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Biol 509 – Term Paper

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**Dystrophin conferral using human endothelium expressing HLA-E in the non-immunosuppressive murine model of Duchenne muscular dystrophy**

Chang-Hao Cui, Shunichiro Miyoshi, Hiroko Tsuji, Hatsune Makino, Seiichi Kanzaki, Daisuke Kami, Masanori Terai, Harumi Suzuki and Akihiro Umezawa (2011).

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Objectives

Duchenne muscular dystrophy (DMD) is a severe, recessive X-linked form of muscular dystrophy, characterized by rapid progression of muscle degeneration that eventually leads to loss in ambulation, paralysis and death. The disorder is caused by a mutation in the gene encoding dystrophin, an important structural component of muscle tissues. The absence of intact dystrophin results in destabilization of the extracellular membrane-sarcolemma-cytoskeleton architecture, making muscle fibers susceptible to contraction associated mechanical stress and degeneration. Several protocols have been developed for cell-based therapies, especially using an mdx mouse model, in which dystrophin is defective due to a single point mutation. This article exploited the immunosuppressive role of HLA-E in a xenogeneic system; using human placental artery-derived endothelial (hPAE) cells in the mouse model.

This study is important because it illustrates a promising source of cell-based therapy for patients with muscular dystrophy. This study focused on the efficient transdifferentiation of hPAE cells into myoblast/myocytes by transplanting the cells into mdx mice.

Experimental Approach and Results

hPAE cells were cultured from placental arteries and cytometric analysis was used to confirm that the cells were of endothelial origin. Confirmation of endothelial markers prompted these researchers to investigate whether hPAE cells produce HLA-E after exposure to tumor necrosis factor α (TNFα) and interferon γ (IFN γ). Gel results showed that hPAE cells started to express HLA-E after exposure to cytokines both at the transcriptional and protein levels. Immunostaining showed that HLA-E was mainly localized in the cytoplasm. Western blot analysis using anti-HLA-E specific monoclonal antibody revealed a single band at 42 kDs, consistent with the molecular weight of HLA-E protein. Immunoprecipitation analysis of the cell supernatant showed a single band at 37 kDa, consistent with the molecular weight of soluble HLA-E (sHLA-E) protein, implying that sHLA-E is secreted.

Knowing that HLA-E (immunosuppression) is produced after exposure to cytokines, they then investigated whether hPAE cells are capable of differentiating into skeletal myocytes in vitro. hPAE cells started to exhibit multinucleated myotubes in culture after induction. Immunocytochemistry indicated that enhanced green fluorescent protein (EGFP)-labeled multinucleated myotubes were positive for desmin (myogenic marker) and myosin heavy chain. Myogenesis of hPAE cells was also analyzed by RT-PCR with primers that can amplify human myogenic genes, but not their mouse counterparts. hPAE cells constitutively expressed the myogenin gene and started to express the desmin and MyHC-IIx/d genes after induction.

Further experiments evaluated the *in vivo* response of hPAE cells by injecting these cells into the thigh muscle of immunocompetent BALB/c mice. Periosteal cells with low expression of HLA-E were injected into these mice for comparison. Histopathological analysis revealed that the injection of periosteal cells induced an immune response at the injected sites but hPAE cells did not, suggesting that hPAE cells fail to elicit pro-inflammatory response in immunocompetent mice. Immunofluorescent analysis revealed that CD45 and CD3 lymphocytes aggregated near the donor periosteal cells after injection into the BALB/c mice. In contrast, CD45 and CD3 lymphocytes were not detected around the vimentin-positive hPAE cells.

Experiment was also carried out to investigate the ability of hPAE cells to generate muscle tissue *in vivo,* by implanting hPAE cells in BALB/c mice with phosphate buffered saline (PBS), injected as control. Myotubes at the hPAE cell injected site expressed human dystrophin as a cluster but those at the PBS injected sites fail to express dystrophin. These results imply that dystrophin is transcribed from the dystrophin gene of human donor cells after hPAE cells differentiated into myotubes and fused to host cell myocytes without immune response.

Expression of dystrophin, aided by HLA-E immune suppression triggered these researches to investigate the involvement of HLA-E by inhibiting the expression of HLA-E by siRNA in hPAE cells. Significant decrease in HLA-E mRNA and HLA-E protein was observed in cells transfected with HLA-E specific siRNA when compared to the control cells transfected with siRNA. Decrease in HLA-E mRNA and protein resulted from the activation of immune response, revealed by the aggregation of CD45 and CD3 positive lymphocytes in BALB/c mice after injection. The opposite result was obtained for the control cells with siRNA-treated cells because they the siRNA was not specific for HLA-E and was unable to inhibit HLA-E in the muscle cell. This result suggests that HLA-E is necessary for inhibition of an immune response *in vivo*. The same result was obtained *in vitro*, when hPAE cells treated with either siHLA-E or control siRNA were co-cultured with primed lymphocytes. HLA-E specific siRNA treated cells were lysed by primed lymphocytes, whereas control siRNA-treated hPAE cells were not, indicating that HLA-E is also necessary for inhibition of the immune response *in vitro.*

Finally, they investigated whether hPAE cells can confer human dystrophin to myocytes. hPAE cells were labeled with EGFP and injected into mdx mice while PBS was injected into the contralateral muscle as control. Human dystrophin was detected in EGFP-positive myotubes as a cluster. This result suggests that human dystrophin is transcribed from the dystrophin gene of human donor cells.

Conclusion

This article illustrates the potential use of hPAE cells in cell-based therapy using an mdx mouse as a model of Duchenne muscular dystrophy. The ability of hPAE to confer dystrophin to myocytes is aided by the HLA-E. HLA-E binding to inhibitory CD94/NKG2 receptor results in the inhibition of NK and CTL dependent lysis. HLA-E immunosuppressive action allows hPAE cells to confer dystrophin to myocytes after induction in an *in vitro* study with BALB/c mice or direct implantation in an *in vivo* study with mdx mice.

 Future research can focus on the isolation of tissue specific stem cells for expansion *in vitro* and transplantation back into the patients in an allogeneic manner.

 The issue I had with this article is the fact that they fail to explain factors that regulate dystrophin expression and what area of the dystrophin gene had the mutation for DMD.