Conclusion
Proton radiotherapy (PRT) prompts different protein expression or activation profiles than photon (X-ray)-based radiotherapy (XRT) in head and neck squamous cell carcinoma (HNSCC) cells. Without regard to time, after a dose of 4-Gy radiation, seven proteins were expressed or activated differently between PRT versus XRT (p all < 0.05) in the four cell lines tested. These proteins were: DNA damage repair related proteins CHK1 and DNA-PKcs; tumor cell proliferation-related proteins AKT, E-cadherin and p70 ribosomal protein S6 kinase (p70S6K); tumor proliferation- and senescence-related protein P16; tumor proliferation- and apoptosis-related protein AKT; and apoptosis-related protein BAD. Further studies using patient tumor pathway mutations of some of these proteins as tumor biomarkers to facilitate the choice of PRT versus XRT, or as radiosensitization targets for combination with PRT for patients with HNSCC are warranted.

Introduction
Currently, the most effective treatment for head and neck squamous cell carcinoma (HNSCC) is photon (X-ray)-based radiotherapy (XRT), with or without chemotherapy, which comes at the cost of acute and late toxicity that can worsen quality of life and contribute to mortality. Proton beams allows highly conformal doses to be delivered to tumors with little or no dose to adjacent normal structures. Proton radiotherapy (PRT) is a less toxic alternative to XRT in patients with HNSCC. XRT is known to kill cancer cells by inducing DNA double-strand breaks. The DNA damage induced by PRT is more complex and has more severe consequences versus XRT. However, the molecular responses of cancer cells to PRT versus XRT remain undefined. This study was undertaken to uncover the molecular changes after PRT versus XRT in HNSCC cells.

Methods and results
Clinical 200-MeV proton beams (18 cm × 18 cm field) or 6-MV X-ray beams (25 cm × 25 cm field) were used. Cells were positioned in the centers of the irradiation fields (in the middle of the spread-out Bragg peak for PRT). Cell protein expressions were determined by Reverse-Phase Protein Array (RPPA) assays (a powerful functional proteomic approach that has been widely used to investigate molecular events of tumors; to identify biomarkers and mechanisms of cancer treatment), which can simultaneously test the expression of 161 proteins involved in signaling pathways including DNA damage repair (DDR), cell cycle regulation, apoptosis, senescence, cell proliferation, and others.

Further preclinical studies targeting DDR related pathways to enhance the HNSCC cells and xenografts response to PRT are ongoing.

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Protein expression profiles after PRT versus XRT in HNSCC cells

Fig. HNSCC cell lines HNS, SqCC/Y1 (human papillomavirus negative, HPV-) and UMSCC-47, UPCI-SCC-152 (HPV positive, HPV+) were used. Cells were collected at 1 hour (Panel A and D), 4 hours (Panel B and E), and 24 hours (Panel C and F) after a doses of 4-Gy irradiation.