the immunostimulatory versus immunosuppressive effects of IL-10 remains alive and well.

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"Ring-Fencing" BRCA1 Tumor Suppressor Activity

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BRCA1 is a crucial human breast and ovarian cancer tumor suppressor gene. The article by Drost et al. in this issue of *Cancer Cell* together with a recent paper in *Science* now provide a clearer picture of how this large and complex protein suppresses tumorigenesis.

Breast and ovarian cancer are major causes of mortality and morbidity in the developed world. Up to 10% of all breast cancers are due to the inheritance of germline mutations in two breast cancer susceptibility loci (*BRCA1* and *BRCA2*). In fact, mutations in *BRCA1* account for up to 80% of families with breast and ovarian cancer predisposition and therefore pose a significant burden to human health. The *BRCA1* gene encodes a large polypeptide that interacts with its constitutive binding partner BARD1. There is a body of evidence indicating that the BRCA1-BARD1 heterodimer is a crucial regulator of the cellular response to DNA damage. Loss of BRCA1 results in genomic instability, probably due to an impaired DNA damage response. It is therefore likely that BRCA1 suppresses tumorigenesis by preventing genetic instability.

Two regions of the BRCA1 protein are thought to be critical to this function: first, an N-terminal RING domain that has E3 ubiquitin ligase activity that is potentiated through its interaction with BARD1; and second, the C-terminal BRCT domain that mediates the specific interaction with the phosphorylated form of DNA repair factors (Figure 1) (Huen et al., 2010). However, the mechanism by which these two regions contribute to tumor suppression has not been clarified. Two recent papers provide this critical information (Shakya et al., 2011; Drost et al., 2011 [this issue of *Cancer Cell*]). In addition the conclusions drawn from these new studies have potential clinical implications for the treatment of patients with breast cancer in which *BRCA1* has been mutated.

Using mice, Shakya et al. (2011) dissected the function of the RING domain associated ubiquitin ligase activity and the BRCT domain. In particular they assessed how these two regions of BRCA1 contributed to embryonic development (the homozygous *Brca1* null mutation is embryonic lethal) and tumor suppression. The E3 ligase activity of the RING domain has previously been implicated in the DNA damage response (Ruffner et al., 2001) and more recently in the maintenance of heterochromatin (Zhu et al., 2011). Furthermore, human cancer predisposing mutations often clustered in this region with some, such as $BRCA1^{C61G}$, abrogating E3 ubiquitin ligase activity. Cellular studies have shown that BRCA1 localized to DNA damage induced foci which also contained polyubiquitinated substrates (Morris and Solomon, 2004). Though compelling, these lines of evidence are correlative and lack genetic evidence directly linking the E3 ubiquitin ligase activity to the role of BRCA1 in DNA repair and in tumor suppression. Shakya et al. (2011) therefore engineered a point mutation within the RING finger domain of BRCA1. This $Brca1^{I26A}$ mutation results in the loss of the E3 ligase activity of BRCA1 but does not compromise either the stability of the protein or its interaction with BARD1. Previous work from the

same lab showed that homozygous ES cells carrying this mutation were not DNA damage sensitive and were competent at DNA double strand break repair through homologous recombination (Reid et al., 2008). To complete their analysis, they have now transmitted the mutation into the mouse germline. Homozygous *Brca1*^{I26A} animals were born normally at expected Mendelian ratios and were not cancer-prone, although they did display moderate male infertility. To determine if the E3 ligase activity of BRCA1 had a tumor suppression function independent of DNA repair, the authors introduced this mutation into three different cancer-prone genetic backgrounds and found no additive influence. Given the correlative evidence described earlier, the key observation that BRCA1 E3 ligase activity was dispensible for both DNA repair and tumor suppression is a complete surprise.

To test the function of the BRCT domain, Shakya et al. (2011) introduced a point mutation (S1598F, corresponding to S1655F in human BRCA1). This was predicted to disrupt domain function since the residue should be critical for recognition of phosphorylated DNA damage response factors. In contrast to the I26 mutation, the authors found residue S1598 to be crucial for both development and cancer suppression. Although both mutations were engineered into two regions that often carry breast-/ovarian cancer-associated mutations, it is important to realize that I26A has not been identified as a germline mutation in *BRCA1* patients.

In this issue of *Cancer Cell*, Drost et al. (2011) engineered mice carrying a well known breast cancer associated mutation of *BRCA1*^{C61G}. This amino acid substitution in the RING domain is known to inactivate E3 ligase activity but also to disrupt the RING-RING interaction with BARD1 (Figure 1) (Brzovic et al., 2001; Ruffner et al., 2001). The consequences of this point mutation in mice contrast with what Shakya et al. (2011) observed. Homozygous mice were not born because of developmental delay and attrition early in development. Drost et al. (2011) then introduced *Brca1*^{C61G} into the KB1P mouse model of mammary tumor development in which both *Brca1* and *p53* are specifically deleted in the mammary tissue (*K14Cre;Brca1*$^{F/C61G}$;*p53*$^{F/F}$). The latency and nature of mammary tumors developed by mice carrying one allele of *Brca1*^{C61G} and one null allele was similar to those carrying two *Brca1* null alleles. However, these cancers showed different responses to two classes of chemotherapeutic agents: cisplatin, a crosslinking agent, and Olaparib, a PARP inhibitor.

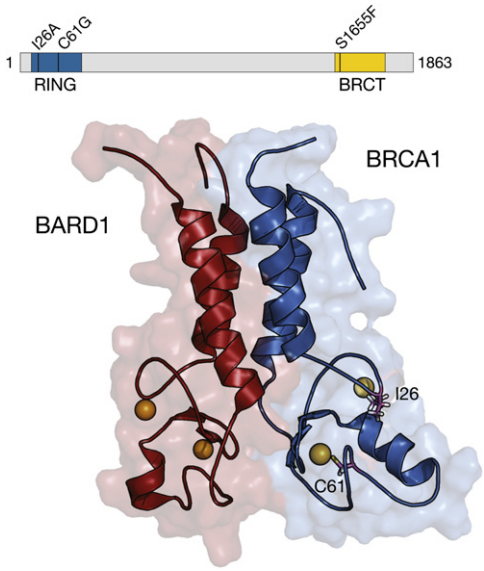

Figure 1. Domain Organization and Structure of BRCA1

Top, Schematic representation of the BRCA1 polypeptide showing the N-terminal RING domain and the C-terminal BRCT domains. Point mutations discussed here are indicated.

Bottom, The NMR structure of the BRCA1/BARD1 dimerized RING domains (Brzovic et al., 2001). The relevant key amino acids discussed here are highlighted.

An important development in treating tumors with *BRCA1* mutations was the demonstration that pharmacological inhibition of poly ADP ribosylase activity (PARP inhibitors) results in potent cytotoxicity of *BRCA1*-deficient cancer cells. This class of drug has been shown to result in striking clinical responses in *BRCA1* mutant patients with advanced breast cancer (Fong et al., 2009; Tutt et al., 2010). PARP-facilitated base excision repair can compensate for the defect in DNA repair caused by BRCA1 deficiency. Removing this backup by PARP inhibition would therefore lead to the accumulation of unrepaired endogenous DNA damage and subsequently cell death. Drost et al. (2011) adoptively transferred tumors carrying the wild-type *Brca1*, *Brca1*^{C61G}, or *Brca1* null into syngeneic recipients; they then treated the mice with either cisplatin or olaparib. They noted that the *Brca1*^{C61G} tumors responded poorly to cisplatin and olaparib treatments compared to *Brca1* null tumors. The *Brca1* null tumors did not develop resistance to cisplatin and were undetectable while being treated with olaparib, whereas the *Brca1*^{C61G} group developed resistance to both drugs. Importantly, they found that the cisplatin-resistant *Brca1*^{C61G} tumors were cross-resistant to olaparib but responded to the bifunctional alkylator nimustine, which preferentially generates interstrand crosslinks similar to cisplatin-sentitive tumors. To extend on their findings, Drost et al. (2011) looked, in more detail, at the DNA repair defect in tumors deficient for *Brca1* or carrying the *Brca1*^{C61G} mutation. They found that both groups of tumors developed similar levels of DNA damage following treatment with the crosslinking agent cisplatinum. However, only *Brca1* null tumors accumulated DNA damage following treatment with olaparib. The difference in response to treatment and development of resistance may be due to residual DNA repair activity of the BRCA1^{C61G} protein. Finally, they excluded that reversion of the *Brca1*^{C61G} mutation was responsible for drug resistance.

Cumulatively, these two studies provide new genetic insight into BRCA1 tumor suppressor activity, with clinical implications. The first salient conclusion is that the RING domain-associated E3 ligase is dispensable for both DNA repair and tumor suppression—an important fact that allows the field to move on. Second, some mutations within the RING domain compromise tumor suppressor function through impaired DNA repair. This could be due to the impact of such a mutation on BRCA1's interaction with BARD1. The disruption of interaction between BRCA1 and BARD1 may affect the stability of both proteins or suggest functions of the BRCA1-BARD1 interaction independent of E3 ligase activity. Perhaps the most provocative finding is that tumors carrying the RING-associated mutation rapidly

acquire resistance to cisplatin and PARP inhibitors. Aside from allowing clinicians to anticipate such a response, if a patient should carry a tumor with this class of mutation, what could be the possible mechanism that gives rise to this? We do not know; however it does seem that these tumors tolerate the accumulation of DNA damage or that they are able to switch on compensatory repair pathways that require a BRCA1 function that is not affected by this mutation, or a combination of both. All in all, these studies provide new murine models for mutant *BRCA1*-driven breast carcinogensis, providing intriguing new results and an excellent platform for future studies.

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Many Tumors in One: A Daunting Therapeutic Prospect

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In this issue of *Cancer Cell*, Snuderl and coworkers demonstrate intratumoral genetic heterogeneity in glioblastoma based on in situ amplification of distinct genomic loci within individual cells in a mutually exclusive pattern. These findings may herald trouble for current targeted therapies but provide insights for future treatment strategies.

Glioblastoma multiforme (GBM) is among the most lethal of all human cancers with a median survival of about 14 months despite aggressive surgical resection and adjuvant chemotherapy with radiation (Stupp et al., 2005). Nevertheless, the clinical course of some GBM patients can be highly variable, which may, in part, be attributable to the recent identification of a least four molecular subtypes of GBM (Verhaak et al., 2010). These molecular subtypes, however, do not explain the diverse array of pathological findings within any given GBM including morphologically distinct tumor cells with various patterns of growth (e.g., bulky tumor to single cell invasion along white mater tracks) and variable effects on the host tissue that are pathognomonic of the disease and responsible for the designation "multiforme" (Bailey and Cushing, 1926).

One potential explanation for the diversity of intratumoral findings is that the phenotype of the GBM clonogenic or stem cell is plastic and variable, affected by both intracellular (e.g., stochastic) and extracellular (e.g., microenvironmental) stimuli. As a result, the variable phenotypes may represent different degrees of aberrant differentiation of a cancer stem cell. A more complex and therapeutically challenging explanation for the clinical and pathologic variability of GBMs, however, is that we are actually dealing with "different tumors" within a given patient.

The term "tumor heterogeneity" can be defined as the presence of subclones of cells, within a given tumor, with different genetic aberrations that mediate divergent biology that define the natural history of that particular tumor (Navin et al., 2011; Yachida et al., 2010). Although not a new idea, the recent advent of high-throughput molecular and genetic methodologies has begun to explore the nature of this phenomenon. To that end, several recent papers have used high-resolution chromosomal copy number analysis and next generation sequencing to show a range of genetically divergent tumor cell clones within leukemia (Stephens et al., 2011), breast cancer (Navin et al., 2011), and pancreatic cancer (Yachida et al., 2010).

In this issue of *Cancer Cell*, Snuderl and co-workers (2011) use fluorescence