Bestrophin-3: localization and function in normal and injured tissues

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ABSTRACT

Bestrophin-3 (Best3) is a protein with multiple functions. It can constitute a calcium-activated chloride channel when overexpressed in cultured cells, but the function of Best3 is not well studied in cells *in situ*. Recently Best3 protein was suggested to play also cell-protective role.

In this thesis the expression and function of Best3 has been studied in mouse and rat tissues by immunohistochemical methods, RT-PCR, siRNA-based downregulation and patch-clamp technique. We showed that Best3 in rat vascular smooth muscle is responsible for a cGMP-dependent, calcium-activated chloride current, important for synchronizing intracellular calcium oscillations in vascular smooth muscle cells. In mouse kidney, brain and intestine, alternative splicing produces only truncated variants of Best3 mRNA and protein which likely do not form ion channels in plasma membrane, but rather have an intracellular localization and function. These variants are expressed in mouse glomerular podocytes, in a subpopulation of astrocytes in neonatal brain after hypoxia-ischemia, and in glia-like cells in myenteric plexus of intestine. In these cells the distribution of Best3 seems to follow that of the intermediate filament nestin. Best3 is also expressed in cells of epithelial type, such as intestinal goblet cells and in brain ependymocytes. The expression of individual splice variants of Best3 changes in response to endoplasmic-reticulum-associated injury and follows separate time courses. Cultured podocytes and astrocytes after endoplasmic reticulum stress also responded with upregulation of Best3 mRNA.

It is suggested that Best3 in some cell types functions as an ion channel, whereas in other cell types it may be responding to endoplasmic reticulum stress-related cell injury. In some locations it exists in truncated splice variants; changes in the ratio between these variants may be important for the cellular response to stress. Alternative splicing may explain the variation in function of Best3.

Keywords: Bestrophin-3, alternative splicing, injury

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I. Matchkov VV, Larsen P, Bouzinova EV, Rojek A, Boedtkjer DM, Golubinskaya V, Pedersen FS, Aalkjaer C, Nilsson H. Bestrophin-3 (vitelliform macular dystrophy 2-like 3 protein) is essential for the cGMP-dependent calcium-activated chloride conductance in vascular smooth muscle cells. Circ Res. 2008 Oct 10;103(8):864-72.

II. Golubinskaya V, Elvin J, Ebefors K, Gustafsson H, Mallard C, Nyström J, Nilsson H. Bestrophin-3 is expressed in mouse glomerular podocytes (manuscript under revision)

III. Golubinskaya V, Osman A, Gustafsson H, Mallard C, Nilsson H. Bestrophin-3 is expressed in a subpopulation of astrocytes in neonatal hypoxic-ischemic brain injury (manuscript)

IV. Golubinskaya V, Gustafsson J, Gustafsson H, Mallard C, Nilsson H. Localization of bestrophin-3 in mouse intestine (manuscript)

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