DIFFERENTIATION OF MURINE EMBRYONIC STEM CELLS INTO INSULIN-PRODUCING CELLS AND FUNCTIONAL CHARACTERIZATION OF THIOREDOXIN-INTERACTING PROTEIN (TXNIP) GENE IN THIS PROCESS

Leal-Lopes, C.1,2; Lojudice, F.H.2; Kossugue, P. M.2; Sogayar, M.C.1,2

1Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo;
2NUCEL/NETCEM, Faculdade de Medicina, Universidade de São Paulo

INTRODUCTION: Alternative therapies involving cell replacement have raised great expectations for treatment of type 1 diabetes (T1D). An interesting therapy for T1D would be the engraftment of insulin-producing cell (IPCs) differentiated from embryonic stem cells (ESCs). Understanding the mechanisms which lead to the differentiation of ESCs into IPCs lays the foundation for the existence of renewable tissue for diabetic patients' therapy. OBJECTIVE: This study aims to analyze the process of pancreatic beta-cell differentiation, at the molecular level, through functional analysis of the Txnip gene. MATERIAL AND METHODS: We promoted murine ESCs (mESCs) differentiation into IPCs. The expression of Txnip at all stages of the protocol was quantified (qRT-PCR). The ability of IPCs to secrete C-peptide was evaluated (ELISA). Lentiviral vectors for Txnip gene expression inhibition were produced and used to establish genetically modified MIN-6 (mouse insulinoma 6) cell lines. These lines were phenotypically characterized for proliferative potential, resistance to apoptosis upon treatment with cytokines, and insulin production. RESULTS AND DISCUSSION: IPCs were obtained from mESCs. These cells are able to secreted C-peptide. We confirmed TXNIP gene downregulation during the differentiation. Four modified MIN-6 cell lines were produced, three containing shRNA sequences for inhibition of the Txnip gene and one a non-target shRNA sequence. We demonstrated that a moderate decrease in Txnip expression increases the proliferative potential, the resistance to apoptosis and the insulin production levels, but extensive inhibition of Txnip gene expression induces loss of beta cell phenotype. TXNIP acts as a negative regulator of an antioxidant system that protects cells from redox stress. Since pancreatic beta-cells are known for their poor antioxidant defenses, we hope to improve the differentiation of mESCs into IPCs by modulation of TXNIP throughout the entire differentiation process. CONCLUSION: This strategy may lead to improvement in the efficiency of mESCs differentiation into IPCs, by regulating their redox status through modulation of TXNIP gene expression. ACKNOWLEDGEMENTS: FAPESP, CNPq, BNDES, CAPES, MS-DECIT. KEY WORDS: Embryonic stem cells, Thioredoxin-interacting protein, Insulin-producing cells