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Direct interaction of BRCA1 with the anticancer drug cisplatin: repair-mediated transcriptional activation of BRCT

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Cisplatin is among the most important drug introduced during the past three decades for the treatment of ovarian cancer. However, treatment-associated risks for second cancer have become the most serious experience by long-term survivors of ovarian cancer. To address potential molecular mechanisms of genetic susceptibility to the drug, we designed, utilized and investigated the functional consequences of the 3/-terminal region of the BRCA1 gene after in vitro platination with cisplatin. The cellular proficiency in repairing the drug-treated genomic DNA was evaluated specifically in the 3,426-bp fragment of the BRCA1 exon 11 of the MCF-7 cells. A decrease in the repair activity of the drug-treated plasmid DNA, using the host cell reactivation assay, was a consequence of increased platination levels. The GAL4-fused BRCT slightly enhanced the rate of the transcriptional activity of the reporter gene in the absence of GAL4 binding sites. A decrease in the transcriptional transactivation of the BRCT, when tested with the GAL4-fused BRCT transcriptional activation system, appeared to be associated with the reduction of the host cell reactivation as well as the increased interstrand crosslinks of the cisplatin-BRCA1 adducts. The present data provide clues and a proposed mechanism by which BRCA1 may be susceptible to cisplatin through repair-mediated transcriptional activation of the BRCT.

C-20

Differential role of Akt1 and Akt2 in cellular response to DNA damage

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Protein kinase B (PKB)/Akt has been implicated in tumorigenesis through promotes cell proliferation and inhibits apoptosis. Three isoforms of Akt family, termed Akt1/PKBα, Akt2/PKBβ and Akt3/PKBγ, are encoded by three separated genes with more than 86% sequence homology and share similar protein structure. Although recent studies reported that Akt/PKB isoform proteins play unique role in vivo, the isotype-specific role of Akt family protein in DNA damage response has not been addressed. In this study, the isotype-specific function of Akt in cellular survival upon DNA damage was examined using previously generated isogenic wild type, Akt1-/- and Akt2-/- mouse embryonic fibroblasts (MEFs). Both Akt1-/- and Akt2-/- MEFs showed similar increase of sensitivity to γ-irradiation (IR) compared to the wild type MEFs. However, Akt2-/- MEFs exhibited marked hypersensitivity to UV irradiation than wild type and Akt1-/- MEFs. Akt2-/- mouse aortic endothelial cells (AECs) also showed similar hypersensitivity to UV irradiation compared to the wild type and Akt1-/- MEFs. The sharp increase of the sub-G1 phase, the cleavage of pro-caspase-3 and PARP, and induction of DNA ladder formation upon UV irradiation indicate that UV-induced cell death of Akt2-/- MEFs due to apoptosis. Finally, it has been shown that the ectopic overexpression of Akt2 increased the resistance to UV-induced cell death. Taken together, these results suggest that Akt isoforms play different role in cellular response to DNA damage and Akt2 is important to cellular survival in response to UV irradiation.

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ATM/ATR kinases signaling pathways mediate DNA damage-induced activation of endothelial nitric oxide synthase.

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Recently it has been reported that nitric oxide (NO) regulated expression of cell cycle-related genes and caused directly intracellular DNA damage. However, the effect of DNA damage on the activity of endothelial nitric oxide synthase (eNOS) is not elucidated. We explored whether DNA damage altered eNOS activation, and also we investigated its underlying molecular mechanism. Ultra violet (UV) irradiation was treated as DNA damaging agent. UV irradiation acutely increased NO production in bovine aortic endothelial cells (BAEC). This increase was accompanied by increased eNOS phosphorylation at serine 1179 (eNOS-Ser1179) without an alteration in eNOS-Thr497 or eNOS-Ser116 phosphorylation. No alteration in amount of eNOS expression was found. Treatment with caffeine, inhibitor of ATM/ATR kinases, inhibited phosphorylation of eNOS-Ser 1179 in the UV-irradiated BAEC. Furthermore, treatment with small interference RNA (siRNA) of ATR completely attenuated UV irradiation-stimulated eNOS-Ser1179 phosphorylation while siRNA of ATM partially reduced its phosphorylation, suggesting an involvement of ATM/ATR kinases. Other DNA damaging agents, hydroxyurea and aphidicolin, also showed the same effects on the involvement of ATM/ATR kinases in eNOS phosphorylation. In conclusion, our results indicate that DNA damage activates eNOS through the ATM/ATR kinases-mediated eNOS-Ser1179 phosphorylation.

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Apolipoprotein AI gene polymorphisms and their association with lipid levels in Iranian hyperlipidemic Patients

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Several studies have been demonstrated the relationship between polymorphisms in the ApoAI gene on chromosome 11q23 and hyperlipidemia. Apolipoprotein AI (apoAI) is a major protein that found in high density lipoproteins (HDL).This study was conducted to elucidate the association between polymorphisms apoAI gene with lipids concentration in Iranian hyperlipidemic subjects.The prevalence of the XmnI (C-2500T), MspI (G-75A) and MspI (+83) polymorphisms of the apoA1 gene was determined by restriction fragment length polymorphism analysis among 76 unrelated patients with primary hyperlipidemia and among 75 normolipidemic subjects.The rare allele frequencies of the XmnI and MspI (+83 bp) polymorphic sites in the patients were significantly higher than the control group ($p<0.05$). Genotypes frequency of MspI (G-75A) polymorphism was not significantly different between two groups. In the hyperlipidemic group, the G allele of MspI (-75) polymorphic site was associated with an increased total cholesterol level ($p=0.015$). The rare allele of MspI (+83bp) polymorphic site was associated with decreased HDL-c and apoAI concentrations in the hyperlipidemic group ($p<0.01$).Various genotypes of XmnI had no significant effect on lipids or apo AI levels in two groups. The results suggest that XmnI and MspI (+83bp) polymorphisms are associated with hyperlipidemia in Iranian hyperlipidemic patients.

C-23

AIMP3 Haploinsufficiency inhibit p53 activation by oncogene including Ras and c-myc, result to Genomic instability

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Generally, oncogenic signals try to lead cells to transformation. But rather, by various tumor suressing mechanism, cell could be guided to other state such as death and senescence. p53 is representative tumor suppressor. And AIMP3 up-regulate p53 in response to DNA damage. Here we suggest AIM3 link oncognic signals such as Ras and c-myc to p53 activation. When expression of AIMP3 was insufficient, various oncogenic signal could not induce p53, increasing the susceptibility of transformation. And the transformed AIMP3+/_ MEFs showed chromosomal abnormality in dividing stages. Thus, AIMP3 plays important roles in activating p53 via several oncogenic stresses and regulating the stability of chromosomes.

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Accumulation of actin and exportin 6 in nuclei in the Doxorubicin-treated human diploid fibroblast

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Actin is a highly conserved, ubiquitous cytoskeletal protein, which contains two leucine-rich nuclear export signal (NES) sequences in the middle of the molecule. It has been known that actin is exclusively kept in the cytoplasm by exportin 1-driven nuclear export. However, we recently reported that actin was accumulated into the nucleus during replicative senescence of human diploid fibroblast (HDF) cells. Besides, nuclear accumulation of actin in old HDF cells. Doxorubincin (DOX), a well-known DNA damaging reagent, also induces nuclear accumulation of actin in HDF cells, but the molecular mechanisms linking DNA damage to nucleus accumulation of actin are still unknown. In this study, we elucidated the possible involvement of Exp6 in the regulation of nuclear actin accumulation. Doxorubicin induces actin accumulation in nuclei with concomitant induction of nuclear accumulation of Exp6. However, mRNA and protein expressions of Exp6 remained unaltered after DOX treatment. Employing Exp6 shRNA construct in DOX-treated HDF cells, we are investigating the relevance of deregulated localization of Exp6 to the DOX-induced accumulation of actin into cucleus.

Poster Session