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ΜΕΛΕΤΗ ΤΗΣ ΤΡΟΠΟΠΟΙΗΣΗΣ ΤΗΣ ΣΗΜΑΤΟΔΟΤΗΣΗΣ ΤΩΝ MAPKs – ERKs ΠΑΡΟΥΣΙΑ ΤΩΝ HCV ΔΟΜΙΚΩΝ ΠΡΩΤΕΪΝΩΝ

SUMMARY

An estimated 3% of the world population is infected by hepatitis C (HCV). HCV is an enveloped positive-strand RNA virus, with a genome of approximately 9.600 nucleotides, encoding for structural (core, E1, E2, p7) and non-structural (NS2, NS3, NS4A, NS4B, NS5A, NS5B) proteins. In most infected individuals, the virus evades the immune system and establishes a chronic infection that can progress to cirrhosis, and hepatocellular carcinoma. HCV proteins are involved in the development of hepatocellular carcinoma as they modulate MAPK signaling pathway. It has been proposed that capsids from a number of viruses like the Ebola virus, can produce signaling events. The HCV nucleocapsid is surrounded by a lipid layer containing glycoproteins E1 and E2. However, defferent forms of HCV particles may exist in the circulation of infected individuals and among them naked capsids have also been reported. Furthermore, novel HCV subgenomes with in-frame deletions of both envelope proteins (E1 and E2), were identified in the liver, as well as in the serum of HCV infected individuals.

Recently, Tsitoura et al, reported the generation of recombinant non-enveloped HCV core particles in the absence of other HCV proteins. More importantly, this report demonstrated that these naked capsids can be taken up by cells and induce cell-signalling phenomena. Based on the previous, we aimed in developing a strategy for the labeling of the HCV non-enveloped capsids by using the enhanced green fluorescent protein (GFP). GFP has been fused to a great number of proteins in order to study their intracellular trafficking and localization. We used the bacilovirus expression system to generate recombinant fluorescent non-enveloped capsid-like particles (fluorescent HCVne) that posses identical properties to the one already described. The chimeric proteins GFP-core191 and GFP-core 173 produced can be efficiently expressed and self assembled to form fluorescent non-enveloped

capsid-like particles that efficiently be taken up by human cells. In addition, we observed the trafficking of these fluorescent HCVne particles in live-microscopy, providing evidence of an attractive tool for viral tracking.

To understand the biological significance of nacked HCVne particles, we investigated the mechanism of entry of these particles. Recent studies have revealed a surprising variety of endocytic routes for enveloped as well as non-enveloped viral capsids including clathrin-mediated and caveolin-mediated endocytosis. Hepatitis C enveloped particles use the clathrin-mediated endocytosis, but to our knowledge no information about the entry mechanism of HCV non-enveloped particles exist. Data presented here, suggest that HCVne particles penetrate into hepatic cells via pH dependent clathrin-mediated endocytosis. During this process different MAPK pathways become activated.

Entry process starts with the attachment of HCVne particles at the cell surface which is followed by a clathrin-mediated internalization, and localization of particles to early endosomes. Internalization occurs relatively fast, with the majority of entering viral particles internalized in early endosomes between 9 to 15 minutes. During the endocytosis of the particles from the cellular surface to early endosomes the pH changes in endosomes seem to be implicated. In addition, phosphorylated proteins and particularly tyrosine phosphoproteins, as well as MAPK-p38 protein are shown to be important during this trafficking.

After entering early endosomes, HCVne particles moved along from endosomal to lysosomal compartments, through involvement of microtubules network. Data presented here shows a colocalization of HCVne particles with late endosomes at 1 hour post incubation teaching maximum colocalization with lysosomes at 4 hours. During early endosome-lysosome transport, defferent phosphorylated proteins are showed to be essential, such proteins are the serine/threonine and tyrosine phoshoproteins, as well as phosphor-ERK1/2 and possibly phosho-p38.

Previously described data showed specific ERK translocation from the nucleus to the cytoplasm after HCVne particles uptake, suggesting a possible activation of ERK1/2 pathway. In this report we described that ERK1/2 present a maximum activation 30 min after internalization that disappears when cells are treated with

specific MEK1/2 inhibitors and is shown to be endocytosis-dependent. In addition, HCVne particles endocytosis produces a sustained ERK1/2 accumulation. Magnitude and duration of ERK1/2 phorphorylation is important for the immediate early genes (IEG) promoter activation as well as for the stability of the produced proteins. In this study we show an increased activation of IEG *c-fos* and *erg-1*, totally or partially attributed to the ERK1/2 pathway. This constitutes an interesting observation as *c-fos* and *erg-1* has been found to be overexpressed with high frequency in aggressive and invasive hepatocarcinomas making these genes an important target for putative therapy.

We have also investigated another important signaling molecule of the MAPK pathway, the ERK5. ERK5, also known as BMK1, is a MAPK that presents approximately 80% of similarities with ERK1/2. This kinase contains the typical ERK phosphorylation motif (TEY) and a unique C-terminal region that is believed to be responsible for its distinct biological activities. ERK5 activity is increased in response to growth factors, oxidative stress and hyperosmolarity, via a direct phosphorylation by MEK5. It is important to note that ERK5 has been implicated in different types of cancers and angiogenesis. We were able to show that ERK5 can be activated by HCVne particles, via MEK5 protein, but not from endogenously expressed core or NS5A proteins. In addition a well known downstream target of this pathway, *mef-2* also seems to be affected.

Although our knowledge about HCV mechanism of entry, replication and infectivity are progressing, a great number of aspects are still left unanswered. The evaluation of the impact of cellular environmental modifications produced by signaling events similar to those produced by the endocytotic properties of naturally occurring HCVne could potentially be of major importance for HCV five cycle and severity of the disease.