Neuropilins and Semaphorins


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Our investigations started with the question, why are there so many VEGF isoforms? One possibility that was considered was that some isoforms may interact with receptors other than VEGFR1 and VEGFR2, the only VEGF receptors known at that time. Cross-linking studies using labeled VEGF₁₆₅ and human umbilical vein endothelial cells (HUVEC) revealed two cross-linking products that were missing in cross-linking experiments in which labeled VEGF₁₂₁ was used. The band unique to VEGF₁₆₅ was isolated and was found to be the product of the neuropilin-1 (npn1) gene.

Npn1 was already known to be a receptor for class-3 semaphorins (semas) such as sema-3A, which function as axon guidance factors during the development of the central nervous system. Subsequent studies showed that another family member, npn2, also functions as a VEGF receptor that binds VEGF₁₆₅ and VEGF₁₄₅, but not VEGF₁₂₁. Npn2 also binds placental growth factor (PlGF2) and VEGF-C. In early development, npn1 is expressed in arteries and npn2 is expressed in veins. The expression pattern of npn1 is similar to that of ephrinB2, but starts earlier.

Class 3 semas interact differentially with different npns. Sema-3A binds npn1, but not npn2, and sema-3F binds to npn2 with high affinity and to npn1 with low affinity. Npns do not have tyrosine kinase domains, but when they bind semas, they form a complex with plexins, which undergo phosphorylation on tyrosine residues located in their intracellular domains and initiate intracellular signaling.

Sema stimulation usually leads to repulsion of axonal growth cones expressing the appropriate semaphorin receptors. Stimulation of HUVEC with sema-3F results in a small contractile response. However, sema-3F inhibits VEGF-induced proliferation of HUVEC. This effect is not due to competition for shared receptors and appears to be an active signal that counteracts the effects of VEGF, because VEGF and sema-3F do not compete for binding to npn2, and because sema-3F also inhibits FGF2-induced proliferation of HUVEC. Sema-3F also inhibited ERK activation by both VEGF and FGF. The inhibitory effect of sema-3F is blocked by siRNA directed against npn2, but not siRNA directed against npn1.

These observations suggested that sema-3F may function as an inhibitor of angiogenesis. Indeed, sema-3F was able to inhibit VEGF and FGF2-induced angiogenesis in-vivo in alginate beads and matrigel plug assays. The efficiency of the inhibition depended on the ratio between the angiogenesis inducer and sema-3F, since in the presence of high VEGF concentrations the sema-3F induced inhibition was ineffective. Tumor formation from HEK-293 tumor cells over-expressing sema-3F was strongly inhibited in xenograft tumor formation assays performed in immune-deficient mice as compared to tumor formation from empty vector transfected HEK-293 cells. Furthermore, tumors that did develop from the sema-3F expressing cells contained low
concentrations of blood vessels and developed much more slowly. However, the efficiency varies between different kinds of tumor cells, and s3f inhibited tumor development from PC3 prostate cancer cells less efficiently, possibly because these cells express higher amounts of pro-angiogenic factors.

There are also some stimulatory semaphorins. Sema-4D is membrane bound and released by proteolysis. It binds to its receptor, plexinB1, which then forms complexes with c-met, receptor for HGF. Sema-6D binds to plexinA1, which can form complexes with VEGFR2 and stimulate it.

Questions

1. What is likely to happen if you block Npn1 to try and treat tumor angiogenesis?
   It is expected that there should be inhibition.

2. How do the Inhibitory effects of semas work?
   Dependent upon Npn1 and plexins – it is not known if they form complexes with VEGF receptors.