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: Angiogenic 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) 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 (fzd7iECKO) resulted in increased retinal tissue sensitivity to hypoxia during the vaso-oblit- erative 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 explore canonical β-catenin and Notch signaling during the two different phases of OIR. Preliminary results showed that transcript expressions of β-catenin (lef1, axin2) and partners of Notch signaling were strongly decreased in fzd7iECKO 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 microvascularity. 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/B16 subject to an high fat diet (HFD) and voluntary exercise protocol of 7weeks, capilarisation of epidymal fat (eWAT) and subcutaneous adipose tissue (sWAT) by histo- logical marking of CD31, and expression of angiogenic factors: Murine double-minute 2 (MDM-2), vascular endothelial growth factor (VEGF-A) and thrombospondin (TSP-1). Morphometric analysis of mice showed a significant reduction of 30% in the ratio of AT/total mass in HFD trained mice at 7weeks of exercise. In these mice, % of mass eWAT/total and sWAT/total signif- cantly reduced by 33 and 39%, respectively, which was associated to a signifi- cant decrease in adipocytes size by 26% in eWAT and 30% in sWAT of HFD mice at 7weeks of exercise. Biochemical study showed that exercise led to an increase of MDM-2 expression and VEGF-A/TSP-1 ratio and in AT of control and HFD mice after 7weeks of exercise pointing the emerging of a angio-adaptive response in favor of capillary growth in AT, was confirmed by a significant increase of the capillary density (capillaries/mm²) in AT of control mice (55 and 36% respectively in eWAT and sWAT) and a capillaries/adipocyte ratio significantly increased in HFD trained mice (respec- tively 16 and 18% in eWAT and sWAT). These results showed for the first time that physical exercise acts as a pro-angiogenic stimulus in AT in favor of capillary growth, thru activation of MDM-2, and VEGF-A/TSP-1 ratio.

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 dilation through prostaoglandin 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 prostaoglandin activity and Cos-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 translational 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 performing immune cells tissue trafficking, understanding the translational regulations of lymphatic vasodilatation is a crucial step toward an innovative inflammatory disease therapy.

0048
Frizzled7 controls postnatal vascular formation through Wnt/β-cate- nin canonical signaling in a Dll4/Notch dependent and independent way

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Growing evidence has shown that Wnt/Fzd proteins are expressed in vas- cular 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 fzd7f/f mice and crossed them with Pdgfbcre mice to generate fzd7fECKO mice. In the fzd7fECKO retinas at P7 dpn, we observed a delay in vascular network formation, a strong increased in tip cells and filo- podia 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 fzd7fECKO mice. Moreover, intraocular injection of siRNA/DVL1 in DVL1KO mice increased DVL1KO phenotype and mimics fzd7fECKO 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-