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3D-2 heterogeneous breast cancer models

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Motivation and Aim: Cell models of breast cancer provide an opportunity for the investigation of molecular mechanisms of tumour progression. Tumour spheroids (3D-models) are frequently used *in vitro* as a model in preclinical studies as we can observe the gradient of the cells receiving oxygen and nutrients. However, most modern 3D-models contain only one type of cells, while in order to be physiologically relevant the models must be heterogeneous. 3D-2 cellular model consists of tumour and stromal cells. Growing heterogeneous spheroids is of current importance today because such models are necessary for the development of new approaches to antitumour therapy and the study of the intercellular interaction in cancer progression.

The aims of the research are to construct heterogeneous 3D-2 cellular models of breast cancer and to analyse the proliferative and invasive ability of cells stimulated by 17β-estradiol and transforming growth factor beta (TGF- β).

Methods and Algorithms: MCF-7 ($ER^+/PR^+/HER2^-$), MDA-MB-231 (ER-/PR-/HER2-) and SK-BR-3 ($ER^-/PR^-/HER2^+$) were used as cancer components of 3D-2 spheroids for different imitating tumour types. We chose patient-derived cancer fibroblasts (BrC4f and BrC120f) and a normal fibroblast (BN120f) as stromal components of 3D-2 models. To visualise the relative position of cells in the spheroids, we obtained tumour cells (MCF-7, MDA-MB-231 and SK-BR-3) expressing red fluorescent protein (mKate) and fibroblasts (BrC4f, BrC120f and BN120f) expressing green fluorescent protein (eGFP). We used low-adhesive plastic and co-cultivated tumour cells and different types of fibroblasts to construct 3D-2 spheroids. Invasion potential of cells which form 3D-2 spheroids was tested on Matrigel™ substrate.

Results: When tumour and stromal cells were mixed, fibroblasts formed the inner frame, and tumour cells formed the outer layer of 3D-2 models. The largest spheroids were obtained from cell culture SK-BR-3. Forming a dense necrotic core and a clear boundary of the spheroid were observed. The smallest spheroids were obtained from MDA-MB-231 cell culture. 3D-models from MCF-7 cells were characterised by a smaller size of necrotic core and the absence of a clear border of the spheroids in comparison with spheroids obtained from SK-BR-3. When we co-cultivated tumour cells with fibroblasts, we found out that the spheres became denser and they got a more precise structure in comparison with the spheres obtained only from tumour cells.

17β-estradiol stimulated the proliferation of MDA-MB-231 cells during mono-culturing, which may be due to the expression of the ERα36 splice variant in cells associated with an aggressive cell phenotype. 17β-estradiol influenced the self-organization of MCF-7 culture spheroids during mono- and co-cultivation with fibroblasts. This was reflected in an increase in their density, which was accompanied by self-organisation into

microtissue. TGF-β stimulated cell proliferation of hormone-independent culture SK-BR-3 in 3D-2 spheroids, which may be connected to the implementation of TGFβ/HER2/EGFR signalling in them.
The analysis of the invasive potential of tumour cells revealed that co-cultivation with stromal cells stimulated cell invasion only for the MDA-MB-231 culture. The stimulation with TGF-β reduced the invasion of cells from 3D-2 models, while the stimulation with 17β-estradiol increased the invasive potential.
Conclusion: As a result, the conditions for construction of 3D-2 heterogeneous spheroids of breast cancer capable of imitating tumour tissue were optimized. 3D-2 heterogeneous breast cancer model is an appropriate *in vitro* model for studying tumour-stroma interactions, invasion potential of tumour cells and research of cytotoxic effect of antitumour drugs.
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