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PROTEIN FUNCTION AND POLYMORPHISM OF THE VISFATIN GENE IN FARM ANIMALS*

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Tkanka tłuszczowa jest nie tylko magazynem energetycznym organizmu zwierzęcego, ale także miejscem wydzielania endokrynnego oraz źródłem adipocytokin, białek o wielokierunkowym działaniu. Adipokiny działają pośrednio lub bezpośrednio, kontrolując m.in. homeostazę energetyczną, homatopoezę, angiogenezę, metabolizm lipidowy i węglowodanowy czy reprodukcję. Do białek tych należą m.in. adipsyna, leptyna, adiponektyna, rezystyna, apelina, chemeryna oraz wisfatyna zyskujące coraz bardziej na znaczeniu w leczeniu chorób cywilizacyjnych. Niniejsza praca przedstawia informacje na temat funkcji białka i zmienności genu stosunkowo niedawno poznanej wisfatyny, o której wiadomo, że jest czynnikiem stymulującym różnicowanie limfocytów pre-B (ang. pre-B-colony enhancing-factor 1, PBEF1), a także posiada aktywność fosforybozylotransferazy nikotynamidu (NAMPT). Wisfatyna katalizuje ważną reakcję szlaku syntezy NAD: kondensację nikotynamidu oraz 5-fosforybozylo-1-pirofosforanu (PRPP) do mononukleotydu nikotynamidowego. Jej nazwa jest związana z tkanką tłuszczową wisceralną (trzewną), gdzie jest wydzielana, ale obecnie wiadomo także, że występuje w szpiku kostnym, makrofagach, mięśniach szkieletowych oraz hepatocytach.

Słowa kluczowe: wisfatyna, funkcja, polimorfizm genetyczny

Visfatin is a factor stimulating the pre-B1 (PBEF1) lymphocyte colony and has the activity of nicotinamide phosphoribosyltransferase (NAMPT), therefore both names are used in the visfatin terminology. It is an enzyme described in the literature as cytokine and adipokine, occurring in two isoforms: iNampt (intracellular) and eNAMPT (extracellular). The extracellular form of peptide was originally described as cytokine (Samal et al., 1994). However, the structure

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of this protein proved similar to the enzyme of nicotinamide phosphoribosyltransferases (Nampt) that was discovered in the 1960s, which is involved in the conversion of nicotinamide into nicotinamide mononucleotide (NMN). It is a precursor of NAD+, a co-enzyme involved in many oxidoreduction reactions, signal paths, cAMP synthesis and other processes. Visfatin is also an insulinomimetic adipokine, secreted mainly from the visceral adipose tissue, but its presence in other tissues, such as bone marrow, liver, muscles, leukocytes and kidneys, has been proven as well (Kitani et al., 2003; Samal et al., 1994). Jasińska and Pietruczuk (2010) have analysed whether the PBEF1 binds with an insulin receptor or another membrane receptor and formulated a hypothesis that visfatin does not form a binding with it in the same active site as insulin. An experiment conducted on Nampt$^{-/-}$ transgenic mice has proven that Nampt affects the secretion of insulin in β cells of the pancreas, participating in NAD synthesis (Revollo et al., 2007). The insulin-mimetic function of visfatin has also been confirmed in studies on MC line mesangial cells. After stimulation with visfatin, these cells increased the binding with the cellular membrane of the GLUT-1 glucose transporter, whereas the application of Nampt inhibitor eliminated this effect. Blocking the expression of the visfatin receptor gene inhibited glucose uptake, which suggests the correctness of both visfatin activity mechanisms (Song et al., 2008).

The process of secretion of the intracellular PBEF1 form, which shows the activity of nicotinamide phosphoribosyltransferase, has been better described and studied in numerous studies. In humans, nicotinamide phosphoribosilotransferase stimulates maturation of vascular smooth muscles, regulates the expression of lipid metabolism genes (van der Veer et al., 2005). There is a positive correlation between body mass index and the percentage of fat in the human body and the concentration of this protein (Berendt et al. 2005). It may affect the body through the central nervous system, as the concentration in cerebrospinal fluid changes with the concentration of visfatin in plasma (Hallschmid et al., 2009).

The intracellular PBEF1 catalyses the reaction between nicotinamide and 5-phosphoribosyl-1-pyrophosphate (PRPP), causing nicotinamide mononucleotide (NMN) to appear in cytosol. NMN in the presence of the Nmnat enzyme is combined with ATP and the product of this reaction is NAD+. The NAD co-factor can be synthesized *de novo* from tryptophan or recovered from nicotinamide, an intracellular NAD degradation product (Luk et al., 2008; Revollo et al., 2007). Nampt is therefore important in the process of producing NAD, as it catalyses the reaction limiting the rate of its synthesis with nicotinamide. The increase in protein concentration increases NAD+ concentration. Nampt influences the expression of genes through its influence on the activity of Sir2 proteins – sirtuins forming the family of NAD+ deacetylases, which are evolutionarily dependent conservative enzymes, the occurrence of which was found in all studied eukaryotic organisms and many prokaryotic

organisms. Through the deacetylation of transcriptional factors, sirtuins affect metabolism, cell differentiation and stress response (Revollo et al., 2004).

Visfatin has also been described as cytokine secreted by activated myeloid lymphocytes stimulating the formation of pre-B cells, which act synergistically with interleukin-7 (IL-7) and stem cell growth (SCF) factor. The relationship between the intracellular location and the cell cycle stage suggests that it can affect cell cycle and cell differentiation. It is believed to be involved in many cancerous processes (Kitani et al., 2003). Visfatin in cells, e.g. neutrophils, has also been observed to inhibit apoptosis, which may be associated with the decreased activity of proteins in Caspases 3 and 8 (Jia et al., 2004).

Visfatin protein structure

Visfatin has two glycosylation sites: in the N-final and C-final segments of the polypeptide chain. Most of the secretable proteins have N-final signal sequences with hydrophobic properties, which has not observed in visfatin (Li et al., 2012). The structure of the PBEF1 protein is highly conservative; this has been demonstrated by analyzing its structure in various species belonging to higher organisms, as well as in bacteria (Li et al., 2012). The amino acid sequence of visfatin in hens shows 94.4% of homology with human visfatin (NP_005737.1), 94.3% with bovine visfatin (NP_001231070.1) and 88.5% with the visfatin identified in fish *Danio rerio* (XP_002661386.1) (www.ncbi.nlm.nih.gov/homologene/4201). Visfatin protein forms homodimers composed of two similar subunits. By comparing the active sites binding the substrate in the visfatin gene between different species ranging from hens to sponges, Kim et al. (2006) observed a high homology of the amino acid sequence in this area.

PBEF1 cellular location

The absence of the N-final signal sequence in the PBEF1 protein suggests that it is not secreted from the cell by classical secretion with the involvement of the endoplasmic reticulum and Golgi apparatus. The post-breeding medium of mouse 3T3-L1 adipocytes has been observed to contain visfatin proteins. In the 3T3-L1 cell line in the cell nuclei, membranes, vesicular structures and mitochondria, the presence of visfatin has not been demonstrated, thus excluding the mechanism of PBEF1 secretion with micro-vesicles. Tanaka et al. (2007) suggest that the transport of this protein is the responsibility of the mechanisms based on structural changes in cell membranes or the existence of unique transporters. Studies conducted on other mouse cell lines of PC-12 and Swiss 3T3 has shown the presence of PBEF1 in both cytoplasm and the cell nucleus. It has also been observed that the concentration in individual structures is dependent on the growth phase. In intensively proliferating cells, it is higher in the cell nucleus, whereas in the absence of stimulation with the nuclear growth factor, it reaches a higher concentration in the cytoplasm (Kitani et al., 2003).

***PBEF1* gene**

PBEF1 coding genes for different species have a similar structure. The degree of human gene homology (NC_006088.3) with cattle is 84.6% (AC_000161.1), with mice – 83% (NC_000078.6) and hens – 84.4% (NC_000007.13 (http://www.ncbi.nlm.nih.gov/). The visfatin gene in humans is located on the 7th chromosome and occupies 34.7 kbp. It consists of 11 exons and 10 introns. The first exon contains a 5'UTR segment and a classic signal sequence while the last – 3'UTR has a repeated TATT motif. The presence of two promoters has been proved and their analysis has shown that differentiated tissue and specific expression is possible. Both of them contain regulator elements depending on the chemical and hormonal factors, such as NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cell), GR (glucocorticoid receptor) and AP-1 (activator protein 1) (Ognjanovic et al., 2001). In hens, the visfatin gene is located on chromosome 1. It stretches across the space of approx. 29 kbp and consists of 11 exons, like in humans. It has been shown that cloning a 1,372-bp promoter segment and deletion of a 1,075-bp segment from the end of 5' or 297 bp from the end of 3' only partially reduced the activity of the protein, which testifies to the presence of numerous promoters in the immediate vicinity of the transcription start site (Li et al., 2012).

In non-translatable 3"UTR region, there is a repeated TATT motif characteristic of cytokines and oncogenes (Samal et al., 1994). However, the mRNA structure does not include signal sequences responsible for the secretion of protein: a leader sequence and a caspase 1 cleavage site (Luk et al., 2008). The *PBEF1* encoding gene also lacks the characteristic GPuGPuTTPyCAPy motif, which occurs in the hematopoietic cytokines at the end of 5' (Ognjanovic et al., 2001).

Gene polymorphism

In the available literature, there are only a few studies on polymorphism in the *locus* of the visfatin gene in farm animals. Tests carried out on pigs on polymorphism of g. 669 T>C in intron 9 have shown that it affects the content of lean meat and fat content of the carcass. A correlation between the gene variant and daily body weight gains has also been observed in the large white and Landrace pigs (Zrůstová et al., 2009). In boar x Meishan cross-breeds, two non-conjugated polymorphic lesions AM999341: g.669T>C in the 9th intron and FN392209: g.358A>G) in promoter sequence have been identified. Both polymorphisms showed a relationship between e.g. muscle, fat deposition, growth and fat cover and quality of pig meat. (Cepica et al., 2010). It has been observed in local Chinese pig breeds that variability in the visfatin gene, i.e. a 35-bp insertion described as g. 381–415 in exon 4 affected the fattening characteristics of animals (body weight, daily weight increases). The individuals burdened with the insertion in both alleles were characterized by higher weight and increases in the first 6 months of life. After six months, an opposite trend has been observed (Wang et al., 2009). On the other hand, 6-nucleotide deletion

of g. 19767-19774 (TAAAAA) in intron 5 had a significant effect on lowering the value of increases and lower body weight. No homozygous animals have been observed for this polymorphic lesion, which may indicate the natural elimination of homozygotes having both mutated alleles, which would possibly have underdevelopment and growth problems (Wang et al., 2012).

The current state of knowledge about the effect of visfatin on the body of hens is poor. It is only known that it is expressed in muscle tissue, pituitary gland, kidneys, spleen, interbrain, liver and adipose tissue. Particularly high levels of this protein have been observed in the liver and skeletal muscles. Moreover, its intensified over-expression was observed in the tissues of older animals. In addition, it was suggested that PBEF1 functions as a myokine, which affects muscle tissue growth in chickens. It may also play a key role in maintaining energy homeostasis through its participation in lipid metabolism (Krzysik-Walker et al., 2008). The hypothesis has been confirmed in a few studies. In the hens of the gushi and anka cross-breeds, the presence of polymorphism of c. 1433G>A (missense mutation) in exon 6 and its relation to body weight have been found, with the G allele having a positive effect on the chicken growth and unfavourable effect the fat deposition (Han et al., 2012). In the same cross-breeds, TT homozygotes, identified by the restrictive enzyme *Xba*I (silent mutation g.17873) in exon 7, have shown better meat utility value parameters at early stages of development (Han et al., 2010). On the other hand, authors' own research (data not published) conducted on Ross 308 line broilers, aimed at determining the effect of g.17873C>T polymorphism on birds' meat utility value have shown a negative influence of the T allele on body weight, birds' leg weight and weight increases, as well as some features of meat quality and texture. The TT genotype chickens showed a significantly lower utility value compared to the C allele carriers. Their meat had a more cohesive texture and was less elastic.

Visfatin/PBEF/Nampt has the properties of cytokine, growth factor and enzyme, but depending on the origin, concentration and experimental model, it exhibits different biological functions with a different mechanism of activity. Despite the increasing number of published experimental results, there are still a number of doubts about its role and it remains a controversial protein in the development of obesity-related diseases.

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Protein function and polymorphism of the visfatin gene in farm animals

SUMMARY

Adipose tissue is not only an energy store for the animal organism but also a place of endocrine secretion and is a source of adipocytokines, proteins with multi-directional effects. Adipokines act directly or indirectly and control, among others, energy homeostasis, homatopoiesis, angiogenesis, lipid and carbohydrate metabolism and reproduction. These proteins include, among others, adipsin, leptin, adiponectin, resistin, apelin, chemerin and visfatin, which are becoming increasingly important in the treatment of civilization diseases. This paper presents information on the function of protein and gene variability of the recently discovered visfatin, which is known to be a factor stimulating the differentiation of pre-B-colony enhancing-factor 1 (PBEF1) lymphocytes and also has the activity of nicotinamide phosphori-bosyltransferase (NAMPT). Visfatin catalyses an important reaction of the NAD synthesis pathway: the condensation of nicotinamide and 5-phosphoribosyl-1-pyrophosphate (PRPP) to nicotinamide mononucleotide. Its name is associated with visceral adipose tissue where it is secreted but it is now also known to occur in bone marrow, macrophages, skeletal muscles and hepatocytes.

Key words: visfatin, function, genetic polymorphism