Understanding the Functional Significance of Variants Identified in Breast Cancer Susceptibility Genes

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BRCA1 and BRCA2 Mutation Increases Breast Cancer Risk

Responsible for most familial breast cancer

Lifetime risk in general population: 13.7%
BRCA1/2 mutation carriers: 35-85%

Increased ovarian cancer risk
BRCA1 and BRCA2: Role in DNA Repair

**BRCA1:** 1863 amino acids
- RING Finger
- E3 ligase
- BRCT-repeat
- Transcriptional activation

**BRCA2:** 3418 amino acids
- BRC Repeats
- Several ss-DNA binding domains
Missense Mutations in *BRCA1* and *BRCA2*

Single amino acid variants:
24% in *BRCA1*; 47% in *BRCA2*
Functional Significance of Single Amino Acid Changes in *BRCA1* and *BRCA2*: Neutral or Deleterious?

- Several predicted missense mutations were found to be neutral changes
- No reliable functional assay
- Prevalence in the general population
- Co-segregation with disease
Linkage Analysis
Prevalence of BRCA1 & BRCA2 Mutations

• 10,000 individuals were screened for BRCA1 and BRCA2 mutations by Myriad Genetics*.

• 55% indicated a personal history of breast or ovarian cancer

• 17% had deleterious mutations

• 13% had one or more variants of unknown clinical significance

• How about other genes?

A Mouse Embryonic Stem Cell-Based Functional Assay

• Embryonic Stem cells for functional analysis
  BRCA1/2 are essential for ES cell viability, maintain stable genome

• Use of Bacterial Artificial Chromosomes (BAC) containing human BRCA1 or 2
  Average insert size of BAC is ~150,000 bases coding & non-coding alterations, expression at physiological levels, easy to modify by recombineering
Recombineering: Recombination-based Genetic Engineering

• Technology developed at NCI
• Utilizes the recombination system of bacteriophage
• Extremely efficient method to manipulate DNA
• Allows precise and rapid alteration of a single nucleotide

Swaminathan et al., Genesis, 2001;
Yang and Sharan, Nucleic Acids Res. 2003;
Sharan et al., Nature Protocols, 2009
Functional Analysis of BRCA2 in Mouse ES Cells
Functional Analysis of BRCA2 in Mouse ES Cells

ES cells are not viable

Mutation is deleterious

ES cells are viable

Mutation can be neutral or hypomorphic
Examining BRCA2 Function in Viable ES Cells

- Test ability to repair damaged DNA
- Effect on overall genomic stability
Functional Evaluation of BRCA2 Variants in Mouse ES cells

Brca2 Ko/+  Brca2 Ko/Ko

BAC Complementation

Sergey Kuznetsov
Y3308X Mutant cells are Viable but Hypersensitive to Genotoxins

Truncation: Loss of 110 aa
Y3308X cells Exhibit Genomic Instability

Brca2 Ko/+  Y3308X
BRCA2: Structure-Function Analysis

Tower domain

Arginine 3052

C-Terminal Domain of BRCA2

Glutamine

Tryptophan

Helical domain

OB3  OB2  OB1
BRCA2 Structure Analysis
Arg3052Trp vs. Arg3052Gln
R3052Q Variant Shows Moderate Sensitivity to DNA Damaging Agents

Arg3052Gln  Arg3052Trp
R3052Q Variant Results in Moderate Genomic Instability
Evaluating Functional Significance of Missense Mutations in ES cells

Unclassified variants

1100 BRCA2

Clinical relevance
Evolutionarily conserved
Functional importance

20 Variants

13 Neutral

4 ES cell lethal

3 Hypomorphic

Functional Analysis of BRCA1 mutants in ES cells

Clinical relevance
Evolutionarily conserved
Functional importance

15 Mutations

RING Domain  Phosphor. sites  BRCT Domain

Chang et al. J Clinical Investigation 2009
Functional Analysis of Deleterious Variants in Mice

BRCA1

RING domain

C64G
C61G
I26A

BRCT domain

A1708E
R1699Q
M1652I

Lethal
Lethal
Viable

Suhwan Chang
E3309X: a Real Life Dilemma

Truncations at codon:

- 3308: Deleterious
- 3309: Deleterious
- 3326: Neutral
Conclusions

• ES cells provide a simple, tractable system to study BRCA1 & BRCA2 variants

• Clinical relevance

• The ES cell-based approach can be used to examine variants identified in other human disease genes
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