INTRODUCTION: Bone fragility is a serious consequence of radiotherapy (RTx) which typically occurs months to years after treatment ends. Fragility can lead to pathological fractures which pose a significant health risk to patients undergoing RTx. There are currently no treatments available to prevent bone fragility resulting from RTx. Previous murine data has shown a short-term increase in osteoclast activity in the weeks following RTx that is hypothesized to contribute to increased bone resorption. However, the specific mechanisms that drive post-RTx osteoclast activation are incompletely understood. The goal of this study was to examine osteoclast activation following RTx and identify how post-RTx osteoclast signaling differs from homeostatic signaling.

METHODS: The murine pre-osteoclast-like cell line RAW 264.7 was utilized to conduct in vitro experiments. To assess osteoclast activation, cells were cultured in media supplemented with 50 ng/mL RANK-L (n=9), 10 ng/mL TNFa (n=9) or 50 ng/mL RANK-L and 10 ng/mL TNFa (n=9). Irradiated cells received a single dose of 4 Gy x-irradiation and were cultured in media supplemented with (n=9) or without (n=9) 50 ng/mL RANK-L. The control group was grown in standard cell culture media (n=9). Cells were analyzed for osteoclast-like differentiation by staining for tartrate-resistant acid phosphatase (TRAP). Osteoclast-like cells were identified by the presence of ≥3 nuclei and TRAP(+) staining. Expression of osteoclast-related gene expression was examined through qRT-PCR. Genes related to cytoskeletal rearrangement (capn1, c-src), extracellular matrix degradation (mmp9, ctsk), and cellular signaling (tyrobp, nfatc1, rac1) were analyzed. Resorption activity was assessed by plating cells on culture plates coated with a bone biomimetic synthetic surface. After 5 days in culture, culture plates were incubated with a 10% bleach solution to lyse cells. Void patterns were quantified using ImageJ.

RESULTS: RTx treatment alone did not yield any osteoclast-like cells. Following combination RANK-L and RTx treatment, RAW 264.7 differentiated into osteoclast-like cells that were larger when compared to RANK-L treatment alone. Combination RANK-L and TNFa treatment also resulted in larger osteoclast-like cells when compared to RANK-L treatment alone. Resorbed area increased with RANK-L treatment alone when compared to the control. No significant change in resorbed area was observed with combined RANK-L and RTx treatment compared to the control.

H$_2$O$_2$ treatment significantly upregulated expression of genes associated with oxidative stress, including ccl5, ehd2, gclm, nos1 and sod1 in RAW 264.7 cells. Of the 9 genes upregulated significantly by H$_2$O$_2$, 8 were also upregulated significantly when compared to the control by RTx treatment. A significant increase in CM-H$_2$DCFDA localization was noted in both the H$_2$O$_2$ and RTx groups 1 hour after treatment when compared to the control.

DISCUSSION: The data suggests that while RTx is not sufficient to activate osteoclasts, RTx in combination with RANK-L upregulates osteoclast activation. Osteoclast-like cells formed from combination RTx and RANK-L treatment were larger than those formed from RANK-L alone. However, resorption activity was not observed in osteoclast-like cells formed from combination RTx and RANK-L treatment. Further experimentation is needed to determine if this activity is exclusive to the RAW 264.7 cell line. If primary cells respond similarly, RTx may hinder the ability of osteoclasts to form a sealing zone or produce enzymes that resorb bone tissue. The data also showed that RTx induces a direct oxidative stress response in pre-osteoclasts. Further studies will focus on the downstream effects of the stress response induced in pre-osteoclasts. It is possible that oxidative stress induces osteoclastogenesis in a mechanism that is reliant on RANK-L, such as increasing RANK expression. Finally, future studies will focus on the osteocyte response to RTx to gain a better understanding of bone homeostasis following radiation treatment.

Osteocytes are a major contributor to RANK-L production and therefore osteocytes may be involved in increased RANK-L production following RTx.

CLINICAL SIGNIFICANCE: Radiation-induced bone fragility is a serious clinical problem that currently has no preventative treatments and limited therapeutic options. Understanding the specific ways in which osteoclast activity changes post-RTx is an important step in designing treatments to restore homeostatic osteoclast function following radiotherapy and prevent bone loss.

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