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TITLE:

Molecular mechanism of action of photoimmunotherapy with antibody-IR700 dye conjugates

INTRODUCTION:

Cancer cell-targeted photoimmunotherapy (PIT) is a platform technology under development for the treatment of various cancers. PIT is a drug and device combination that utilizes an antibody conjugated to the IR700 (IRDye 700DX[®]) light-excitable dye, which upon antigen binding and illumination with non-thermal red light, leads to necrotic cell death. The objective of these studies was to characterize the molecular pharmacology and the underlying mechanism of action of PIT.

METHODS:

In vitro PIT studies using human and mouse cancer cells were performed to evaluate conjugate (antibody + dye) binding, dose response and light dosimetry response. Mouse xenograft experiments assessed necrotic markers in tumor tissue and surrounding normal tissue post-PIT treatment. Oxidative damage to lipids was detected with a reporter dye. Mass spectrometry analysis was performed to assess protein oxidation, crosslinking and axial ligand dissociation from the dye.

RESULTS:

Binding of the conjugate to the target cancer cells was required for PIT treatment to induce cell death as measured by antibody competition studies, however, internalization of the conjugate was not required for cytotoxic effects. Administration of non-thermal red light to the target cells bound with the conjugate led to morphologic changes and membrane disruption within minutes, and necrotic cell death was observed in a conjugate- and light-dose dependent manner. Requirement of conjugate binding to the cancer antigen provided high level of tumor specificity as demonstrated by lack of damage to normal surrounding tissue after PIT treatment. Light excitation of the conjugate generated ¹O₂ with high quantum yield. *In vitro* PIT treatment led to cell membrane lipid peroxidation, mediated by ¹O₂ as shown by abrogation with sodium azide, a selective ¹O₂ quencher. In addition, protein oxidation, cross-linking and aggregation of the conjugate was observed. In low oxygen concentrations and in the presence of ascorbate, photo-induced dissociation of axial ligands from the dye was detected.

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CONCLUSIONS:

These studies suggest the molecular mechanism of action of PIT includes generation of $^1\text{O}_2$ that leads to lipid peroxidation and cell membrane disruption. Additional mechanisms may include protein oxidation and non-oxygen dependent mechanisms such as axial ligand loss of the IR700 dye. These results describe a biophysical process that damages and disrupts the cell membrane integrity resulting in necrotic cell death and immunogenic cell death.