

P3.26**Local and systemic transplantation of human adipose-derived stem cells into the GRMD dog**

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Progressive muscular dystrophies (PMD) are an untreatable group of disorders characterized by progressive degeneration of skeletal muscle caused by the absence or defective muscular proteins. The possibility to restore the faulty muscle protein and improve muscle function through cell therapy is a promising approach for the treatment of PMD. Different animal models are available for pre-clinical studies; however the only animal model that reproduces the full spectrum of human pathology is the Golden Retriever Muscular Dystrophy (GRMD) dog, a model for Duchenne Muscular Dystrophy. Affected animals carry a mutation that predicts a premature termination codon in exon 8 and a peptide that is 5% the size of normal dystrophin. These dogs present clinical signs within the first weeks of life involving the limbs as well as masticatory muscles. Diaphragmatic and intercostal muscles impairment leads to progressive respiratory failure. Here we have injected human adipose-derived stem cells (hADSC) intravenously, without immunosuppression, into GRMD dogs. We show that hASCs are able to reach, engraft and fuse to the host GRMD dystrophic muscle. Interestingly, we found a strong band of human dystrophin by WB, but a modest number of labeled fibers by IF. Additional studies are currently underway to better define the localization of human dystrophin at the GRMD muscles. We are also comparing the effect of local injections of canine versus human ASCs. These results may have important applications for future therapy in patients with different forms of muscular dystrophies.

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P3.27**The amazing regenerative potency of human satellite cells - analysis in single fibers**

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Satellite cells are quiescent but ready-to-go stem cells located beneath the basal lamina of skeletal muscle. They effectively repair injured muscle until old age but their regenerative potential could not be utilized yet for therapeutic strategies in muscular dystrophies. While our increasing knowledge on murine satellite cells has shed light on the importance of Pax3, Pax7 and Notch-signaling, the understanding of the human counterpart has remained very poor. We aim to characterize the human satellite cell.

We analyzed single human muscle fibers ($n = 540$) that were dissected manually immediately after muscle biopsy ($n = 42$) and subsequently cultured in coated wells. Fibers were stained for satellite cell markers (Pax7, Myf5, CD56, c-Met, Syndecan-4, CD34) and desmin in two day intervals thereafter up to a period of 28 days. Biopsy material was obtained from patients aged 27 to 87 years without known neuromuscular disorders during surgery after due approval. We found that early after initiation of culture proliferating, migrating desmin-positive cells could be identified *within* single fibers if the basal lamina remained intact. Only after six to ten days in culture

the first cells evaded the fiber and extensive proliferation of desmin-positive cells followed (after 14 days 1000/fiber). Interestingly, the number of desmin-positive cells/ fiber reached similar numbers independent of the age of the donor, although the number of Pax7 positive cells as a marker of quiescent satellite cells diminished with age. We therefore conclude that the generation of proliferating desmin positive cells within human muscle fibers is at least partly independent from the Pax7 positive cell population. Furthermore, studying satellite cell fate within intact human muscle fibers adds a new approach to this interesting area of research. Disclosure: Supported by German Ministry of Research and Education (SatNet).

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P3.28**Do factors released from dystrophic muscle enhance myogenic differentiation of mesenchymal stem cells from human umbilical cord tissue?**

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Progressive muscular dystrophies are a group of disorders characterized by progressive and irreversible muscle degeneration for which there is no therapy. Human umbilical cord tissue (HUCT) has been considered as an important source of mesenchymal stem cells (MSC) with the ability to differentiate into distinct cell types. However, there is limited information concerning the most favorable conditions to induce differentiation of MSC into muscle cells. It has been proposed that factors released from injured muscle provide the signals that contribute to the establishment of a favorable micro-environment to start the regeneration process. However, it is not known whether local signals released after damage are specific for muscle progenitor cells or whether they also promote homing as well as the myogenic commitment and differentiation of human MSC. Here we investigated, for the first time, if dystrophic muscle releases factor(s) capable of inducing the myogenic differentiation of MSC from HUCT *in vitro*. For this purpose, a conditioned medium from *mdx* muscle, the murine model of Duchenne muscular dystrophy, was prepared and its myogenic effect on MSC was evaluated after incubating cells in culture medium containing either 0,5% FBS alone (negative control) or supplemented with 0,5 mg/mL of conditioned medium. A medium supplemented with 3% of horse serum was used as a positive control. We observed that the incubation of MSC isolated from HUCT in the conditioned medium prepared from *mdx* muscle, was much more efficient in the differentiation of MSC in muscle cells and fusion into multinucleated myotubes (with a transient increase of MyoD and myogenin expression), as compared to cells incubated under control conditions. These results suggest that inflammatory and growth factors with myogenic effects, present in this conditioned medium, may be involved in such MSC differentiation. These results may have important applications for future therapies in patients with different forms of muscular dystrophies.

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