Background

BRCA1 mutation carriers face high risks of breast and ovarian cancer due to mutations in DNA repair genes. Typical treatments of these cancers rely on killing cells by mechanisms such as induction of DNA damage, altering cell cycle checkpoints, or cell division. A study has examined BRCA1 mutation carriers treated for breast cancer and found evidence of neutropenia as patients experienced decreases in their blood counts.1 Neutrophils are a key cell type in the innate immune system; they serve as the first line of defense as they are recruited to respective sites of infection.2 However, they are short lived; and thus, they need to be continuously generated in steady-state conditions from hematopoietic stem cells and progenitor cells in bone marrow to ensure containment of invading pathogens. When an infectious or inflammatory challenge presents itself, and neutrophils are used up in large quantities, the hematopoietic system has to rapidly adapt to increased demands by switching from steady-state granulopoiesis to emergency granulopoiesis (Fig. 1).3 Here, we investigate whether deficiency in BRCA1 impairs emergency granulopoiesis.

Methods

Cell Culture and Treatment
U937 cells were plated onto three 12-well plates. Each plate corresponded to a time point of 24 hours, 48 hours, and 72 hours. 5.0x10^6 cells were placed into each well. Treatments used were 20, 40 ng, 50ng, 60ng and 80ng of IL-1β, and an untreated control was used for each plate.

Sample Collection and Analysis
At 24 hours, 48 hours, and 72 hours, cells were washed, collected, and placed in 400μL of Trizol. RNA extraction was performed, and cDNA was made using RNA, nuclelease-free water, and iScript reverse transcriptase mix. A qPCR was then run using FANCC, BRCA1 and GAPDH probes to measure FANCC and BRCA1 expression.

Results

Treatment of U937 cells with different concentrations of IL-1β

![Graph showing relative expression of BRCA1 and FANCC with different concentrations of IL-1β.]

Figure 3. Relative expression of BRCA1 and FANCC in U937 cells post (A) 20 ng IL-1β treatment, (B) 40 ng IL-1β treatment, (C) 50 ng IL-1β treatment, (D) 60 ng IL-1β treatment, and (E) 80 ng IL-1β treatment.

Conclusion

- FANCC expression in U937 cells does not significantly change over time with increasing concentrations of IL-1β.
- BRCA1 expression was lowest at 48 hours post IL-1β treatment.
- BRCA1 expression had a greater decrease at 20 ng to 50 ng IL-1β.
- It appears that the general trend for both FANCC and BRCA1 expression is different than what has previously been reported (4).

Future Work

- Transfect U937 cells with an IRF8 vector before treating with IL-1β, and assay for FANCC expression as shown in previous literature.
- Assay for FANCC expression in U937 PMA treated cells to investigate emergency granulopoiesis in macrophage-like cells and mouse bone marrow derived macrophages.
- Assay for proteins involved in BRCA1 activation including, but not limited to RAD51, RNA pol. II, BARD1, and p53 to examine additional components to the BRCA1 gene.
- Assay for HIP1, a protein studied in the lab and shown to have no change in expression following BRCA1 over-expression, for use as an additional control.

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References