FORMATION AND FUNCTION OF SPHINGOSINE 1-PHOSPHATE – TO THE VASCULATURE AND BEYOND

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Metabolism of sphingomyelin, an abundant membrane phospholipid by the sphingomyelinase pathway results in the formation of sphingosine 1-phosphate (S1P), a pleiotropic lipid mediator. Our laboratory discovered that S1P acts via the G protein-coupled receptors of the EDG family, which are now called S1P1-5 receptors. S1P receptors are present ubiquitously in vertebrates and regulate many physiological and pathological functions, including, vascular development, angiogenesis and immune cell trafficking. In mammals protein-bound S1P is abundantly present as a circulating lipid mediator in plasma. However, cellular sources and mechanism of export of S1P in vivo is not known, even though it is widely assumed that S1P is a platelet-derived lipid mediator. Recent data from our lab addressing this issue will be discussed.

In the vascular system, S1P1 receptor activates the PI-3-kinase/Akt pathway to regulate endothelial cell migration and adherens junction assembly. The small GTPase Rac is critical for this receptor to activate the actin- and microtubule cytoskeleton dynamics. In contrast, the S1P2 receptor antagonizes this pathway by activating the small GTPase Rho/ and Rho-associated kinase (ROCK). Ultimately, this pathway induces the tumor suppressor phosphatase PTEN to inhibit cell migration and adherens junction assembly. In addition, mural cell recruitment to nascent vascular sprouts is also regulated by S1P1 signaling in vascular endothelial cells. Indeed, antagonistic function of these two S1P receptors is thought to mediate complex regulation of vascular formation, homeostasis and pathology.

The S1P1 receptor is essential for vascular maturation during development. Activation of this receptor on endothelial cells activate the Rac-dependent polarized translocation of N-cadherin and activation of this cell-cell adhesion molecule. Inside-out cadherin signaling, which involves lateral clustering and phosphorylation results in proper adhesion to mural cells. We propose that this event is critical for vascular stabilization during development and also in tumor angiogenesis. Down-regulation of S1P1 receptor expression by RNA interference approaches or pharmacological means led to inhibition of tumor angiogenesis, presumably due to the inhibition of vascular stabilization.

In addition, S1P may also play a role in tumor biology. Analysis of the Apc<sup>Min/+</sup> model of intestinal tumorigenesis indicated that S1P metabolic enzymes (Sphingosine kinase (Sphk), S1P phosphatase and S1P lyase) and receptors are expressed in the adenomatous polyps. Deletion of the Sphk1 gene resulted in the significant reduction in epithelial cell proliferation and tumor size. Interestingly, S1P levels were not significantly reduced in the polyps, presumably because of the action of Sphk2 enzyme. Deletion of the genes for S1P receptors (S1pr2, S1pr3 and S1pr1<sup>−/−</sup>) did not influence polyposis, suggesting that extracellular S1P signaling is not critical. Interestingly, sphingosine levels were elevated in the polyps. Treatment of intestinal epithelial cells in vitro with sphingosine resulted in cell-cycle arrest at the G1/S-boundary. These data suggest that intracellular sphingolipid metabolism plays a role in tumorigenesis in the intestinal tract. We speculate that Sphk inhibitors may be useful as ant-cancer agents.

These and other studies support the concept that S1P is a critical bioactive lipid mediator that acts in multiple ways to choreograph mammalian physiology and homeostasis. Further knowledge in this signaling system may yield novel opportunities in the control of various human diseases, including vascular during vascular malformation.

LECTURES
THE JUNCTIONAL PROTEIN VE-CADHERIN ENHANCES TGF-β SIGNALING IN ENDOTHELIAL CELLS

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Transforming growth factor-beta (TGF-β) is a multifunctional dimeric polypeptide growth factor that regulates proliferation, differentiation, migration, extracellular matrix production and survival of many different cell types. TGF-β mediates its cellular effects through serine threonine receptors coupled to phosphorylated intracellular effectors, termed Smads, which in turn regulate target gene transcription. In endothelial cells (ECs), one TGF-β type II receptor (TβRII) and two distinct TGFβ type I receptors, i.e. the EC-restricted ALK-1 and the broadly expressed ALK-5, have been described. Whereas ALK-1 activation induces the phosphorylation of Smad1/5/8, ALK-5 promotes Smad2/3 phosphorylation. Although identification of deleterious mutations within multiple components of TGF-β signaling provides a compelling basis for implicating aberrant TGF-β signaling in the vascular disorder termed Hereditary Hemorrhagic Telangiectasia (HHT), the precise molecular mechanism of disease development remains unclear. Hence, we might anticipate that a better understanding of TGF-β signaling regulation in ECs will point to possible mechanisms implicated in the development of HHT.

Vascular fragility and hemorrhages which characterize impaired TGF-β signaling in human vascular disorders are also frequently associated with alterations of the organization and function of endothelial intercellular junctions. Adherens junctions (AJs) are protein structures that maintain the stability of the vascular tree and control vascular permeability to solutes and circulating cells. VE-cadherin (VE) is the main component of endothelial AJs that mediates endothelial cell-to-cell homophilic adhesion and controls vascular homeostasis through the association with both catenins (α- and β-catenin), phosphatases (DEP-1) and growth factor receptors (VEGFR-2) by modulating their intracellular signaling properties. Because of the striking resemblance of the vascular phenotype of TβR−/− and VE−/− mice, we hypothesized that TGF-β signaling might be a novel pathway regulated by VE. Comparing TGF-β responsiveness of mouse isogenic ECs differing for VE expression only and HUVEC treated with VE or control siRNA, we found that VE expression potentiates: i) TGF-β-dependent Smad3 phosphorylation, ii) TGF-β/Smad3 endogenous target genes expression, and iii) ECs sensitivity to TGF-β-induced growth inhibition. We further assessed that VE clustering at intercellular junctions is in part responsible for its positive regulatory activity on TGF-β signaling. Finally, upon stimulation with TGF-β, TβRs associate with VE and concentrate at cell-to-cell contacts where they might be activated by VE clustering in vivo. Taken together, these data reveal VE/β-catenin signaling as a positive regulator of TGF-β pathway that increases the response of resting ECs to TGF-β, providing prospects for better understanding the role of AJ proteins in TGF-β-mediated effects in EC pathophysiology.
Some of the most devastating consequences of Hereditary haemorrhagic telangiectasia (HHT) result from cerebral vascular malformations that manifest themselves in various types of arteriovenous shunts. The purpose of the presentation is to describe through our experience the various aspects of the cerebro medullary lesions encountered, their clinical manifestations and endovascular treatment.

The charts and angiographic films of 50 patients diagnosed with HHT according to the Curaçao criteria were evaluated concerning age of onset of symptoms, or, if not applicable of first consultation. The files were reviewed for clinical presentation, family and personal history, while the patients’ angiograms were analysed with respect to the number of lesions (single, multiple), the location (superficial supratentorial, deep supratentorial, infratentorial, spinal), and type of lesion (fistulous AVF, nidus-type AVM, micro AVM). A total of 75 central nervous system manifestations of HHT were found. Lesions included seven spinal cord AVFs that were all present in the paediatric age group (mean age: 2.2 years), 34 cerebral AV fistulae, all but two affected patients were less than 6 years (mean age 3.0), 16 nidus type AVMs (mean age 23.1 years) and 18 micro-AVMs (mean age: 31.8 years) were found.

The commonest angiographic features in adults are nidus (81.8%) and multiplicity (45.5%), while in the paediatric group venous ectasia and giant pouches (91.3%), AVF(69.6%) and multiplicity(52.2%). In spinal cord lesions macrofistulas are demonstrated in 83% of HHT with no multiplicity. No specific difference with non HHT AVMs were found as far as AVM angiarchitecture in adults. However phenotypes in children below 6 years of age were significantly different from those of older patients with AVFs and large venous ectasias.

Intracranial haemorrhage was the presenting symptom in 8.8% and the risk of haemorrhage in the natural history was 0.7% per year. At follow up the risk of hemorrhage of untreated lesions in adult seems lower than AVMs without HHT context. On the contrary the natural history of AVFs in young children carries a high percentage of hemorrhagic episodes.

A total of 31 children under the age of 16 were included in a retrospective analysis. All children were treated in Bicetre. 20 children presented with 28 arteriovenous (AV) fistulae including seven children with spinal AV fistulae and 14 children with cerebral AV fistulae (one child had both a spinal and cerebral fistulae); 11 children had small nidus type AVM. Follow-up ranged between 3 and 168 months (mean: 66 months). A total of 115 feeding vessels were embolised in 81 single sessions resulting in a mean overall occlusion rate of the malformation of 77.4% (ranging from 30 to 100%). Two of 30 patients (6.5%) died following the embolization procedure, two patients (6.5%) had a persistent new neurological deficit, eight patients (26.7%) died following the procedure, in 11 patients (36.7%) an amelioration of symptoms could be achieved, six patients (20%) were completely asymptomatic following the endovascular procedure. In the surviving patients morphological complete occlusion was possible in twelve patients (38%), therapy is still not completed in six patients. The endovascular approach employing glue as the embolizing agent represents a safe and efficient way to control the neurovascular phenotypes of HHT. None of the patient followed with control angiogram, showed new lesion or recurrence of the embolized part.

Since members of the same family can present with completely different phenotypes of this disease there seems to be no obvious relationship between the type of mutation and the phenotype of the disease. There seems to be an age-related continuum of vascular abnormalities (from large fistulous areas to small AVMs and micro-AVMs) associated with HHT. The most likely postulated determining factor for the HHT phenotype is the timing of the revealing event in relation to the maturity of the vessel. HHT-related CAVMs often present as multiple lesions, cortical in location, micro AVMs or AVF. HHT in SCAVM is expressed as single macro AVF, especially in the paediatric group. AVF in children are highly suggestive of HHT.

We do not recommend screening in HHT adult patients for CAVM while in the paediatric population in HHT families, clinical and MRI screening could be recommended before 6 months of age for cerebrospinal localisation. Since the natural history of neurovascular manifestations of HHT in children is associated with a high morbidity and mortality, therapeutic intervention is mandatory in this age group.