CHANGES IN RADIOSENSITIVITY OF VARIOUS CELLULAR STAGES OF MEGAKARYOPOIESIS IN MOUSE BONE MARROW CULTURE

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Introduction

Methods to culture for mouse megakaryocyte progenitor cells have been established in many laboratories (Metcalf et al. 1975 and Williams and Jackson, 1982). The results indicated that multiple cellular stages of bone marrow cells during megakaryopoiesis can be observed in cultures. The establishment of these cultures has provided a reliable system for the quantative analysis of radiosensitivity of each cellular stage in megakaryopoiesis. In this experiment, the effect of radiation on early and late stages in megakaryopoiesis was examined by using 3 and 72 hr cultures of mouse bone marrow, respectively.

Materials and Methods

Balb/C female mice, 10 weeks old, were used for all the experiments. Bone marrow cells were aseptically obtained from femurs by flushing the bone marrow cavities with NCTC 109 medium. The bone marrow cells in the suspension were seeded at $3x10^5$ cells in 0.4 ml of plasma culture which contained 10% lymphocyte conditioned medium. The cultures were fixed with 5% glutaraldehyde every day for 15 days after the seeding. After 15 mins of fixation, the cells in the cultures were stained for acetylcholinesterase activity. The acetylcholinesterase positive cells were scored as megakaryocyte colony. The 3, 24, 72 and more than 120 hr cultures received a dose of 1, 2, 3, 6, 12 and 20 Gy from a 200 KeV x-ray machine (Shimazus Co.) with a dose rate of 1 Gy/min.

Results

The kinetic for colony formations of megakaryocyte progeniter cells (CFU-M) from mice in culture are shown in figure 1. It can be seen that the CFU-M appeared on day 2 after seeding of the bone marrow cells and reached a maximum at 120 hr culture, then decreased to about 50% of the maximum at 168 hr. The kinetic indicated the process of maturation of the megakaryocyte progeniter cells of the mice, because morphologically

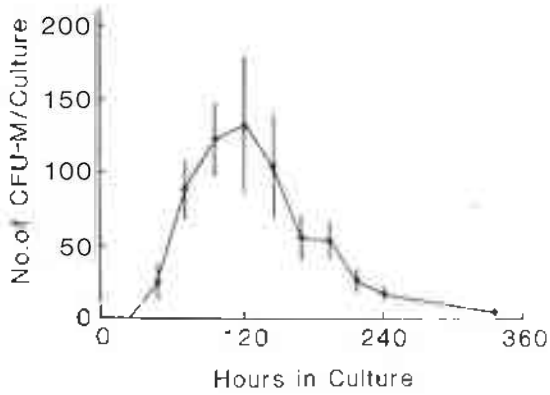

Figure 1. The kinetic of colony formations of megakaryocyte progeniter cells of mouse bone marrow in plasma culture.
The mouse bone marrow cells in the suspension were seeded at $3x10^5$ cells in plasma culture dishes which contained 10% lymphocyte conditioned medium. These cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air for 15 days. The cultures were fixed with glutaraldehyde every day after seeding. After the fixation, the cells in the cultures were stained for acetylcholinesterase activity to score the megakaryocyte.

the majority of the colonies showed immature megakaryocytes at 50 hr culture, complete shapes of megakaryocytes at 120 hr culture and denaturation of the cytoplasm at 168 hr culture.

The response to radiation in the colony formations during megakaryopoiesis of mice is shown figure 2. As shown by the open circles in the figure, the dose survival response of CFU-M in 3 hr culture has no shoulder with a single slope, giving a mean lethal dose (D_0 dose) of 1.5 Gy. The shape of the dose survival response in 24 hr culture was identical to that in 3 hr culture. On the other hand, the dose survival response of CFU-M in 72 hr culture consists of a bi-phasic slope, as shown by the closed circles of figure 2. The D_0 doses of the initial slope and the terminal slope are 11 and 22.5 Gy, respectively. The dose responses

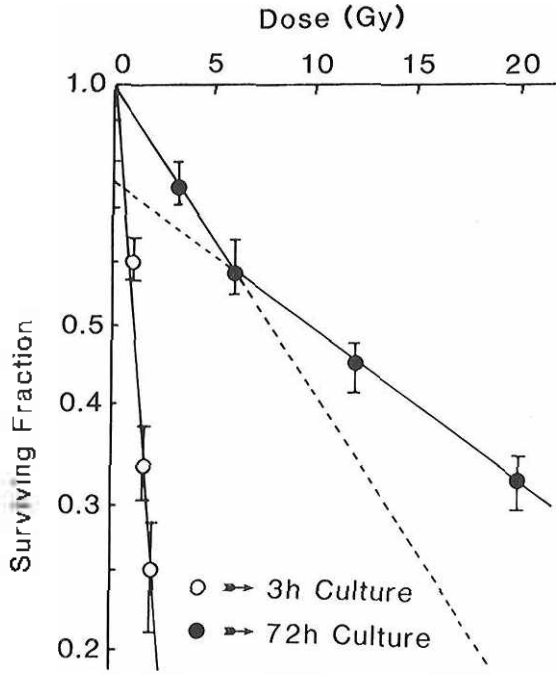

Figure 2. The dose survival curves at different stages of megakaryopoiesis in mouse bone marrow culture.
The 3 and 72 hr cultures were exposed to doses of X-ray, then specimens for the cells were made as already described in figure 1. The specimens were observed for scoring megakaryocyte colonies. The survival fraction after irradiations was expressed as the ratio of the colony number of post-irradiation over that of unirradiated control. The survival fractions were expressed with open circles in 3 hr culture and with closed circles in 72 hr culture.

were also tested for more than 72 hr culture. However, the effect of cell killing by radiation was not detectable in such old culture tested the mentioned doses. The results indicated that the radiosensitivity of mouse megakaryocyte decreased with the progress into the differentiated stages.

Discussions

Results indicated that the megakaryopoiesis in the culture of mouse bone marrow cells can be observed morphologically and the decrease of radio-sensitivity of megakartocyte can be observed with the progress of megakaryopoieses.

In the culture of mouse megakaryocyte progeniter cells, the maturation of the progeniter cells can be observed after 92 hr culture. However, the generation of thrombocyte is not detected during 15 days of the culture. It means that this culture system may lacking a stimulating factor to generate thrombocytes.

As shown in figure 2, the radiosensitivity of CFU-M in 3 hr culture differs extremely from that of 72 culture. In the survival response of CFU-M in 3 or 24 hr cultures, the D_0 dose is 1.5 Gy which is almost comparable to D_0 dose (1.2 Gy) of CFU-M reported by Nakeff et al. (1979) based on *in vivo* irradiation. The dose survival response of CFU-M in 3 or 24 hr cultures also reveals a single slope. It implies that the megakaryocyte progeniter cells at early steps of the development may be synchronously growing and relatively homogeneous on radiosenstivity. On the other hand, the dose response curve of CFU-M in 72 hr culture shows a bi-phasic slope. The bi-phasic response suggests that there are at least two kinds of cell population in terms of radiosensitivity.

These results suggest that relatively undifferentiated cells of megkaryocytes are more radiosentive compared to well differentiated ones. (**Key Words**: Differentiation, Megakaryocyte, Radiosensitivity)

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