

THE FUTURE OF CANCER THERAPY: THE GENOME EDITING ERA

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University Magna Græcia, Catanzaro (Italy)
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1° Level Building G

ABSTRACT FORM

Mail the form to: aicc.umg@unicz.it

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

CRISPR/Cas9 for the Study of the Interactions between Viruses and Host

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Poster

The CRISPR/Cas9 system has many applications in virology: it has been used to achieve viral DNA inactivation from latently infected cells, allowing viral eradication, or to inactivate specific proteins involved in virus-host cell interaction. Herein we applied the CRISPR/Cas9 technique to generate knock-out cell lines useful for the study of cellular determinants critical for HIV-1 infection. As a preliminary screening, the editing efficiency was evaluated by T7 endonuclease I assay, and then confirmed by western blot and flow cytometry analyses. We targeted β_2 microglobulin (β_2m), human thioesterase 8 (ACOT8) and histone deacetylase 6 (HDAC6) genes. β_2 microglobulin is required for the membrane translocation of HLA molecules where HLA-C interacts with HIV-1 Env and modulates viral infectivity (Zipeto & Beretta, Retrovirology 2012). We edited β_2m in 293T, HeLa-Lai (expressing HIV-1 Env), TZM-bl (CD4 and CCR5 expressing HeLa, highly sensitive to HIV-1 infection) and parental HeLa cells. We showed in 293T cells that HIV-1 proteins transfection did not translocate HLA-C at the cell surface in absence of β_2m . We obtained similar result in β_2m negative HeLa-Lai cells, showing that HIV-1 Env interacts with HLA-C at the plasma membrane after its surface translocation. Besides, we demonstrated that HIV-1 pseudoviruses produced in β_2m negative 293T cells were significantly less infectious than those produced in parental ones (Serena et al., Scientific Reports, 2017). ACOT8 thioesterase interacts with HIV-1 Nef protein preventing its degradation (Serena et al, Scientific Reports 2016). To better understand the role of ACOT8 in HIV-1 infectivity, we developed ACOT8 knock out 293T and TZM-bl cell lines. We observed in TZM-bl cells, susceptible to HIV-1 infection, that ACOT8 absence did not affect the infectivity. The role of ACOT8 in pseudoviruses production is being tested using 293T edited cells. HDAC6 is an important regulator of membrane dynamics involved in HIV-1 infection (Valenzuela-Fernandez et al, Molecular biology of the cell, 2005). We inactivated the HDAC6 gene in 293T cells. These cells will be used to test the HIV-1 infectivity and syncytia formation. In conclusion, the CRISPR/Cas9 system represents a new, powerful tool in basic and applied research in virology.