Using X-Ray Footprinting to Investigate Dose Rate Effects On Oxidative Damage to Proteins

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The "FLASH" effect is a new term to describe the discovery that delivery of very high dose rate radiation to treat tumors will spare healthy tissue to a greater extent than conventional radiation dose rates, even when the total dose and damage to the tumor tissue is maintained [1]. Evidence for this effect has been successfully demonstrated experimentally in mice [2], pigs and cats [3], and in a first human patient [4], and has been shown using various energy sources including electrons, protons, and photons. However, the mechanism of the FLASH effect is complex and not well understood. The effect likely involves multiple factors, including tissue oxygen levels, radical recombination mechanisms, and immune and inflammatory responses. New approaches are needed to isolate the contributions of each of these and explore, at a fundamental level, the mechanisms underlying the FLASH effect. Here, we describe the application of the technique of X-ray footprinting mass spectrometry (XFMS) to determine the relative levels of radiation mediated peptide oxidation as a function of oxygen concentration. XFMS is a relatively new structural biology method which uses protein oxidation as a means to evaluate protein structure, interactions, and dynamics in solution [5], and has been used to characterize proteins ranging in size from small single-domain forms to MDa complexes, as well as RNA folding and DNA-protein interactions. The method takes advantage of hydroxyl radical creation in aqueous buffers upon ionizing radiation. Hydroxyl radicals are one of the most reactive of ROS, and form permanent modifications on protein side chains, which can then be quantified using standard bottom-up LC-MS. While the method is most often used to gain information on protein structure by comparing regions of oxidation as a function of solvent accessibility, here we present its novel use to quantify oxidative damage to short peptides after different FLASH and conventional radiation regimes as a function of oxygen availability.

References

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