proliferation checkpoint genes, such as *CDKN1A*, in their repressed state and, thereby, cancer cells even tighter in their undifferentiated phenotype. In this regard, mutant EZH2 exhibits a similar effect in GCB-DLBCL, with its hyperactivity increasing gene repression of B cell maturation-related factors. In both aberration types, a key position at the gate toward B cell differentiation is held by bivalent genes, whose hypermethylation or hyper-repression with H3K27me3 further blocks the B cells maturation process and locks the cells in a germinal center configuration. From a therapy perspective, it would be of great interest if GCB-DLBCL cases with a high degree of epigenetic switches and hence reduced reversibility were less sensitive to EZH2 inhibitor treatment. Herein, lymphoma patients with EZH2 hypermethylation phenotype might profit from combinational therapies of EZH2 inhibitors (McCabe et al., 2012) and DNA demethylating agents (Herranz et al., 2006) (Figure 1).

Unlike in GCB-DLBCL, biological consequences of PcTG hypermethylation in other cancer types are not easy to dissect. Here, PcTG silencing in cancer types derived from tissue progenitor cells might contribute to a transformation process toward cancer stem cells by effectively preventing cell differentiation. Likewise, somatically-derived cancer types might convert to a more undifferentiated state with support of the formation of a stem cell-like epigenetic setting. Accordingly, in addition to DLBCL, other tumor types might profit from therapeutic strategies involving EZH2 inhibitors and DNA demethylating agents.

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Luring BRCA1 to the Scene of the Crime

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To preserve genome stability, BRCA1 must be recruited to sites of DNA damage, where BRCA1 facilitates repair of double-strand DNA breaks (DSBs). In this issue of *Cancer Cell*, Li and Yu report that BRCA1 recruitment involves a novel interaction between its partner protein BARD1 and poly(ADP-ribose) chains at the DSB.

Germline mutations of *BRCA1* are a primary cause of hereditary breast and ovarian cancer. The central role of BRCA1 in the cellular response to double-strand DNA breaks (DSBs) is thought to be a key aspect of its tumor suppression activity (Li and Greenberg, 2012). The DSB response entails recruitment of various signaling and repair factors, including BRCA1, to nuclear sites of damaged DNA. These factors selectively accumulate at one or both of two cytologically distinct subcompartments: (1) the "DSB end" itself and (2) the flanking "DSB chromatin" marked by γH2AX, a phosphorylated form of the H2AX histone variant (Bekker-Jensen et al., 2006). Of note, BRCA1 can be recruited to both subcompartments, suggesting that it performs multiple, as yet undefined, functions in DSB repair. Its localization to DSB chromatin occurs through an elaborate pathway that entails H2AX phosphorylation, H2A polyubiquitination, and ubiquitin-mediated recruitment of the RAP80 complex (Li and Greenberg, 2012). Ultimately, γH2AX-dependent assembly of BRCA1 requires its direct interaction with Abraxas, a polypeptide within the RAP80 complex.

Early on, Celeste et al. (2003) reported that BRCA1 recruitment to DSBs is a kinetically biphasic process comprised of an early γH2AX-independent stage and a late γH2AX-dependent stage. Subsequently, Mailand et al. (2007) observed that BRCA1 localization to DSB flanking chromatin, but not the DSB end subcompartment, is ablated upon

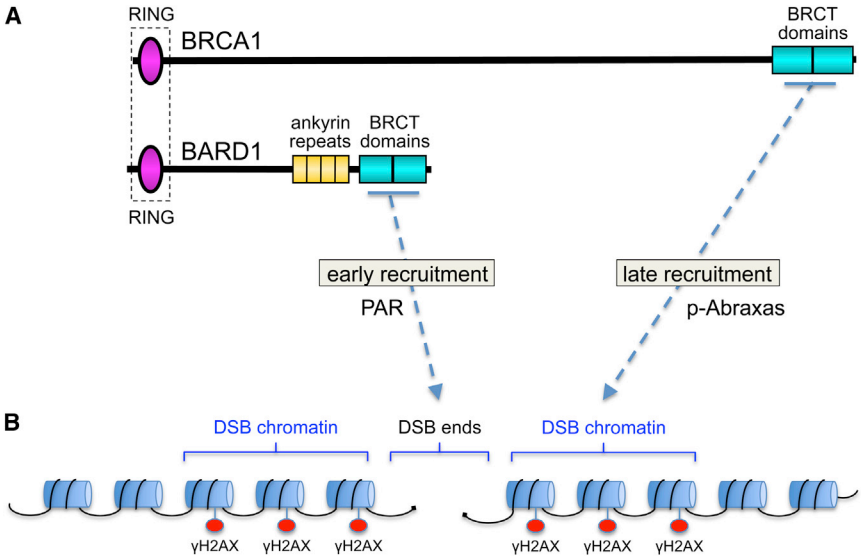

Figure 1. Biphasic Recruitment of the BRCA1/BARD1 Tumor Suppressor to Distinct Subcompartments of a DSB

(A) An illustration of the BRCA1/BARD1 heterodimer showing the RING and tandem BRCT motifs of both proteins as well as the internal ankyrin repeats of BARD1. Heterodimerization of BRCA1 and BARD1 is mediated by sequences encompassing their respective RING domains (dotted rectangle).

(B) A diagram of the chromosomal region spanning a DSB, including the "DSB end" subcompartment and the flanking "DSB chromatin" subcompartment containing γH2AX histones. In the DNA damage response, early recruitment of BRCA1/BARD1 to DSB ends is mediated by BARD1 BRCT recognition of PAR (as reported here by Li and Yu, 2013), while late γH2AX-dependent recruitment to the flanking DSB chromatin entails BRCA1 BRCT recognition of the phosphorylated Abraxas protein.

inactivation of the γH2AX pathway. These results implied the existence of a distinct, but unknown, mechanism for rapid assembly of BRCA1 at DSB ends. To elucidate this process, Li and Yu (2013; in this issue of *Cancer Cell*) combined laser microirradiation and live cell imaging to specify the requirements for early (<1 min post damage) and late (2–10 min post damage) BRCA1 recruitment.

As illustrated in Figure 1, BRCA1 possesses an amino-terminal RING domain and two carboxy-terminal BRCT repeats. Structural studies show that two tandem BRCT repeats can form a globular domain that acts as a phospho-recognition surface. Indeed, the BRCT repeats of BRCA1 bind, in a mutually exclusive manner, to at least three different phosphoproteins implicated in DSB repair, one of which is the Abraxas polypeptide that mediates BRCA1 assembly to DSB chromatin (Li and Greenberg, 2012). In vivo, BRCA1 exists as an obligate heterodimer with BARD1, a related protein with the same configuration of RING and BRCT motifs (Wu et al., 1996). Although proteins that bind the BARD1 BRCT repeats have not yet been reported, structural studies suggest that these repeats also form a phospho-binding pocket.

Germline *BARD1* mutations that segregate with tumor susceptibility have recently been identified in some families with hereditary breast cancer (e.g., De Brakeleer et al., 2010), indicating that BARD1, like BRCA1, also serves as a tumor suppressor. Indeed, most functions of BRCA1 and BARD1, including their tumor suppression activity, appear to be mediated by the BRCA1/BARD1 heterodimer (Shakya et al., 2008). Thus, Li and Yu examined the requirements of both proteins for BRCA1/BARD1 recruitment to DSBs (Li and Yu, 2013). As expected, late recruitment was dependent on the BRCT domains of BRCA1 and abrogated by tumor-associated mutations that ablate its interaction with phosphorylated Abraxas. Notably, however, early recruitment of BRCA1 was independent of its BRCT repeats, but instead required the BRCT repeats of BARD1. Importantly, early BRCA1 recruitment was ablated by tumor-associated missense mutations of BARD1, as well as artificial BARD1 mutations designed to disrupt the predicted phospho-binding pocket of its BRCT domain. Together, these data argue that rapid assembly of the BRCA1/BARD1 heterodimer at DSBs is dependent on the phospho-recognition properties of the BARD1 BRCT domain.

A bigger surprise emerged when Li and Yu identified the molecular factor that mediates γH2AX-independent recruitment of BRCA1/BARD1 (Li and Yu, 2013). One of the earliest steps in the DNA damage response, occurring within seconds of DSB formation, is recruitment and activation of PARP1, a nuclear enzyme that catalyzes PARylation—the polymerization of ADP-ribosyl groups (from donor NAD+ molecules) to form poly(ADP-ribose) (PAR) chains and their covalent conjugation to select substrate proteins (Rouleau et al., 2010). Knowing that PAR polymers are rich in phosphate groups (two per ADP-ribosyl monomer), Li and Yu (2013) surmised that PAR might act as a receptor for phospho-dependent binding by the BARD1 BRCT domain. Through a series of stringent analyses, they established that the BARD1 BRCT domain does indeed interact with PAR and does so with affinities comparable to those of other known PAR-binding motifs. Moreover, they found that early γH2AX-independent recruitment of BRCA1/BARD1 is ablated upon disruption of the BARD1/PAR interaction by various means, including enzymatic inhibitors of PARP1 and mutations that abolish the phospho-recognition properties of the BARD1 BRCT.

Tandem BRCT repeats, such as those of BRCA1 and BARD1, are found in a number of cellular proteins. Previously, all the phospho-ligands reported to bind these BRCT domains have been phosphorylated isoforms of proteins, such as phospho-Abraxas. The discovery that a nucleic acid polymer such as PAR can functionally interact with the BARD1 BRCT domain indicates that the potential phospho-recognition properties of BRCT proteins may be more diverse than anticipated. Structural studies should uncover the molecular determinants of the BARD1/PAR interaction and how they relate to those that form canonical BRCT/phosphoprotein complexes. In addition, it will be interesting to ascertain whether the BARD1 BRCT domain can also bind in a traditional manner to other as yet unknown phosphoprotein ligands,

and whether these interactions are in turn influenced by PAR formation.

With these new findings, a comprehensive and satisfying picture has emerged to account for the biphasic pattern of BRCA1 assembly at DSBs (Figure 1). In this scenario, the BRCA1/BARD1 heterodimer is rapidly recruited to DSB ends in an H2AX-independent manner by virtue of the interaction of its BARD1 BRCT domain with PAR. Subsequently, the heterodimer associates in a more durable fashion with the flanking DSB chromatin through the well-defined γH2AX pathway upon interaction of its BRCA1 BRCT domain with phosphorylated Abraxas. It is important to note that the vast majority of BRCA1 and BARD1 mutations associated with hereditary breast cancer are either (1) truncating or missense mutations that ablate BRCT phospho-recognition or (2) missense mutations that abolish BRCA1/BARD1 heterodimerization. Thus, a common denominator of most tumor-predisposing defects is disruption of either the early and/or late pathways of BRCA1/BARD1 recruitment. A key objective of future studies will be to determine the specific functions of BRCA1/BARD1 within each of the two DSB subcompartments and to ascertain whether these functions are relevant for tumor suppression.

The exquisite sensitivity of BRCA1- and BRCA2-defective tumors to PARP inhibitors was originally thought to reflect a synthetic lethal effect whereby BRCA1/2 mutant cells, which are intrinsically defective for DSB repair, are rendered inviable by simultaneous inactivation of single-strand DNA break repair, which is normally dependent on PARP1 (Rouleau et al., 2010). However, recent studies suggest that this model is incomplete and that other actions of PARP may also be relevant (Helleday, 2011). As noted by Li and Yu (2013), most BRCA1-deficient tumors have lesions of the BRCA1 BRCT domains that would disrupt the late γH2AX-dependent, but not the early PAR-dependent, pathway of BRCA1/BARD1 recruitment. Thus, the heightened sensitivity of these cells to PARP inhibition might reflect the fact that both modes of BRCA1/BARD1 recruitment are ablated simultaneously. As such, this work may have important clinical implications for the anti-cancer activity of PARP inhibitors.

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Chromoplexy: A New Category of Complex Rearrangements in the Cancer Genome

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Widespread structural alterations of cancer genomes are increasingly observed in a broad spectrum of tumors. In a recent issue of *Cell*, Baca and colleagues describe large chains of rearrangements that coordinately affect multiple chromosomes in prostate cancer. This phenomenon of chromoplexy may define cancer subtypes and drive punctuated tumor evolution.

The application of next-generation sequencing technologies has resulted in systematic efforts to characterize the mutational spectrum, genomic alterations, and clonal evolution of a wide range of tumors. In particular, whole-genome sequencing approaches can reveal extensive structural rearrangements throughout the tumor genome, which can be difficult to detect using more limited exome sequencing approaches. The whole-genome sequencing