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Gene expression and signaling; the viral and cellular models

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A Novel Mechanism of Protein Degradation

Protein degradation is a very basic process in every living cell. A large fraction of onco- and tumor suppressor proteins are regulated at the level of their stability. Therefore investigation of the mechanisms of proteasomal protein degradation has attracted much attention. For example p53 level is controlled by the rate of its proteasomal degradation, a process regulated by the Mdm2-ubiquitin degradation pathway. We in collaboration with L Sacks' group reported on a novel pathway regulating p53 stability. This pathway is regulated by NAD(P)H: quinone oxidoreductase 1 (NQO1). Pharmacological and genetic inhibition of NQO1 induces p53 proteasomal degradation and inhibits p53-mediated apoptosis. p53 binds NQO1 and dicoumarol a competitive

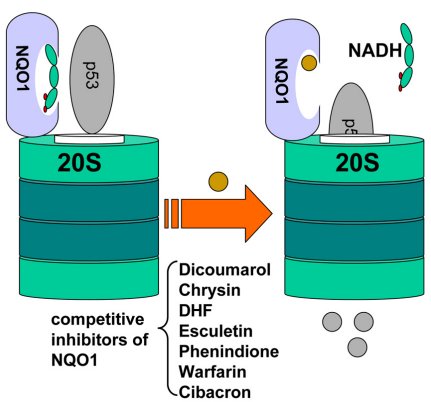

Fig.1 *A schematic model for the process of p53 degradation by 20S proteasome and its regulation by NQO1. NQO1 requires NADH for its activity. When NADH is removed the associated proteins undergo degradation. The various competitive NQO1 inhibitors used by us are listed.*

NQO1 inhibitor dissociates the complex and destabilizes p53. Under this condition degradation of p53 is regulated by a mechanism that is independent of both Mdm2 and ubiquitin. Interestingly, the hot-spot p53 mutants often expressed in tumors are partially resistant to this pathway. A limited number of other short-lived proteins such as p73 and ODC are regulated by NQO1 as well. This process was recapitulated *in vitro* using purified 20S particles. Together with C Kahana's lab found that NQO1 is intimately associated with the 20S proteasome. These findings suggest a role for 20S particles in the process of protein degradation in animal cells that is regulated by NQO1 and possibly by additional proteins yet to be identified (Fig 1). Epidemiological data indicate that this pathway may play a role in oncogenesis.

DNA Damage Signaling

We study the molecular basis of cell response to DNA damage, in particular double strand break (DSB). We have characterized a novel signaling-pathway that is initiated by DSB and triggers activation of the non-receptor tyrosine kinase c-Abl. This kinase in turn tyrosine phosphorylates the intimately associated p73, a member of p53 tumor suppressor family. This process often elicits apoptosis unless the damage has been repaired or the DNA fragments are excluded. The latter process generates micronuclei (MNi). We found that c-Abl regulates MNi formation. The emerged model is that upon irradiation the DNA repair machinery is activated to repair the DSB. In parallel, but with much slower kinetic c-Abl is activated. c-Abl inactivates the repair machinery and induces MNi to exclude the unrepaired DNA fragments. So that a low number of breaks can be repaired before c-Abl becomes activated. Mild damage is partially repaired and the unrepaired extra DNA fragments are excluded out of the nucleus by micronucleation. A sever damage that failed to be repaired and to form MNi gives rise to apoptosis (Fig 2). This model

defines a new phase in cell life cycle between survival and apoptosis whereby cells make the last effort to survive.

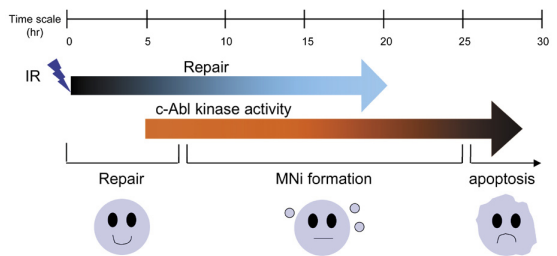

Fig.2 *Scheme of time kinetics of cell response to DNA damage by IR. C-Abl activation is slower and inhibits the repair. The color intensity defines the level of activation. For explanation see the text.*

The Molecular Mechanisms of Virus Host-Cell Interaction

Viruses in order to propagate have to invade cells and to occupy the desired cellular machinery. How, a given virus with a very limited number of genes can do so is a very basic question but the answers await understanding of the virus genes from one hand and the cellular machineries from the other hand. Hepatitis B virus (HBV) is a common infectious agent worldwide, hence provides an excellent model. HBV is a liver specific virus. Our study revealed that this tropism is determined at the level of both receptor and post-receptor levels, and identified the molecular basis of these processes. The mechanism of post-receptor tropism is regulated at the level of transcription. HBV enhancers and promoters are activated by a number of transcription factors that are mainly express in liver cells such as HNF3 and nuclear receptors (i.e. HNF4α, RXRα and PPARα). We have recently found that the HBV transcripts are categorized into early and late groups based on their timing of expression. Each regulated by a separate enhancer. However the activity of the late enhancer depends on the presence of functional early enhancer. These findings improve our understanding of HBV transcription program on the genome-wide level.

HBV encodes a regulatory protein named pX, having transcription coactivator function. We found that pX exploits the cellular transcription machinery at all possible levels, namely chromatin, enhancer and the basal promoter binding proteins. A number of cellular proteins were identified to interact with pX. An interesting new candidate that we named HBXAP was recently identified by us to be a novel chromatin remodeling protein. Given the fact that pX regarded as the HBV oncogene we looked for the possible involvement of HBXAP in cancer. Preliminary data indicate that the HBXAP gene is amplified in a number of human tumors. These studies are under progress with two major aims; to decipher the function of pX in transcription and in oncogenesis.

Selected Publications

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