Transcriptional evaluation of induced pluripotent cells from patients with Cockayne syndrome after induction of DNA damage triggered by oxidative stress

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Abstract

Cockayne Syndrome (CS) is characterized by symptoms related to premature ageing with severe involvement of the central nervous system. The molecular basis of the disease is related to deficiency in the transcription-coupled repair (TCR), mainly with mutations in the ERCC8 and ERCC6 genes (coding for CSA and CSB proteins, respectively). The phenotype of CS cells is presented as high sensitivity to ultraviolet (UV) light, causing DNA damage, which in turn prevents transcription recovery after irradiation. They are also more susceptible to DNA damage caused by oxidative stress, which maybe responsible for endogenous DNA lesions. Although it has been proposed that the CS transcriptional pattern following DNA lesions might be responsible for the cellular and clinical phenotype of patients, this pattern has not been investigated yet for stem cells. In the present work, we are investigating the transcription pattern through RNAseq in CS induced pluripotent stem cells (iPSCs) following DNA damage by oxidative stress. Preliminary tests for cell survival determination allowed the standardization of a Potassium bromate (KBrO3) concentration for DNA damage challenge experiments. Experiments were conducted on a wild type cell strain (F9048), and on a CSB mutant (GM10903, Coriell), both reprogrammed to iPS. Libraries were prepared for RNAseq with mRNA from both cell strains, extracted 24 h after KBrO3 and mock treatments. Sequencing was conducted on an Illumina NextSeq, with paired-end reads. The run yielded 637 million clusters, with an average of 52 million paired-end reads per sample. Data analysis was performed with the HISAT2-StringTie-Ballgown protocol (Tuxedo 2), against Ensembl GRCh38 genome. RSeQC was used for quality control and determination of median transcript integrity number (medTIN), and distribution of reads along each transcript to exclude library preparation bias. CS cells presented 109 differentially expressed genes, all observed exclusively on these cells following DNA damage challenge. Interestingly, only one gene (VLDLR-AS1) was identified as differentially expressed in wild type cells under the same treatment, suggesting CS cells are more sensitive to transcriptional variation after oxidative stress. An enrichment of GO terms for the regulation pathway of insulin growth factor (IGF) was found among the differentially expressed genes on CS cells, but not on wild type cells, corroborating previous findings. Furthermore, over half of the differentially expressed genes on CS are associated with the GO biological process "response to stimulus", mainly "response to stress". Five differentially expressed genes were classified as GO neuron projection regeneration (ULK1, SPP1, APOE, ADM, JUN), and possibly contribute to the severe nervous system involvement in the patients phenotype.

Funding: CAPES and FAPESP