THE FUTURE OF CANCER THERAPY: THE GENOME EDITING ERA

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ABSTRACT FORM

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Abstracts submitted for Italian Association of Cell Cultures meeting "The future of cancer therapy: the genome editng era" must be written in English.

A CRISPR/Cas9 based approach to study the implication of HTLV regulatory proteins in the NF-κB modulation

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Human T-cell leukemia virus type 1 (HTLV-1) infects approximately 20 million people worldwide and 5% of them may develop adult T-cell leukemia (ATL), a fatal T-cell malignancy with no effective treatment currently available. The homologous HTLV-2 does not cause ATL, but is associated with milder neurologic disorders. Both viruses encode a potent viral oncoprotein, termed Tax, which deregulates several cellular pathways, including NF-κB. In addition to Tax, the HTLV-1 proviral genome encodes from the antisense strand, a basic leucin zipper factor, HBZ, which plays an essential role in the oncogenic process leading to ATL. Comparative studies of the functional activity of Tax-1 and HBZ, and the HTLV-2 homologous, Tax-2 and APH-2 (HTLV-2 antisense protein), may provide clues to explain the dissimilar pathobiology of HTLVs. Herein, we compared the effect of the viral regulatory proteins HBZ and APH-2 on Tax-modulated NF-κB cell signaling. Our data demonstrated that APH-2 suppressed, more efficiently than HBZ, the Tax-dependent NF-κB activation. By confocal microscopy, we observed that, differently from HBZ, the APH-2 protein is recruited into cytoplasmic structures where co-localized with Tax. The co-expression of APH-2 and Tax impaired the degradation of the NF-κB inhibitor IκB-α, restraining the transcriptional factor p65 into the cytoplasm. APH-2, but not HBZ, was present in complex containing the TRAF3 protein, an upstream inhibitor of the alternative NF-κB pathway. Applying the CRISPR/Cas9 technique, we generated TRAF3 knock-out cell lines. Several $TRAF3^{-/-}$ clones were selected and NF-κB promoter activity was analyzed by luciferase assays. The results showed that, in absence of induction, the NF-κB promoter is slightly activated, in the $TRAF3^{-/-}$ cell line compared to the parental cell line. The absence of TRAF3 adaptor factor did not inhibit the Tax-mediated NF-κB activation. Ongoing studies using $TRAF3^{-/-}$ clones will allow to clarify the effect of the HTLV antisense protein on the alternative NF-κB pathway activation.