RUBINSTEIN-TAYBI SYNDROME CELLS AS A MODEL SYSTEM TO INVESTIGATE THE ROLE OF CBP and p300 PROTEINS IN DNA REPAIR MECHANISMS

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The Rubinstein–Taybi syndrome (RSTS OMIM #180849, #613684) is a genetic disorder characterized by postnatal growth deficiency, microcephaly, intellectual disability and increased risk of tumors. Although the exact molecular etiology of RSTS is not clearly understood, it is widely accepted that RSTS is associated with mutations and chromosomal rearrangements - microdeletions and translocations- with breakpoints at 16p13.3, where the gene encoding the cAMP Response Element-Binding Protein (*CREBBP* or *CBP*) resides. About 60% of RSTS individuals carry a heterozygous mutation/deletion of CREBBP, while a small percentage (~3%) of RSTS are caused by mutations in the *EP300* gene (located at 22q13.2) encoding for p300. CREBBP and p300 are highly homologous proteins that possess lysine acetyl transferase (KAT) activity, and play a key role in transcription regulation. However, they have also distinct cellular functions and cannot always replace one another. Both proteins are involved in other aspects of DNA metabolism, including cell cycle checkpoints (by acetylating p53), and DNA repair (by acetylating factors involved in nucleotide (NER) and base excision repair (BER) mechanisms, such as FEN,1 XPG, DNA polymerase β (pol β), some DNA glycosylases (e.g. TDG, NEIL2, OGG1), as well as PARP-1, and WRN and RECQL4 helicases. In agreement with these findings, we have recently shown that depletion of both CBP and p300 by RNA interference, significantly impairs NER. Thus, deficiency in CBP and/or p300 may result in genome instability and cancerogenesis, thereby explaining, at least in part, the higher incidence of malignancies observed in RSTS patients.

To better elucidate the involvement of CBP and p300 in response to DNA damage, and in particular in DNA repair, we have analyzed lymphoblastoid cell lines derived from RSTS patients carrying monoallelic deletion, or mutations of CREBBP gene, in order to define their usefulness for these studies. Western blotting analysis has shown, as expected, a reduced expression of CBP, and in one case also of p300 protein in RSTS lymphoblasts, as compared to the lymphoblastoid cell lines from normal donors. p53 and p21 protein levels were also significantly reduced in RSTS cells, while no significant differences were observed in the protein expression of PCNA, and other related DNA repair proteins, such as XP proteins (A-G), PARP-1, XRCC1 and DNA pol β. Despite a similar proliferation rate, RSTS cells showed signs of histone H2AX phosphorylation, suggesting the presence of DNA damage. In addition, RSTS cells were more sensitive to treatment with the oxidative agent $KBrO_3$. Experiments have been performed to assess the recruitment of XRCC1 and DNA pol β to DNA damage sites, and the efficiency of DNA repair is being analysed by the Comet test. Preliminary results suggest a different and delayed kinetics in the repair of oxidative lesions in RSTS cells, requiring further investigations in order to explain the behaviour of these cell lines.