Introduction: Chondrogenesis is controlled by cellular interactions with surrounding matrix proteins and growth factors that mediate cellular signaling pathways. Recently we identified a novel cartilage extracellular matrix protein 1 (ECM1), a molecule previously been linked to lipoid proteinosis and lichen sclerosus. The purpose of this study is to define the role of ECM1 in chondrogenesis and the molecular mechanism involved.

Materials and Methods: Expression pattern of ECM1 in the course of chondrogenesis of C3H10T1/2 cells, assayed by Western blotting; effects of ECM1-conditioned medium and knockdown of ECM1 via siRNA approach on the expression of collagen X, assayed by real-time PCR; induction of ECM1 by PTHrP in chondrocyte hypertrophy in ATDC5 cells, assayed by real-time PCR; expression of ECM1 in the growth plate of wildtype and PTHrP knockout mice, assayed by immunohistochemistry (IHC); ECM1 is required for PTHrP action in chondrocyte hypertrophy assayed by in vitro chondrogenesis.

Results: ECM1 is differentially expressed in the course of chondrogenesis: in order to define the role of ECM1 in chondrocyte differentiation, we first examined its expression pattern in the course of chondrogenesis using micromass 10T1/2 cells induced by BMP2. As shown in Fig. 1A, a robust induction of ECM1 is observed during the chondrocyte differentiation, specially at the late stage of chondrogenic process.

ECM-conditioned medium inhibits whereas knockdown of ECM1 accelerates hypertrophic chondrocyte differentiation: We next determined whether ECM1 regulates chondrocyte differentiation. Micromass cultures of 10T1/2 cells were treated with BMP2 with either control or ECM1 conditioned medium for 3 Days for Sox9 and collagen II and 7 Days for collagen X. As revealed in Fig. 1B, ECM1 dramatically inhibited expression of both early and later marker genes for chondrogenesis. It resulted in a 78% for Sox9, 82% inhibition for collagen type II and approximately 90% repression on collagen X.

Next we examined the effects of ECM1 knockdown on the expression of collagen X. Endogenous expression of ECM1 was suppressed via siRNA silencing, which markedly enhanced the expression of Collagen X in chondrogenesis. These results clearly indicate that ECM1 is a potent negative mediator for hypertrophic chondrocyte differentiation.

Discussion: Our findings demonstrate for the first time that ECM1, a direct downstream molecule of PTHrP in cartilage, is a novel negative regulator of hypertrophic chondrocyte differentiation and suggest that ECM1 may also plays an important role in the pathology of arthritis.