T.P.41
The role of the transcriptional factor Pax3 on myogenesis and the effect on the expression of myogenic regulatory factors
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Pax3 is a member of paired box (Pax) family and considered to play an important role in the initial stage of skeletal muscle development. However, its precise function of myogenesis in vitro and in vivo remains unknown. For example, how Pax3 affect the expression of myogenic regulatory factors (MRFs) has not yet been clarified. We have investigated the effect of TSA, one of most potent HDACIs, on myogenesis using the C2C12 skeletal muscle cell line. We found that the change of the expression of Pax3 was not directly correlated with other MRFs (Hagiwara et al., BBRC (2011)). To investigate the role of the transcriptional factor Pax3 on myogenesis and its effect on the expression of MRFs, we performed Pax3 cDNA transfection to the C2C12 cell line by using Effectene® Transfection Reagent (QIAGEN). We analyzed the effect of Pax3 transfection by immunostaining, Western blot, and microarray analyses. In Pax3 transfected C2C12 cells, the formation of myotube is decreased compared to non-transfected cells. At 48 h after Pax3 transfection, the expression MyoD was reduced by Western blot analysis. By microarray analysis, the expression MyoD was reduced and the expression of the cell cycling regulators were changed most significantly. These results suggest that Pax3 inhibit muscle differentiation by suppressing the expression of MyoD and enhancing the expression of the cell cycling regulators. Pax3 may have a role to preserve muscle cell precursors undifferentiated.

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T.P.42
Antisense oligonucleotide induced over-expression of progerin in human myoblasts: A possible model of muscle aging
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Antisense oligonucleotides (AOs) may be used as splice-modifying tools, with great therapeutic potential in diseases such as Duchenne muscular dystrophy and spinal muscular atrophy. They can also be used experimentally to induce specific alternative splicing patterns to produce various transcript isoforms in non-disease tissues. The aim of this study was to produce a model of accelerated muscle aging by inducing expression of the progerin isoform transcript of the lamin A gene (LMNA) in normal human myoblasts. This internally truncated transcript is missing the last 150 bases of exon 11, and gives rise to the mutant protein progerin that is associated with the lethal premature ageing disease, Hutchinson–Gilford progeria syndrome (HGPS). In HGPS, which arises from a silent de novo mutation in exon 11 of LMNA, progerin is found mostly in tissues of mesodermal origin and it has been suggested that it may also play a role in the ‘natural’ ageing process in tissues. We designed 25–30 base long 2′-O-methyl AOs (n = 14) targeting the donor splice site of exon 11 and acceptor site of exon 12 of LMNA and transfected primary human myoblasts with these AOs for 48 h. RT-PCR showed that AOs that annealed to the area around the exon 12 donor site did induce the Δ150 lamin A transcript, but most AOs also caused a variable degree of exon 11 skipping. This contrasts with a previous study using 2′-O-methoxy-ethyl AOs in human fibroblasts that produced only the Δ150 lamin A transcript, and may reflect the influences of different oligomer chemistries on altered splicing. Transfection of human fibroblasts with our AOs showed similar results as with myoblasts. The present findings indicate that AO-induced over-expression of progerin in human myoblasts is possible and suggest that this may be a suitable model for studies of the role of progerin in muscle aging and sporadic inclusion body myositis in which there are features of accelerated muscle aging.

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DUCHENNE AND OTHER MUSCULAR DYSTROPHIES – POSTER PRESENTATIONS

T.P.48
The use of standard and complementary therapies in DMD and BMD
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The Neuromuscular Clinic at the Royal Children’s Hospital, Melbourne, Australia manages the care of children and adolescents with a variety of neuromuscular conditions, including approximately 125 subjects with Duchenne or Becker Muscular Dystrophy (DMD/BMD). Increasingly families are reporting their use of complementary therapies. These include massage, osteopathy, chiropractic manipulation and nutritional supplements. In consultation with our physiotherapists and dietician, an online questionnaire has been developed to survey families on their use of additional mainstream allied health therapies, complementary therapies, structured physical activities and nutritional supplements. From this information we hope to understand families’ use of mainstream and alternative therapies and better inform our clinical practice. From the information we obtain we expect that we may be able to develop future studies to investigate the relevance of these therapies for subjects with DMD/BMD.

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