INDUCED PLURIPOTENT STEM CELLS AS AN INNOVATIVE MODEL FOR PHARMACOLOGICAL STUDIES IN PATIENTS WITH AICARDI-GOUTIÈRES SYNDROME

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Background and aim: Aicardi-Goutières Syndrome (AGS) is an autosomal recessive disease; the most common clinical features are mental retardation, dystonia, microcephaly and chilblain lesions. It is associated with mutations in different DNA sensing genes, the most common are RNASEH2B and TREX1. AGS has features of both autoinflammatory and autoimmune disease and therapy includes the administration of corticosteroids (e.g. methylprednisolone) in combination with purine antimetabolites (azathioprine), or antimalarials (mepacrine). Induced pluripotent stem cells (iPSCs) are cells that are capable of self-renewal and can differentiate into many cell types. Being reprogrammed from somatic cells, easily retrievable from patients and maintaining the genetic characteristics of the patient and the disease, iPSCs can be used for studies on personalized therapies, screening of candidate drugs and to develop a cell therapy in regenerative medicine using patient specifics cells that reduce the rejection probability. In this study we investigated the effect induced by a panel of 6drugs, already used in therapy for AGS patients or that could be used for their immunomodulatory activity, in order to develop a model of in vitro cytotoxicity on iPSC of AGS patients.

Materials and methods: iPSCs were derived from 3AGS patients with different mutations: AGS1(TREX1mutation), AGS2(RNASEH2B mutation) and AGS7(IFIH1mutation) and the results were evaluated with respect to a control iPSC line derived from a healthy donor (iPSC-C). Total RNA was extracted using TRIZOL reagent, reverse transcribed and gene expression of 3stem cell genes (SOX2, OCT4, C-MYC) and drug target genes (HPRT1, NR3C1, MB21D1, CRBN) was analysed by real time PCR. The cytotoxicity of mercaptopurine (MP) ($9.77*10^{-10}$ M-$4.00*10^{-6}$ M), thioguanine (TG) ($9.77*10^{-10}$ M-$4.00*10^{-6}$M), mepacrine (Mepa) ($2.44*10^{-8}$M-$1.00*10^{-4}$M), dexamethasone (Dex) ($2.44*10^{-8}$M-$1.00*10^{-4}$M), lenalidomide (Len) ($2.44*10^{-8}$M-$1.00*10^{-4}$M) and thalidomide (Tal) ($2.44*10^{-8}$M-$1.00*10^{-4}$M) was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay after 72h of exposure.

Results: All 3iPSC-AGS lines expressed stemness cell genes (SOX2, OCT4, C-MYC) at the same level of the control line (iPSC-C). Genes associated with the tested drugs pharmacodynamics (HPRT1for MP and TG, NR3C1for Dex, MB21D1for Mepa and CRBN for Len and Tal) were expressed in iPSC-AGS lines, at levels comparable to iPSC-C, except for the MB21D1gene which was significantly more expressed in AGS7than iPSC-C. The 3iPSC-AGS lines and the control one (iPSC-C) were found to be sensitive to MP, TG and Mepa, with differences between AGS2and iPSC-C in the case of TG (EC50 AGS2= $3.34*10^{-7}$M and EC50 iPSC-C = $1.23*10^{-7}$M, p-value two-way Anova test = 0.015). For Mepa, in AGS7, low drug concentrations increased viability ($1.56*10^{-6}$M, $3.91*10^{-7}$M and $9.77*10^{-8}$M, p-value two-way Anova test <0.001and p-value Bonferroni's multiple comparisons test <0.05). All iPSCs considered were instead resistant to Dex, Len and Tal at the tested concentrations.

Discussion and conclusion: A different cytotoxic effect was obtained for TG which was less cytotoxic in AGS2than iPSC-C in the absence of altered HPRT1gene expression. It is conceivable that the RNASEH2B mutation induces a reduced sensitivity to TG due to its effect on DNA replication and repair. The expression of MB21D1was greater in AGS7than in the control, AGS7also showed apparent resistance to the lower concentrations of Mepa. The molecular relation between this two observations still needs to be elucidated. In conclusion, this study analysed the use of iPSC-AGS for drug-induced cytotoxicity analysis, highlighting a possible use of patient specific iPSCs in drug screening.