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NFATp represses chondrogenesis

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Keywords

Cartilage, chondrosarcoma, differentiation, mesenchymal stem cells, NFATp

Context

NFAT was originally identified as a transcription factor which binds the interleukin (IL)-2 promoter in activated T cells. It is present as an inactive phosphorylated protein in the cytoplasm of resting cells. Upon cell activation there is an increase in intracellular calcium which results in the dephosphorylation of NFAT by the phosphatase calcineurin. This in turn leads to translocation of NFAT into the nucleus where it binds DNA and other transcription factors such as activator protein 1 (AP-1) and drives transcription of various genes involved in the immune response. The immunosuppressive drugs cyclosporine and FK506, used in transplant surgery, inhibit calcineurin and therefore also immune responses which depend on NFAT activation. There are four members of the NFAT family (NFAT-1, -2, -3 and -4). Mice deficient in NFAT-1 (NFATp/NFATc2) show modest splenomegaly with hyperproliferation of T and B lymphocytes and enhanced Th_2 responses. In addition to this immunological phenotype, these mice develop ambulatory problems and showed a decreased range of joint motion from the age of six months. To determine the reason for joint abnormalities in NFATp-deficient mice.

Significant findings

Mice deficient in NFATp were healthy and showed normal embryological skeletal development at birth, but at 6 months old showed progressive difficulty in ambulation and a decreased range of joint motion. X-ray analysis of these mice showed abnormal bone deposition in some articulating joints. Histological analysis of the hip joint from 3 months revealed proliferation of chondrocytes, disruption of the articular cartilage and initiation of chondrogenesis in extra-articular regions arising from cells distinct from the articular chondrocytes and reminiscent of the process of endochondral ossification. RT-PCR analysis of passaged monolayer cells isolated from articular or extra-articular cartilage of NFATp-deficient mice showed increased levels of PCR products for collagen type II and type X relative to wild-type cells. Conversely, when expression plasmids containing NFATp were transfected into NFATp-deficient chondrocytes, RT-PCR products for collagen type II and collagen type X were decreased relative to that for cells transfected with control plasmid. RT-PCR analysis of passaged monolayer

chondrocytes detected mRNA for each NFAT family member. These cells were classified as chondrocytes because a high percentage expressed the cartilage markers collagen type II and COMP. No NFATp protein could be detected by standard techniques in chondrocytes or cartilage, suggesting that in normal cartilage NFATp is expressed at very low levels. Differentiation of human mesenchymal stem cells *in vitro*resulted in induction of NFATp mRNA under chondrogenic conditions but not under conditions which initiate osteogenesis. Further histological examination revealed increased chromatin content, noticeable mitotic figures, multiple nucleoli and invasion of muscle tissue consistent with transformation. Growth of some cells also showed loss of contact inhibition, and karyotypic analysis of a few cell lines also revealed aneuploidy.

Comments

This paper is of particular interest as it describes The nuclear factor of activated T cells (NFAT)p as the first transcription factor to be involved in the control of chondrogenesis from adult precursor cells but which has no effect on embryonic skeletal development. The dysregulation of chondrogenesis in NFATp-deficient mice leads to abnormal deposition of cartilage and bone in the extra-articular tissues. This deposition may be relevant to the pathological progression of osteoarthritis. As NFATp has been identified as a repressor of hondrogenesis, inhibitors of NFATp activation should promote synthesis of cartilage matrix (as well as influencing the immune response) and could therefore be useful drugs for treatment of arthritic disease.

Methods

Mutant mice were generated by targeted gene disruption of the NFATp locus; this resulted in production of reduced amounts of NFATp which lacked DNA binding functionality. Paraffin or plastic embedded sections were stained with hematoxylin and eosin, safranin O and fast green or toluidine blue. Chondrocytes were isolated from the femoral head cartilage or from the extra-articular tissues, where chondrogenesis was initiated spontaneously, and grown to passage 2. RNA was prepared from these cells for use in reverse transcriptase polymerase chain reaction (RT-PCR) of chondrocyte genes (collagen type II, collagen type X, cartilage derived morphogenetic protein) either before or after transfection with NFATp expression plasmids. Immunohistochemistry was carried out using antibodies to collagen type II or cartilage oligomeric matrix protein (COMP) for confirmation of cartilage phenotype. Cell proliferation was measured by tritiated thymidine incorporation.

Additional information

An interesting commentary by Caplan and Dennis is provided in the same issue of *J Exp Med* (Caplan AI, Dennis JE *J Exp Med* 2000, **191:**1-4)

References

1. Ranger AM, Gerstenfeld LC, Wang J, Kon T, Bae H, Gravallese EM, Glimcher MJ, Glimcher LH: The nuclear factor of activated T cells (NFAT) transcription factor NFATp (NFATc2) is a repressor of chondrogenesis . J Exp Med . 2000, 191: 9-21.