623 POSTER

Origin of apoptosis and development of main proapoptotic and antiapoptotic signal transduction pathways

V.A. Halytskiy. *Institute of Gerontology of AMS Ukraine, Immunology and Pathophysiology Department, Kiev, Ukraine*

Mitochondria, as is known, are essential for Bcl-2/p53-mediated apoptosis of multicellular organisms cells, that is no coincidence. In our opinion, apoptosis is a reflection of symbiotic interrelations between nuclear cells and microorganisms afterwards transformed into mitochondria; it is an evolutionary altered reaction of endosymbionts to the influence of environment factors capable to damage eukaryotic host cell. Initial biological sense of this reaction consisted in ensuring most quick release of mitochondria predecessors from symbiosis, provided the probability for irreversible injury and following death of host cell was very high, thus escaping endosymbionts death. Mitochondria predecessors could establish subsequently new symbiosis with other nuclear cells, and so on. Predecessors of some caspases as well as proteins, including p53, which are host cell injury sensors (injury presence is detected through activation of components of cellular DNA-reparation systems – proteinkinases DNA-PK, ATM, ATR) and proteins, including Bax, which open mitochondrial membrane pores, had been initially encoded by endosymbiotic microorganisms genome and functioned in the interests of endosymbionts. (Subsequently, genes of these proteins were translocated into nucleus and expressed since then by cell). But, having been directed to host cell destruction, the above-mentioned reaction of endosymbionts was extremely unfavourable for host cells, as it deprived them of a possibility to continue struggling for existence in each challenging situation. In response to this menace, eukaryotic cells synthesized various proteins, which can suppress activity of the mechanisms responsible for host cell destruction, first of all, by preventing mitochondrial membrane pores formation and cytochrome C or factor AIF passage to cell cytoplasm. Thus, antiapoptotic proteins can have a cellular origin. It is also possible that endosymbionts, in their turn, endeavoured to synthesize proteins preventing cellular antiapoptotic agents activity; these proteins were predecessors of such proapoptotic factors as Bad. Bcl-2/p53-dependent mechanism is, in our opinion, the most ancient apoptotic pathway; it was formed basically in time of establishment of symbiosis between nuclear cells and mitochondria predecessors in Proterozoic aeon middle, approximately 1.8 billion years ago. Other apoptotic pathways were formed afterwards. They did not require mitochondria involvement, that is very significant.

624 POSTER

DNA damage-inducible interaction of a novel protein with p53

S.L. Wang, T.H. Lao. *National Defense Medical Center, Biochemistry, Taipei, Taiwan*

Mutations in the p53 tumor-suppressor gene occur in more than 50% of human cancers of diverse types. In addition, 70% of families with Li-Fraumeni syndrome have a germline mutation in p53, predisposing these individuals to multiple forms of cancer. Similarly, a mouse model with homozygous deletion of p53 shows early onset of multiple tumor types. These studies emphasize the importance of p53 function in tumor development. p53 has a number of biological activities including cell-cycle arrest, apoptosis and DNA repair. However, the exact mechanism by which p53 suppresses tumor formation still remains elusive. A key aspect to understanding p53 function is the identification and analysis of proteins that interact with it.

We have employed the Sos Recruitment System (SRS), a modified cytoplasmic yeast two-hybrid, to screen p53 interacting proteins. Using the SRS library screen, we have identified a novel p53-interacting protein 1 (Pip1). Pip1 is a specific p53-interacting protein in the SRS. The interaction of p53 and Pip1 was further confirmed by *in vitro*, *in vivo* binding assays. After screening a genomic and three cDNA libraries, a full-length Pip1 cDNA was obtained. The ORF of full-length-Pip1 cDNA encodes a protein of 428 amino acids with calculated molecular weight of 46 kDa and the results of current database search indicated that the Pip1 is an unidentified protein, which contains a conserved ring-finger domain that is present in a diverse family of regulatory proteins involved in different aspects of cellular function. In the *in vivo* binding study, the interaction of p53 and Pip1 can only be detected in the presence of ionizing radiation, suggesting that this interaction might be involved in DNA-damage-induced p53-signalling pathway. The cellular localization of Pip1 is affected by p53 in transiently transfected cells. In the absence or low level of p53, Pip1 is exclusively localized in cytosol, whereas Pip1 is mainly observed and colocalized with p53 in nucleus when nuclear p53 was coexpressed with Pip1. This observation not only provides another evidence of the interaction of these two proteins but also renders us some clues for the function of Pip1 on p53. On the other hand, we found that Pip1 downregulates the transactivation activity of p53 on both p21 and mdm2 promoters. More importantly, depending on the cellular context, Pip1 can suppress p53-induced apoptosis and attenuate the G2/M checkpoint initiated by p53, whereas, as a control in the absence of p53, Pip1 has no effect on cell cycle profiles.

Taken together, our preliminary results of both binding and functional studies strongly suggest that Pip1 might function as a negative regulator, as mdm2 does, in DNA-damage-induced p53-signaling pathway.

625 POSTER

A naturally occurring constitutively active variant of the epidermal growth factor receptor increases cell motility

M.W. Pedersen[1], V. Tkach[2], N. Pedersen[3], V. Berezin[4], H.S. Poulsen[5]. *[1] Copenhagen University Hospital, Department of Radiation Biology, Copenhagen, Denmark; [2] Intitute of Molecular Pathology, Protein Laboratory, Copenhagen, Denmark; [3] Copenhagen University Hospital, Department of Radiation Biology, Copenhagen, Denmark; [4] Intitute of Molecular Pathology, Protein Laboratory, Copenhagen, Denmark; [5] Copenhagen University Hospital, Department of Radiation Biology, Copenhagen, Denmark*

Tumour cell motility is one of the rate-limiting steps of invasion, which defines progression toward a more malignant phenotype. Elevated expression of epidermal growth factor receptor (EGFR) in many cancers is associated with progression of superficial to invasive forms of the disease. The naturally occurring type III mutant epidermal growth factor receptor (EGFRvIII) is a tumor-specific, ligand-independent, constitutively active variant of the epidermal growth factor receptor. EGFRvIII is expressed frequently by a number of human solid tumours including those of the lung, breast, prostate, brain, and ovary.

The present study was designed to investigate the effect of EGFRvIII expression on cell motility and compare it to that of ligand activated EGFR using transfected fibroblasts. We show here using time-lapse video recording that expression of EGFRvIII greatly enhance the motility of fibroblasts independently of ligand stimulation. Expression of EGFRvIII in addition caused a marked increase in the number of cellular protrusions (lamellipodia) and a reduction in the number of stress fibers and focal adhesions. The EGFR tyrosine kinase inhibitor, AG1478 and the MEK inhibitor, U0126, blocked these cellular effects of EGFRvIII.

Two cell lines expressing different levels of EGFR were used for comparison. The low expressing cell line responded to EGF treatment by increasing motility in a manner very similar to the motility induced by EGFRvIII. In contrast, the high expressing cell line responded to EGF by detachment from the extracellular matrix and decreased motility. Cellular detachment was correlated to a high phosphorylation of PLC-γ, whereas increased motility was correlated to a high level of ERK phosphorylation. Overall these results indicate that tumor associated EGFR mutations might be critical for tumor cell motility, invasion and thus progression of disease.

626 POSTER

Raltitrexed up-regulate dihydropyrimidine dehydrogenase activity, resulting in antagonism of anti-tumor effect in its combination with 5-fluorouracil

Y. Ogata[1], Y. Nozoe[1], Y. Araki[1], T. Sasatomi[1], M. Kanazawa[1], K. Matono[1], S. Torigoe[1], Y. Sato[1], H. Fukumori[2], K. Shirouzu[1]. *[1] Kurume University, Department of Surgery, Kurume City, Japan; [2] Taiho Pharmacetical Co. Lit., Cancer Research Laboratory, Saitama City, Japan*

We have studied the influence of raltitrexed, a specific thymidylate synthase (TS) inhibitor on dihydropyrimidine dehydrogenase (DPD) activity in cultured cancer cells and transplanted tumors of nude mice that highly produce DPD. Besides, we have investigated the combination effect of raltitrexed and 5-fluorouracil (5-FU) on their *in vitro* anti-tumor effects and their relations to DPD activity and DPD mRNA level were increased in HuTu-80 small intestine carcinoma cells and its transplanted tumors. On the other hand, raltitrexed had no influence on DPD activity in MIAPaCa2 pancreatic carcinoma cells. On the single or combination treatment of raltitrexed and 5-FU, the respective effect on the growth of tumor cells was studied and the results showed that in MIAPaCa2, the combination index (CI) was 0.65, showing a synagistic effect but in HuTu-80, it was 1.62 and showing an antagonistic effect. We concluded that raltitrexed might up-regulate DPD activity in tumor cells, resulting in antagonism when it is combined with 5-FU.