doi:10.1017/S1431927616006449
Microsc. Microanal. 22 (Suppl 3), 2016

Structural Oncology – Determining 3D Structures of Breast Cancer Assemblies

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Under normal cellular conditions, the breast cancer susceptibility protein (BRCA1) protects the genome by acting as a tumor suppressor. Cells harboring mutations in the *BRCA1* gene lose the ability to properly repair DNA damage and transcribe their genome. These effects can contribute to genomic instability and cancer induction [1]. Indeed, mutations in the *BRCA1* gene are heavily linked to the development of hereditary breast and ovarian cancers [2]. A major question in the field remains, how do mutations in BRCA1 disrupt molecular processes?

Currently, there is little information available for how BRCA1 interacts with other proteins. Conventional biochemical separations do not yield BRCA1 complexes suitable for structural analysis. To address this issue, we have recently developed a tunable microchip system that facilitates the rapid recovery of native protein assemblies from the nuclear material of patient-derived breast cancer cells (Figure 1). We employed the new microchip system to isolate and visualize BRCA1-transcriptional complexes, for the first time. We collected cryo-EM images of BRCA1 assemblies under low-dose conditions and processed the images using the RELION software package [3]. Resulting 3D structures of the assemblies were interpreted by using a combination of antibody-labeling and molecular modeling techniques. This information allowed us to interpret the interactions between the RNAP II core complex and BRCA1 structural elements in the presence of DNA fragments. K63-linked ubiquitin moieties were also resolved in the structure, indicating a signal for DNA damage repair. Complementary biochemical experiments supported these findings to reveal the first 3D insights of BRCA1 protein assemblies in the context of human disease [4].

Additional molecular modeling experiments on the BRCA1 C-terminal domain (BRCT) suggested a mechanism for peptide interactions within the BRCT binding site (Figure 2a, b). Phosphorylated peptide repeats present in the RNAP II core (pSer5) have the proper physical attributes to fit within the BRCT binding site. However, models of a prevalent BRCA1 clinical mutation ($BRCA1^{5382insC}$) showed that the mutated BRCT domain does not support these associations (Figure 2c, d). Biochemical analysis of protein interactions with $BRCA1^{5382insC}$ also indicated decreased affinity for its nuclear partner BARD1 (Figure 2e). Overall, these findings demonstrate new strategies to delineate the multifaceted role of BRCA1 in RNA metabolism while defining opportunities to dissect native BRCA1 protein interactions. This research is supported by funds from the Commonwealth Health Research Board [2080914], the Concern Foundation [303827], and NIH/NCI [R01CA193578] to D.F.K.

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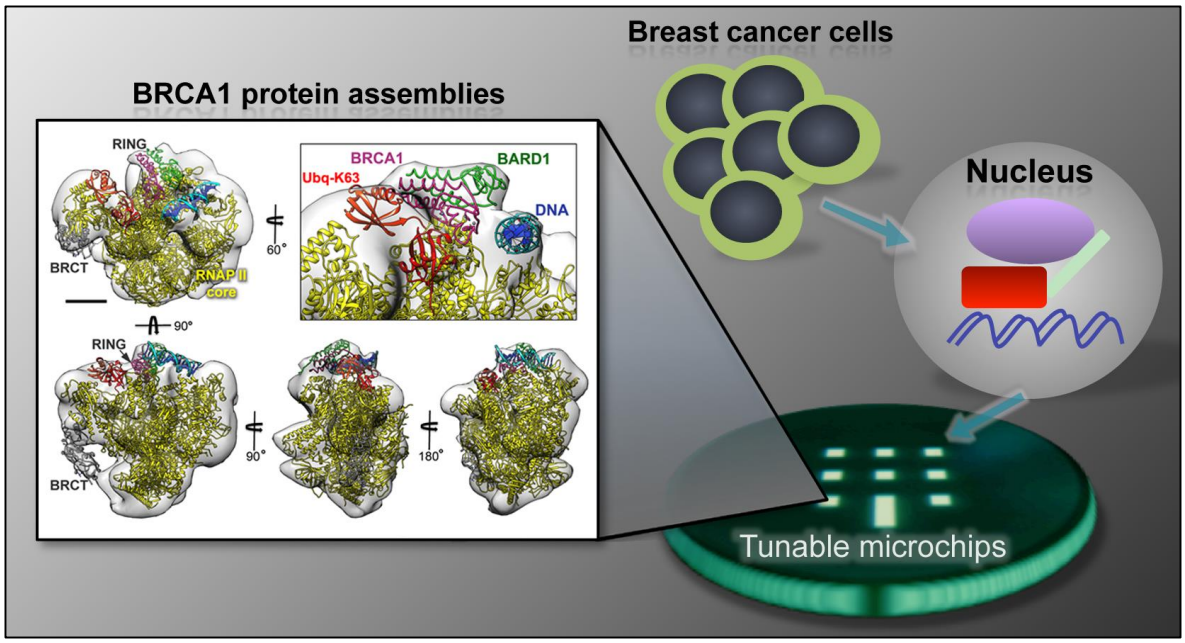

Figure 1. Hereditary breast cancer cells were lysed and the nuclear material from the cells was enriched in RNAP II core complexes along with BRCA1, and BARD1. The BRCA1-transcriptional assemblies were incubated upon tunable microchips decorated with antibodies against the BRCA1 C-terminal domain (BRCT). Cryo-EM images were collected and the resulting EM density maps revealed the first structural information of BRCA1 assemblies formed in breast cancer patients.

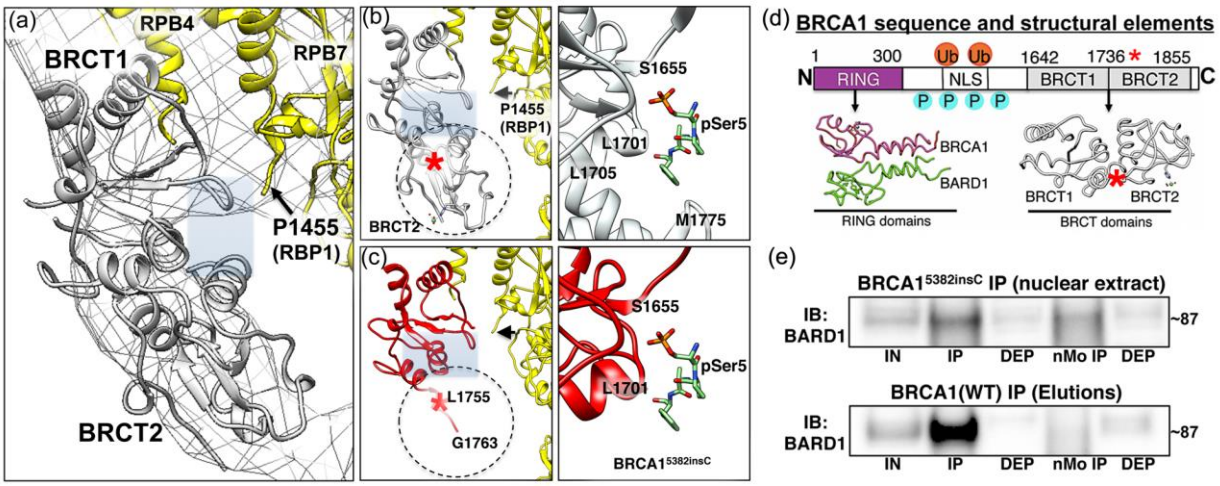

Figure 2. (a) Structural findings revealed the RNAP II core (yellow) was proximal the BRCT (gray). **(b)** Models for the wild type BRCT show potential binding interactions with peptide repeat (pSer5) found in RNAP II. **(c)** Models for the mutated BRCA1^{5382insC} reveal the manner in which a prevalent breast cancer mutation (red star) disrupts these associations. **(d)** Primary sequence and structural elements of the BRCA1 protein show the location of the 5382insC mutation. **(e)** Co-IP experiments indicate the BRCA1^{5382insC} mutation (top panel) affects interactions with its binding partner BARD1 in comparison to wild type BRCA1 (bottom panel). IN (input material); IP (immunoprecipitation); Dep (unbound material); nMo (normal mouse IgG control). Adapted from Gilmore *et al*., 2015 [4].

https://doi.org/10.1017/S1431927616006449 Published online by Cambridge University Press