Honors College of Arts and Sciences | Ohio State University

Variants of Uncertain Significance in BARD1 and BRCA1

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AIM

This research is designed to identify whether certain variants of uncertain significance (VUS) in the protein BARD1 are functional in homology directed repair (HDR). This data will allow for a better clinical classification of BARD1 variants in patients.

INTRODUCTION

Over the last twenty years, the ease and accessibility of full genome sequencing has greatly increased. As more patients get their genome sequenced, more DNA variants are identified. A problem is that a large number of these variants have an unknown clinical significance. Clinicians are unsure of whether these variants are pathogenic or benign. We refer to these variants as variants of uncertain significance (VUS).

This project assesses VUS in the protein BARD1. BARD1 forms a heterodimer with BRCA1 and together they function in DNA repair at double stranded DNA breaks (DSB).[1,2,3] Both BARD1 and BRCA1 are commonly mutated in cancers, specifically breast and ovarian cancers.[4,5,6] Identifying the significance of BARD1 VUS will allow clinicians to better assess a patient's risk following full genome sequencing.

BARD1 variants are tested in DNA repair assays to determine whether they are functional in repairing DNA. Variants that are nonfunctional in DNA are more likely to be clinically pathogenic. We are confident in our assay because when we test clinically known BARD1 pathogenic variants, they are nonfunctional in our HDR assay.

Figure 1. DNA DSB Repair Assay with GFP

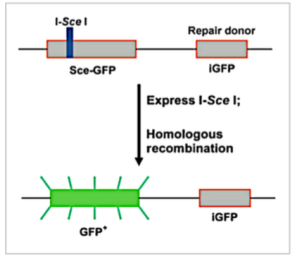

Figure 1. When I-SceI initiates a DSB, if the cell repairs via HDR, a functional GFP protein in produced. Image from [9].

METHODS

Homology-directed Repair (HDR) Assay

HeLa DR cells are integrated with two non- functional copies of GFP coding sequence. One copy of the GFP sequence contains a recognition site for the endonuclease *I-SceI*. When *I-SceI* is transfected into the cell, it induces a DSB, and if the cell repairs via HDR by copying the second GFP sequence, the repaired gene becomes functional (Figure 1). Cells are subsequently analyzed by flow cytometry to determine the percentage of cells producing GFP, or the amount of cells that successfully underwent HDR.[8,9]

BARD1 variants are tested by transfecting a high-expressing plasmid containing the variant BARD1 and an siRNA that targets and silences endogenous BARD1. The endogenous protein knock-down and variant protein expression is confirmed via Western blot.

Clonogenic Assay

To confirm the results of our GFP assay, four variants of differing GFP percentages were tested in a clonogenic assay. Endogenous BARD1 is knocked down, and a variant BARD1 protein is expressed. Cells are exposed to varying levels of DNA damaging agents, and colonies are allowed to form. Colonies are stained with crystal violet and counted (Figure 2). Variants that are better able to repair DNA have greater survival following DNA damage and form more colonies.

Figure 2. Clonogenic Assay Stained Colonies

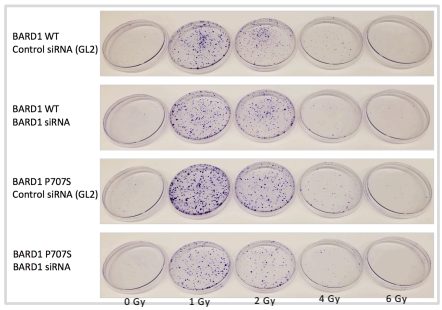

Figure 2. Plates expressing BARD1 variant P707S were exposed to IR. From left to right there is an increasing amount of X-ray irradiation exposure, measured in Gray (Gy). These plates were stained with crystal violet and colonies were counted.

RESULTS & DISCUSSION

76 BARD1 variants were tested in the GFP assay, and the GFP percentages normalized to wild type BARD1 are depicted in Figure 3. The results of the GFP assay allowed the identification of 16 BARD1 variants that are deficient in HDR (Figure 3). Variants are classified as nonfunctional at HDR if they show a GFP percentage of 0.6 or below compared to wild type. These variants are depicted with an * in Figure 3.[6]

Of the 16 HDR deficient variants, four were selected for testing in the clonogenic assay. The four were chosen because they covered a range of HDR deficiencies. The results of the clonogenic assay showed a correlation between HDR deficiency and cell survival following external DNA damage. These results reinforce the fact that the HDR assay is an appropriate measure of DNA repair. There was no correlation between the proportion of HDR deficiency and the amount of cell survival. For example, a variant may be 80% deficient in HDR, but that does not predict the percentage of cells that will die following IR or Cisplatin treatment. Overall, this data allows for a better clinical classification of BARD1 variants in patients.[6]

Figure 3. BARD1 Variants Functionality in Homology-Directed Repair Assay

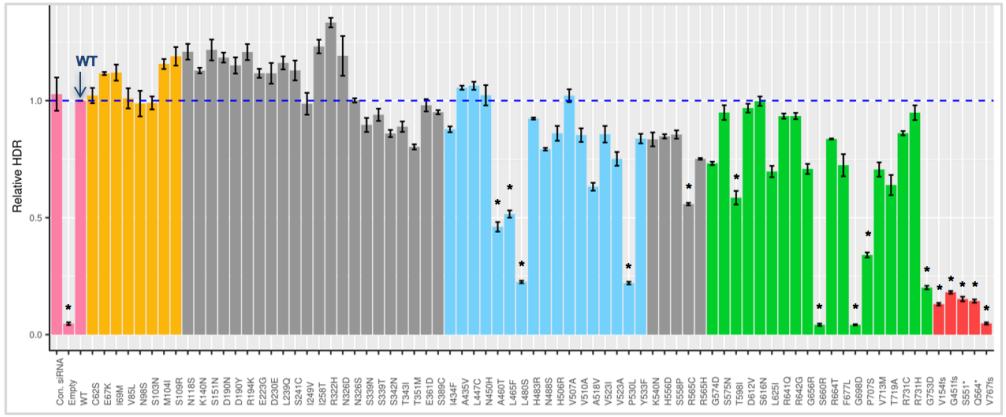

Figure 3. The x-axis denotes the variant BARD1. The blue dotted line represents WT HDR levels. Bars below the dotted line are deficient in HDR. Bars denoted with a * are statistically significant, meaning that the relative HDR of the variant is 0.6 or below comparted to wild type. These variatns are nonfunctional in HDR, meaning they do not repair DNA properly. This gives insight into how these variants may be classified clinically.[6]

Figure 4.

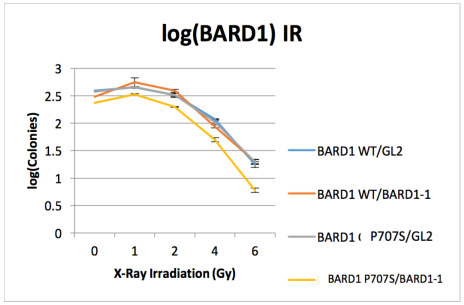

Figure 4. The graph shows that increased IR decreased colony number across all variables, but BARD1 P707S with endogenous BARD1 knocked down (yellow) shows the most sensitivity.

CONCLUSIONS

16 BARD1 variants that are nonfunctional in homology-directed repair were identified. Variants deficient in HDR also showed less cell survival following exposure to DNA damaging agents. This allows for better clinical classification of these variants.

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