PhD Open Days

Minicircle-derived RNA interference as a novel gene-based anti-angiogenic therapy

PhD program in Biotechnology and Biosciences (BIOTECnico)

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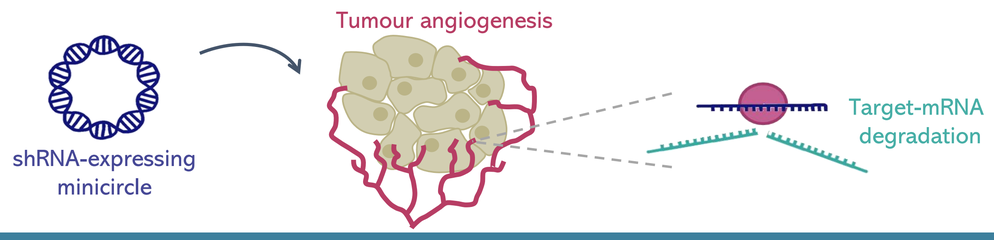
1 Motivation

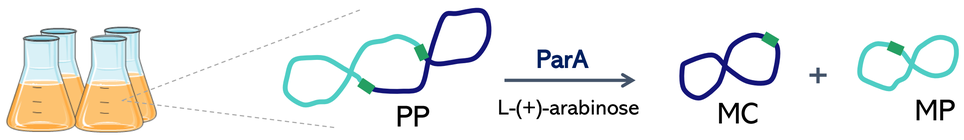
- **Gene-based therapies** continue to advance, aiming at developing novel medicines for a broad range of diseases, including cancer.
- **Tumour angiogenesis** is one hallmark of cancer progression and depends on the interaction between different types of cells and many signalling molecules, such as **vascular endothelial growth factor (VEGF)**.
- Blocking VEGF expression using **RNA interference (RNAi)** might be a promising strategy to slow down tumour growth.
- **Expression vectors encoding short-hairpin RNAs (shRNAs)** can silence gene expression in vivo as effectively as synthetic siRNAs.
- A promising delivery system are **minicircles (MC)**, which are safe non-viral vectors capable of high levels of transgene expression.

We hypothesized that MC-derived RNAi molecules that target key players in the VEGF regulatory pathway could be a novel gene-based therapy for angiogenic disorders characterized by vascular overgrowth.

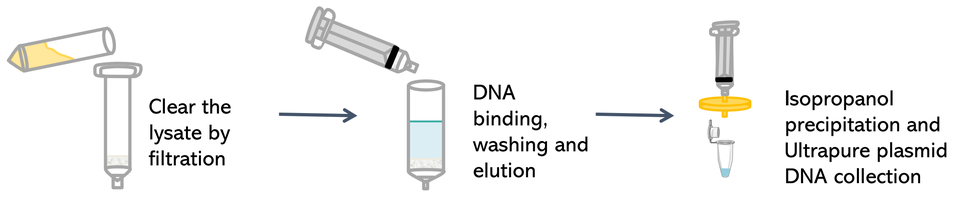


2 Materials and Methods

- *E. coli* BW2P was used for the production and *in vivo* recombination of the parental plasmid (PP) into the miniplasmid (MP) and MC vector.

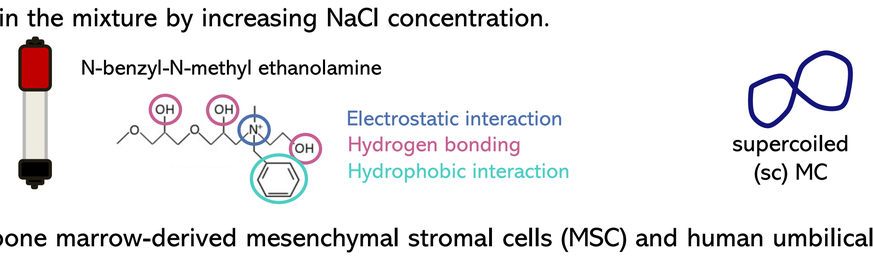


- Cell harvesting and plasmid purification (HiSpeed® Plasmid Maxi Kit Qiagen).

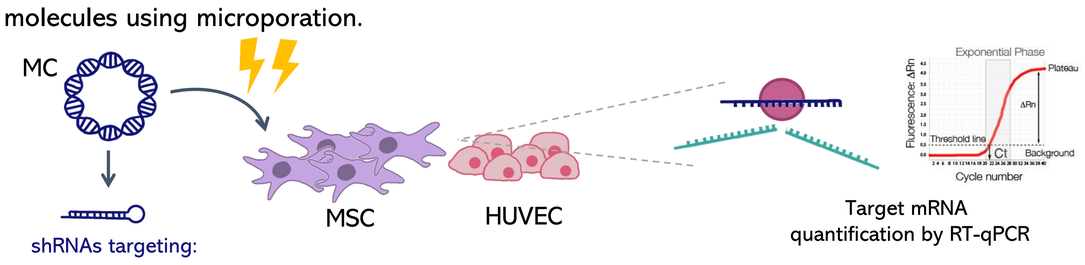


- Digestion with Nb.BbvCI: nick one of the MP strands and of the non-recombined PP.

- Multimodal chromatography: separation of the supercoiled (sc) MC from open-circular (oc) species in the mixture by increasing NaCl concentration.



- Human bone marrow-derived mesenchymal stromal cells (MSC) and human umbilical vein endothelial cells (HUVEC) were transfected with sc MCs and synthetic siRNAs targeting VEGF regulatory pathway molecules using microporation.



- VEGF-A – Stimulation of EC functions and vascular permeability
- HIF-1α – Inducer of VEGF expression under hypoxia
- VEGFR2 – Major signal transducer in EC

Fold change = 2^-ΔΔCt

%KD =(1– 2^-ΔΔCt) × 100

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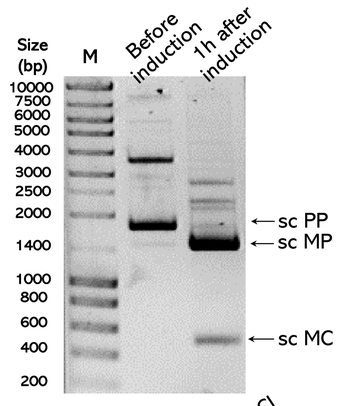
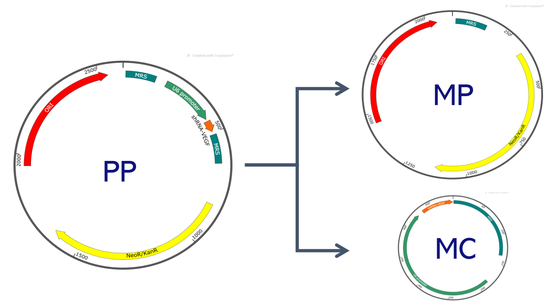
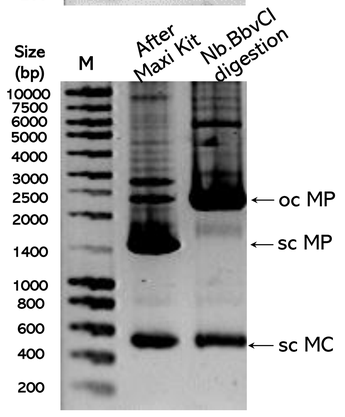
3 Results and Discussion

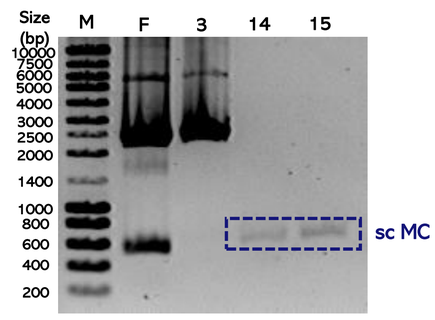
Production and purification of shRNA-expressing minicircles

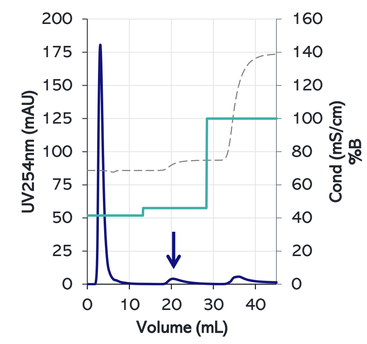




- After L-(+)arabinose induction, the PP (2604bp) becomes absent originating the MP (2108bp) and MC (496bp).



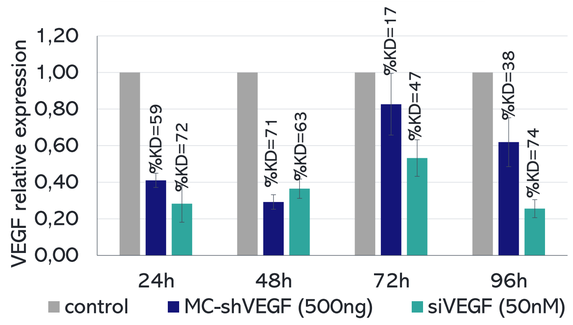




- Nb.BbvCI digestion readily converted the sc MP into the corresponding oc form, which allowed the isolation of the sc MC by multimodal chromatography.

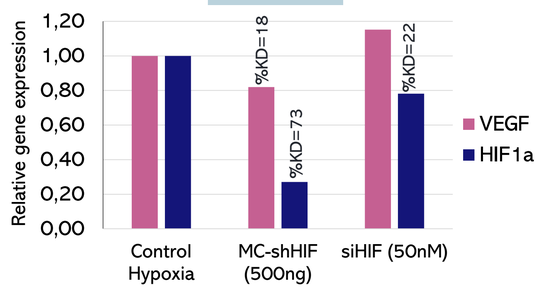
Evaluation of the therapeutic potential of shRNA-expressing MC

n=3: three different MSC donors

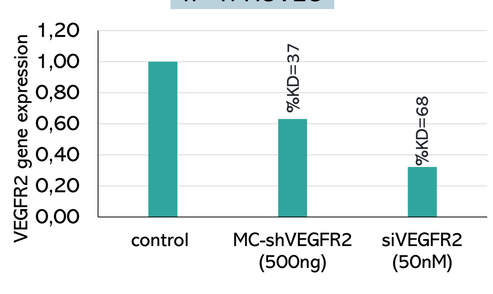


- RT-qPCR results revealed that the silencing effect of MC-shVEGF is stronger after 48h (KD=71%), decreasing in the following timepoints. This result was expected since the peak of expression of the MC is reached 2 days post-transfection.

n=1: MSC



n=1: HUVEC



- A preliminary experience under hypoxic conditions (i.e. 2% O_2) showed that MC-shHIF1α induced a KD of 73% and 18% of HIF-1α-mRNA and VEGF-mRNA 48h post-transfection, respectively.
- A preliminary experience of microporation of HUVEC indicated that MC-shVEGFR2 induced a KD of 37% after 48h.

4 Conclusions and Future Perspectives

This study provides important insights regarding the implementation of an MC-derived RNAi-based system that targets pro-angiogenic molecules, aiming at slowing down tumour angiogenesis.

Future studies:

- Further assessment of MC-shHIF1α and MC-shVEGFR2 silencing potential, by optimization of MC quantity and timepoints post-transfection.
- Evaluation of the MC-induced silencing at the protein level.
- Evaluation of the effect of MC silencing on the pro-angiogenic potential of the transfected cells by performing *in vitro* functional angiogenesis assays.

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