The Role of TGF-β in Radiation-Induced Pain Driven by Osteoclast-Neuron Crosstalk

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INTRODUCTION: Chest wall pain affects ~40% of patients with lung cancer who receive thoracic stereotactic body radiotherapy (SBRT).[1] While radiation increases osteoclast (OC) resorptive activity and induces rapid bone loss with subsequent fracture,[2] the molecular mechanism responsible for radiation-induced pain is unclear. A clinical trial completed at our institution used risedronate, a bisphosphonate that reduces OC activity and bone loss,[3] to prevent rib fractures in lung cancer patients receiving SBRT.[4] Although treatment with risedronate showed no reduced fracture rate, the risedronate group had a significant reduction in Grade 2+ chest wall pain. Our group has shown that neurons treated with conditioned media from irradiated OCs demonstrate elevated expression of pain biomarkers Calcitonin Gene-Related Peptide (CGRP) and Substance P (SP). However, this increased expression is prevented when neurons are exposed to conditioned media from irradiated OCs treated with risedronate. To investigate the molecular mechanism responsible for risedronate reducing radiation-induced pain, proteomics analysis of small extracellular vesicles isolated from conditioned media of irradiated OCs identified TGF-βR1 as a central molecule implicated in both bone loss and pain signaling. Therefore, the objective of this *in vitro* study was to determine if TGF-βR1 inhibition decreased pain biomarker expression when exposing sensory neurons to conditioned media from irradiated OCs.

METHODS: Animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the Wake Forest University School of Medicine (IACUC Protocol #A23-056). RAW264.7 murine macrophages were differentiated into mature osteoclasts using RANKL (35 ng/mL). After 3 days of differentiation, cells were re-fed with fresh growth media containing RANKL (35 ng/mL). The treatment group was irradiated with 10 Gy using the Precision X-ray SmART+ system (220kVP X-Rays). Control osteoclasts were not irradiated. Thoracic dorsal root ganglia (DRG) from T1-T13 were dissected from WT C57BL/6 mice (8-12 weeks old) and prepared following the protocol by Park et al.[5] DRG cells were washed, counted, and seeded on coverslips pre-coated with Poly-D-lysine and laminin for 48 hours in a 24-well plate using neuronal growth medium. Neuronal cultures were treated with conditioned media from irradiated osteoclasts ± a TGF-βR1 inhibitor (galunisertib, 20 μM). 48 hours after treatment with conditioned media ± TGF-βR1 inhibitor, DRG were lysed in RLT Buffer + β-mercaptoethanol (β-ME). RNA expression of pain biomarkers was quantified via RT-qPCR. Pain biomarker expression was calculated using the delta-delta Ct method (fold change) and analyzed via two-way ANOVA with Tukey post-hoc test for multiple comparisons. Statistical significance was assessed at an alpha threshold of 0.05. All analyses were performed with GraphPad Prism (GraphPad Software, San Diego, CA, USA).

RESULTS: TGF-βR1 inhibitor effectively inhibited TGF-βR1 ($p < 0.001$) [**Figure 1A**]. Pain biomarker expression (CGRP and SP) increased with 10 Gy (**Figure 1B-C**). The TGF-βR1 inhibitor was associated with decreased expression of CGRP ($p=0.09$) and SP ($p=0.04$) [**Figure 1B-C**].

DISCUSSION: Proteomics analysis of small extracellular vesicles from irradiated OCs identified TGF-βR1 as a central mediator of bone loss and pain signaling between irradiated OCs and sensory neurons. Conditioned media from irradiated OCs increased expression of TGF-βR1, CGRP, and SP in sensory neurons. Increased expression of pain biomarkers was prevented when neurons were treated with a TGF-βR1 inhibitor. Therefore, TGF-βR1 inhibition combined with anti-resorptive therapy may serve as a therapeutic strategy for preventing both radiation-induced fracture and chest wall pain in patients undergoing thoracic SBRT.

SIGNIFICANCE/CLINICAL RELEVANCE: Galunisertib, a TGF-βR1 inhibitor actively used in clinical trials for cancer treatment, may reduce radiation-induced pain. Anti-resorptive therapy combined with TGF-βR1 inhibition delivered acutely may be efficacious in reducing both radiation-induced fracture and debilitating pain, which may further serve to eliminate SBRT dose constraints and optimize tumor control.

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IMAGES AND TABLES:

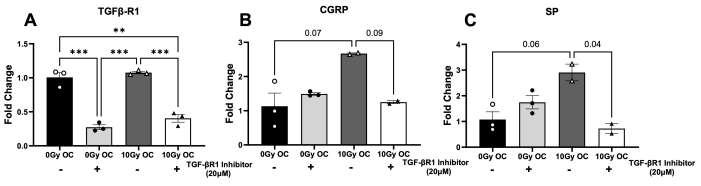

Figure 1A-C. Fold change expression of TGF-βR1, CGRP, and SP. Expression of TGF-βR1 (A) and pain biomarkers, CGRP and SP (B-C), were increased in sensory neurons treated with conditioned media from irradiated osteoclasts, but this increase was prevented when sensory neurons were treated with a TGF-βR1 inhibitor.