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Peptide KE in Human Proteome

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Peptide KE exhibits immunoprotective, geroprotective, and oncostatic activities and stimulates functional activity of fibroblasts. The KE motif is present in amino acid sequences of some cytokines and peptide hormones functionally similar to KE peptide. However, the relationship between the presence of KE motif and protein functions on the scale of known human proteome has not yet received sufficient attention. The incidence of bioregulatory peptide KE in proteins of various functional groups constituting human proteome is studied. The study is carried out with the use of the available data on the human proteome (UniProt portal) comprising 20,417 proteins. The levels of KE motifs were maximum in cytoplasmic and nuclear proteins, while the presence of KE in the membrane and all other proteins was the minimum. KE peptide molecules released from nuclear proteins during limited proteolysis can bind to DNA and regulate gene expression.

Key Words: *peptide KE; human proteome; nuclear proteins; gene expression*

Peptide KE (Lys-Glu) is characterized by immunomodulating, geroprotective, and oncostatic activities and stimulates functional activity of fibroblasts. Peptide KE is a peptide thymomimetic [1-3,5] that is present in the structure of Thymalin drug. At the molecular and cellular level, the main immunomodulating effects of KE are stimulation of thymocyte differentiation into T lymphocytes and of stem cells into T and B lymphocyte precursors, activation of immune response of T lymphocytes and macrophages, and reduction of the level of their apoptosis under conditions of natural and accelerated aging of the immune system [4,10,12,14]. The geroprotective effect of KE consists in the increase in the relative content of euchromatin and telomere length in lymphocytes of individuals of various ages [9] and prolongation of the life span in animals [8]. The oncostatic effects of KE include a decrease in the counts of cultured lymphoma and hepatoma cells, suppression of HER2/neu gene expression and decrease in tumor diameters in transgenic mice with mammary adenocarcinomas [13]. Peptide KE crosses the cell membrane, enters the nucleus, and regulates gene expression [6,7]; KE regulates the synthesis of some proteins. A model of KE dipeptide interactions with the gene promoter zones involving hydrogen bonds has been created by physicochemical methods with the use of bioinformatics [11].

Experiments on plants have demonstrated that KE peptide regulates the expression of growth, development, and differentiation genes in gene families *CLE* (*CLE1-8*), *KNOX* (*KNAT1*, *KNAT2*, *KNAT3*, and *KNAT6*), *LET* (*LET6*, *LET12*), and *GRF* (*GRF1-4*) of *Nicotiana tabacum* callus culture. *CLE* (CLAVATA3/Endosperm surrounding region-related) family genes are involved in the development of seeds, maintenance of stem cell pool in plantules. *KNOX* (KNOTTED-like homeodomain) and *LET* genes encode transcription factors involved in differentiation of plant stem cells [6].

Amino acid sequence of KE is found in many cytokines and peptide hormones, such as IL-1p, IL-2, IL-3, IL-4, IL-5, IL-6, IFNα, splenin, splenopentin,

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thymosin, thymopoietin, motilin, parathyrin, and somatotropin-releasing hormone. This fact gives us grounds to suggest that KE molecule released during limited proteolysis of proteins becomes a regulator of the functional activity of cells. In addition, KE peptide stimulates the expression of *IL2* gene mRNA in mouse spleen lymphocytes [10]. The distribution of KE motif in proteins, including human proteins, has not been studied up to date.

We analyzed the incidence of KE peptide in proteins of various functional groups constituting human proteome.

MATERIALS AND METHODS

The data on the complete human proteome from UnitProt open portal was used for the analysis of peptide KE incidence in human proteins. By the moment of our study, it contained 20,417 proteins and 16,626 of these had status "reviewed" that means their known location and/or function. Using Python programming language with a sorter algorithm we had created, we distributed all these proteins into 4 groups by their predominant (reflected in the proteome legend) location in the cell: nuclear (N), membrane (M), cytoplasmic (C), and other (O), including proteins with other location, namely: mitochondrial, excretory, endoplasmic reticulum, Golgi complex, and lysosomal.

The number of entries of KE motif in amino acid sequences of proteins in each of the above groups was evaluated. The study was carried out using standard means of Python programming language (Count method, Collections modulus). The proteome proteins were ranked by the counts of KE entries. Two polar samples, 1000 proteins each, were formed for detailed analysis: proteins with the greatest numbers of KE entries and random selected proteins containing no KE motif in their structure.

The samples were compared using Student's *t* test from the standard means of R programming language (https://r-project.org/). The differences between the groups were assumed to be significant at $p<0.001$.

RESULTS

The reviewed proteins of human proteome are distributed by four locations into approximately equal groups (Fig. 1). The data on the quantity of KE motifs in the structures of the known human proteins are presented in Table 1.

The incidence of lysine (K) and glutamine (E) in the studied pool of 16,626 reviewed proteins containing at least one KE motif in their amino acid sequences was 0.0567 and 0.0700, respectively. In case of random combination of amino acid residues, the incidence of KE motif in the protein pool was expected to be equal to the product of these two values, that is, 0.00397 (397×10^{-5}). The actual incidence of KE for all these proteins was 0.0049 (490×10^{-5}), that is by 23.4% higher ($p<0.001$) than the random variable (Table 1). This difference was particularly high for cytoplasmic (53.9%; $p<0.001$) and nuclear proteins (45.8%; $p<0.001$).

The proteins with KE motif in their structure are "attracted" to the nucleus and cytoplasm and are rather scanty in the cell membrane (Fig. 2). Cytoplasmic and nuclear proteins predominate among proteins containing numerous repeats of KE motif, constituting almost $^3/_4$ of these proteins. The proteins containing no KE motifs were located mainly in cell membranes or other

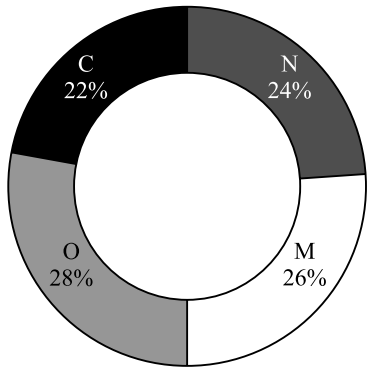

Fig. 1. Distribution of 16,626 human proteins by their location in the cell. Here and in Fig. 2: N (nuclear), M (membrane), C (cytoplasmic), O (other) proteins.

TABLE 1. Entries of KE Motif in the Reviewed Human Proteins

Protein group	Number of proteins containing KE motifs	Sum of amino acid residues in all proteins of the group	Sum of KE motifs in proteins of the group	Incidence of KE motifs in pooled proteins, $\times10^{-5}$	Difference *vs.* random distribution, $\times10^{-5}$
Cytoplasmic (C)	3662	2,504,433	15,319	611	+214
Nuclear (N)	3951	2,411,090	13,972	579	+182
Others(O)	4635	2,310,456	8543	415	+18
Membrane (M)	4378	2,460,642	9600	347	-50
All proteins	16,626	9,686,621	47,434	490	+93

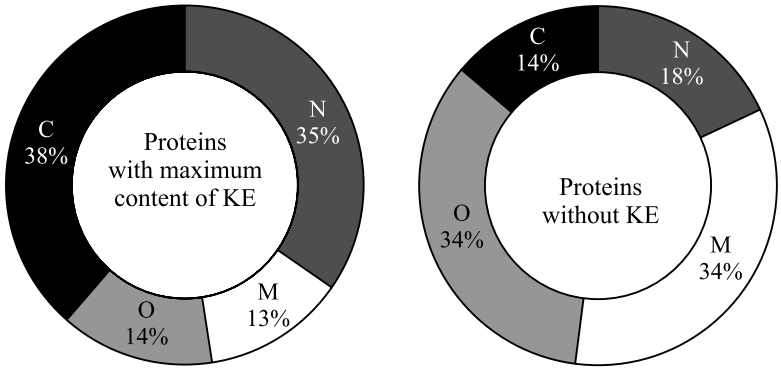

Fig. 2. Predominant location of human proteins in cell depending on the content (absence) of KE motif in their structure.

cell structures (more than $^{2}/_{3}$ of the proteins reviewed) (Fig. 2).

Our results indicate a high probability of KE peptide involvement in transcription and translation processes in the cell nucleus and cytoplasm. Cytoplasmic and nuclear proteins with high content of KE motifs after partial proteolysis become sources of KE molecules — gene expression regulators [15]; products of these genes determine the rate of proliferation and differentiation and maintain functional activities of immune cells and fibroblasts.

On the whole, these data confirm the role of short peptides in transcription control. The higher the content of KE motif in the protein structure, the higher is the probability of formation of the respective peptide in partial proteolysis of the protein molecule and the probability of its involvement in transcription processes. Numerous repeats of KE motifs in the protein composition are a rich source of KE peptide molecules, capable of binding DNA and regulating the expression of cell differentiation and functional activity genes.

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