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CAF reprogramming inhibits ovarian cancer progression

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Development and progression of cancer are often facilitated by deregulation of gene expression and accumulation of somatic mutations. Therefore, major research efforts have focused on cancer cells to identify these disease-related genes and mutations, and investigators have developed therapies that target such deregulated genes and mutations and used them for a variety of cancers. In addition to cancer cells, the complex and relatively unexplored tumor microenvironment plays an important role in cancer progression. However, therapeutic regimens targeting tumor stromal cells, particularly cancer-associated fibroblasts (CAFs), have yet to be developed.

Because the tumor-supportive roles of CAFs are increasingly being recognized, researchers have begun to evaluate the potential of inhibiting tumor progression via stromal ablation. However, multiple studies demonstrated that ablation of activated myofibroblasts in tumors actually promotes tumor progression and reduces survival.[1,2] Although inhibiting tumor progression by targeting stroma via global depletion of CAFs may not be viable, reprogramming CAFs by targeting deregulated genes presents the opportunity for developing novel cancer therapy. CAF reprogramming allows silencing of CAF-derived tumor-promoting factors while preserving the overall structural integrity of the extracellular matrix. We believe that reprogramming CAFs in the tumor microenvironment can inhibit tumor progression, thereby presenting an alternative approach to the treatment of cancer.

Transcriptome profiling of laser-microdissected ovarian tumor samples enabled us to identify stroma-exclusive genes that are deregulated in tumors. This cannot be achieved using bulk tumor samples containing various amounts of epithelial and stromal components. Using laser-microdissected tissue samples, we identified a CAF gene expression signature for high-grade serous ovarian cancer (HGSOC).[3] Immunolocalization of microfibrillar-associated protein 5 (MFAP5) in tissues samples obtained from HGSOC patients and cell culture studies demonstrated that whereas MFAP5 was expressed by cancer cells in a small group of patients, CAFs had markedly higher MFAP5 expression than that in cancer cells, and MFAP5 was exclusively expressed by CAFs in the majority of HGSOC patients.[4,5] We further evaluated the functional roles of MFAP5 in ovarian cancer progression. We observed that MFAP5 was actively secreted by CAFs into the extracellular matrix, where MFAP5 binds to the $\alpha v\beta_3$ integrin receptors on the surface of ovarian cancer cells. Engagement of $\alpha v\beta 3$ integrin with MFAP5 on cancer cells subsequently activates the calcium-dependent focal adhesion kinase/extracellular signal-regulated kinase/cAMP response element-binding protein signaling pathway, which leads to upregulation of troponin C type 1 (TNNC1) expression. TNNC1 facilitates the formation and rearrangement of the F-actin cytoskeleton and enhances the motility of ovarian cancer cells via induction of traction force.[5] TNNC1 is commonly expressed by muscle cells, including those in skeletal and cardiac muscles, and is involved in the contraction of these muscles. Further survival analysis demonstrated that TNNC1 is an independent biomarker for poor prognosis for HGSOC. Besides stromal-epithelial crosstalk mediated by CAF-derived MFAP5, we found a positive correlation between MFAP5 expression and microvessel density,[4,5] suggesting that CAF-derived MFAP5 modulates tumor progression via increased angiogenesis. However, the underlying molecular mechanism of how MFAP5 stimulates angiogenesis remains to be elucidated. Taken together with the fact that high stromal MFAP5 expression is closely associated with poor outcome of HGSOC and functional studies demonstrated the stimulatory effects of MFAP5 on the motility and invasive potential of ovarian cancer cells, reprogramming of CAFs by targeting MFAP5 may be a novel therapeutic strategy for ovarian cancer that may improve survival in HGSOC patients.

To determine the effect of reprogramming of CAFs by targeting MFAP5 on ovarian cancer progression, we injected murine MFAP5-targeting small interfering RNA (siRNA) incorporated into chitosan nanoparticles into ovarian cancer-bearing mice. We observed that this treatment successfully silenced MFAP5 expression in CAFs, decreased the expression of MFAP5's downstream target gene TNNC1 in tumor cells, and inhibited ovarian tumor growth, invasion, and metastasis.[5] These findings suggested that delivery of siRNAs specific to CAF-

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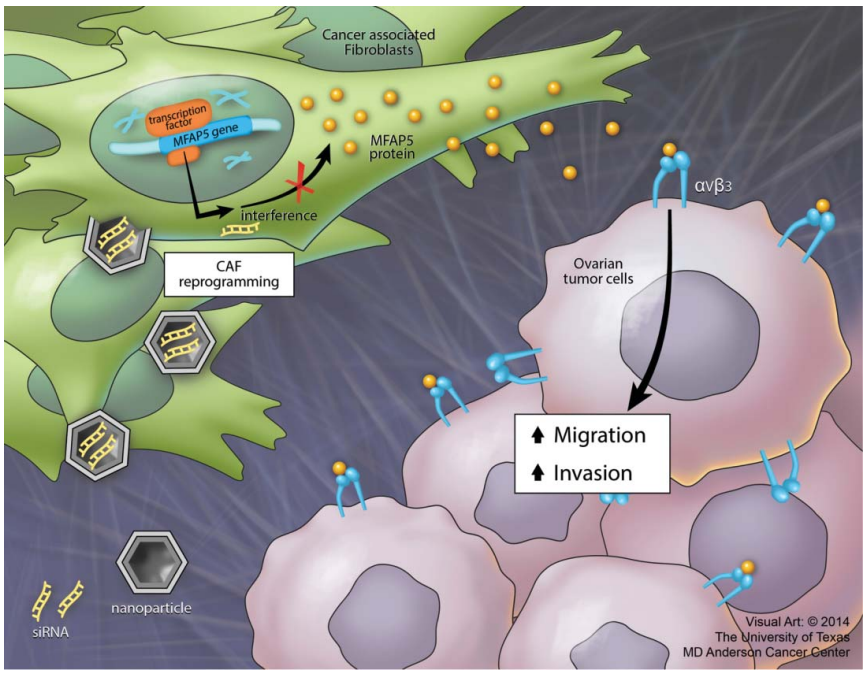

Figure 1. CAF reprogramming using MFAP5-tareting siRNA delivered by chitosan nanoparticles significantly reduces the level of CAF-derived MFAP5 protein in the tumor microenvironment. Shutting down MFAP5-modulated activation of αvβ3 integrin and subsequent calcium-dependent FAK/CREB/TNNC1 signaling result in the inhibition of ovarian cancer metastasis.

expressing genes could reprogram CAFs and lead to inhibition of tumor progression (**Fig. 1**). CAF reprogramming can be further improved with targeted delivery of siRNA sequences using vehicles that specifically target CAFs. This improved specificity can certainly increase treatment efficacy, reduce treatment dosages required for gene silencing, and possibly reduce side effects. A number of such targeted delivery platforms are being developed and evaluated.[6]

In summary, CAF-derived MFAP5 plays important roles in modulating cancer progression and has proven to be a potential therapeutic target for ovarian cancer. These findings suggest that CAF reprogramming via targeted gene silencing in these cells is a new paradigm for ovarian cancer treatment. Also, it may synergize with other treatment regimens that target cancer cells to increase treatment efficacy.

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