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13. ABSTRACT (Maximum 200 Words)

Studies have shown that apoptosis and survival pathways in response to DNA damage play a critical role in breast cancer development and progression. 90% of breast cancer cases are sporadic where mutations of BRCA1/2 have not been detected. Other breast cancer genes must exist. Our group has approached the issue in two ways, a genomic and a proteomic approach. We have established and utilized a novel retrovirus-based genetic screen system to search for genes that would confer resistance to DNA dmage induced apoptosis. Multiple clones have been isolated from this genetic screen. Among the genes identified are both novel and known proteins that may be important in DNA-damage responses. To further elucidate the pathways mediated by BARD1, we looked for factors interacting with BARD1 using mass spec sequencing. Several factors have emerged from this study and are being examined. The information obtained from our studies should prove useful for developing new and effective screening strategies, drug targets, and treatment for breast cancer.

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Introduction

The initiation and progression of breast cancer is likely the result of dysregulation of both oncogenes and tumor suppressor genes (1, 2). Mutations of these genes can affect many aspects of the cells including defects in cellular survival and proliferation, genomic integrity, and sensitivity to DNA damage. However, the identity and the mechanisms of genes that regulate DNA damage induced cell death are much less well studied. We have proposed to establish a genetic system to screen for genes that regulate survival in cultured cells through high-efficiency mutagenesis using Enhanced Retroviral Mutagens (ERM) (3). Due to the random nature of retroviral integration, endogenous genes involved in cell survival signaling cascades may be activated or inactivated by ERM. The targeted gene loci are marked by retroviral integration thereby allowing quick isolation of the candidate genes. The overall objective of this proposal is to identify and study genes that allow the survival of normal and cancerous breast cells. The physiological roles of these genes and their interactions with known signaling pathways will be investigated. Genetic screens will be performed to search for survival genes in response to DNA damage. And the function and signaling mechanisms of BARD1 in breast cancer cell survival will also be examined. The proposed studies should help in our understanding of the molecular basis underlying cell survival signaling and breast cancer as well as provide new therapeutic targets for the cure of this disease.

Body

For Task 1, we have proposed to isolate mammalian genes involved in breast cancer cell survival. This will be achieved by establishing Enhanced Retrovirus Mutagen (ERM)-mediated genetic screen and analyzing isolated clones, by establishing secondary screens using human breast epithelial cells to confirm the role the cloned genes in DNA-damage-induced apoptosis, and by identifying the candidate genes targeted by ERM

In the last report, we described the identification of a number of genes involed in cell survival induced by DNA damage, through the novel retrovirus-based genetic screen system (3). We are currently cloning these genes into expression systems to verify their function.

For Task 2, we have proposed to biochemically characterize breast cancer genes including how BARD1 may be involved in breast cancer cell survival. BARD1 was cloned originally as an interactor of BRCA1 and has been implicated as a critical factor in BRCA1 tumor suppression (8). Missense, point mutation and loss-of-function mutations of BARD1 have been found in breast cancers (9). Our preliminary results suggest that BARD1 may modulate cell survival in response to DNA damage. One aspect of the study was to identify the factors that may interact with BARD1. To accomplish this, we took a proteomic approach. Briefly, large amount of human HeLa cells were grown and harvested. Nuclear extracts were made from these cells and then immunoprecipitated in large-scale with an anti-BARD1 antibody. The immunoprecipitates were subsequently resolved by SDS-PAGE, and visualized by Coomassie Blue Staining. Desired bands were then excised from the gels, digested, and sequenced via Mass Spectrometry.

In our last report we identified CPSF-160, CPSF-73, and Symplekin (10-12) as part of the BARD1 complex. We have performed the large-scale analysis multiple times and identified additional factors. One of these is Death inducer obliterator-1 (DIO-1). DIO-1 is an interesting protein whose sequence suggests that it may be a key mediator linking signaling events to transcriptional control. DIO-1 contains a canonical transcription activation domain at the N-terminus, which is followed by a nuclear localization signal (NLS) and a PHD finger motif found in many proteins implicated in chromatin remodeling function (13,14). Several PHD finger proteins including DIO-1 are known mediators of apoptosis. Mis-expression of DIO-1 in chick limbs caused massive cell death, supporting a critical role of DIO-1 in cell survival regulation and development (13). It has also been shown that DIO-1 mRNA is upregulated in response to a variety of stress conditions.

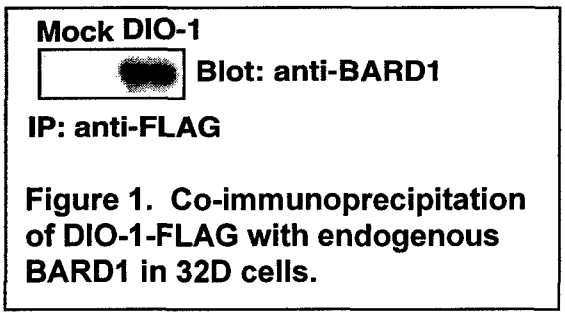

Figure 1. Co-immunoprecipitation of DIO-1-FLAG with endogenous BARD1 in 32D cells.

We therefore reasoned that BARD1 might in fact mediate cell survival signaling through regulation of DIO-1. To approach this, we first carried out experiments to test if BARD1 and DIO-1 indeed can interact directly in vivo. Lysates from 32D cells overexpressing FLAG-tagged DIO-1 were immunoprecipitated with anti-FLAG antibodies and probed for BARD1. As shown in Figure 1, exogenously expressed DIO-1 could indeed bring down endogenous BARD1. We are currently in the process of generating anti-DIO-1 antibodies for further in vivo studies.

As noted earlier, BARD1 is a BRCT domain containing protein. BRCT domains are found in a number of proteins, most notably BRCA1 and BRCA2. The biochemical properties of this domain therefore may offer important clues to the function of BARD1 and its associated complexes. Using an oriented peptide library array strategy developed in the lab (OPAL) (15), we carried out studies that determined the binding specificity of a number of BRCT domains and demonstrated that the BRCT domain represents a new class of phospho-binding domains. This work has resulted in a published manuscript.

Key Research Accomplishments

Optimization of a system to identify BARD1 associated factors
Successful identification of a number of novel factors from the proteomic approach
Identification of DIO-1 as a potential BARD1 interacting factor
Identification of phosphopeptides that bind BARD1 BRCT domain

Reportable Outcomes

We have used a proteomic approach to identify factors in the BARD1 complex. A new factor we found to interact with BARD1 is DIO-1. This interaction potentially links DNA-damage response and cell survival and is under active investigation. We also identified phosphopeptides that specifically bind the BARD1 BRCT domain.

Publications

Rodriguez M, Yu X, Chen J, and **Songyang Z.** (2003) Phospho-peptide binding specificities of BRCT domains. *J. Biol. Chem.* 278:52914-8.

Conclusions

We are continuing to modify and optimize our proteomic strategies. We have now identified an increasing number of proteins that interact with BARD1. The diverse networks of protein-protein interaction we found has placed BARD1 at the center of mediating survival pathways in response to DNA damage. The information obtained from our studies should prove especially useful for the development of new and effective screening strategies, drug targets, and treatment for breast cancer.

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