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Quantification of cell fusion events between breast cancer cells and breast epithelial cells

Recent data suggest that cell-cell fusion contributes to the development of hybrid cell clones showing genetic and behavioral alterations. But less is known about forces triggering cell fusion processes between breast cancer cells and tumor surrounding cells. The influence of chronic inflammation and tumor microenvironment is increasingly being recognized as an etiology for cancer progression and cell fusion. Several inevitable steps of cell fusion- containing chemoattraction, cell adhesion, priming and post fusion processes- are well-investigated. However, information on how and which components particularly affect cell-cell fusion events and thereby disease outcome remains elusive.

Available facilities to quantitate these cell-cell fusion processes *in vitro* could provide a novel method to identify cancer promoting components of the stroma. In the present study cell-cell fusion can be measured by using a cre/ loxP double fluorescence reporter vector system. Therefore, the two human breast cancer cell lines MDA-MB-435 and HS578-T were stable transfected with a double fluorescence reporter vector using lentiviral transduction and fluorescence activated cell sorting subsequently. In addition to it, a third human breast epithelial cell line M13SV-1 expressing stable the cre recombinase was generated.

Cell fusion experiments are promoted by co-culturing cells under appropriate conditions and for different periods of time. Fused cells can easily be quantitated by measuring recombination events by flow cytometry. Regarding the complex nature of tumor milieu the aim of the project is to investigate the potential influence of several inflammatory and anti-inflammatory cytokines for alterations in fusogenic behaviour of cell lines.

Increased fusion activity was already observed while adding the proinflammatory cytokine TNFα or epithelial growth factor (EGF). It could also be assumed that hypoxia is a stimulus for cell-cell fusion. In contrast the chemoattractant SDF-1α, which is involved in many cancer metastatic mechanisms, had not any influence in

our experiments so far. Interestingly, both tested breast cancer cell lines showed partially different fusion activity after stimulation with several cytokines.

In order to investigate further potential influences on cell-cell fusion the following recombinant human supplements mimicking inflammation processes or promoting cell adhesion will be tested in the cell culture media (rh GRO-alpha, rh Leptin, rh TGF-beta3, rh CTGF, rh Galectin-1, rh MIP-4/ CCL18, rh MCP3/ CCL7, rh MCP1/ CCL2, rh IFNgamma, rh IL-1alpha/ IL-1F1, rh IL-4, rh IL-8, rh IL-10, rh IL-12, rh IL-16, rh IL-21, SDF-1α/ CXCL12a, rh TNFα, rh IGF-I, rh IGF-II).

Receptor status data of used human cell lines will be used as reference for comparison of cell fusion experiment outcomes during later tests. In order to further characterize the receptor status of cell lines FITC conjugated anti-human antibodies (CD9, CD18, CD27, CD29, CD105) will be tested among others by flow cytometer. Since tumor associated macrophages play a crucial role in linking chronic inflammation and cancer, the influence of listed factors on cell-cell fusion could be investigated in addition between breast cancer cell lines and stable cre expressing macrophages.

Summarized the present study establishes an efficient tool for quantification of cell-cell fusion processes *in vitro* by cre mediated recombination of a loxP double fluorescence reporter vector system. The examination of signalling molecules of the tumor microenvironment highlights a new opportunity to identify a potential link between components priming and triggering cell-cell fusion and therefore promoting carcinogenesis.