65 Measurement of modified thymine derivatives with GC/MS

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Modified bases were produced in DNA by exposure of DNA to ionizing radiation. The technique of gas chromatography/mass spectrometry (GC/MS) was used to identify and quantify thymine glycol in DNA. Thymine glycol was decomposed with hot formic acid treatment and we could not detect thymine glycol with a conventional method. DNA was incubated with E. coli Endo III to excise thymine glycol. After treatment DNA was precipitated with excess ethanol. Supernatant fraction was collected and lyophilized. Sample was derivatized with BSTFA. Thymine glycol could be measured and quantified with addition of deuterated thymine glycol as an internal standard. Another modified thymine bases such as 5-OHMe-U and 5-formyl-U were also measured.

66 Chaperones Hsp70 and Hsp40 suppress intracytoplasmic aggregate formation and ameliorate neurite retraction and cell death in cultured neuronal cells expressing mutant SOD1

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Mutations of the superoxide dismutase 1 (SOD1) gene cause familial amyotrophic lateral sclerosis (FALS). The intracytoplasmic aggregate formation containing mutant SOD1 is the hallmark of FALS. Since we previously demonstrated that heat shock proteins (HSPs) reduced aggregate formation and cell death in a cell model of spinal and bulbar muscular atrophy, we assessed the effects of HSPs on a cell model of FALS. Transient expression of mutant SOD1 (G93A) in neuro2a cells resulted in the intracytoplasmic aggregate formation, cell death, and neurite outgrowth inhibition. Endogenous Hsp70 and Hsp40 were markedly upregulated in the cells bearing aggregates and were colocalized with these aggregates. Overexpression of exogenous HSPs, especially the combination of Hsp70 and Hsp40 reduced intracytoplasmic aggregates, but did not change the expression level of insoluble high molecular protein complexs that were suggested to be mutant SOD1 oligomers. HSPs markedly ameliorated neurite retraction. HSPs also prevented cell death, but to an extent far less than the effect on neurite outgrowth and aggregate formation. These findings suggest that HSPs inhibit large aggregate formation but do not suppress mutant SOD1 oligomer development, and may ameliorate the functional aspects rather than eventual cell death.

67 Characterization of X-ray-induced Mutation in Werner Syndrome Cells

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Werner syndrome (WS) is a rare premature aging syndrome. Because the WS gene, *WRN*, is a member of the RecQ helicase family, it is assumed to be a caretaker of genomic integrity. In the present study, we examined X-ray-induced mutation frequency at *HPRT* locus in WS780 cells and analyzed types of mutations by multiplex PCR. The results indicated that the mutation frequency induced by X-irradiation was higher in the WS cells than in the control cells. It is notable that the majority of mutations observed in the WS cells consists of deletion mutations. We then examined in vitro assay for end-joining ability of DNA double-strand breaks in the nuclear extracts prepared from the WS cells. Sequencing analysis revealed that the deletions possibly caused by illegitimate recombination between two short homologies were occurred more frequently in the WS cells than the control cells. These results suggest that a defect in the WRN function promotes the illegitimate recombination and that this recombination might lead to the induction of large deletion mutations in WS cells.