Beta-carotene acts as a regulator of skeletal muscle cells

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Skeletal muscle development- #2487

P23- 342- Beta-carotene acts as a regulator of skeletal muscle cells

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The enzyme beta, beta-carotene-15,15'-monooxygenase (BCMO1) cleaves provitamin A carotenoids into active vitamin A principally in liver and intestine. The BCMO1 gene is expressed at low level in the muscle tissue but little is known about its function. In the chicken muscle, we observed that various BCMO1 expression levels are associated with different carotenoids contents. To investigate the potential role of BCMO1 on skeletal muscle, we assessed the impact of beta-carotene (BC, the prototype substrate of the BCMO1 enzyme) supplementation in vitro on proliferative avian myoblasts. Proliferation was evaluated by BrdU incorporation and by flow cytometry. The BrdU incorporation index was reduced and the proportion of G0/G1 cells increased following BC supplementation. Cell differentiation was evaluated by immunolabelling of sarcomeric myosin heavy chain (MHC). In this proliferative environment, the proportion of MHC positive cells increased following BC supplementation. The effects of BC were inhibited in the presence of DEAB, an inhibitor of retinaldehyde dehydrogenase, supporting the hypothesis that the BCMO1 enzyme is active in myoblasts and can contribute to retinoic acid production from BC. Our data suggest that provitamin A carotenoids could be used as nutritional regulators of skeletal muscle growth.

carotenoids, myogenesis

Skeletal muscle development- #2517

P23- 343- The role of Wnt signalling in embryonic stem cells differentiation into mesoderm

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Pluripotent stem cells (PSCs) can differentiate into all cell types building mammalian body. There are many indications that Wnt proteins may influence the pluripotency and differentiation of PSCs. It has been shown that canonical Wnt signalling is involved in both- maintaining pluripotent state of PSCs (ten Berge et al. 2011) and their mesodermal specification (Liu et al. 1999). Here we describe the role of Wnt-11, acting through non-canonical signalling pathway, during in vitro differentiation of murine PSCs.

The aim of our research was to design a protocol for directed and efficient differentiation of PSCs, such as embryonic stem cells (ESCs), into skeletal myoblasts. Since Wnt-11 is crucial for the formation of dermomyotome, a source of skeletal muscle precursors during embryogenesis, we assessed the influence of this factor on in vitro myogenic differentiation of ESCs. First, we determined the level of endogenous expression of Wnt-11 in undifferentiated and differentiating ESCs at mRNA and protein level. Next, we checked if ESCs are able to respond to exogenous Wnt-11 by investigating the expression of both Wnt-11 receptors: Frizzled-5 and Frizzled-7. Subsequently, we determined the influence of exogenous Wnt-11 on ESCs morphology, proliferation, and differentiation. We determined the level of expression of germ layers markers: endoderm (GATA-4), ectoderm (Pax6), and mesoderm (Brachyury, Mesogenin) as well as myogenic markers such as Pax3, Pax7, and MRFs. Finally, we assessed the Wnt-11 influence on the ability of ESCs to fuse with skeletal myoblasts. We found that Wnt-11 influences ESCs by promoting their differentiation into mesoderm.

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ESCs, differentiation, mesoderm, Wnt signalling

Skeletal muscle development- #2532

P23- 344- Molecular control of muscle mass: roles of the proteins GASP/WFIKKN

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Muscle mass is the result of a dynamic balance between protein synthesis and degradation. In the last decades, myostatin, a member of the transforming growth factor-? superfamily, has been shown to be a key molecular element of skeletal muscle development, acting as a negative regulator. Myostatin knockout mice display both hypertrophy and hyperplasia of muscle fibers and a leaner body composition due to reduced fat mass. Several strategies to block myostatin signaling pathway have been carried out to develop therapeutic applications, in particular to increase muscle mass in various diseases including muscular dystrophy. One research focus of our laboratory concerns two myostatin inhibitors, the GASP proteins. GASP-1 and GASP-2 are the only representatives of a unique family of multidomain proteins, with a tissue expression characteristics markedly different, suggesting that these proteins possess a variety of biological roles, in addition to regulation of muscle development.

To better understand the mechanism of action of the GASP proteins, we have generated and characterized mouse lines overexpressing ubiquitously Gasp-1, Tg(Gasp-1) or Gasp-2Tg(Gasp-2). Our results revealed that the Tg(Gasp-1) mice present a muscular phenotype due to hypertrophy without hyperplasia. No reduction in fat mass has been observed. To investigate genetic interaction networks, we initiated an in silico approach combined with the Affymetrix microarraytechnology. On 20000 genes, more than 3000 were identified as up or down regulated in primary myoblasts overexpressing Gasp-1 compared to wildtype cells. The 10 more relevant genes were selected for validation using quantitative real-time RT-PCR analysis. Phenotypic studies of the Tg(Gasp-2) mouse line are currently in progress.

Myostatin, GASP/WFIKKN, muscle differentiation, DNA chip

Skeletal muscle development- #2579

P23- 345- Molecular mechanism for nuclear envelope localization of MTOC activity in muscle cells.