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Letter to the Editor

Role of chromosome segregation genes in BRCA1-dependent lethality

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Mutations in BRCA1 tumor suppressor account for ~45% of hereditary breast cancer and predispose individuals to ovarian and prostate tumorigenesis. BRCA1-deficient cells contain numerous chromosome aberrations (duplications, translocations, inter-sister gaps) and defects in gene regulation. While BRCA1 binds numerous factors to engage in a wide variety of activities (ubiquitin ligase, DNA repair/damage signaling, chromatin remodeling, transcription activation), how BRCA1 mutations affect genetic instability at the molecular level remains unclear.[1,2]

Expressing the C-terminal BRCT domain of BRCA1 in budding yeast severely limits cell growth. This small colony phenotype revealed a physiological role for BRCA1 function even in this simple eukaryote and provided for rapid characterization of clinically relevant BRCA1 alleles.[3,4] Subsequent yeast cell studies linked BRCA1 responses to DNA damage-induced stalled transcription complexes.[5,6] However, a direct role for BRCA1 defects in promoting chromosomal instabilities has yet to be demonstrated.

To identify novel yeast genes that participate in BRCA1 phenotypes, the C-terminal BRCT domain of BRCA1 (herein termed BRCA1) was transformed into a collection of yeast mutant strains representing a diverse array of cell processes.[7] From this collection, mutations in five genes (*MCM21*, *CTF19*, *CTF7*, *TOF1* and *CDC6*) produced BRCA1-dependent conditional synthetic lethality (Fig. 1). The first four genes reveal a novel link between BRCA1 and factors integral to chromosome segregation. Mcm21p and Ctf19p form part of the COMA outer kinetochore plate complex that promotes full kinetochore assembly.[8] Kinetochores tether chromosomes to spindle microtubules and produce chromosome motion during mitosis. Ctf7p (Eco1p) and Tof1p both promote sister chromatid pairing (cohesion)—a fundamental component of proper chromosome segregation. [9-11] Intriguingly, DNA damage activates Ctf7p-dependent cohesion which then promotes DNA repair.[10,11] Consistent with the identification of *ctf7* and *tof1*, we also found that *smc3* mutants exhibit severely compromised growth when expressing BRCA1 (data not shown). Smc3p is a structural cohesin component that maintains sister chromatid pairing from S-phase until anaphase.[9] The fifth gene, *CDC6*, promotes DNA replication initiation. Cdc6p is recruited by Origin Recognition Complexes (ORCs) which subsequently bind MCM helicase to form a DNA pre-replicative complex (pre-RC).[12]

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Chl1p is a DNA helicase that functions in sister chromatid-pairing reactions.[10] Chl1p was of interest because its human homolog BACH1/BRIP/FANCJ binds BRCA1.[13,14] We quantified colony diameters of wildtype and *chl1* cells expressing either BRCA1 or mutant brca1 (nonsense mutation producing a truncated protein) after 3 days at 30°. Wildtype cells expressing BRCA1 produced an average colony diameter 39% (41% upon independent assay) the size of cells expressing the mutated brca1 control. *chl1* cells containing the mutant brca1 control produced an average colony diameter 73% (79% upon independent assay) the size of wildtype cells containing mutant brca1, revealing a *chl1*-dependent small colony phenotype. If not lethal, the combination of BRCA1 and *chl1* should produce greatly diminished colony diameters. Instead, *chl1* cells expressing BRCA1 produced an average colony diameter 50% larger (31% upon independent assay) than expected and surprisingly larger than wildtype/BRCA1 colony diameters. Parallel studies similarly revealed that *chl1* cells expressing BRCA1 produce an average colony diameter ~20% larger than expected when compared to a random control vector (Suppl. data).

Here, we report that BRCA1 function specifically perturbs kinetochore and cohesion pathways—possibly by altering assembly/dissolution reactions, respectively. This model is supported by evidence that BRCA1 predominantly localizes to heterochromatic centromeric DNA upon which both kinetochores and cohesins assemble.[15] Of the six genes identified in this study, Ctf7p and Chl1p are of particular interest. Yeast and human Ctf7 (EFO2/ESCO2) and human BRCA1 share many binding partners (RFC, PCNA, helicases) that can exhibit acetyltransferase-related activities.[1,9] Defects in human EFO2/ESCO2 (and hSMC3) produce a variety of maladies including SC phocomelia, Roberts syndrome and Cornelia de Lange syndrome.[10,16] In humans, BACH1/BRIP/FANCJ (Chl1p in yeast) binds and participates in BRCA1-dependent DNA double-strand break repair.[10,13] Notably, *chl1* interacts genetically with each of *ctf7, tof1*, *ctf19* and *mcm21*,[14,17,18] suggesting that Chl1p links BRCA1 to kinetochore/cohesion activities. We further speculate on how BRCA1 exacerbates *cdc6*-dependent alleles. Previous findings suggest that Ctf7-dependent cohesion establishment is coupled to DNA replication.[9] Thus, BRCA1 may uncouple establishment from replication initiation in *cdc6* mutants. Alternatively, Cdc6p binds ORCs which maintain sister chromatid pairing in parallel to cohesins,[19] suggesting that BRCA1 effects ORC-dependent sister chromatid pairing.

Note

Supplemental material can be found at:
www.landesbioscience.com/supplement/SkibbensCC7-13-Sup.pdf

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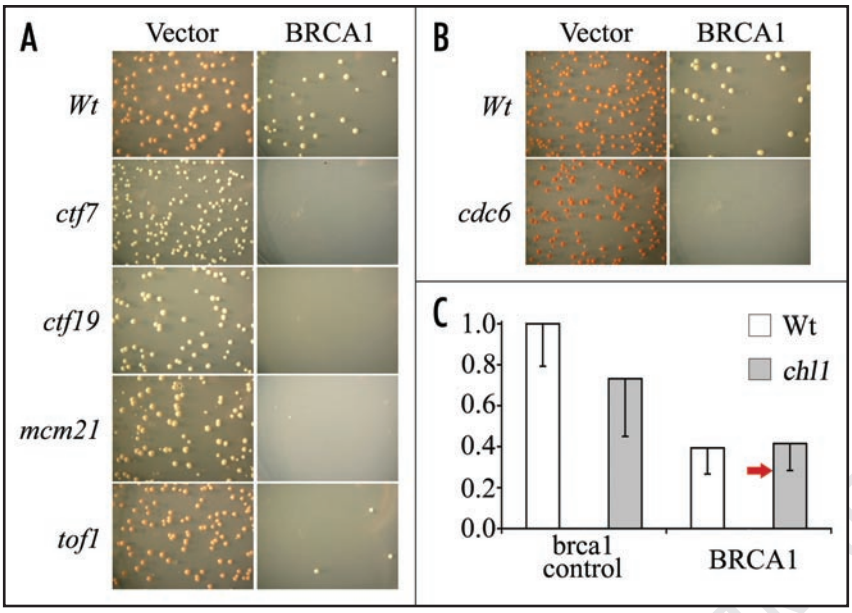

Figure 1. Growth of wildtype (*Wt*) and mutant yeast strains transformed with BRCA1 or vector alone. (A) Colony size after 6 days of growth at 37°. (B) Colony size after 7 days of growth at 30°. Lethality was also observed in *ctf19*, *mcm21* and *cdc6* mutant strains expressing BRCA1 at 12° (Suppl. data). Note that the extended periods of growth chosen to highlight BRCA1-dependent lethality negate the small colony phenotype. Field of view = 53 mm. (C) Loss of *CHL1* partly rescues this growth defect. Independent assays reveal that *chl1* cells expressing BRCA1 produce colony diameters roughly 40% larger than the expected small colony phenotype (red arrow) and larger than wildtype cells expressing BRCA1, compared to a truncated brca1 control plasmid.