106 Detection of X-ray induced genetic changes in human cells by using microsatellite and minisatellite sequences.

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Repetitive sequences such as microsatellite and minisatellite sequences are very changeable and highly polymorphic. It is not yet known whether these repetitive sequences change in human somatic cells with increased frequences like in germ line cells or not. However, cultured human cells which are deficient in mismatch repair activity are known to show hyper-mutability in microsatellite sequences. We are now trying to detect X-ray induced genetic changes in the mismatch-repair deficient cells. We have established a method to isolate DNA without large effort from many clones by using a microtiter plate. Using this technique, we are now analyzing many clones which were formed after X-ray irradiation.

107 Analysis of Mutations in the Human HPRT Gene Induced by Heavy-ion Irradiation II

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PURPOSE: Knowledge of the specificity of mutations induced by a variety of mutagens has enabled better understanding of the molecular mechanisms of mutagenesis. However, only a few studies have been published on the characteristics of heavy-ion-induced mutations. We have analyzed the hypoxanthine phosphoribosyltransferase (HPRT) mutations in human cells induced by C- and Ne-ions adjusted to different LETs.
MATERIALS AND METHODS: The human lymphoblastoid cell lines, WI-L2-NS and TK6, were used in this experiment. The survival curves for both C- and Ne-ions were determined by limiting dilution method and mutant clones were isolated as resistance to 6-TG (5mg/ml).
RESULTS: The difference in radiosensitivity between these two cell lines were also observed with high energy (low LET) of C- and Ne-ions. Our preliminary results suggest that the LET dependence of RBE effect on cell viability is similar to that for human embryo (HE) cells in our previous experiment. Both methodology and result for the analysis of HPRT mutant clones at the DNA sequence level will be reported at the meeting.

108 Towards the establishment of an *in vitro* system for the rejoining of DNA double strand breaks by cell extracts

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The DNA double strand break (dsb) has been implicated as the critical lesion induced by ionizing radiation leading to chromosome aberrations, mutations and cell death. We have attempted to refine the measurement and analysis of the rejoining of dsbs with the use of nuclear extracts applied to defined plasmid molecules carrying specific enzymatically-induced dsb. The plasmid pZErO-2 containing the *ccdB* gene under the control of the *lac* gene promoter was used as a substrate. The *ccdB* gene is lethal to *E. coli* on being overexpressed, which makes it easy to select plasmids with the mutated *ccdB* gene caused by mis-rejoing. We have compared the activities of extracts from an ataxia-telangiectasia cell line (AT2KYSV) with those from a normal cell line (N2KYSV). The extract from AT2KYSV cells showed much higher frequency of mis-rejoining than the N2KYSV extract. Sequence analysis of the mutant plasmids revealed deletion mutations which occurred mostly between short direct repeats (3 – 6 base pairs).