Mechanisms of Nuclear Translocation of βIPKC

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INTRODUCTION: Embryonic stem cells (ESC) proliferate while maintaining the ability to differentiate into various cell types (self-renewal). Previous studies from our laboratory suggest that low molecular weight forms of βIPKC are expressed in the nucleus of undifferentiated murine ESC and that most βIPKC targets are nuclear proteins involved in proliferation/differentiation processes. We also noted that during differentiation there is a change in subcellular localization of βIPKC, which is either expressed in the cytoplasm of differentiated cells, or absent. Thus, this study aims to evaluate the mechanisms involved in βIPKC translocation to the nucleus.

MATERIAL AND METHODS: We determined whether βIPKC constructs would localize to the nucleus as did endogenous βIPKC in ESC, Hela and HEK293T cells. Cells were transfected with βIPKC constructs fused to either GFP or HA tagged. Subcellular localization of the constructs was determined by fluorescence microscopy and immunoblotting. A putative nuclear localization signal (NLS) in the catalytic domain of βIPKC was evaluated by site directed mutagenesis.

DISCUSSION AND RESULTS: All the cell lines examined showed nuclear translocation of overexpressed βIPKC only when the regulatory domains C1 and C2 were deleted (βIPKCΔNPSC1C2). Interestingly, Hela cells showed an intense nuclear accumulation of βIPKCΔNPSC1C2 as compared to ESC and HEK293T cells. Furthermore, βIPKC-K618A, containing a mutation at the putative NLS in the catalytic domain of βIPKC, decreased nuclear translocation.

CONCLUSIONS: Under basal conditions Wt βIPKC did not translocate to the nucleus. In some cells, the catalytic domain of βIPKC translocates more to the nucleus than in others. This domain contains a putative bipartite NLS that seems to play a key role in this process. Understanding the mechanisms and stimuli that regulate the nuclear translocation of βIPKC in ESC could help us elucidate the signaling pathways that control the processes of ESC proliferation and self-renewal.

Keywords: protein kinase C, embryonic stem cells and nuclear localization signal

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