also discuss our future plans.

S-1-6 Some issues in risk estimation for neutrons

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For low LET radiations, the cancer risk has been estimated mainly based on the epidemiological data of the atomic bomb survivors in Hiroshima and Nagasaki. However, the risk from neutrons cannot be directly estimated from human data since no epidemiological data is available. For the cancer risk from neutrons, the RBE of neutrons has been investigated based on experimental data by comparing with the dose response of gamma radiation. The risk estimate using such a method is influenced by uncertainty of risk estimates of gamma radiation in low dose region because the slope of the dose response curve from gamma irradiation decreases to zero. The most important issue in the neutron risk estimate is inverse dose-rate effects that show a reduced risk as dose rate increases. There are some evidence for inverse dose-rate effects in endpoints such as mutations and transformations, but mechanistic explanations are needed to estimate how carcinogenic effects are dependent upon dose and dose rate. We examined how mutagenic effects depending upon dose rate have an influence on multistage process in carcinogenesis.

Radiation Induced Genetical Instability: What Is the Mechanism That a Cell Memorizes Being Irradiated?

S-2-1 Dysfunctional Mammalian Telomeres Join to Double-Strand Breaks

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Telomeres are nucleoprotein structures that maintain genomic stability by protecting the ends of linear chromosomes. In striking contrast to natural chromosomal termini, broken chromosome ends produced by DNA double strand breaks (DSBs) are highly recombinogenic, and represent a major threat to the integrity of the cell's genome due to their potential for causing chromosomal rearrangements that contribute to genomic instability and tumorigenesis. We've demonstrated that effective end-capping of mammalian telomeres has a seemingly paradoxical requirement for proteins more commonly associated with DNA DSB repair. Ku70, Ku86, and DNA-PKcs all participate in DSB repair through non-homologous end-joining. Mutations in any of these genes cause spontaneous chromosomal end-to-end fusions that maintain large blocks of telomeric sequence at the points of fusion that are not a consequence of telomere shortening. We've also shown that nascent telomeres produced via leading-strand DNA synthesis are especially susceptible to these end-to-end fusions. Here we report that impaired end-capping also allows dysfunctional telomeres to misjoin to broken chromosome ends created by radiation-induced DSB.

S-2-2 Ionizing radiation induced chromosomal instability: Detection and quantification of the early events

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Carcinogenesis is a multistep process by which a normal cell gradually develops into a transformed malignant cell. The presence of structural and numerical chromosomal aberrations in tumour cells suggests that chromosomal instability (CIN) plays and important role in carcinogenesis. Numerous investigations have demonstrated that ionizing radiation is able to induce CIN. Research aimed to elucidate the precise role of ionizing radiation induced CIN in carcinogenesis requires methods capable to measure true CIN, i.e. the continuous formation of de novo chromosomal abnormalities. In a pilot study, we have successfully established a novel method to detect and quantify CIN within in situ fixated colonies of human epithelial cells. This strategy allows to visualize the occurrence of CIN within a colony (the progeny of a single cell) and to quantify the rate of CIN as events per number of cell generations. Preliminary results suggest that CIN initiated by telomere dysfunctioning, might be involved in the immortalization of these cells. More details of the colony based in situ fixation method will be presented and our preliminary results discussed.

S-2-3 Radiation-Induced Delayed Chromosome Aberrations

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To know the mechanism for delayed formation of chromosome aberrations by radiation, we established the experimental system to analyze delayed chromosome aberrations using a microcell-mediated chromosome transfer. We investigated chromosome alterations by FISH in two different conditions. In one condition, an irradiated human chromosome was transferred into unirra-

diated recipient mouse cells. In the other condition, an unirradiated human chromosome was transferred into irradiated recipient mouse cells. The results indicated that the irradiated human chromosome was unstable in the unirradiated recipient cells to be rearranged with the recipient mouse chromosomes and that the unirradiated human chromosome also showed an unstable nature in the irradiated recipient cells. Telomere FISH analysis indicated that end-to-end fusions were involved in the formation of delayed chromosome aberrations. These findings suggest that delayed chromosome aberrations can be formed by the interaction between a damaged chromosome and an intact chromosome possibly mediated by telomeric instability.

S-2-4 DNA double strand break repair and chromosome instability in mammalian cells

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Chromosomal double strand breaks (DSBs) are usually repaired in mammalian cells through either of two pathways: end-joining (EJ) or homologous recombination (HR). To clarify the relative contribution of each pathway and the ensuring genetic changes, we developed a system to trace the fate of DSBs in the human genome by introducing restriction enzyme I-SceI site into thymidine kinase gene (TK) of human lymphoblastoid cell line (TK6). A DSB in the TK gene stimulated EJ as well as inter-chromosomal HR, but EJ contributed to the repair of DSBs over 100 times more frequently than HR. Molecular analysis revealed that EJ mainly causes small deletions limited to the TK gene. Seventy percent of the small deletion mutants analyzed showed 100 to 4,000 bp deletions with a 0 to 6-bp homology at the joint. Another 30%, however, were accompanied by complicated DNA rearrangements, presumably the result of sister chromatid fusion and breakage-fusion-bridge (BFB) cycle. BFB cycle could be an important mechanism for explaining delayed chromosomal instability caused by DSBs. HR, on the other hand, always resulted in non-crossing-over gene conversion.

S-2-5 Genomic instability in F1 mice born to irradiated spermatozoa and p53 dependent genomic cross-talk

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Radiation induced genomic instability is characterized by two features; untargeted and delayed mutation. We have shown that irradiation of sperm results in induction of maternal allele mutation at a minisatellite and the pink-eyed locus in F1 mice. Detailed analysis of embryos fertilized by irradiated sperm indicated that DNA synthesis of both male and female pronuclei is suppressed p53 dependently. This p53 dependent S checkpoint operates also in tissue culture cells. In the cells irradiated with less than 2.5 Gy, p53 aids ATM dependent phosphorylation of PCNA which then slows down the replication fork movement. Under this condition, PCNA forms foci in irradiated cells. In the absence of p53, PCNA was distributed in the nucleus homogeneously and DNA replication progresses at a rate equivalent to that of unirradiated cells. The relation between slowing-down of replication fork and recombination will be discussed.

Biological Effects of Low Dose and Low Dose-rate Irradiations

S-3-1 Mechanisms of DNA double-strand break repair

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DNA double-strand break (DSB), which is often induced by ionizing radiation, is the most serious damage in genome. There are at least two pathways which can repair DSBs: non-homologous end-joining (NHEJ) and homologous recombination (HR). When DSBs are generated by radiation, ATM kinase, which is activated by auto-phosphorylation and dissociation of their homodimer., phosphorylates histone H2AX. Subsequently, many repair proteins are recruited to the DSB sites and they activates damage responding pathways. A key role protein, Nbs1, physically interacts with phospho-H2AX for recruitment of a multifunctional repair complex, Mre11/Rad50/Nbs1, to DSB sites. This physical interaction requires the fork-head associated (FHA) domain of Nbs1 protein and the complex was found to be essential for HR. In this presentation, we will discuss about the processing and signaling pathways responding to radiation-induced DSBs.

S-3-2 NHEJ pathway plays an important role in low dose-rate effects (LDRE)

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It has been generally accepted that LDRE results from the SLD repair. As the dose rate is lowered and the treatment time protracted, more and more SLD can be repaired during the exposure. Recently we found that SLD repair due to DSB repair medi-