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Targeted 2D- and 3D-cell cultures reveal mechanistic effects of extracellular matrix and stromal cells on the metastatic niche formation and metabolism of cancer cells

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Primary tumor progression and metastasis are highly dependent on the cancer cell population and its interaction with surrounding stimuli, i.e. extracellular matrix (ECM) and stroma. Therefore, a comprehensive knowledge about processes involved in the remodeling of the ECM during niche formation and the metabolic coupling of cancer cells and stromal cell populations is needed in order to improve cancer treatment. Due to their ease in use and reproducibility, cell culture-based models have been widely used in cancer research. However, common models often lack important aspects of tumor biology, in particular, regarding ECM and stroma. For that reason, this work aimed at novel two- (2D) and three-dimensional (3D) models in mono- and co-culture that enable deeper insights into the role of ECM and stroma in cancer cell biology.

First, the effects of ECM compounds on the growth of 2D-cultures and 3D-spheroids of MDA-MB-231 human breast cancer cells were addressed. This showed that 3D-cultures of this cell line establish stable spheroids only in the presence of ECM supplementation but not in their absence. In parallel, MCF10A human breast epithelial cells were tested to evaluate their transferability towards co-culture modeling with distinct media compositions. This revealed highly altered spheroid morphologies and sizes when adapting mutual mono-culture protocols. In particular, MDA-MB-231 showed increased spheroid growth in enriched media commonly used for MCF10A cells, while the non-cancerous epithelial cells established proliferating subspheroids upon supplementation with basal membrane extract (BME).

Next, the role of ECM on biosynthesis and secretion of the bone metastasis marker, bone sialoprotein (BSP), were studied. Therefore, cultures in cell-repellent wells containing basal membrane extract served as robust 3D-culture model. This showed that BSP expression levels were higher in the presence of BME, Type-I collagen, and proteolytic activity associated with ECM remodeling, implying mechanistic interactions between matrix metalloprotease (MMP) activity, BSP expression and matrix acidification. Experiments with cycloheximide on samples processed with and without permeabilization indicated an induced protein neogenesis with consecutive secretion consistent with latent transforming growth factor beta or matrix MMPs being activated during ECM modulation.

After altered cellular activity of MDA-MB-231 cancer cells based on ECM stimuli was ensured, findings were transferred to HT-29 human colon cancer cells to enhance the significance of the readouts. Changing the cell culture model to an array-based 3D-culture system improved cell-cell interactions and mechano-transduction processes that were rather limited in spheroid cultures based to their compact structures and a lack of stronger physical support. Chip cultures revealed metabolic alterations in both, ECM producing fibroblasts and cancer cells, indicating mechanisms complying with the reverse Warburg effect. During this metabolic coupling process, the aerobic glycolysis is increased in stromal fibroblasts and membrane shuttling of metabolites can favor cancer progression and metastasis.

All 3D-studies were facilitated by progress in optical tissue clearing of intact fixed 3D-cancer cell cultures. Thus, the present work contributed to these novel protocols for cancer cell spheroids and 3D-cell-array cultures.

Altogether, links between ECM-mediated cancer progression and metabolic coupling as well as metastatic niche formation were described in this work using novel cell culture models. Technological advances in both, cell culture methodology and downstream sample analysis were achieved, which yielded new insights on intercellular signaling induced by ECM components or stromal cells. Therefore, considering co-culture compositions and ECM compounds, a promising tool for preclinical trials was established to help improving cancer research.