Optimization of Dimedone Switch Method for detection of S-sulfhydrated proteins in human induced pluripotent stem cells derived from Down syndrome individuals

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The gene for cystathionine beta-synthase (CBS), the enzyme producing hydrogen sulfide (H_2S), located on chromosome 21, is present in extra copy in Down syndrome (DS). Dysregulation of H_2S signalling has been linked to DS, but the precise underlying mechanisms are yet to be fully identified. The present study established and characterized induced pluripotent stem cell (iPSC) lines reprogrammed from peripheral blood mononuclear cells (PBMC) collected from a blood sample of a paediatric DS patient (T21) and an age-matched apparently healthy donor (Eup). We observed that T21 cells present an increase in H_2S levels, which was accompanied by a 2-fold change increase in the levels of CBS protein, in comparison to the Eup lines. Also, we propose to identify the S-sulfhydration targets of H_2S in the iPSC lines. For that, using the T21 cell line we did the optimization of the assay that detects protein persulfidation described by Zivanovic et al., 2019. So, the first step involves the reaction of 4-chloro-7-nitrobenzofurazan (NBF-Cl) with all cysteines and amino groups in proteins. Next, the persulfidated cysteines were specifically labeled by Cysteine Sulfenic Acid Probe named DCP-Bio1. The NBF-Cl signal is characterized by a fluorescence signal (emission 488nm) that can be detect on SDS-PAGE gels and correspond to the total protein. The persulfidated cysteines labelled by DCP-Bio1 were detected by Streptavidin-HRP substrate. Our observations showed that 5 mM NBF-Cl was insufficient to react with all cysteines and amino groups and we determined that the optimal NBF-Cl concentration during cell lysis was 10 mM. Furthermore, we successfully verified the specific detection of persulfidated cysteines, which were found in the presence of 50 µM DCP-Bio1 when compared to the untreated sample. Overall, we established the optimal conditions of the dimedone switch method in the cellular models of DS which will allow us to detect S-sulfhydrated proteins. The obtained data will allow to better understand the biology of H_2S and its contribution to the neurodevelopment impairment in Down Syndrome patients.

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