Cell-based assays

Vectalys is an R&D company with a state-of-the-art technology platform for customized viral vector production.

The company has developed a unique process enabling the production of high titer high purity lentiviral vectors for optimal knock-down or over expression in relevant models: primary and stem cells for target gene validation and specific tissue for animal models.

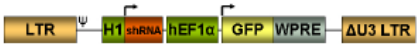
Introduction

On the occasion of a collaborative project, Vectalys developed a lentiviral shRNA delivery approach to study the function of a putative target involved in the metastatic pathway *in vitro* as well as *in vivo*. The target is a novel tyrosine kinase (TK) receptor identified as a potent suppressor of anoikis. This receptor is expressed as five isoforms sharing the same extracellular (EC) and transmembrane (TM) domains. Only two isoforms however contain the TK domain responsible for the metastatic activity. The lack of *in vitro* cellular models displaying full-length isoforms incited us:

> to establish such an *in vitro* cellular model (HEK293T) expressing the full-length cDNA or ΔTK domain to evaluate the specific function of the TK domain,

Transgene expression cassette inserted between the 2 LTR.

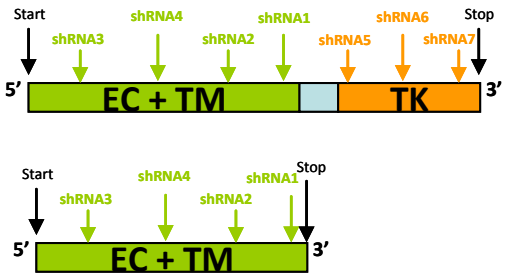
> to construct lentiviral shRNA-expressing vectors and select one able to efficiently silence the receptor and ideally be specific to the TK domain.



shRNA expression cassette inserted between the 2 LTR.
GFP is co-transduced but independently transcribed.

Material and methods

Firstly, we designed shRNA sequences divided into two groups: the first inhibiting all five isoforms (targeting the shared EC or TM domain) and the second specifically inhibiting the two TK isoforms (figure below).



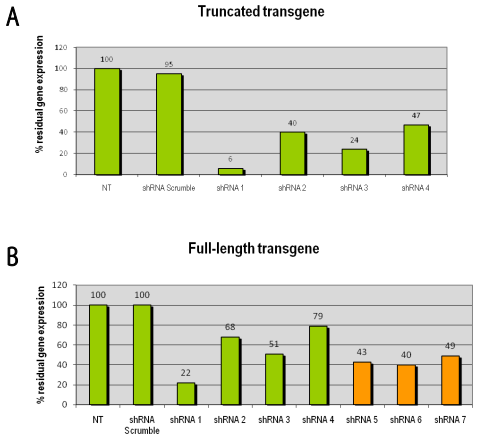
Position of the 7 shRNAs on the full-length transgene (A) or truncated form (B).

We produced all the lentiviral vectors expressing cDNAs or shRNAs. HEK293T cells over-expressing the full-length or truncated cDNA were constructed by transduction at MOI 20. These two cell types were then transduced at MOI 20 by shRNA-expressing lentiviral vectors. Overexpression and silencing efficacy were measured by RTqPCR after transduction with shRNA or cDNA-expressing vectors.

Finally, we quantified the integrated copy number of each shRNA construct within the host cell genome by qPCR.

Results

Quantitative analysis of shRNA efficiency



Efficacy of shRNAs targeting the full-length transgene (A) or the truncated transgene (B). The % residual gene expression was obtained by RT-qPCR using SYBR Green and calculated by comparison with the expression levels in cells not transduced (NT) by shRNA. shRNA scramble is a negative control shRNA with a random sequence.

Quantification of shRNA integrated copy number

A	shRNA copy number / transduced cell	% residual transgene expression
NT	0	100
shRNA scrumble	1,9	100
shRNA 1	5	22
shRNA 2	4	68
shRNA 3	1,1	51
shRNA 4	1,2	79
shRNA 5	2,9	43
shRNA 6	2,5	40
shRNA 7	0,9	49

B	shRNA copy number / transduced cell	% residual transgene expression
NT	0	100
shRNA scrumble	2,9	95
shRNA 1	8,3	6
shRNA 2	6,7	40
shRNA 3	3,1	24
shRNA 4	3,2	47

Absolute efficiency of the 7 shRNAs targeting the full-length transgene (A) or the 4 shRNAs targeting the truncated form (B). In order to measure the shRNA copy number per host cell genome, we performed qPCR using SYBR Green by amplification of an integrated vector segment.

Our results show that:

> All designed shRNAs show a silencing effect with different inhibition scores. The order of efficiency of group 1 shRNAs is identical whether isoforms are short or full-length. shRNA1 displays the highest knock-down capacity, with only 6% and 22% of residual gene expression respectively for the truncated and full-length transgenes. The inhibition of the truncated transgene is always higher than that of the full-length form. This could be explained by a difference in mRNA folding that perhaps modifies target accessibility.

> The three TK-specific shRNAs inhibit the transgene to comparable levels, however, lower than that reached with shRNA1.

> Values in table show that the high efficacy of shRNA1 is mainly due to a high number of integrated copies. However, it is not possible to calculate the efficiency per shRNA copy since this depends on the integration site of the lentiviral DNA into the host genome and its transcriptional activity status.

> It is therefore possible to increase silencing efficiency by increasing the integrated copy number. This is achieved by concentrating lentiviral vectors after production to increase the MOI.

Conclusion

Vectalys constructed a cellular model expressing the candidate gene which then allowed us to screen a set of shRNAs in order to study the gene function by silencing assays.

These cellular models are being used for the development of animal models. Cells over- or under-expressing the truncated or full-length cDNA are injected into recipient mice, with the aim of developing a solid tumor and study the gene function *in vivo*.

This project has demonstrated gene transfer by viral technology as a powerful tool for use in candidate gene validation. This technology allows the rapid generation of cell models using any cell type and is applicable to any candidate gene.

References.

Gayon R and Iché A. **Functional validation of a novel cancer target by lentiviral shRNA delivery.** Poster presented at the annual RNAi Europe conference, 2007