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Article

# miRNA-Mediated KHSRP Silencing Rewires Distinct Post-transcriptional Programs during TGF-b-Induced Epithelial-to-Mesenchymal Transition

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SUMMARY

Epithelial-to-mesenchymal transition (EMT) confers several traits to cancer cells that are required for malignant progression. Here, we report that miR-27b-3p-mediated silencing of the single-strand RNA binding protein KHSRP is required for transforming growth factor b (TGF-b)-induced EMT in mammary gland cells. Sustained KHSRP expression limits TGF-b-dependent induction of EMT factors and cell migration, whereas its knockdown in untreated cells mimics TGF-b-induced EMT. Genome-wide sequencing analyses revealed that KHSRP controls (1) levels of mature miR-192-5p, a microRNA that targets a group of EMT factors, and (2) alternative splicing of a cohort of premRNAs related to cell adhesion and motility including Cd44 and Fgfr2. KHSRP belongs to a ribonucleoprotein complex that includes hnRNPA1, and the two proteins cooperate in promoting epithelial-type exon usage of select pre-mRNAs. Thus, TGF-b-induced KHSRP silencing is central in a pathway leading to gene-expression changes that contribute to the cellular changes linked to EMT.

## INTRODUCTION

Epithelial-to-mesenchymal transition (EMT) is a reversible transdifferentiation process in which epithelial cells lose their characteristics and instead acquire mesenchymal properties. EMT has been implicated in several physiological and pathological processes, including embryonic development, wound healing, organ fibrosis, and cancer progression (see Kalluri and Weinberg [2009] for a review).

Through EMT, cells gain the ability to migrate and resist apoptosis as well as the potential to enter stem cell-like states (De Craene and Berx, 2013; Ye and Weinberg, 2015). Phenotypical hallmarks of EMT include morphological changes from a cobblestone-like epithelial shape to a spindle-like fibroblast

phenotype, loss of epithelial CDH١ (also known as E-cadherin) at cell junctions, and increased expression of mesenchymal CDH٢ (also known as N-cadherin). The opposite transdifferentiation process, mesenchymal-to-epithelial transition (MET), is also possible (De Craene and Berx, ٢٠١٣; Ye and Weinberg, ٢٠١٥).

For many years, it has been proposed that cancer cells undergo EMT to facilitate invasion and dissemination although recent evidence proposed that EMT is instead a prerequisite for acquisition of chemoresistance (Fischer et al., ٢٠١٥; Zheng et al., ٢٠١٥).

EMT is initiated by signals that cells receive from their microenvironment, and transforming growth factor b (TGF-b) is considered to act as a primary inducer of this process (Moustakas and Heldin, ٢٠١٢; Katsuno et al., ٢٠١٣). TGF-b belongs to a large family of structurally related factors, including activins and bone morphogenetic proteins (BMPs), which regulate cell growth, survival, differentiation, and migration (Shi and Massague´, ٢٠٠٣). TGF-b ligands signal through receptor serine/threonine kinases that, in turn, phosphorylate cell-specific SMAD proteins that form complexes with the common SMAD٤, translocate into the nucleus and regulate gene expression by modulating gene transcription as well as by promoting maturation of select microRNAs (miRNAs) from precursors (Blahna and Hata, ٢٠١٢).

Recently, we have demonstrated that the single-strand RNAbinding protein KHSRP (also known as KSRP) is a component of the TGF-b/BMP signaling pathway in multipotent C٢C١٢ cells. TGF-b/BMP-activated SMAD proteins associate with KHSRP