

TITLE

High Throughput Screening of the BRCA1-Associated BRCC36 Metalloprotease

ABSTRACT

Breast Cancer is the most common malignancy among women in the United States, and inherited breast cancer contributes more than 20,000 cases annually. BRCA1, or Breast Cancer Associated gene 1 is a breast and ovarian cancer-specific tumor suppressor. Women, who have a germline loss-of-function allele of *BRCA1* are 85% likely to develop breast cancer by age 70. We have recently identified a BRCA1-associated protein complex that acts to bring BRCA1 to the chromatin structure surrounding DNA lesions. A critical component in this complex is the BRCC36 zinc-metalloprotease, a K63-specific deubiquitinating enzyme (DUB). Our lab has shown that BRCC36 DUB activity is required for G2 checkpoint control and Ionizing Radiation (IR) sensitivity in U2OS cells. Recent results also indicate that knockdown of a component of the BRCC36 complex confers lethality in a BRCA1-dependent manner. The results from these and other studies have led us to propose that BRCC36 represents an important target for small molecule inhibition in breast cancer chemotherapy.

In this proposal, we seek to identify small molecules that inhibit the proteolytic activity of BRCC36. We have been able to purify milligram quantities of BRCC36 in both bacteria and baculoviral-expression systems. In addition, we have expertise in the lab to produce the substrate ubiquitin-AMC, a ubiquitin derivatized at the C-terminus with 7-amido-4-methylcoumarin. Monitoring the cleavage of this highly fluorogenic AMC marker will allow us to screen for small molecule inhibitors of BRCC36 and identify lead compounds, which may be developed into pharmacological reagents.