European Cells and Materials Vol. 18. Suppl. 2, 2009 (page 43) ISSN 1473-2262

Osteogenesis within muscle: craniofacial vs. limb muscle cells

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INTRODUCTION: Ectopic bone formation in skeletal muscle has been observed as a result of pathological and experimental conditions[1]. These observations have led researchers to investigate the existence of osteoprogenitor cells within skeletal muscles[2]. Most of the work in this field has described cells isolated from non-craniofacial muscle. However, the involvement of neural crest cells (NCC) in the development of craniofacial skeletal muscles (CSkM; masseter) may have an effect on the multipotentiality of the cells isolated from these muscles. It is therefore hypothesized that CSkM contains a more highly active multipotential cell population than non-craniofacial muscles. Therefore, the aim of this study is to investigate and compare the osteogenic differentiation abilities of cells isolated from craniofacial and limb muscles.

METHODS: Cells derived from mouse masseter (CSkM) and hind-limb (LM) muscles were isolated by enzymatic digestion, then serially plated based on their adhesion properties (PP1 being cells that adhered within 1 hour, PP2 cells that adhered between 1 and 48 hours and PP3 being cells that adhered after 48 hours). Cells were differentiated along the osteogenic and myogenic lineages using osteogenic medium (OS), or myogenic medium (MM). Osteogenic differentiation was assessed by Alkaline Phosphatase (ALP) activity, and calcium deposits. Myogenic differentiation was assessed by the presence of multinucleate myotubes.

RESULTS: The growth dynamics of the PP1 fraction of cells (both CSkM and LM) demonstrated an initial rapid growth rate compared to the other fractions. PCR identification of relevant mRNA transcripts showed that the PP1 fraction was $Nanog^{+}$, $Sca\text{-}1^{+}$, $CD34^{+}$, $Desmin^{-}$; whilst the PP3 fraction was $Nanog^{+/-}$, $Sca\text{-}1^{+}$, $CD34^{-}$, $Desmin^{+}$. The myogenic differentiation abilities were also different among different pre-plates. More myotubes were observed in late adhered cells (PP3) compared to early-adhered ones (PP1 and PP2) (Figure 1). Cells from CSkM and LM were examined for their mineralisation capabilities after treatment with OS medium for 21 and 28 days. All cells showed increased mineralisation compare to the positive control mouse osteoblast (MOB). Generally, early adhered cells from both CSkM and LM showed greater mineralisation than late adhered cells.

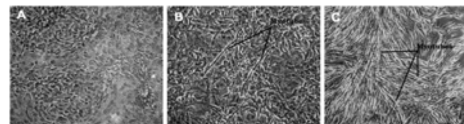

Fig1: Phase contrast images of myotube formed by CSkM after day 21 (A) PP1 (B) PP2 (C) PP3

In order to investigate the effect of cell age on differentiation potentials, cells from craniofacial muscle at passages 1 and 14, were exposed to OS, MM, and standard growth media for one week. After one week, RNA was extracted and RT-PCR was performed to examine the expression of osteogenic (Runx-2 and ALP) and myogenic (MyoD and Desmin) genes. In general, osteogenic genes were expressed in early-adhered cells and myogenic genes were expressed in late adhered cells. In passage 14 cells, early adhered cells continue to express osteogenic genes. On the other hand, the expression of myogenic genes in late adhered cells decreased compared to the same cells at early passage number. Finally, ALP expression was observed in PP1 and PP2 in both control and differentiated cells.

DISCUSSION & CONCLUSIONS: Different populations of cells that may have different osteogenic and myogenic differentiation abilities were isolated from CSkM and LM based on their adhesion properties. Early isolated cells (PP1 and PP2) from both muscles showed higher osteogenic and lower myogenic differentiation abilities compared to the late preplate (PP3). There are some differences in gene expression between early and late adhered cells. Early adhered cells are $Nanog^{+}$, $Sca\text{-}1^{+}$, $CD34^{+}$, $Desmin^{-}$. Where as the late adhered cells are $Nanog^{+/-}$, $Sca\text{-}1^{+}$, $CD34^{+/-}$, $Desmin^{+}$. There are no clear differences in osteogenic differentiation abilities between cells isolated from both muscles.

REFERENCES: [1]Urist MR, *et al.* Clin Orthop Relat Res. 196 Jul Aug;59:59-96. [2]Bosch P, *et al,* Orthop Res. 2000 Nov;18 (6):933-4.