Epigenetic Alterations in High and Low LET Radiation Induced Genomic Instability

Umut Apyar, William F. Morgan and Janet E. Baulch

Radiation-induced genomic instability is thought to play an important role in carcinogenic processes. However, the mechanisms by which genomic instability is initiated and perpetuated remain to be elucidated. Radiation-induced mutations, double-strand breaks, or changes in gene expression alone have not been able to account for persistent genomic instability. This study tests the hypothesis that epigenetic aberrations are perpetuated in chromosomally unstable clones exhibiting radiation induced genomic instability. GM10115 Chinese hamster ovary (CHO) cells have human chromosome 4 on the background of the hamster genome. In previous work by Morgan and colleagues, these cells were irradiated and stable and unstable clones were characterized for stability using fluorescence *in situ* hybridization (FISH) for human chromosome 4. Prior to establishing the epigenetic profile of these clones in the current study, the chromosome stability of these clones was reconfirmed by FISH. The clones were then evaluated for specific locus, repeat element and global DNA methylation and for changes in miRNA mediated gene expression.

Results of this study demonstrate no effect of irradiation or persistent genomic instability on specific locus methylation for *nuclear factor-kappa B* (*NFκB*), *tumor suppressor in lung cancer 1* (*TSLC1*) or *cadherin 1* (*CDH1*) gene promoter regions. However, a potential mutation or deletion event was detected in the *NFκB* promoter for two of the unstable clones. This genomic event is currently being characterized. Significant changes were observed in long interspersed nuclear element 1 (LINE-1) or Alu repeat element DNA methylation for all unstable clones. Analysis of non-specific global methylation indicated that both DNA hypomethylation and hypermethylation may be observed in both stable and unstable irradiated clones.

Microarray analysis of gene expression levels identified possible down-regulation of the genes encoding inhibitor of differentiation protein 3 (*ID3*) and the histone 1 H2 linker protein (*HIST1H2*) in all four unstable clones. A consensus between two clones was also observed for additional changes in gene expression. Genes that showed significant changes in expression affect pathways including apoptosis, chromatin remodeling and DNA methylation. MicroRNA arrays did not identify any miRNA that were common to all four unstable clones. However, six miRNA were commonly altered in expression level in two of the unstable clones. Among these, altered miRNA expression could be validated by qRT-PCR for mmu-miR-466g, hsa-miR-30a and hsa-miR-195. Hsa-miR-30a and hsa-miR-195 were down-regulated and potential mRNA targets shown to be up-regulated include zinc finger protein 91 (*ZFP91*) and Max binding protein gene (*MNT*).

The results of this study show that epigenetic alterations can be perpetuated in unstable clones with RIGI. Further it suggests that epigenetically regulated alterations in

apoptosis, chromatin remodeling and DNA methylation pathways may contribute to maintenance of the genomic instability phenotype.

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