EFFECT OF MILD HYPOTHERMIA ON THE TRANSCRIPTOME OF CHO-CELLS PRODUCER OF RECOMBINANT GLYCOPROTEIN: SECRETION PATHWAY

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Introduction. Recombinant glycoproteins (RGP) are needed in large quantities because they are widely used as bio-pharmaceutics in the treatment of many diseases (1). It has been reported that temperature downshift (TDS) from 37°C to 28-34°C increases the specific productivity (Q_p) of RGP and extended culture duration (1). Specially, the Q_p of tissue plasminogen activator (tPA) increased 1.8 folds produced in Chinese hamster ovary (CHO) cells "TF70R" (2). In order to understand at molecular level, the effect of TDS over the Q_p increase, transcriptomic analysis by massive sequencing (RNAseq) was performed, with special interest in the secretory pathway of RGP.

Methods. The TF70R cells were cultured in CD-OPTICHO (GIBCO) media, in spinners flask, 60 rpm and CO_2 5%. Biphasic cultures where maintained at 37ºC by 48 h, then were changed to 30°C. The mRNA from samples collected at 0, 24 and 48 h after TDS, were purified with Qiagen RNeasy kit, transformed to cDNA and evaluated with Illumina Genome Analyzer GAII. Data were aligned with mouse genome database (3). The differential expression was determined with the NOIseq program (4) with reliability of 0.9. Data were ordering in gene ontology (GO) terms.

Results. A total of 15775 genes were mapped. The transcripts coding for tPA did not changed significantly compared with the time before the TDS. Importantly, *rbm3* was over-expressed at 24 h after TDS, indicating the cold stress sensing. A total of 389 genes were found deregulated significantly, being 108 or 141 upregulated and 63 or 77 repressed, at 24 h or 48 h, respectively. The gene groups more deregulated were cell cycle function (34%), metabolism (18%) and those related with the cytoskeleton (11%) (fig. 1). During RGP biosynthesis through the classical secretory pathway, the proper functioning of the chaperones and enzymes related, is fundamental. Mild hypothermia caused deregulation of 28 genes related with the recombinant tPA and endogenous secretion trough the classical via. Particularly, *hspa5* coding for chaperone BiP was overexpressed at 24 and 48 h. This gene is finely regulated during stress response and is important in the peptide folding. The genes coding for calreticulin and EDEM (involved in folding and degradation glycoproteins at the Endoplasmic Reticulum) were overexpressed at 24 and 48 h. Moreover, genes coding for clathrin, OCRL, LRP1 and Wipi1 that are part of the ensemble of secretory vesicles were also overexpressed.

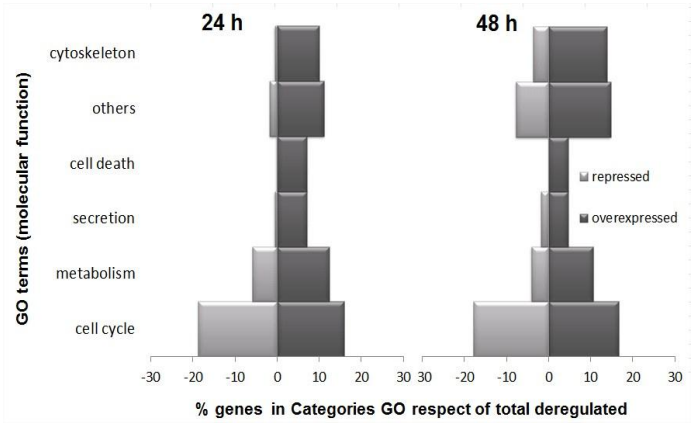

Fig.1. Percentaje of deregulated genes grouped in GO categories based on it molecular function, at 24 and 48 h.

Conclusions. Mild hypothermia caused deregulation of 389 genes at 24 and 48 h, from 15775 mapped. The transcript coding for tPA remains constant in all samples analyzed, indicating that the tPA Q_p increase is due to other factors. The TDS caused differential expression of 28 genes (mainly overexpressed) related with the classical secretory pathway. Hence, the up-regulation of *hspa5*, and genes coding for calreticulin, EDEM, clathrin, OCRL, LRP1 and Wipi1, and others, suggest a cell strategy increasing chaperones and regulators that may be related with the productivity increase.

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