Clinica Chimica Acta 446 (2015) 105–110

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Invited critical review

Afamin — A pleiotropic glycoprotein involved in various disease states

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ARTICLE INFO

Article history:
Received 12 February 2015
Received in revised form 28 March 2015
Accepted 2 April 2015
Available online 16 April 2015

Keywords:
Diagnosis
Prognosis
Metabolic syndrome
Tumor marker
Neuroprotection
Pregnancy

ABSTRACT

The human glycoprotein afamin was discovered as the fourth member of the albumin gene family. Despite intense research over the last 20 years, our knowledge of afamin's physiological or pathophysiological functions is still very limited. Circulating afamin is primarily of hepatic origin and abundant concentrations are found in plasma, cerebrospinal, ovarian follicular and seminal fluids. In vitro binding studies revealed specific binding properties for vitamin E. A previously performed analytical characterization and clinical evaluation study of an enzyme-linked immunosorbent assay for quantitative measurement of afamin in human plasma demonstrated that the afamin assay meets the quality specifications for laboratory medicine. Comparative proteomics has identified afamin as a potential biomarker for ovarian cancer and these findings were confirmed by quantitative immunoassay of afamin and validated in independent cohorts of patients with ovarian cancer. Afamin has also been investigated in other types of carcinoma. Most of these studies await further evaluation with validated quantitative afamin assays and require validation in larger patient cohorts. Transgenic mice overexpressing the human afamin gene revealed increased body weight and increased blood concentrations of lipids and glucose. These transgenic mouse data were in line with three large human population-based studies showing that afamin is strongly associated with the prevalence and development of the metabolic syndrome. This review summarizes and discusses the molecular, biochemical and analytical characterization of afamin as well as possible clinical applications of afamin measurement.

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1. Introduction

Human afamin is a glycoprotein that is present in biological fluids such as plasma, and cerebrospinal, ovarian follicular and seminal fluids. Afamin was discovered in 1994 by Lichtenstein et al. as the fourth member of the albumin gene family [1]. Despite intensive research in the last

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http://dx.doi.org/10.1016/j.cca.2015.04.010

20 years, our knowledge of afamin's physiological or pathophysiological functions is still very limited. Most published work has so far focused on the molecular and biochemical characterization of afamin and on discovery studies using proteomics and epidemiological approaches to search for (patho)-physiological functions. Suitable cell lines expressing human afamin have not yet been reported and the precise molecular structure is also unknown, which explains our limited knowledge of afamin functions, particularly at the mechanistic molecular level.

2. Biology of afamin

2.1. First discoveries and characterizations

Human afamin (AFM) was discovered by sequence analysis and cloning in 1994 as the fourth member of the human albumin gene family, which includes albumin (ALB), α-fetoprotein (AFP) and vitamin D-binding protein (DBP) [1]. Afamin was previously described independently by three other research groups as α-albumin in the rat [2], as α-1T-glycoprotein in humans [3], and was first reported as a tryptophan-poor α_1-glycoprotein 50 years ago in Clinica Chimica Acta [4]. Thus, the year 2014 marked not only the 20th anniversary of its first molecular characterization, but also the 50th anniversary of its initial protein-chemical discovery and description in 1964.

All four genes of the albumin gene family map to the chromosomal region 4q11–q22 and are tandemly linked in the sub-centromeric region 5′ALB–5′AFP–5′AFM–5′DBP3′-centromere, and hence are transcribed in the same, centromere-bound, direction [1,5]. The linear chromosomal arrangement of the four genes and the structural differences between them are congruent with the following evolutionary divergence of the gene family: starting with the first duplication of an ancestral progenitor gene, a single evolutionary line led to the contemporary DBP, leaving ALB/AFP/AFM on the other line of descent. The second duplication occurred in this ALB lineage, giving rise to ALB and the AFP/AFM progenitor, while the third one gave rise to the AFP–AFM pair. Recently, a new, fifth member of the albumin gene family was discovered, located adjacent to the 3′ end of the AFM gene but structurally related to AFP, which is why it was named α-fetoprotein-related gene (ARG) [6]. The ARG gene is expressed perinatally at very low levels in the liver of mice, rats, dogs and horses, but is considered an inactive pseudogene in humans and other primates.

The nucleotide sequence of the human afamin gene spanning 24.454 bp was first reported by Nishio et al. revealing a gene structure of 15 exons separated by 14 introns [7]. Based on structural similarity, α-albumin appears to be most closely related to α-fetoprotein. The complete structure of this family of four tandemly linked genes provides a well-characterized 200 kb locus in the 4q sub-centromeric region of the human genome.

Human afamin is a glycoprotein with an apparent molecular weight of 87 kDa and 55% amino acid sequence similarity (34% identity) to albumin [1] but, in contrast to albumin, is highly and in a complex manner glycosylated [3,8]. The afamin sequence completely lacks tryptophan [1], in line with its initial description of a tryptophan-poor glycoprotein [4]. The polypeptide chain is composed of a 21-amino acid leader peptide, followed by 578 amino acids of the mature protein. Afamin has five predicted N-glycosylation sites, and treatment with N-glycanase reduces the apparent molecular mass of afamin to 65 kDa [1,3]. Like other members of the albumin multigene family, afamin consists of three structural domains containing 17 Cys–Cys disulfide bridges [1].

At the same time as human afamin was discovered, the rat ortholog of afamin was described by Bélanger et al. [2]. In the rat, liver expression of afamin starts at birth and continues in adult animals, in contrast to α-fetoprotein which is heavily expressed in yolk sac, fetal and newborn liver, but not in the adult liver. Therefore, in rats afamin can be considered the adult form of α-fetoprotein. In extrahepatic rat tissues such as kidney and brain, afamin is not expressed. Rat afamin reveals six predicted N-glycosylation sites [2]. Allard et al. estimated the rat serum concentration of afamin at approximately 20 mg/L [9].

2.2. Gene regulation of afamin

The tight linkage between the members of the albumin gene family and their liver-specific expression has prompted the suggestion that these genes share common regulatory elements. The α-fetoprotein enhancer region that activates α-fetoprotein and albumin in fetal liver development does not, however, affect the expression of all gene family members, including afamin, later in hepatic development [10]. Two hepatocyte nuclear factor 1 (HNF1)-binding sites binding HNF1α and HNF1β have been identified in the afamin promoter. Expression studies in mice suggest that the different responsiveness of albumin gene family members is crucial for their liver expression [11]. More recently, a microarray study showed increased expression of transcription factor islet-1 levels (which is associated with increased β-cell function) to increase afamin expression 35-fold [12].

In summary, our understanding of afamin's gene regulation is very limited and needs to be further explored using suitable cell lines and/or animal models.

2.3. Biochemical and functional characterization

Human plasma afamin has been shown to be a specific binding protein for vitamin E [13]. Radio ligand-binding assay followed by Scatchard and Hill analyses demonstrated in vitro afamin's specific binding affinity for both α-tocopherol and γ-tocopherol, two of the most important forms of vitamin E. Maximum 18 binding sites for vitamin E per molecule afamin was estimated. The binding dissociation constant was determined to be 18.0 ± 7.1 μM, indicating that afamin might play a role as vitamin E carrier in plasma and other body fluids under physiological conditions. Furthermore, a Hill coefficient of 1.8 was obtained indicating a slight positive cooperativity that can best be explained by the fact that incoming, hydrophobic α-tocopherol molecules increase the hydrophobicity of the protein–ligand complex and thus make this complex more accessible to forthcoming ligands. Due to the large binding capacity of afamin for vitamin E, it might take over the role of vitamin E transport in body fluids under conditions in which the lipoprotein system is not sufficient for vitamin E transport. Specific binding of vitamin E was also confirmed by means of surface plasmon resonance technology with afamin immobilized on carboxymethyl dextran surface chips [13].

In view of the described experimental evidence, homology modeling and docking calculations were performed on the predicted tertiary structure and demonstrated coincidence between calculated and in vitro results (Fig. 1) [8,13].

Several studies reported quantitative and qualitative analyses of afamin's substantial glycan decorations. First reports date back to the initial discovery of afamin by Haupt and Heide in 1964, who reported a carbohydrate content of 13% [4]. This value is in remarkable agreement with more recent reports by Jerkovic et al., who found 15% carbohydrates by quantitative high performance liquid chromatography [8], but stands in contrast to data from Lichenstein et al., who estimated 24% carbohydrates based on less accurate comparisons of electrophoretic separation between fully glycosylated and enzymatically deglycosylated afamin [1].

Two reports on compositional glycan analysis of afamin demonstrated that >90% of glycans are sialylated biantennary complex structures bound to five N-glycosylation sites [3,8]. One of these amino acid residues, Asn362, possesses a rare consensus sequence for N-glycan of Asn-X-Cys. Afamin also contains five potential O-linked glycosylation sites; there is, however, no evidence of O-glycosylation [8]. Immunoblot analyses of normal human plasma using afamin-specific antibodies after 2-dimensional gel electrophoresis revealed substantial molecular heterogeneity of afamin believed to be due to different glycosylation