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THE ROLE OF COLLAGEN PROTEINS IN THE FORMATION AND RESORPTION OF BONE TISSUE


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КОЛЛАГЕН ОҚСИЛЛАРИНИНГ СУЯК ТЎҚИМАЛАРИНИНГ ШАКЛЛАНИШИ ВА РЕЗОРБЦИЯСИДАГИ РОЛИ

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РОЛЬ КОЛЛАГЕНОВЫХ БЕЛКОВ В ОБРАЗОВАНИИ И РЕЗОРБЦИИ КОСТНОЙ ТКАНИ

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Резюме. *Ушбу мақолада коллаген оқсилларини таркибий ташкил этиш, коллагеннинг ҳар хил турлари ва уларнинг роли, коллаген оқсилларини синтез қилишнинг кўп босқичли жараёни, шунингдек суяк метаболизмининг ўзига хос белгилари, суяк тўқимасини моделлаштириш ва қайта қуриш жараёнларини акс эттириш ва улардан фойдаланиш мақсадга мувофиқлиги масалалари ҳамда суяк тўқималарининг шаклланиши ва резорбцияси интенсивлигини баҳолаш кўриб чиқилган.*

Калит сўзлар: *коллаген, фибриллар оқсиллар, коллаген синтези, суяк маркерлари, моделлаштириш, қайта қуриш.*

Abstract. *This article presents the issues of the structural organization of collagen proteins, various types of collagen and their role, the multi-stage process of synthesis of collagen proteins, as well as specific markers of bone metabolism reflecting the processes of modeling and remodeling of bone tissue, and the possibility of using them to assess the intensity of formation and resorption of bone tissue.*

Key words: *Collagen, fibrillar proteins, collagen synthesis, bone markers, modeling, remodeling.*

The basis of connective tissue is the intercellular substance. It provides transport of various nutrients, as well as mechanical support to cells. The intercellular substance consists of glycoproteins, proteoglycans and hyaluronic acid. Collagen content prevails among glycoproteins. Collagen is a fibrillar structural protein of the extracellular substance of connective tissue, containing several domains and consisting of 3 polypeptide chains arranged in a triple helix. The primary structure of the polypeptide chains of collagen can be represented in the form of a formula (Gli-X-Y), where X and Y can be any amino acids, but most often the amino acid proline is in place of X, and hydroxyproline or hydroxylysine is in place of Y. In the formation of a specific spatial configuration of the collagen molecule, the presence of these amino acids plays a key role. Collagen is a polymorphic protein. There are more than 28 types of collagens and more than 40 genes encoding collagen [27]. They differ from each other in the primary structure of the polypeptide chain, in function and in location. A special formula is used to designate each type of collagen, which uses Roman numerals indicating the type of collagen and Arabic numerals indicating the collagen chain. For example, type 1 collagen is written with the formula [α1(I)2 α2(I)]. The index in parentheses indicates the number of identical chains in the collagen molecule. Due to the polymorphism of the collagen molecule, there are different classifications of collagen proteins. According to the most common classification, there are 5 groups of collagen proteins:1. Fibrillating collagens. This includes collagen types I II III V XI XXIV XXVII; 2. Collagens associated with fibrils: IX XII XIV XVI XIX XXI XXII;3. Collagens forming reticular structures: IV VI VIII X XXVIII; 4. Transmembrane collagens: XIII XVII XXIII XXV; 5. Collagens with multiple interrupting domains of the triple helix: XV XVIII.

90-95% of the organic matrix of bone tissue is type I collagen, which provides bone strength. Each type I collagen molecule consists of 2 α1 chains and 1 α2 chains. Each of these chains consists of a helical domain, with C- (carboxytherminal) and N- (aminotherminal) terminal propeptides at the end necessary for the formation of a triple helix of a tropocollagen molecule. In the process of collagen biosynthesis, during the formation of the tropocollagen molecule, the N-terminal (amino terminal) and C-terminal (carboxytherminal) propeptide are cleaved off with the help of specific proteases. These propeptides are necessary for the formation of the triple helix of the collagen molecule and for the further formation of collagen

fibrils. When the terminal propeptides are cleaved from the procollagen molecule, they are released into the blood. The detection of these terminal propeptides (C- and N-terminal propeptides) are of important clinical and diagnostic importance, since they reflect the exchange of collagen molecules, i.e. functional activity of osteoblasts. PINP (aminotherminal propeptide of procollagen type I) and PICP (carboxytherminal propeptide of procollagen type I) serve as biochemical markers of bone formation.

These markers have proven themselves well in monitoring the effectiveness of anti-osteoporotic treatment. In addition, they are of great value in the diagnosis of diseases such as osteoporosis, Paget's disease, renal osteodystrophy, as well as in some oncological and rheumatic diseases.

Type II collagen is an important component for the normal development of bones and teeth. It is most present in cartilage, intervertebral discs, as well as in the vitreous body. Its molecule consists of three identical α chains, each of which consists of 1060 amino acid residues with an extended continuous domain and short non-spiral fragments.Collagen fibrils are relatively thinner than type I collagen fibrils.

Type III collagen is a homotrimer (α1(III). Each of the α chains contains up to 1029 amino acid residues. It is the predominant protein in the skin and interstitial blood vessels. It is present in bone tissue only in trace amounts.

Collagen IV is a key structural component of the basement membranes. Its molecules form hexameric structures formed by the "butt-to-butt" connection of the N-ends of collagen trimers. These hexamers are additionally stabilized by transverse covalent crosslinking between lysine and meteonin residues. The spirals of type IV collagen, connecting with each other, form a "web" characteristic of this type of collagen, which plays an important role in regulating the permeability of the basement membranes.

Type V collagen is present in the skin, in fetal bone tissue, in the mature cornea and interstitial kidneys. It is considered as a factor in the initiation of the assembly of type I collagen molecules.

The main component of descemet membranes of the corneal endothelium is type VIII collagen. It is also present in large quantities in the subendothelial layer of blood vessels. Type VIII collagen chains (α1 and α2) are structurally similar to type X collagen chains.

Type X collagen is secreted by hypertrophied chondrocytes during endochondrial ossification. It is represented by a homotrimer, in which each of the α chains contains short spiralized sections of 154 amino acid residues, limited by non-collagen domains from the N- and C-terminus, with a length of 37 and 161 amino acid residues, respectively.Unlike fibrillar collagens, terminal fragments are not cleaved off during posttranslational modification. And the presence of non-collagen domains provides a characteristic structure for collagens of types IV, VIII and X.

There are transmembrane collagens, which belong to the class of transmembrane proteins. These collagens have a short cytoplasmic terminal fragment (N-terminus) and, connected to a hydrophobic membrane site, an extracellular long interrupted spiralized domain. The presence of extracellular domains in the molecules of transmembrane collagens ensures their cellular adhesion.

Type VIII collagen is found mainly in focal contacts, type XVII collagen in hemidesmosomes, type XXV collagen in neurons, type XXIII collagen is expressed by prostate carcinoma cells.

Collagens XV and XVIII are classified as chondroitin sulfate and heparan sulfate proteinoglycans, respectively. They are mainly localized in the area of the basement membranes. Type XV collagen is most common in skeletal muscles, the heart and the placenta. They are expressed by the adrenal glands, kidneys and pancreas.

Type XVIII collagen pre-mRNA in humans undergoes 2 types of splicing. During translation, 2 isoforms of this protein are formed: short and long. The short chain is expressed in various organs and tissues, but the synthesis of the long isoform is characteristic only of the liver. The biological role of these types of collagen has not been fully studied. But it is assumed that they are involved in the regulation of the functions of specialized basement membranes.

Collagen synthesis is a complex multi-stage process that includes both intracellular and extracellular stages. Polypeptide chains of the molecule of the heterotrimer [a1(I)]2a2(I) collagen predominant in bone tissue are encoded by the COL1A1 and COL1A2 genes [15] and synthesized on polyribosomes of the granular endoplasmic reticulum (EPR) in the form of precursors of prepro-α chains. Prepro-α-chains contain a "signaling" peptide at the N-terminus, which is necessary for the orientation of chain synthesis into the cavity of the endoplasmic reticulum, and cleaves off after performing its function. These pro-α chains then undergo hydroxylation and glycosylation processes, which plays a key role in the spatial stabilization of the triple helix of the collagen macromolecule [25]. This process is adjacent to the course of translation and continues until the separation of the polypeptide chain from the ribosomes [3]. Hydroxylation of proline is necessary for the formation of its stable three-spiral structure at subsequent stages of collagen biosynthesis. Hydroxylated lysine residues (along with non-hydroxylated ones) are necessary for the formation of covalent bonds between collagen molecules during the assembly of collagen fibrils. In Scurvy disease, a pathology caused by a lack of vitamin C, collagen synthesis is disrupted at the stage of hydroxylation of proline and lysine residues. As a result due to impaired hydroxylation of peptide chains, less stable and durable collagen fibers are formed. This is associated with the fragility of blood vessels in scurvy and the occurrence of multiple spot hemorrhages. This process is adjacent to the course of translation and continues until the separation of the polypeptide chain from the ribosomes. About 100 amino acid residues of proline at the Y position, a small number of proline residues at the X position and 10 lysine residues at the Y position in repeats of [Gli-X-Y]n are hydroxylated by the corresponding enzymes: prolyl-4-hydroxylase, prolyl-3-hydroxylase and lysylhydroxylase [3].

After hydroxylation, glycosylation occurs, as a result of which galactose or 2O-3-glucopyranosyl-O-3-β-galactopyranose is attached to the oxygroups by an O-glycoside bond. The reaction is catalyzed by the enzymes UDP-galactosacollagengalactosyltransferase and UDP-glucose-collagenglucosyltransferase, respectively. Both hydroxylation and glucosylation occur in the structures of the agranular endoplasmic reticulum, and their product is the procollagen molecule. The synthesized procollagen molecule contains additional sites – N- and C-terminal

propeptides containing 100 and 250 amino acid residues, respectively, representing globular non-spiralized domains. The composition of C-terminal propeptides contains 7-8 cysteine residues. The inter-chain disulfide bonds formed between cysteine residues are important in the formation of the three-stranded helix of the procollagen molecule [3]. The stabilization of the procollagen triple helix is also carried out by interacting with a specific protein called chaperone located in the endoplasmic reticulum. Next, the procollagen molecule passes into the Golgi complex and is secreted into the extracellular space. Thus, this is the end of the intracellular stages of collagen biosynthesis. This is followed by the intracellular stage of collagen formation and maturation. It begins with the enzymatic cleavage of N- and C-terminal propeptides [3,5]. Cleavage of the N-terminal propeptide is carried out by a group of enzymes: disintegrin-like matrix metalloproteinases (ADAMTS – a disintegrin and metalloproteinase with thrombospodin motifs) encoded by ADAMTS genes-2, -3, -14 [1,15]. The C-terminal propeptide is cleaved off using bone morphogenetic protein-1 (BMP-1, bone morphogenetic protein-1, KMB-1), which has proteinase activity. The above enzymes have a Zn2+-binding catalytic domain in their composition. At the ends of the molecule, non-spiralized areas with a short length (9-35 amino acid residues in fibrillar collagens), the so-called telopeptides, are preserved.

These propeptides, PINK (aminotherminal propeptide of procollagen type I) and P1CP (carboxytherminal propeptide of procollagen type I), serve as biochemical markers of bone tissue formation [7,16]. Thus, it follows from this that the quantitative determination of the level of free propeptides in biological fluids is of great clinical importance, as it helps to determine the functional activity of osteoblasts.

The loss of terminal globular protein domains by the procollagen molecule is a key factor leading to the spontaneous ability of tropocollagen to fibrillogenesis. Tropocollagen molecules self-assemble into microfibrils and fibrils. These formations are not mature and do not have sufficient strength. As fibrils form, they are strengthened by additional intra- and inter-chain covalent cross-linking. This is done using the extracellular copper-containing enzyme lysyl oxidase. To date, 5 varieties of this enzyme are known, the function of which is the oxidative deamination of the ε-amino groups of lysine in the region of N- and C-terminal telopeptides [11,13] with the formation of allysine and hydroxyallysine, which are reactive aldehydes by chemical nature [3,5]. These reactive aldehydes take part in the construction of covalent bonds between themselves and with other residues of allysine (aldimine condensation) or hydroxyallysine (ketoimine condensation) of neighboring tropocollagen molecules located at an accessible distance, forming so-called "cross-links" (cross-links). The presence of these covalent "cross-links" provides the collagen fibrillation with significant mechanical strength and spatial stability.

In the process of bone tissue remodeling, in order to maintain its integrity and strength, a balance between the processes of collagen synthesis and breakdown is very important. The nature and intensity of the collagen degradation process is influenced by many factors: the activity of matrix metalloproteinases (MMPs), the activity of growth factors, the presence of proteins binding growth factors and modulating their action, the number and functional state of cellular receptors mediating the action of growth factors, the presence of adhesive molecules responsible for intercellular interactions. Currently, there are 4 known pathways of collagen catabolism in mammals: 1) phagocytosis with the participation of integrin α2β1, in which insoluble collagen is transported to lysosomes, where it is degraded by cathepsins; 2) collagenolysis with the participation of cathepsin K during osteoclastic bone resorption; 3) extracellular hydrolysis of fibrils, denaturation of the triple helix and proteolysis of α-chains using collagenases and gelatinases; 4) extracellular degradation of collagen fibrils using collagenase, endocytosis, and further hydrolysis by lysosomal cathepsins. The destruction of collagen fibers can also occur when exposed to reactive oxygen species. MMP collagen remodeling is crucial for homeostasis and tissue regeneration. Matrix metalloproteinases belong to the family of Zn2+- and Ca2+-dependent endopeptidases involved in the remodeling of connective tissue through the destruction of its organic components at physiological pH values. MMPs got their name for their ability to specifically hydrolyze the main proteins of the intercellular matrix. The structure of all MMP is represented by a signaling peptide necessary for successful secretion from the cell; a propeptide site, upon cleavage of which MMP is activated; a catalytic domain having coordination bonds with the zinc cation of the catalytic center, and a hinge region. The catalytic domain includes two Zn2+ ions and three Ca2+ ions. All enzymes, except MMP-7, have a terminal hemopexin-like domain that serves as a substrate binding center.

As a result of the action of catabolic factors, mainly osteoclastic resorption, amino- and carboxytherminal fragments called N- (NTX-I) and C-terminal telopeptides (CTX-I), connected by transverse "crosslinking", respectively, are cleaved from the type 1 collagen molecule during its decomposition. The cysteine protease cathepsin K, indicated in in vitro experiments, plays a crucial role in this. CTX-I is represented by two forms: α-CTX and β-CTX and is a peptide fragment of the α1 chain of type 1 collagen, consisting of 87 amino acid residues, containing a specific amino acid sequence Glu-Ala-Gis-Asp-Gli-Gli-Arg. β-CTX in the amino acid sequence presented above contains β-isomerized aspartic acid (syn.: β-CrossLaps –β-isomerized carboxy-terminal cross-linking region of collagen type I: β-isomerized carboxytherminal section of type I collagen with cross-links) and is a specific marker of bone resorption [2,13,14], reflecting the intensity of the breakdown of type I collagen in relatively old bone tissue. The determination of the level of β-CTX in various biological fluids can be performed by solid–phase enzyme immunoassay (ELISA, ELISA - enzyme-linked immunosorbent assay) using specific monoclonal antibodies to epitopes of two α1 chains of Glutylase-Ala-Gis-βAsp-Gli-Gli-Arg bound by covalent bonds between lysine residues. The α-CTX level allows us to estimate the rate of degradation of newly formed bone.

Thus, it follows from this that the quantitative determination of the level of free propeptides in biological fluids formed as a result of collagen degradation is of important clinical importance, since it helps to determine the functional activity of osteoblasts, and can be used in patients with osteoporosis to assess the intensity of bone formation and resorption.

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РОЛЬ КОЛЛАГЕНОВЫХ БЕЛКОВ В ОБРАЗОВАНИИ И РЕЗОРБЦИИ КОСТНОЙ ТКАНИ

Ким О.В., Юсупов Ш.А.

Резюме. *В данной статье представлены вопросы структурной организации коллагеновых белков, разнообразные типы коллагена и их роль, многоступенчатый процесс синтеза коллагеновых белков, а также специфические маркеры костного метаболизма, отражающие процессы моделирования и ремоделирования костной ткани, и возможности применения их для оценки интенсивности образования и резорбции костной ткани.*

Ключевые слова: *Коллаген, фибриллярные белки, синтез коллагена, маркеры костной ткани, моделирование, ремоделирование.*