

Characterizing a TET-repressible ErbB2-expressing fibroblast line as a possible model for testing ErbB2 targeted therapies

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ErbB2 is a receptor tyrosine kinase underlying bad prognosis in several human malignancies. Trastuzumab, a humanized anti-ErbB2 antibody, is a specific targeted therapy against these tumors, with a history of both success and a high rate of therapy resistance.

Our objective was to characterize an ErbB2 expressing cell line that can serve as a model for antitumor therapies to better understand the keys of successful therapy in animal models. We've investigated the SADR cell line which is a derivative of NIH3T3 fibroblasts stably transfected with the ErbB2 gene under the negative control of tetracycline.

We studied the sensitivity of SADR cells to short (5 minutes) and long (3 days) trastuzumab (0.1 mg/ml) treatment in terms of proliferation, cell cycle, ErbB2 expression and phosphorylation, apoptosis, and cell morphology in native and in ErbB2-deprived (tetracycline treated) SADR cells. As in initial experiments we found a drift in the rate of proliferation inhibition, we also examined how the parameters in question changed during the aging of the cell line: experiments were made with cells from 3rd, 10th and 20th passages. Proliferation was assessed both from mitochondrial activity (MTT assay) and from cell culture impedance (xCELLigence system). DNA, ErbB1, pErbB2 and Annexin V were fluorescently labeled and measured in flow cytometry. For further characterization, we also assessed mRNA expression using a whole mouse genome chip.

The proliferation rate upon ErbB2 deprivation by tetracycline decreased at all passages, coherent with the proliferation advantage from excess ErbB2. In these cells, trastuzumab did not decrease the proliferation further, nor did it affect the cell cycle, thus confirming specific trastuzumab action targeting ErbB2. In native SADR cells not treated with tetracycline, trastuzumab caused a G1-block that was correlated with the passage number, also enhanced short term ErbB2 phosphorylation, and decreased ErbB2 expression in the long run. While in the earliest passage minimal effects were observed upon trastuzumab treatment, by the 20th passage all ErbB2-related effects have manifested. Apoptotic rate was low (under 1%), and treatment did not alter that. Long term trastuzumab treatment decreased the focus formation and increased spreading. The mRNA expression showed that both trastuzumab treatment and ErbB2 deprivation increase the expression of genes with antiangiogenic effect (thrombospondin, adamts5, connective tissue growth factor) and downregulate the level of those enhancing cell adhesion and focus formation. These confirm the observed morphological changes and the in vivo inhibition of tumor progression as well.

The SADR cells appear to be not only a therapy target model, but also a model of addiction to the ErbB2 oncogene at the cellular level. The experiments also draw attention to the stability problems of transfected cellular systems.

