Scientific Report Balsano-Will

Background

Viruses are able to target and modulate host cell signaling and diverse regulatory cascades, thus leading to a effective viral propagation. In the last years, professor Will's group has demonstrated that SPOC1 (also known as PHF13), that they have previously identified as a regulator of DNA damage response and chromatin structure [1], is crucial in restricting the gene expression and progeny production of Adenovirus after viral infection [2]. To evade this antiviral cellular response, virus promotes the SPOC1 proteasomal degradation. Preliminary data collected and published by prof. Will indicate that a similar evasion mechanism could be also adopted by other viruses, including hepatitis C virus (HCV) [2]. Since the main role of SPOC1 is to repair double strand DNA breaks, it is conceivable to imagine that protein degradation induced by virus could represent a main risk for cellular transformation because may lead to an excessive DNA damages accumulation.

Objective

The project aimed to identify the role of the SPOC1 (Survival-time associated PHD Protein in Ovarian Cancer1/PHF13), a known regulator of double stranded DNA repair and of chromatin structure, on HCV infection, its replication and changes in infected cells. The study results may lead to the identification of novel markers and potential therapeutic targets for HCV infection and its related hepatocarcinogenesis.

Methods

The human hepatoma cell line Huh7.5.1 were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 mg/ml streptomycin and 2 mM L-glutamine (Lonza).

J6/JFH1 cDNA (kindly provided by C. Rice, Rockefeller University) was used to generate the HCV cell model. The chimeric J6/JFH1 virus was generated and used according to Lindenbach BD and collegues [3]. Huh7.5.1 cells were infected (MOI = 0.1) 1 day after seeding. After 3 days cells were harvested and processed for RNA and protein extractions and evaluation by immunofluorescence.

To evaluate the impact of SPOC1 on HCV viral infection, before the infection, Huh7.5.1 cells were stably transfected with a 4TO plasmid containing the sequence for SPOC1 wt expression (pcDNA4TO-SPOC1 constructs) [2], to obtain cells overexpressing SPOC1. Plasmid was kindly provided by professor Will. Transfection was performed through lipofectamine 2000 (Life

Technologies). For SPOC1 protein detection was used a primary antibody generated in rabbit by professor Will group. Viral proteins were detected by Western Blot and immunofluorescence using an antibody against NS5A viral protein (Austral Biologicals Antibodies), and RT-PCR.

Results

Based on the results previously published by prof. Will, during its period in Rome, we have performed Huh 7.5.1 cell transfection to overexpress the protein SPOC1 with the plasmids he kindly provided us. The success of the transfection was confirmed by Western Blot. The increase of the protein expression (about two times), was revealed using specific antibodies that his group have generated. SPOC1-overexpressing cells were then infected with the HCV chimeric viral strain J6/JFH1. After three days we evaluated by immunofulorescence, RT-PCR and Western Blot the amount of presence of HCV. Interestingly we appreciated a significant reduction of viral proteins and transcripts, about 2,5 times lower compared to cell not overexpressing SPOC1.

Conclusions

Thanks to the collaboration with prof. Will, that has revealed several physiological properties of the SPOC1 protein, and has generated useful and not-commercial tools to investigate it, we uncovered the great impact of SPOC1 in counteracting viral replication.

The presence of prof. Will in Rome, in particular, allowed us to shortening the optimization time of the scientific protocols needed to use his tools, and thanks to his great expertise, gave us the opportunity to put the basis for a scientific publication that could shed light on new molecular mechanisms through which HCV virus replicate inside the host cells. Today, the collaboration is going on and we are investigating the impact of SPOC1 overexpression against the HCV-related cell transformation.

References

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