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Micronuclei levels in doxorubicin- and mitomycin C-treated MRC-5 and HeLa cells with knockdown of the histone *H1-5* gene

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H1 binding stabilizes nucleosomes and promotes higher-order chromatin compaction while its depletion leads to chromatin relaxation [1] and potentially increased DNA vulnerability. Salinas-Pena et al. [2] have challenged the idea of functional redundancy among H1 variants, emphasizing the distinct role of H1.5 in chromatin organization and genome stability. However, its involvement in cellular responses to mutagens remains poorly understood.

Here we examined the genotoxic effects of doxorubicin (DOX) and mitomycin C (MMC) in MRC-5 and HeLa cells following *H1-5* knockdown (KD) via CRISPR-Cas9.

To ensure that observed DNA damage was not confounded by cytotoxicity, cell viability was assessed prior to genotoxicity testing using MTT and trypan blue exclusion tests. Based on these data, non-cytotoxic concentrations were used in the subsequent micronucleus (MN) and comet assays.

H1-5 KD significantly increased spontaneous MNi frequency: from 9.5 ± 0.7 ‰ to 18.0 ± 2.8 ‰ in MRC-5, and from 14.0 ± 1.4 to 31.0 ± 1.4 ‰ in HeLa cells. DOX (0.0175–0.07 μg/ml) and MMC (0.01–0.1 μg/ml) treatments led to dose-dependent increases in MNi, with consistently higher levels in KD than WT cells. Comet assay results showed a similar pattern, with increased DNA damage (% DNA in tail) in KD cells.

Our preliminary findings suggest that *H1-5* knockdown may enhance spontaneous and drug-induced DNA and chromosome damage in normal and cancer cells. This may reflect a role for histone H1.5 in maintaining genome integrity, though further validation is needed. Understanding of how histone depletion affects the genotoxic response to antitumor agents may help in the development of strategies to minimize adverse effects in cells with *H1-5* gene alterations.

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