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 quantitative 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 progenitor 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 progenitor cells of the mice, because morphologically

![Figure 1. The kinetic of colony formations of megakaryocyte progenitor 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.](image)

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
Radio-sensitivity of megakaryocyte can be observed with the progress of megakaryopoiesis.

In the culture of mouse megakaryocyte progenitor cells, the maturation of the progenitor 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 Nakoff et al. (1979) based on \textit{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 progenitor cells at early steps of the development may be synchronously growing and relatively homogeneous on radiosensitivity. On the other hand, the dose response curve of CFU-M in 72 hr culture shows a bi-phasic slope. These results suggest that there are at least two kinds of cell population in terms of radiosensitivity.

"Key Words: Differentiation, Megakaryocyte, Radiosensitivity"

\section*{Literature Cited}

