Splicing shift from membrane bound VEGF receptor 2 to soluble VEGFR2 using morpholino oligomer suppresses suture induced corneal angiogenesis and lymphangiogenesis, and suppresses corneal graft rejection

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Introduction

Vascular endothelial growth factor-A (VEGF-A) is a potent initiator of angiogenesis and VEGF receptor 2 (VEGFR2) is its primary angiogenic receptor. In addition to membrane-bound VEGFR2 (mVEGFR2), the VEGFR2 gene produces a soluble isoform protein, which contains only a partial extracellular domain, because of alternative polyadenylation. Previously, we succeeded in shifting mVEGFR2 to soluble VEGFR2 (sVEGFR2) by manipulating splicing using morpholino oligomers (VEGFR2-MOe13). In this study, we examined whether VEGFR2-MOe13 could suppress suture induced corneal angiogenesis & lymphangiogenesis, and graft rejection in a mouse corneal transplantation model.

Methods

For the suture induced corneal model, we harvested the corneas at two time-points (7 and 14 days) after suture placements. 15µl of standard morpholino (40ng/µl), VEGFR2-MOe13 (40ng/µl) or DPBS were injected subconjunctivally after suture placement. CD31 and LYVE-1 immunostaining was used to evaluate neovascularization and lymphangiogenesis area in cornea, respectively. For the corneal transplantation model, we injected DPBS, standard morpholino or VEGFR2-MOe13 into the conjunctiva 1, 2, 3 and 4 weeks after corneal transplantation (graft: C57Bl/6, recipient: Balb/c). Graft rejection was evaluated by corneal opacity every week up to 8 weeks. At 8 weeks, we evaluated neovascularization & lymphangiogenesis by CD31 and LYVE-1.

Results

VEGFR2-MOe13 suppressed suture-induced neovascularization by 52.2% (7days) and 29.6% (14days) compared to DPBS ($p<0.001$ and 0.05, respectively). Seven days after suture placement, VEGFR2-MOe13 did not suppress lymphangiogenesis. However, 14days after suture placement, VEGFR2-MOe13 suppressed lymphangiogenesis by 27.8% compared to DPBS ($p<0.05$). In the corneal transplantation model, VEGFR2-MOe13 increased graft survival compared with DPBS and standard morpholino (log rank test: p=0.0186 and 0.0610, respectively). Concordantly, VEGFR2-MOe13 decreased neovascularization & lymphangiogenesis significantly at 8 weeks.

Conclusion

We demonstrated a splicing shift from mVEGFR2 to sVEGFR2 using VEGFR2-MOe13 suppressed suture induced corneal neovascularization and lymphangiogenesis. We also succeeded in suppressing mouse corneal graft rejection using VEGFR2-MOe13. This may become a new approach for developing anti-angiogenic therapy.

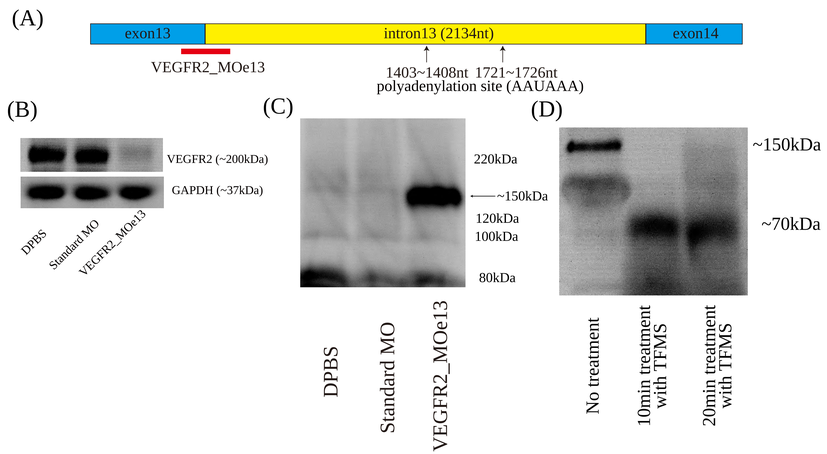

Figure 1 VEGFR2_MOe13 decrease mbVEGFR2 and increase sVEGFR2
(A) VEGFR2_MOe13 was designed at exon13-intron13 junction. (B) Western blot for mVEGFR2 from HUVEC. VEGFR2_MOe13 decrease mVEGFR2 protein. (C) Western blot for sVEGFR2 from conditioned culture medium. VEGFR2_MOe13 increase sVEGFR2 protein in culture medium. (D) Deglycosilation of sVEGFR2 to confirm 150kDa band is sVEGFR2. After de-glycosilation, 150kDa band becomearound 70kDa which is similar to caluculated molecular weight 76kDa.

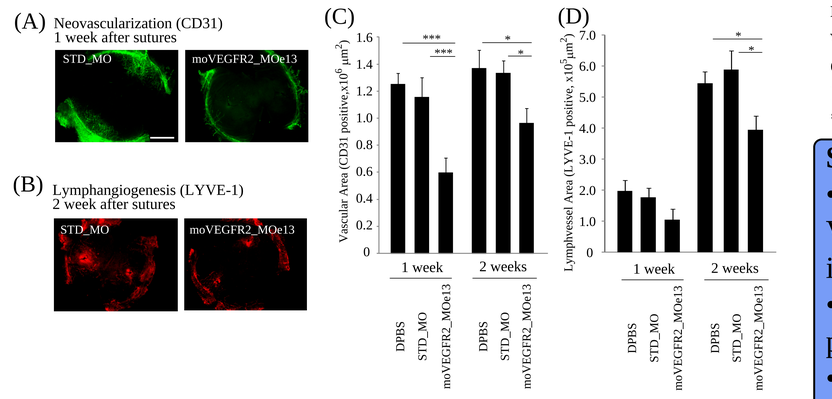

Figure 2 moVEGFR2_MOe13 injection to conjunctiva suppress neovascularization and lymphangiogenesis in corneal suture model.
(A) Representative image of corneal neovascularization one week after suture. (B) Representative image of corneal corneal lymphangiogenesis two weeks after sutures. (C, D) Mean area of corneal neovascularization and lymphangiogenesis respectively (n=13-16). Risk factors (p-value) were calculated by two-tail student`s t-test (*: $p<0.05$, **$p<0.01$, ***$p<0.001$). Error bar is ±s.e.m.

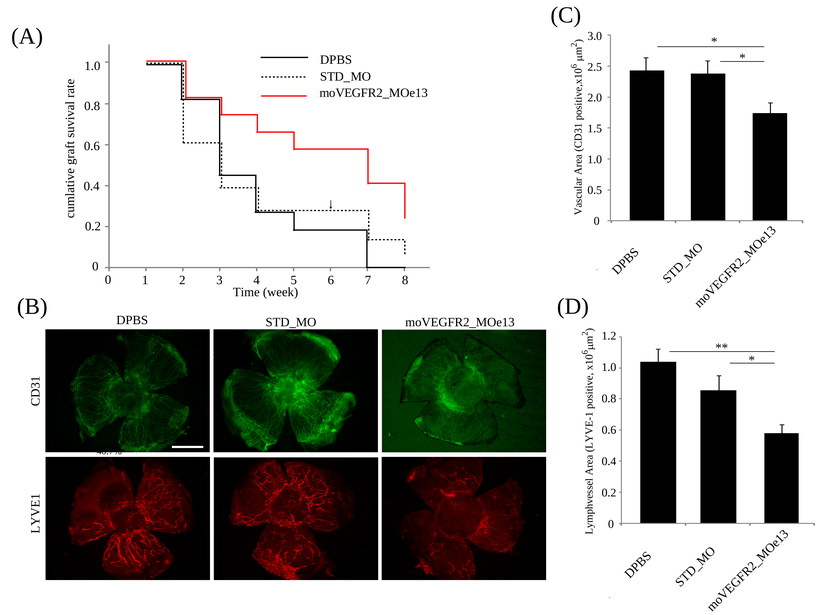

Figure 3 moVEGFR2_MOe13 suppresses rejection in mouse cornea transplantation model. (A) Cumulative graft survival rate. moVEGFR2_MOe13 increased graft survival rate compared with DPBS and STD_MO (log rank test: p=0.0186 and 0.0610, respectively). Arrow indicates censored data. (B) Representative image of corneal neovascularization and lymphangiogenesis at 8weeks. Scale bar is 1mm. (C, D) Mean area of corneal neovascularization and lymphangiogenesis at 8weeks respectively (n=11-17). Risk factors (p-value) were calculated by two-tail student`s t-test (*: $p<0.05$, **$p<0.01$). Error bar is ±s.e.m.

Summary

- Blocking of exon13-intron13 junction in VEGFR2 by VEGFR2_MOe13 leads to mbVEGFR2 decrease and sVEGFR2 increase.
- moVEGFR2_MOe13 can suppress neovascularization and lymphangiogenesis in cornea suture model.
- moVEGFR2_MOe13 reduced corneal rejection in mouse cornea transplantation model
- This has applications not only for anti-angiogenesis or eye disease by targeting VEGFR2 but in other conditions where regulatory manipulation of splicing and polyadenylation could have therapeutic valence.

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