The Runt-related transcription factor 1 in prostate cancer-associated fibroblasts

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Although most carcinomas arise from a series of genetic alterations in epithelial cells, cancer is a disease that affects not just the transformed cells themselves, but the entire tissue. During tumor development, reciprocal signaling patterns between the cancer cells and the surrounding tissue are altered in a way that affects tissue architecture and cell fate (1). As a result of signals derived from the tumor and a response to tumor development that resembles the response to a wound (2), 3), epithelial tumors of a number of organs, including prostate, are often surrounded by an activated stroma that can constitute as much as half of the tissue mass. This activated stroma is characterized by changes in extracellular matrix proteins, increased angiogenesis, and a heightened inflammatory reaction (4). The cells within this activated stroma include endothelial cells and pericytes that form the tumor vasculature, infiltrated inflammatory cells, and the predominant population, the activated myofibroblasts (3, 5). Activated myofibroblasts resemble normal fibroblasts, cells that secrete extracellular matrix proteins and thereby create a 3D environment that provides tissue with strength and flexibility (4). However, in the tumor microenvironment, the fibroblasts undergo a myodifferentiation process in which they express smooth muscle actin, proliferate, and produce extracellular matrix proteins, including collagen (2). The cancer-associated fibroblasts of the activated stroma have been shown in mouse models to promote tumor growth and progression (6, 7). In patients, the presence of activated fibroblasts is associated with aggressive behavior (8), angiogenesis (7, 9), inflammation (9), and poor prognosis (10). Preventing the host stroma from contributing to cancer growth could represent an important therapeutic strategy. Realizing this goal requires a better understanding of the development of a reactive stroma. In PNAS, Kim et al. advance our understanding of the myofibroblasts that surround tumors and the molecular basis for their function (11).

One of the outstanding questions about cancer-associated fibroblasts is their origin. The original studies on these cells focused largely on local resident fibroblasts as the predominant cellular contributor to myofibroblasts (12). More recent studies have expanded the options (reviewed in ref. 2). Other cells in the local environment have been reported to serve as myofibroblast progenitors, including pericytes, vascular smooth muscle cells, and even endothelial cells undergoing endothelial-to-mesenchymal transition. Bone marrow-derived mesenchymal stem cells are another possible source, and a population of fibroblast-like cells has been identified in circulating blood. Alternatively, the cancer cells themselves have been found to undergo epithelial-to-mesenchymal transition and contribute to the pool of cancer-associated fibroblasts. Thus, there are multiple possible cellular origins of the myofibroblasts in tumors, including mesenchymal and nonmesenchymal cells, and resident as well as recruited cells.

Kim et al. test the possibility that resident mesenchymal stem cells contribute to prostate cancer-associated fibroblasts (11). They isolated a resident mesenchymal stem cell population from a sample of normal prostate. The cell line displayed the morphology of normal fibroblasts in culture and exhibited the capacity to differentiate into neurogenic, chondrogenic, and osteogenic lineages. The cells from this population expressed a number of cell surface markers previously associated with mesenchymal stem cells (13). Based on the presence of cells with these cell surface markers, the authors identified candidate mesenchymal stem cells in the stromal compartment of multiple examples of normal human prostate. Because TGF-β1 in the tumor microenvironment is an important signal for the generation of cancer-associated fibroblasts (12), Kim et al. cocultured mesenchymal stem cells they isolated with prostate cancer cells engineered to express TGF-β1 in a 3D organoid coculture model and mouse xenografts. The mesenchymal stem cells responded by activating TGF-β1-responsive genes in culture, and by expressing α-smooth muscle actin in mice. The findings reported in the paper, therefore, provide new information on the existence of a mesenchymal stem cell population within the normal prostate that could serve as a source for the myofibroblasts present within prostate tumors. Further studies would allow a determination of the extent to which mesenchymal stem cells with these characteristics do, in fact, contribute to the activated myofibroblasts that constitute prostate tumors in mice and humans.

The authors then tackled the question of the molecular basis for the activation of these mesenchymal stem cells. resident within the prostate are activated by TGF-β. TGF-β induces RUNX1, and the RUNX1 contributes to mesenchymal stem cell proliferation. TGF-β subsequently induces mesenchymal stem cell differentiation and this process is not dependent on RUNX1.

![Fig. 1](https://example.com/fig1.png)

**Fig. 1.** A model for prostate cancer stroma formation suggested by the results of Kim et al. Mesenchymal stem cells resident within the prostate are activated by TGF-β. TGF-β induces RUNX1, and the RUNX1 contributes to mesenchymal stem cell proliferation. TGF-β subsequently induces mesenchymal stem cell differentiation and this process is not dependent on RUNX1.
mesenchymal stem cells. TGF-β can both promote the proliferative expansion of mesenchymal stem cells (14) and then later facilitate the adoption of a myofibroblast phenotype (15). Kim et al. discovered that organoid coculture with TGF-β-expressing prostate cancer cells resulted in higher levels of a number of transcription factors in the prostate mesenchymal stem cells, including the Runx-related transcription factor 1 (RUNX1) (11). The RUNX transcription factors are expressed in specific cell lineages and are important transcriptional regulators of stem cell commitment to form blood or bones (16, 17). Kim et al. show that, in the absence of RUNX1, the response to TGF-β activation changed dramatically. With RUNX1 knockdown, the mesenchymal stem cells exhibited a sharply reduced capacity for proliferation, as evidenced by many fewer cells in the S phase of the cell cycle, lower levels of some cell cycle promoting transcripts, and higher levels of transcripts encoding some cell cycle inhibitors. Decreased cell proliferation was also observed when the RUNX1 knockdown mesenchymal stem cells were introduced into nude mice. Along with reduced proliferative capacity, the RUNX1 knockdown cells exhibited an even more pronounced activated myofibroblast gene expression profile in coculture and higher levels of smooth muscle actin in xenografts (Fig. 1). The authors conclude that RUNX1 contributes to the maintenance of the proliferative status of TGF-β–activated mesenchymal stem cell progenitors before their differentiation to myofibroblasts.

The discovery that RUNX1 levels modulate the cellular response to TGF-β provides an important clue to the process by which TGF-β promotes multiple different cell fates. The findings correlate well with previous studies showing that RUNX transcription factors are induced by TGF-β signaling (18) and are important mediators of the effects of TGF-β (18). Further, RUNX transcription factors have been shown to form a physical complex with the Smad transcription factors that mediate TGF-β signaling (19, 20). The TGF-β response element contains recognition sites for RUNX and Smad transcription factors in close proximity, and the binding of both factors has been reported to enhance expression of some target genes (18, 20). RUNX2 can also affect the subnuclear targeting of Smads to the nuclear matrix (19). Future experiments could elucidate how signals from TGF-β and RUNX1 are interpreted and integrated by prostate mesenchymal stem cells. For instance, do RUNX1 and Smad proteins physically interact within prostatomesenchymal stem cells? Does RUNX1 affect the subnuclear localization of Smads in prostate mesenchymal stem cells? Do both transcription factors, individually or as a complex, bind the promoters of a set of TGF-β–responsive genes and does this affect the set of genes that are activated during myofibroblast differentiation or the strength of the transcriptional response? Can these interactions explain how TGF-β activation in the context of reduced RUNX1 levels results in a decreased proliferative response, but a stronger myodifferentiative response?

Other important questions raised by the findings of Kim et al. concern the physiological relevance of RUNX1 in the context of prostate tumors. Do high stromal RUNX1 levels result in an expansion of cancer-associated fibroblasts in prostate tumors, and if so, do high RUNX1 levels correlate with poor prognosis? Conversely, are there patients with low RUNX1 levels, and do the cancer-associated fibroblasts in these patients have reduced proliferation or a more extreme commitment to a myofibroblast phenotype? Do these patients have a better prognosis? The results of these experiments will provide valuable information for assessing whether targeting RUNX1 in mesenchymal stem cells would prevent the development of an activated, tumor-promoting stroma, and potentially provide a treatment for prostate cancer patients.