Lymphangiogenesis


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Lymphatic capillaries differ from blood vessel capillaries in that they lack smooth muscle cells and pericytes and have very poorly developed basement membrane. Many types of leukocytes, particularly dendritic cells, migrate through lymphatics.

Lymphangiogenesis is controlled by VEGF-C and VEGF-D binding to VEGFR3. VEGF-C and -D must be activated by proteolytic cleavage. The first cleavage activates for binding to VEGFR3, the second cleavage in amino terminal domain activates for binding to VEGFR2. So under conditions of extensive proteolytic activity, VEGF-C and -D are angiogenic.

VEGFR3 is initially expressed in all blood vessels. Deletion of the *Vegfr3* gene is embryonic lethal, because there is failure to reorganize the primary capillary plexus into a hierarchy of large and small vessels. This is an important blood vascular function in the early embryo, not a lymphatic function. The heterozygote embryos survive and the lymphatics develop by sprouting from embryonic veins. The first lymphatic endothelial cells are marked by the homeobox transcription factor PROX1. During this time, the VEGFR3 levels in blood vessels decrease as they increase in lymphatic vessels. After this time, VEGFR3 provides good marker for lymphatic endothelial cells.

When lymphatic endothelial cells are put in culture, they maintain expression of PROX1 and VEGFR3, which allows them to be separated from blood vascular endothelial cells. With pure populations of the two types of endothelial cells, it is possible to make comparisons of gene expression. There is about 1-1.5% difference in gene expression between them. Blood vascular endothelial cells express more proteolytic enzymes (urokinase, MMPs) and STAT6. Lymphatic endothelial cells express more TIMP3, protease inhibitors, lymphatic chemokine, PROX1, and Net transcription factor, and they fail to express STAT6.

*Vegfc* null embryos start to swell at mid-gestation. Without VEGF-C, there is no sprouting of lymphatics. Heterozygotes have a phenotype in which blood vessels are normal, but lymphatics are delayed in development and remain severely hypoplastic in the skin. So there is haploinsufficiency just as is the case for VEGF-A.

The FoxC2 transcription factor is mutated in some patients with lymphedema and swelling of extremities. FoxC2 is controlled by the VEGFR3 signal transduction pathway, and it blocks basement membrane production and expression of PDGF-B by lymphatic endothelial cells. The absence of PDGF-B expression prevents recruitment of smooth muscle cells and pericytes around lymphatic endothelial cells. The absence of perivascular cells and basement membrane is important to the normal functioning of lymphatics, because their presence impedes fluid from entering lymphatics. In *FoxC2* null mice, the lymphatics produce basement membrane and release PDGF-BB, which causes smooth muscles cells to surround the lymphatics; these alterations impede fluid entry into the lymphatics and results in lymphedema. In veins, the regions of valves show expression of FoxC2, which prevents smooth muscle cell recruitment in the areas of valves. Patients with
FoxC2 mutations have smooth muscle cells in the regions of blood vessel valves, which disrupt functioning of the valves and lead to varicose veins.

If adenoviral vector-mediated gene transfer is used to overexpress VEGF-C, there is excessive sprouting of lymphatics resulting in many new lymphatics. This could be a treatment for lymphedema, and a phase I trial has been started to test this possibility.

Expression of angiopoietin 1 also causes lymphatic sprouting. Angiopoietin 1 can stimulate both Tie1 and Tie2 in both blood and lymphatic endothelial cells.

What happens when you shut off this pathway after lymphatics have already developed? This is achieved with VEGF-C/D trap, an Fab fragment that binds VEGF-C and VEGF-D. If the keratin 14 promoter is used to drive expression of the VEGF-C/D trap, it begins to appear in skin at embryonic day (E) 15. This results in regression of lymphatics due to apoptosis of lymphatic endothelial cells, and blood vessels are normal.

VEGFR3 is upregulated in some pathologies, such as Kaposi sarcoma (spindle cells are high in VEGFR3) and breast carcinoma, resulting in necklace vessels. There is not much lymphangiogenesis in most tumors, but when VEGF-C is expressed, sprouting and dilation of lymphatics occurs.

Mice in which the insulin promoter drives expression of VEGF-C get lymphatics in the pancreas. If these mice are crossed with RipTag mice, the double transgenics show extensive metastasis, whereas RipTag mice do not develop metastasis. The double transgenic mice can be used to test treatments for metastasis, and two things that suppress metastasis are VEGF-C/D trap and blocking monoclonal antibodies to VEGF-C.

Cell lines that are selected for metastasis have upregulation of VEGF-C. Xenografts with these cell lines get extensive lymphatic vessels around tumors and metastasis. If they are treated with AdVEGFC/D-trap early, the development of excessive lymphatics and the metastasis are blocked. But if treatment is started after day 25, metastasis is not prevented.

When tumors grow and enlarge, they sometimes begin to produce VEGF-C or -D, and that causes sprouting and hyperplasia of lymphatics from tumor vessels and promotes metastasis.

Questions

1. Is Ang1 a ligand for Tie1?
   Ang1 does not bind to Tie1 extracellular domain in solution. But if you overexpress Tie1 on cell surface and stimulate with Ang1 (recombinant form called CompAng1), you can stimulate tyrosine kinase activity of Tie1. So there is a missing component in the complex. Tie2 increases the activation of Tie1, so they form a complex. By using kinase negative mutants of the receptors, you can see that Tie2 participates in Tie1 signaling. In normal endos, Ang1 (or Ang4) stimulates Tie1 and in transfected cells that do not have Tie2. Ang2 does not give activity for Tie1 phosphorylation, but rather inhibits the phosphorylation stimulated by Ang1.

2. Does lymphangiogenesis come from lymphatics or veins?
   It comes from lymphatics- you don’t see it coming from veins and don’t see contribution by bone marrow cells. If you block VEGF-C you block about 2/3 of metastasis, but there is always a little left and this could be due to active migration of tumor cells into lymphatics.

3. Are there lymphatic vessels in the eye?
   You can provoke lymphangiogenesis in the cornea by induction of VEGF-D, so it is secondary.