Topic 9 – Angiogenesis, microcirculation, growth factors, progenitor cells – A

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0269
Role of Frizzled7 during pathological angiogenesis: a model of retinopathy
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Background: Pathological angiogenesis in the retina is the cause of many human diseases, including retinopathy of prematurity and proliferative diabetic retinopathy. A better understanding of the mechanisms involved in the abnormal proliferation of blood vessel observed in these blinding pathologies appears to be essential to develop potential therapeutic drugs against new molecular targets. Growing evidence has shown that Wnt/Frizzled (Fzd) proteins are directly involved in vascular development. Recently, we have evidenced that Fzd7 receptor is required for postnatal angiogenesis of retina by controlling endothelial cells (EC) proliferation and migration through a β-catenin canonical pathway. This study aimed to investigate the role of Fzd7 during aberrant angiogenesis in a model of ischemic retinopathy.

Methods/Findings: We used mice model of oxygen-induced retinopathy (OIR) to explore the involvement of Fzd7 during initial vaso-obliteration (VO) and subsequent neovascularization (NV) phases. First we observed Fzd7 was expressed in pathologic neovessels. Second, by transgenic approaches, we observed that specific deletion of Fzd7 in the endothelium (fzd7ΔECKO) resulted in increased retinal tissue sensitivity to hypoxia during the vaso-obliterative phase of the OIR model. Moreover, Fzd7 deletion in EC reduced the ectopic growth of pathologic neovessels into the vitreous during the second phase of the OIR model demonstrating that Fzd7 was involved in pathological NV formation in mice retina after ischemia. To determine the molecular mechanisms by which Fzd7 may regulate ischemic retinopathy, we explored β-catenin and Notch signaling during the two different phases of OIR. Preliminary results showed that transcript expressions of β-catenin (lef1, axn2) and partners of Notch signaling were strongly decreased in fzd7ΔECKO retina mice as compared to control.

Conclusion: By controlling pathogenesis of ischemic retinopathy, Fzd7 could be an efficient and specific target to develop anti-angiogenic drugs in the treatment of ischemic retinopathies.

0259
Physical exercise induced adipose tissue angio-adaptation in a context of “diabesity”
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Adipose tissue (AT) homeostasis and growth are dependent on microvasculature. This capillary network has a large remodeling capacity, a process called angio-adaptation. In response to metabolic alterations linked to obesity, signaling pathways involved in the maintenance of vascular homeostasis of AT appear to be affected. In this context, we studied in C57/B6J mice to an high fat diet (HFD) and voluntary exercise protocol of 7weeks, capil whichever of epididymal (eWAT) and subcutaneous adipose tissue (sWAT) by histological marking of CD31, and expression of angiogenic factors: Murine-endothelial cell (EC) properties and are directly involved in blood vessel development by regulating endothelial cell (EC) properties. In this study, we analyzed the effects of frizzled7 deficiency in EC on vessel formation in the postnatal mouse retina. We generated fzd7−/− mice and crossed them with Pdgfb−/− mice to generate fzd7−/−ECKO mice. In the fzd7−/−ECKO retinas at P7 dpn, we observed fzd7−/−ECKO mice phenotype showing that Fzd7 may control angiogenesis in vivo.

0170
VEGF-D translational regulations promotes tumor lymphatic vessels plasticity
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The lymphatic vasculature has a major role in physiology. It drains the interstitial fluids and performs immune surveillance by transporting immunity cells. The lymphatic vessels are also involved in pathological conditions such as chronic inflammatory diseases, lymphedema and cancer. Lymphangiogenesis, the growth of new lymphatic vessels, is induced by the lymphangiogenic growth factors VEGF-C and VEGF-D. Recent studies suggest that VEGF-D is also involved in lymphatic dilatation through progastaglandin signaling pathways.

In this study, we demonstrated the molecular mechanism involved in lymphatic dilatation. We identified a stress-induced translational regulation of VEGF-D expression through an IRES activation under heat shock conditions. We demonstrated that VEGF-D IRES activity was dependent of progastaglandin pathway as Cox-2 inhibitors are able to abolish the IRES-promoted VEGF-D protein synthesis, and lymphatic vessels dilatation. Using plasmon surface resonance on biotinylated VEGF-D mRNA coupled to mass spectrometry, we identified the IRES transacting factor (ITAF) that allows the recruitment of the ribosome to promote VEGF-D transnslational initiation: the Nucleolin.

Our results bring new insights on the VEGF-D regulation and on the lymphatic vessels plasticity. As lymphatic vessels play a crucial role in inflammation by forming immune cells tissue trafficking, understanding the regulations of lymphatic vasodilatation is a crucial step toward an innovative inflammatory disease therapy.

0048
Frizzled7 controls postnatal vascular formation through Wnt/β-catenin canonical signaling in a Dil4/Notch dependent and independent way
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Growing evidence has shown that Wnt/Fzd proteins are expressed in vascular cells and are directly involved in blood vessel development by regulating endothelial cell (EC) properties. In this study, we analyzed the effects of frizzled7 deficiency in EC on vessel formation in the postnatal mouse retina. We generated fzd7−/− mice and crossed them with Pdgfb−/− mice to generate fzd7−/−ECKO mice. In the fzd7−/−ECKO retinas at P7 dpn, we observed a delay in vascular network formation, a strong increased in tip cells and filopodia numbers and a decrease in proliferation compared to WT. Interestingly, we also analyzed the retinal vascular development in mice KO for Dishevelled1 (DVL1), a partner involved in Wnt/Fzd signaling downstream Fzd receptor. DVL1KO mice showed a similar but mildly vascular phenotype compared with fzd7−/−ECKO mice. Moreover, intracocular injection of siRNA/DVL3 in DVL1KO mice increased DVL1KO phenotype and mimics fzd7−/−ECKO mice phenotype showing that Fzd7 may control angiogenesis through DVL1 and 3. To a better understanding of how Fzd7 regulates vessels formation in vivo, we analysed the effects of LiCl injection (activator of β-
catenin pathway) or Dll4 intraocular injection in fzd7iECKO and WT mice on vascular retinal phenotype. Our results showed that LiCl totally overrules the effects of Fzd7 deficiency in EC whereas Dll4 only partly rescues the phenotype observed in fzd7iECKO mice. The tip cells impairment in fzd7iECKO mice is overruled by Dll4 but not the delay of vascular growth. We then assessed whether fzd7 deletion impaired the expression of partners of β-catenin and Notch pathways by RT-qPCR. In vivo, deletion of fzd7 in EC induces a decrease in both partners of β-catenin and Notch pathways expression compared to control. Moreover this impairment of β-catenin and Notch pathways in vivo is totally rescue by LiCl injection. This study shows that fzd7 plays a crucial role during angiogenesis in postnatal mouse retina. Fzd7 controls vascular growth through a β-catenin signaling in a Notch independent way but also regulates the tip cells selection through a β-catenin signaling in a Notch dependent manner.

Blood vessels, muscle cells and peripheral nerves work together to regenerate ischemic muscles

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Background: In elderly and diabetic patients, the increased risk of developing ischemic disease is associated with impaired regenerative properties of most tissues including skeletal muscle, which make those patients a challenging population to treat. We recently demonstrated that in skeletal muscle, Hedgehog (Hh) signaling promotes ischemia-induced angiogenesis by maintaining peripheral nerve and by promoting myogenesis. The objective of this study is to investigate the functionality of Hh dependent regulation of peripheral nerve survival and myogenesis in aged mice.

Methods and Results: In the present study, we used 12 week old (young mice) and 20 to 24 month old C57BL/6 mice (old mice) to investigate the activity of Hh signaling in the setting of ischemic skeletal muscle regeneration. In this model, delayed ischemic muscle repair observed in old mice was associated with an impaired upregulation of Gli1. Sonic Hedgehog expression was not different in old mice compared to young mice while Desert Hedgehog (Dhh) expression was downregulated in skeletal muscle of old mice both in healthy and ischemic conditions. Rescue of Dhh expression by gene therapy in old mice increased nerve density and promoted ischemia-induced angiogenesis, nevertheless it failed to promote myogenesis. After further investigation, we found that, in addition to Dhh knockdown, Smo reduced (Smo) expression was significantly downregulated in old mice. We used mice in which Hh signaling is specifically disrupted in myocytes (HSA Cre:Smo<sup>flx/flx</sup>) and demonstrated that Smo knockdown is sufficient to impair ischemia-induced myogenesis.

Conclusion: The present study demonstrates that Hh signaling is impaired in aged mice which leads to impaired peripheral nerve survival and myogenesis in acute ischemic stress condition. Moreover, this study brings the new concept that it is necessary to restore both peripheral nerve integrity and myogenesis in elderly to promote revascularisation of ischemic tissues.

Endoglin in adhesion between endothelial and mural cells

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The interaction and interplay between endothelial cells (ECs) and mural cells (such as vascular smooth muscle cells -VSMCs- and pericytes) play a pivotal role in vascular biology. Endoglin is an RGD-containing ligand of β1 integrins highly expressed by ECs during angiogenesis. Our working hypothesis is that endothelial endoglin acts as an adhesion molecule via integrin recognition motifs, allowing the interaction between ECs and mural cells. We find that suppression of endoglin expression or addition of soluble endoglin inhibits the adhesion between ECs and VSMCs as shown by tubulogenesis assays on matrigel. The interaction and interplay between endothelial cells (ECs) and mural cells (such as vascular smooth muscle cells -VSMCs- and pericytes) play a pivotal role in vascular biology. Endoglin is an RGD-containing ligand of β1 integrins highly expressed by ECs during angiogenesis. Our working hypothesis is that endothelial endoglin acts as an adhesion molecule via integrin recognition motifs, allowing the interaction between ECs and mural cells. We find that suppression of endoglin expression or addition of soluble endoglin inhibits the adhesion between ECs and VSMCs as shown by tubulogenesis assays on matrigel. The EC-VSMC adhesion was totally abolished by an anti-integrin αβ1 inhibitory antibody, whereas it was markedly enhanced by the integrin activators MnCl<sub>2</sub> or CXCL12. The CXCL12-dependent cell adhesion was abolished in the presence of soluble endoglin or a derived pentapeptide containing the RGD motif. Adhesion of cells overexpressing different endoglin mutant constructs, allowed the specific mapping of the endoglin RGD motif as involved in adhesion to VSMCs. Binding of soluble endoglin to VSMCs was markedly enhanced by MnCl<sub>2</sub> and CXCL12 and this increase was inhibited by the RGD peptide. Moreover, transgenic mice overexpressing soluble endoglin show podocyturia and lower number of glomerular podocytes, suggesting that soluble endoglin induced the detachment of podocytes from glomerular capillaries. These results suggest a critical role for endoglin in integrin-mediated adhesion of mural cells and provide a better understanding on the mechanisms of vessel development and maturation in normal physiology as well as in pathologies such as preeclampsia, cancer or hereditary hemorrhagic telangiectasia.