

Summary

The serum- and glucocorticoid-inducible protein kinase Sgk1 has been implicated in a variety of functions in different tissues and subcellular compartments. It has been proposed that the kinase regulates the activity of plasma membrane transporters and ion channels, has an influence on the cell cycle and might have a role in the pathogenesis of different diseases. Sgk1 has been identified as an activity regulated gene in the rodent hippocampus. The expression of Sgk1 mRNA is upregulated in hippocampal neurones after neuronal activity and in oligodendrocytes in the white matter after general stress.

To clarify the inconsistent data concerning the subcellular localization of Sgk1, HEK293 cells were transfected with Sgk1-EGFP and analyzed by confocal microscopy. The fusion protein was mainly localized to the mitochondria irrespective of any stimuli that have been reported to influence the subcellular localization of the kinase. *In vitro* import assays using purified mitochondria and subcellular fractionations of mouse liver tissue confirmed the predominant mitochondrial localization of Sgk1. Biochemical experiments demonstrated that the N-terminus of Sgk1 confers a strong association with the outer mitochondrial membrane and that the kinase is localized on the cytosolic surface of the organelles. Microscopy of EGFP-tagged deletion variants identified amino acids 17-33 as the mitochondrial targeting sequence of Sgk1. Pulse-chase experiments demonstrate that the identical residues also determine the short half-life of the Sgk1 protein. This makes it likely that the kinase is stabilized upon arrival at its mitochondrial destination and degradation mainly occurs in the cytosol.

NDRG1 is the only known unambiguous phosphorylation target of Sgk1. Here it could be shown that Sgk1 mediated phosphorylation of NDRG1 is more efficient when Sgk1 is localized to mitochondria. Moreover, NDRG1 has been identified as a substrate for Sgk1 in the mouse hippocampus after neuronal activity and after application of the glucocorticoid receptor agonist dexamethasone. Analysis of the spatial mRNA expression pattern of Sgk1 in the mouse brain revealed only minor differences between dexamethasone treated mice and control animals. This makes it likely that agonists of the glucocorticoid receptor can activate the kinase and that catecholamines are responsible for the stress

induced expression of Sgk1 in oligodendrocytes. Comparison of the expression patterns of Sgk1 and NDRG1 render it obvious that NDRG1 is a phosphorylation target of Sgk1 in the granule cells and pyramidal cells of the hippocampus.

Attempts to identify a cellular function for Sgk1 disclosed no role for Sgk1 in the fission or fusion of mitochondria nor in the regulation of gene expression, as overexpression of Sgk1 in HEK293 cells had no influence on the morphology of mitochondria or the transcriptome.

Using five different antibodies including one that has been generated during this thesis it was not possible to detect the Sgk1 protein in primary mouse tissues or untransfected cell lines. Only after the administration of dexamethasone in animals, or after strong overexpression in HEK293 cells Sgk1 could be detected in Western Blot analysis. Thereby in HEK293 cells a second specific band at approximately 40 kD occurred. It could be demonstrated that this second species originates from the usage of an alternative translational start.

The results of this thesis challenge the data of several publications, especially with respect to subcellular localization and postulated cellular functions of Sgk1. Moreover, they cast any antibody-dependent expression analysis of Sgk1 into doubt. On the other hand, the presented data may give rise to further substantiated analysis that shed more light on the cellular function of Sgk1.