3

Feil G[1], Zimmermann R[1], Maurer S[1], Boehmler A M[2], Krug J[1], Wanke I[1], Bock C[1], Moehle R[2], Seitz G[2], Stenzl A[1], Sievert K[1]
1. Department of Urology, University of Tuebingen, 2. Department of Medicine II, University of Tuebingen

MESENCHYMAL STEM CELLS FOR FUNCTIONAL TREATMENT OF URINARY INCONTINENCE IN A RAT MODEL

Hypothesis / aims of study

Urinary incontinence is caused by functional and morphological defects of the rhabdosphincter, which is usually associated with damaged sphincteric myofibers after surgery for cancer or BPH or with age-dependent decrease of myofibers by apoptosis. However, none of the different therapies can heal defects or reconstruct the muscle. As reported recently, the injection of autologous stem cells directly into the rhabdosphincter is a promising treatment modality for a functional therapy of urinary incontinence (1). Aim of our study was to examine myogenic differentiation of mesenchymal stem cells (MSC) and to monitor the integration of differentiated MSC into the urethral sphincter in a rat model.

Study design, materials and methods

So far, injection of differentiated MSC into the urethral sphincter was performed in 13 Lewis rats (7 females, 4 males, body weight 150-630 g). For this purpose MSC were isolated from bone marrow from 2 male Lewis donor rats by adherence on the bottom of a culture flask and grown to confluence in minimum essential medium supplemented with 20% FCS, 2% sodium pyruvate, 1% glutamine, and 1% penicillin/streptomycin. To induce differentiation of MSC into myoblasts once-passaged cells were exposed to 5-azacytidine for 24 hours (2) and then propagated. Cell differentiation was examined in 2 and 3 times passaged cells with and without exposure to 5-azacytidine by immunocytochemistry with monoclonal antibodies against alpha-smooth muscle actin (clone 1A4, clone CGA7), skeletal muscle MyoD (clone MoAb5.8A), and skeletal slow muscle myosin (clone NOQ7.54D). For in vivo tracking differentiated MSC were detached by trypsin/EDTA and stained with PKH26 (1mM), a red fluorescent cell linker. PKH26-labeled cells were directly injected into the bladder neck of the animals. To monitor integration of the applied cells into the host tissue animals were sacrificed 2, 4, 7, and 45 days after cell injection. For histology the bladder and urethra were excised en bloc and fixed in 18% sucrose. Cryosections were performed and stained with hematoxylin.

Results

Immunocytochemistry showed positive reactions with the anti-alpha-smooth muscle actin antibodies 1A4 and CGA7 both in two and three times passaged cells after exposure to 5-azacytidine. 1A4 identifed about 50% to 90% and CGA7 some 20% of the cells. In contrast, anti-skeletal muscle MyoD antibody MoAb5.8A only detected single cells in both cell passages. There was no immunoreactivity with anti-skeletal slow muscle myosin antibody NOQ7.54D. Immunostaining of undifferentiated MSC with these antibodies was negative each time. Histologic examinations revealed well-defined groups of fluorescent cells in bladder neck tissues. Injected cells could be demonstrated in host tissues 2, 4, 7, and 45 days after injection.

Interpretation of results

Early myogenic differentiation of MSC in vitro after exposure to 5-azacytidine indicates the development both of smooth muscle and skeletal muscle cells. Transplantation of MSC in the bladder neck was technically feasible in the rat model. Staining of cells with PKH26 allows long-time monitoring of morphological changes of grafted cells.

Concluding message

The data obtained are providing the basis for further studies of differentiation of MSC into myoblasts and myofibers in vitro and in the rat model as well as for investigations of human MSC in a xenograft animal model regarding the functional regeneration of the rhabdosphincter by transplantation of autologous MSC.

References
(1) Stem cell therapy for urinary incontinence. Urologe A 2004; 43(10): 1237-41
(2) Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. Muscle Nerve 1995; 18(12): 1417-26