

In Vivo-Like Three-Dimensional Extracellular Matrix Systems: a Study of Stroma Progression During Tumorigenesis

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In the course of epithelial tumor progression, the tumor microenvironment undergoes a number of dynamic and regulated alterations that occur in parallel to transformation. In many cases, as proliferating epithelial cells progress and become aggressive, the host microenvironment evolves also, inducing basement membrane discontinuation, severe immune responses, and the formation of new blood vessels. These microenvironmental host responses are accompanied by additional dynamic alterations on the mesenchyme, known as 'stromagenesis,' that occur in the near vicinity of the progressing tumor (1). The mesenchymal stromagenic alterations that occur parallel to tumor progression are similar to those that accompany certain developmental processes, pathological fibrotic reactions, and fibrotic wound-healing responses.

Our laboratory studies the molecular characteristics and signaling mechanisms of stromagenic fibroblasts and their extracellular matrices (ECMs) during tumor progression. We are currently studying stromagenesis focusing on both stroma dynamics and signal transduction. For these studies, we use an *in vivo*-like stromal-derived 3D system (2), which takes full advantage of traditional *in vitro* tissue culturing yet mimics the stromal microenvironment *in vivo*. By utilizing this 3D culturing system, we hope to engage in developing stromal therapeutic targets that could potentially contain epithelial neoplasia at a chronic and innocuous state.



***In vivo*-like 3D stromagenic systems.**

Amatangelo, Brown, Quiros, Valianou, Cukierman, in collaboration with Klein-Szanto,[§] Bassi,[§] Cheng,[§] Haluszka,[§] Tokar,[§] S. Cohen,[§] Hoffman,[§] Watson[§]

Stromagenesis is a host reaction of connective tissue that is induced in cancer, which produces a progressive and permissive mesenchymal microenvironment, thereby supporting tumor progression (1). The stromal microenvironment is complex and comprises several cell types, including fibroblasts, the primary producers of the non-cellular scaffolds or ECMs. The events that support tumor progression during stromagenesis are, for the most part, unknown due to the lack of suitable, physiologically relevant experimental model systems. In this report, we introduce a novel

in vivo-like 3D system derived from tumor-associated fibroblasts at diverse stages of tumor development that mimic the stromagenic features of fibroblasts and their matrices observed *in vivo*. Harvested primary stromal-fibroblasts, obtained from different stages of tumor development, do not retain *in vivo* stromagenic characteristics when cultured on traditional two-dimensional substrates. However, they are capable of effectively maintaining the tumor-associated-stromal characteristics within 3D cultures (Figure 1). We have shown that *in vivo*-like three-dimensional matrices appear to have the necessary topographical and molecular information sufficient to induce desmoplastic-stroma differentiation of normal fibroblasts (2). The above-mentioned study was conducted on a murine 3D stroma system that

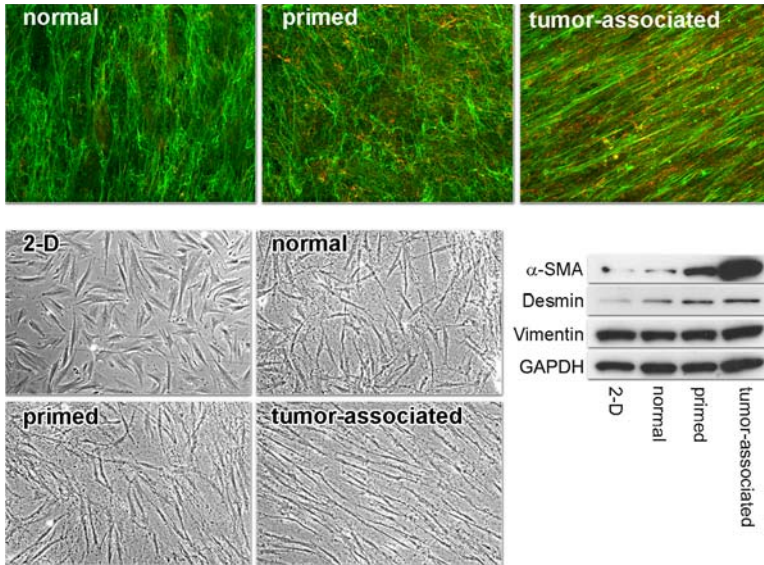


Figure 1. Normal, primed and tumor-associated 3D matrices derived from these classes of fibroblasts. In top panels; fibronectin is shown in green and collagen type I in red. In the middle panels; normal fibroblasts were plated onto tissue culture plates (2-D) or within normal, primed or tumor-associated 3D matrices. The right panel represents a Western blot probed for the indicated desmoplastic markers of the lysates obtained from a parallel to the middle panel experiment. Note the differences in matrix production, as well as the effect that the various matrices have on normal fibroblasts within the matrices.

is derived from squamous cell carcinoma associated fibroblasts. We are also in the process of establishing stroma-derived 3D systems from stromal human ovarian and pancreatic cancers.

Stromagenic 3D-adhesion signaling and cell motility. Beacham, Valianou, Amatangelo, Lech,* Zinshteyn, Cukierman

Our working hypothesis posits the existence of architectural, biochemical, and/or mechanistic aberrations that manifest as modified adhesions within 3D vs. 2D substrates, as well as within the tumor-associated stroma. The aberrant structures within the tumor-associated stroma support or even incite tumor progression. To

test this, the identification of representative signal-transduction cascades among 3D-adhesion structures within the three stromagenic stages, normal, primed, and activated (1), is being conducted in our laboratory. First, we have established some signaling differences among cells plated on classic 2D tissue cultures or within control fibroblasts-derived (NIH-3T3) 3D matrices. We observed that re-plating normal cells within fibroblasts-derived 3D matrices induces morphological and signaling changes; e.g., we have documented a down regulation of activated small GTP binding protein Rac within 3D matrices and that this matrix induced Rac activity down regulation is responsible for a directional and persistent motility (3,4).

Publications

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