Matrilin-3 Stimulates Chondrogenesis by Activating Epidermal Growth Factor Signaling in Chondroprogenitor Cells.

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Introduction: Our previous studies have shown that the cartilage extracellular matrix (ECM) protein matrilin-3 (MATN3) is capable of inducing chondrogenic differentiation of chondroprogenitor cells [1, 2]. Consistent with these findings, we discovered recently that MATN3 is capable of stimulating expression of chondrogenesis genes such as collagen II (Col2a1) and aggrecan (Acan) in ATDC5 chondroprogenitor cells in a manner that depends on epidermal growth factor receptor (EGFR) [3]. In the present study, we show for the first time that MATN3 can stimulate phosphorylation and rapid activation of EGFR.

Methods: Matrilin-3 overexpression. The ATDC5 murine chondroprogenitor cell line was stably transfected with a construct containing the MATN3 gene as previously described [2]. Cell Culture conditions. ATDC5 cells were seeded at 2 × 10^5 cells per well. Cells were serum starved overnight. Cells were either left untreated or treated with 200 ng/ml of recombinant MATN3 (rMATN3) protein. Cell lysates were collected to be used for western blot analysis at: 0, 10, 30 and 60 min following treatment. Cells that were stably transfected with the MATN3 gene construct were collected immediately after the serum starvation stage for western blot analysis. Western blotting. SDS-PAGE was conducted, under reducing conditions, on all cell lysates. Protein bands were successfully transferred to a nitrocellulose membrane and blotted using monoclonal antibodies against pEGFR, pAKT, AKT and β-actin.

Results: Chondroprogenitors that were treated with recombinant MATN3 exhibited increased pEGFR levels as early as 10 min after treatment (Fig. 1A). Similarly, an increase in pAKT levels became obvious 10 min after treatment with rMATN3 (Fig. 1A). ATDC5 cells that were stably transfected with the MATN3 transgene exhibited higher basal levels of pEGFR relative to untransfected ATDC5 cells (Fig. 1B).

Discussion: The present study, for the first time, presents direct evidence that treating ATDC5 chondroprogenitors with the ECM protein MATN3 activates EGFR signaling in these cells. Since MATN3 expression induces spontaneous chondrogenic differentiation in ATDC5 chondroprogenitor cells [2], and silencing EGFR prevents such induction, we put forward a hypothesis that EGFR signaling is required for matrilin-3 to induce chondrogenesis in chondroprogenitors [3]. Matrilin-3 contains several EGF-like domains, it is possible that this ECM protein activates EGFR signaling (Fig. 2) through the EGF-like domain(s). Indeed, mutations in the first EGF domain of MATN3 have been linked to Spondyloepimetaphyseal dysplasia (SEMD) and hand osteoarthritis. It will be tested whether such disease associated mutations in MATN3 affect EGFR dependent activation of chondrogenesis.

Significance: Previous studies have implicated the involvement of MATN3 in chondroprogenitor cell development [1, 2]. This study elucidates the biological mechanism by which MATN3 regulates chondrogenic differentiation of these cells, which play a key role in skeletal development.
Figure 1. Matrilin-3 treatment initiates epidermal growth factor pathway activation in chondroprogenitor cells. Western blot of ATDC5 chondroprogenitors treated with recombinant human matrilin-3 protein for 0, 10, 30 and 60 minutes in serum-free medium (A). Western blot of MATN3 transfected and untransfected ATDC5 cells (B). Western blotting results for phospho-EGFR, phospho-AKT, total AKT and beta-actin are shown.

Matrilin-3

| EGFR |

Chondroprogenitor cell

Signal cascade

Transcriptional regulation:
Chondrogenesis markers

Figure 2. Matrilin-3 regulates chondrogenesis marker expression by activating the EGFR pathway. Proposed mechanism by which matrilin-3 induces chondrogenesis of progenitors.