Video Article

Biomimetic 3D culture of normal and transformed human breast cells

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Abstract

The Bissell laboratory has for over 30 years been developing methods for the culture of human breast or murine mammary cells under physiologically-relevant conditions, primarily in three-dimensional biological matrices (3D) that provide a basement-membrane-like microenvironment for the cells. The conclusion that such 3D cultures facilitate observations that are more relevant, as compared to those made using 2D cell culture on tissue-culture plastics, is based on repeated observations of cellular polarity, architecture, functions, signaling cascades, and phenotypes analogous to those observed in vivo and in the clinic. Thus 3D cultures are the research method of choice if one is to reach clinically applicable conclusions; conclusions based on conventional 2D systems are often erroneous. The most accessible biological matrices for 3D cultures are derived from Engelbreth-Holm-Swarm murine sarcomas (EHS) {commercialized under the tradenames Cultrex (Trevigen) and Matrigel (BD)}, or collagen I alone or in a mixture with EHS. The critical qualities of these matrices include extracellular signaling agents and physical and mechanical properties of the matrices, all of which are finely tuned to generate biomimetic context for cell culture. More recently, we and others have generated synthetic matrices whose nanofibrillar and viscoelastic properties can be tuned for optimal cell culture, and to which specific adducts can be applied at will, thus allowing dissection of the various microenvironmental cues controlling cell growth, differentiation, gene and epigenetic activity, and cancerous transformation. The visualized experiment presented here is the basic method for culturing human breast cells by embedding them either in a gel of growth-factor reduced, laminin-rich extracellular matrix (lrECM); or by culturing them in a lrECM slurry on top of a gelled layer of lrECM. The merits of each will be explained in the video and protocol. This protocol may be adapted readily for use with other matrices such as collagen I or hyaluronin-based synthetics, or with other epithelial cell types.

Disclosures

No conflicts of interest declared.