SESSIONE: *Fight Against Cancer*

AN APPROACH AGAINST CANCER: TRANSLATIONAL READTHROUGH INDUCING DRUGS (TRIDs) FOR RESTORING P53 EXPRESSION IN STOP MUTATED CELLS

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Stop mutations are gene mutations characterized by the substitution of a single nucleotide in the coding sequence of a gene, which causes the onset of a premature stop codon (PTC) within the reading frame of the mRNA, resulting in the formation of a truncated and non-functional protein. This type of mutation accounts for approximately 11% of genetic diseases, including conditions such as Cystic Fibrosis, Duchenne Muscular Dystrophy, Choroideremia, Schwachman-Diamond syndrome, and certain types of hereditary cancers involving mutations in the TP53 gene. About 10% of TP53 mutations are stop mutations [1, 2]. TP53 encodes a protein made up of 393 amino acid residues called p53, which acts mainly as a transcription factor, regulating numerous pathways such as the cell cycle arrest, DNA damage repair, apoptosis, autophagy, and metabolism when cells are under certain stress conditions. TP53 mutations create a favorable environment for tumor formation, and mutant p53 may exhibit loss of function, dominant-negative repression, or gain of oncogenic function, contributing to tumor stability and progression [2]. Today there is no therapy for the pathologies caused by this type of mutation, but an approach that has proven to be particularly effective is represented by molecules with readthrough activity (TRIDs; Translational Readthrough Inducing Drugs) which intervene on the ribosome allowing the overcoming of the PTC and the restoration of the synthesis and subsequent functionality of the protein [3]. In this work, we investigate the effects of TRID molecules with readthrough activity on the TP53 gene in tumor cells, which harbors the PTC R213X, the most common TP53 stop mutation, that generates a truncated and non-functional p53. We analyzed the restoration of p53 protein expression before and after induction of DNA damage by Western blot, its nuclear localization with fluorescence microscopy, the mRNA expression of p53, and its targets p21 and GADD45 to evaluate the functionality of the protein by Real-Time RT PCR. After 24 hours of treatment with TRIDs, we observed a partial nuclear localization of p53, an increase in mRNA expression of its targets and a restoration of protein expression after the induction of DNA damage. These results represent a promising path for developing targeted cancer therapies against stop mutations, a new approach to impede tumor proliferation, and a solid foundation for the formulation of novel personalized therapy modalities not only against cancer.

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