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14. ABSTRACT The purpose of this study was to investigate the role of coffee and caffeine in the function of the DNA repair protein BRCA1 and to determine whether or not coffee and/or caffeine prevent BRCA1 hereditary breast cancer. We have bred the necessary genetically engineered mice for the animal study to determine whether coffee, decaffeinated coffee, or caffeine prevents BRCA1 hereditary breast cancer. We applied for a no cost extension to complete these studies during the next year so we will know whether or not caffeine prevents breast cancer in the animal model. We have also analyzed the 1853 mutation in the BRCA1 mutant cell line HCC1937 and shown that is unaffected by DNA damaging agents including caffeine although the full length wildtype BRCA1 protein is affected. We also show that the BRCA1 mutant proteins 1853 and Cys61Gly show a loss of nuclear localization and are defective in DNA repair and radiation response. We are preparing a manuscript to report this finding.					
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Introduction:

Not all women who inherit mutations in the BRCA1 gene develop breast cancer and recent evidence indicates some diet or lifestyle changes can reduce cancer risk. There is evidence that exercise and improved diet can reduce cancer risk in BRCA1 mutation carriers (1). Traditional Chinese medicine employed coffee as a breast cancer preventive, and a recent study reported that increased consumption of caffeinated coffee results in a 30-70% reduction in cancer in BRCA1 mutation carriers (2). Prior studies of sporadic breast cancer have shown minimal if any effect of coffee on breast cancer risk (2) suggesting that the effect may be limited to hereditary breast cancer. Coffee and caffeine have been linked to increased estrogen levels (2), inhibition of DNA repair and cell cycle, and inhibition of BRCA1 BRCT interactions (3). All of the anti-genotoxic and medicinal qualities of coffee cannot be attributed to caffeine (4), so it is important to study coffee and caffeine as separate variables. We proposed to determine whether we can show a biological effect of coffee or caffeine on molecular, cellular, and animal models of BRCA1 breast cancer. Then we can determine whether the effect is due to estrogen modulation, cell cycle/DNA repair effects, or BRCT interactions. This should allow us to understand if and how coffee or caffeine reduce breast cancer risk in BRCA1 mutation carriers.

Body:

We have bred the necessary genetically engineered mice for the animal study to determine whether coffee, decaffeinated coffee, or caffeine prevents BRCA1 hereditary breast cancer. We applied for a no cost extension to complete these studies during the next year so we will know whether or not caffeine prevents breast cancer in the animal model. We have also analyzed the 1853 mutation in the BRCA1 mutant cell line HCC1937 and shown that is unaffected by DNA damaging agents including caffeine although the full length wildtype BRCA1 protein is affected. We also show that the BRCA1 mutant proteins 1853 and Cys61Gly show a loss of nuclear localization and are defective in DNA repair and radiation response.

BRCA1 is a large, nuclear phospho-protein which regulates the repair of double strand breaks (DSB) in DNA. It contains an N-terminal RING domain, which functions as an E3 ubiquitin ligase when bound to its partner protein BARD1. BRCA1 also contains a C-terminal BRCT repeat domain, which functions as phospho-protein binding module. BACH1, a helicase involved in DNA repair, is one important interacting partner which binds to the BRCT repeats of BRCA1. Mutations affecting both domains are associated with hereditary cancer, suggesting that the function of both domains is necessary for the ability of BRCA1 to suppress tumor formation. Once the DNA damage response is initiated, BRCA1 is recruited to gammaH2AX foci in a manner dependent on MDC1, where it participates in the regulation of DNA repair. As the repair process concludes, gamma H2AX is removed from the surrounding region. We were interested in studying whether mutations affecting the RING domain and mutations affecting the BRCT repeat domain have different biochemical consequences for BRCA1 function. We therefore analyzed several characteristics of wild-type, RING mutant (C61G), and BRCT mutant (1853stop) BRCA1 protein expressed in the BRCA1 mutant human breast carcinoma cell line HCC-1937, which expresses almost no endogenous BRCA1. We found that loss of the BRCT repeat domain decreased the half-

life of BRCA1 protein and resulted in mostly cytoplasmic localization. In contrast, the RING domain mutant BRCA1 appeared to have similar protein stability and nuclear localization as wild-type. However, mutation of either domain appeared to disrupt co-localization of BRCA1 with its protein binding partners BARD1 and BACH1. Furthermore, both types of mutations prevented the recruitment of BRCA1 protein into gammaH2AX foci following ionizing radiation.

In order to study the biochemical properties of mutant BRCA1 in comparison to wild-type protein we utilized recombinant human adenoviruses expressing BRCT truncated (Ad-1853), RING mutated (Ad-C61G), or wild-type (Ad-BRCA1) protein. These adenoviruses contain an R-G-D modification in the fiber knob surface binding protein, which increases the efficiency of infection and downstream expression of the transgene. Transduction of the *BRCA1* mutant HCC-1937 human breast carcinoma cell line with these viruses at a multiplicity of infection (MOI) of 100 viral plaque forming units per cell yielded approximately equal protein expression of all three forms of BRCA1 48 hours following viral transduction.

Analysis of control cells infected with Ad-GFP showed that almost no endogenous BRCA1 protein can be detected in this cell line. Expression of BARD1 and BACH1, which is another BRCA1-interacting protein involved in DNA repair, were found to be approximately equal in all transduction groups (data not shown). Prior to harvest, quantitation of GFP expression in Ad-GFP transduced cell cultures by microscopy indicated that the transduction efficiency is approximately 80% for this cell line under these conditions.

We determined the subcellular localization of these forms of BRCA1 by immunofluorescence microscopy. A dual anti-BRCA1 antibody protocol was utilized in order to ensure the specificity of the staining protocol. C61G BRCA1 was organized into discrete nuclear foci (Figure 1), similar to the expected focal nuclear pattern observed with the wild-type protein (Figure 1a). However, 1853stop BRCA1 was primarily localized in the cytoplasm (Figure 1b). Similar to immunoblot results, we found that virtually no endogenous BRCA1 could be detected by this staining protocol (Figure 1d). Figure 1 is presented on the next page with its legend.

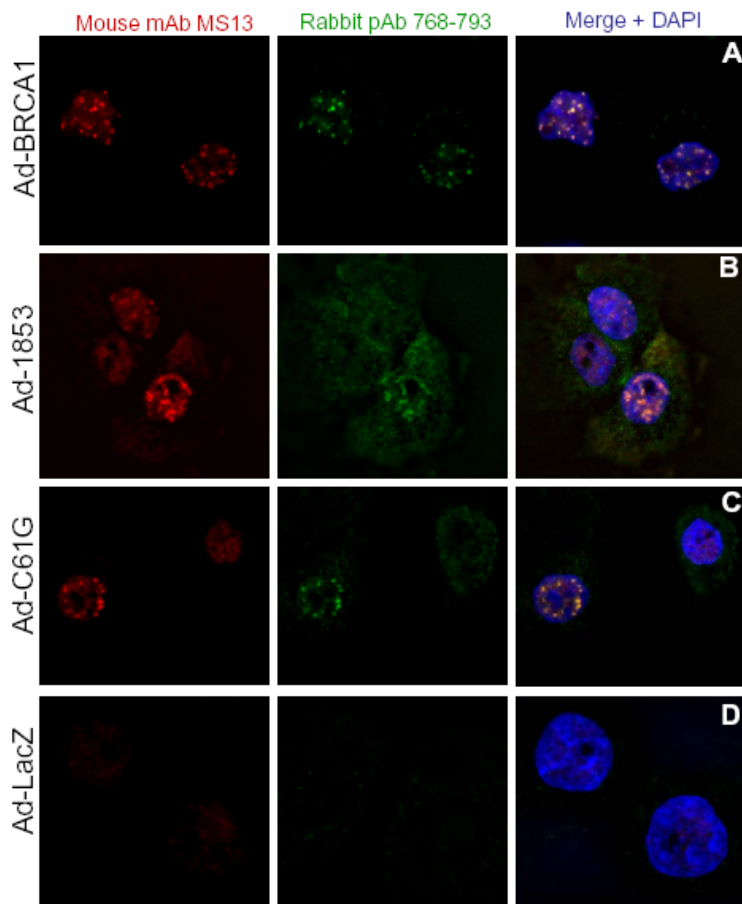


Figure 1. Truncation of the BRCT Repeats Inhibits Nuclear Accumulation of BRCA1. HCC-1937 cells were transduced at MOI=100 with adenoviral vectors expressing the indicated forms of BRCA1 or with a control virus expressing β -gal (Ad-LacZ). Cells were cultured for 48 hours and then fixed for immunostaining of BRCA1 protein with both an N-terminal directed mouse monoclonal (red) and an exon 11 directed rabbit polyclonal (green) antibody. Overlap of both signals produces a yellow signal in the merged images, which demonstrates specific staining for BRCA1. Nuclei are stained with DAPI. Both wild-type and C61G BRCA1 proteins stain in nuclear foci, while 1853stop BRCA1 appears to be primarily localized in the cytoplasm.

Although caffeine does not change the localization of the proteins it does inhibit the DNA repair function of the proteins.

Key Research Accomplishments:

- Developed breast cancer model and bred genetically engineered mice with BRCA1 hereditary breast cancer to test effect of coffee
- Constructed and expressed BRCA1 mutants in HCC BRCA1-defective cells
- Demonstrated that both C61G and 1853ter have DNA repair defect and are not properly localized in the cell

Reportable Outcomes:

1. Manuscript in preparation based on the cellular studies funded by this proposal: Nelson AC and JT Holt. Distinct Mechanisms of BRCA1 Mutations in Breast Cancer. Manuscript in Preparation
2. Presentation: Andy Nelson graduate student presented:
July, 2007 Invited Speaker, 22nd Annual National MD/PhD Student Conference, Keystone, CO

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