Shifting expression of membrane Vascular Endothelial Growth Factor Receptor-2 (mVEGFR2) to soluble Vascular Endothelial Growth Factor Receptor-2 (sVEGFR2) reduces CNV volume and tumor growth.

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Purpose

- Angiogenesis plays a key role in both age-related macular degeneration (AMD) and cancer growth.
- VEGFR2 is present in both membrane-bound (mVEGFR2) and soluble (sVEGFR2) forms due to alternative splicing.
- mVEGFR2 is pro-angiogenic, while sVEGFR2 is anti-angiogenic.
- We have developed a morpholino-based gene therapy technique that shifts expression from mVEGFR2 to sVEGFR2.
- We present the success of this technique in treating a murine model of choroidal neovascularization (CNV) and a xenograft tumor model.

Methods

CNV Model:

- CNV was induced in C57 mice using laser photocoagulation.
- On day 1 and day 4 after photocoagulation, either control morpholino, sVEGFR2-inducing morpholino, or DBPS was injected intravitreously.
- On day 7, CNV volumes were measured by confocal microscopy.

Xenograft Tumor Model:

- NMRI nu/nu mice received subcutaneous injections of HCT 116 colon cancer cells.
- One week after the injection of cells, treatment began with control morpholino, sVEGFR2-inducing morpholino, or HBSS.
- Tumors were injected with treatment twice weekly for a period of three weeks.
- Tumor size was measured twice weekly.
- Fluorescent staining was performed on tumor sections. Isolectin conjugated Alexa488 (Invitrogen, # #I21411) and anti-LYVE-1 antibody (Abcam, #14917) were used for blood vessel and lymph vessel staining, respectively.

Results

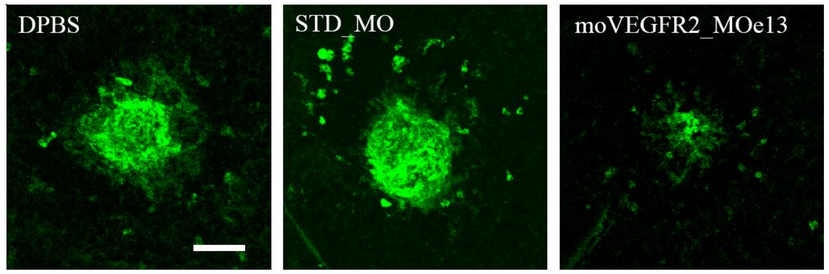

Fig. 1 Representative images of laser induced CNV. Scale bar is 100mm.

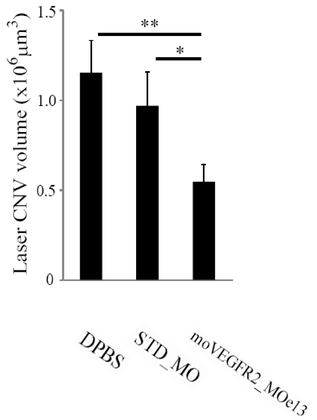

Fig. 2 The average volume of laser-induced CNV (n=11-17).

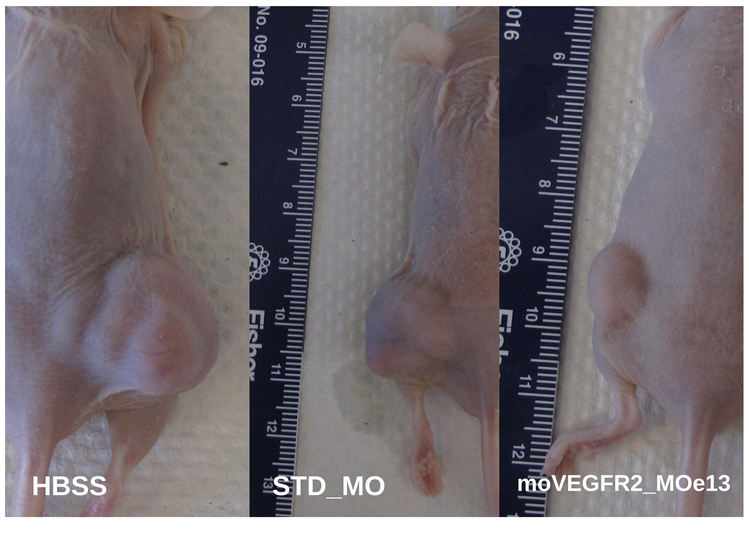

Fig. 3 Representative images of tumors at 21 days after initiation of treatment.

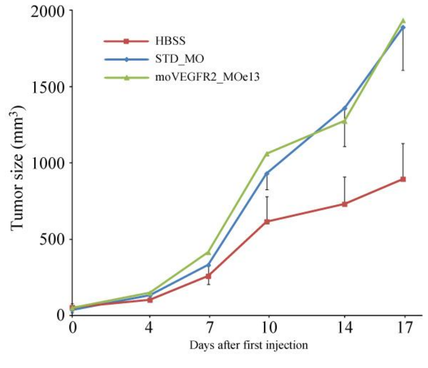

Fig. 4 The average volume of tumors (n=5).

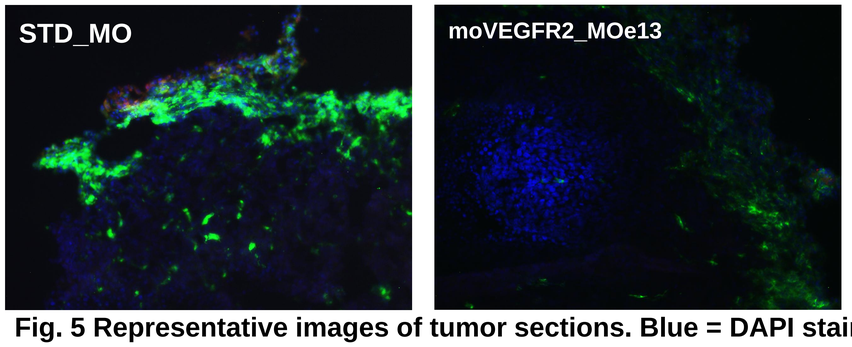

Fig. 5 Representative images of tumor sections. Blue = DAPI staining. Green = blood vessels (Isolectin). Red = lymph vessels (LYVE-1)

Summary of Results

- There was a 50% reduction of CNV in the mice treated with the sVEGFR2-inducing morpholino compared to the mice treated with control morpholino (n=11-17).
- There was a 53% reduction in tumor volume in the tumors treated with the sVEGFR2-inducing morpholino compared to the mice treated with control morpholino (n=5).
- Harvested tumors from the sVEGFR2-inducing morpholino group showed a reduced expression of mVEGFR2 and increased expression of sVEGFR2 as confirmed by RT-PCR.

Conclusions

- Anti-sense morpholinos targeting the exon13-intron13 junction of VEGFR2 shift expression from mVEGFR2 to sVEGFR2.
- Here we have shown that this results in a decrease in CNV volume in a laser CNV model.
- We have also demonstrated that increased sVEGFR2 can reduce tumor growth in a xenograft model.
- This is an interesting example of a therapy developed for ocular diseases being used for cancer therapy.
- The splicing shift described in this study shows promise as a therapeutic method for neovascular-related disorders.

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