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"Mechanisms of Signal Transduction in Cell Adhesion and Differentiation"

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Organized by:
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7) **Activation of angiotensin II signalling pathways in high glucose-treated rat retinas**

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Diabetic retinopathy (DR) is a common and serious complication in diabetic patients and the leading cause of blindness in working people. In the past, research has been primarily focused on the vascular aspects of this disease, but recent evidences clearly demonstrate that the neural components of the retina may be also affected before overt vasculopathy. Moreover, the observed modifications in neuronal/glial cell interactions represent a crucial feature in the pathogenesis of DR and could be responsible for some of the functional deficits in vision that begin soon after the onset of diabetes. These changes could be linked to the metabolic dysfunction related to excess of glucose, which can alter synthesis and release of factors modulating neuroglia and blood vessel structures and functions. Among these mediators, the retinal renin-angiotensin system (RAS) could play a pivotal role in view of its action on vasculature and tissue remodelling. In this work using rat retinal tissue cultures we investigated whether high glucose exposure could activate RAS signalling-dependent pathways. Retinas were explanted from Sprague-Dawley rats and exposed to normal glucose (5.5 mM), high glucose (30mM), or iso-osmolar mannitol (5.5 mM glucose + 24.5 mM mannitol) for 48 hours. Tissue cell lysates were used for the detection of tyrosine phosphorylated proteins and of molecules involved in intracellular Ang II signalling. Western blot analysis revealed an increase of protein phosphorylation in rat retinal tissue cultures exposed to high glucose, but not in isoosmolar mannitol, especially due to specific bands identified as phospholipase Cg (PLCg), proline-rich tyrosine kinase 2 (Pyk2), c-src kinase and ERK 1/2. Moreover, an increase in c-src and lyn kinase activities was also observed. The analysis of downstream signalling in the nuclear compartment showed that cAMP-responsive element binding (CREB) protein is phosphorylated (S133) in response to high glucose suggesting activation of transcription factors. Among the signalling pathways triggered by metabolic dysfunction induced by excess of glucose in rat retina, our data suggest a role for RAS in retinal neuroglial dysfunction, which, in turn, may act on microvasculature, contributing to the pathogenesis of DR. In a long-term perspective, a pharmacological intervention targeted to modulating RAS activity may represent an innovative therapeutic approach to this invalidating diabetic complication.

8) **KRIT1, the disease gene responsible for type 1 Cerebral Cavernous Malformations (CCM1), acts as a modulator of RAP1A and ICAP1A functions**

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Cerebral Cavernous Malformations (CCM; OMIM 116860) are vascular malformations, mostly located in the central nervous system, characterized by tortuous, abnormally enlarged and often leaky capillary cavities (caverns), which are devoid of normal vessel wall elements, such as pericytes. These vascular malformations can arise sporadically or may be inherited as an autosomal dominant condition with incomplete penetrance. They occur as single or multiple lesions of various sizes which can be clinically silent or give rise to clinical symptoms ranging from headaches and focal neurological deficits to seizures and fatal intracerebral hemorrhage. This disease can be diagnosed by Magnetic Resonance Imaging (MRI), and it has been recognized as a common clinical entity: its prevalence in the general population has been estimated to range from 0.1 to 0.5%, although only 20-30% of affected individuals develop symptomatic disease. To date, there are not direct therapeutic approaches for CCM treatment.

The nature of CCM lesions suggests a perturbation in normal development or stabilization of small blood vessels; however, the mechanisms of their occurrence and an explanation for their focal nature remain elusive. Recently, it has been demonstrated that at least 47% of families affected with CCM harbor a mutation in the gene KRIT1 (CCM1; OMIM 604214). Although comprehensive analysis of the KRIT1 gene in patients with CCM has led to the identification of more than 90 disease-associated mutations, neither the molecular mechanisms involving the KRIT1 protein nor the molecular consequences of KRIT1 mutations are understood so far: their characterization remains therefore a fundamental challenge for basic and translational research.

Noteworthy, two interacting partners of KRIT1, the small GTPase RAP1A and the Integrin Cytoplasmic domain Associated Protein-1A (ICAP1A), have been shown to be crucial players in cadherin and integrin-mediated cell adhesion and signaling. On the other hand, it is well established that a fine-tuned crosstalk between cadherins and integrins plays a fundamental role in regulating the dynamics of vascular tissue