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Molecular basis of virus-cell interaction and cell response

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Study of molecular aspects of virus-host cell interaction has led to the discovery of vital cellular processes. The notion that viruses have developed tools to effectively recruit the required cellular machinery for their survival and propagation is the basic rationale behind our working hypothesis. We utilize the hepatitis B virus (HBV) as a model, because it became evident that HBV is the prototype of a new virus family with a novel life cycle. Additionally, this virus is associated with wide range of clinical manifestations, including liver cancer (HCC).

The earliest phases in infection

Attachment: HBV does not infect cultured cells therefore our knowledge on the mechanism of the early stages of virus-cell interaction is rather poor. We have developed a protocol to pharmacologically sensitize cells to HBV infection. We also developed a novel tool to measure virus-cell attachment by light microscopy at a single cell resolution (Fig. 1). We identified the QLDPAF sequence within the HBV surface protein (preS1) as the receptor binding viral domain epitope and a secondary attachment site that recognizes a distinct receptor on the cell membrane. Our results provide evidence for multivalent HBV attachment with synergistic interplay. This cell system was utilized to show that the HBV regulatory protein named pX plays an essential role in infection. Up to this point the role of pX in HBV life cycle was not known.

Transcription: Recently, we revealed a novel and much longer HBV transcript (leRNA). leRNA is the first transcript to be expressed and therefore provides the first evidence for HBV enhancer-1 to be activated immediately after transfection. leRNA might potentially encode a novel fused X-Core 40kDa protein, previously detected in HCCs. Unexpectedly, we found that the majority of the leRNA molecules retain in the nucleus, suggesting that HBV programs an RNA nuclear export mechanism yet to be identified.

Towards production of a synthetic virus

For gene therapy, tools need to be developed to deliver genetic information to the target cells in safe and efficient ways. Viruses are important but unsafe delivery vectors. This problem can be minimized by generation of 'synthetic viruses' namely the different virus components are separately synthesized in heterologous systems and then are being reassembled to the desired final product. To this end we have generated the different constituents of HBV and confirmed their functionality (Fig. 1). An interesting outcome was that the recombinant HBV Core particles freely enter the cells (core transduction).

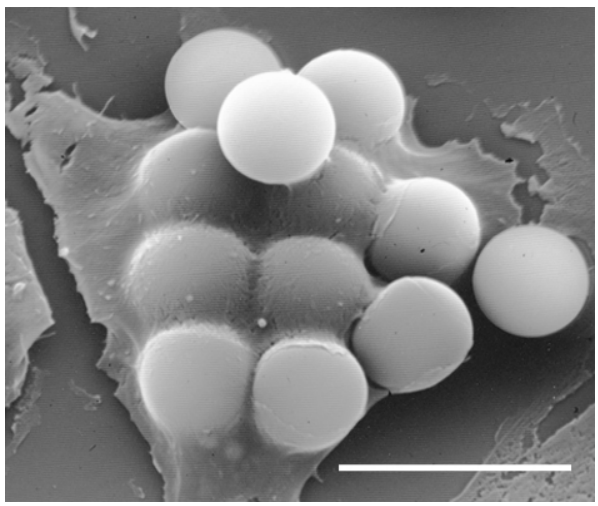

Fig. 1 *Interaction and internalization of giant synthetic viral particles with susceptible cells. Viral empty particles were conjugated to synthetic beads and applied on the cells. Note that attachment is accompanied by endocytosis.*

HBV and HCC

Epidemiological and other studies have established a relationship between the HBV carrier-state and development of HCC. How HBV induces malignancy is an open question. We have evidence for the role of the virus enhancer and the regulatory protein pX in this process. We hypothesized that the HBV enhancer might have an intrinsic oncogenic function and therefore it might be considered as onco-DNA. Interestingly, in HBV associated HCC p53 is mostly wt. This led to the possibility of functional inactivation of p53 by pX. A number of studies reported by us and others support this possibility. Recently, we have isolated a novel gene XAP-8, whose product interacts with pX. XAP-8 is a nuclear protein that plays a role in chromatin

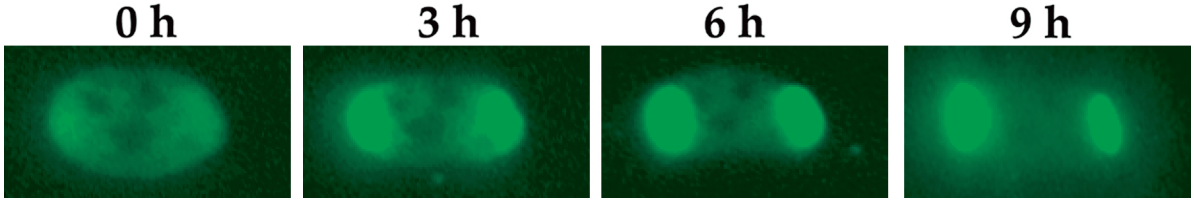

Fig. 2 *Mobility of GFP-p73 towards the nuclear poles.*

remodeling. Interestingly, XAP-8 gene was amplified in a number of primary tumors, suggesting XAP-8 is a novel oncogene candidate.

p73-cAbl and novel apoptotic pathways

The HBV enhancer interacts with c-Abl. c-Abl, unlike its transforming variants that induce leukemia, is a poor tyrosine-kinase, but it is activated by DNA-damaging agents and γ-irradiation to induce apoptosis. Previously, we have found that p73 involved in this process as well. c-Abl and p73 are physically associated in cellular extracts through the c-Abl- SH3 domain and the p73 PxxP motif. Studies revealed that p73 is a putative c-Abl substrate both *in vivo* and *in vitro*. Interestingly, phosphorylated p73 translocates to the nuclear matrix and even undergoes exonucleosis via formation of micro-nuclei. Furthermore, p73 displays unique nuclear mobility and accumulates at the nuclear poles (Fig. 2). The significance of this nuclear molecular dynamics that is regulated by protein and possibly DNA redistribution for HBV life cycle is currently under study.

A novel pathway in p53 degradation

Recently (in collaboration with J. Lotem and L. Sachs) we have found a novel pathway that regulates p53 stability that involves an enzyme NADH quinone oxidoreductase 1 (NQO1). Inhibition of NQO1 by dicoumarol caused degradation of both endogenous and γ-irradiation-induced p53. NQO1 inhibition also induced p53 degradation and blocked p53-mediated apoptosis in γ-irradiated normal thymocytes and in M1 cells. Interestingly, dicoumarol induced degradation of not only wild-type p53 but also of mutant p53, hinting at an Mdm2 independent p53 degradation mechanism. Preliminary results are in support of this possibility. These results raise the possibility of combining dicoumarol with cytotoxic agent in therapy against cancer cells that express high levels of gain of function p53 mutants.

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