Epidermal Growth Factor Receptor Suppresses IL-1 Beta Expression And Maintains Matrilin-3 Dependent Regulation Of Chondrogenesis Markers In Chondroprogenitors.

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Introduction: Chondroprogenitors are a newly discovered population of progenitor cells that exist in articular cartilage with the capacity to differentiate and replenish tissue that is damaged due to arthropathy or injury. Recent studies suggest that the cartilage extracellular matrix (ECM) protein matrilin-3 (MATN3) can regulate chondroprogenitor cell differentiation as well as chondrocyte homeostasis [1, 2]. Moreover, MATN3 promotes the expression of cartilage anabolic markers (i.e. type II collagen, aggrecan) while inhibiting the expression of catabolic matrix proteases (i.e. MMP13, ADAMTS 4 and 5) as well as the hypertrophic marker type X collagen [1, 3]. However, the biological mechanism(s) by which these effects are mediated remains largely unclear. Remarkably, a mis-sense mutation in one of the four epidermal growth factor-like (EGF-like) domains in the MATN3 protein is associated with the development of hand osteoarthritis (HOA). It suggests that the EGF-like domains have biological significance that is necessary to maintain the normal regulatory capabilities of MATN3 protein. In this study, we test the hypothesis that epidermal growth factor receptor (EGFR) signaling is required to mediate MATN3 regulation of cells in the chondrogenic lineage.

Methods: Chondroprogenitor cell lines. We immortalized two murine chondroprogenitor cell lines by stably transfecting ATDC5 cells with either wildtype (WT)-MATN3 or a HOA associated mutant MATN3 gene construct containing a threonine to methionine missense mutation in one EGF-like domain. Silencing EGFR. EGFR was completely silenced in parental ATDC5 cells as well as those stably transfected with WT-MATN3 and HOA-MATN3 using a small interfering RNA (siRNA). Cells were seeded at 4.0 x105 cells/well in 12-well plated in DMEM containing 5% FBS, 10μg/ml transferrin, 3.0 x10-8M sodium selenite. Cells were serum starved for 5 hours then transiently transfected with murine EGFR ON-TARGETplus siRNA (DHARMACON) using Lipofectamine 2000. Gene expression analysis. mRNA was isolated from cultured cells, which was then reverse transcribed to cDNA. Real-time RT PCR was utilized to measure gene expression of EGFR, IL-1 receptor antagonist, IL-1 beta, aggrecan, collagen II and X. Expression data was normalized using Ribosomal RNA 18S. Statistical analysis. Data represents mean values ±SD (error bars). Statistics were conducted using a one-way ANOVA and post hoc analysis. There is a minimum of 3 biological replicates within each experimental group. Statistical significance is indicated as follows: *p < 0.05, **p < 0.01, ***p < 0.005.

Results: EGFR mRNA expression was significantly up-regulated in WT-MATN3 expressing chondroprogenitors compared to parental ATDC5 cells. However, its level remained low in the cells expressing HOA-MATN3 (Fig 1A). Thus, MATN3 stimulated EGFR levels and the HOA mutant failed to do so. Successful knockdown of EGFR (Fig 1A) elevated mRNA expression of IL-1 beta (Fig 1B) and its downstream target IL-1 receptor antagonist in chondroprogenitors (Fig.1C). Thus, the absence of EGFR led to activation of IL-1 beta pathway. WT-MATN3 stimulated aggrecan and collagen II, but EGFR knockdown prevented MATN3 stimulation of these chondrogenesis markers (Fig 1D, 1E). In contrast, HOA-MATN3 failed to stimulate aggrecan and Col II, even in the presence of EGFR. Thus, MATN3 stimulation of chondrogenesis depended on EGFR, while the HOA mutant abolished the dependency (Fig 1D, 1E). While EGFR knocked-down chondroprogenitors elevated hypertrophic marker Col X expression, Col X was suppressed in MATN3 expressing cells (Fig 1F). Thus, suppression of Col X by MATN3 does not require the presence of EGFR.

Discussion: EGFR signaling has been shown to be a key regulator of cartilage development and homeostasis [4, 5] although its exact role in cartilage biology is not fully understood. Here we show for the first time that the absence of EGFR leads to the activation of the IL-1 pathway, which in turn results in down-regulation of chondrogenesis markers Col II and aggrecan and up-regulation of hypertrophic marker Col X. Furthermore, MATN3 appears to regulate chondrogenesis through up-regulation of EGFR, since knocking down EGFR abolishes MATN3 stimulation of Col II and aggrecan. The HOA-MATN3 mutant was incapable of regulating these markers in the presence or absence of EGFR. This strongly suggests that MATN3 mediate these regulatory effects through EGFR signaling, while HOA-MATN3 cannot do so due to its EGF-like domain mutation. Our result indicates that the lack of regulation of chondrogenesis through EGFR signaling may contribute to pathogenesis of osteoarthritis, as we demonstrated in chondroprogenitors harboring the hand OA MATN3 mutation.

Significance: Our study reveals an underlying mechanism connecting EGFR signaling with the ECM protein MATN3 and the IL-1 pathway in chondrogenesis and hypertrophy of chondroprogenitors, which play a major role in development of osteoarthritis.

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Figure 1. Genetic silencing of EGFR stimulates IL-1 beta and prevents matrilin-3 induced stimulation of chondrogenesis markers in chondroprogenitors. Relative mRNA levels of EGFR (A), IL-1 beta (B), IL-1 receptor antagonist (C), aggrecan (D), collagen II (E) and collagen X (F) in parental ATDC5 cells as well as those stably transfected with WT or HOA-MATN3 under EGFR silenced (and unsilenced) conditions.