Role of the Glycoprotein Dickkopf-3 in Cerebral Blood Vessels of Spontaneously Hypertensive Rats

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Hypertension is the most prevalent risk factor for human stroke and cardiovascular complications in hypertensive patients are related to endothelial damage or dysfunctional angiogenesis. Genes involved in angiogenesis may represent suitable candidates as drug targets in stroke. Dickkopf-3 (Dkk-3), a member of the dickkopf protein family, acts as a pro-apoptotic factor on cancer cells and as an angiogenic factor involved in embryogenesis and a differentiation factor in remodelling the tumor vasculature in endothelial cells. We have found that the dkk-3 gene maps within a chromosomal region linked to the stroke-prone phenotype in spontaneously hypertensive (SHRsp) rats. We have shown that Dkk-3 is a potent inducer of VEGF expression in endothelial cells. Studies performed in tumor cell lines have shown that overexpression of Dkk-3 leads to an increased phosphorylation of Smad3, a transcription factor that lies along the signaling pathway stimulated by transforming growth factor-β1 (TGF-β1). The established role of TGF-β1 in angiogenesis provides a further potential link between Dkk-3 and the development of stroke. It is known that Dkk-3 leads to the activation of ATF3 and Smad3 phosphorylation, which are molecular partners mediating the intracellular signaling of the TGF-β1), an anti-inflammatory and neuroprotective cytokine. Dkk-3 gene maps near to the chromosome 1 region linked with the stroke prone phenotype in the genome of SHRsp, which develop vasogenic stroke after high-salt diet. This raises the intriguing possibility that Dkk-3 gene may be considered a candidate gene for the risk to develop stroke under hypertension. Here we analysed the expression of Dkk-3 in brain tissues of non-stroke prone SHR and SHRsp rats maintained for one month on a high-salt diet. Immunohistochemistry showed a Dkk-3 upregulation in striatal blood vessels of SHR rats on a high-salt diet as compared to SHR rats on normal diet, whereas Dkk-3 was not expressed in blood vessels of SHRSP rats on high- or normal-salt diet. Real-time-PCR analysis showed a significant increase of Dkk-3 mRNA levels in the whole brain of high-salt diet/SHR rats *vs.* normal-diet/SHR rats, with no changes in SHRsp rats. Dkk-3 induction was associated to VEGF up-regulation in striatal blood vessels of high-salt diet/SHR rats, as assessed by immunohistochemistry. No induction of Dkk-3 and VEGF was observed in the blood vessels of SHRsp rats. Finally, we found an up-regulation of VEGF in cultured human endothelial cells after incubation with human recombinant Dkk-3. These data support the hypothesis that Dkk3 expression on blood vessels, in hypertensive conditions, might be responsible for VEGF induction, a key component in the homeostatic mechanisms triggered in response to hypertension.