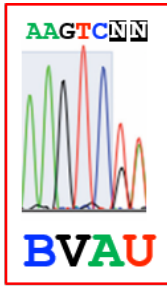


BRCA Variants Analysis Unit Home

BRCA VARIANTS ANALYSIS UNIT



Goal

The main goal of the BRCA Variants Analysis Unit is to undertake a comprehensive analysis of BRCA variants of unknown clinical significance listed in the BIC database. We will use the ES cell-based functional assay to examine the effect of the variants on the known functions of BRCA1 and BRCA2. This analysis will help determine the functional consequences of these polymorphisms on BRCA1/2 function.

The BRCA Variants Analysis Unit is headed by Dr. [Shyam Sharan](#), Ph.D.

Background and Significance

Breast cancer is the most frequently diagnosed cancer in women in the United States. Among the various risk factors responsible for the development of this cancer, the best-established indicator is inheritance of a mutant *BRCA1* or *BRCA2* gene. The protein encoded by the human *BRCA1* and *BRCA2* genes consist of 1863 and 3418 amino acids, respectively. Germline mutations in these genes account for 20-60% of the familial breast cancer cases. The lifetime risk of developing breast cancer for *BRCA1/2* mutation carriers is greater than 70%, which makes it extremely important to accurately identify risk groups. Sequencing based tests are now available and more than 250,000 people have been tested for *BRCA1/2* mutations in US alone. More than 800 *BRCA1* and 1100 *BRCA2* variants of unknown clinical significance are listed in the Breast Cancer Information Core (BIC) database (<http://research.nhgri.nih.gov/bic/>). To date very few BRCA variants have been functionally classified. Some of these are listed in the Leiden Open Variation Database (<https://databases.lovd.nl/shared/variants/BRCA2/unique>). At present, the segregation of the mutation with the disease in the family and presence of the mutation in the general population are used to distinguish between a deleterious missense mutation and a silent polymorphism. Unfortunately, such information is not available for most mutations. A few functional assays have been developed that measure the ability of BRCA1 and BRCA2 variants to repair damaged DNA or determine the effect of BRCA1 variants on its transcriptional activity.

We have developed a functional assay that is based on the finding that BRCA1 and BRCA2 play a critical role in the survival and proliferation of mouse embryonic stem (mES) cells. This assay is designed to not only examine the effect of BRCA variants on DNA repair but also on cell viability and homeostasis, cell differentiation and overall genomic stability. Over the years we have used it to study a number of BRCA variants. In every case our results have been in agreement with existing epidemiological and other functional data. Because we use BACs to introduce human variants in mES cells, we are able to not only examine variants in the coding region of the gene but also variants in the promoter region, 5' and 3' UTR as well as splice site variants.

The data so far accumulated strongly suggest that this assay can be used to understand the functional consequences of any mutation identified in human *BRCA1* and *BRCA2* genes. This functional evaluation will be of tremendous value not only to physician and genetic counselors but also basic researchers who are interested in understanding the biological functions of these genes. Moreover, our assay will serve as a model to generate functional assay for other human disease genes. With the identification of a large number of single nucleotide polymorphisms in humans, tools are needed to evaluate their functional significance. Our approach can be used as a general method for study of genes that result in phenotypes detectable in ES-cells.

Functional Assay

To study the functional significance of human BRCA variants, we have developed a mouse embryonic stem cell based functional assay. The assay is based on the observation that *Brca1* as well as *Brca2*- deficient mES-cells are not viable.

We have generated ES cells with a disrupted allele of *Brca1* or *Brca2*. The second *Brca1* or *Brca2* allele is conditionally targeted by flanking the entire gene with two loxP sites. Two halves of the human HPRT mini gene are inserted next to the two loxP sites in AB2.2 ES-cells lacking a functional *HPRT* gene and are therefore sensitive to HAT selection. When the two loxP sites recombine in the presence of CRE, a functional *HPRT* mini gene is generated, enabling, in theory, the selection of recombinant ES-cells in HAT media. However, these ES cells die because of the lack of BRCA1 or 2, which are essential for ES cells survival.

We have shown that the lethality of *Brca-null* cells can be rescued by introducing a bacterial artificial chromosome (BAC) expressing a wild-type human BRCA1 or BRCA2 gene. We have also shown that introducing a BAC with known BRCA variants that are deleterious do not rescue *Brca*-null lethality while neutral variants do. Furthermore, deleterious variants either failed to rescue ES cell lethality or resulted in viable cells with defects in cell survival, proliferation or are hypersensitive to genotoxic stress. In contrast, mutations that have been shown to be neutral in people are always indistinguishable from the wild-type gene.