Development of a Multiplex Quantitative Immunofluorescence Assay to Determine DNA Damage Repair Deficient Models In Vitro and In Vivo and the Response to Cytotoxic Agents

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Introduction

The use of DNA damage agents remains a mainstay in cancer therapy. Defects in a tumor cell, including deletions and mutations of key DNA repair proteins, can alter the response and dictate which of the DNA damage repair (DDR) processes will be critical in repairing the damage, enabling the ability to predict effectiveness of treatment by identifying sensitive populations (1,2). Our group is developing a quantitative multiplex immunofluorescence assay (qIFA) for biomarkers of active homologous recombination (HR) and nucleotide-excision repair (NER) pathways. The complete DNA damage repair panel (1 DDR1) consists of reporters for pH2AX, pERCC1, GPR50, Rad51, and ERCC1. The assay in development uses highly specific antibody reporters, some of which have been directly conjugated to fluorophores, allowing the use of multiple antibodies from the same host species when following a properly controlled SDS.

Materials and Methods

Formalin Fixed, Paraffin Embedded Slide Preparation in Vivo:bruA720 ovarian cancer cells (NCI Frederick Repository) were treated with vehicle or 24-h Cisplatin (NCI 101197, Invitrogen) for 4 or 24 hours. Cells were prepared as FFPE blocks according to the standard protocol of the Pathology and Histotechnology Laboratory at NCIC Frederick. The FFPE A720-senograft samples obtained from a previous study were used for new development of the DDR1 qIFA panel. Information about the tumor staging, drug dosing and biopsy collection can be found in the referenced source (1). Tumors biopsied 1 or 2 post-dose were used. Slides were cut to 1 um sections and stained using a Bird-see-AutoStainer (Leica Microsystems) with optimized antibody dilutions and antigen retrieval methods. Image Acquisition and Analysis: Slides were observed on a wide-field fluorescence microscope (Nikon 208, Andor Camera). Analysis Software Imaging other FFPE slides, a number of FFPE cells were analyzed for nuclear area positive (gH2AX, pNbs1) or number of foci per nucleus (Rad51, ERCC1) using Definiens analysis software. Nuclei were classified using an algorithm and associated with those passing criteria foci counted blue. Cell area was green (Figure 7, top right). Mean percent of nuclei with foci and the # of foci in each nucleus is shown.

Conclusions

• We have developed a DNA damage repair immunofluorescence multiplex assay to assess DNA damage markers pH2AX, pNbs1, and Rad51 and nucleotide excision repair protein ERCC1 on the same tissue section.
• Using this technology, we can rapidly perform high-content analysis for characterization of drug treatment response and identification of sub-populations which respond differently to treatment.
• To achieve an assay with multiple markers, conjugation of primary antibodies directly to fluorophores was accomplished, further development of conjugated antibodies in the panels is required. Extending the use of anti- species secondarys will reduce background noise encountered when using an anti-mouse secondary on a xenograft sample.
• The Definiens Tissue Studio package was used to quantitate results of both nuclear area and positive nuclear foci. Analysis of large data containing approximately 100,000 nuclei. This software is being further developed for analysis of its in vivo assays.

References


Antibody Validation

Antibody Description Reporter Source
pH2AX Histone H2AX phosphorylated at Ser139 790 direct Invitrogen-Complement Group 1
ERCC1 ERCC1 full length protein competition assay. AlexaFluor 660 Novus 
Ab + FL Protein

Confocal Imaging

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Analytical Model: Use of Definiens Software for High-Content Data Analysis

An ovarian cancer cell line negative for BRCA1 expression (UWB1.289) and its isogenic, BRCA1 reconstituted (UWB1+BRCA1) were exposed to IR, fixed and stained for Rad51 at 24 h post exposure. Differences in DNA damage repair pathway activity altered Rad51 foci capacity.

In Vivo Use of DDR1 qIFA Multiplex

Vehicle Topotecan A375 xenografts were exposed to vehicle or 1.5 mg/kg topotecan (2 hr). Tumors were biopsied then formalin fixed and paraffin embedded. (H&E) sections of the FFPE block were stained with immunofluorescence staining for DDR1, DDR1 antibody stained in FFPE tumor sections, and DDR1 antibody competition assay. DDR1 antibody was then used as an antibody concentration assay. DDR1 full length protein competition assay.

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