Osteogenesis imperfecta – molekularna osnova i lijekovi budućnosti

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Molecular basis of osteogenesis imperfecta and future medical treatment

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Osteogenesis imperfecta (OI) or brittle bone disease is a metabolic bone disease characterized by bone fragility, low bone mass, and increased rate of bone fractures and deformities. Clinical presentation in OI patients shows wide variability ranging from mild to severe and lethal OI types. Advances in molecular biology and studies on animal OI models found at least 16 new genes involved in OI pathogenesis. The majority of mutations are autosomal dominant affecting COL1A1 and COL1A2 genes responsible for collagen synthesis. The remaining 10%-15% of mutations in OI are autosomal recessive and affect genes involved in various metabolic bone processes. Progress in understanding bone metabolism and genetic engineering offers new potential therapeutic opportunities that are under different stages of investigation.

Key words: osteogenesis imperfecta, type I collagen, molecular genetics, gene therapy, stem cell

INTRODUCTION

Bone tissue is a type of dense connective tissue consisting of living cells embedded in a mineralized organic matrix (1). It has mechanisms to grow and change its shape and size to suit varying stressors including the ability to resist mechanical forces. Bone cells include osteoblasts, osteocytes and osteoclasts. Osteoblasts synthesize bone matrix and participate in its mineralization (2). Osteocytes are thought to be sensory cells that are involved in signaling processes inside the bone. They are connected to other osteocytes through their projections, which extend through the canaliculi. Osteoclasts are large multinucleated cells responsible for bone resorption. The mineralized matrix of bone tissue has an organic and an inorganic component. Organic component of bone includes collagen, proteoglycans, matrix proteins, cytokine and growth factors (2). The vast majority of the organic matrix is type I collagen that provides tensile strength (3). Inorganic (mineral) composition of bone is primarily formed from salts of calcium and phosphate. The majority of salts are hydroxyapatite and osteocalcium phosphate, which provide compressive strength. Thus, the collagen and mineral together are a composite material with an excellent tensile and compressive strength, which can bend under strain and recover its shape without damage (4).

Bone metabolism (bone turnover) relies on complex signaling pathways and control mechanisms to achieve proper rates of growth and differentiation. These controls include the action of several hormones, including parathyroid hormone (PTH), vitamin D, growth hormone, steroids, and calcitonin, as well as several bone marrow-derived membrane and soluble cytokines and growth factors (4).

A number of bone turnover markers (BTMs) can now be determined using commercial tests. Bone turnover markers

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are generally subdivided into two categories: biomarkers of bone formation, and biomarkers of bone resorption and osteoclastogenesis. Bone formation markers derive from the osteoblastic activity and include bone alkaline phosphatase (BSAP), osteocalcin (OC), N-terminal propeptide (PINP), and C terminal propeptide of type I procollagen (PICP) (5). The markers of bone resorption and osteoclastogenesis result from degradation of type I collagen such as intermolecular crosslinks pyridinoline (PYD) and deoxypyridinoline (DPD), C-terminal telopeptide (CTX), N-terminal telopeptide (NTX) and matrix-metalloproteinase (MMP)-generated (CTXMMP or ICTP) type I collagen fragments, the enzymes secreted by osteoclasts, i.e. tartrate-resistant acid phosphatase 5b isoform (TRAP-5b), and receptor activator of nuclear factor NF-κB ligand (RANKL). Several prospective cohort and case-control studies suggest that increased BTM levels predict fractures independently of age, BMD, and prior fracture.

Collagens are ubiquitous molecules in all multicellular animals (4). As an important ingredient of the extracellular matrix, bones, cartilages and other structures in the body, many cells have an ability to produce collagen molecules. Collagens are divided into several types: fibrillar collagens (types 1, 2, 3 and 5), fibril-associated collagens (types 6 and 9), sheet forming and anchoring collagens (types 4, 7 and 15), transmembrane collagens (types 13 and 17), and host defense collagens (collectins, class A scavenger receptors on macrophages). Fibrillar collagens are most abundant in the extracellular matrix of connective tissues such as tendons and cartilage, making the majority of its volume (4).

Two genes are involved in the synthesis of type I collagen molecule: *COL1A1* located on chromosome 17 and *COL1A2* on the long arm of chromosome 7. Two pro-alpha 1 chains are transcribed from *COL1A1*, and one pro-alpha 2 chain from *COL1A2*, which in the end form a triple helix when the synthesis and post translation modifications are done (6).

The gene transcript initiates the formation of collagen on the ribosomes of the rough endoplasmic reticulum. After translation, three individual pre-pro-collagen chains are formed: two alpha 1 chains and one alpha 2 chain, which are transported into the reticulum where additional processing is done and the collagen fibrils are fold into a triple helix (4).

Within the endoplasmic reticulum, lysine and proline residues of the propetide are hydroxylated by enzymes to promote the stability and glycosylation of the triple helix. Additionally, three alpha chains are packed together in such a way to make repeating glycine-x-y patterns, which give significant stability to the triple helix. Glycine is the smallest amino acid that can fit in the tightly packed axes without causing chain distortion.

After the collagen molecule leaves the endoplasmic reticulum, it is then called procollagen and is transported to the Golgi apparatus, where it is further modified and prepared for exocytosis. During transport out of the cell, certain procollagen processing and fibril assembly has already started in compartments between the Golgi apparatus and the cell membrane. Recent studies suggest that the procollagen molecule is transported in two ways, slow and fast. In the reticulum, procollagen is recognized by a receptor gatekeeper and goes through a pore that transverses both the endoplasmic reticulum and plasma membranes. This allows fast exit from the cell that requires little energy and is driven by diffusion gradient. The slow transport goes through the Golgi apparatus *via* vesicle (6).

Once in the extracellular matrix, additional propeptides are cleaved on both ends of the molecule, and such process results in the formation of type I collagen that crosses links with the other type I collagen molecules to form fibrils and finally collagen fibers.

Collagen is one of the main protein components of the extracellular matrix, along with proteoglycans, hyaluronic acid, laminins and fibronectin. Together they form a highly organized support medium that is important in regulating cell and tissue development and function. Different combinations of the main proteins allow the extracellular matrix to act with specificity in different tissues of the body. Helix form of the collagen molecule has excellent force dispersal properties (6).

Type I collagen molecules have an excellent resistance to tension and make a primary substance in the bone. Fibers of type I collagen are also the most common protein in the extracellular matrix, tendons and ligaments, as well as capsules of many organs and dentine cementum.

Mutations of the collagen genes cause several other diseases; Caffey disease, Ehler-Danlos syndrome, osteogenesis imperfecta and dermatofibrosarcoma protuberans are caused by mutations in the *COL1A1* gene, whereas *COL1A2* gene is linked to the Ehler-Danlos syndrome and osteogenesis imperfecta (7).

ETIOLOGY OF CHANGES IN OSTEOGENESIS IMPERFECTA

Osteogenesis imperfecta (OI) is an inherited connective tissue disease characterized by variability of clinical presentation (7, 8). Presumably, large variability in clinical presentation is a consequence of the high number of genes involved in the disease pathogenesis and diversity of genetic mutations. Most frequently, mutations occur in the genes responsible for encoding alpha-1 and alpha-2 chains of type I col-

lagen, or in the genes responsible for posttranslational modification of type I collagen. In most cases (85%-90%), OI is caused by an autosomal dominant genetic defect, in which only one gene copy contains pathogenic variant (9-11). Around 10%-15% of OI cases are caused by recessive genetic mutations inherited from both healthy parents that do not have OI, however, they both carry mutation in their DNA (12). Recent molecular and clinical investigations found more than 16 genetic and molecular phenotypes of OI.

The most common mutations that cause OI are autosomal dominant mutations in *COL1A1* (17q21.31-q22) or *COL1A2* (7q22.1) genes responsible for the synthesis of alpha-1 and alpha-2 chains of type I collagen (10, 11). Mutations in *COL1A1* and *COL1A2* cause OI type I-IV. In general, OI-causing mutations can be divided into quantitative and structural defects. Mutations that cause quantitative defects can result in a microsense frame shift or premature stop codon, which leads to the production of mutant mRNA. However, there are mRNA products from the other gene copy that was not affected by the mutation. The end result of protein synthesis from this mRNAs is a mixture of normal and abnormal collagen chains, forming half of the normal amount of healthy collagen type I. Clinical presentation of quantitative defect variant is usually a mild OI phenotype I. Structural defects result in the synthesis and secretion of abnormal collagen molecules. Mutations alter chain sequence in the triple helical domain and result in a wide phenotypic range from lethal type (OI type II), severe (type III) to moderate type IV. Most frequently, these mutations cause substitution of one of the glycine residues in alpha chains, which have a crucial role in helix formation.

Osteogenesis imperfecta type V is caused by an autosomal dominant mutation of *IFITM5* gene. It is the only dominantly inherited type of OI that is not due to collagen structural abnormalities. Mutation generates the alternative in-frame start codon and adds 5 residues to the N-terminus of bone restricted IFITM-like protein (BRIL). BRIL is highly expressed in osteoblasts and developing bone. Alterations in BRIL function lead to insufficient bone mineralization (13).

Osteogenesis imperfecta type VI is caused by a mutation of *SERPINF1* gene, which encodes pigment epithelium-derived factor (PEDF), a glycoprotein involved in various processes including neurotrophic and antiangiogenic processes and bone mineralization (14).

Mutations of the genes encoding cartilage-associated protein (*CRTAP*) and prolyl 3-hydroxylase 1 (P3H1 encoded by *LEPRE1*) were the first identified causes of recessive OI, now known as OI type VII (*CRTAP*) and VIII (*LEPRE1*). The lack of *P3H1* or *CRTAP* causes severe to lethal bone dysplasia. Also, mutation of *PPIB*, discovered later, causes lack of cyclophilin B (CyPB), resulting in severe to lethal bone dysplasia named OI type IX. *CRTAP*, *P3H1* and CyPB form a complex within the ER that post-translationally modifies specific proline residues (14). Also, the complex has a function as a peptidylprolyl*cis-trans* isomerase (PPIase) and as a chaperone, preventing type I collagen chains from forming premature aggregates in the ER15.

Osteogenesis imperfecta type X is caused by a mutation in the *SERPINH1* gene, which encodes heat-shock protein 47 (HSP47). The function of HSP47 is to recognize the folded state of type I procollagen trimer in the ER and help maintain it. The *FKBP10* gene encodes for FK506-binding protein 10 (FKBP10 or FKBP65), an ER-localized PPIase and a chaperone molecule. FKBP65 has multiple ligands, including type I collagen and elastin. It is involved in the folding of collagen type I. Mutations of this gene cause OI type XI and can also cause Bruck syndrome type I (12, 14).

In addition, changes in several other genes involved in osteoblast differentiation can cause OI, e.g., SP7 (OI type XII), *BMP1* (OI type XIII), *TMEM38B* (OI type XIV), *WNT1* (OI type XV), *CREB3L1* (OI type XVI), *SPARC* (OI type XVII) and *MBTPS2* (OI type XVIII) (12, 14).

EPIDEMIOLOGY, PATHOLOGY AND PATHOGENESIS OF OSTEOGENESIS IMPERFECTA

The prevalence of OI is one case *per* 20 000 births (15). The actual prevalence could be even higher because milder variants often remain unrecognized. According to population based data, OI is described worldwide and its prevalence is relatively equal and stable.

The pathogenesis of bone disease in OI can not only be explained by quantitative or qualitative type I collagen abnormalities and different animal models were created in order to elucidate bone metabolism at the molecular and cellular level. Genetic heterogeneity including the large number of genes involved in OI pathogenesis and their pathogenic variants, as well as additional unknown modifying mechanisms have made OI pathophysiology still incomprehensible (8, 15).

Bone is a dynamic tissue in which the process of bone formation and resorption is constantly being carried out, whereby in physiologic conditions these two processes are in equilibrium. In OI patients, bone resorption is more intensive than formation, which ultimately leads to reduction in total bone mass. Animal model studies have shown insufficient osteoblast productivity, although their m-RNA-measured collagen synthesis is constantly present (8). *In vivo* and *in vitro* studies have revealed altered osteoblast differentiation in OI bone, leading to an increase in immature os-

TABLE 1. Genetic basis, pathogenesis and clinical features of OI (19)

OI type	Gene	Pathogenesis	Clinical features	Disease severity
I	*COL1A1 COL1A2*	Defects in collagen synthesis, structure, or processing	Increased fracture rate, blue sclerae, conductive hearing loss	Mild
II	*COL1A1 COL1A2*	Defects in collagen synthesis, structure, or processing	Intrauterine fractures, minimal calvarial mineralization, rib and long bone fractures and deformities	Lethal
III	*COL1A1 COL1A2*	Defects in collagen synthesis, structure, or processing	Frequent fractures, bone deformities, short stature, kyphoscoliosis, immobility	Moderate to severe
IV	*COL1A1 COL1A2*	Defects in collagen synthesis, structure, or processing	Recurrent fractures, osteoporosis, long bone deformities	Mild to moderate
V	*IFITM5*	Defect in bone mineralization	Calcification of interosseous membrane of the forearm and hyperplastic callus formation	Variable
VI	*SERPINF1*	Defect in bone mineralization	Recurrent fractures, high levels of alkaline phosphatase	Moderate to severe
VII	*CRTAP*	Defects in collagen modification	Chondrodysplasia, rhizomelia, patient usually born with deformities and fractures of the limb bones	Lethal/severe
VIII	*LEPRE1/ P3H1*	Defects in collagen modification	Rhizomelia, coxa vara and popcorn metaphysis seen on x-ray	Severe to lethal
IX	*PPIB*	Defects in collagen modification	Bowed femurs with anterior bowing of the tibiae	Severe
X	*SERPINH1*	Defects in collagen folding and cross-linking	Macrocephaly, an open anterior fontanel, high and prominent forehead, hypoplasia of the midface with shallow orbits, blue sclerae, dentinogenesis imperfecta, scoliosis, joint laxity, nephrocalcinosis and chronic lung disease	Severe
XI	*FKBP10*	Abnormal cross-linking of collagen molecule	Joint contractures	Moderate to severe
XII	*SP7*	Defects in osteoblast development with collagen insufficiency	Fractures in lower extremities, delayed dental eruption, midface hypoplasia	Mild
XIII-XVIII	*BMP, TMEM38, WNT1, CREB3L, SPARC, MBTPS2*	Collagen C-propeptide cleavage defect, calcium channel defect, defects in ER-stress transducer regulator molecule	Sporadic reports, increased fracture rate, osteoporosis	Moderate to severe

OI, osteogenesis imperfecta

teoblast synthesis. Premature osteoblast/osteocyte precursor cells cause osteoclastogenic induction and an increase in osteoclast number (16).

Osteoblasts have a capability to differentiate into osteocytes, the cells involved in the control of various bone growth stimuli from the outside. Osteocytes produce bone control molecules that can modulate osteoclast activity. RANKL is a supporting molecule in osteoclast precursor differentiation, while sclerostin molecule acts as a negative regulator of bone mass in osteoblasts. Ultrastructural examination of OI bone tissue also showed an increase in the number of osteocytes, with some lacunae containing multiple osteocytes.

Bone mineralization in OI is increased, with small but plentiful mineral crystals associated with low mechanical strength (17). Completely disorganized cytoskeleton was found in patients with lethal or severe outcomes. It is not clear whether cytoskeletal organization is a primary or secondary modulator in OI (18). Taken all together, the outcome of all previously described interactions is the formation of fragile bone susceptible to fractures.

CLINICAL PRESENTATION

Osteogenesis imperfecta is a multisystem disorder that mostly affects tissues composed of type I collagen, i.e. bones, teeth, and ligaments (15, 19). The major features in OI patients are listed below:

Bone fragility and increased fracture rate depending on disease type. As a consequence of fractures and inappropriate bone healing, scoliosis, barrel chest and long bone deformities are frequently seen.

Craniocervical junction anomalies are found in 30% of all OI patients and include basilar invagination, basilar impression and platybasia as most frequent in OI.

Growth retardation and short stature are more pronounced in recessive than in dominant forms of the disease.

Dentinogenesis imperfecta: abnormalities in teeth color and quality.

Hearing loss related to combined conductive and sensorineural deficits.

Cardiopulmonary complications are a major cause of death in OI patients. During childhood, there are rib fractures leading to respiratory insufficiency, whereas in adulthood dilatation of aortic root and hypertension are frequently reported.

Blue sclera is used as a feature that differentiates type I from type IV.

Additional features: triangular face and macrocephaly.

Based on clinical and radiological findings, OI is originally classified into four types, I-IV (Sylence 1979) (20). Advantages in molecular genetics have delineated 18 molecularly defined OI types (I-XVIII). Their molecular and clinical features are presented in Table 1 (19).

GENETIC COUNSELING

In order to get complete care in OI patient, molecular diagnosis and pathogenic variant detection should be routine procedures in clinical practice. Genetic testing and counseling should be provided to all OI affected individuals, their parents and offspring, as well as individuals at risk (15, 19).

Detailed family history and clinical evaluation of parents and proband is a key prerequisite for appropriate genetic testing and evaluation (21, 22). Even subtle clinical features in parents such as discrete limb length abnormalities can be reflection of parental mosaicism. After clinical OI diagnosis, the next step in patient evaluation is molecular diagnosis obtained by the next generation sequencing techniques (NGS). At present, there are various approaches using different gene panels involved in OI pathogenesis. For economic reasons, in families with a single proband it is rationale to make first screen for *COL1* genes, followed by complete sequence analysis of other OI genes in case of negative results. If the results of sequence analysis are negative, additional testing using array or MLPA that identifies exon or whole-gene deletions/duplications is required (19).

In cases where more than one family member is affected, it is recommended to investigate the whole OI gene panel in the proband using NGS methods as the first step (19).

Genetic testing in parents is made after positive results in the proband in order to determine carrier status and inherited risks in future pregnancies. In most cases, pathogenic variants are absent in parents suggesting *de novo* genetic changes that occurred before conception in either one specific sperm or egg that contributed to the pregnancy. Also, pathogenic variants can originate from parental mosaicism that makes the recurrence risks in future pregnancies higher than it would be expected. The presence of mosaic carrier status in the parent can reach recurrence risks equivalent to the fully heterozygous status (23). Parental mosaicism should be considered in families with recurrent OI and negative results of NGS panel testing. Although NGS techniques are a valuable method for parental mosaicism detection, in some cases it can be missed due to the low grade of mosaicism and technical difficulties (23). In these cases, it is recommended to perform additional NGS analysis using more than one tissue (blood, buccal, saliva, sperm).

Although molecular genetic testing methods can offer precise and quick detection of pathogenic variant, genetic counseling and providing information on disease type and prognosis is complicated in routine clinical practice. Definitive genotype-phenotype correlation that could explain and predict all clinical features in OI patients does not exist (24). Autosomal dominant mode of inheritance carries a 50% risk of genetic transmission in future pregnancies, but in practice it is not always easy to predict phenotype in the offspring. Autosomal dominant mode of inheritance and its variability in clinical expression, presence of additional modifier genes, and interfamilial variability even among family members with the same mutation complicate genetic counseling in OI patients. Disease phenotype in OI patients is mostly determined by classes of mutation, e.g., mild OI type caused by first class of mutation (quantitative collagen defects) and severe OI type caused by second type (structural collagen defects). Besides the above-mentioned classes of mutations, phenotype features depend on the affected alpha type I collagen chain, position of the mutation, substituting amino acid, and/or combination of these variables (24).

Although rare, autosomal recessive forms of OI should be considered when unaffected parents have more than one child affected, in cases of parental consanguinity, and in cases with negative results of standard NGS panel covering *COL1A1* gene. In these cases, it is recommended to perform analysis in the proband using whole OI gene panel (19). Autosomal recessive forms of OI are associated with 25% recurrence risk in parents of affected proband. Even in family members that share the same mutation, wide phenotypic variability has been demonstrated. Consequently, precise molecular and clinical diagnoses are a prerequisite for counseling in these patients.

Prenatal and preimplantation genetic diagnosis can be applied in pregnancies/fetuses at risk. Prenatal diagnosis should be offered in all pregnancies with positive family history, i.e. OI in parent and sibling (19). Molecular genetic testing is available for all OI types if the mutation is known in

the family. The source of DNA for prenatal analysis are CVS at 10 weeks or amniocytes at 10-15 gestational weeks (19).

Prenatal ultrasound diagnosis in low risk pregnancies may identify fetus with findings suggestive of OI. Molecular genetic testing can be offered but inability to identify mutation does not exclude OI diagnosis in the patient.

FUTURE TREATMENT OF OSTEOGENESIS IMPERFECTA

Advances in molecular medicine, genetic engineering and transplantation have led to new potential therapies based on stem cell transplantation and genetic engineering (25, 26).

Cell based therapy using stem cells

Major characteristics of stem cells are capability of differentiation into multi-lineage cells and self-renewal capacity. Based on their origin, stem cells are divided into different categories: embryonic stem cells, induced pluripotent stem cells, and adult stem cells. Mesenchymal stem cells are adult stem cells located in various animal and human tissues. Their easy accessibility and ability of multi-lineage differentiation in mesodermal tissues such as osteoblasts, osteocytes and chondrocytes makes them attractive for bone disease treatment (26). Preclinical trials of bone marrow/mesenchymal stem cell transplantation in various OI transgenic murine OI models (OI type III, IV) using different cell delivery systems (intraosseous, intravenous, liver injection) were performed (26). Transplantation methods in preclinical animal trials achieved low donor bone engraftment in affected animals of 0.3%-28% but transplanted cells succeeded to differentiate into osteoblasts (27). Results of bone engraftment depended on the type of transplant (whole bone marrow, mesenchymal stem cells, fetal blood mesenchymal stem cells), transplantation technique, and age of animal model. Despite the low bone engraftment, biomechanical improvements in bone matrix quality, decreased fracture rates and lethality in affected animals were achieved (28).

Favorable results in animal OI models encouraged scientists to apply these methods in humans. First therapeutic use of allogeneic bone marrow transplantation from HLA matched siblings in OI patients was made by *Horowitz et al.,* who found an increase in growth velocity and mineral content and reduction in fracture rates (29). Initial positive effects of this therapy were not sustained over time and patients underwent treatment with isolated bone marrow stem cells (BM-MSCs) 18 months after initial transplantation. Post transplantation BM-MSCs application strengthened previously achieved favorable outcomes in these patients. First *in utero* transplantation was performed in a female OI fetus using HLA mismatched male fetal MSCs. Post transplantation monitoring in this patient found three bone fractures, normal growth rate and normal psychomotor development in the first two years. The latest transplantation experiences came from Götherström *et al*., who made prenatal and postnatal transplantation of human fetal mesenchymal stem cells (hfMSC) in two OI patients with severe type. Prenatal and postnatal transplantation improved growth velocity in these patients, and no new fractures were observed over time. Both patients showed no alloreactivity to donor hfMSCs or possible toxic effects of transplantation procedure (30).

Despite previously mentioned favorable results using transplantation methods, there is still a large number of ambiguities. A small number of treated patients and consequently the impossibility of statistically based conclusions prevent the application of these methods in routine clinical practice.

In order to define optimal therapeutic approach, additional basic and clinical investigations are necessary. From the current point of view, the researcher key points are the time of transplantation, appropriate type and source of stem cells regarding their proliferation, differentiation and viability after transplantation. Another area of interest is bone microenvironment after transplantation, signal pathways that provide osteogenic and mineralization potential, as well as immunogenic response of the host.

Gene therapy

Correction of the gene defect would be the most desirable therapy in OI patients in the future. According to current literature data, several gene therapeutic strategies are oriented towards mutant collagen transcripts (31).

It is well known that the pathogenic gene variants producing structurally abnormal collagen chains usually cause severe phenotypes in OI patients. One of the recent gene therapy approaches based on mutant gene silencing includes suppression of mutant allele resulting in their haploinsufficiency and consequently conversion of severe OI phenotype with structural collagen defect into less severe quantitative defect (31). Gene therapy strategies aiming at silencing mutant collagen transcripts use several methodological approaches, i.e. antisense oligodeoxyribonucleotides (ODNs), short interfering RNA (siRNA), and hammerhead ribozymes. Antisense ODNs are small molecules that can hybridize to target RNA and cause their degradation and also inhibit protein translation. The siRNA is able to incorporate in nuclease-containing multiprotein complex RISC (RNA-induced silencing complex) and then guided to target mRNA and consequently degraded. Hammerhead ribozymes present another type of synthetic RNA molecules that could bind to mutant RNA and cause their degra-

dation. All of these strategies were tested using *in vitro* or *ex vivo* model and to a lesser extent animal OI models (30, 31). Results of *in vitro* and *ex vivo* investigation of a siRNA tool to allele specific *COL1A1* gene silencing in mouse for classic OI showed reduction in mutant RNA transcription to up to 50%, while mutant protein was reduced by about 40%. Allele dependent silencing of *COL1A2* in single heterozygous individual using small interfering RNAs in OI patient decreased overall *COL1A2* transcription by around 70%, mostly due to silencing of T allele (32).

Although promising, at this moment, the therapeutic approach described is still in the experimental use. The complexity of its use derives from the fact that it requires specific design of silencing molecules (siRNA) complementary to target sequence/allele, creation of available carrier that could export it into target cells, and well defined technical details regarding application (32).

These techniques require additional investigations to determine their effect on other parts of the gene with similar or partially complementary sequences, as well as the possible undesirable effects.

Future perspectives

Based on the previously described experimental therapies, a combination of gene and cell therapy could be one of the future treatment options.

Research in the field of stem cell biology revealed induced pluripotent stem cells (iPSCs) to be superior to mesenchymal stem cells. iPSCs can be derived directly from adult tissues, offer large number of cells, and eliminate the necessity of adequate HLA donor. Discovery of the new agents for stem cell mobilization, signaling pathways in their microenvironment will allow higher amount of transplantable cells and available conditioning in treated patient.

The current level of science development offers several techniques of genome editing that could be used in gene correction. CRISPR/Cas9-based technologies can be used in editing single or multiple genes in various tissues. Using specific RNA endonucleases with a synthetic guided RNA it is possible to recognize the site- specific DNA mutant loci and cleavage. Another potential genome editing tools in OI patients could be transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs). These techniques usually require vector (viral or plasmid). Despite rapid progress in the field of genome editing techniques and awareness of their clinical significance, guidelines for its use and safety in preclinical and clinical trials are still lacking. Although these techniques offer great opportunities, their application in clinical practice is burdened with potential risks of unfavorable immune response and genotoxicity (33).

Heterogeneous genetic basis in OI and raising number of pathogenic gene variants complicate the process of gene editing in these patients. Also, the variability of OI clinical presentation imposes doubts in an unique therapeutic approach in OI patients.

It is expected that OI treatment would be personalized over time and oriented to single patient and his specific needs based on genetic, biochemical and pathophysiological characteristics and clinical presentation, as well as safety concerns (34).

Currently, bisphosphonates are the most commonly used drugs in both pediatric and adult OI population (35).

Abbreviations

OI-osteogenesis imperfecta, PTH-parathyroid hormone, BTMs-bone turnover markers, BSAP-bone alkaline phosphatase, OC-osteocalcin, PINP- N-terminal propeptide, PICP-C terminal propeptide of type I procollagen, PYD- pyridinoline, DPD-deoxypyridinoline, CTX-C-terminal telopeptide, NTX-N-terminal telopeptide, MMP-matrix-metalloproteinases, TRAP-5b-tartrate-resistant acid phosphatase 5b isoform, RANKL-receptor activator of nuclear factor NF-κB ligand, BMD-bone mineral density, mRNA-messenger RNA, BRIL-IFITM-like protein, PEDF-pigment epithelium-derived factor, *CRTAP*-cartilage-associated protein, P3H1-prolyl 3-hydroxylase 1, CyPB-cyclophilin B, PPIase-peptidyl-prolyl*cis-tran s*isomerase, HSP47-heat-shock protein 47, NGS-next generation sequencing, MLPA-multiple ligand probe amplification, CVS-chorionic villi sampling, DNA-deoxyribonucleic acid, BM-MSCs-bone marrow stem cells, hfMSC-human fetal mesenchymal stem cells, ODNS-antisense oligodeoxyribonucleotides, SiRNA-short interfering RNA, RISC-RNA-induced silencing complex, iPSC-s-induced pluripotent stem cells, TALENs-transcription activator-like effectors nuclease, ZFNs-zinc finger nucleases

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SAŽETAK

Osteogenesis imperfecta – molekularna osnova i lijekovi budućnosti

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Osteogenesis imperfecta (OI) ili bolest krhkih kostiju je metabolička bolest kostiju obilježena krhkim kostima, niskom koštanom masom i povišenom stopom lomova i deformiteta kostiju. Klinička prezentacija bolesnika s OI veoma je raznolika, od blagog do teškog i smrtonosnog tipa OI. Napretkom molekularne biologije i istraživanjima na životinjskim modelima OI nađeno je najmanje 16 novih gena uključenih u patogenezu OI. Većina mutacija su autosomno dominantne i zahvaćaju gene COL1A1 i COL1A2 koji su odgovorni za sintezu kolagena. Preostalih 10%-15% mutacija u OI su autosomno recesivne i zahvaćaju gene uključene u razne metaboličke procese u kostima. Sve bolje razumijevanje metabolizma kostiju i genetski inženjering nude nove potencijalne terapijske mogućnosti koje su u različitim fazama ispitivanja.

Ključne riječi: *osteogenesis imperfecta*, kolagen tip I, molekularna genetika, genska terapija, matična stanica