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Monoclonal Antibody Activity in Human Umbilical Endothelial Cells That Possess Opposing Growth Factor Signaling Receptors

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Monoclonal Antibody Activity in Human Umbilical Endothelial Cells That Possess Opposing Growth Factor Signaling Receptors

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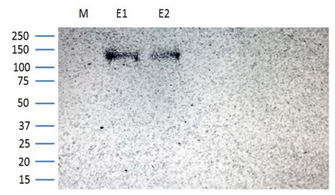
Abstract

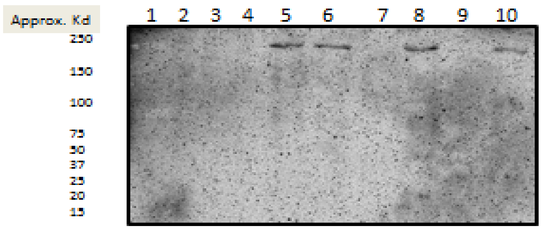
Peripheral vascular disease (PVD) refers to the clinical manifestations of reduced blood flow to the legs, usually secondary to atherosclerosis. Depending on the extent and severity of the blood flow reduction, patients with PVD are often severely limited by pain in their legs with ambulation (termed claudication) which can progress to limb threatening ischemia requiring surgical revascularization or amputation. In some patients with PVD, blood flow to the legs is maintained by the development of collateral blood vessels that "bypass" the flow limiting atherosclerotic lesions. Because none of the currently available therapeutic agents enhance blood flow in patients with PVD, there is an enormous effort in industry and academic laboratories to develop approaches to augment the growth and development of collateral blood vessels. We have shown that HPTPβ, a protein tyrosine phosphatase (PTP) expressed primarily in vascular endothelial cells, is a negative regulator of the VEGFR2 and Tie2 signaling pathways, two pathways known to promote new blood vessel growth (angiogenesis) and to augment collateral blood flow in animal models of PVD. Based on these studies we hypothesized that inhibition of HPTPβ would improve blood flow to ischemic tissues by enhancing the activation of VEGFR2 and Tie2. To test this hypothesis, potent and selective HPTPβ inhibitors have been developed. Several of these inhibitors had nanomolar IC_{50} and were at least 100 fold selective for HPTPβ over other phosphatase enzymes. In addition, a number of the inhibitors also enhanced the activation and biological activity of Tie2 and VEGFR2 in endothelial cells and augmented Tie2 activation *in vivo*. Consistent with our hypothesis, these inhibitors also enhanced new blood vessel development in an *ex vivo* model of angiogenesis, the rat aortic ring model and *in vivo* in a rat model of PVD. If successful, HPTPβ inhibitors could provide breakthrough therapy for patients with PVD and other ischemic cardiovascular diseases such as coronary vascular disease and cerebral vascular disease.

Protein Blot for PTPBeta

R15E6 Western blot using purified histidine-tagged Human PTP-Beta fusion protein, Hek-293 cells. 200ng of protein loaded per lane, I˚Ab, R15E6,5ug/ml and secondary used at 1:10,000, goat anti-mouse IgG1-HRP. Image collected on Alpha imager using a CCD camera. E1 and E2 are HPTPbeta eluted from Nickle-column.



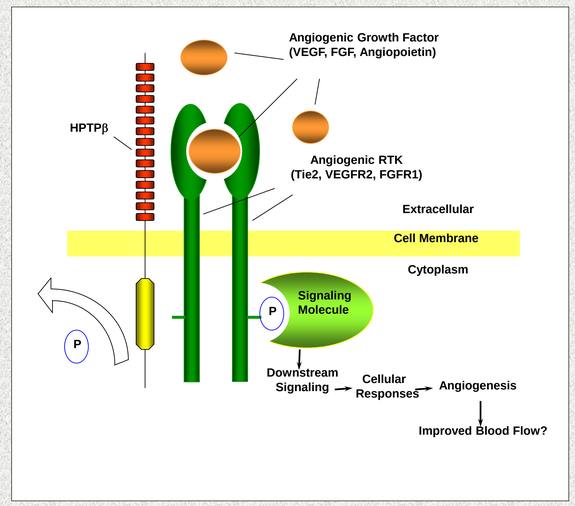
Tie-2 Activation Assay: Immunoprecipitation With R15 AND 33.1 Mabs and blotting with directly labeled HRP-Rabbit Anti-Phosphotyrosine Antibody



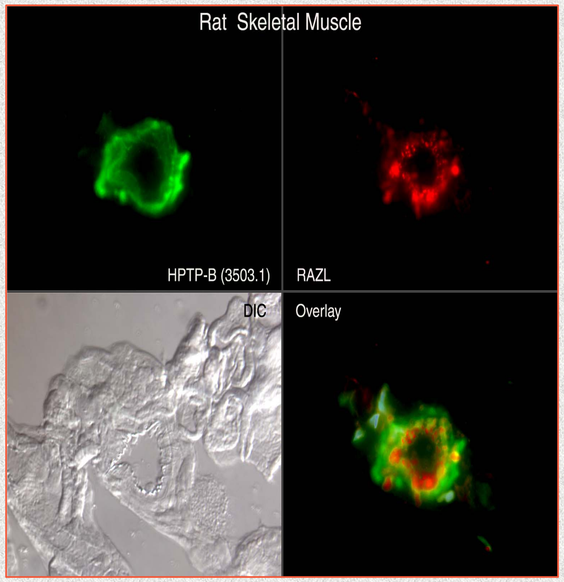
Legend for lanes assignments:

1= Marker
2=Cell lysate A431 (positive control?)
3=Control dish (DMSO vehicle), IP with R15 Mab
4=Control dish (DMSO vehicle), IP with 33.1 Mab
5=Ang-1+AKB-9778 3ug/ml, IP with R15 Mab
6=Ang-1+AKB-9778 3ug/ml, IP with 33.1 Mab
7=Ang-1+R15Mab 10nM, IP with R15Mab
8=Ang-1+R15Mab 10nM, IP with 33.1Mab
9=Ang-1+R15Fab 10nM, IP with R15Mab
10=Ang-1+R15Fab 10nM, IP with 33.1Mab

Protein Phosphatase Beta Limits Angiogenic Response

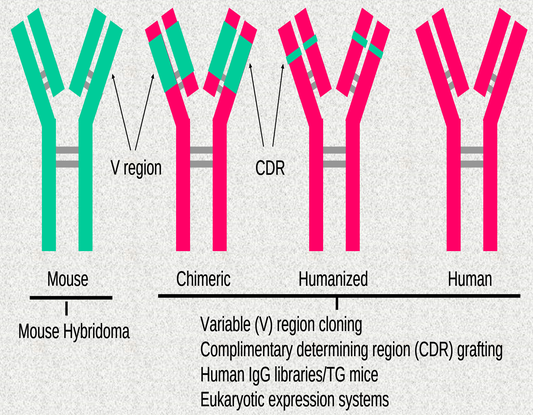


Expression of PTPBeta Protein in Endothelial Cells

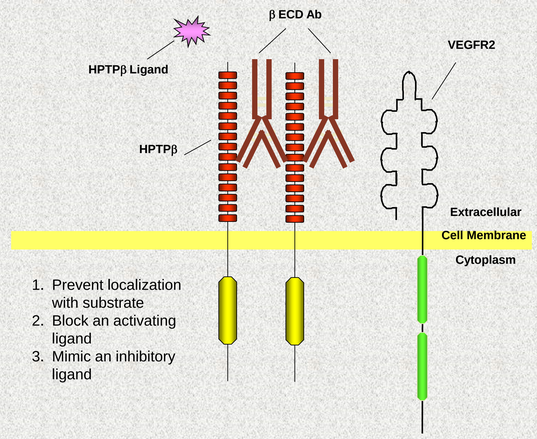


Future Directions:

Fully human antibodies are derived from human cDNA libraries or from mice engineered to express the human IgG gene and therefore contain no murine sequences.



Binding of an HPTPβ Antibody Could Modulate Phosphatase Activity



1. Prevent localization with substrate
2. Block an activating ligand
3. Mimic an inhibitory ligand