P37 Utilization of DR1 as true RARE in regulating the Ssm, a novel retinoic acid target gene in the mouse testis

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Objectives: Various nuclear receptors form dimers to activate target genes via specific response elements located within promoters or enhancers. RXR serves as a dimerization partner for many nuclear receptors including RAR and PPAR. Dimers show differential preference towards directly repeated response elements with 1-to-5 nucleotide spacing (DR1-5), and DR1 is a promiscuous element which recruits RAR/RXR, RXR/RXR, and PPAR/RXR. In the present investigation, we aimed to identify novel target genes of nuclear receptors in mice by using genomic element trapping.

Materials and Methods: To find target genes that are directly bound and regulated by nuclear receptor (PPARδ/RXRα), we employed a yeast-based system to trap functional DR1s in the mouse genome in the presence of nuclear receptor ligands (carbaprostacyclin and 9-cis-RA). One clone, named p39, was analyzed and its full-length cDNA sequence and partial promoter region were identified. We performed electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP) to confirm two DR1 sites of p39 are directly bound by nuclear receptors. We also examined tissue-specific expression of the target gene using Northern blot hybridization, quantitative RT-PCR (qRT-PCR) and *in situ* hybridization.

Results: This target gene, namely *Ssm*, recruits all three combinations of nuclear receptors *in vitro* but *in vivo* regulation is observed by trans-retinoic acid-activated RAR/RXR dimer. Indeed, chromatin immunoprecipitation experiment demonstrates binding of RARβ and RXRα in the promoter region of the *Ssm*. Interestingly, expression of *Ssm* is almost exclusively observed in the secondary spermatocytes in the mouse testis, where RA signaling is known to regulate developmental program of male germ cells.

Conclusions: The results show that *Ssm* is a RAR/RXR target gene uniquely using DR1, and exhibit stage-specific expression in the mouse testis with potential function in later stages of spermatogenesis.

Key word: Nuclear Receptor, RAR/RXR, Retinoic acid, Secondary Spermatocyte specific marker (SSM), Testis

P38 Neurogenic differentiation of mesenchymal-like stem cells from human adipose tissue

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Objectives: For the survey of an alternative source of human stem cells we investigated the ability of human mesenchymal-like stem cells from adipose tissue (AT-MSC) to differentiate into neural cells under experimental cell culture conditions. Furthermore we examined whether estrogen increases neuronal differentiation.

Materials and Methods: We isolated AT-MSC from human liposuction tissue and cultured 5-10 of passages in Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS). To induce neural differentiation of AT-MSC, cells were cultured in the presence of DMSO and BHA. To confirm the neural characteristics, immunocytochemistry stain for β-tubulin III, GFAP and Gal-C were performed. RT-PCR was performed for detecting NeuroD1, GFAP and MBP mRNA. To determine the effect of estrogen on neural differentiation and axonal growth, E_2 and ICI-118,780, estrogen antagonist, was added to the neural induction medium.

Results: Following neural induction, AT-MSC changed toward neural morphology, forming compact cell bodies with multiple extensions. AT-MSC cultured in the presence of DMSO/BHA expressed proteins of β-tubulin III, GFAP and Gal-C. The expression rate was about 51% for neuron, 18% for astrocyte and 10% for oligodendrocyte. In the presence of estrogen, the rate of neuron was increased to 61% ($p<0.05$). Increase in neurons was abrogated by an ICI about 50%. Comparison of mean neurite length demonstrated that E_2 reduced neurite growth when compared to neural induced and ICI treated group ($P<0.05$). However, increase in neurite branching was observed in the presence of E_2 ($P<0.05$).

Conclusions: In the present study, we showed that AT-MSC were capable of differentiating into neural cells. Furthermore, AT-MSC treated with estrogen can be increased growth and development of neurons. These results indicate that MSC derived from adipose tissue may be an alternative source of cell therapy for CNS defects and estrogen treatment would be more effective for lesion restoration.

Key words: adipose tissue, estrogen, mesenchymal stem cell, neurogenic differentiation, transdifferentiation