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Effect of X-Ray Irradiation on Proliferation and Differentiation of Osteoblast

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Summary

We exposed the osteoblast-like cell line, MC3T3-E1, to 1-to 10-Gy X-ray. Irradiation at doses of 5-Gy dose or more decreased the DNA content of cells at the proliferation stage, confluence, and post-proliferation stages. The alkaline phosphatase activity, conversely, was increased by irradiation, and the calcium content of irradiated cells was greater than that of nonirradiated. These findings suggest that irradiation induces terminal differentiation and calcification of osteoblasts.

Key words: Irradiation—Osteoblast—Differentiation.

Introduction

Bone is constructed by endochondral ossification or membranous ossification, and it is well known that radiation therapy in children impairs bone formation [1,2]. We recently investigated the effect of irradiation on endochondral ossification using rabbit growth plate chondrocytes [3,4]. Irradiation is also known to decrease the width of long bones which are formed by membranous ossification[2]. Although it is thought to affect osteoblast function, direct action of irradiation on osteoblast differentiation has not been fully elucidated. In the present study, we examined the effects of irradiation on proliferation and differentiation of osteoblast-like cell line MC3T3-E1.

Materials and methods

1. Materials. MC3T3-E1 cell line was purchased from RIKEN Cell Bank, alpha modified minimum essential medium (αMEM) from Flow Laboratories (McLean, VA), and fetal bovine serum from Mitsubishi Kasei Co. (Tokyo).
2. Culture. The cells were seeded at the density of 5,000/cm^2, and cultured with αMEM containing 10% fetal bovine serum. After confluence, 50μg/ml of ascorbic acid and 5mM of β-glycerophosphate were added to observe matrix calcification.
3. Irradiation. Cultured cells were exposed to X-ray irradiation on day 2, at the most actively proliferating stage, on day 4, when confluent growth was seen, and on day 8, at which stage most of the cells had stopped proliferating and showed alkaline phophatase activity (ALPase). The cells were exposed a single time to irradiation at the dose rate of 25cGy/min generated from a Hitachi X-ray Generator (model 1505) operating at 130kV and 3mA (additional filtration of 0.1mm copper and 0.5mm aluminum). The culture plates were placed on a turntable that completed one revolution every 10 seconds. An Ionex dosimeter, type 2500-3, placed in the center of the table was used to monitor the dose exposure.
4. Determination of ALPase activity. ALPase activity was measured by a modification of the method of Bessey et al. using para-nitrophenyl phosphate (pNP) as a substrate, as described previously [5,6]. One unit was defined as the activity catalyzing the hydrolysis of 1μmole pNP / μg DNA / 30 min.
5. Determination of DNA content. The DNA content was measured as previously described [7,8].
6. Determination of calcium content. The calcium content was determined using an atomic absorption spectrophotometer (Model AA-640, Shimadzu, Kyoto), as described previously [9].

Result & Discussion

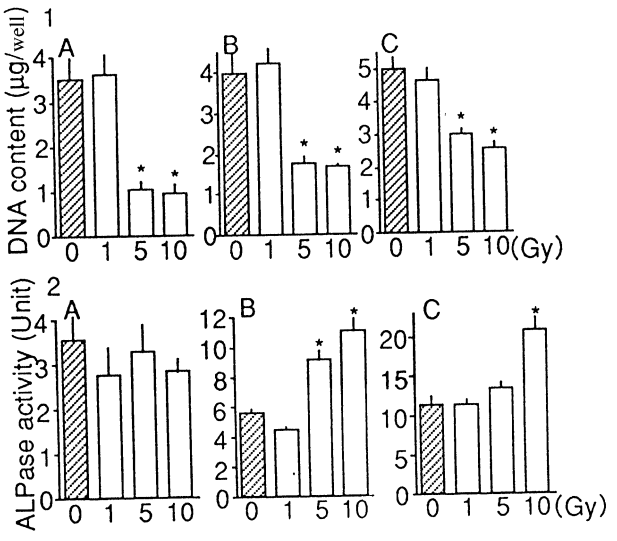

Figure 1
Effects of irradiation on DNA content (1) and ALPase activity (2)
Cells were exposed to irradiation at various doses on day 2(A), day 4(B), or day 8(C). The DNA content (1) and ALPase activity (2) were determined 4 days after irradiation. Each bar shows the mean ±S.D. for 3 samples. *: $p<0.05$;1unit : hydrolysis of 1μmole pNP /μgDNA /30 min

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A 5- to 10-Gy dose of irradiation decreased the DNA content (Fig. 1-1). X-ray irradiation on day 8, when most of the cells had ceased proliferation, also decreased the DNA content 4 days after irradiation. This indicates that irradiation at 5 Gy or more not only inhibited cell proliferation but also induced cell death.

As shown in Figure 1-2, irradiation on day 2 did not have an influence on the ALPase activity 4 days after irradiation. On the other hand, irradiation on day 4 or day 8, at which stages cell proliferation activity was lower, increased the ALPase activity in a dose-dependent fashion.

In this culture, uptake of ^{45}Ca in the extracellular matrices was increased after day 24, and on day 30 there were nodules in which calcification was observed (data not shown). A 10-Gy dose of irradiation increased the calcium content. The later the stage at which the cells were exposed, the less prominent was the increase of calcium content (Fig. 2). Interestingly, the greatest increase of the ALPase activity on day 30 was observed when the cells were exposed to irradiation on day 8 (data not shown). It is reported that some proteins in bone matrix, such as osteopontin, osteocalcin, and type I collagen, have an influence on osteoblast calcification [11,12]. One reason that the increase of ALPase activity showed no direct relation to the effect of irradiation in promoting calcification may be that the mineralization-related proteins are affected by irradiation. Investigation of the effect of irradiation on the expression of these mineralization-related proteins could clarify the mechanisms by which irradiation promoted calcification.

Irradiation induces terminal differentiation in some other culture systems, such as, human neuroblastomas [12-14] and fibroblasts [15]. In these systems, induction of differentiation is accompanied by cessation of proliferation. In the osteoblast, it seems indicated that inhibition of the cell proliferation by irradiation may induce osteoblast differentiation.

We showed in this study that X-ray irradiation of the osteoblastic cell line MC3T3-E1 inhibited cell proliferation and enhanced the differentiated phenotype. This suggest that the inhibitory effect of irradiation on proliferation may take a part in impairment of bone formation after irradiation *in vivo*. Furthermore, because the more differentiated osteoblast is unlikely to proliferate, we speculate that stimulation of osteoblast differentiation by irradiation may increase the likelihood of inhibited bone growth.

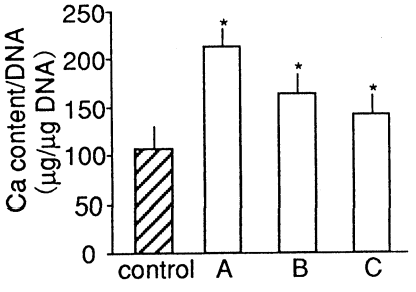

Figure 2
Effects of irradiation on calcium content of the culture
Cells were exposed to a 10-Gy dose of irradiation on day 2(A), day 4(B), or day 8(C), and the calcium contents were determined on day 30. Each bar shows the mean ±S.D. for 3 samples. * : $p<0.05$ compared to the calcium content of the control cultures.

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