In Cell Stress Conditions, VEGFR2 Exerts Pronounced Effects on Cell Growth in Dysplastic Barrett's Epithelial Cells

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INTRODUCTION

Vascular endothelial growth factor (VEGF), a potent inducer of angiogenesis, recently has been shown to exert direct pro-proliferative and pro-survival effects on cancer cells through binding to its tyrosine kinase receptors (VEGFR1 and VEGFR2). In earlier studies, we showed that VEGF/VEGFR2 signaling exerts direct pro-proliferative effects on transformed Barrett's and adenocarcinoma cells in an autocrine fashion, with no significant effect on apoptosis. Moreover, compared to non-dysplastic Barrett's cells, these neoplastic cells were highly sensitive to the growth suppressive effects of VEGF/VEGFR2 inhibition suggesting a potential role of VEGF therapies in Barrett's cancers. To explore the potential contribution of VEGFR signaling to cell growth of dysplastic Barrett's cells, we knockdown the VEGFR1 or VEGFR2 and studied the effects on cell morphology, cell number, proliferation, and apoptosis. Our results suggest that the VEGFR2 receptor is involved in pro-proliferative and anti-apoptotic events in dysplastic Barrett's cells.

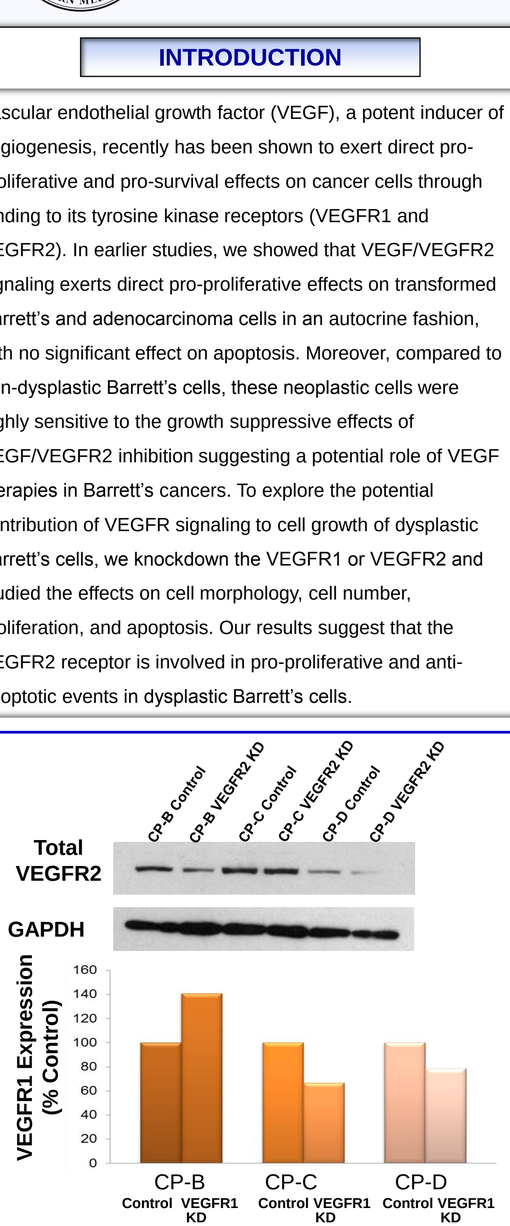


Figure 1: Knockdown of VEGFR1 or VEGFR2 in Dysplastic Barrett's Cell Lines CP-B, CP-C, and CP-D. Knockdown (KD) of VEGF1/VEGFR2 was performed through stable infection with retroviral shRNA vectors. We observed knockdown of VEGFR2 in CP-B and CP-D, but not in CP-C as shown by the western blot. Knockdown of VEGFR1 was primarily seen in CP-C by qRT-PCR. We used CP-B and CP-D to assess effects of VEGFR2 KD and CPC-C for effects of VEGFR1 KD in all further studies.

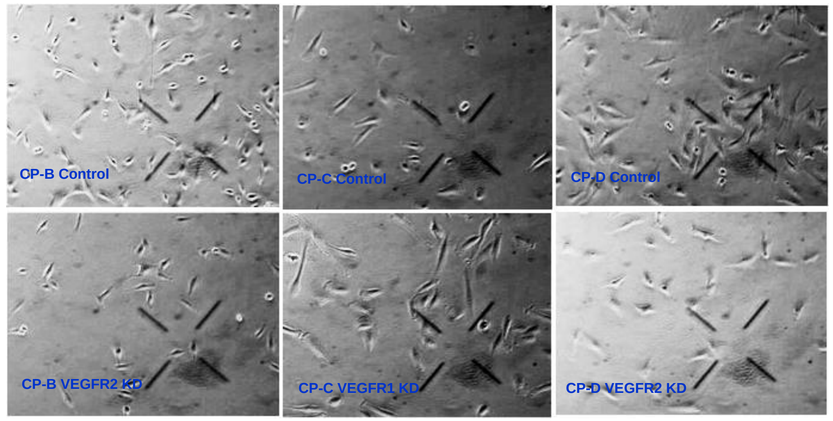
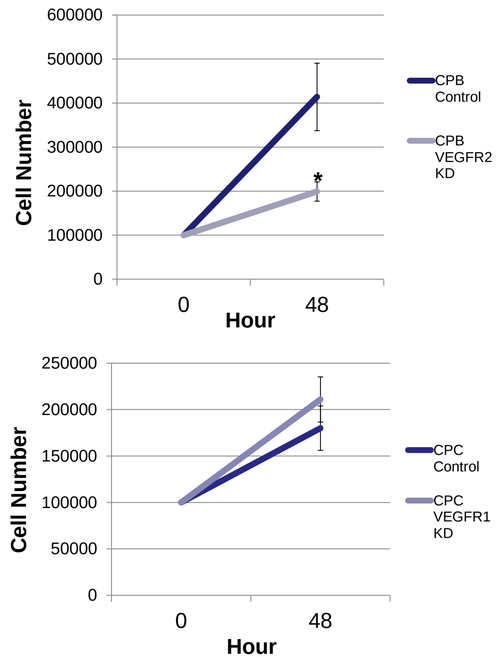


Figure 2: Cell morphology is unchanged after VEGFR1 or VEGFR2 knockdown. Cells were plated in complete medium and cell morphology was assessed by optic microscopy using DIC.



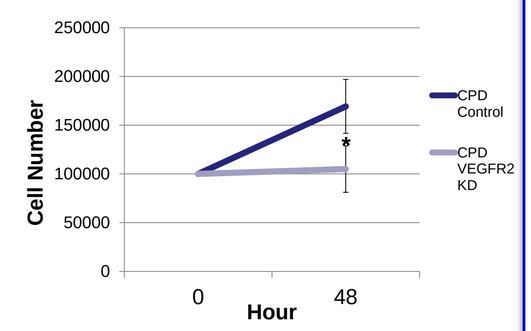


Figure 3: Dysplastic Barrett's cells with VEGFR2knockdown show decreased rate of proliferation under stressed culture condition **as compared to controls.** Cells were plated at 100,000 /well in a 12 well plate and counted at 48 hours after growth in 1% full growth medium. CP-B and CP-D containing VEGFR2KD had significantly lower cell numbers compared to corresponding controls. However, these changes were not observed when cells were grown in full growth medium (data not shown). CP-C cells containing VEGFR1KD had no significant difference in cell number compared to control in either of the two media. *, p ≤ 0.05 compared to control

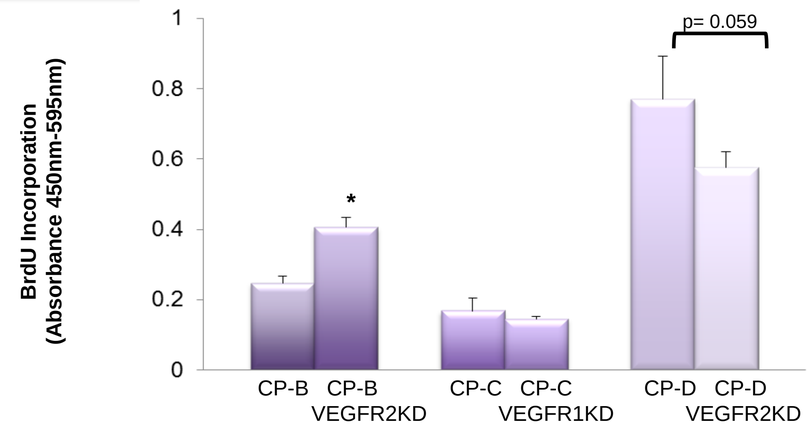


Figure 4: Dysplastic Barrett's CP-B cells containing VEGFR2 knockdown significantly increase cell proliferation where as CP-D cells containing VEGFR2 knockdown demonstrate a decrease in proliferation. No difference in proliferation is observed in CP-C cells containing VEGFR1 knockdown. A BrdU assay was performed to assess for cell proliferation. *, p ≤ 0.05 compared to control

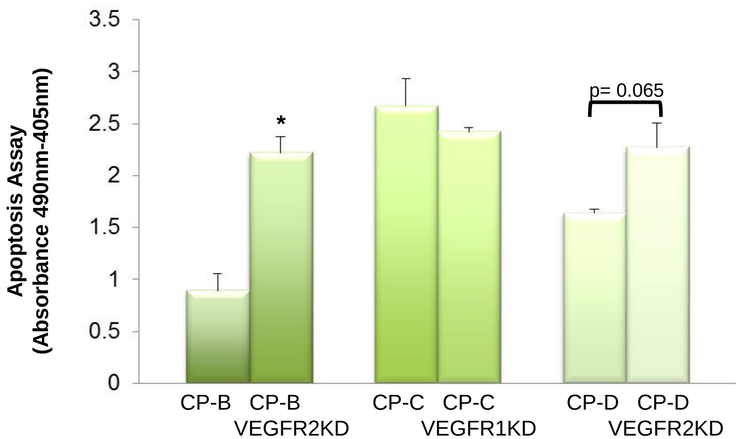


Figure 5: Dysplastic Barrett's CP-B and CP-D cells containing VEGFR2 knockdown increase apoptosis. No difference in apoptosis is observed in CP-C cells containing VEGFR1 knockdown. A cell death Elisa assay was performed to assess for apoptosis. *, p ≤ 0.05 compared to control

CONCLUSIONS

- ❑ VEGFR2, but not VEGFR1, contributes to cell growth of dysplastic Barrett's cells, but only under conditions of cell stress.
- ❑ Dysplastic Barrett's cells demonstrate both pro-proliferative and pro-survival effects of VEGFR2 signaling.

SPECULATION

These findings support a potential role of anti-VEGFR2 therapies in the treatment of high grade dysplasia in Barrett's esophagus.