Characterization of βIPKC targets in the Nucleus of Murine Embryonic Stem Cells

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Embryonic stem cells (ESC) are pluripotent cells that proliferate while retaining the ability to differentiate into several cell types (self-renewal). For effective use of ESCs in cell therapy one needs to understand the specific molecular processes of differentiation and self-renewal. The family of serine / threonine kinases, Protein kinase C (PKC), has been shown to be involved in proliferation / differentiation of ESCs. However, the exact function of each isoenzyme is not yet clear. In previous studies we saw that among the PKCs expressed in ESC, βIPKC is expressed in the nucleus. In addition, upon differentiation some cells ceased to express βIPKC and in others βIPKC was now found dispersed throughout the cytoplasm. To identify other βIPKC targets we developed an improved 2D-PAGE method to isolate nuclear proteins in ESCs using cellular fractionation. We used ESCs treated with a specific βIPKC inhibitor peptide, βIV5-3, for 15 minutes and compared the nuclear phosphoproteome of control cells and cells treated with βIV5-3. We observed that the phosphorylation of 75 spots decrease upon treatement with, βIV5-3. We previously used total cell lysates and detected 17 βIPKC targets of which more than 60% were nuclear proteins,. Analyzing nuclear extracts we now identified 48 proteins. of which only 3 were detected when using total lysates Functionally the proteins detected had similar functions confirming that βIPKC is involved in splicing processes and transcription processes Thus, our results further confirm the idea that βIPKC may be involved in the regulation of protein transcription in undifferentiated ESC.

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