EVALUATION OF MOLECULAR AND CYTOGENETIC DEFECTS IN DYSKERATOSIS CONGENITA

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Dyskeratosis congenital (DC) is a rare multi-system disorder phenotipically characterized by skin pigmentation, nail dystrophy and leukoplakia. DC patients frequently develop bone marrow failure which predisposes them to cancer development. The molecular mechanism involved in tumorigenesis is not yet elucidated. N this study, a cell line of B Lymphocytes from Dyskeratosis congenita GM03650, characterized by X-linked Dyskerin1 gene (DKC1) mutation, was used to evaluate the effects of this gene mutation on chromosome instability. Considering the role of DKC1 gene, telomerase activity and telomere maintenance were taken into account. The results of the telomere length assay (Roche) show that telomeres in mutant cells are not shortened, compared to the telomeres measured in a lymphoblastoid cell line of a health individual, that we used as control. Since the TTAGGG sequences of telomere were intact, a possible involvement of protein dysfunction was considered. The analysis of gene expression highlighted an alteration in the expression of the telomeric cohesin (SA1), the centromeric cohesin (SA2) and the SMC1 gene from the SMC complex of cohesins. All these proteins are involved in the cohesion maintenance between sister chromatids to a proper replication, recombination and segregation of chromosomes. The effects of cohesin up-regulation on DNA replication and recombination have been studied in both cell lines by the Co-FISH, using a PNA telomeric probe fluorescently labelled. The microscopic analysis highlighted a statistically significant increase in the frequency of intra-strand exchanges (2.9 *vs* 0.36; DC *vs wt* cell line), while terminal exchanges were not increased. On the other side, the cytogenetic FISH analysis performed wih a centromeric probe highlighted a significant increase in tetraploidy frequency (7.5 fold in binucleate cells) in the mutant, compared to the *wt* cell line. Interestingly, tetraploidy was increased in both mononucleate and binucleate cells. The molecular mechanism of this cytogenetic defect induction in the DC cell line will be clarified by further studies.