirable calcification of soft tissues [20, 36]. Low fetuin-A levels in inflammatory conditions could increase the risk of calcification.

Fetuin-A is a carrier of insoluble calcium phosphate. It forms soluble complexes with calcium and phosphate in the circulation, being an important inhibitor of calcium salt precipitation and vascular calcifications. Indeed, vascular calcification is related to increased risk of cardiovascular mortality.

Fetuin-A has a property to stabilize mineral complexes in serum and at the same time to mediate their transport and clearance [20, 37].

The selective accumulation of fetuin-A from plasma in bone is mediated by its high affinity of for bone minerals [38]. In bone, fetuin-A/AHSG accounts for 25% of the noncollagenous proteins [39]. The circulating levels of fetuin-A regulate the cell-dependent process of osteogenesis.

Fetuin-A/AHSG inhibits the formation *de novo* and precipitation of basic calcium phosphate (BCP) for a short period of time [40]. That is why, an undesirable calcification in circulation may be inhibited by fetuin-A without obstructing bone mineralization. This inhibitory action on tissue calcification is mediated through clustering acidic amino acids in domain D1.

A study on fetuin-A deficient mice suggested that it acts as a calcium chaperone [41] which mediates the transport of minerals from the circulation and the extracellular space [42]. It may be regarded as an opsonizing serum protein, which stimulates phagocytosis of formed microparticles by dendritic cells [43]. A specific depletion of fetuin-AHSG from the blood essentially reduces the inhibitory activity of serum proteins on apatite formation.

Recent studies have verified that the inhibition was mediated by the transient formation of "calciprotein particles", soluble spheres containing fetuin-A, and BCP [44].

These hypothetical calciprotein particles (CPPs), consisting of calcium phosphate and fetuin-A, were proposed to explain the mechanism of the inhibitory effect of fetuin on calcium phosphate crystal formation. Primary CPPs (50 to 80 nm) can grow up to 500 nm and produce secondary CPPs. The secondary CPPs are stable. They are covered by a layer of fetuin-A and serum albumin, and can be readily cleared from circulation [40, 44]. Furthermore, CPPs shape and size modulate their cytotoxic effects [45]. The studies on molecular modelling suggested that the inhibition of calcium-phosphate deposition is mainly mediated by the cluster of acidic residues on the β-sheet of the amino-terminal cystatin-like domain 1 of fetuin-A. The CPPs consisted of about 25% fetuin-A in volume fraction, while the remaining volume was filled with calcium phosphate [46].

In patients with chronic renal failure, liver cancer and liver cirrhosis on long-term dialysis low fetuin-A level might be associated with higher CVD mortality [47]. Nonetheless, currently there are no available methods to regulate the levels fetuin-A in circulation. The upregulation of fetuin-A levels may be a solution in cases of undesirable calcification

[46]. However, various functions of fetuin-A have to be taken into account, and its role in insulin resistance in particular.

Fetuin-A and inflammation

Fetuin-A have been shown to be a negative acute phase reactant. The inverse relationship with concentration of CRP in the serum has been demonstrated [48]. The glycoprotein is downregulated by interleukin-1 beta (IL-1ß), increased adipose tissue expression of IL-1ß associated with inflammation, and insulin resistance in obesity. Fetuin-A production is divergently regulated by various proinflammatory mediators: its role was found to be both positive and negative in injury and infection. It directly inhibits pathogen-associated molecular pattern (PAMP) – induced release of high mobility group box protein 1 (HMGB1) by innate immune cells [49]. It also facilitates anti-inflammatory actions of cationic polyamines like spermines.

In stroke, due to cerebral ischemia, there is an induced transient increase in blood-brain barrier permeability. This permits the entry of circulating proteins like fetuin-A and peripheral immune cells (such as macrophage/monocytes) [49].

Peripheral administration of fetuin-A in rats attenuates ischemia-elicited HMGB1 release and subsequent cytokine expression, thereby conferring a temporal protection against cerebral ischemic injury [50].

In addition, tumour necrosis factor-α (TNF-α) leads to a decrease of the expression of fetuin-A in vitro [28, 51]. On the contrary, fetuin-A may induce low-grade inflammation, associated with metabolic syndrome and atherogenic lipid profile [52]. These studies demonstrated an important role of fetuin-A in regulating injury or infection-related inflammatory reactions.

A number of studies have proposed that synthesis of fetuin-A may be divergently regulated in various clinical conditions. This warrants the assumption that this hepatokine may play the role of a positive or negative acute phase protein during different disease states.

Elevated fetuin-A level was found in the studies related to trauma and/or ischemic injury or stroke; these facts suggest a positive acute phase protein role of fetuin-A in these conditions [53]. However, in conditions like endotoxemia [54], systemic inflammation [50], sepsis, as well as other clinical conditions with inflammatory component including rheumatoid arthritis [55], pancreatitis [56], chronic kidney disease [57], decreased fetuin-A levels were noticed; suggesting an anti-inflammatory or protective role of fetuin-A. This protective role of fetuin-A in lethal systemic inflammation is possibly due to inhibited release of active HMGB1.

Fetuin-A as a hepatokine in non-alcohol induced fatty liver disease

The liver is a central regulator of whole-body glucose and lipid homeostasis. Fetuin-A is a hepatokine that has been proved to have a role in the pathohgenesis of metabolic disease and has been identified as a marker and mediator of fatty liver development [58].

Fat accumulation in the liver in non-alcohol induced fatty liver disease (NAFLD) was associated with a higher level of fetuin-A in several studies [28, 59]. Currently, involvement of the liver in the ongoing rise of morbidity in T2DM and cardiovascular disease is discussed [60]. The liver produces and secretes in the circulation a specific class of proteins – hepatokines, which are engaged in the regulatory effects of clearance and glucose and lipid metabolism. Production of this glycoprotein in the liver increases in steatosis and inflammation, but is not in expanded and dysregulated adipose tissue [28].

It has also been shown that fetuin-A plays an important role in lipid-induced insulin resistance in humans [28]. The high concentration of saturated fatty acids via NF-kB activation and increased blood glucose via growth-factor responsive mitogen-activated protein kinase (ERK 1 or 2) signalling pathways activation induces hepatic synthesis of this hepatokine [61].

Along with the inhibitory effect on insulin [62], fetuin-A modulates an adaptation for saturated fatty acids, preventing the activation of toll-like receptor 4 (TLR 4) which, in turn, induces inflammatory reaction and insulin resistance [63].

It was shown in a number of studies that the increased production of fetuin-A in patients with hepatic steatosis was closely associated

with ectopic fat accumulation in the liver. A significantly elevated serum fetuin-A level was demonstrated in patients with morphologically confirmed NAFLD. The study of Haukeland et al. [64] demonstrated the correlation of hepatic expression of fetuin-A with the important enzymes of lipid metabolism, like sterol regulatory element-binding protein 1c (SREBP1c), fatty acid synthase (FAS), carnitine palmitoyl-transferase 1 (CPT-1), as well as the enzymes of gluconeogenesis: phosphoenol pyruvate kinase 1 (PEPCK-1) and glucose-6-phosphatase (Glu-6-P). However, the mRNA expressions of fetuin-A in the liver were not significantly different in NAFLD patients when compared with controls.

The reduction of body weight in obese NAFLD patients leading to a reduction in hepatic fat content resulted in lowering its serum concentration [57]. It was also shown that even short–term, 7-day lasting physical exercise, brought a positive response in lowering the serum concentration of this hepatokine despite the fact, that there was no effect on body weight or hepatic triglyceride content as assayed by proton magnetic resonance imaging (MRI) [65].

Fetuin-A is an inhibitor of cysteine cathepsins

Fetuin-A is an inhibitor of cysteine cathepsins, which have a key role among the lysosomal proteases. Cysteine cathepsins belong to the family of papain-like cysteine proteases, which are widely distributed among living organisms [66-67]. Both positive and negative fetuin-A relations with proteases were demonstrated, including meprin metalloproteinases [68], matrix metalloproteinase -3 and -9, [69] m-calpain (the cysteine proteases) [70], cathepsin L, and trypsin.

Human fetuin-A, being a negative acute phase protein involved in inflammatory diseases, is a potential endogenous regulator of meprin activity. The metalloproteases meprin-α and meprin-β are unique proteolytic enzymes in inflammation, angiogenesis, neurodegeneration, cancer and fibrosis. Fetuin-A and cystatin C are suggested to be physiological modulators of meprin activity.

The interactions of fetuin-A with proteinases are suggested to be involved in the processes of tumorigenesis and tumor progression. In the study of the adherence of Lewis lung tumour cells (LLC) to fetuin-A it was shown that LLC interact in a Ca^{2+}-dependent manner, leading to growth of the tumor cells. These studies prove the important role of fetuin-A in serum as a major promoter of growth, that may induce the establishment and growth of a tumor [71].

Conclusions

The experimental and human studies point out the important role of the predominantly liver-derived hepatokine fetuin-A in the regulatory effects of clearance and metabolism of glucose and lipids. Apparently, fetuin-A plays a crucial role in the pathogenesis of a number of metabolic disorders and clinically recognized conditions. We present some of the clinical conditions, mostly related to the fetuin impact on Ca^{2+} metabolism, anti- and pro-inflammatory effects, and regulation of proteolysis. Altogether, data on the mineralization research and correlations with inflammatory cytokines, chemokines and various activation biomarkers demonstrate that fetuin-A levels in plasma and tissues could be used as a useful marker in clinical practice in the future.

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