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Cytogenetic Instabilities in Atomic Bomb-Related Acute Myelocytic Leukemia Cells and in Hematopoietic Cells from Healthy Atomic Bomb Survivors

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1. Introduction

On August 6 and 9, 1945, nearly 65 years ago, atomic bombs (ABs) were dropped on two Japanese cities, Hiroshima and Nagasaki. The explosive powers of these two bombs are said to be equal about 12.5 kilotons of TNT for Hiroshima bomb and 22 kilotons for Nagasaki bomb. The dissipation of energy is believed to have been in the ratios consisting of 50% of bomb blast, 35% of thermal rays and 15% of radiation. Heat and bomb blast caused death in AB survivors within two weeks after bombing. On the other hand cause of death after two weeks were closely related to radiation. The initial radiation caused by the Hiroshima bomb composed mostly gamma rays including about 10 % of neutrons, on the while Nagasaki bomb emitted only gamma rays, In the both cities, a 50% death rate was estimated for those who were exposed at 1.2 km on the hypocenter. LD50 value in human was estimated as around 4 Gy from Hiroshima AB victims. Bone marrow depletion by radiation was the most critical damage leading to death. There victims who died early had extensive bone marrow damage, manifested by leukopenia and thrombocytopenia. Epilation began one to four weeks post-exposure.

Epidemiological studies have established that leukemia develops more frequently among atomic bomb (AB) survivors than in the general populations(Pierce *et al.*1996). Observed cancers and the approximate dates at which significant increases became evident were leukemia in 1950, thyroid 1955, breast and lung cancer in 1965, and gastric and colon cancer in 1975, and multiple myeloma in 1975. The incidence of leukemia peaked 8-10 years after the bombing, and a higher incidence was found in survivors exposed to higher radiation doses and those exposed at a younger age. The incidences of chronic myelocytic leukemia (CML) and acute lymphocytic leukemia(ALL) were decreased to the control level after 15-20 years and 25-30 years after the bombing, respectively, while the relative risk of AML development is still increased in persons who were exposed to the bombing at the age of 20-39 years（Preston *et al.*1994). The peak leukemia prevalence among Hiroshima survivors has passed, but even in recent years the death rate from leukemia among proximately exposed survivors remains higher than the mean death rate of the non-exposed and for all Japan. Among those exposed within 2 km of the hypocenter of Hiroshima, there was a marked increase in CML and the

ratio of acute type to chronic type was 1.6, whereas almost the same ratio as the all-Japan ratio of 3.8 was observed in those exposed beyond 2 km (Kamada and Tanaka 1983). The Leukemia Registry Study has revealed that the younger the individual at the time of bombing , the greater was the risk of leukemia during the early period and the more rapid was decline thereafter. On the other hand, in the group aged 45 or more at the time on bombing, the increase in risk occurred later and persisted during the period 1960-1975. Acute type leukemia contributed to these trends. More recent reports have indicated that the incidences of acute myelocytic leukemia (AML) and MDS were still high even 40-60 years after the bombing (Richardson *et al*., 2009; Iwanaga *et al*., 2011). These results suggest that mechanism for development of radiation-induced AML seems to be different those of ALL and CML.

In summary, AB-related leukemia and solid tumor exhibit the following characteristics: (1) Leukemia and cancer risk increased with dose. (2) The leukemia risk increased with decreasing age at the time of bombing. (3) Unlike leukemia, the latency period increase with decreasing age at the time bombing, with a marked radiation effect evident when survivors reached the age level at which the cancers frequently occur. AB-survivors population is very unique and can be used for clarifying the mechanisms responsible for radiation-induced leukemia.

2. Dosimetry system of AB radiation

The former T65D system of dosimtry had been used as the most accurate method of estimating the doses received by individual survivors although this has now been replaced by the DS86 and DS01 system. Original T65D system was based on the nuclear testing conducting at Nevada, USA, and was devised in 1965 as a formula that incorporated various parameters such as distance from the hypocenter, and transmission factors for shielding materials, etc. AB survivors are defined as those directly exposed in the city at the time of the bombing within 5 km of the hypocenter. In order to permit more detail calculations, the DS86 and DS01 system was formulated in 1986 and 2001 based on elementary physical processes, and enabled computer analysis of the different processes involved from the time of emission until arrival at various human organs, including factors of calculations of weather and soil. Dose of gamma rays was increased four times, on the other hand, neuron dose is decreased more than 10% of former values. The DS86 system was used for present study. The DS86 assessment of Hiroshima AB radiation contained low energy neutrons of less than 1.0 MeV. If the hypothesis that the RBE of low energy neutrons is higher than that of fission neutrons with 2.3 MeV is true, Hiroshima AB radiation would have created a significantly higher degree of biological effects. Therefore our experiments using ^{252}Cf-neutrons and monochromatic neutrons was performed in Hiroshima University, The relative biological effectiveness (RBE) of dicentric chromosomes aberrations was increased to 10.7 at 0.75 MeV from 3.9 at ^{252}Cf- neutrons and reached to 16.4 as highest RBE value at 0.37 MeV, but the value was decreased to 11.2 at 0.186 MeV (Tanaka *et al.* 1994, 1999). RBE of low energy neutron is higher than that of fission neutrons. It is still now in question how radiation containing low energy neutron effects on developments of leukemia and cancer.

3. Chromosome aberrations in AB-related leukemia

We have investigated chromosome aberrations in leukemias developing individuals with a history of heavily exposure to AB radiation(Kamada *et al.*,1991, Nakanishi *et al*., 1999) and

have reported cytogenetic and molecular biological alterations in AB-related leukemias (Kamada and Tanaka 1983; Tanaka *et al.*, 1989; Tanaka *et al.*, 1991; Kamada *et al.*, 1991). In the present study, to clarify the mechanisms responsible for radiation-induced leukemia, we analyzed cytogenetic and molecular biological alterations in AB-related leukemias in comparison with those in *de novo* (non-AB-related) leukemia. Acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML) and other hematological disorders were excluded from the present analysis. In our previous cytogenetic molecular biological studies of 132 patients with AB-related leukemia observed during 1978-1999, 33 patients with acute myeloid leukemia (AML) had been exposed to DS86 doses of exceeding 1Gy. Eight more AB patients were included in the present study, after our previous publication in 1999 (Nakanishi *et al.*, 1999). This revealed that among 132 patients with AB-related leukemias observed during 1978-1999, 33 patients with AML had been exposed to doses of more than 1 Gy on the basis of the Dose System 1986 (DS86). Leukemia patients were divided into three groups according to their exposure status; those who received more than 1 Gy bone marrow dose, 0.01-0.99 Gy and non-exposed groups (Table 1). Chromosome aberrations in the 33 patients were compared with those in 588 control patients with *de novo* AML who had been born before August 1945. Thirty-two and 58 AB-related patients who had been exposed to 0.01-0.99 Gy and less than 0.01Gy were also analyzed for chromosome aberrations using the same methods.

Bone marrow cells or peripheral lymphocytes from 33, 32, 58 and 588 leukemia patients with and without AB radiation exposure, respectively, were cultured for 24 h and harvested for chromosome preparations. These preparations were stained by the Giemsa banding procedure and karyotyped according to ISCN (2009). Mean numbers of aberrant chromosomes were scored in karyotypes of the main leukemia clone in each patient. One thousand metaphases were observed by microscopy, and spontaneous chromosome aberrations such as chromatid breaks, gaps, and hyperploid cells, which are useful markers of radiation-induced chromosome instability, were also detected. FISH analyses were performed using cosmid and YAC probes such as *MLL* and *CD3* on 11q22-23 regions of chromosome 11 for mapping of chromosome breakpoints in each patient.

The leukemia subtypes of FAB classification in the patients in each group were analyzed. Among 33 AB-related patients exposed more than 1Gy, 13 had been diagnosed as having MDS (RA or RAEB) by the FAB classification before the development of AML. The others had been diagnosed as having AML (AMLM1- 8 patients; AMLM2- 8 patients; AMLM4- 1 patient; AMLM5- 1 patient; AMLM6- 1 patient). None of the patient had AML M3.

Chromosome complexity was indicated by the number of aberrant chromosomes found in the main leukemic clone. Of the 33 AB-related patients who had been exposed to > 1Gy, only one (3.1%) had a normal karyotype, compared with 45.7% of AB-related patients exposed to 0.01-0.99 Gy, and 62.5% exposed to < 0.01 Gy. On the other hand, 44.1% of the non-exposed control leukemia patients (246 out of 558) had a normal karyotype. The numbers of aberrant chromosome per metaphase were 3.69, 1.89, 2.0 and 0.93 in AB-related leukemia patients who had been exposed to>1Gy, 0.01-0.99 Gy, <0.01Gy and non-exposed, respectively. These results indicate that AB-related patients who had been exposed >1.0 Gy had more complex chromosome aberrations in their leukemic cells. Both-AB-leukemia groups with exposure to 0.01-0.99 Gy and <0.01 Gy also had higher numbers of aberrant chromosomes per cell. Only 3.1% of patients with AB-leukemia who had been exposed to >

1 Gy had normal karyotype, while 45.7% and 62.5% of those exposed to 0.01-0.99 Gy and <0.01 Gy did so. About half of the AML patients showed normal karyotype.

Specific chromosome aberrations that were found at high frequencies are also listed in Table 1. Deletion of chromosome 20[del(20)] and loss of chromosome 20 were found in 10 patients, and der(11)or del(11) at 11q13-11q22 of chromosome 11 in 10 patients. On the other hand, non-exposed patients had a higher number of t(8;21) and t(15;17) translocations, which is specific to AML FAB type M2 and M3,respectively. The groups exposed to 0.01-0.99 Gy and <0.01Gy had no representative chromosome aberrations and lower percentages of t(8;21) and t(15;17) than the patient with *de novo* AMLs.

DS86 dose (Gy)	Number of patients	Percentage with a normal karyotype	Number of aberrant chromosomes per metaphase	Specific chromosome aberrations
AB patients exposed to >1.0Gy	33	3.1%	3.69	del(20)(q11) der(11)(q23) der(11)(q13)
0.01-0.99Gy	32	45.7%	1.89	-
<0.01Gy	58	62.5%	2.0	-
Non-exposed patients	558	44.1%	0.93	t(8;21) t(15;17)

Table 1. Comparison of chromosome aberrations found in patients with AB leukemia and patients with *de novo* leukemia

The incidences of several leukemia-specific chromosome aberrations were compared between the 33 patients with AB-related leukemia who were exposed to >1 Gy or non-exposed, and the 558 patients with *de novo* leukemias (Fig.1). Deletion of chromosome 5 or loss of chromosome [del(5)/-5] showed the highest incidence, but the incidences did not differ between the two groups. Deletion of chromosome 7 or monosomy of chromosome 7[del(7)/-7] showed the second highest incidence, and these aberrations were found more frequently in *de novo* leukemias. Deletion (20)/-20 and deletion and derivative of chromosome 11 at 11q13-11q22 were observed in higher frequencies in patients with AB-related leukemia. Deletion or monosomy of chromosome 13 [del(13)/-13] was also more frequent in AB-related leukemias [2 of 33 in del(13) and 3 of 33 in monosomy 13]. Translocations of t(8;21), t(15;17), inv(16) and t(9;22) were detected only in *de novo* leukemias. Further FISH analysis using target gene probes were performed, and the results indicated that the breakpoints associated with derivative translocations at 11q22-23 of chromosome 11 [der(11)t(11;α)(q22-23;α)]lay outside the *MLL* gene. FISH analysis using whole chromosome painting of chromosome 8 and target gene probes demonstrated severe complex chromosome aberrations containing chromosome segmental jumping translocations(SJT) at 8q24 of chromosome 8 and 11q22-23 of chromosome 11 (Tanaka *et al,* 2001). *MYC* oncogene signals located on some regions on the long arm of chromosome 8 and several other chromosomes, which were considered to translocate to several other chromosomes from original region on chromosome 8 (8q24). The phenomenon is known as segmental jumping chromosomal translocation(SJT) (Tanaka *et al.,* 1997). In conclusion, AB-

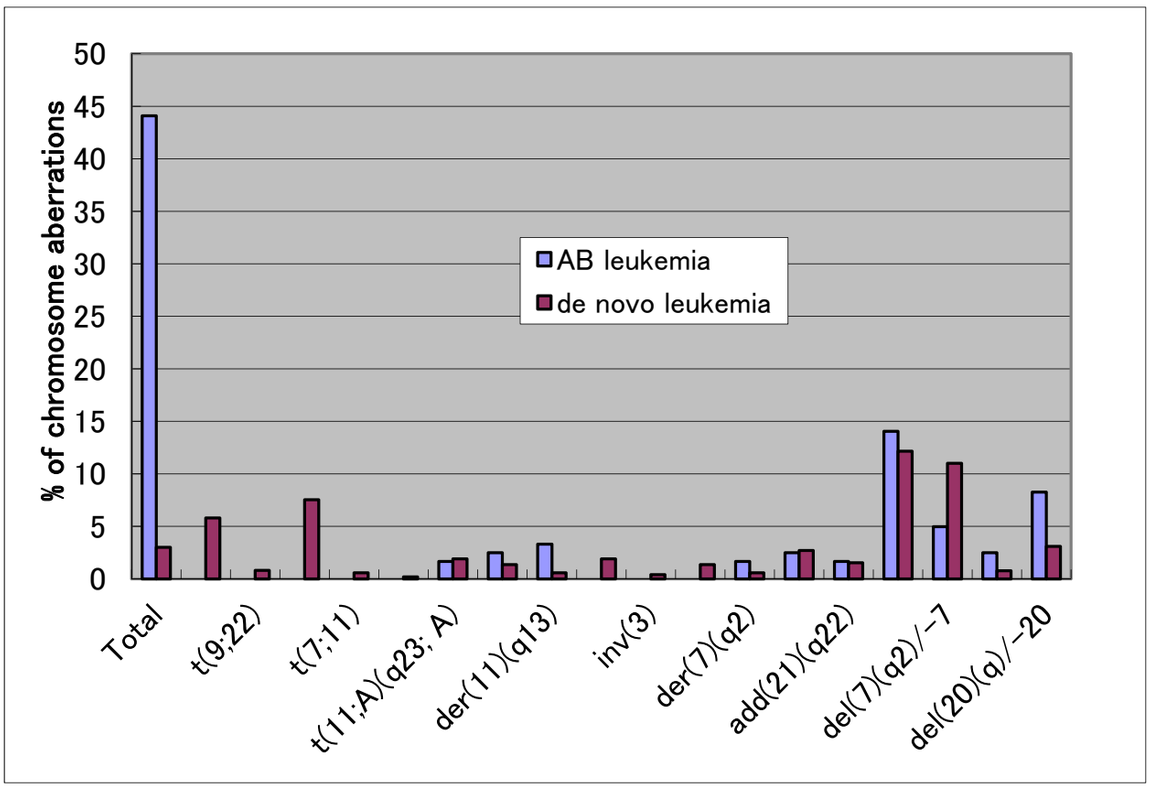

Fig. 1. Comparison of frequencies of each type of chromosome aberration between AB-related leukemias and *de novo* leukemias

related leukemia patients showed more complex chromosome aberrations and did not have any leukemia-specific chromosome aberrations such as t(8:21) and t(9;22).

4. Oncogene alterations in leukemias developed from atomic bomb survivors

Alterations in the *AML1* and *MLL* oncogenes in AML patients were also analyzed by PCR-SSCP and FISH, respectively, and compared with those in the *de novo* in AMLs. Stored DNAs were available from 9 patients with AB-related leukemia, including 6 patients who had been exposed > 1.0 Gy. These leukemia DNAs were analyzed for *AML1* oncogene alterations by PCR-SSCP. Exons 1 and 3 in the *AML1* gene, which overlaps the RUNT domain of the *AML1* gene were analyzed.

Runt domain contains exons 3, 4 5, 6 and 7. PCR-SSCP and DNA sequence analyses of the *AML1* gene on human chromosome 21 were performed in 8 AML patients who had been heavily exposed to the AB radiation. Genomic DNAs were amplified by PCR in a total volume of 20 µl containing PCR buffer. PCR of exons 1 and 3 of the *AML1* gene was performed using forward/reverse primers for the flanking intron. To identify *AML1* gene mutations, PCR-SSCP analysis was performed on a Gene Phor system (Amersham Pharmacia Biotech). After gelelectrophoresis, the gels were silver-stained to visualize the bands. All PCR products with abnormal bands detected by PCR-SSCP were confirmed by independent amplification and PCR-SSCP analysis. For identification of *AML1* gene mutations, PCR products that showed abnormal bands were subcloned into a pCR2.2 vector

(Invitrogen), and 10 independent clones were sequenced in both directions using a BigDye Terminator Cycle sequencing kit (Perkin-Elmer) and analyzed on an ABI Prism 310 Genetic Analyzer (Perkin Elmer). To confirm mutations, PCR products from the cDNA were also sequenced. First-strand cDNA was synthesized using total RNA and random hexamers with SuperScriptsII reverse transcriptase (Gibco). The cDNA products were amplified with the primers and subcloned PCR products were sequenced as described above. PCR of exon 3 of the *AML1* gene performed using the following flanking intronic , forward/reverse primers; 5′-AGCTGTTTGCAGGGTCCTAA-3′/ 5′-GTCCTCCCACCACCCTCT-3′. The cDNA products were amplified with the following primers; 5′-GCAGGGTCCTAACTCAATCG-3′/ 5′-GCTCGGAAAAGGACAAGCTC-3′, and subcloned PCR products were sequenced as described above.

Two patients had gene rearrangement in exon3 of *AML1*. These samples were used for subsequent DNA sequence analysis and showed C to A, G to A and G to T mutations, respectively. When we further analyzed exon 1 of *AML1*, another three patients who had been exposed to >1.0 Gy were found to have an abnormal band in PCR-SSCP analysis. Exon 1 is located outside the RUNT domain of the *AML1* gene. These might be heteromorphic abnormality because they had the same G to T transition. Two of 6 patients with AB-related AML had abnormality in exon 3 of *AML1*.

The *MLL* gene on the long arm of chromosome 11 was analyzed in 3 patients by the Southern blot method. All of the 6 patients with AB-related AML were shown to have the chromosome breakpoint at 11q22-23 and 11q25, lying outside the *MLL* gene by FISH analysis using several region-specific cosmid probes. Two of the 6 patients had been exposed to >1 Gy. The karyotypes in the 6 patient showed r(11), der(11)(q22), del(11)(q23) and inv(11)(p15q21) in one patient each and add(11)(q21) in 2 patients.

5. Incidence of micronucleus (MN)in lymphocytes and bone marrow cells from healthy AB survivors

It has not yet been clarified whether chromosomal instability is preserved in lymphocytes and bone marrow cells from healthy AB survivors. Chromatid-type aberrations and MN are suitable markers for chromosomal instability because they appear several days after exposure. Both of bone marrow and lymphocytes samples from these AB-survivors were obtained after 40-50 years after AB radiation exposure. Micronucleus (MN) was evident incidences in the lymphocytes and bone marrow erythroblasts in healthy AB survivors. Furthermore, MN in bone marrow erythrocytes or lymphocytes in 35 and 20 healthy AB survivors without leukemia or hematological disease, respectively, were analyzed using stored smear slides. Cytochalasin B (0.5 μg/ml) was not used for the present lymphocyte MN assay. MN was observed in 1,000 PHA-stimulated lymphocytes, and in 500 bone marrow erythroblasts on smear slides. Cells with MN were scored by microscopy. Exposure doses in most of the healthy AB survivors and patients with AB-related leukemia had been estimated by DS86 dosimetry. Individual approximate exposure doses in the remaining AB survivors were estimated on the basis of the chromosome aberration rate in peripheral blood.

Thirty five AB survivors who had been exposed 2km from the hypocenter in Hiroshima city and whom exposure doses (DS86)had been estimated, were used for MN analysis of bone

marrow erythroblasts. Bone marrow smear slide was stained with May-Grünwald Giemsa solution were used for this purpose. Eleven age-matched controls were also studied for comparison. Frequencies of MN in bone marrow erythroblasts were increased by exposure to doses up to 3Gy and after the dose they were slightly decreased. Twenty healthy AB survivors for whom exposure doses had been estimated were used for lymphocyte chromosomal instability. The incidences of MN in 1,000 lymphocytes on peripheral blood smears stained with May-Grünwald Giemsa solution from these AB-survivors were observed in the present analysis. The age-matched non-exposed controls had 1-8 MN per 1,000 lymphocytes, whereas AB-survivors who had been exposed to up to 3 Gy showed a dose-dependent increase of MN in lymphocytes, although small errors in measurement could have occurred in the absence of addition of cytochalacin B. The AB-survivors who had been exposed to more than 4-5 Gy had a slightly lower number of MN than those who had been exposed to 3 Gy. The incidence of MN in lymphocytes clearly increased in a dose-dependent manner. Healthy AB survivors had a higher incidence of MN in lymphocytes and bone marrow erythroblasts, depending on their DS86 exposure dose. These results indicate the presence of AB radiation-induced chromosome instabilities in both bone marrow erythroblasts and lymphocytes of healthy AB survivors.

A healthy AB survivor who developed colon cancer after these examinations was serially analyzed for the chromosome aberration rate and MN incidence. The healthy AB-survivor was a 65 year old woman who had been exposed to 3Gy to Hiroshima bomb, developed colon cancer in 1997. She had been serially observed chromosome aberration rates in lymphocytes for 22 years from 1975 to 1997(Fig.2). Translocation was detected by G-banding using trypsin solution. The frequency of translocation changed from 22-35% during this period, and then increased rapidly to 46 % at the time of colon cancer development. The incidences of both spontaneous chromosome aberrations such as chromatid breaks and hyperploidy and MN were higher than in the control. Chromatid-type aberrations such as breaks and gaps were also serially followed up during this period. Spontaneous chromosome aberrations, including the total rates of chromatid-type aberrations and hyperdipolidy and hyperploidy, are shown in Fig.2. The frequency of spontaneous chromosome aberrations remained at around 6-14% in 1975-1991 and it increased at the time of cancer development. MN incidence in lymphocytes was also followed up serially during this period and showed a similar pattern (Fig.2). The incidence of MN per 1,000 lymphocytes was 4-7 and increased to 16 at the time of cancer development. Serial observations in an AB-survivors showed that all of the cytogenetic markers of chromosomal instability, including translocations, spontaneous chromosome aberrations and MN increased at the time of colon cancer development.

6. Plausible mechanisms for development of chromosome instability in AB-related leukemia and healthy AB survivors

In vitro culture studies have demonstrated radiation-induced chromosome instability in human and rodent cells after several cell divisions (Kadhim *et al.,* 1992; Holmberg *et al.* 1993). These chromosome abnormalities are known as delayed chromosome aberrations (Tanaka and Ihda 2008; Tanaka *et al.* 2008). MN and chromatid-type aberrations are suitable cytogenetic markers for detecting chromosome instability because they are eliminated by cell division and appear only for a short time after radiation exposure, being associated

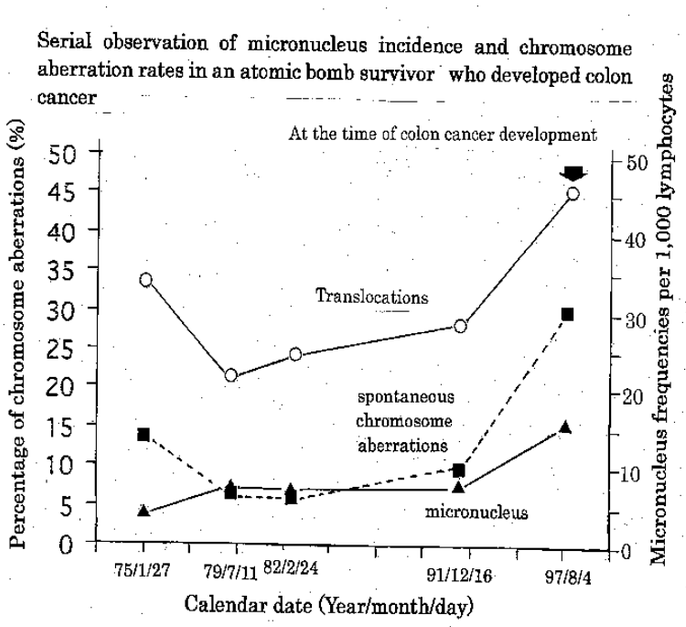

Fig. 2. Serial observation of MN incidence and chromosome aberration rates in an AB survivor who developed colon cancer

with the approximately 3-year life span of lymphocytes. Chromosome aberrations appear a long time after radiation exposure,and are considered to be an indirect radiation exposure. The frequency of translocations found in AB-survivors depends on the radiation exposure dose, but such aberrations are not suitable for proving chromosomal instability. AB-survivors who were exposed to higher radiation doses had a higher number of MN in both erythroblasts and lymphocytes 30-40 years after the bombing. The incidence of MN in lymphocytes clearly increased in a dose- dependent manner. However, conflicting result has been reported for dicentric chromosomes in peripheral lymphocytes in healthy AB survivors (Hamasaki *et al.* 2009). These results provide clues as to why the individuals who were exposed to radiation showed higher chromosomal instability.

First, it can be hypothesized that a small percentage of DNA damages is not repaired completely and persists for a long time after exposure to radiation. At several days after exposure, un-repaired sites are recognized by DNA-binding proteins in an error-prone manner. The key issue in this model is the type of mechanism responsible for long-term preservation of cells with un-repaired DNAs (Suzuki *et al.*, 2003). Gene recombination associated with chromosomal instability will occur at the time. A second hypothesis is that inflammation occurs in the organs of AB-survivors exposed to higher radiation doses(Neriishi *et al.*, 2003). Macrophages, NK-cells, lymphocytes, monocytes release active oxygen species into areas of inflammation. Such oxygen stress can induce chromatid-type of aberrations and MN in lymphocytes located in the vicinity. Similar findings were observed

in mice experiments (Lorimore *et al.,* 2001). Interestingly, the most common abnormality for delayed chromosome instability observed in mice were chromatid-type aberrations (Watson *et al.* 1996, 2001; Ullrich and Davis 1999), which imply released reactive oxygen species or secreted factors related to the chromosome instability. The present serial analyses of MN and spontaneous chromosome aberrations in a patient with AB-related colon cancer also revealed that chromosome instabilities increased rapidly at the time of cancer development. In this case also, it is considered that phagocytic cells that had accumulated in cancer-prone tissues released active oxygen species, which induced chromosome aberrations and MN in lymphocytes. Of course,however, the first hypothesis also still remains to be examined.

AB-related leukemias showed more complex chromosome aberrations than *de novo* leukemias. Twenty-seven of the 33 patients with AB-relatedAML showed complex chromosome aberrations involving more than three chromosomes including chromosomes 11 and 20, and the number of chromosomes per cell in AB-relatedleukemias was 3.69, compared with 0.93 of *de novo* leukemia. Only one of the 33 patients (3.1%) had a normal karyotype, compared with 44.1% of *de novo* leukemia. It is considered that phagocytic cells that had accumulated in cancer-prone tissues released active oxygen species, in turn inducing chromosome aberrations in bone marrow leukemic cells. Of course, the hypothesis that malignant clones might have developed from hematopoietic stem cell with higher chromosomal instability remains to be investigated.

7. Different mechanisms for development of AB-related AML and *de novo* AML

Most of the 33 patients with AB-related AML had a preceding long term MDS stage. Therefore, no leukemia type-specific translocations of such as t(8;21), t(15;17), t(11;A)(q23;A) and inv(16) were found in these 33 patients. The best approach is to compare types of chromosome aberrations between the patients with AB-related MDS and those with AB-related AML developed from MDS. Translocations on chromosome 11 at 11q13 to 11q23, and deletion/loss of chromosome 20 were frequently found in AB-related leukemias (Table 1). The fact that AB-related leukemias did not have chromosomal breakpoints in the *MLL* gene indicates that AB-related leukemia develops from MDS. MDS has shown to have a chromosome breakpoint at 11q22, not 11q23 (Tanaka *et al.,* 2001). Most cases of 11q23 AML do not have a MDS stage (Tanaka *et al.* 2001). The reason why AB-related leukemia did not have a translocation breakpoint within the *AML1* gene on 21q22 would be the same as that for11q22 abnormality in MDS. It is well known that deregulation of the *AML1* gene results from gene mutations as well as chromosomal translocations such as t(8;21), t(3;21), t(12;21) and so on (Harada *et al.* 2003).

It has been reported that secondary MDS developing after chemotherapy has been shown to have a high incidence of *AML1* gene mutation and that MDS shows a higher incidence of mutations in the *AML1* gene (Harada *et al.*, 2003),in which the frequencies of AML1 point mutations were 17% (15/88 cases) in sporadic MDS/AML and 50% (11/22 cases) in secondary MDS/AML (4/20). In present study, 2 of 6 patients with AB-related AML had point mutations in exon 3 on the *AML1* gene. On the other hand, PCR-SSCP analysis of non-AB-related AML patients has shown that only 2 showed transformation from MDS and that none of 14 MDS patients diagnosed as having RAEB-T had abnormalities(i.e. 2 of 30 non-AB-related AML patients showed transformation)(Harada and Harada, 2009), thus indicating that AB-related

AML might have a higher incidence of *AML1* gene mutation. AMLs that developed in residents at living near the Nevada nuclear weapons testing site were reported to have chromosome aberrations involving the *AML1* gene at 21q22 on chromosome 21 (Hromas *et al.,* 2000). The two of the present 6 patients with AB-related AML who had been exposed to more than 1Gy had point mutations in exon 3, which encodes the RUNT domain of AML1(CBFβ 2) protein. It will be necessary to compare the break points in the *AML1* gene (exons 1 and 3) and the base changes in the DNA sequences between these two AB-related leukemias and the published DNA sequences in MDS. AML 1(CBFβ 2) protein having point mutation could not bind with both histon deacetylation (HDAC) and histon acetylation HAT, which results in suppression of transcription and transformation of MDS to AML (Ding *et al*., 2009). C- terminal *AML1* point mutations (exons 3-5) were exclusively found in sporadic MDS/AML, while N-terminal(exons 6-8) mutations were found in chemotherapy related-secondary MDS/AML (Harada *et al*., 2003). Therefore the role of exon 3 mutations of *AML1* gene in the pathogenesis of radiation-induced leukemia has been questioned.

These results suggest that AB-related leukemia is derived from abnormal pluripotent hematopoietic stem cells, which are preserved for a long term and show higher genetic instability such as microsatellite instability (Nakanishi *et al.,* 2001)(Fig.3), whereas *de novo* AML develops from committed hematopoietic stem cells and shows simple chromosome aberrations. t(8;21)-positive leukemic cells arise from committed stem cells, on the other hand del(20), t(11,?) might be derived from pluripotent stem cells, as in the case for deletion of chromosome 5/monosomy 5 at the pluripotent stem cell level (Nilson *et al*., 2007). Both of these leukemias developed from MDS. Thus the *AML1* gene might be activated only at the hematopoietic stem cell level.

Genetic instability is preserved in hematopoietic stem cells damaged by radiation.

As most AB-leukemias developed from MDS,it is more appropriate to compare AB-leukemia with *de novo* AML having a MDS stage before development. Observation of AB-leukemias in bone marrow smears showed that their hematological features were similar to those of MDS found in aged patients. Recent leukemia study showed that a higher incidence of MDS was observed in Nagasaki AB survivors (Iwanaga *et al.,* 2011), which implicates that AB-related MDS would have more chance to acquire a higher chromosome instability in long-term after exposure to AB-radiation. This also suggested that AB-radiation might induce earlier development of MDS. Chromosome aberrations found in AB-related leukemia included a higher frequency of del(20) and 11q22-11q23 abnormalities than those in *de novo* leukemias. Monosomy and deletion of chromosome 5 (-5/del(5)) occurred at almost the same frequencyas those in *de novo* leukemias. On the other hand monosomy 7 and deletion of chromosome 7(-7/del(7)) occurred at lower frequencies in AB-related leukemia than in *de novo* leukemias (Arif *et al.,* 1997). This indicated that the type of chromosome aberrations in AB-related leukemias showed different from those in *de novo* AML and MDS. AB radiation might induce different type of cancer as well as cancer development with a shorter latency. A similar finding has been observed using analyses of array CGH and cell surface marker by FACS sorting for low-dose-rate radiation-induced murine leukemias, lymphomas and tumors by The Institute for Environmental Science (Tanaka *et al.,* 2007; Hirouchi *et al*., 2009, 2011).

In conclusion, chromosome and oncogene features revealed in present study might be related to the higher incidence of AML transformed from MDS in AB survivors a long time

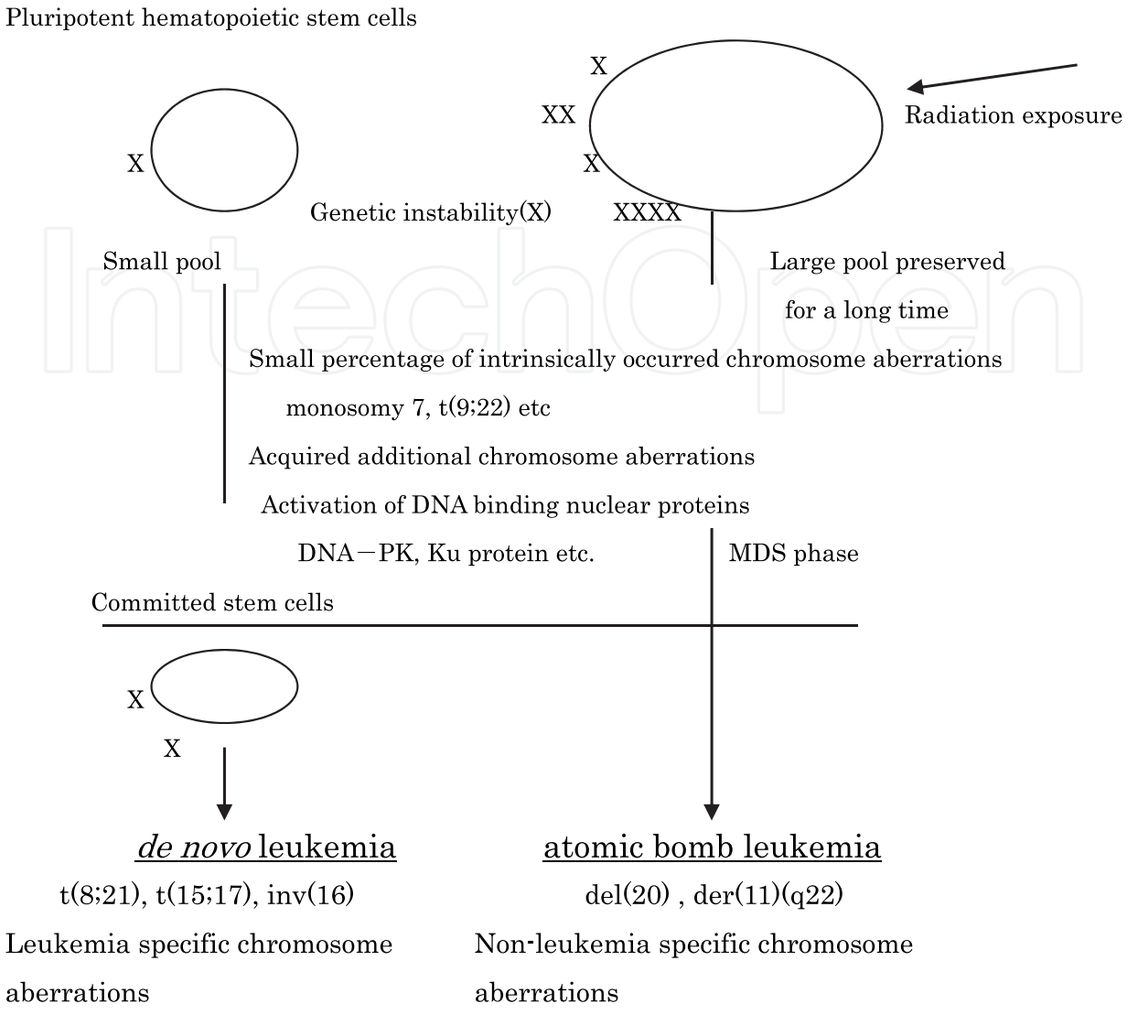

Fig. 3. Hypothetical schema of the development of radiation-induced leukemia from pluripotent hematopoietic stem cells

after exposure, and these findings are important for understanding the mechanisms responsible for radiation-induced leukemia.

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9. References

Arif M., Tanaka K., Chendil D., Asou H., Kyo T., Dohy H., Kamada N. (1996) Hidden monosomy 7 in acute myeloid leukemia and myelodysplastic syndrome detected by interphase fluorescence in situ hybridization. *Leukemia. Res.* Vol. 20, pp.709-716.

Ding Y., Harada Y., Imanaga J., IMURA A., HARADA H.(2009) AML1/RUNX1 point mutation possibly promotes leukemic transformation in myeloproliferative neoplasms. *Blood*Vol. 114, No.25, pp.5201-5205.

Hamasaki K., Kusunoki Y., Nakashima E., Takahashi N., Nakachi K., Nakamura N., Kodama Y. (2009) Clonality expanded T lymphocytes from atomic bomb survivors in vitro show no evidence of cytogenetic instability. *Radiat. Res.* Vol. 172, No 2, pp.234-243.

Harada H., Harada Y., Tanaka H., Kimura A., Inaba T. (2003) Implication of somatic mutations in the *AML1* gene in radiation-associated and therapy-related myelodysplastic syndorome / acute myeloid leukemia. *Blood* Vol. 101, pp. 673-680.

Harada Y. and Harada H.(2009) Molecular pathways mediating MDS/AML with focus on AML1/RUNNX1 point mutations. *J Cell Physiol.* Vol. 220, No.1, pp.16-20.

Hirouchi T., Takabatake T., Yoshida K., Nitta Y., Nakamura M., Tanaka S., Ichinohe K., Oghiso Y., Tanaka K., (2009) Upregulation of c-myc gene accompanied by PU.1 deficiency in radiation-induced acute myeloid leukemia in mice. *Exp. Hematol.* Vol. 36, pp. 871-881.

Hirouchi T., Akabane M., Tanaka S., Tanaka I. B., Todate A., Ichinohe K., Oghiso Y., Tanaka K. (2011) Cell surface marker phenotype and gene expression profiles of murine radiation-induced acute myeloid leukemia stem cells are similar to those of common myeloid progenitors. *Radiat. Res*. Vol.176, pp. 311-322.

Holmberg K., Falt S., Johansson A., Lambert A. (1993) Clonal chromosome aberrations and genomic instability in X-irradiated human T-lymphocyte cultures. *Mutat. Res.* Vol. 286, pp. 321-330.

Hromas R., Shopnick R., George H., Bowers C.,Varella-Garcia M., Richkind K.(2000) A novel syndrome of radiation-associated acute myeloid leukemia involving AML1 gene translocation. *Blood* Vol. 15,pp. 4011-4013.

Iwanaga M, Hsu W-I., Soda M., Takasaki Y., Tawara M., Joh T., Amenomori T., Yamamura M., Yoshida Y., Koba T., Miyazaki Y., Matsuo T., Preston D. L., Suyama A., Kodama K., Tomonaga M. (2011) Risk of myeloid dysplastic syndrome in people exposed to ionizing radiation: a retrospective cohort study of Nagasaki atomic bomb survivors. *J. Clin. Oncol.* Vol. 29, No.4, pp. 428-434.

ISCN (2009) An International System for Human Cytogenetic Nomenclature. Eds. Shaffer L. G., Slovak M. L. and Campbell L. J., Karger, Basel

Kamada N. and Tanaka K. (1983) Cytogenetic studies of hematological disorders in atomic bomb survivors . *Radiation-induced Chromosome Damage in Man,* (T. Ishihara and M. S. Sasaki Eds.), pp.455－474, Alan R. Liss, New York

Kamada N. and Tanaka K, Oguma N., Mubuchi K.(1991) Cytogenetic and molecular changes in leukemia among atomic bomb survivors. *J. Radiat Res.* Vol.32,Suppl.2, pp.257-265.

Lorimore S. A., Coates P. J., Scobie G. E., Milne G., Wright E. G. (2001) Inflammatory-type responses after exposure to ionizing radiation *in vivo*; a mechanism for radiation-induced bystander effects? *Oncogene* Vol.20, pp. 7085-7095.

Nakanishi M.,Tanaka K., Shintani T., Takahashi T., Kamada N.(1999) Chromosomal instability in acute myelocytic leukemia and myelodysplastic syndrome patients among atomic bomb survivors. *J. Radiat. Res.* Vol.40,pp.159-167.

Nakanishi M., Tanaka K., Takahashi T., Kyo T., Dohy H., Fujiwara M., Kamada N.(2001) Microsatellite instability in acute myelocytic leukemia derived from A-bomb survivors. *Int. J. Rad. Biol.* Vol. 77, pp. 687-694.

Neriishi K, Nakashima K, Delongchamp R. R. (2003) Persistent subclinical inflammation among A-bomb survivors. *Int. J. Radiat. Res.* Vol. 77, pp.475-482.

Nilson L., Edén P., Olsson E., Månsson R., Åstrand-Grundström I., Storömbeck B., Theilgand-Mönch K., Andeson K., Hast R., Hellstörme-Lindberg E., Samuelsson J., Berg G., Nerlov C., Johansson B., Sigvardsson M., Borg Å, Jacobsen S. E. W.(2007) The molecular signature of MDS stem cells supports a stem-cell origin of 5q-myelodysplastic syndromes. *Blood* Vol. 110, No.8, pp.3005-3014.

Pierce D. A., Shimizu Y., Preston D. L., Vaeth M., Mabuchi K. (1996) Studies of the mortality of atomic bomb survivors. Report 12, Part 1. Cancer: 1950-1990. *Radiat. Res.* Vol.146, No.1, pp.1-27.

PrestonD. L., Kusumi S., Tomonaga M., Izumi S., Ron S., Kuramoto A., Kamada N., Dohy H., Matsuo T., Masui T.(1994) Cancer incidence in atomic bomb survivors. Part 1. Leukemia, lymphoma and multiple myeloma, 1950-1987. *Radiat. Res.* Vol. 137(Suppl.) S86-97.

Richardson D., Sugiyama H., Nishi N., Sakata R., Shimizu Y., Gran E. J., Soda M., Hsu W. L., Suyama A., Kodama K., Kasagi F.（2009）Ionizing radiation and leukemia mortality among Japanese Atomic Bomb Survivors, 1950-2000. *Radiat. Res.* Vol.172, No.3, pp. 368-382.

Suzuki K., Ojima M., Kodama K., Suzuki M., Oshimura M., Watanabe M. (2003) Radiation-induced DNA damage and delayed induced genomic instability. *Oncogene* Vol.22, pp. 6988-6993.

Tanaka I. B., Tanaka S., Ichinohe K., Matsushita S., Matsumoto T., Otsu Y., Sato F. (2007) Cause of death and neoplasia in mice continuously exposed to very low-dose rates of gamma rays. *Radiat. Res.* Vol. 167, pp. 417-437.

Tanaka K., Hoshi M., Kamada N., Sawada S. (1994) Effects of 252 Cf neutrons, transmitted through an iron block on human lymphocyte chromosome. *Int. J. Radiat. Biol.* Vol. 66, pp.391-397.

Tanaka K., Arif M., Eguchi M., Kyo T., Dohy H., Kamada N.(1997) Frequent jumping translocations of chromosomal segment involving the ABL oncogene alone or in combination with CD3-MLL genes in secondary leukemias. *Blood*Vol. 89, pp. 596-600.

Tanaka K., Gajendiran N., Endo S., Hoshi M., Kamada N. (1999) Neutron energy-dependent initial DNA damage and chromosomal exchange. *J. Radiat. Res.* Vol.40, Supl. pp. 36-44.

Tanaka K. , Eguchi M., Eguchi-Ishimae M., Hasegawa A., Ohgami A., Kikuchi M., Kyo T., Asaoku H., Dohy H. and Kamada N. (2001) Restricted chromosome breakpoint sites on 11q22-23.1 and 11q25 in various hematological malignancies without *MLL/ALL-1* gene rearrangement. *Cancer Genet. Cytogenet.* Vol. 124, pp. 27-35.

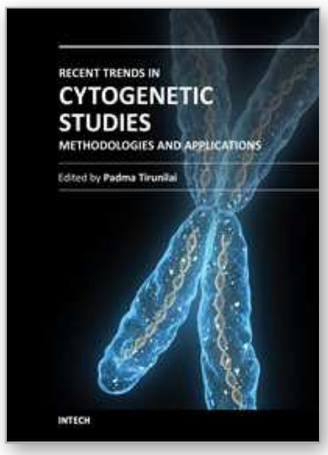
Tanaka K. and Ihda S. (2008) Radiation-induced chromosome instability and luekemogenesis in human. *J. Genetic Toxicol.* Vol. 1, No. 1,pp.1-19.

Tanaka K., Kumaravell T. S., Ihda S., Kamada N. (2008) Characterization of late –arising chromosome aberrations in human B-cell lines established from alpha-ray- or gamma-ray-irradiated lymphocytes. *Cancer Genet. Cytogenet.* Vol 187, pp. 112-124.

Ullrich R. L. and Davis C. M. (1999) Radiation-induced cytogenetic instability *in vivo*. *Radiat. Res.* Vol. 152, pp. 170-173.

Watson G. E., Lorimore S. A., Wright F. G. (1996) Long-term *in vivo* transmission of alpha-particle-induced chromosomal instability in murine haemopoietic cells. *Int. J. Radiat. Biol.* Vol. 69, pp. 175-182.

Watson G. E., Pocock D. A., Papworth D., Lorimore S. A., Wright E. G. (2001) *In vivo* chromosomal instability and transmissible aberrations in the progeny of hematopoietic stem cells induced by high- and low-LET radiations. *Int. J. Radiat. Biol.* Vol. 77, pp. 409-417.



Recent Trends in Cytogenetic Studies - Methodologies and Applications

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Recent Trends in Cytogenetic Studies - Methodologies and Applications deals with recent trends in cytogenetics with minute details of methodologies that can be adopted in clinical laboratories. The chapters deal with basic methods of primary cultures, cell lines and their applications; microtechnologies and automations; array CGH for the diagnosis of fetal conditions; approaches to acute lymphoblastic and myeloblastic leukemias in patients and survivors of atomic bomb exposure; use of digital image technology and using chromosomes as tools to discover biodiversity. While concentrating on the advanced methodologies in cytogenetic studies and their applications, authors have pointed out the need to develop cytogenetic labs with modern tools to facilitate precise and effective diagnosis to benefit the patient population.

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