Expression of Trk Receptors during Cartilage Differentiation

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The products of the tyrosine kinase *trk* family of proto-oncogenes bind neurotrophins (NTFs) and are components of their high-affinity receptors. The *trk* gene encodes for the TrkA transmembrane glycoprotein, which functions as a receptor for nerve growth factor (NGF). Brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) are the preferred ligands for TrkB, and neurotrophin-3 (NT-3) for TrkC (reviewed in reference 1). The presence of Trk receptors in nonneuronal cells of developing tissues and organs indicates that these molecules may play a role in embryonic development that would be distinct from a neurotrophic function. Cartilage development involves mesoderm-derived cells (chondrocytes) that contribute to appositional growth of the limb skeleton. Chondrocytes synthesize macromolecules characteristic of cartilage such as collagen type II and sulfated proteoglycans. The majority of studies dealing with cartilage cell differentiation have been carried out on mesenchymal cells isolated from limb buds.[2] However, the molecular mechanisms regulating cartilage formation are not fully understood.

In the present study we analyzed the expression of Trk receptors during mouse limb development *in vivo* and in micromass cultures *in vitro.* Because little is known about their regulation, we studied the effects of various growth factors on expression of Trk receptors *in vitro.* F1(CBA × NMRI) and Balb C mouse embryos (E10–E18) were used. Immunohistochemistry was performed according to Mitsiadis *et al.*[3] Five-μm sections were incubated with TrkA (B. B. Rudkin), TrkB (Santa Cruz Biotechnology, USA), and TrkC (D. Martin-Zanca) affinity purified antibodies against the extracellular domain of the receptors, and cTrk (Santa Cruz Biotechnology, USA) an-

tibody against the intracellular domain of all Trk receptors. During early stages of limb development (E10–E11), a weak staining for Trk receptors was found in mesenchymal cells, whereas a strong cytoplasmic or nuclear labeling was detected in differentiating chondrocytes in the central core of the limb bud, at E12–E15. This staining persisted until E18 and was found in the epiphysial plate of the bone (data not shown). TrkC reactivity was observed in the Golgi apparatus of several chondrocytes, whereas the chondrocytes were negative for TrkB. *In situ* hybridization was performed according to Mitsiadis *et al.*[3] using a ^{32}P-labeled cDNA probe that detects all *trk* genes (D. Martin-Zanca). These studies confirmed that the *trk* genes are expressed in the developing cartilage (data not shown). Micromass cultures for the *in vitro* studies were performed according to Wroblewski and Edwall-Arvidsson.[4] Initially (1–2 days), the Golgi apparatus of almost all cells was positive for TrkA, TrkB, and cTrk antibodies (FIG. 1). When cartilage foci started to form (3–7 days), cells of the foci continued to express TrkA and cTrk, but not TrkB. TrkA and cTrk reactivities were localized to the nuclei of some cells (FIG. 2). When cells were cultured in serum-deprived medium supplemented either with insulin growth factor I (IGF-I, 100 ng/mL) or IGF-II (100 ng/mL), expression of Trk receptors was not affected. In contrast, fibroblast growth factor 2 (FGF-2, 10 ng/mL), which inhibits/delays chondrogenic differentiation,[4] down-regulated the expression of TrkA in cartilage.

In conclusion, our results show that the Trk receptors are differentially expressed during chondrogenesis, and that TrkA expression is regulated by FGF-2. There is an apparent correlation between the nuclear localization of TrkA and the initiation of chondrocytes differentiation. These observations open the way for studies on Trk signal transduction in nonneuronal cells.

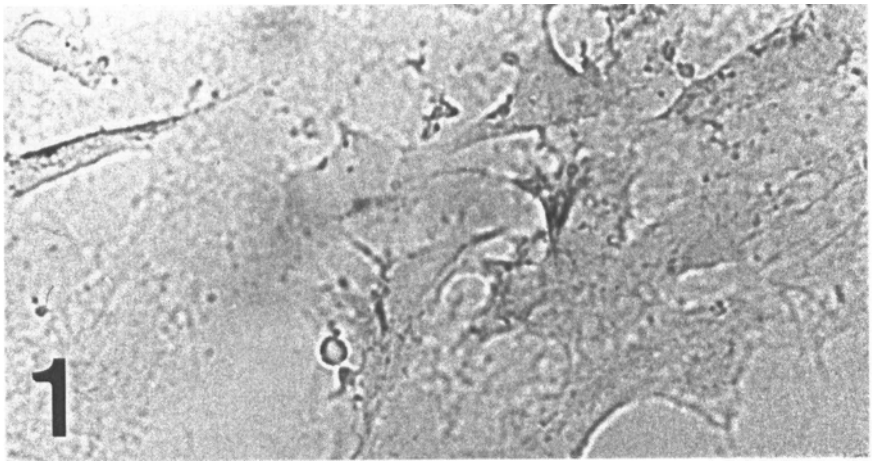

Figure 1. Immunohistochemical localization of the Trk receptors (cTrk antibody) in undifferentiated limb-derived mesenchymal cells in micromass cultures. Note their expression in the Golgi apparatus of the cells.

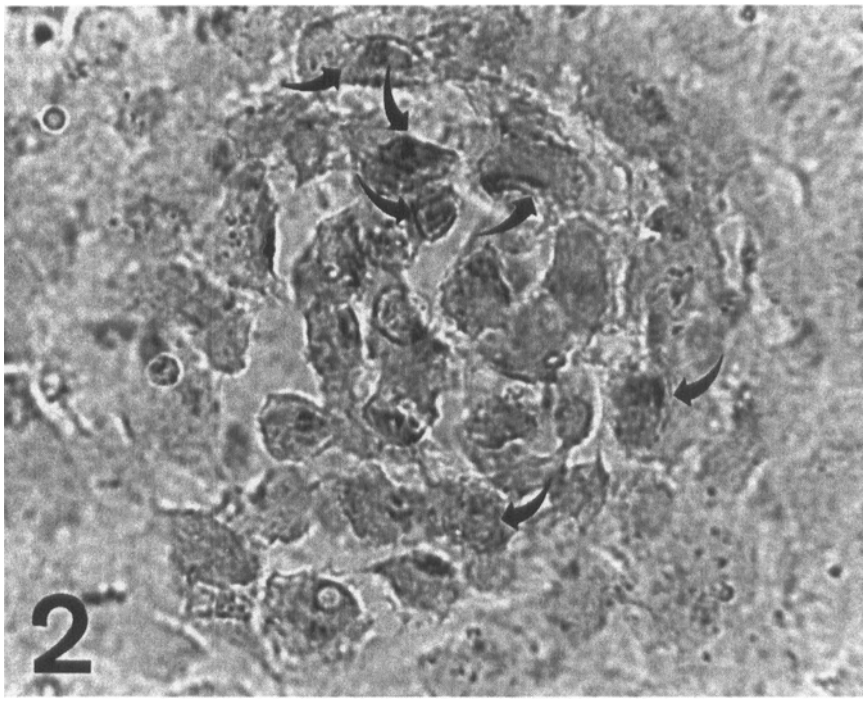

Figure 2. Immunohistochemical localization of the TrkA receptor in differentiating chondrocytes in micromass cultures of cells isolated from limb buds. Note the nuclear staining in some cells of the cartilage foci (*arrows*).

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