IJAE Vol. 115, n. 1/2 (Supplement), 2010

Morphological and molecular events during H9c2 differentiation: role for pPKCδ/SC35 interaction

Susi Zara[1], Viviana di Giacomo[2] Monica Rapino[3], Valentina Di Valerio[2], Amelia Cataldi[2]

[1] Department of Drug Sciences "G. d'Annunzio" University, Chieti-Pescara, Italy
[2] Department of Medicine and Ageing Sciences, "G. d'Annunzio" University, Chieti-Pescara, Italy
[3] Institute of Molecular Genetics CNR, Unity of Chieti, Italy

H9c2 cells are rat embryonic myoblasts with skeletal muscle properties which reach differentiation when cultured in HS low concentration (1%). Their terminal differentiation consists of fusing and forming multinucleated myotubes. At ultrastructural level the organization of differentiated cells undergoes modifications in terms of morphological features: they appear thin and elongated, often fused to form multinucleated syncytia (myotubes) and well organized actin filaments are recognizable and detected by immunogold labeling. The decreased expression of cyclin A, required cell cycle regulator for the onset of DNA replication, and the increased expression of myogenin, marker of skeletal muscle differentiation, accompany such morphological modifications.

Among the molecules involved in a cascade of reactions controlling the functioning of nuclear complexes and leading to cell differentiation, a role has been assigned to Protein Kinases C. In our experimental model a ten fold greater signal of pPKCδ in 1%HS cells, with respect to 10%FBS cells, is shown. In order to investigate a potential activation of SC35 splicing factor by pPKCδ, pPKCδ-SC35 co-immunoprecipitation has been performed and revealed only in differentiated cells, supporting a possible interaction between the two molecules. Immunogold co-localization of SC35 factor with pPKCδ, carried out by FEI-SEM and TEM, evidences in 1% HS cells an increased level of pPKCδ and its accumulation in the nucleus, which represents reasonable evidence of protein activation along with an increased expression of SC35. In order to check the specificity of PKCδ involvement in H9c2 differentiation, PKCδ silencing has been performed and both PKCδ and myogenin expression have been detected by immunofluorescence analysis, evidencing a decrease of myogenin expression in 1% HS PKCδ siRNA- transfected H9c2 cells in parallel to the reduced percentage of PKCδ positive cells.

Thus these results suggest that pPKCδ/SC35 interaction represents a crucial event resulting in downstream changes in transcription of components which determine the morphological modifications related to cardiomyocyte differentiated phenotype.