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Development of a cell-based corin assay

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Corin is a transmembrane serine protease expressed in cardiomyocytes. Single Nucleotide Polymorphisms (SNPs) and mutations in the Corin gene leading to reduced corin activity were identified in patients with hypertension, cardiac hypertrophy and heart failure. Corin exerts its cardio protective effects via the proteolytic cleavage and activation of pro-atrial natriuretic peptide (pro-ANP) to ANP. Binding of mature ANP to the ANP receptor (GC-A) augments intracellular cGMP levels in target organs such as kidneys and peripheral blood vessels, ultimately leading to reduced blood volume and pressure. We recently described a reporter cell line stably expressing both the receptor guanylyl cyclase GC-A and the cGMP-dependent cation channel CNGA2, which was used as a real-time cGMP biosensor. Additional expression of the Ca2+-sensitive photoprotein aequorin allowed natriuretic peptide detection by luminescence measurements. However, upon ANP stimulation luminescent signals were found to be transient. Therefore, we generated a second reporter cell line which expressed the calcium-sensitive GFP variant GCaMP6 instead of aequorin. In contrast to the luminescence-based reporter cell line, ANP stimulation of our novel GCAMP6 reporter cell resulted in stable, long-lasting fluorescence signals. Using this reporter system, we could show that recombinant soluble wild type corin (solCorin), but not the active site mutant corin (S985A) is able to convert proANP to ANP, resulting in left-shifted concentration-response curves. Equally, the cardiomyocyte derived HL-1 cell line converted proANP to ANP while HL-1 corin knockout cells did not. Furthermore, transient overexpression of corin conferred proANPase activity to HEK293 cells. Our findings underline the role of corin as the proANPase. The fluorescence-based ANP reporter cell line is well-suited for the characterization of corin modulators, structure-function studies of corin variants, and might be used for the identification of novel corin activators by HTS.

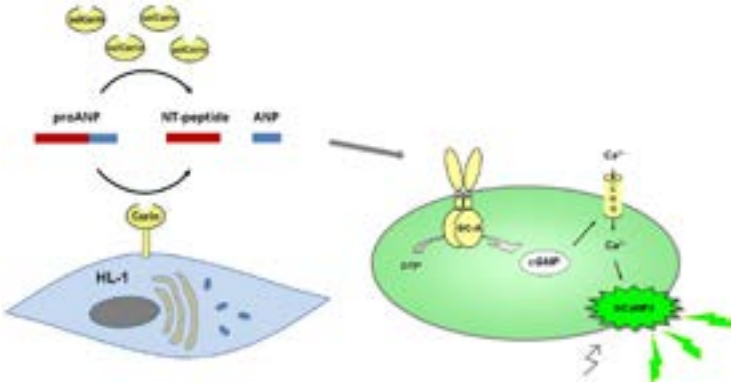

Figure 1: Schematic presentation of the cell-based Corin reporter assay. ANP generated by corin-mediated proANP cleavage is detected by fluorescence measurements using the ANP receptor GCaMP6 reporter cell line.

Biography

Frank Wunder is a senior scientist at Bayer AG in Wuppertal, Germany. His PhD. thesis at the Center for Molecular Neurobiology Hamburg (ZMNH) was mainly focussed on the molecular biology of potassium channels. He joined the Bayer pharmaceuticals division in 1993. Wunder has many years of experience in the development of new screening assays, with major focus on cell-based assays for GPCRs, voltage- and ligand-gated ion channels and molecular targets involved in the nitric oxide/cGMP pathway. He has successfully implemented novel screening platforms for cAMP and cGMP. He gained additional expertise in pharmacological models of cardiovascular disease, such as primary cell cultures, isolated heart preparations, and *in vivo* models.

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