

Functional analysis of Protein Disulfide Isomerase from the tick embryonic cells BME-26

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Protein disulfide isomerase (PDI) has been described as a multifunctional protein that participates in protein folding, assembly and post-translational modification. PDI is known as an essential catalyst for disulfide bond formation and isomerization in the ER. Although, PDI is mainly found in ER, some isoforms has been identifying on the surface of lymphocytes, platelets, endothelial cells, hepatocytes. In cancerous cells its function has been related to involvement in the maintenance of cell adhesion. In order to investigate the involvement of this enzyme in cell viability and in cell proliferation, we identify PDI on the cell surface in embryonic tick cells (BME-26). Specific Primers were designed for all isoforms that coding region and the products were amplified by PCR and cloned in pGEM-T vector. After DNA sequencing, for confirmation of identity, the amplicons were cloned into prokaryotic expression pCold-DNA II vector. The RmPDIs were produced using the E. coli Rosetta-Gami B (DE3) pLyS. Three PDIs isoforms from BME-26 cells were identified. Thus, the further analyzes were proceeding and their biological functions studied. The dsRNA of each BmPDIs gene and one dsRNA combination of BmPDI-1/BmPDI-3 were separately added to the culture medium. Additionally, the knockdown of this enzyme was preceded and these cells were treated with a PDI inhibitor (DTNB) to study the function of this enzyme in BME-26 cells. Furthermore, we observed that an increase of the expression of the three isoforms PDI when BME-26 cells were treated with DTNB. Altogether, the results, indicates that the BME-26 cells had a phenotype pattern that is different when treated with DTNB and after silenced to these isoforms. These results are suggesting a possible relationship between PDIs and migration and BME-26 cell proliferation. Next steps of this work include the production of specific antibodies and its immunolocalization on BME-26 cell surface.

Keywords: Tick, Protein Disulfide Isomerase, antibodies.

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