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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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 IC50 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 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. Imitate an inhibitory ligand

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Protein Blot for PTPBeta

Legend for lanes assignments:
1: Marker
2: Control (vehicle)
3: Control (DSMO vehicle), IP with R15 Mab
4: Control (DSMO vehicle), IP with 33.1 Mab
5: Ang-1+R15 Mab 10nM, IP with R15 Mab
6: Ang-1+33.1 Mab 10nM, IP with 33.1 Mab
7: Ang-1+R15 Mab 10nM, IP with R15 Mab
8: Ang-1+R15 Mab 10nM, IP with 33.1 Mab
9: Ang-1+33.1 Mab 10nM, IP with R15 Mab
10: Ang-1+33.1 Mab 10nM, IP with 33.1 Mab

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