Radiotherapy is considered to cause detrimental effects on bone tissue eventually increasing bone loss and fracture risk. However, there is a great controversy on the real effects of irradiation itself on osteoblasts, and the mechanisms by which irradiation affects osteoblast differentiation and mineralization are not completely understood. We explored how X-ray radiation influences differentiation and bone-specific gene expression in mouse calvarial osteoblasts. Irradiation at 2 Gy not only increased differentiation and mineralization of the cells, but also upregulated the expression of alkaline phosphatase, type I collagen, osteopontin, and osteocalcin at early stages of differentiation. However, irradiation at higher doses (>2 Gy) did not stimulate osteoblast differentiation, rather it suppressed DNA synthesis by the cells without a toxic effect. Additional experiments suggested that transforming growth factor-beta 1 and runt-related transcription factor 2 play important roles in irradiation-stimulated bone differentiation by acting as upstream regulators of bone-specific markers. [BMB Reports 2012; 45(10): 571-576]

INTRODUCTION

Radiotherapy is a useful treatment for oral cancer in combination with surgery. However, irreversible damage occurs to intact bone tissue after therapeutic irradiation depending on the quantity of ionizing radiation used. Persistent irradiation damage causes osteoradionecrosis followed by a loss of bone mass and an increase in bone fracture risk (1, 2). Numerous studies have attempted to determine the mechanisms by which irradiation affects bone differentiation and mineralization. Considerable findings have shown that irradiation leads to detrimental effects on cells, as evidenced by decreased viability and/or DNA synthesis in bone-like (3-5), leukemia (6), and mesenchymal stromal cells (7). Irradiation also results in single or double-stranded DNA breaks and eventually blocks cell cycle progression (6). Therefore, it is believed that irradiation predominantly induces DNA damage with attendant growth inhibition and that this is responsible for suppressing osteoblast differentiation and mineralization. However, there is a great controversy on the actual effects of irradiation on bone, in which irradiation stimulates osteoblast differentiation (5, 8). This stimulation appears to be associated with increasing the levels of bone-specific regulatory markers such as alkaline phosphatase (ALP), transforming growth factor-beta 1 (TGF-β1), and runt-related transcription factor 2 (Runx2) (3, 8, 9). mRNA expression of bone differentiation-related proteins, such as type I collagen (COL I), osteocalcin (OCN), and osteopontin (OPN), is dynamically affected by irradiation, and the resulting effects differ according to the quantity of radiation employed and the times of testing after irradiation (5, 10, 11). In addition, the cellular mechanisms by which X-ray radiation affects differentiation and mineralization of osteoblasts are still unclear. Furthermore, almost all previous experiments used bone-like cell lines instead of primary osteoblast cultures.

In this study, we investigated how X-ray radiation influences differentiation and mineralization of mouse calvarial osteoblasts. We determined mRNA and/or protein expression patterns of ALP, COL I, OCN, OPN, TGF-β1, and Runx2 to explore the possible mechanisms involved in irradiation-stimulated osteoblastic differentiation.

RESULTS

Ionizing irradiation increases ALP activity and stimulates mineralization in calvarial osteoblasts based on quantity

We initially explored the effects of X-ray radiation on mineralization of osteoblasts. Exposure of the cells to low-dose radiation increased the formation of bone-like nodules at 21 days after radiation (Fig. 1A). The number of bone-like nodules was augmented up to 131% and 134% in the 1 and 2 Gy-irradiated groups, respectively, compared to that in the non-irradiated controls (Fig. 1B). However, irradiation at >2 Gy did not stimulate cells mineralization. In parallel, the deposition of calcium ion was significantly higher in the groups exposed to 1 or 2 Gy radiation than those exposed to 4 or 8 Gy radiation as compared to that in the control groups (Fig. 1C). The groups irradiated with 2 Gy, but not 4 Gy, revealed a significant in-
Osteoblastic stimulation by irradiation
Soon-Sun Park, et al.

Ionizing irradiation stimulates mRNA expression of bone-specific markers in primary osteoblasts
The results from real time reverse transcription polymerase chain reaction (RT-PCR) analyses revealed that mRNA expression of osteoblast differentiation-related genes, such as ALP, COL I, and OPN, was stimulated 7 days post-irradiation, and that 2 Gy radiation led to the most prominent increase in ALP, COL I, and OPN mRNA levels (Fig. 2). The irradiation-mediated increase in ALP and COL I mRNA levels also occurred at 14 days after 2 Gy irradiation. However, exposure of the cells to 4 Gy irradiation caused a significant decrease in mRNA expression specific to ALP and OCN at 21 days after radiation.

Ionizing irradiation increases TGF-β1 and Runx2 mRNA expression at the early stages of osteoblastic differentiation
Results from the RT-PCR analysis showed that irradiation increased TGF-β1 and Runx2 mRNA expression levels at 3 days post-irradiation (Fig. 3A). These findings were supported by the results of real time-PCR in which X-ray radiation dramatically facilitated mRNA expression of both TGF-β1 and Runx2 at 3 days post-irradiation (Fig. 3B, C). When mRNA expression levels were determined at 7 days post-irradiation, TGF-β1 level had increased only in 4 Gy-irradiated cells, whereas the Runx2 mRNA level was augmented in cells irradiated with 1 or 2 Gy. Runx2 and TGF-β1 mRNA expression was not affected by X-ray radiation at 14 days post-irradiation, whereas the levels were significantly suppressed in 4 Gy-irradiated cells 21 days after radiation.

Ionizing irradiation at low doses promotes secretion of cytokines from osteoblasts without cytotoxicity
TGF-β1 levels were determined in conditioned media at various...