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Review

Brit1 regulates DNA damage repair and chromosome dynamics to suppress tumor phenotypes

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ABSTRACT

Cell cycle control in mammalian cells consists of inherent mechanisms that prevent aberrant proliferation leading to cellular transformation. Such mechanisms include DNA damage detection and repair to regulate chromosome integrity, as well as cell cycle checkpoints. Recent evidence suggests that Brit1 is involved in such mechanisms of cell cycle control. Initially identified as a suppressor of telomerase activity, Brit1's function as a potential tumor suppressor was supported after its role as a mediator of the DNA damage response (DDR) pathway was detailed. DNA damage in the form of single or double strand breaks leads to Brit1 localization to the damaged site. This is followed by Brit1-mediated recruitment and activation of regulatory proteins that transduce the damage signal to activate cell cycle checkpoints. Specifically, following a DNA double stranded break (DSB), Brit1 co-localizes with γ-H2AX, followed by subsequent recruitment of ATM, ATR, 53BP1, MDC1 and NBS1 proteins. It was shown that Brit1 interaction with SWI/SNF is required for this recruitment process and IR-induced foci (IRIF) formation. Beyond its role in the DNA damage response, Brit1 has also been shown to play a central role in maintaining centrosome copy number, and regulating the timing of chromosome condensation through its interaction with condensin II. Accumulating research suggests that Brit1 is a tumor suppressor, as evidenced by an inverse relationship between Brit1 expression and chromosomal abnormalities observed in human breast cancer cell lines and ovarian and prostate tumors. Furthermore, Brit1 has been shown to be a prognostic marker used to predict breast tumor grade.

KEYWORDS: Brit1, MCPH1, microcephaly, DNA damage response, centrosome amplification

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INTRODUCTION

Maintaining an efficient DDR system in a cell is vitally important, as propagation of damaged DNA promotes genomic instability and cellular transformation [1]. Amongst the various types of genetic lesions, DNA DSBs are the most dangerous in terms of their effect on stability [2]. Sources of DSBs include exogenous factors such as ionizing radiation (IR) or endogenous factors such as reactive oxygen species (ROS)-mediated damage, meiotic recombination, and immunoglobulin recombination [3, 4]. Thus, cells have evolved an elaborate system of sensor, transducer and effector proteins that collectively form several pathways that translate the DSB signal into cellular responses. These responses manifest in the form of cell cycle checkpoint activation followed by homologous recombination (HR) or non-homologous end-joining (NHEJ) DNA repair or apoptosis [5, 6]. At the

core of this system is the ability of sensor and transducer proteins to recruit downstream molecules to the site of DSBs. An abundant amount of work on the ataxia telangiectasia mutated (ATM) and ATM-Rad3-related (ATR) proteins have shown that these two proteins are upstream transducers of DDR signaling arising from both single- and double-strand DNA breaks [7]. ATM deficiency causes immunological disorders including leukemia, while ATR deficiency can lead to developmental problems such as microcephaly [8, 9]. Given the importance of the ATM and ATR pathways, it is logical that regulators of these pathways would also be of importance. Brit1 has been shown in recent years to be involved in DDR processes by acting at least in part as a recruitment factor for various other DDR-related proteins [10], as well as promoting cell cycle checkpoint activation [11]. In addition, Brit1 has been shown to be involved in regulating chromosome dynamics and mitotic progression [12, 13]. In this review, we will highlight some of the accumulating work addressing the role of Brit1 in these fundamental cellular processes, as well as discuss the potential effect that Brit1 deficiency may have on cellular transformation.

Brit1 is a mediator of DNA damage response and repair

The *BRIT1* (BRCT-repeat Inhibitor of hTERT expression) gene was discovered in a genetic screen to identify proteins that repress the expression of hTERT, the catalytic subunit of telomerase [14]. Telomerase is strongly suggested to increase cellular lifespan, and increasing evidence show that its persistent activity is a driving force for tumor progression [15, 16]. The amino acid sequence of Brit1 was later matched to the disease gene, microcephalin (*MCPH1*), which is one of at least six genes shown to be misregulated in microcephaly, a neuro-developmental disorder in which affected patients have reduced brain and head sizes [17]. In addition, sequence analysis showed that Brit1 contains repeats of the BRCT (BRCA1 C-Terminus) domain, which recognizes phosphorylated proteins in the DDR pathways [18, 19]. BRCT domains are found in several proteins involved in DNA damage repair processes ranging from initial damage response to cell cycle checkpoint activation [20]. Such BRCT-containing proteins include BRCA1, 53BP1 and MDC1 [21-24]. The presence of tandem BRCT-repeats in Brit1 suggested that it may also be involved in DDR and repair. Indeed, early studies of Brit1 function provided evidence that Brit1 is required for cell cycle arrest at the intra-S and G2/M checkpoints initiated by IR, and limiting premature mitosis in the presence of genotoxic stress [11, 25]. At least a partial explanation for Brit1's role in checkpoint activation was provided when it was shown in the same study that Brit1 positively affected BRCA1 and Chk1 expression, two proteins well known to be involved in checkpoint activation [11]. In addition to Brit1 IRIF formation in response to IR-induced DSBs, Brit1 is crucial for the subsequent recruitment and IRIF formation of several other DDR proteins, including 53BP1, MDC1, NBS1 and p-ATM, all formerly implicated to play vital roles in DNA damage repair and response [10, 11, 26-29]. Brit1 interaction with the chromatin remodeler, SWI/SNF, provides a mechanism whereby Brit1 promotes protein recruitment and IRIF formation [30]. Together, these studies on Brit1's involvement in DDR provide the rationale for later studies depicting Brit1's role in limiting tumor progression.

Brit1 is required for foci formation after DNA damage

DNA double strand breaks may result from environmental factors that include IR and chemical agents, or endogenous processes such as immuno-globulin class switching, meiotic recombination and stalled replication forks during DNA replication [31]. Regardless of the cause, a physiological outcome that ensues in nearly all cell types is recruitment and foci formation of hundreds of proteins to the damaged site for the purpose of DNA repair [32]. Amongst these foci forming proteins, ATM and ATR are two transducer molecules that sit atop two major inter-connected signaling cascades that result in checkpoint activation and DNA repair [33]. Early breakthrough studies of Brit1's role in DDR show that Brit1 is a chromatin binding protein. In the presence of IR, Brit1 foci were observed to co-localize with γ-H2AX foci, and digestion with micrococcal nuclease demonstrated that Brit1 binds directly

to chromatin [11]. Astonishingly, later studies delineating the position of Brit1 within the hierarchical signaling pathway showed that Brit1 is upstream of both the ATM and ATR pathways [10]. Immunofluorescent imaging after IR treatment showed that Brit1 foci formed as early as two minutes after IR. Though Brit1 colocalizes with γ-H2AX, depletion of Brit1 using siRNA did not abolish γ-H2AX foci formation. However, Brit1 knockdown inhibited IRIF formation by several DDR proteins including 53BP1, MDC1, NBS1, as well as p-ATM. This supports earlier work showing that Brit1 promotes activation of NBS1 via ATM-mediated phosphorylation at S343 [11]. These two pieces of data provide evidence that Brit1 acts just downstream of or in parallel with γ-H2AX. It is interesting to note that though γ-H2AX is phosphorylated by several kinases of the PI-3 kinase and PIKK family [34], loss of Brit1 alone appears to inhibit downstream recruitment of DNA damage repair proteins to the vicinity of γ-H2AX foci, thus effectively blocking the repair signal at γ-H2AX. The inability of DDR proteins to form IRIFs was explained by chromatin fractionation, which confirmed that these molecules were unable to bind to chromatin in the absence of Brit1. In the same study, the role of Brit1 in UV-mediated DNA damage repair was investigated. It was shown that Brit1 foci co-localized with ATR and RPA foci, however Brit1 knockdown impaired ATR and RPA foci formation. Further support for Brit1 in the ATR pathway was provided when targets of ATR-mediated phosphorylation, RPA and RAD17, were not phosphorylated in the absence of Brit1. Together, these data suggest that Brit1 is an early mediator of DDR, positively influencing IRIF formation and activation of several molecules in both ATM- and ATR-mediated repair pathways [10, 35].

Brit1 recruits DNA damage response proteins via SWI/SNF interaction

Though Brit1 was clearly shown to be an early DDR protein, it remained unclear how Brit1 promoted IRIF formation by downstream proteins. This was until an analysis of Brit1 binding partners revealed that Brit1 bound to the BAF170 and BAF155 subunits of SWI/SNF [30]. The SWI/SNF ATP-dependent chromatin-remodeling complex uses ATP hydrolysis to relieve nucleosomal compaction and physically shifts histone octamers along the histone-wounding DNA [36, 37]. It was shown in this study that the N-terminal region of Brit1 containing a single BRCT domain was required for direct binding to BAF170 and BAF155. Binding to these core subunits mediated Brit1 binding to SWI/SNF, an interaction that is enhanced by IR. It was further observed that mutation of BAF170 at an ATM-targeted sequence, S969A, abolished this enhanced interaction, but did not affect basal interaction [30]. This suggests that Brit1 binding is dependent on ATM phosphorylation of BAF170. It is interesting to speculate why a single phosphorylation site on BAF170, which is outside the proposed binding site (residues 571-645), enhances its interaction with Brit1. A possibility would be that S969 of BAF170 confers a stronger interaction between the two proteins, or more interestingly, phosphorylated S969 may be a target for another BAF170 binding protein that acts as a bridge between Brit1 and SWI/SNF. This study goes on to show that loss of Brit1 reduces binding of SWI/SNF subunits BRG1, BRM and BAF170, as well as other repair proteins, Ku70 and Rad51, to the damaged site. It is known that chromatin relaxation promotes recruitment of DDR proteins to the damaged site for both HR- and NHEJ-mediated DNA damage repair [38], and that SWI/SNF directly interacts with γ-H2AX to promote DSB repair independent of phosphorylation events by ATM and PIKKs [39]. The results from studying Brit1 and SWI/SNF interaction help to explain why loss of Brit1 reduces HR- and NHEJ-mediated DNA repair; that is, Brit1 recruits SWI/SNF to relax chromatin, which in turn promotes DDR protein recruitment to the damaged site. To show that chromatin relaxation alone was sufficient for IRIF formation, it was shown that while Rad51 and p-RPA foci didn't form in Brit1-depleted cells, they were able to form when chromatin was relaxed by chemical-treatment. Further support for Brit1's role in HR-mediated repair was provided when it was shown that Brit1 directly interacts with Condensin II, and that HR repair was impaired in Condensin II-depleted cells [40].

Brit1 promotes checkpoint activation

Recruitment of DNA damage response proteins is required for checkpoint activation and subsequent

repair [7, 41]. Downstream of the ATM and Mre11/Rad51/Nbs1 (MRN) complex sensor proteins is BRCA1, a checkpoint protein that appears to promote downstream signaling by acting as a scaffold for proteins in the ATM and ATR pathways [42]. Early studies investigating Brti1's role in DDR showed that IR-treatment of Brit1-knockdown cells did not lead to noticeable changes in cell cycle phase distribution. This suggests that Brit1 is required for checkpoint activation following DNA damage. This is supported by observations in Brit1-deficient cells that there is an increase in sub-G1 population indicative of cell death due to lack of DNA damage repair, and by an increased number of cells entering mitosis [11]. A partial explanation for how Brit1 promotes checkpoint activation was proposed while examining the phosphorylation status of checkpoint regulating proteins, BRCA1 and Chk1. It was found that expression of these two proteins was reduced in Brit1-depleted cells, while introduction of a knockdown-resistant form of Brit1 rescued BRCA1 and Chk1 expression levels [11]. Regarding telomerase expression, Brit1 may suppress telomerase expression indirectly through its regulation of BRCA1. It has been shown that BRCA1 is able to repress telomerase expression in prostate and breast cancer cell lines, partially through inhibition of c-Myc transcriptional activity [44]. More recently shown was that loss of BRCA1 leads to chromatin bridges in mammary epithelial cells, an effect of telomere dysfunction [45].

How Brit1 regulates BRCA1 and Chk1 expression is yet to be fully understood, though it appears that Brit1 regulates Chk1 at the level of protein and mRNA stability, while BRCA1 may be regulated via promoter activity or mRNA maturation [46]. With respect to control of expression, regulation may be intimately tied to Brit1's association with SWI/SNF, allowing not only DDR proteins more direct access to the chromatin surrounding DSBs, but also allowing relevant transcription factors better access to the promoter regions of genes that express DDR proteins. Additional studies suggest that Brit1 may also function downstream of ATR. In response to DNA damage, Chk1 phosphorylation of Cdc25A leads to destruction of Cdc25A. This serves to inhibit mitotic entry in the presence of DNA damage induced by either UV or IR [47-49]. However, in Brit1 deficient cells, UV-treated cells displayed stable levels of Cdc25A [25]. Since Cdc25A is responsible for removing inhibitory Y15- and T14-phosphates from Cdk1, it follows that UV-treated cells with deficient Brit1 displayed low levels of Cdk1 phosphorylation and high Cdk1-cyclin B1 activity with increased premature chromosome condensation (PCC) in the G2 phase of the cell cycle. These results can be explained by low Chk1 expression in Brit1 deficient cells. However, it is interesting that this effect was observed in the presence of normal levels of Chk1, suggesting that regulation of the ATR pathway may occur at the level of Chk1 expression as well as by a downstream mechanism that is yet to be determined. Besides promoting expression of BRCA1 and Chk1, Brit1 also increases phosphorylation and activation of NBS1 in the presence of IR as part of ATM pathway activation. The accumulating evidence suggests that Brit1 contributes to the ATM pathway by at least promoting NBS1 phosphorylation, while contributing to the ATR pathway by increasing BRCA1 expression in addition to either increasing Chk1 expression or affecting the downstream proteins regulating mitotic entry. Together, it appears that since BRCA1, Chk1 and NBS1 are important DDR proteins, Brit1's role in arresting cells at the intra-S and G2/M phases may be due in part to its ability to affect the expression and activation of these proteins. Translating these studies to neurophysiological disorders displaying microcephaly, it is known that the ATR/Chk1 pathways are often disrupted [50, 51]. Therefore, the finding that Brit1 has a positive effect on Chk1 provides an explanation for why the Brit1 gene, *MCPH1*, is defective in these disorders. Additionally, the ability of Brit1 to regulate the expression of BRCA1 and Chk1 and their downstream activities makes Brit1 an important upstream regulator of DDR, and thus a potential tumor suppressor gene.

Brit1 and chromosome dynamics

A functional DNA damage response is one crucial mechanism for maintaining genomic stability. Initial work by Rai *et al.* [10] showed that Brit1 knockdown cells displayed genomic instability.

This work provided the impetus for examining Brit1 localization and interacting proteins to better resolve the mechanisms that lead to genomic instability. In addition to its role in DNA damage response, Brit1 has also been shown to maintain genomic stability by regulating centrosome numeracy and chromosome condensation.

Brit1 and centrosome regulation

Centrosomes are cellular structures that serve as the microtubule organizing centers (MTOC) from which microtubule nucleation and elongation toward chromosomes occur [52, 53]. Gamma-tubulin proteins within centrosomes associate with microtubule subunits, and are required for microtubule nucleation [54]. Maintaining centrosome numbers and thus a normal distribution of microtubules is crucial for preventing deregulated mitosis exemplified by chromosome breakage or missegregation, and defective cytokinesis leading to multinucleated cells [55-57]. Thus, centrosome amplification is a cause of chromosomal instability (CIN), a phenotype associated with cancer [58-60].

As would be expected for the crucial function of maintaining microtubule spindles, there are several known mechanisms that regulate centrosome copy number. Using p53-deficient mouse embryonic fibroblasts (MEFs), Fukasawa *et al.* [61] showed that these cells were able to synthesize several functional centrosome structures in a single cell cycle. This overamplification of centrosomes led to chromosomal missegregation and eventually profound defects in mitosis. The BRCA1 and BRCA2 DNA damage repair proteins were also found to inhibit centrosome amplification. Using MEFs containing BRCA1 deletions at exon 11, Xu *et al.* [62] showed that these cells contained an elevated number of centrosomes compared to not only wild-type MEFs, but also p53-/- MEFs. BRCA2 however was shown to interact with nucleophosmin, and as a complex, bind to centrosomes to maintain numerical integrity [63]. Recent work done by Rai *et al.* [12] further contributes to our understanding of centrosome maintenance through their work with Brit1 and MDC1. Beyond their association with γ-H2AX and role in DNA damage response, it was shown that Brit1 and MDC1 associate with centrosomes [64]. Depletion of MDC1 in culture led to centrosome amplification in association with multipolar spindles, and chromosomal misalignment during metaphase. These effects were further pronounced in the presence of IR. Brit1 depletion also led to mitotic defects in the form of spindle malformation at the equatorial plane and chromosomal misalignment during metaphase. Furthermore, Brit1 depletion was characterized by the prolonged presence of cytokinesis bridges and multinucleated cells, indicative of failed cytokinesis. Therefore, it is suggested that the supernumerary chromosomes observed in Brit1 deficient cells is due to failed cytokinesis. The group went on to show an inverse relationship between Brit1 and MDC1 versus Aurora A and Plk1 expression. Aurora A and Plk1 are two kinases involved in centrosome dynamics and cytokinesis [65, 66], with overexpression being linked to centrosome amplification [67]. The finding suggests that Brit1 and MDC1 depletion leads to mitotic defects in part due to loss of negative regulation of Aurora A and Plk1 expression. Other groups also support Brit1 localization at the centrosome. Jeffers *et al.* [68] show that IR-induced centrosomal localization is dependent on Brit1's N-terminal BRCT1 domain. However, low levels of persistent centrosomal binding occurring independently of cell cycle can occur in the absence of BRCT1, suggesting BRCT2 and BRCT3 domains are sufficient. Brown *et al.* [69] show that when Brit1-/- DT40 chicken cells are treated with IR, centrosome amplification occurs, but is rescued by exogenous expression of Brit1. Together, these results suggest that Brit1 persistently localizes at centrosomes, and in the presence of IR, localization is elevated to inhibit centrosome amplification. It has been suggested that centrosome amplification can be viewed as a mechanism that ensures cell death as a barrier to propagation of DNA-damaged cells [70]. It would be interesting to further analyze the balance between preventing centrosome amplification to repair damaged DNA, and promoting amplification to prevent proliferation of DNA-damaged cells that have perhaps evaded DNA damage or spindle checkpoints.

Brit1 and chromosome condensation

Chromosome condensation is an important early step in mitosis. Condensin I and Condensin II are

two v-shaped molecules of the SMC (structural maintenance of the chromosome) family that use ATP-binding and -hydrolysis to manipulate nucleosomes into a condensed state [71, 72]. PCC in the G2 phase of the cell cycle and delayed decondensation in G1 occur when this regulatory process is compromised [17]. PCC leads to a large percentage of prophase-like cells due to the early initiation of chromosome condensation. In microcephaly patients, where Brit1 is deficient, PCC is commonly observed, and can be used as a diagnostic marker for microcephaly [73, 74].

Work by Trimborn *et al.* [75] first suggested a possible link between abnormal chromosome condensation in microcephaly and Condensin II. Since Brit1 is defective in microcephaly, the later discovery of a physical interaction between Brit1 and Condensin II supported this link. More specifically, Wood *et al.* [40] showed that Brit1, via a conserved sequence in the middle region of the protein (residues 376-485), interacts with Condensin II. Loss of Brit1 relieves negative regulation of Condensin II and allows it to prematurely bind to chromatin, thus resulting in early condensation as was shown in Brit1-/- MEF cells [40]. Interestingly, deletion analysis of Brit1 showed that the Brit1 N-terminal domain is required to reduce the amount of prematurely condensed chromosomes, however the Condensin II binding domain of Brit1 is not required. This suggests that an additional protein(s) may be involved in the concurrent regulation of Condensin II. Indeed, a follow-up study by Leung *et al.* [13] identified SET, a regulator of histone acetylation [76], as a direct binding partner to the N-terminal region of Brit1. They found that SET depletion in MEF and human cells resulted in the PCC phenotype identical to that observed in Brit1-/- MEFs, and this phenotype was rescued upon co-depletion of Condensin II. The interaction between SET and Brit1 provides an attractive explanation for how microcephaly patients with Brit1 deficiency acquire the PCC phenotype. Together, these recent studies suggest that in addition to its role in DNA damage response, Brit1 is also able to interact with nucleosome-remodeling factors to regulate chromosome condensation.

Potential role of Brit1 in tumor suppression

Increased susceptibility to cancer at the cellular level can manifest from aberrant telomerase expression, CIN and impaired response to DNA damage, to name a few. Telomerase is the most widely expressed tumor-associated gene, detectable in the vast majority of cancers [77, 78]. Telomerase activity in association with stable telomere length is normally restricted to germ cells and stem cells. However, persistent activity promotes limitless replicative potential of its resident cells by preventing the normal phenomenon of telomere shortening, thus leading to tumor development [79, 80]. Therefore, developing targeted therapeutics against telomerase has been a topic of intense research due to its criticality and specificity to cancer cells [15, 81, 82]. Centrosome amplification leading to CIN is another universal defect observed in a variety of cancers including the breast, pancreas and prostate [60, 83, 84]. Centrosome amplification and deregulated chromosome condensation are independent driving forces for chromosome missegregation and the tumor phenotype [85]. A defective DNA damage response where DNA DSBs are allowed to propagate unchecked and contribute to genomic instability, is yet another cause of cancer predisposition [31]. Since Brit1 has been observed to play a role in regulating each of the above processes, it is reasonable to hypothesize that Brit1 deficiency could impair regulation, leading to cancer progression.

Indeed, Rai *et al.* [10] demonstrated that Brit1 deficiency is observed in a significant percentage of primary ovarian cancers, which correlates with increased genomic instability. Similarly, low Brit1 DNA copy number was observed in 72% of the 54 breast cancer cell lines tested [10], while 70% of breast cancer specimens assayed had decreased Brit1 protein levels [12]. Moreover, retrospective analysis of breast cancer biopsies showed that Brit1 expression inversely correlated with onset of cancer metastasis [10].

Following these observations, Liang *et al.* [86] engineered Brit1 knockout mice to analyze the effects that loss of Brit1 has on genomic stability and response to IR. Similar to microcephaly patients, Brit1-/- mice were able to grow to adulthood, however their body weight was ~20% less than their wild-type (WT) counterpart. Brit1-deficient mice from another group exhibited reduced life spans, which is consistent with microcephaly [43, 87, 88]. Mice with Brit1 deficiency appeared to have a compromised DNA damage response, as

they were hypersensitive to IR, dying 9 days post-IR treatment compared to their heterozygous and WT counterparts, who survived at least 4 weeks post-IR. Hypersensitivity to IR in the Brit1-/- may be explained by an impaired ability to repair damaged DNA by HR; Brit1-/- MEFs had an elevated number of chromatid breaks versus control cells after IR treatment. Since Brit1 was previously shown to recruit DDR factors to promote DNA repair [10], the group further investigated the underlying cause of defective DNA repair by staining IR-treated meiotic chromosomes for DDR proteins. They found that RAD51 and BRCA2 foci formation, which was shown to require Brit1 [89], was reduced in Brit1-/- cells, thus providing a possible explanation for failed meiotic recombination in Brit1-/- spermatocytes. In parallel with this work, Trimborn *et al.* [87] engineered mutant (Mcph1gt/gt) mice expressing defective Brit1 with a gene-trap ablating the c-terminal region of the protein. Primary fibroblasts from the mutant mice exhibited significantly increased levels of PCC and delayed chromosome decondensation compared to WT mice, which supports the common phenotype observed in microcephaly patients. These mutant mice also exhibited reduced life span compared to heterozygous and WT mice. Together, these mice models expressing either defective Brit1 [87] or not expressing Brit1 [86] clearly show a negative effect on chromosome dynamics, and possible infidelity in DNA damage response.

However, it has not yet been demonstrated in an animal model that the cellular deformities associated with Brit1 deletion contribute to tumorigenesis. This lack of a direct cause-effect relationship between Brit1 deficiency and cancer seems reasonable since microcephaly patients are not predisposed to cancer. Still, the question that needs to be answered is why Brit1 expression levels are lowered in several types of cancers - of which the majority share defective features shown to be regulated by Brit1, and why the rate of onset to metastasis is accelerated in Brit1 deficient cells [10]. It is interesting to observe that much of the published data supporting Brit1 as a tumor suppressor gene was generated using cancer cell lines with Brit1 knockdown [10], which displayed genomic instability and other tumor-promoting phenotypes similar to that observed in cancer cells. Therefore, one could reason that specific genetic lesions obtained during the course of tumorigenesis, along with Brit1 deletion, could together promote the cancerous phenotype. Inactivating Brit1 may not directly cause tumor formation, however a synthetic lethal interaction involving Brit1 may be targeted within the tumor allowing the full effect of Brit1 deletion to manifest. Supporting such a hypothesis is recent work showing that Brit-/- mice crossed with the p53-/- background resulted in enhanced susceptibility of Brit1 deficient mice to cancer [86]. This data, together with the recent clinically relevant finding that levels of Brit1 expression can be used as a diagnostic marker for breast tumor grade [90], provide the rationale to develop a more complete understanding of the comprehensive factors that contribute to the tumorigenic phenotypes associated with Brit1 deficiency.

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