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Gene 33/Mig6 Regulates Apoptosis and the DNA Damage Response through Independent Mechanisms

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Abstract
#5524

Gene 33/Mig6 Regulates Apoptosis and the DNA Damage Response through Independent Mechanisms

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ABSTRACT

Gene 33 (Mig6, ERRFI1) is an inducible adaptor/scaffold protein whose expression can be induced by both stress and mitogenic signals. It contains multiple domains for protein-protein interaction and is involved in a broad spectrum of cellular functions. Gene 33 promotes apoptosis in a cell type-dependent manner. A recent study has linked Gene 33 to the DNA damage response (DDR) induced by hexavalent chromium [Cr(VI)]. Here we show that Gene 33 induces apoptosis via both c-Abl/p73 and EGFR/AKT-dependent pathways in lung epithelial and lung carcinoma cells. Ectopic expression of Gene 33 also triggers DDR in an ATM-dependent fashion and through pathways with or without association with apoptosis. We observed significant presence of Gene 33 in the nucleus and chromatin. We show that the nuclear localization of Gene 33 is dependent on its 14-3-3 binding domain. We find that the chromatin localization of Gene 33 is, at least in part, dependent on its EBD motif. Our data also show that Gene 33 may regulate chromatin targeting of c-Abl and EGFR. Moreover, we observed strong association of Gene 33 with histone H2AX and that Gene 33 promotes interaction between ATM and histone H2AX without triggering DNA damage. Our study reveals novel nuclear functions of Gene 33, which mediate DDR and apoptosis through independent mechanisms. Given our previous finding that Gene 33 depletion promotes Cr(VI)-induced DNA damage, our data suggest that Gene 33 may foster DNA repair by activating DDR.

BACKGROUND

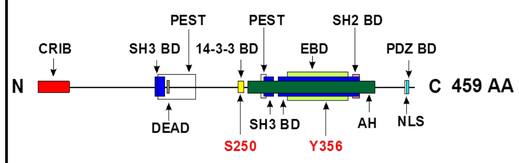


Figure 1. Linear structure of Gene 33.

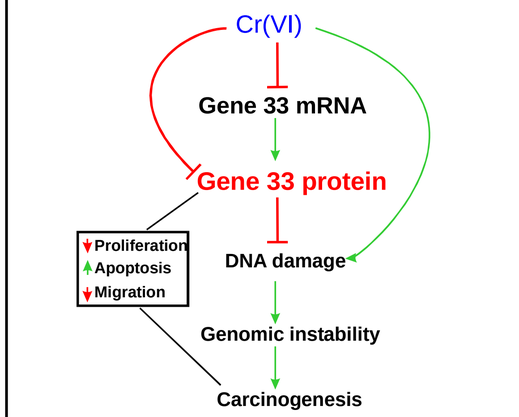


Figure 2. A potential role of Gene 33 in Cr(VI)-induced lung carcinogenesis. Cr(VI) reduces the levels of Gene 33 mRNA and Protein, which inhibits Cr(VI)-induced DNA damage that lead to genomic instability, cell transformation and carcinogenesis.

RESULTS

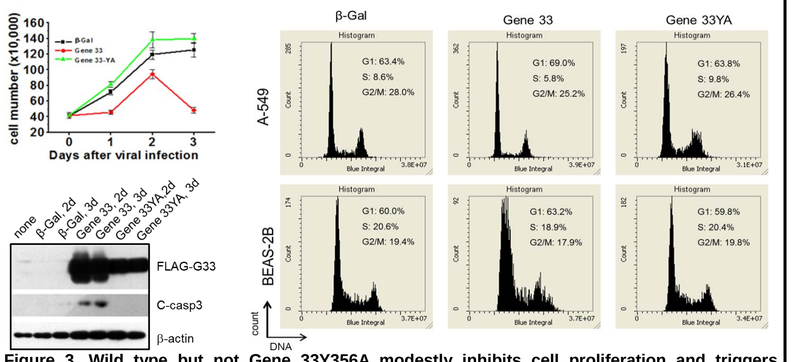


Figure 3. Wild type but not Gene 33Y356A modestly inhibits cell proliferation and triggers apoptosis. Cell counting and IB detecting the indicated proteins in A549 cells after infected with adenoviral vectors encoding β-Gal, wild type Gene 33, or EBD mutant (YA) Gene 33. Laser scanning cytometry measuring cell cycle distributions of A549 and BEAS-2B cells after infected with the adenoviral vectors.

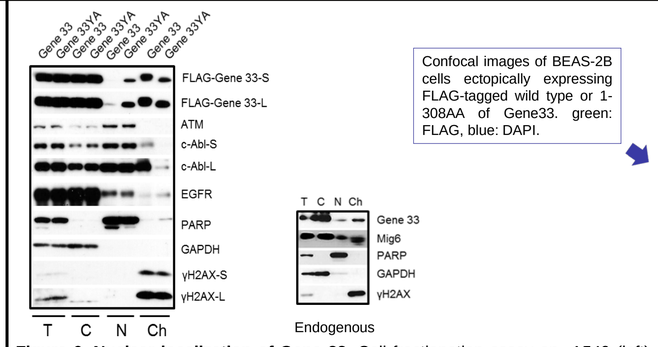
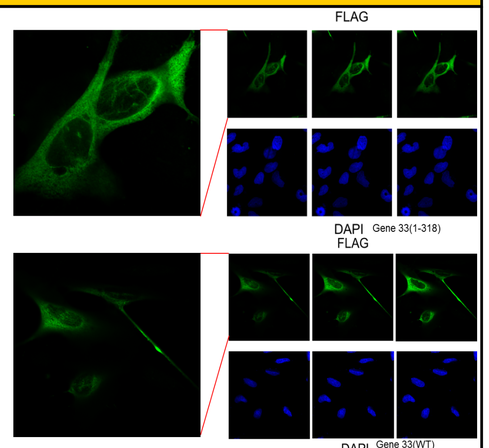


Figure 6. Nuclear localization of Gene 33. Cell fractionation assay on A549 (left) BEAS-2B (middle) cells with or without infected with the indicated adenoviral vectors. T: total, C: cytoplasm, N: nucleus, Ch: chromatin, Gene 33YA: Gene 33 EBD mutant, S: short exposure, L: long exposure, Mig6: Mig6 antibody. Right: confocal imaging showing nuclear signals of Gene 33.

Confocal images of BEAS-2B cells ectopically expressing FLAG-tagged wild type or 1-308AA of Gene33. green: FLAG, blue: DAPI.



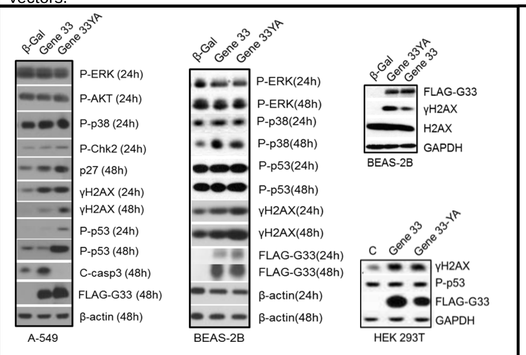


Figure 4. Ectopic expression of Gene 33 induces apoptosis in an EBD-dependent but p53-independent manner and provokes DDR in an EBD-independent fashion. IB detecting the indicated proteins in A549 and BEAS-2B cells infected with the indicated adenoviral vectors, and in 293T cells transfected with the indicated expression plasmids.

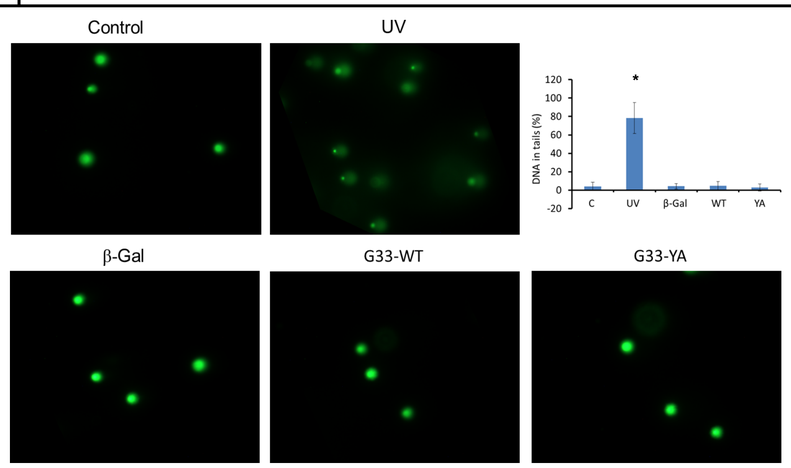


Figure 7. Ectopic expression of Gene 33 does not trigger DNA damage. A549 cells were either untreated (Control), or treated with UV for 30s and then recovered for 2h (UV), or infected with the indicated adenoviral vectors for 24h. Cells were then used to detect DNA damage by the comet assay. Representative images of the comet assay were shown and the DNA damage index (percentage of DNA in tail) was determined using the the OpenComet comet assay software (*P < 0.05).

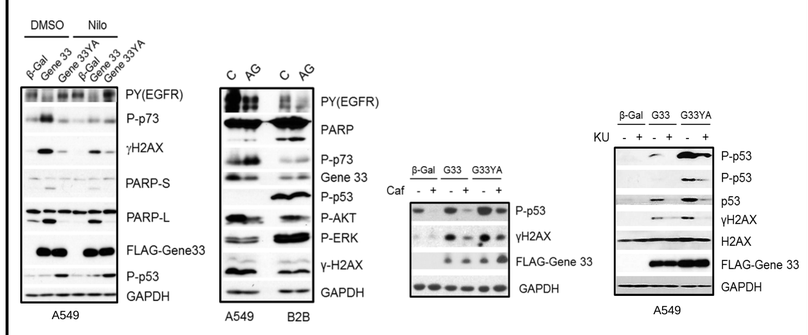


Figure 5. Gene 33 induces apoptosis in a c-Abl/p73-dependent and DDR in an ATM-dependent fashion. A549 or BEAS-2B cells were infected with the indicated adenoviral vectors and treated with the c-Abl inhibitor Nilotinib (Nilo), the EGFR kinase inhibitor AG1478 (AG), the ATM/ATR inhibitor caffeine (Caf), or the ATM kinase inhibitor KU55933 (KU). The indicated proteins were detected by IB.

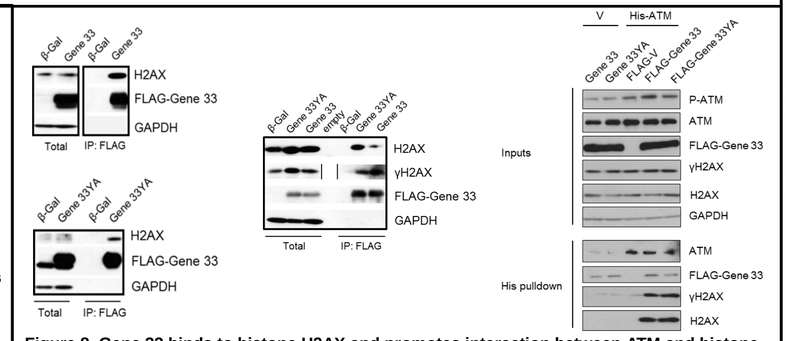


Figure 8. Gene 33 binds to histone H2AX and promotes interaction between ATM and histone H2AX. BEAS-2B cells were infected with the indicated adenoviral vectors followed by IP and IB as indicated. Far right: 293T cells were transfected with expression plasmids encoding FLAG-tagged wild type Gene 33 or Gene 33 with EBD mutation (Gene 33YA) with or without co-transfection of His-tagged ATM followed by HIS pulldown and IB as indicated.

CONCLUSIONS

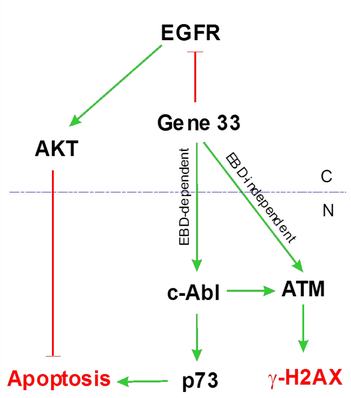


Figure 9. A proposed model on the regulation of apoptosis and DDR by Gene 33. Gene 33 binds and activates c-Abl thereby triggering the p73-dependent pro-apoptotic pathway. Gene 33 may also promote apoptosis by inhibiting EGFR and AKT-mediated pro-survival pathway. Both of these pathways are dependent the EBD motif of Gene 33. Gene 33 activates DDR (manifested as γH2AX) via both a c-Abl/EBD-dependent pathway and a c-Abl/EBD-independent pathway through direct activation of c-Abl and promoting ATM-histone H2AX interaction, respectively. Both pathways are dependent on ATM to activate DDR. Apoptosis- and DDR-associated activities of Gene 33 occur, at least in part, in the nucleus and/or chromatin.

C: cytoplasm, N: nucleus.