15

Cell cycle control/cell trafficking

15-01/P

A NOVEL TUMOR SUPPRESSOR ROLE FOR RNASE L: REGULATION OF THE AU-RICH RNA BINDING PROTEIN, HUR

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Ribonuclease L (RNAse L) has been designated as candidate tumor suppressor gene and has a pro-apoptotic role. However, the mechanism on cellular division has not been yet demonstrated. We have found that expression of the RNA binding protein, HuR, which plays a role in cell cycle control and tumorigenesis, was downregulated in an RNAse L-overexpressing HeLa line. RNase L also downregulated known HuR mRNA targets, cyclin D1 and uPA, whereas these mRNAs were upregulated in HuR-overexpressing HeLa cells. Embryonic fibroblast lines derived from *RNASEL*-knock out mice had higher levels of HuR than the wild type cells. We found that the effect of RNAse L was temporal. It occurred at stages when the cells are confluent and asynchronized or in late G_1 and (G_1/S) arrested synchronized cells. In contrast, the effect of RNAse L was not seen with sub-confluent cells. Both RNAse L and HuR co-localized either in the cytoplasm or in the nucleus where RNAse L was active or inactive on HuR mRNA, respectively. This phenomenon was observed with both normal and transformed cells. We compared the effect of wild type RNAse L with that of the missense variant of RNAse L, R462Q, associated with increased risk of familial prostate cancer, and other mutants, in transient transfection assays. We found that the mutants had reduced capacity to downmodulate HuR. In addition, HuR mRNA was relatively stable in RNAse $L^{-/-}$ fibroblasts when compared to wild-type cells. Further confirmation of RNase L downregulation of HuR mRNA was obtained using GFP reporter mRNA fused with the 3′UTR of mature- and pre-mRNA of HuR and of control stable mRNAs. Though RNAse L reduced control reporter activity, it was selectively more potent towards the HuR 3′UTR. The data suggest that RNAse L can regulate cell cycle events through modulation of HuR in subcellular compartment-dependent manner.

15-02/P

INTERFERON-BETA-INDUCED S-PHASE LENGHTENING CORRELATES WITH P53 STATUS IN HUMAN PAPILLOMA VIRUS-POSITIVE CARCINOMA CELLS

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Interferon (IFN)-β induces a significant antiproliferative action in cervical carcinoma cells expressing E6 and E7 oncoproteins from high risk genotypes of Human Papilloma Virus (HPV). The knowledge of the signalling by which IFN can counteract the activity of the oncogenic viral proteins E6 and E7 appears of primary importance for new IFN-based therapeutic strategies to circumvent cancer disease progression or improve clinical outcome. In SiHa and ME-180 cells, where p53 function is inhibited by HPV E6 oncoprotein, we observed that IFN-β induces S-phase slowing which promotes apoptotic cell death. By using RNA interference to inhibit the HPV E6 oncoprotein expression we obtained selective silencing of E6 and a consequent accumulation of p53 which was accompanied by transactivation of the p53 target genes. Interestingly, in asyncronous and synchronized E6 silenced cells, IFN-β was unable to inhibit cell proliferation *via* cell cycle S-phase slowing, suggesting that IFN-β effect on DNA replication is related to p53 inhibition due to E6 oncoprotein. Actually, E6 silencing experiments performed in SiHa cell clones expressing a dominant negative p53 or p53 siRNA, demonstrated that the S-phase slowing by IFN-β is linked to the impairment of p53 function due to E6 oncoprotein expression. The correlation between the IFN-induced S-phase alteration and p53 status prompts us to deeply investigate the mechanisms of this alteration and the cellular context leading IFN to take advantage of peculiar biological and molecular features of neoplastic cells.

15-03/P

EXOCYST REGULATION OF TUMOUR NECROSIS FACTOR SECRETION IN MACROPHAGES

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The exocyst complex is a group of eight interacting membrane and soluble proteins that function to target and dock secretory vesicles during intracellular protein trafficking. We have previously mapped the secretory pathway for tumour necrosis factor (TNF) in macrophages, showing that TNF is transported to the cell surface for secretion *via* the recycling endosome (RE). Here we investigated the expression and function of the exocyst complex in TNF secretion by macrophages. Multiple exocyst subunits are expressed in LPS-activated mouse macrophages; some subunits are colocalized on the RE along with transferrin and exocytic TNF. One subunit, Sec15, was GFP-tagged and expressed in macrophages for imaging in live and fixed cells. Overexpression of GFP-Sec15 perturbed TNF secretion assayed by ELISA and by cell surface staining. This resulted in accumulation of TNF precursors in the RE or tubular extensions thereof. Knockdown of Sec15 protein also perturbed TNF trafficking and secretion. Our results demonstrate that the exocyst complex functions to regulate TNF trafficking through the RE for secretion and that it does so in conjunction with Rab11 and selected membrane fusion proteins (SNAREs). The exocyst complex is thus identified as a key regulator of TNF secretion and as a potential anti-inflammatory target.