Modulation of c-Myb during chondrogenesis

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Introduction
Chondrogenesis is a multi-step cellular event: first, the mesenchymal cells differentiate into chondrocytes which proliferate, mature, and undergo hypertrophy and matrix calcification. This process is important in embryonic skeletal development as well as in postnatal skeletal growth.

Transcription factor c-Myb is known as associated with control of cell proliferation, survival, and cell death. It plays a critical role in hematopoietic development with c-Myb null mice dying around day 15 of embryogenesis. Our recent work has shown that c-Myb could be important in cell differentiation of undifferentiated cells, not only in proliferation. Transcription factor Sox9 is critical for chondrocyte differentiation and function (Long & Ornitz, 2013). Homozygous mice for Sox9 (Sox9−/−) are perinatally lethal.

Aims
1. To determine whether overexpression of c-Myb in micromass cultures would affect initiation of cartilage nodules.
2. To test whether inactivation of c-Myb would cause downregulation of creating cartilage.
3. To follow, if there is any connection between c-Myb and Sox9 transcription factors in signalling pathways.

Material and methods
Micromass culture: The mesenchymal micromasses were established from mouse front limbs in embryonic day 12 (ED12) and then were transiently transfected using the Fugene transfection reagent. We performed gain-of-function experiments used cells transfected by construct carrying c-Myb. Downregulation was achieved by c-Myb siRNA. Cells were lysed and analyzed by qPCR after 24 hour.

qPCR: Mesenchymal cells were cultivated 24 hour and then were lysed. RNA was isolated and used for first-strand cDNA synthesis. The samples were processed using qPCR and analyzed by the ΔΔCT method with normalization against actin mRNA levels, used as an internal control.

Alcian-blue staining: Micromasses were cultured for 7 days, fixed with 10% paraformaldehyde and were stained in AB (AB – 5% Alcian-blue in 95% ETOH) in 0.1M HCI, overnight at RT. Data were analyzed in pixels of the total area using Adobe Photoshop 6.0 software.

Results: Overexpression c-Myb/Sox9 enhanced chondrogenesis

Results: Modulation of c-Myb influenced Sox9 expression

Results: Electroporation of c-Myb increased amount of c-Myb positive cells

Fig. 3: Sox9 expression was increased to 122 % after c-Myb overexpression. In comparison, the level of Sox9 expression was decreased after downregulation of c-Myb using siRNA.

Conclusions:
The critical role of c-Myb during embryogenesis, especially in proliferation, was confirmed by many studies (Oh & Reddy, 1999; Sandberg et al., 2005). Endochondral ossification is a highly organized process and gives rise to the majority of bone in the skeleton, which evolves via successive step of mesenchymal condensation, chondrogenesis and chondrocyte maturation.

Our results showed that the initiation of cartilage nodules was higher when c-Myb was overexpressed. This could be explained by a novel function of c-Myb in cell differentiation. Increased Sox9 expression after c-Myb overexpression and decreased Sox9 level after c-Myb siRNA silencing suggest a conclusion, that c-Myb could be an important regulator in transition between proliferation and differentiation of cells.

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