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Review article

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Skeletal anomalies in patients with neurofibromatosis type 1

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Abstract

Introduction Neurofibromatosis type 1 (NF1) is one of the most common hereditary tumor syndromes. The average incidence of NF1 in the world is 1:3000 of the population. The characteristic signs of the disease are neurofibromas and café-au-lait macules on the skin. 60 % of patients with NF1 develop specific skeletal anomalies: scoliosis, chest deformity, pseudarthrosis, requiring surgical treatment and long-term rehabilitation. It is necessary to develop prognostic criteria for the development of severe skeletal anomalies in NF1 and take early measures to prevent their progression. Congenital pseudarthrosis of the tibia is diagnosed in 5 % of children with NF1, accounting for 80 % of all cases of this pathology in the general population. Spinal scoliosis is detected in 60 %, osteoporosis in 50 %, chest deformity in 37.6 %, microgenia in 53 %, increased head circumference in 25 %, sphenoid wing dysplasia in 12 %, facial asymmetry in 10 % of patients with NF1. **The aim** of the review is to focus on the pathogenesis of skeletal anomalies development in NF1 that result in disorders of the musculoskeletal system in NF1 in order to take early measures for the prevention and treatment of the disease. **Materials and method** The review is based on numerous studies found in the databases: PubMed, Scopus, Web of Science, published mainly over the past 5 years. The suitable studies were searched by keywords and their combinations «neurofibromatosis type 1» with the words «skeletal abnormalities», «musculoskeletal system», «pseudarthrosis», «scoliosis», «pathogenesis», «deformation», «treatment», «frequency», «prevalence», «genotype-phenotype correlation», « modifier genes». **Results and discussion** The pathogenesis of skeletal anomalies is due to both the loss of heterozygosity of the *NF1* gene in pseudoarthrosis and the effect of neurofibromin deficiency on the development of connective tissue. Currently, the only effective drugs for the treatment of tumor syndrome in NF1 are inhibitors of mitogen-activated kinase (MEK), which suppress the increased activity of Ras oncogenes. A promising issue is the study of the effect of MEK inhibitors on the progression of skeletal anomalies in patients with NF1 in the treatment of tumor syndrome. Therefore, dynamic observation by an orthopedic surgeon with an objective assessment of the observed changes is of great importance in the management of patients. It is necessary to widely introduce molecular genetics methods for confirming the diagnosis of NF1 in the clinic in cases of a combination of skeletal anomalies with individual signs of the disease, since the manifestations of NF1 are steadily progressing with age, even in the presence of erased and atypical forms of the disease. Since the analysis of scientific literature has shown the possible influence of modifier genes on the pathogenesis of NF1, the search for mutations in these genes is promising. **Conclusion** Most patients with NF1 develop orthopedic pathology, which is associated with the role of the *NF1* gene in the development of connective tissue. The increased mutability of this gene causes the loss of heterozygosity in the development of congenital pseudoarthrosis of the tibia. At the same time, *NF1* driver mutations are detected in 10 % of sporadic malignant neoplasms. Therefore, the role of somatic mutations in the *NF1* gene in the development of skeletal anomalies in the general population is probable. The methods of NF1 therapy that are under investigation may become the basis for the complex treatment of oncological and orthopedic patients.

Keywords: modifier genes, chest deformity, neurofibromatosis type 1, pseudoarthrosis, scoliosis

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INTRODUCTION

Neurofibromatosis type 1 (NF1) is the most common autosomal dominant hereditary tumor syndrome. The average incidence of NF1 in the world population is 1:3000. The disease develops as a result of inherited germinal heterozygous mutations in the oncosuppressor gene *NF1*. About 50.0 % of cases of the disease are sporadic, caused by de novo mutations, which indicates a high mutability of the *NF1* gene. The gene product, the protein neurofibromin, negatively regulates the activity of Ras oncogenes due to the hydrolysis of their active GTP-bound forms into inactive GDP-bound ones. Accordingly, mutations in the *NF1* gene lead to Ras stimulation and enhanced cell proliferation with the formation of benign (neurofibromas, gliomas) and malignant tumors [1]. Diagnostic criteria for NF1 include: 1) six or more café-au-lait macules on the skin (CALM) with a diameter greater than 5 mm in prepubertal and greater than 15 mm in postpubertal age; 2) freckling in the axillary or groin areas; 3) two or more cutaneous neurofibromas or one plexiform neurofibroma; 4) two or more iris hamartomas (Lish's nodules); 5) glioma of the optic nerve; 6) specific bone dysplasias; 7) NF1 found in the first degree relatives [2]. The diagnosis of NF1 is established in the presence of two out of 7 diagnostic criteria, which also include specific skeletal anomalies [3], such as sphenoid wing dysplasia, thinning of the cortical layer of long bones, and congenital pseudoarthrosis of the tibia (PTI) [2].

Patients with NF1 are characterized by coffee-with-milk spots (café-au-lait macule – CALM), which are tumor-like formations that develop as a result of inactivation of the second *NF1* allele in melanocytes.

The latter, like Schwann cells, originate from common precursors of the neural crest [4]. Tumor-like formations characteristic of NF1 include iris hamartomas, which occur in 70.0 % of patients [5], as well as fibrous hamartomas of long bones, which cause the development of CPT [6]. The clinical picture of NF1 is characterized by a variety of manifestations and their prevalence. In 99.0 % of patients with NF1, CALM is common, 90.0 % have freckles, 81.0 % have behavioral problems (up to 40.0 % with attention deficit and hyperactivity disorders) [7], 5.4 % suffer epilepsy [8], 2.0 % develop Arnold-Chiari malformation [9]. In NF1, the incidence of neurofibromas is 40.0–60.0 %, intellectual impairment is encountered in up to 30.0–65.0 %, plexiform neurofibromas in 30.0–50.0 %, gliomas of the optic nerves in 15.0–20, 0 %, and malignant peripheral nerve sheath tumors (MPNST) in 8.0–13.0 % [5].

Sixty percent (60 %) of patients with NF1 have disorders of the musculoskeletal system (MSS), among which spinal deformities and CPT are the most common. Treatment of severe forms of these pathologies is surgical methods used due to the ineffectiveness of conservative therapy [10]. CPT is diagnosed in 5.0 % of patients with NF1 [7]. Craniofacial developmental anomalies are detected in the majority of patients, including a reduction in the lower jaw (53.0 %) [11], hypertelorism (63.5 %) [12], increased head circumference (25.0 %), facial asymmetry (10.0 %). Dysplasia of the wing of the sphenoid bone, characteristic of the disease, is detected in 5.0–12.0 % of cases with NF1 [13]. Growth retardation indicates the MSS disorders. Children with NF1 grow normally until puberty. Afterwards, the rate of body growth decreases compared to healthy subjects. Short stature is encountered in 18.0–30.0 % of adult patients with NF1, who are characterized by a higher incidence of serious complications, such as severe scoliosis, CNS tumors, and plexiform neurofibromas [14].

Osteoporosis develops in 50.0 % of patients with NF1 and results in a significant increase in the incidence of fractures [1]. The situation is complicated by the fact that there is a deficit in the performance of a number of functional tasks, a significant imbalance, an impairment of muscle strength and coordination of the upper limbs in NF1. Characteristic features are a lack of reaction time and motor skills, a moderate deficit in manual dexterity and balance, and gait disturbance [3]. Chest deformity is detected in 37.6 % [12], spine deformity (scoliosis, kyphosis) from 21.0–49.0 % [7] to 60.0 % of patients with NF1 [10]. Prognostically unfavorable are dystrophic anomalies (rotation, wedge-shaped or serrated deformity of the vertebrae, expansion of the spinal canal, fusiform transverse processes), as they lead to the progression of the pathology with severe curvature of the spine. Therefore, early surgical treatment is indicated for this pathology. Genetic studies of all CPT cases in the general population showed that most of them (80.0 %) are associated with NF1 [10].

The **purpose** of this review is to determine the mechanisms in the development of MSS pathology in NF1 at the molecular level, to identify the possible influence of mutations in the *NF1* gene and modifier genes on the development of skeletal anomalies. New data could become the basis for the development of effective methods for the prevention and treatment of the disease upon consideration of the data obtained from molecular genetic studies.

MATERIAL AND METHODS

PubMed (https://pubmed.ncbi.nlm.nih.gov), Scopus (https://www.scopus.com), Web of Science (https://webofscience.com) were used for the search of the studies published over the last 5 years. The search was carried out using the following key words in their combinations *neurofibromatosis type 1* and "skeletal abnormalities", "musculoskeletal system", "pseudarthrosis", "scoliosis", "pathogenesis", "deformation", "treatment", "frequency", "prevalence", "genotype-phenotype correlation", "modifier genes".

RESULTS AND DISCUSSION

Pathogenesis of skeletal anomalies in neurofibromatosis type 1

The exact mechanisms of focal bone lesions in NF1 are still unknown, but there is no doubt that changes in signaling pathways caused by neurofibromin deficiency are involved in their development. Normally, *NF1* is expressed by osteoblasts, osteoclasts, chondrocytes, fibroblasts, and vascular endothelial cells. Under the negative regulatory control of neurofibromin, Ras proteins are required for the normal formation of craniofacial structures, since the jaws and the base of the skull develop mainly from the neural crest [13]. Neurofibromin plays a role in the growth and metabolism of the skeletal muscle, therefore, decreased muscle mass and weakness are observed in NF1 [3]. However, bone deformities in NF1 are frequently secondary due to the germination of plexiform neurofibromas and their mass pressure on the surrounding tissues [15].

The mechanism of development of MSS pathology in NF1 may be due to the relationship of the *NF1* gene with various molecules, since neurofibromin contains several functional domains. The main one is the GAP-related domain (GRD), which regulates the MAPK and PI3K/AKT/mTOR pathways by influencing their Ras activator. However, in neurofibromin deficiency, the transcription factor ZNF423 is repressed and the factor associated with the epithelial-mesenchymal transition is activated, which indicates the role of *NF1*

in transcriptional regulation [16]. On one side of the GRD are the cysteine-serine-rich domain (CSRD) and the tubulin-binding domain (TBD). The CSRD domain binds to dimethylarginine dimethylaminohydrolase 1 (DDAH1). TBD interacts with tubulin and LRPPRC (leucine-rich pentatricopeptide motif-containing protein). On the other side of GRD, there are several domains: SEC14 (binds to phospholipids and LIM kinase 2); PH (pleckstrin homology domain interacts with valosin-containing protein and LIM kinase 2); CTD (carboxy-terminal domain - C-terminal domain binds to dihydropyrimidinase-bound protein 2 (DPYSL2), focal adhesion kinase (FAK) and DDAH1); SBD (syndecan-binding domain interacts with syndecan). SFC14 and PH domains cause inhibition of LIM kinase 2 by RHO-associated protein kinase, which modulates the actin cytoskeleton [17]. Thus, the complex structure of neurofibromin and the presence of several domains binding to different molecules is the cause of the complex pathogenesis of NF1 and the development of skeletal disorders, what is reflected in the pronounced clinical polymorphism of the disease [18, 19].

The development of osteoporosis in half of patients with NF1 is caused by defective bone metabolism of osteoblasts. Moreover, congenital neurofibromin deficiency plays a role in hematopoietic cells and osteoclasts, which become insensitive to bisphosphonates [1]. The study of CPT cells showed inactivation of the second *NF1* allele in all patients, that indicates the role of activation of Ras signaling pathways in the development of this pathology [20], as well as the prospects for the use of effective methods of treating tumor syndrome (using MEK inhibitors) in CPT management. Moreover, the loss of heterozygosity of *NF1* is detected in vertebral tissue samples obtained in surgical treatment of scoliosis in NF1 [21]. Transcriptome profiling has shown that pseudoarthrosis cells in NF1 have increased expression of EREG (encodes epiregulin) and EGFR (epidermal growth factor receptor). This leads to the inhibition of osteogenic differentiation. Sequencing of the mRNA of separate osteocytes enables to determine that overexpression of EREG is due to mutations of the second allele of the *NF1* gene without changes in the expression of transforming growth factor beta (TGFß1). In experiments on mice, this phenomenon was confirmed, which is conservative for animals. However, blocking epiregulin function with AG-1478 or EGFR with posiotinib did not restore normal cell differentiation, which indicated the need to find other ways to influence this pathology [22]. The most promising is the use of MEK inhibitors, that have shown their effectiveness not only in the treatment of osteoporosis in NF1 [1], but also in tumor syndrome [23].

Role of modifier genes in the development of manifestations in neurofibromatosis type 1

Although the clinical manifestations of NF1 are characterized by considerable variability, the association of specific symptoms of the disease with a particular type of mutation has not been proven. Moreover, an identical mutation in the *NF1* gene can cause a mild course of the disease in some patients and severe manifestations in others, even among members of the same family [18, 19]. Due to the complex structure of the *NF1* gene and the interaction of its protein product with various molecules [17], the role of modifier genes in the pathogenesis of NF1 has been assumed. This assumption was made in 1993 by Easton et al. based on the analysis of the correlation of NF1 symptoms in monozygotic twins and other relatives [24]. The identification of modifier genes may serve as a key to the development of effective NF1 therapies. To solve this problem, both the study of specific genes and sequencing of the entire genome have been carried out, taking into account the characteristics of protein-coding genes and non-coding RNA [19]. Thus, specific microRNAs miR-34a, miR-10b [25], miR-24 [26], and miR-107 [27] have been identified as modifiers of tumorigenesis in NF1. The expression of miR-204 is reduced in the tissues of sporadic MPNSTs and in patients with NF1. Experiments with MPNST cell lines in vitro and mice in vivo have shown that restoring the levels of miR-204 significantly reduces tumor cell proliferation, migration, and invasion. This microRNA inhibits Ras-signaling and is a biomarker for the diagnosis of MPNST, as well as a candidate target for the development of targeted therapy for these tumors [28]. Compared with neurofibromas, a significant increase in the expression of miR-21, the target of which is the protein of programmed cell death PDCD4, was determined in MPNST cells. Transfection of miR-21 inhibitor into MPNST cell lines significantly increased caspase activity and suppressed cell growth with stimulation of PDCD4 expression. As a result, apoptosis was induced, what allows us to consider miR-21 as a target for targeted MPNST therapy [29].

A significant increase in the expression of chemokines was determined in the tissues of neurofibromas in patients with NF1, CXCR4 by 120 times and CXCL12 by 512 times. This indicates the importance of the *CXCR4* and *CXCR12* genes in the development of tumors in NF1 [30]. As a result of the comparative genome-wide association search (GWAS) in patients with NF1 with different amounts of NF1, the effect of the allelic variant of the *RPS6KA2* gene (rs12190451) on the development of CALM was found. The *RPS6KA2* gene is phosphorylated and activated by ERK1/2 kinases via RAS-MAPK signaling pathways. In this regard, it is assumed that *RPS6KA2* serves as a modifier gene for the development of CALM in NF1 [4]. Experiments on mice have shown the role of the *ATM* gene in initiating the formation of neurofibromas [31]. Meta-analysis of GWAS results in NF1 using forward and reverse genetics strategies allowed constructing protein-protein interaction networks to search for potential genes involved in the pathogenesis of NF1. As

a result, 10 potential modifier genes in the development of NF1 were identified: *AKT1* (encodes the homologue protein of the viral oncogene V-Akt of mouse thymoma), *BRAF* (encodes serine-trenoin kinase B-Raf), *EGFR* (epidermal growth factor), *LIMK1* (containing LIM-motif protein kinase), *PAK1* (P21-activated kinase 1), *PTEN* (Phosphatase and TENsin homolog), *RAF1* (serine-trenoin kinase Raf-1), *SDC2* (syndecan), *SMARCA4* (SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily A, Member 4) and *VCP* (valosin-containing protein) [18].

The role of modifier genes in the development of NF1 is evidenced by the features of the "NF1 microdeletion syndrome", when not only *NF1*, but also neighboring genes are lost. The manifestations of the disease are more severe associated with congenital heart defects, early manifestation and a large number of cutaneous neurofibromas, pronounced mental retardation [32]. The *HCA66* gene is involved into the microdeletion region of chromosome 17q11.2, the protein product of which interacts with the oncosuppressor Apaf-1 (apoptic protease activating factor-1). Accordingly, when *HCA66* is inactivated, cells become less susceptible to apoptosis [19]. Other candidate modifier genes located in the microdeletion region may be *CENTA2* (encodes the Arf-GAP protein with a double phosphate domain), *RAB11FIP4* (encodes a protein interacting with the Rab11 family), *C17orf79* (open reading frame of chromosome 17), *UTP6* (encodes a small nucleolar subunit) [32].

The influence of modifier genes on the specific manifestations of NF1 is reflected in the association of the MSS pathology with other signs of the disease. Thus, comorbidity with severe skeletal abnormalities was determined in children with plexiform neurofibromas. Preclinical studies in mice have shown that *NF1* deficiency in bone progenitor cells disrupts pyrophosphate homeostasis in a MEK-dependent manner, which alters bone mineralization. An increased expression of *ANKH*, along with *Enpp1*, was detected in murine and human neurofibromas. The *ANKH* gene encodes a transmembrane protein expressed in joints and plays a role in the development of osteoblasts and osteoclasts [15]. In *NF1*-deficient stromal cells of the bone marrow of mice (mBMSC), a decrease in the expression of the alkaline phosphatase gene *ALPL* was noted [22]. Low expression of the *COL14A1* collagen gene was detected in neurofibrom tissues [31]. NF1 patients with low growth are more likely to develop severe complications, such as brain tumors, large plexiform neurofibromas and severe scoliosis. At the same time, there is a correlation between optic nerve gliomas in combination with the MSS pathology with the development of these complications [14]. Mutations in the *NF1* gene are detected on average in 10.0 % of all sporadic malignant neoplasms in people who do not suffer from NF1. Moreover, these mutations can be drivers of carcinogenesis and initiate tumor resistance to chemotherapy. Therefore, since the *NF1* gene features an increased mutability [17], it can be assumed that somatic mutations in it may be the cause of the MSS pathology in the general population. To confirm this assumption, molecular genetic studies of cells of tissue samples are necessary taken from patients during surgery of such pathologies as pseudoarthrosis, fibrotic dysplasia, scoliosis, since biallelic inactivation of *NF1* is detected in such cases [20, 21].

Current management of skeletal anomalies in neurofibromatosis type 1

At a meeting of the International Bone Abnomalities Consortium sponsored by the Children's Tumor Foundation in 2011, the concepts of CPT management were developed. The surgical approach consists in the recovery of the "fibrous hamartoma" and periosteum to a healthy bone, rigid stabilization of congenital pseudoarthrosis and bone grafting with an autogenous iliac crest. In children, it is preferable to use the Ilizarov apparatus to ensure subsequent bone lengthening [6]. In experiments on mice with NF1, effective treatment of CPT was shown using a combination of MEK inhibitors and local administration of bone morphogenetic protein (BMP2) to the area of pseudoarthrosis. It is planned to introduce this method into the clinical setting [33]. In severe scoliosis with a Cobb angle of more than 45º in NF1, surgical correction by posterior approach and fixation with high-density metal implants of the third generation is effective [34]. The use of standard methods for surgical correction of scoliosis in patients with NF1 results in some complications such as rod breaks, proximal transfer of kyphosis and curvature progression [35].

Since the same mechanisms are involved in the pathogenesis of skeletal anomalies in NF1 as in the development of a tumor syndrome (neurofibromin deficiency [1, 13], and there is a loss of heterozygosity of the *NF1* gene [20, 21]), the same approaches can be used to treat the MSS pathology as in the treatment of neoplasms in NF1. The most promising approach is aimed at inhibition of RAS signaling pathways using the most selective agents, since some drugs have not shown a significant effect in the clinical setting. Thus, tipifarnib, a farnesyl transferase inhibitor that blocks RAS, had no effect on the progression of plexiform neurofibromas compared with placebo in a clinical trial in 62 patients with NF1 [36]. However, MEK inhibitors have shown marked efficacy in the treatment of both plexiform neurofibromas and low-grade gliomas. The first drug from this group to be approved by the FDA was selumetinib [23], which is a small molecule that acts as an ATP-independent inhibitor of mitogen-activated protein kinase (MEK kinase 1 and 2), which is a key mediator of activation of the RAS/RAF/MER/

ERK pathway (enhanced in NF1) [37]. In 2016, data from Dombi et al. on the results after treatment with selumetinib of 24 children with NF1 were reported. The most common toxic effects were acne, asymptomatic elevation of creatine kinase, and gastrointestinal lesions. Oral administration of the drug at a dose of 25.0 mg per 1.0 m^2 of body area was carried out in 28-day cycles. As a result, a decrease in the volume of neurofibromas was observed in 71.0 % of children. Similar data were obtained in the experiments on mice (a decrease in the size of neurofibromas in 67.0 %) [38]. Moreover, the effectiveness of selumetinib in a combined therapy with LDN-193189 (an inhibitor of the BMP2 receptor type 1) was shown on the MPNST (NF1-/-) cell line, while the single use of LDN-193189 did not yield the proper antiproliferative effect. The results obtained suggest that the use of selumetinib is possible in the complex chemotherapy of MPNST [39]. In 2020, Baldo et al. studied 17 children with PN during 12 months of selumetinib administration and assessed the reduction in size (more than 20.0 % of the volume) of tumors in 16 of 17 patients with NF1 [37]. In two children, an unusual complication was subsequently noted, unilateral edema of the lower limb without changes in lymphatic drainage and blood circulation [40]. In 2020 Santo et al. described the effectiveness of selumetinib in the treatment of PN in 18 out of 19 patients with NF1 (95.0 %) in the first 60–90 days [41]. In 2020, a study by Gross et al. in a phase 2 of an open-label clinical trial in children with NF1 with a continuous scheduled (28-day cycles) use of selumetinib described a consistent reduction in the size of inoperable neurofibromas in 70.0 % of patients (35 out of 44) [42].

Selumetinib was shown to be effective in brain tumors in patients with NF1. Six groups of patients aged 3 to 21 years were treated with selumetinib at a dose of 25 mg/m^2 twice a day, 26 courses for 28 days. In 36.0 % (9 out of 25) of patients with grade 1 pilocytic astrocytoma, in 40.0 % (10 out of 25) of patients with low-grade glioma, a persistent clinical effect was shown. Accordingly, on average, 38.0 % of patients with NF1 showed the effectiveness of the drug in the treatment of brain tumors [43]. Spinal neurofibromas in NF1 cause progressive spinal cord compression and neurological dysfunction. Treatment with selumetinab (12 cycles) of 24 patients with NF1 aged 6 to 60 years with spinal neurofibromas (20 patients had spinal cord deformity) showed its clinical efficacy in 18 studied individuals (75.0 %) [44]. Attempts to use other groups of drugs for the treatment of NF1 showed significantly lower results, although different from placebo. Thus, a decrease in the volume of plexiform neurofibromas in patients with NF1 treated with imatinib mesylate (an inhibitor of kinases) was achieved in 17.0 % of cases [45], with the use of peg-interferon alfa in 5.0 % [46]. The anti-inflammatory and antifibrosing drug pirfenidone (designed to combat idiopathic pulmonary fibrosis) suppressed the growth of PN in 15.0 % of adult patients with NF1 [47], but did not show effect on this type of tumor in children with NF1 [48].

Since osteoclasts in NF1 are insensitive to bisphosphonates, a personalized approach using MEK inhibitors is needed for the treatment of osteoporosis. Long-term use of vitamin D and calcium is also ineffective. However, NF1+/- osteoclast precursors exhibit significant hypersensitivity to M-CSF, which binds to their c-Fms receptors and enhances their migration, adhesion, and bone resorption capacity [1]. The introduction of PLX3397, a pharmacological inhibitor of c-Fms receptors, reduces the activity of osteoclasts. Accordingly, PLX3397 may be a promising drug for the treatment of osteoporosis in patients with NF1 [49]. MEK inhibitors were effective in enhancing tibial fracture healing [50] and treating pseudarthrosis in mouse models with NF1 (in combination with the recombinant human bone morphogenesis protein rhBMP-2) [51]. A potential inhibitor of bone mineralization is the increased accumulation of pyrophosphates in response to ERK activation in chondrocytes of patients with NF1. In experiments on mice with NF1, the effectiveness of asphotase alfa for the treatment of osteoporosis was revealed, since this enzyme reduces the concentration of pyrophosphate in the bones [52].

A promising direction is the etiotropic treatment of NF1 without genome editing using a recombinant adeno-associated virus (rAAV) containing an expression cassette to replace mutant alleles and restore neurofibromin function. However, due to the large size of the cDNA of the *NF1* gene (8500 bp), the use of standard vector delivery systems is not possible. Therefore, truncated variants of the *NF1* gene that retain functional domains can be used as an alternative [53]. In 2019, the efficiency of restoring Ras-GTPase activity through GRD expression using a panel of adeno-associated virus (AAV) vectors was shown on MPNST cell lines and human Schwann cells. As a result, there was a pronounced suppression of Ras by the *NF1*-specific pathway [54]. By transfection of the isolated domains GRD, CSRD, LRD, CTD of the neurofibromin protein, their normal function was partially restored in an experiment on a neurofibroma cell line. Moreover, recombinant transgene sequences can be designed to encode truncated functional proteins that are easily packaged into viral vectors [55]. It can be expected that the results obtained will become the basis for the introduction of *NF1* gene therapy into the clinical practice, since this method of treatment has shown its effectiveness for a number of monogenic diseases. Alternative methods of *NF1* gene therapy are also being developed. In particular, in nonsense

mutations that account for up to 20.0 % of the causes of NF1 development [40], approaches are used to suppress the termination of translation of premature termination codons (PTC). To do this, PTC pseudouridylation, inhibition of nonsense-mediated mRNA decay, and superquarrel tRNAs are carried out. The simplest way is to use aminoglycosides, which contribute to the translation of a protein of normal length up to 35 % of the norm due to incorrect pairing of aminoacyl-tRNA with a premature termination codon. Other antibiotics that inhibit RTC include negamycin (binds to the small subunit of the ribosome), spiramycin, josamycin, and tylosin. Suppression of PTC in mammalian cells without affecting translation termination at normal termination codons causes PTC124, known as ataluren. This agent has shown its effectiveness in restoring the translation of normal proteins in models of various monogenic diseases [56].

The investigation of the role of modifier genes in the pathogenesis of NF1 can become the basis for the development of a targeted therapy of the disease, including in the treatment of skeletal disorders. The most promising application is microRNA molecules as objects, whose role as modifier genes in NF1 was described earlier [25–29]. Elevated levels of miR-27a-3p and miR-27b-3p are detected in cutaneous neurofibromas and MPNST cell lines in NF1, which contribute to the proliferation, migration and invasive ability of tumor cells. Both microRNAs have a direct impact on the mRNA of the *NF1* gene [57]. The ability of miR-641 to inhibit *NF1* expression has been shown. The levels of this microRNA are elevated in non-small cell lung cancer, which causes resistance to chemotherapy that targets EGFR (epidermal growth factor receptors). Accordingly, the targeted effect on this microRNA can regulate the expression of *NF1* [58]. Similarly, miR-103a-3p causes chemoresistance to cisplatin in non-small cell lung cancer by acting on *NF1* [59]. Elevated levels of miR-27a-3p in gliomas contribute to resistance to temozolomide due to the targeted inhibitory effect on *NF1* [60]. Analysis with a luciferase reporter showed that the *NF1* gene is a direct target for miR-514a, the increased production of which in melanoma cell lines inhibits the expression of *NF1*, what correlates with increased cell survival. MiR-514a belongs to a cluster of microRNAs involved in the transformation of melanocytes and contributing to the development of melanoma. MiR-514a is expressed in 69.0 % of all melanoma cell lines and only in 3.0 % of other malignant formations [61]. Gastric cancer is characterized by increased expression of miR-107, which inhibits the mRNA of the *NF1* gene by binding to an area inside the 3'-UTR. This microRNA causes the progression of gastric cancer, and its levels correlate with the size of the tumor and the depth of the invasion [27]. The *NF1* gene is also a direct target of miR-125a-3p, which promotes differentiation and apoptosis of human monocytic leukemia cell line cells [62]. In squamous cell lung cancer, fibroblasts stimulate tumor development and exhibit increased expression of miR-369, which has a targeted effect on the mRNA of the *NF1* gene. Due to this, migration and invasion of cancer cells is potentiated, as MAPK signaling pathways are activated [63]. The obtained data on the role of various microRNAs in controlling the expression of the *NF1* gene can become the basis for both targeted therapy of specific malignant tumors and for the treatment of NF1 by stimulating the expression of the normal NF1 allele [53].

CONCLUSION

Skeletal disorders develop in most patients with NF1 and are specific for the disease. This indicates the role of the *NF1* gene and the molecules interacting with its protein product in the regulation of the musculoskeletal system development. In the cells of pseudoarthrosis site and deformed vertebrae in scoliosis, biallelic inactivation of the *NF1* gene is detected. Since the *NF1* gene has an increased mutability, it is assumed that in the general population, somatic inactivation of *NF1* can cause the MSS pathology. This assumption is due to the fact that somatic mutations in the *NF1* gene in patients not suffering from NF1 are detected on average in 10 % of sporadic malignant neoplasms and are drivers of carcinogenesis. Moreover, the same drugs (MEK inhibitors) that are successfully used in the treatment of tumor syndrome in this disease have shown their effectiveness in the treatment of skeletal anomalies in NF1. Since NF1 is characterized by pronounced clinical polymorphism even in patients with identical mutations and members of the same family, the influence of modifier genes on the development of disease manifestations is probable. This is evidenced by the results of a number of authors. Identification of the influence of specific genes on the development of NF1 can become the basis for the development of new methods in the treatment of both the tumor syndrome and skeletal anomalies. Currently, an integrated approach has been used for the treatment of orthopedic pathology in NF1. In congenital pseudarthrosis of the tibia and severe scoliosis, surgical treatment along with pharmacotherapy has been used. For treatment of osteoporosis in NF1, the use of asphotase alfa, an inhibitor of c-Fms receptors (PLX3397), has been under consideration. The use of MEK inhibitors with local injection of rhBMP-2 into the area of pseudarthrosis as well as the introduction of NF1 gene therapy into the clinic, seems promising.

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