Hiroshima Journal of Medical Sciences
Vol. 33, No. 4, 659~662, December, 1984
HIJM 33-92

Chromosome Analysis of Bone Marrow Fibroblast Colony-Forming Cells (CFU-F) in Heavily Exposed Atomic Bomb Survivors*)

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(Received September 18, 1984)

Key words: Chromosome aberration, Atomic bomb survivor, Fibroblast, CFU-F

ABSTRACT

A chromosome analysis was performed on cultured bone marrow fibroblasts (CFU-F) from two atomic bomb survivors exposed within 1 km of the hypocenter, whose estimated radiation dose is 357 rad and 365 rad respectively. In CFU-F of both cases, stable types of chromosome aberrations were detected. The rate of these chromosome aberrations related well to the chromosome aberration rate of peripheral T-lymphocytes of the same case. These findings suggest the possibility that chromosome aberration exists in somatic cells other than hematopoietic cells of heavily exposed atomic bomb survivors.

INTRODUCTION

An elevated incidence of leukemia and malignant tumors has been observed as late radiation effects among atomic bomb survivors. Furthermore, a positive correlation has been demonstrated between chromosome aberrations of somatic cells of atomic bomb survivors and their radiation exposure dose[1]. Today some 40 years after exposure to the atomic bomb even among healthy proximal exposed survivors with normal hematological values, a high frequency of chromosome aberrations has been seen in bone marrow cells and T- and B-lymphocytes[11]. In addition, as chromosome aberrations observed in T lymphocytes of proximal exposed survivors appear in a high frequency among chromosomes closely related to chromosome aberrations demonstrated in leukemia cells[17], interest is now focussed on the relation between the mechanism of leukemogenesis and chromosome breaks and translocations at the oncogene sites.

With the recent advances made in the techniques of blood cell culture, the presence of chromosome aberrations has been demonstrated even in the hematopoietic stem cell level[16]. It is considered that the proliferation of blood cells is under some control from the interaction between progenitor cells and their hematopoietic environment. Directing our attention to myelofibroblasts as one of the components of the hematopoietic environment, we conducted a chromosome analysis on colony forming unit of fibroblasts (CFU-F) of proximal exposed survivors.

SUBJECTS AND METHODS

The subjects of the present study are two healthy female A-bomb survivors exposed within 1 km from the hypocenter in Hiroshima with no abnormal hematological findings at present. Their age at time of exposure was 20 and 22 and their estimated T65D radiation exposure dose is 357 rad and 365 rad, respectively.

METHOD OF COLONY ASSAY AND CHROMOSOME ANALYSIS

5×10^5 bone marrow mononuclear cells iso-

*) 重田千晴，田中公夫，川上正仁，大北　威：近距離原爆被爆者における骨髄線維芽細胞 (CFU-F) の染色体分析

Table 1. Chromosome analysis of cultured bone marrow fibroblasts (CFU-F) in atomic bomb survivors

Case	Estimated radiation dose (T65D)	No. of cells analysed	No. of cells with abnormal karyotype	Chromosome aberration rate (%)	Chromosome aberration rate in T-lymphocyte (%)	Abnormal karyotype in fibroblast (CFU-F)
1	357 rad	21	3	14.8	14.3	46, XX, t (14p;15p), inv (5) (p−q+) 46, XX, 6q− 46, XX, −20, +mar
2	365 rad	7	2	28.6	15.8	46, XX, t (Cq−;Fq+) 47, XX, t (Cq−;Fq+), Cq−, +mar

lated by density gradient centrifugation were suspended in 5 ml of 20% FCS·α medium and then placed in plastic culture dish 6 cm in diameter for culture in 5% CO_2 at 37°C for 12–14 days to obtain CFU-F of bone marrow origin[2]. To CFU-F colony adhered to the culture dish, 0.25% trypsin·EDTA solution was added and after incubation for 5 min at 37°C the cells were pipetted off. After rinsing the cells with α medium, fibroblast growth factor (50 ng/ml) was added to 20% FCS·α medium and then cultured for 2–3 days in 5% CO_2 at 37°C within a slide chamber. Thereafter, 0.01 γ/ml of colcemid was added for culture for 8–10 hr and the cells which adhered and proliferated in the culture dish were subjected to hypotonic treatment in a mixture of 0.075 M KCl and 1% sodium citrate (4:1). After fixing and air drying with flaming, the specimen was spread and then stained with Giemsa stain. All the available metaphases were photographed for karyotype analysis.

RESULTS

The results of chromosome analysis of bone marrow CFU-F colony obtained by the foregoing method are shown in Table 1. In case 1, of the 21 observed cells chromosome aberrations were observed in 3 cells (chromosome aberration rate of 14.3%) and in the remaining case, of the 7 observed metaphases chromosome aberrations were observed in 2 cells (chromosome aberration rate of 28.6%). In case 1, the abnormal karyotypes were 46, XX, t (14p; 15p), inv (5) (p−q+); 46, XX, 6q−; 46, XX, −20, and +mar, while in Case 2, the abnormal karyotypes were 46, XX, t (Cq−;Fq+); 47, XX, t (Cq−;Fq+), Cq− and +mar. All the detected abnormal karyotypes were abnormalities of stable type such as translocation, inversion, and deletion (Fig. 1).

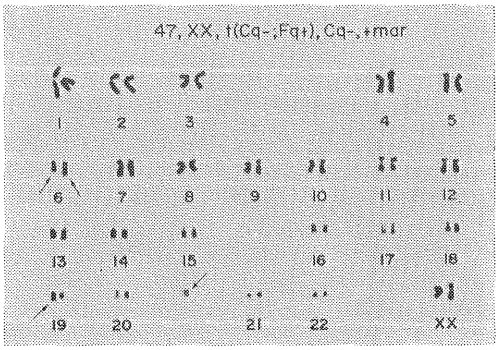

Fig. 1. Karyotype of bone marrow fibroblast in case 2

DISCUSSION

Even today some 40 years after exposure to the atomic bomb, chromosome aberrations are observed in a high frequency in the bone marrow cells, T- and B-lymphocytes and skin fibroblasts of proximal exposed survivors whose hematopoietic tissue is assumed to have received a serious damage from radiation exposure. However, abnormal values are not necessarily observed in the routine blood tests of these survivors harboring such chromosome aberration in the hematopoietic cells and the biological implication of the organic abnormalities of these cells still remains unknown.

With the developments made in *in vitro* colony assay methods, it has become possible to pursue hematological disorders and malignant transformation at the hematopoietic stem cell level. For example, in chronic myelogeneous leukemia chromosome aberration has been detected at the pluripotent stem cell level[16], suggesting a relationship between chromosome

aberration and leukemogenesis. Our chromosome analysis of bone marrow erythroid colony of the two proximal exposed survivors has shown that the chromosome aberration rate shows a good correlation with the chromosome aberration rate observed in the peripheral T-lymphocytes of the same case[15]. These findings indicate that the bone marrow cells and T- and B-cells of atomic bomb survivors have already at the stem cell level chromosome aberrations and suggests at the same time a possibility of a deep relationship to the process of leukemogenesis.

On the other hand, much importance is attached also to the role played by bone marrow stromal cells in the environment of proliferation and maturation of bone marrow hematopoietic progenitors. In recent years studies have been made on fibroblasts, one of the stromal cells, with attention given to the character of their progenitor cells as CFU-F and to their function in hematopoiesis[2,4-6,8,14], but little has been reported on cytogenetic aspects of CFU-F. We conducted a chromosome analysis with the use of CFU-F of a Ph^1 positive chronic myelogeneous leukemia case exposed at a distance of 1.9 km, but Ph^1 chromosome could not be detected in the fibroblast of this case. Excluding one case reported by Hentel et al.[9], other workers have also reported only observing normal karyotype in their chromosome analysis of CFU-F of Ph^1 positive chronic myelogeneous leukemia cases[3,7,12,18]. In view of the fact that marker chromosomes could not be detected in CFU-F of bone marrow origin of chronic myelogeneous leukemia patients and of the results of Fialcow on G6PD isoenzyme[10], it is the opinion of the majority that the origin of CFU-F and of hematopoietic stem cells differs. This detection of chromosome aberrations in both the colony of hematopoietic stem cell origin and in stromal cells considered to have a different origin suggests the possibility that chromosome aberrations exist in somatic cells outside the hematopoietic organs. In proximal exposed survivors it is known that the incidence of not only leukemia but also other malignant tumors is high[13]. The very interesting findings suggesting a relation between carcinogenesis of other organs and chromosome aberrations indicate that further studies should be made.

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