

➤ IGF1-R

IGF1-R is a type II tyrosine kinase receptor consisting of two extracellular α -subunits, two transmembrane β -subunits, and an intracellular tyrosine kinase domain. It is ubiquitously expressed and has multiple functions. However, IGF1-R over-expression transforms mouse 3T3 fibroblasts, and high levels of IGF1-R expression have been detected in human tumors, making it a bona fide target for the development of novel anti-cancer treatment.

➤ MEF-IGF1-Rrep

MEF/Toff/IGF1-R cells were generated by stable transfection of MEF/Toff cells (Clontech, Heidelberg, Germany) with the plasmid pTRE-IGF1-R-2myc6his and pSV-hygro. Clones were selected with hygromycin and subclones were selected by picking clones that form colonies when grown in softagar (Graeser et al., unpublished). The cells express the human IGF1-R cDNA under the control of a Tet-inducible promoter. IGF1-R is expressed in the absence of doxycycline and expression is inhibited in the presence of doxycycline.

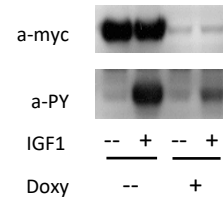
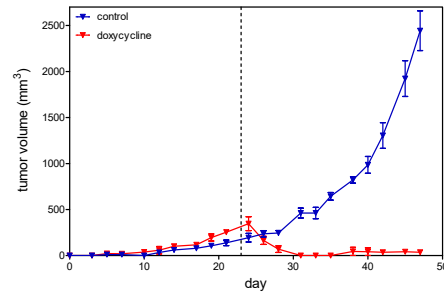


Figure 1: Characterization of the cell line. The cells were kept either in the presence or absence of the regulator, doxycycline, and IGF1 (100 ng). IGF1-R expression was checked via its myc-tag, and autophosphorylation using an anti-phosphotyrosine antibody.

➤ Tumor growth *in vivo*

MEF-IGF1-Rrep cells harvested from tissue culture flasks were implanted into the subcutaneous space of the left flank of the mice. At an average size of 400 mm³, the mice were randomized into two groups, and one group received doxycycline in the drinking water. As shown in Figure 2, the repression of IGF1-R expression induced by the treatment resulted in tumor regression.



➤ Immunohistochemistry

As shown in figure 3, the addition of doxycycline to the mice in the drinking water ablates exogenous IGF1-R expression in the tumors.

Figure 3: Immunohistochemistry of MEF-IGF1-Rrep tumors. On the left, a tumor of an untreated mouse is shown. The tumor on the right panel was extracted from a mouse treated with doxycycline. IGF1-R positive cells are brown, the nuclei are counterstained with hematoxylin (blue).

