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Generation of stable breast cancer cell lines with overexpression of αvβ3 integrin

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Motivation and Aim: Integrins are transmembrane receptors that mediate adhesion of cells to extracellular matrix. αvβ3 integrin is known to be involved in angiogenesis and metastasis, and is aberrantly expressed on the endothelium of tumor blood vessels and on the membrane of many types of tumor cells [1, 2]. This receptor is a promising target for anticancer therapy, especially due to their ability to recognize and bind to RGD motif. One of the approaches is the modification of drugs and diagnostic agents with RGD peptides for their targeted accumulation in tumors [3]. The development and testing of such drugs requires the availability of a model cell line with enhanced expression of αvβ3. The aim of the study was to establish cell lines of mouse breast carcinoma 4T1 and human breast adenocarcinoma MDA-MB-231 with stable overexpression of integrin αvβ3.

Methods and Algorithms: To induce endogenous gene expression we used CRISPR-Cas9 guide RNA-directed synergistic activation mediator (SAM) system [4]. Integration of transgenes encoding components of the SAM complex into the cell genome using lentiviral vectors ensures stable activation of target gene expression. We constructed 11 recombinant plasmids encoding various sgRNA to the promoter regions of the *H. sapiens* ITGAV and ITGB3 and *M. musculus* itgav and itgb3 genes. T293 cells were used for production of lentiviral vectors. First, MBA-MB231 and 4T1 cell were transduced with dCas-VP64 and MS2-p65-HSF1 encoding lentiviruses and selected for stable expression. Then the selected cell lines were transduced with a mixture of lentiviral vectors expressing sgRNAs. Next, the antibiotic-resistant clones were selected and monoclonal cell lines were obtain by the limiting dilution method. The expression of αv and β3 integrin subunits was quantified by qRT-PCR and presence of αvβ3 integrin on the surface of modified cells was confirmed by flow cytometry with FITC labeled Anti-Integrin alpha V beta 3 antibody.

Results: We generated two genome-modified cell lines of human breast adenocarcinoma MDA-MB-231_27 and mouse breast carcinoma 4T1_27 with over expression of αVβ3 integrin. According to qRT-PCR data, the expression of αv and β3 subunits was upregulated 16.5-fold and 9-fold in MDA-MB-231_27, 4-fold and 1.5-fold in 4T1_27 cell line respectively.

Conclusion: Two stable breast cancer cell lines with overexpression of αvβ3 integrin were generated for testing diagnostic and/or therapeutic efficiency of RGD-conjugates.

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