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A THERAPEUTIC APPROACH FOR RHABDOSPHINCTER REGENERATION BY AUTOLOGOUS ADULT STEM CELLS

Hypothesis / aims of study

Human mesenchymal stem cells have the potential to differentiate into both smooth and skeletal muscle cells (1). As stress urinary incontinence is associated with damaged sphincteric myofibers or with an age-dependent decrease of myofibers by apoptosis autologous mesenchymal stem cells are applicable for a functional treatment of stress urinary incontinence (2). The aims of the stydy were firstly, to investigate survival and myogenic differentiation of human mesenchymal stem cells in a rat model and secondly, to demonstrate a linkage to the host's nerve system.

Study design, materials and methods

Human mesenchymal stem cells were isolated from bone marrow aspirates by plastic adherence and propagated in vitro. To induce myogenic differentiation human mesenchymal stem cells were exposed to 5-azacytidine (AZA) in cell passage (P) 1 (3). Native mesenchymal stem cells in P1 and P3 as well as AZA-exposed stem cells of subsequent P2 or P3 were directly injected into the rectus abdominis muscle of athymic nude rats. For in vivo tracking mesenchymal stem cells were labeled with PKH26 red fluorescent cell linker. Integration and myogenic differentiation of stem cells in rat muscle tissue was monitored histologically from 4 days up to 16 weeks after cell injection. Immunohistochemistry was performed on cryosections for the detection of the muscle markers desmin and skeletal muscle myosin heavy chain with the specific antibodies clone D33 and clone NOQ7.54D, respectively. To investigate innervation of newly formed skeletal muscle cryosections were stained with alpha-bungarotoxin conjugate that binds to the acetylcholine receptors of motor end plates.

Results

Native and AZA-exposed mesenchymal stem cells of all passages could be demonstrated in all animals investigated. Histology of animals in the short-term experiments up to 8 days revealed well-defined clusters of transplanted mesenchymal stem cells (red PKH26 fluorescence) in the rectus abdominis muscle. After 4 and 8 weeks of cell injection, a continuous dissemination of transplanted mesenchymal stem cells was detected. Histology of animals in the long-term study revealed PKH26-positive myofibers that were in parallel with the native skeletal muscle fibers. Immunohistochemistry for myogenic desmin and skeletal muscle myosin heavy chain demonstrated striated myofibers and skeletal muscle myosin in PKH26-positive myofibers. Staining for acetylcholine receptors showed motor end plates adjacent to newly formed PKH26-positive myofibers.

Interpretation of results

The results demonstrated a long time survival of transplanted mesenchymal stem cells over a period of 16 weeks. The findings support that mesenchymal stem cells are capable of integrating into the host's tissue and differentiating into skeletal muscle cells, dependent on the muscle environment. The proximity to the skeletal muscle is able to trigger the differentiation of transplanted and integrated mesenchymal stem cells. Our data suggest a development of single stem cells into myotubes and subsequently the formation of myofibers that are well integrated into the host tissue. Innervation of the newly formed muscle indicates a functional integration of transplanted and myogenic differentiated mesenchymal stem cells.

Concluding message

The experimental athymic rat model demonstrates the potential of human bone marrow-derived mesenchymal stem cells to differentiate into striated muscle. The data offer a new option for regeneration of damaged rhabdosphincters as a functional treatment of stress urinary incontinence based on autologous adult stem cells. Further in vivo studies in a large animal model are performed to identify a functional improvement of rhabdosphincters by urodynamics.

References

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Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Bone marrow was obtained from healthy individuals after informed consent according to the guidelines of the Ethics Committee of the University of Tübingen (project number 268/2003V). The studies in the animals were performed in conformity with guidelines for the care and use of animals.**