Cryo-EM Reveals a Unique BRCA1 Complex in Metastasis

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Currently, there remains a critical need for mapping proteins implicated in the progression of cancer. Visualizing the biological complexes that support metastatic disease allows for a greater understanding of personalized and targeted therapies. The Breast Cancer Susceptibility Protein (BRCA1) and its binding partner, the BRCA1-associated Ring Domain protein (BARD1), is one such complex implicated in the progression of breast and ovarian cancers. Due to the size of the protein complex (~300 kDa), cryo-Electron Microscopy (cryo-EM) provides the technical framework to better study the structure-function relationship of BRCA1 assemblies within the context of disease. Using silcon nitride (SiN) microchips with integrated microwells, we are able to illuminate the unique features of the BRCA1 protein complex in metastatic breast cancer.

We developed a protocol to capture native BRCA1 complexes from human metastatic breast cancer cells (MDA-MB-361 line; ATCC) for cryo-EM imaging and quantitative analysis on tunable silicon nitride microchips (Figure 1). We collected images on the FEI Twin BioSpirit TEM under low-dose conditions (<10 electrons/Å^2) at 120 kV. At a magnification of 68,000×, the final sampling in the output images was 4.4 Å/pixel at the specimen level. Implementing standard routines in the SPIDER software package [1] individual complexes were selected from the images and standard reference-free alignment routines were implemented to yield class averages (Figure 2a). Using 3D classification routines in the RELION software package [2], we were able to calculate an initial BRCA1-BARD1 structure and further evaluate particle heterogeneity of the sample (Figure 2d, e). The similarity of three classes in the preliminary data support the idea that the structure of BRCA1-BARD1 assemblies is not highly flexible in its dimeric form as the particles were rather evenly distributed (Figure 2e). Additional classes were not statistically significant according to the RELION output.

The C-terminal region of the BRCA1 contains two motifs collectively known as the BRCT (BRCA1 C-terminal domain). Interestingly, the resulting EM maps revealed additional density (Figure 2d) in this region. This domain serves as the platform for additional protein substrates and is imperative for coordinating genomic repair. The additional density in the BRCT domain likely represents a p53 substrate based upon complementary biochemical analysis. Importantly, mutations in these regions lead to an increased risk of breast cancer [3]. Our work for the first time suggests that beyond genomic mutations, post-translational modifications to the BRCA1-BARD1 complex may have a role in the cancer susceptibility [4].
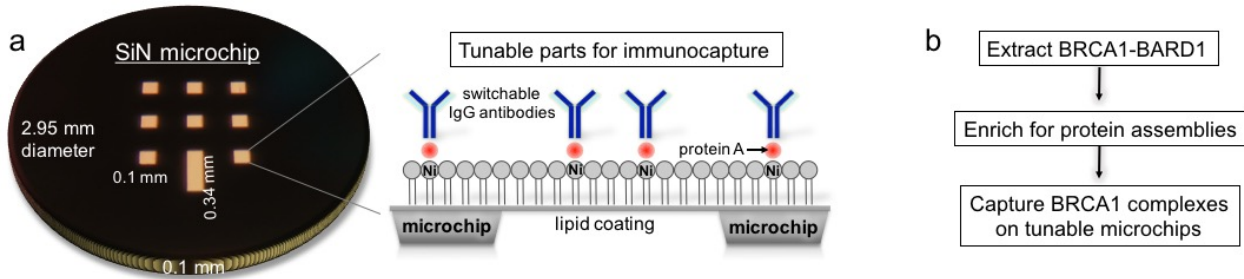
References:

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Figure 1. Tunable microchips used to capture BRCA1-BARD1 complexes from metastatic breast cancer cells. **(a)** Schematic of the customizable layers applied to the microchip that include a lipid coating, protein A (red) and switchable antibodies (blue). **(b)** Flow chart showing the rapid sample preparation using the tunable microchip technique.
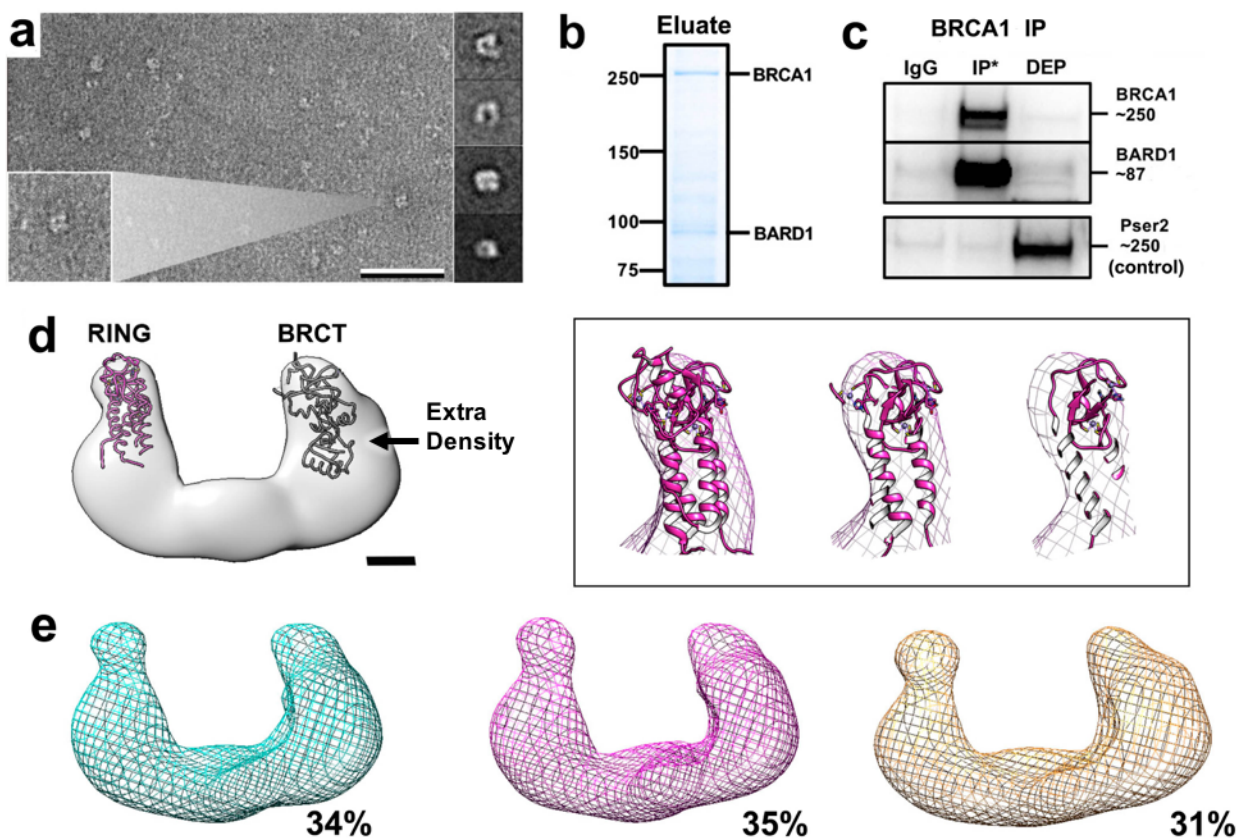
Figure 2. Cryo-EM analysis of BRCA1-BARD1 complexes derived from metastatic cancer cells. **(a)** EM image with inset and 2D class averages of BRCA1-BARD1 complexes from metastatic human cancer cells. Scale bar is 60 nm. **(b)** BRCA1 migrates at ~250 kDa while BARD1 migrates at ~87 kDa according to SDS-PAGE. **(c)** Co-IP experiments confirm BRCA1-BARD1 interactions in the enriched nuclear material. RNAP II phosphorylated at Pser2 repeats (~250 kDa) served as a negative control. Species-specific IgG control experiments show low background signal. Antibody-specific control (IgG); Input material (IN); unbound / depleted material (DEP); interacting proteins (IP). **(d)** Atomic models of the RING and BRCT domains fit within the EM density maps. Scale bar is 2 nm. The quality of the fit of the RING domain is shown through cross sections of the model within the map. **(e)** 3D classification of BRCA1-BARD1 complexes exhibit few variations, suggesting sample stability and homogeneity. The percentage of particles in each class is indicated.