ECTOPIC LOCALIZATION OF NEURAL CREST CELLS: ETIOLOGICAL FACTOR OF SCOLIOSIS*

A.M. Zaidman[1], E.L. Strokova[1], E.V. Kiselyova[2], T.A. Ageeva[3], L.A. Suldina[2], A.A. Strunov[2], A.I. Shevchenko[2]
Ya.L. Tsivyan Novosibirsk Research Institute of Traumatology and Orthopedics, Russia

Objective. To identify cell phenotypes in vertebral body growth plates from patients with idiopathic scoliosis.
Material and Methods. Cells were isolated from vertebral body growth plates both on convex and concave sides of the deformity in 50 patients operated on for idiopatic scoliosis. Cells were cultured and identified by methods of common morphology, neuromorphology, electron microscopy, immunohistochemistry, and PCR analysis.
Results. Cultured cells obtained from the convex side of the deformity were identified as chondroblasts. Cells isolated from growth plates on the concave side of the deformity were described as neuro- and glioblasts. Cells formed synapses, contained neurofilaments, and expressed neural and glial proteins, respectively.
Conclusion. Ectopic localization of neural crest-derived cells in vertebral body growth plates is the etiological factor for scoliotic disease.
Key Words: scoliosis, growth plate, neural crest, protein expression, neurofilaments, synapses, chondrocytes.

**Zaidman AM, Strokova EL, Kiselyova EV, Ageeva TA, Suldina LA, Strunov AA, Shevchenko AI. [Ectopic localization of neural crest cells: etiological factor of scoliosis]. Hirurgia pozvonocnika. 2015;12(4):88–97. In Russian.*

The neural crest is one of the most remarkable rudiments in the body of an embryo.
BM Carlson

For centuries, scientists were interested in etiological factors of idiopathic scoliosis. Despite numerous studies on various levels, the causes of scoliosis remain unknown [9, 18, 24, 27, 28]. Nowadays, genetic nature of idiopathic scoliosis is widely discussed [12, 20, 21, 26, 29], however, there is no data on identity and localization of gene/genes responsible for the disease [11].

Long-term and multifaceted studies of spinal deformities, from early to severe forms [1, 2], revealed that asymmetry of the spine growth is associated with impaired functioning of growth plates chondroblasts on the concave side of the spinal deformity. While the chondroblasts on the convex side go through all stages of differentiation and subsequent osteogenesis, the growth plate chondroblasts on the concave side remain in the early stages of histogenesis, contributing to growth asymmetry. In order to identify genetic features which may be associated with impairment of chondroblasts histogenesis on the concave side of the deformity, we examined real-time expression of the key genes associated with the growth process: growth genes (GHR, EGFR, IGFIR, TGFBRI), genes for synthesis and matrix structure (ACAN, CUM, VCAN, COL1AI, COL2AI, HAPLNI), and genes responsible for sulfation and transmembrane transport of sulfates (DTDST, CHSTI, CHTST3). Analysis of the data revealed that while the level of synthesis of matrix proteins and core proteins of proteoglycans remains unchanged in the growth plates, the process of sulfation, link-protein synthesis, and function of growth transcription factors are disrupted, with the latter not responding to normal differentiation and proliferation signals. Factor analysis revealed marked differences in chondroblasts phenotypes in patients with idiopathic scoliosis in comparison with the norm. It has, therefore, been concluded that the identified gene expression profile may be caused by the presence of different cell phenotypes in the vertebral body growth plates of patients with idiopathic scoliosis [3].

The aim of the study was to identify cell phenotypes in the vertebral body growth plates in patients with idiopathic scoliosis.

Material and Methods

Identification of cell phenotypes in the vertebral body growth plates in patients with idiopathic scoliosis was conducted by differentiated culturing of cells from the convex and concave sides of the deformity [6].

Cells from the vertebral body growth plates of 50 children (11–15 years of age) with III–IV degree idiopathic scoliosis operated on in the Clinic of Pediatric Orthopedics of Novosibirsk NIITO were studied.

Samples of the material were collected into sterile tubes containing 0.9% normal saline and 20 μg/mL of gentamicin.

Hyaline cartilage of the growth plates was washed in saline, minced in a Petri dish with minimal volume of RPMI

medium to a size of 1–2 mm2, placed in 1.5 % solution of collagenase in a siliconized dish and incubated in CO2-incubator at 37 °C for 22–24 h. The resulting cell suspension was passed through a nylon filter to remove tissue fragments and the cells were pelleted by centrifugation for 10 min at 2000 rpm. The precipitated cells were identified and their total count was determined using Gorjaev's chamber.

The isolated cells were cultured in DMEM F12 medium supplemented with 15% FBS (fetal bovine serum), streptomycin – penicillin (250) and amphotericin B in CO2 incubator at 37 °C. Plastic Petri dishes (60) were used for culturing. Coverslips for subsequent morphological studies during the cultivation were stacked at the bottom of the culture dishes. Isolation, seeding and cultivation of the cells were carried out separately for different heights and sides of the deformity.

The cells were cultured for 21 days. The medium was replaced once in 3 days. The cells were not passaged. For morphological studies, the cells were removed over a period of 5 to 21 days, fixed in 70° alcohol and stained with hematoxylin-eosin and Alcian blue according to Cajal and Nissl.

Electron microscopy studies. On day 14 of the cultivation the choronocytes culture was passaged though the mixture of trypsin and versene solutions (1:1). The substances were inactivated with DMEM F12 medium supplemented with 15 % FBS (fetal bovine serum) and streptomycin – penicillin (250) and amphotericin B. The cell suspension was centrifuged for 10 min at 1200 rpm and resuspended in 1 ml of the medium. Siliconized sterilized chips for electron microscopy were placed in 12-well plate (one per well). The cells were seeded in a concentration of 10 µl per substrate and 5 µl per chip. The adhesion of the cells was monitored for 2 hours. A total of 2 ml of the medium were added to the wells with chips and films and the system was left for a day.

The samples were fixed on the chips for scanning electron microscopy for 15 min by 2.5 % glutaraldehyde diluted with the culture medium, transferred to a solution of 2.5 % glutaraldehyde in 0.1 M cacodylate buffer and fixed for one hour. After washing with two changes of 0.1 M cacodylate buffer, the samples were fixed in 1 % osmium tetroxide, prepared in distilled water, washed with two changes of water, placed in a special holder, and dehydrated by incubation in increasing alcohols concentrations (30, 50, 70, 100 %) for 10 minutes. The samples were then dried using the critical point method and examined in a scanning electron microscope before and after coating with 1 nm of chromium in argon atmosphere. The analysis was performed at 1,000–30,000 magnification and accelerating voltage of 30 kV.

Immunohistochemical studies. The cells were fixed on special plastic substrates in 2.5 % glutaraldehyde solution in 0.1 M Na-cacodylate buffer (pH 7.4) for one hour and the material was washed in three changes of 0.1 M Na-cacodylate buffer (pH 7.4). The samples were postfixed in 1 % osmium tetroxide solution supplemented with 0.8 % potassium ferrocyanate in the same buffer for 1 h. The cells were then washed three times with distilled water and left overnight in a refrigerator at 4 °C in 1% aqueous solution of uranyl acetate. Next day, the samples were washed with water and dehydrated in increasing concentrations of alcohols (5 min in 30 and 50 %, 10 min in 70, 96 and 100 % ethanol solutions, respectively). The cells were then further dehydrated in acetone (twice, 20 min) and impregnated with resin mixture consisting of 4 components (Epon 812, DDSA, MNA and DMP-30) as following: resin:acentone 1:2 (v/v) for 1 h, resin:acentone 1:1 (v/v) for 2 h, resin:acentone 2:1 (v/v) for 2 h, pure resin for 2 h, pure resin for 1 h. The samples were poured into foil molds and incubated overnight in a CaCl2 desiccator to remove air bubbles from the resin. The samples were then placed in an oven at +60 °C for 3 days for polymerization.

The immunohistochemical studies were conducted in accordance with recommendations of the antibodies manufacturer. Prior to the study, the sections were dewaxed and tissue antigens were unmasked in RT Link-module in citrate buffer (pH 9.0) at 95 °C for 60 min. Endogenous peroxidase was then blocked with a 3 % H2O2, proteins were blocked with serum.

The tissue sections and cells were incubated with antibodies at 22 °C for 30 minutes. The following antibodies were used: GFAP (N1506 clone, rabbit polyclonal, DAKO); S100 (IR504 clone, rabbit polyclonal, DAKO); Synaptophysin (DAKSYNAP clone, mouse monoclonal, DAKO); Neurofilament Protein (NF, 2F11 clone, murine monoclonal, DAKO). All antibodies were diluted according to the manufacturer's instructions. The polymer detection system EnVision FLEX was used to visualize immunomorphological reaction. At the final stage, the cell nuclei were stained with hematoxylin.

Fluorescence immunohistochemistry studies. The cultured cells were fixed in 4 % formalin for 10 min. The formalin was removed from the cells by PBS for 10 min. After washing, the cells on the slides were kept in 4 % Triton X100 for 15 min and washed with PBS for 30 minutes. The hybridization was performed by BSA solution (bovine serum albumin) in a humid chamber for 5–30 min. The cells were stained with primary Neuronal Class III -Tubulin (TUJ1) and Anti-Neurofilament 200 antibodies. The secondary antibodies used in the experiment were 1 green 488 and 2a red 568. The nuclei were stained with DAPI.

Results

The cultured cells, isolated from external compartment of the growth plates located below and above the deformity, formed a monolayer consisting of bright round cells adjacent to each other (Fig. 1). Nucleus with 1–2 nucleoli and dispersed chromatin were located in the center of the cell. The cytoplasm contained chondroitin sulfates A and C and glycogen granules. The immunohistochemistry revealed the expression of aggrecan, Sox 9, chondroitin sulfates A and C, and types I and II collagen (Fig. 2). Electron microscopy revealed round cells with large light nuclei with intussusceptum.

The cytoplasm was rich in organelles and intermediate microfilaments. Mitochondria were large, predominantly oval, with short transverse cristae; endoplasmic reticulum had expanded cisterns; Golgi apparatus was represented by dictyosomes and numerous vesicles located near the plasma membrane. Accumulation of intermediate filaments was observed near nucleus (Fig. 3).

Several types of cells were isolated from the growth plates from the concave side of the deformity (scoliosis peak, Fig. 4). There were large multipolar cells with one long axon and numerous branching short processes (Fig. 5a). This type of cells had a spherical nucleus with 1–2 nucleoli located at the center of the cell, and granular reticulum, Nissl substance, was detected in the cytoplasm and processes. There were oval cells with a large centrally located nucleus and a narrow rim of Nissl-positive cytoplasm passing into long processes on both poles of the cells. Unibipolar and pseudounipolar cells were located nearby. The cytoplasm and processes of these cells contained Nissl substance (Fig. 5b). Immunohistochemically, these cells expressed NF-200 and III-tubulin proteins (stained with Neural crest III-tubulin and Anti-Neurofilament 200 antibodies) and NF-1 gene (Fig. 6). The second type is large cells with round nuclei and numerous branched cytoplasmic processes. These cells expressed astrocytic protein (stained with S-100 antibodies, Fig. 7a). The preparations contained round and oval cells with clear boundaries and multiple processes. At the center of such cell there was a large round nucleus, surrounded by cytoplasmic rim. These cells and their processes are Cajal and Gomori positive (Fig. 8). These cells expressed glial acidic protein (stained with GFAP antibodies; Fig. 6, 7b).

According to ultrastructural data (transmission microscopy), the first type of cells were elongated cells with a round nucleus and 1–2 electron-dense nucleoli (Fig. 8). One long process (axon) with membrane protrusions (axon hillocks) departed from the soma. Such protrusions (spikes) were located on

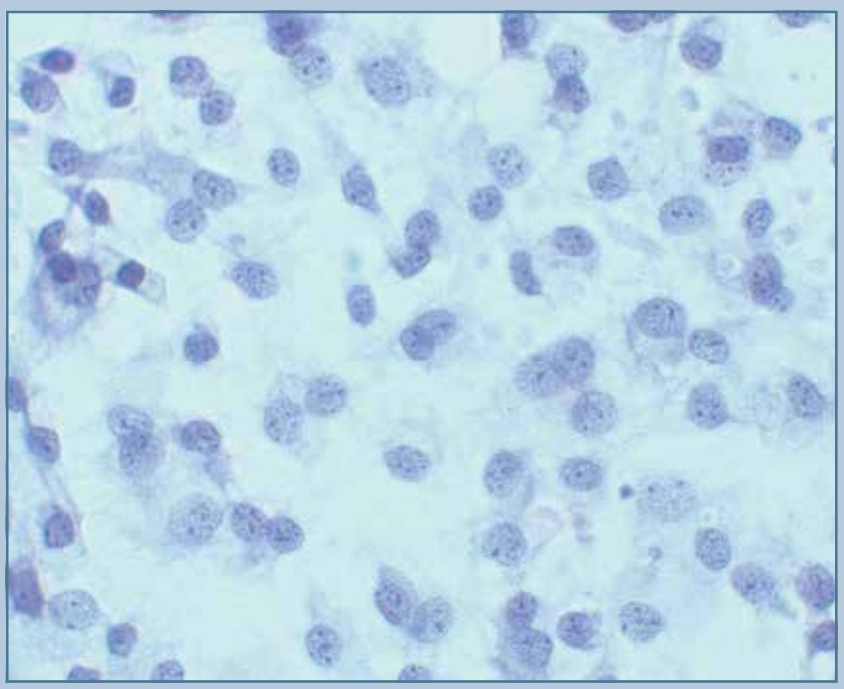

Fig. 1
Monolayer of the cultured chondroblasts from the convex side of the spinal deformity; hematoxylin-eosin, 10 × 60

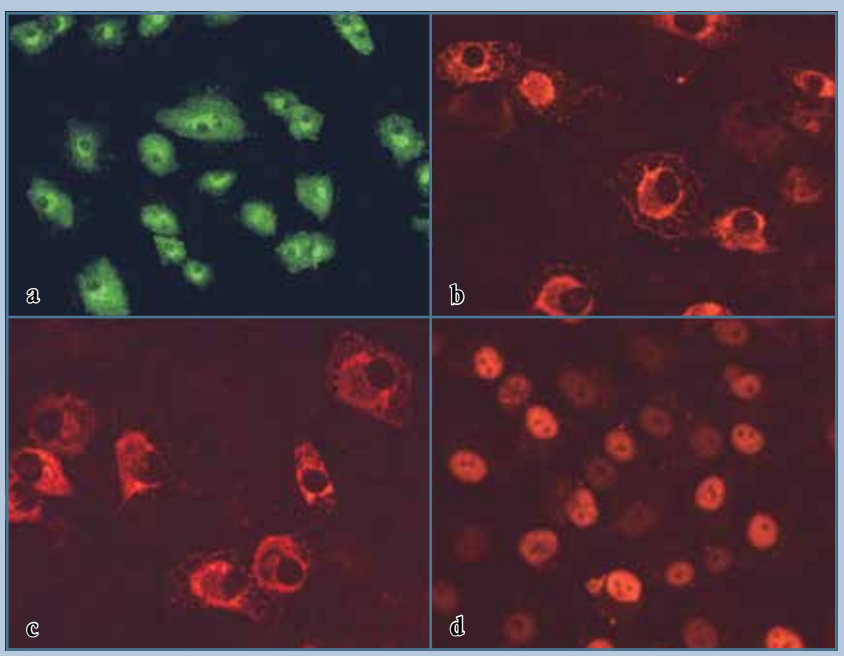

Fig. 2
Immunohistochemical protein identification: **a** – collagen type I (green); **b**, collagen type II (red); **c**, aggrecan (red); **d**, sox 9 (red)

the cell bodies as well. A large number of small light vesicles (vacuoles) were observed inside the spikes. Contacts (forming synapses) were detected between the axon and the soma. Small and large vacuoles were located on both sides of the sinus membranes. The cytoplasm of the cells was rich in organelles. The endoplasmic reticulum consisted of long, narrow cisterns with extended regions. Well-developed Golgi apparatus was represented by numerous dictyosomas and vesicles. There were exocytose vesicles. Mitochondria were elongated with branched thin cristae and dense matrix. The cells processes contained neurofilaments. Granules were detected in the cytoplasm and processes and were, most likely, of protein nature. In addition, there were stacks of rough endoplasmic reticulum, which resembled Nissl bodies. The second type of cells has large number of processes that form contacts. These cells contained a light nucleus with 1–2 nucleoli and heterochromatin bodies. In the cytoplasm, the endoplasmic reticulum is represented by branched canals. Golgi apparatus has numerous vesicles and exocytose vesicles. Mitochondria have different shapes with expanded cristae. The cytoplasm contains a large number (accumulation) of intermediate filaments. Neurofilaments were not detected in these cells (Fig. 9).

Scanning electron microscopy of the cells, obtained from the growth plates from the concave side of the deformity, revealed that the cells have elongated shape and long processes forming contacts. Synapses and a large spike are formed between the cells. The cytoplasm and processes contained dense granules, 300 to 500 nm in diameter (Fig. 10, 11). Numerous spikes on neurons processes (uncoated preparation).

Discussion

The results demonstrated that cell phenotypes in the vertebral body growth plates of patients with idiopathic scoliosis depend on localization. The cultured cells, isolated from the growth plates on the convex side of the deformity, were identified as chondroblasts. The evaluation criteria for chondrogenic cell differentiation were morphological structure, including ultrastructural organization, synthesis of organ-specific proteoglycans and expression of genes associated with the growth process [3].

The cultured cells, isolated from the growth plates on the concave side of the deformity, were identified as neuro- and glioblasts. Morphologically, neuroblasts are a multi-, uni-, bipolar, and pseudouniploar cells, which form multiple contacts with both the processes and the cell bodies. The cytoplasm

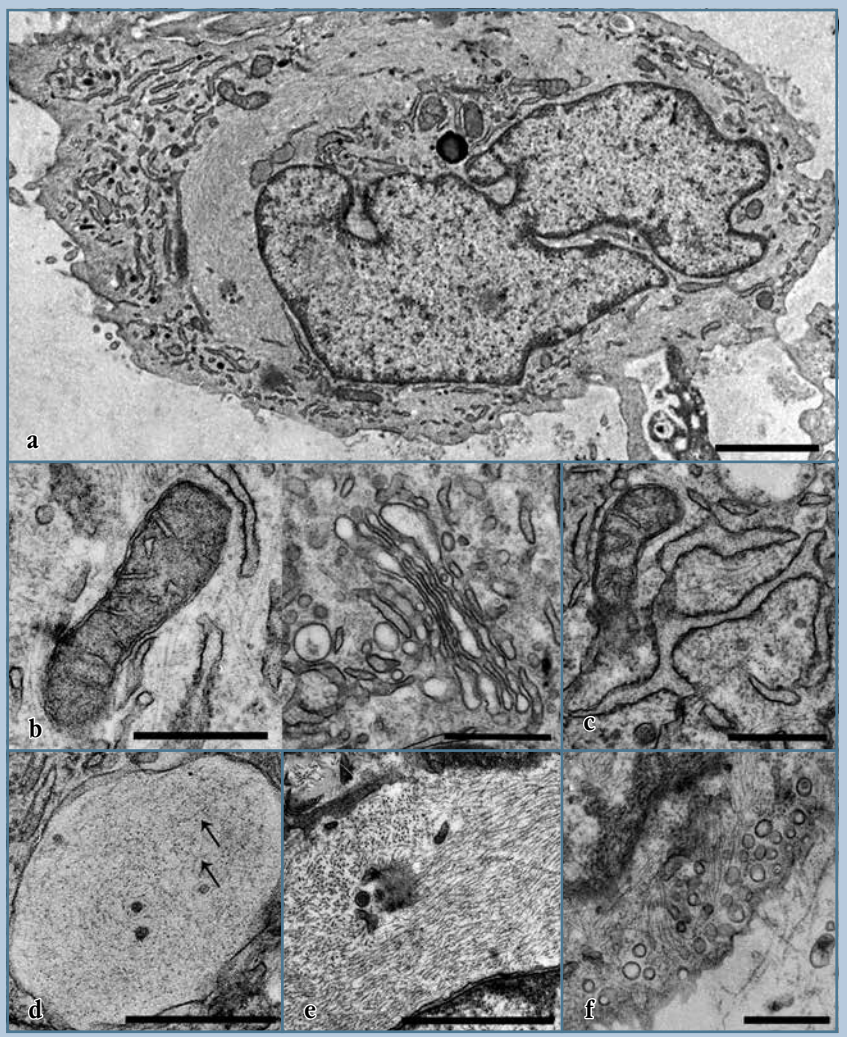

Fig. 3
Ultrastructure of the chondroblasts from the convex side of the growth plate at the peak of the deformity in a patient with idiopathic scoliosis (scale: **a**, 2 μm, **b–f**, 0.5 mm): **a**, general view of the cell containing the nucleus with intussusceptum and large concentration of intermediate filaments in the cytoplasm; **b**, mitochondria with short transverse cristae, narrow and expanded rough endoplasmic reticulum cisterns; **c**, dictyosomes of the Golgi apparatus; **d**, vacuole with short thin filaments (arrows) inside; **e**, accumulation of intermediate filaments near the nucleus; **f**, numerous vesicles near the plasma membrane of the cell

of the cells contained Nissl substance, neurospecific proteins III-tubulin, NF-1 and NF-200 gene were expressed.

The data of electron microscopy suggested neural genesis of the cells under study: they have extended network of neurofilaments, formed and forming sinuses with vesicles, typical elongated mitochondria. The processes and cell bodies had numerous axon hillocks-spikes, containing vesicles. Multiple contacts were identified between the processes and the cells. The second type was round cells that contained a large number of processes that form networks. The process and the cells were Gomori and Cajal positive and expressed glial proteins. Based on morphological and ultrastructural data this type of cells was classified as glioblasts.

Naturally, the question arises how can the cells of neural genesis be localized in the growth plates of patients with idiopathic scoliosis? To answer this question one should consider the early stages of embryogenesis.

It is known that the spine is formed from the mesenchyme [5]. However, at the stage of the neural tube formation, the neural crest cells separate and migrate along three routes [4, 6].

One of the routes of the neural crest cells migration is a truncal route that passes through the front (rostral) somite's section and, ultimately, these cells form the sensory ganglia [25]. The migrating cells undergo epithelial-mesenchymal transformation by switching expression from neural cell adhesion proteins to mesenchymal adhesion proteins [7].

While rounding, the neural crest cells acquire the phenotype of mesenchymal cells and do not differ from the surrounding cells [22]. This process is

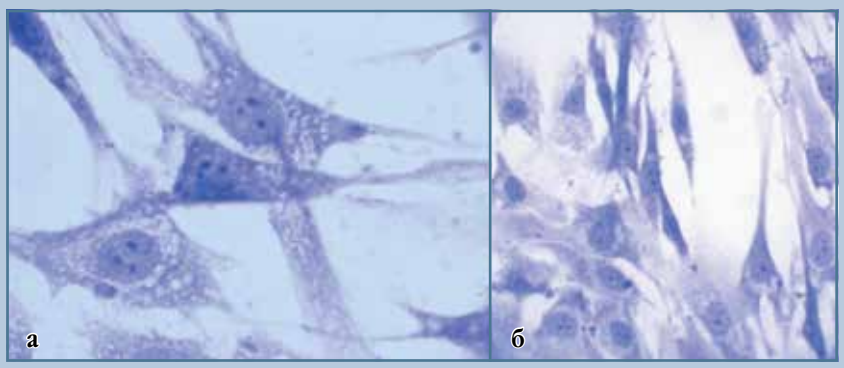

Fig. 5
Cell culture: idiopathic scoliosis, concave side of the peak of the deformity; Nissl stain; magnification 400 **(а)** and 200 **(б)**

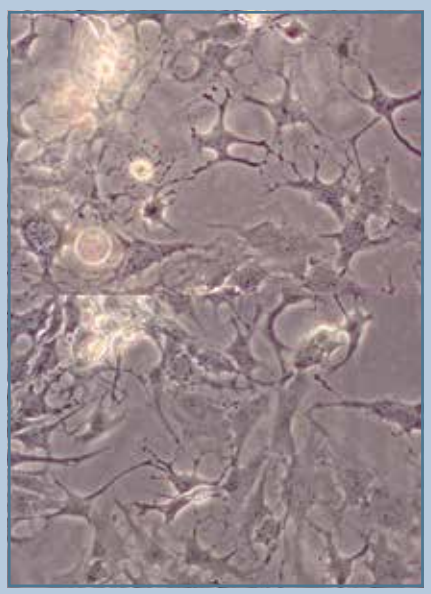

Fig. 4
The cells of neural origin in the cell culture from the concave side; native preparation; 10 × 40

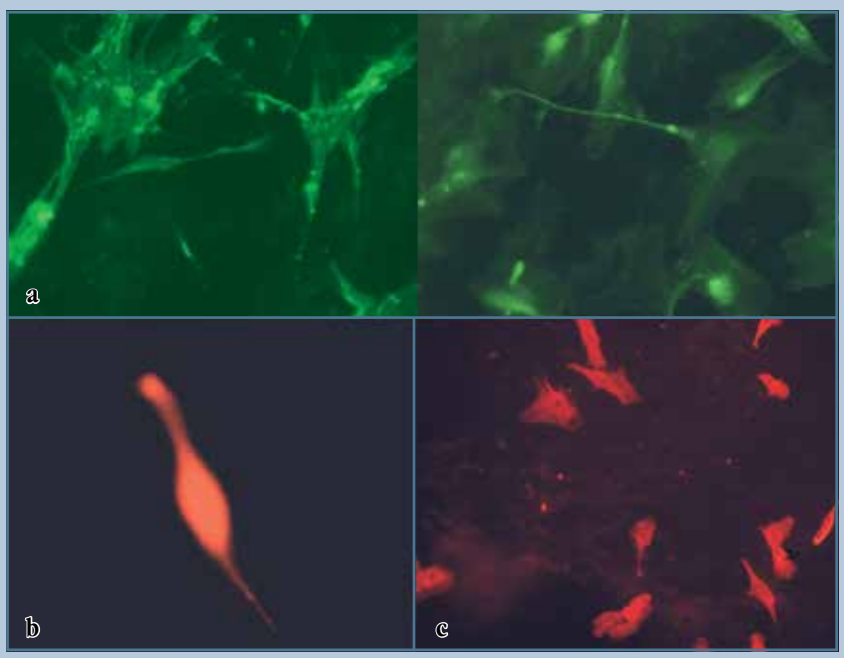

Fig. 6
Immunohistochemical straining for neutral antibodies of the cultured cells from the concave side of the spinal deformity in idiopathic scoliosis: **a**, NF-1 (green); **b**, III -tubuline (red); **c**, NF-200 (red)

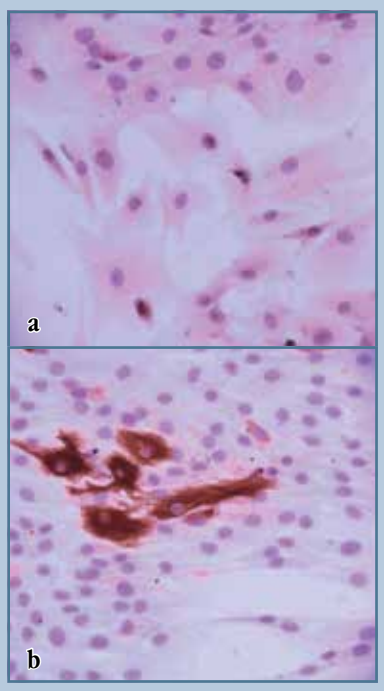

Fig. 7
Immunohistochemical staining for glial proteins: **a**, S100 astrocytic protein, magnitude 200; **b**, GFAP, glial protein, magnitude 200

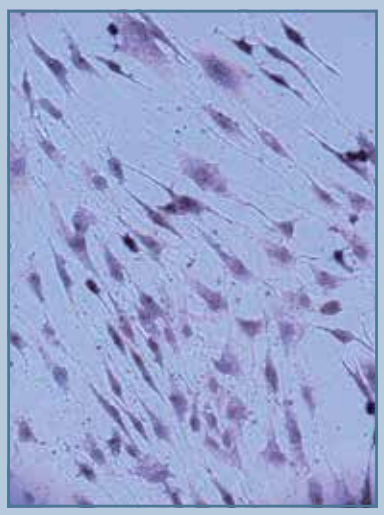

Fig. 8
Cell culture: idiopathic scoliosis, concave side at the peak of the deformity; Cajal stain, magnification 200

defined by subsequent migration of the neural crest cells along mesenchymal substrate, which lines the route from the neural tube to the somites. The formation of the substrate is associated with expression of Pax 3 gene and synthesis of two isoforms of versican (V1, V0) [13]. The trajectory of the neural crest cells movement is regulated by asymmetric distribution of induction (V0, V1 versican) and inhibition factors [19]. The neural crest cells movement is inhibited via high molecular mass polymer proteoglycan, aggrecan, and is associated with restriction in dispersion of the neural crest cells by glycosaminoglycans side chains [23]. Chondrogenic differentiation of the mesenchymal cells in the somites occurs during the neural crest cells migration. These data demonstrate the interdependence of chondro- and gangliogenesis [16]. Moreover, the removal of the somite results in inability to form sensory ganglia and disruption of the somite segmentation causes malformed ganglia [8]. Therefore, the migration of the neural crest cells through the somites is logical and, moreover, there is concurrent regulation of morphogenesis of the spine and sensory ganglia [14].

The neural crest cells migration through the somites is one of the stages in the formation of the spine and sensory ganglia. Given the tropism of these two structures, it is unclear to what extent the function of the sensitive ganglia is disrupted in idiopathic scoliosis. The answer to this question requires further research. The presence of neural crest-derived cells in the vertebral body growth plates of patients with idiopathic scoliosis is, undoubtedly, the result of disruption of one of the stages of the spine morphogenesis.

One can presume that disruption of spatial and temporal patterns of the neural crest cells migration results in some of them settling and being deposited in the somite. It may occur due to Pax 3 mutation, with subsequent disruption of versican synthesis along the migration route [13]. These data were confirmed by Krull et al. [14]. Disruption of versican secretion and inhibition of versican sulfation interrupted the neural crest cells migration. The interaction of the neural crest cells with the interstitial matrix follows "cell – cell – matrix" principle [10]. Any disruption in the synthesis and/or interactions of the neural crest cell receptor (integrin) with migration substrate molecules may prevent further morphogenetic events in the somite [25].

Inhibition of the neural crest cells migration may be associated with many factors, but there is an obvious question: why do vertebral body growth plates in the areas of the neural crest cells deposits contain poorly differentiated chondroblasts? The neural crest cells are known to assume the phenotype of the cells at the site of their final migration, matching the environment to which they have migrated, but their genotype remains unchanged [17]. Understandably, the neurogenic cells, located in the vertebral body growth plates, are not primed for the growth process. It explains the lack of chondrogenic cells differentiation in the growth plate and areas of their localization, which leads to asymmetry and local disruption of growth with the subsequent development of the spinal deformity. Ultimately, disorders of the spine morphogenisis during the embryogenesis emerge as scoliosis during the growth periods, with all clinical and morphological manifestations of the disease.

Based on our own and literature data it is possible to make some assumptions about variability of clinical manifestations of scoliosis. First of all, about the causes of progression of the spine deformity and preferential development the thoracic spine deformity in idiopathic scoliosis.

Since the migration of the neural crest cells occurs at intervals of 10–1 μ and the distance between them is a diameter of a cell [15], the number of deposited cells can be different and it determines the degree of chondrogenesis disorder and growth asymmetry. If the number of the deposited cells is small, the spine deformity occurs at the stage of intensive growth (growth phase I), but it subsequently levels out due to an

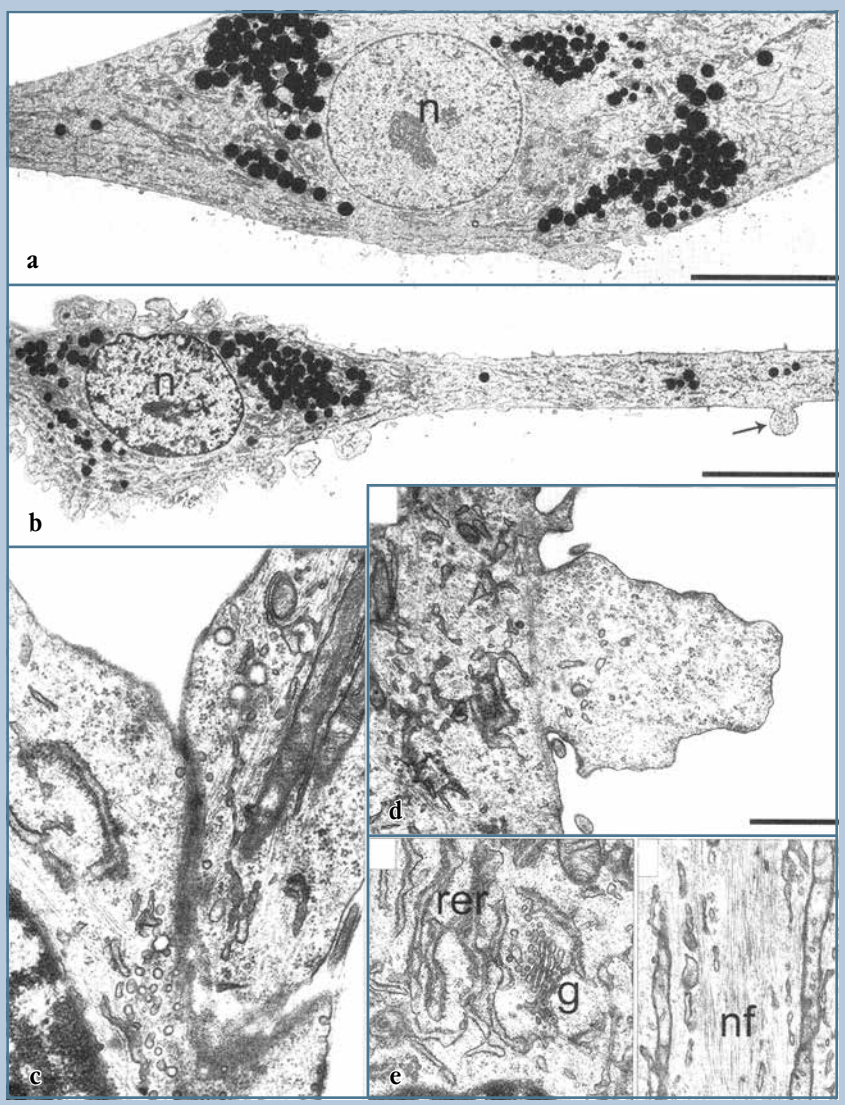

Fig. 9
Micrograph of the cultured cells, isolated from the vertebral body growth plates (concave side, peak of the deformity) of patients with idiopathic scoliosis (scale: **a**, 10 μm, **b–e**, 1 μm) **a**, neuron-like elongated cells with a long process (axon), numerous electron-dense granules around the nucleus and in the cytoplasm of the cells, spikes which are formed on axons (arrow); **b**, fragment of the cytoplasm of a cell from the culture with well-developed rough endoplasmic reticulum and Golgi complex; **c**, fragment of an axon with extensive network of neurofilament penetrating processes of the cells and elongated mitochondria; **d**, forming contact between the axon of one cell and the body of another, numerous vesicles at the point of contact; **e**, spikes that are formed on the body and processes of the cells

unchanged growth plate or remains at the early stages of development (I–II degree idiopathic scoliosis). These data are based on previous experimental studies by A.M. Zaidman (unpublished data). A focal damage to a growth plate in a growing animal led to a slight deformity, which subsequently leveled during the linear growth period or did not progress. If the half of a growth plate was damaged, however, an animal developed pronounced deformity that progressed until the end of growth.

One of unexplained questions of vertebrology is the preferential development of the thoracic spine deformity in idiopathic scoliosis. The analysis of the trajectory of the neural crest cells migration can provide an answer to this question. Since the neural crest cells migration along the trunk route passes only through the thoracic somites, the impaired movement and deposition of the cells subsequently emerge as development of growth asymmetry and deformity of the thoracic spine. Presumably, the development of the lumbar deformity can be attributed to the impaired migration of the neural crest cells along route II and the formation of motor ganglia.

In conclusion, the analysis of the differentiated growth of the cell cultures from vertebral body growth plates of 50 patients with III–IV degree idiopathic scoliosis revealed causes of impaired growth and development of the spinal deformity, and suggested explanations for variability of clinical manifestations of idiopathic scoliosis based on the degree of the disorder and the morphogenetic processes in the vertebral body growth plates.

Conclusions

1. Ectopic localization of the neural crest-derived cells, which are not genetically pre-programmed to undergo chondrogenic differentiation and growth process, in the vertebral body growth plates is the etiological factor of scoliosis.

2. Local disruption of chondrogenesis in the vertebral body growth plates of patients with idiopathic scoliosis is the cause of growth asymmetry and development of the spinal deformity in idiopathic scoliosis.

3. The extent of the structural changes in the spine and the prognosis of progression of the deformity depend on the degree of disruption of morphogenetic processes in the vertebral body growth plate which occurred during the embryogenesis.

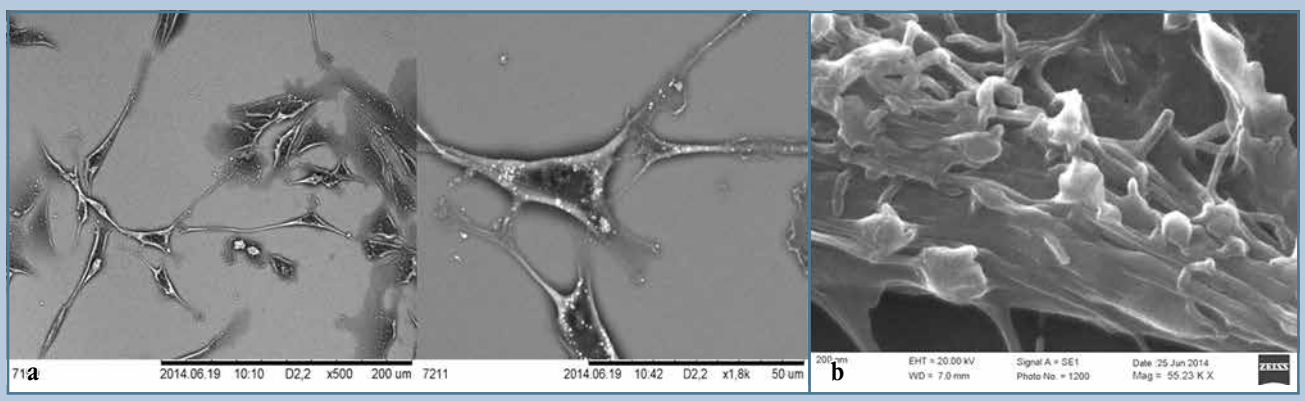

Fig. 10
Scanning microscopy: neurons in the cell culture derived from the vertebral body growth plate (concave side of the peak of the deformity) of a patient with idiopathic scoliosis, uncoated preparation: **a**, general view of the cells at small magnification, the cells have elongated shape and long processes, the scale of 200 μm; **b**, contacts formed between two cells, synapse and large spike are visible, the cytoplasm of neurons and the processes contain small, dense granules of 300 to 500 nm in diameter, the scale of 50 μm

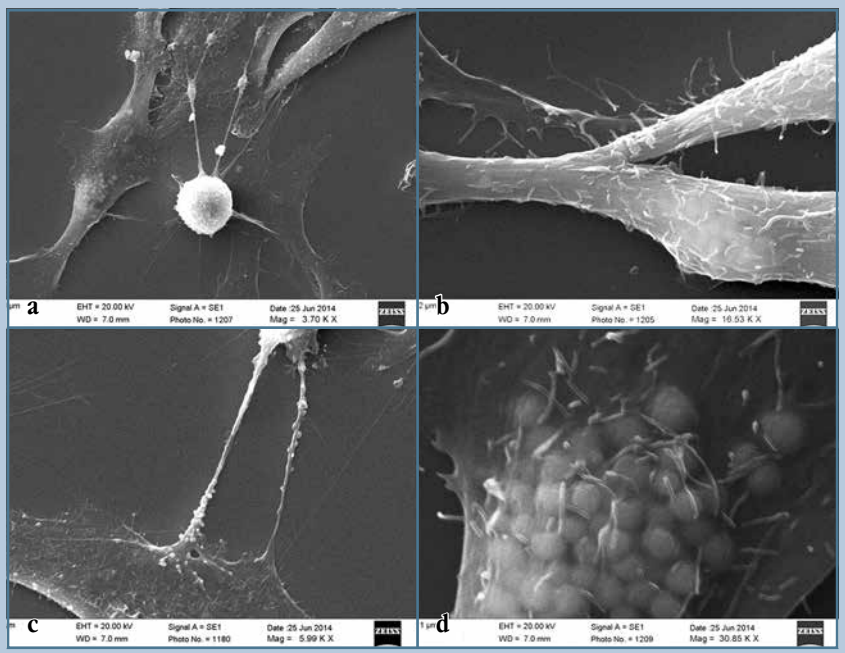

Fig. 11
Scanning microscopy: appearance of neurons in the cell culture derived from the vertebral body growth plate (concave side of the peak of the deformity) of a patient with idiopathic scoliosis, coated preparation (scale: a, 1 μm, b–d, 2 μm): **a**, general view of the cells; **b**, fragment of a neuron process at high magnification; **c**, neuron process (axon) with spikes; **d**, a fragment of a neuron at high magnification, the surface contains short processes, granules in the cytoplasm are visible through the plasma membrane

Литература/References

1. **Zaidman AM.** [Idiopathic Scoliosis: Morphology, Biochemistry, Genetics. Novosibirsk, 1994. In Russian].
2. **Zaidman AM, Mikhailovsky MV, Sadovoy MA.** [Neurofibromatosis and Scoliosis. Novosibirsk, 2011. In Russian].
3. **Zaidman AM, Strokova EL, Novikov VV, Vasyura AS, Mikhailovsky MV, Sadovoy MA.** [Gene expression in growth plate chondrocytes of patients with idiopathic scoliosis. Hir Pozvonoc. 2014;(4):88–98. In Russian].
4. **Carlson BM.** [Patten's Foundations of Embryology. Moscow, 1983. In Russian].
5. **Knorre AG.** [Embryonic Histogenesis. Leningrad, 1971. In Russian].
6. **Tokin BP.** [General Embryology. Moscow, 1977. In Russian].
7. **Bronner-Fraser M, Garcia-Castro M.** Manipulations of neural crest cells or their migratory pathways. Methods Cell Biol. 2008;87:75–96. DOI: 10.1016/S0091-679X(08)00204-5.
8. **Bundy J, Rogers R, Hoffman S, Conway SJ.** Segmental expression of aggrecan in the non-segmented perinotochordal sheath underlies normal segmentation of the vertebral column. Mech Dev. 1998;79:213–217.
9. **Dayer R, Haumont T, Belaieff W, Lascombes P.** Idiopathic scoliosis: etiological concepts and hypotheses. J Child Orthop. 2013;7:11–16. DOI: 10.1007/s11832-012-0458-3.
10. **Erickson CA, Perris R.** The role of cell-cell and cell-matrix interactions in the morphogenesis of the neural crest. Dev Biol. 1993;159:60–74. DOI: 10.1006/dbio.1993.1221.
11. **Gorman KF, Julien C, Moreau A.** The genetic epidemiology of idiopathic scoliosis. Eur Spine J. 2012;21:1905–1919. DOI: 10.1007/s00586-012-2389-6.
12. **Guo X, Chau WW, Hui-Chan CW, Cheung CS, Tsang WW, Cheng JC.** Balance control in adolescents with idiopathic scoliosis and disturbed somatosensory function. Spine. 2006;31:E437–E440. DOI: 10.1097/01.brs.0000222048.47010.bf.
13. **Henderson DJ, Ybot-Gonzalez P, Copp AJ.** Over-expression of the chondroitin sulphate proteoglycan versican is associated with defective neural crest migration in the Pax3 mutant mouse (splotch). Mech Dev. 1997;69:39–51. DOI: 10.1016/S0925-4773(97)00151-2.
14. **Krull CE.** Inhibitory interactions in the patterning of trunk neural crest migration. Ann NY Acad Sci. 1998;857:13–22.
15. **Krull CE, Collazo A, Fraser SE, Bronner-Fraser M.** Segmental migration of trunk neural crest: time-lapse analysis reveals a role for PNA-binding molecules. Development. 1995;121:3733–3743.
16. **LeDouarin NM, Teillet MA.** Experimental analysis of the migration and differentiation of neuroblasts of the autonomic nervous system and of neurectodermal mesenchymal derivatives, using a biological cell marking technique. Dev Biol. 1974;41:162–184. DOI: 10.1016/0012-1606(74)90291-7.
17. **Logan M, Martin JF, Nagy A, Lobe C, Olson EN, Tabin CJ.** Expression of Cre Recombinase in the developing mouse limb bud driven by a Prxl enhancer. Genesis. 2002;33:77–80. DOI: 10.1002/gene.10092.
18. **Machida M, Dubousset J, Yamada T, Kimura J.** Serum melatonin levels in adolescent idiopathic scoliosis prediction and prevention for curve progression – a prospective study. J Pineal Res. 2009;46:344–348. DOI: 10.1111/j.1600-079X.2009.00669.x.
19. **McGonnellIM,GrahamA.**Trunkneuralcresthasskeletogenicpotential.CurrBiol. 2002;12:767–771. DOI: 10.1016/S0960-9822(02)00818-7.
20. **Miller NH.** Idiopathic scoliosis: cracking the genetic code and what does it mean? J Pediatr Orthop. 2011;31(1 Suppl):S49–S52. DOI: 10.1097/BPO.0b013e318202bfe2.
21. **Ogilvie JW.** Update on prognostic genetic testing in adolescent idiopathic scoliosis (AIS). J Pediatr Orthop. 2011;31(1 Suppl):S46–S48. DOI: 10.1097/BPO.0b013e3181fd87eb.
22. **Peris R, Perissinotto D.** Role of the extracellular matrix during neural crest cell migration. Mech Dev. 2000;95:3–21. DOI: 10.1016/S0925-4773(00)00365-8.
23. **Pettway Z, Domowicz M, Schwartz NB, Bronner-Fraser M.** Age-dependent inhibition of neural crest migration by the notochord correlates with alteration in the S103L chondroitin sulfate proteoglycan. Exp Cell Res. 1996;255:195–206. DOI: 10.1006/excr.1996.0170.
24. **Porter RW.** The pathogenesis of idiopathic scoliosis: uncoupled neuro-osseous growth? Eur Spine J. 2001;10:473–481.
25. **Roffers-Agarwal J, Gammill LS.** Neuropilin receptors guide distinct phases of sensory and motor neuronal segmentation. Development. 2009;136:1879–1888. DOI: 10.1242/dev.032920.
26. **Salehi LB, Mangino M, De Serio S, De Cicco D, Capon F, Semprini S, Pizzuti A, Novelli G, Dallapiccola B.** Assignment of a locus for autosomal dominant idiopathic scoliosis (IS) to human chromosome 17p11. Hum Genet. 2002;111:401–404. DOI: 10.1007/s00439-002-0785-4.
27. **Stokes IA, Burwell RG, Dangerfield PH.** Biomechanical spinal growth modulation and progressive adolescent scoliosis – a test of the "vicious cycle" pathogenetic hypothesis: summery of an electronic focus group debate of the IBSE. Scoliosis. 2006;1:16. DOI: 10.1186/1748-7161-1-16.
28. **Veldhuizen AG, Wever DJ, Webb PJ.** The aetiology of idiopathic scoliosis: biomechanical and neuromuscular factors. Eur Spine J 2000;9:178–184. DOI: 10.1007/s005860000142.
29. **Wise CA, Gao X, Shoemaker S, Gordon N, Herring JA.** Understanding genetic factors in idiopathic scoliosis, a complex dislase of childhood. Curr Genomics. 2008;9:51–59. DOI: 10.2174/138920208783884874.

Address correspondence to:
Zaidman Alla Mikhaylovna NNIITO, Frunze str., 17, Novosibirsk, 630091, Russia,
AZaydman@niito.ru

Alla Mikhailovna Zaidman, MD, DMSc, Prof.; Elena Leonidovna Strokova, researcher, Novosibirsk Research Institute of Traumatology and Orthopaedics n.a. Ya.L. Tsivyan; Tatyana Avgustovna Ageeva, MD, DMSc, Novosibirsk State Medical University; Elena Vladimirovna Kiseleva, PhD in Biology; Anton Aleksandrovich Strunov, PhD in Biology; Lyubov Aleksandrovna Suldina, MD fellow; Shevchenko Aleksander Igorevich, PhD in Biology, Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia.