was 10-fold less potent for Vps34 (450 nM) but 1000-fold less potent for p110γ (4,428nM) giving a 10-fold selectivity in favor of Vps34. At this stage there are no structural data confirming the binding mode of PT210 in Vps34 or p110γ, but the results suggest that even more potent and selective inhibitors may be within reach.

So how might such emerging inhibitors be used? First, they could be employed as improved tools to ask outstanding questions about the precise roles of Vps34 in cells. With respect to therapy, since autophagy is a double-edged sword in cancer (White and DiPaola, 2009) the jury is still out as to whether inhibiting autophagy would be a good or a bad thing. The potential therapeutic effects of pharmacologic Vps34 modulation may well be context-dependent, and thus there could perhaps be a need for biomarkers for patient selection. Improved Vps34 inhibitors would allow us to determine in a better way than before whether it is time for the newly unveiled ancestral PI3K to join some of the upstart younger generation as a new cancer drug target. Furthermore, the recent emergence of GOLPH3 as an oncoprotein involved in vesicular trafficking (Scott and Chin, 2010) suggests that this area might be of broader therapeutic significance, giving rise to an even more extended target family.

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Loss of 53BP1 Is a Gain for BRCA1 Mutant Cells

Elizabeth M. Kass,[1] Mary Ellen Moynahan,[2] and Maria Jasin[1,*]
[1]Developmental Biology Program
[2]Department of Medicine
Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA
*Correspondence: m-jasin@ski.mskcc.org
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Mutations in *BRCA1* predispose to tumorigenesis presumably from the inability to accurately repair DNA double-strand breaks by homologous recombination. Two new papers shed light on how loss of the DNA damage response protein 53BP1 reverses phenotypes of BRCA1 mutant cells, with potential clinical implications.

Defects in homologous recombination (HR) cause chromosome instability and are associated with tumor predisposition (Moynahan and Jasin, 2010). The inability to accurately repair DNA double-strand breaks (DSBs) by HR ultimately forces cells to rely on alternative nontemplate-based repair pathways, including nonhomologous end joining (NHEJ), resulting in the accumulation of chromosome aberrations, a hallmark of tumor cells. *BRCA1*, mutations of which are associated with a markedly increased risk of breast and ovarian cancer, was the first tumor suppressor gene identified to have an important role in HR.

Recently, a surprising observation was reported for mice deficient in BRCA1 and the DNA damage response protein 53BP1, in that loss of 53BP1 rescued the embryonic lethality, tumor susceptibility, and premature aging of mice homozygous for *Brca1* exon 11 deletion without fully eliminating the chromosome instability (Cao et al., 2009). Although deficiency of other DNA damage response factors such as p53 and Chk2 had

previously been reported to rescue the lethality of *Brca1*$^{\Delta 11/\Delta 11}$ mice, tumorigenesis was nonetheless observed on these backgrounds. Two new papers pursue the protective mechanism imparted by 53BP1 loss in BRCA1 mutant cells and explore its potential therapeutic implications.

In the April 16th issue of *Cell*, Bunting and colleagues provide evidence for the restoration of error-free homology-directed repair in *Brca1*$^{\Delta 11/\Delta 11}$ mouse cells by deletion of 53BP1. Previous work had indicated that 53BP1 promotes NHEJ while suppressing HR, probably mediated by its interaction with dimethylated lysine 20 of histone H4 (Xie et al., 2007). When 53BP1 is inhibited, the normal competition between the two prominent DSB repair pathways (Moynahan and Jasin, 2010) is disrupted and cells have increased HR. In the new report, 53BP1 deficiency in *Brca1*$^{\Delta 11/\Delta 11}$ B cells is shown to result in a reduction in spontaneous and induced asymmetric radial chromosome structures, which are characteristic of HR deficiency and require NHEJ to form (Bunting et al., 2010).

Importantly, the reduction in radials is also seen upon treatment with an inhibitor of the single-strand break repair protein poly(ADP-ribose) polymerase (PARP). PARP inhibitors selectively kill cells that are deficient in HR and are currently being used in clinical trials to treat cancer patients with *BRCA1* or *BRCA2* mutation (Rouleau et al., 2010). Bunting et al. observed that 53BP1 loss renders *Brca1*$^{\Delta 11/\Delta 11}$ cells insensitive to PARP inhibitor-induced cell death.

In a complementary paper recently published in *Nature Structural and Molecular Biology*, Bouwman and colleagues provide evidence that 53BP1 loss rescues phenotypes of cells completely devoid of BRCA1 (Bouwman et al., 2010). Utilizing a transposon-based insertional mutagenesis screen in conjunction with a selectable *Brca1* conditional allele, they identified 53BP1 as a suppressor of the proliferation defect of *Brca1* null mouse cells. Depletion of 53BP1 also reduced, although did not eliminate, chromosome aberrations and reversed cisplatin sensitivity induced by *Brca1* inactivation, consistent with a restoration of HR proficiency. Further, Bouwman and colleagues found that 53BP1 depletion reduced checkpoint activation elicited by unrepaired DNA damage resulting from *Brca1* inactivation.

How might 53BP1 disruption restore HR levels in BRCA1-deficient cells? The central step in HR is DNA strand exchange catalyzed by the Rad51 protein. Because Rad51 functions by forming a nucleoprotein filament with single-stranded DNA, a critical, early step of HR is DNA end resection for single-stranded DNA generation (Moynahan and Jasin, 2010). Whereas BRCA2, Rad51 paralogs, and other proteins are thought to promote Rad51 nucleoprotein filament formation, BRCA1 is implicated in an upstream step of HR, possibly end resection itself, because BRCA1 promotes other repair pathways with single-stranded DNA intermediates (Stark et al., 2004). Bunting et al. provide evidence that 53BP1 suppresses end resection: during resection, RPA loads onto the single-stranded DNA and is phosphorylated; deletion of *53BP1* dramatically increases RPA phosphorylation.

A prediction of this model is that, whereas loss of 53BP1 would rescue defects in homology-directed repair in *Brca1* mutant cells, its absence would not rescue cells with mutations in downstream HR genes. Both groups addressed this point, Bouwman et al. with *Brca2* mutation and Bunting et al. with mutation of a Rad51 paralog gene, *Xrcc2*, and found that 53BP1 loss has no effect on either of these downstream mutations as predicted. In general, NHEJ components suppress HR, and evidence suggests that this may be through inhibition of end resection (Moynahan and Jasin, 2010). Nonetheless, unlike 53BP1, loss of the NHEJ component Lig4 does not abrogate the PARP inhibitor-induced radial chromosomes or cell death of *Brca1*$^{\Delta 11/\Delta 11}$ cells, indicating a specific role for 53BP1 (Bunting et al., 2010).

Given that 53BP1 loss is associated with increased end resection as measured by RPA phosphorylation and that ATM kinase activity has been shown to promote end resection (Jazayeri et al., 2006), Bunting et al. decided to examine the effects of ATM inhibition. Chemical inhibition of ATM resulted in decreased RPA phosphorylation, reduced Rad51 foci formation after ionizing radiation, and, significantly, resensitized *Brca1*$^{\Delta 11/\Delta 11}$ *53BP1*$^{-/-}$ cells to the antiproliferative effects of PARP inhibition, suggesting a critical role for ATM in promoting HR after loss of 53BP1.

Besides mechanistic insights, both studies have potentially intriguing therapeutic implications. Breast cancers arising in *BRCA1* mutation carriers are often high grade, lack expression of both estrogen and progesterone receptors, and do not overexpress ERBB2/HER2, hence are often referred to as triple-negative breast cancers. An analysis of breast tumor samples from two independent cohorts showed a significant association between triple-negative breast tumors and low levels of 53BP1 expression (Bouwman et al., 2010). Furthermore, tumors from *BRCA1/2* mutation carriers frequently showed reduced 53BP1 staining as compared to non-*BRCA1/2* tumors. The decrease in 53BP1 may be clinically important given that patients with triple-negative breast tumors that demonstrated low 53BP1 staining had decreased survival.

Resistance to platinum-based chemotherapeutic agents is not uncommon in ovarian tumors with *BRCA1* mutations and may be mediated in part by secondary mutations that restore function of the BRCA1 protein (Swisher et al., 2008). Based on the resistance to both crosslinking agents and PARP inhibition presented in these papers for mouse cells, loss of 53BP1 may be an alternative mechanism by which *BRCA1* tumors in patients develop resistance to therapy. In light of their findings, Bunting et al. suggest new areas for therapeutic investigation, including use of an ATM inhibitor in combination with a PARP inhibitor for resistant tumors (Bunting et al., 2010). ATM deficiency is already known to significantly associate with triple-negative and *BRCA1/2* breast tumors in one of the cohorts examined for 53BP1 (Tommiska et al., 2008). It would be of interest to ascertain whether the breast tumors that show 53BP1 loss also demonstrate ATM loss or whether these are mutually exclusive, as would be necessary for the combination therapy to be beneficial.

In summary, these new studies shed light on a potential mechanism by which loss of 53BP1 allows cells lacking functional BRCA1 to overcome defects in HR and hypersensitivity to various DNA-damaging agents and provide convincing evidence that 53BP1 may be an important

pharmacological target for future breast cancer therapies.

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