Development of cell lines with overexpression of the αvβ3 integrin by CRISPR/Cas9 SAM-activation

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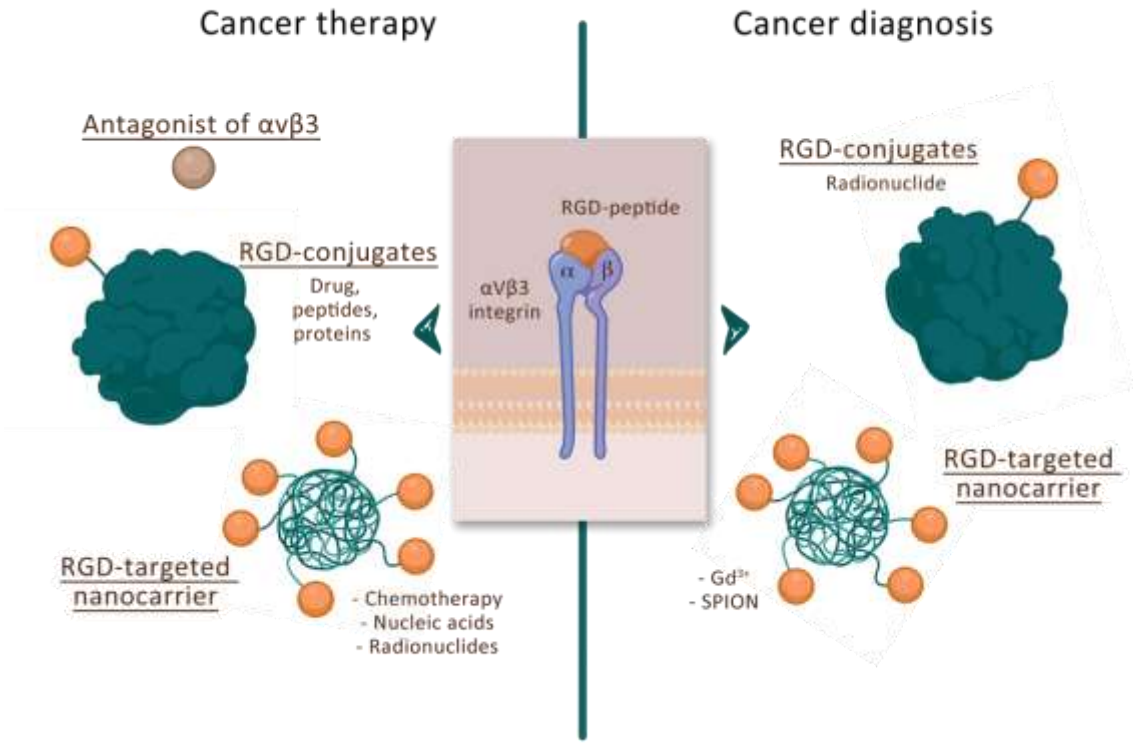
Relevance and Aim

RGD-based targeting strategies for cancer diagnosis and therapy



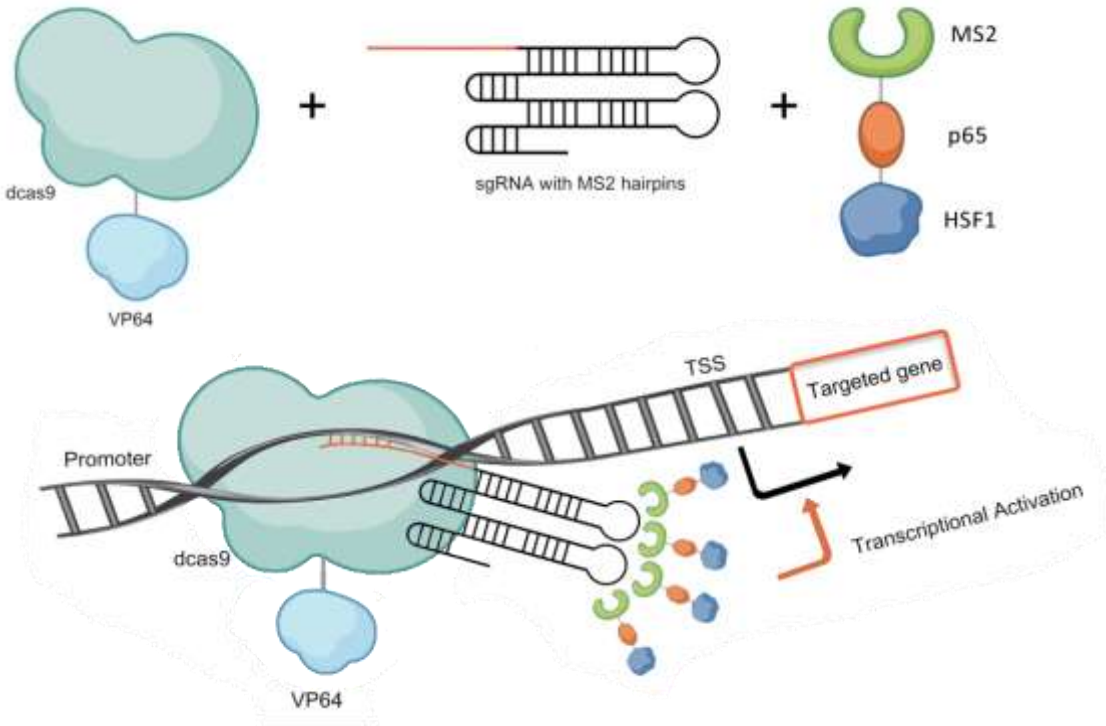
Danhier F, Le Breton A, Préat V. RGD-based strategies to target alpha(v) beta(3) integrin in cancer therapy and diagnosis. Mol Pharm. 2012 Nov 5;9(11):2961-73

αvβ3 integrin is known to be involved in angiogenesis and metastasis. It is widely present on the endothelium of tumor vessels and on the membrane of many types of tumor cells. This receptor can be used as a target for cancer diagnosis and therapy. One of the approaches is to modify drugs and diagnostic agents with RGD peptides for its targeted accumulation in tumors. The development and testing of such drugs requires the availability of model cell lines with increased expression of αvβ3.

The aim of the work is to obtain cell line of human breast adenocarcinoma MDA-MB-231 with increased expression of αvβ3 integrin.

Methods

SAM – synergistic activation mediator



Konermann S, Brigham MD, Trevino AE, et al. Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. *Nature*. 2015;517(7536):583-588.

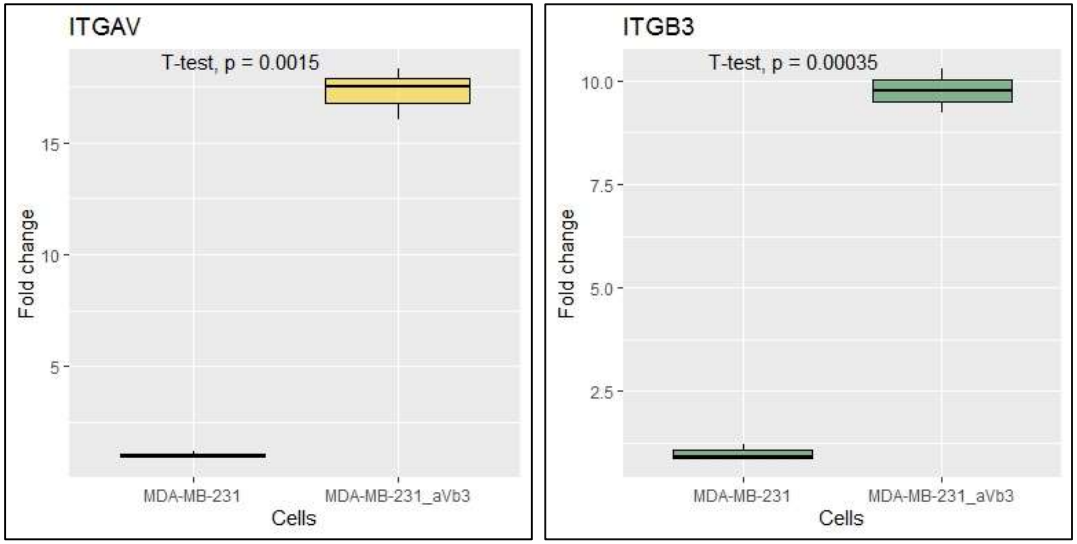
We constructed 7 recombinant plasmids encoding sgRNA to the promoter regions of the H. sapiens *ITGAV* and *ITGB3* genes. Lentiviral particles containing DNA components of the SAM complex - dCas-VP64, MS2-p65-HSF1 and 7 different sgRNA to the promoter regions of the genes encoding the αv and β3 subunits of human integrin were produced.

Transduction of cells with lentiviruses, selection of antibiotic-resistant clones and separation of the monoclonal line were performed. αv and β3 integrin subunits gene expression activation was confirmed by qRT-PCR and flow cytometry.

Results

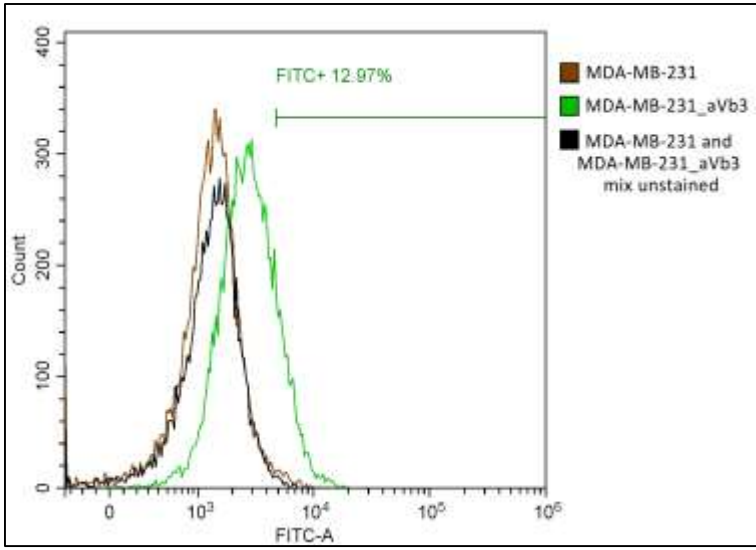
Endogenous expression of activated genes

	MDA-MB-231	MDA-MB-231_aVb3
ITGAV, fold change	1.01±0.14	17.27±1.16
ITGB3, fold change	1.01±0.19	9,76±0.52



Expression of *ITGAV* and *ITGB3* in monoclonal cell line MDA-MB-231_aVb3. Results are expressed as mRNA fold change normalized to *Actb* using the Pfaffl method. The values plotted as the mean per group ± SD from three independent experiments

Flow cytometric analysis of CD51/CD61 (integrin aVb3) expression of both modified and non-modified cells MDA-MB-231. Cells were stained with FITC Mouse Anti-Human CD51/CD61. Fluorescent histogram depicting CD51/CD61 expression was derived from gated events with the side and forward light-scatter characteristics of viable cells.



Conclusion

Modified cell lines with increased expression of αvβ3 provide an opportunity to better characterize the effectiveness of the RGD-targeted drug. The strength of this approach is that the control (not modified) and experimental cell lines differ in only one parameter.