Understanding BRCA1(-complexes) in DNA repair and cancer

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Chapter 6

Discussion and future perspectives

This thesis addresses the complexity of BRCA1 biology as well as the search for new therapeutic targets for the treatment of BRCA1-deficient tumours. BRCA1 is a tumour suppressor protein with multiple roles in the cell that contribute to genomic stability. Despite years of research, many questions about BRCA1 biology remain unanswered. This chapter discusses how this thesis contributes to further unravelling BRCA1 biology and which questions require further research.

BRCA1 AND CANCER

Somatic *BRCA1* mutations are found in many cancers of different origin. Differently, hereditary *BRCA1* and *BRCA2* mutations dramatically increase the risk of developing specifically breast or ovarian cancer (1) and to a lesser extent pancreatic or prostate cancer (2-4). It is currently thought that the oestrogen responsiveness of breast and ovary tissues cause this increase in cancer risk in carriers of hereditary BRCA mutations. Indeed, many studies show that for BRCA mutation carriers, the prognosis worsens when the tumour is oestrogen receptor-positive compared to an oestrogen receptor-negative tumour (5,6). However, the exact mechanism behind this predisposition to specific types of tumours remains unclear.

The BRCA1 survival paradox

Homologous recombination (HR) deficiency is present in approximately 6 percent of all cancers, with loss-of-function mutations in BRCA1 being one of the primary causes (7). BRCA1-mutated tumours invariably show biallelic disruption of *BRCA1*, indicating that dysfunctional BRCA1 promotes cellular growth in a tumour setting (8). In contrast, BRCA1 loss in mice is embryonically lethal, indicating that BRCA1 is essential for life (9). This intriguing paradox can partially be explained by co-occurrence of *TP53* mutations in *BRCA1*-deficient tumour samples, allowing for their survival (10). However, not all *BRCA1*-deficient tumours possess *TP53* mutations. Moreover, *BRCA1/TP53* double-knockout mice are still embryonically lethal, albeit at a later stage (9). Indeed, our lab and others have encountered significant difficulties in generating *BRCA1* knockout cell lines in a *TP53*-deficient background. All together, these findings strongly suggest the presence of additional mutations in *TP53*-deficient tumours to off-set the reduced fitness upon BRCA1-loss. The search for driver mutations in multiple *BRCA1*-defcient clones could be the start of finding the additional mutations required for BRCA1-deficient cell survival.

BRCA1 AS TUMOUR SUPPRESSOR

BRCA1 plays a role in multiple processes in the cell, namely in the double-strand break (DSB) repair pathway HR, cell cycle check-point activation, replication fork protection, ssDNA gaps suppression, R-loop dissolution and mitosis (discusses in more detail in chapter 1). Currently, the importance of each separate BRCA1 function for tumour suppression, focussing mainly on the role of BRCA1 in HR and ssDNA gap suppression, is under debate.

What is the main vulnerability of BRCA1-deficient cells?

Recent studies highlight post-replicative gaps as the main cause for toxicity upon BRCA1 loss or mutations (11,12). Additionally, the sensitivity of BRCA1-deficient cells to certain therapeutic agents is explained by the increase of ssDNA gaps in these cells (11,12). However, other publications, including chapter 3 of this thesis, show that faulty DSB repair should not be dismissed as the main toxicity in BRCA1-deficient cells. Chapter 3 of this thesis describes how EXO1 loss induces S-phase poly(ADP-ribosyl)ation (PARylation), likely reflecting ssDNA gaps in the form of unprocessed Okazaki fragments, in both BRCA1- and BRCA2-deficient cells. Yet, the loss of EXO1 is only toxic to BRCA1-deficient cells (13). EXO1 loss in BRCA1-deficient, but not BRCA2-deficient cells, leads to a significant decrease in the DSB repair pathway single-strand annealing (SSA). The loss of both SSA and HR, leads to an accumulation of DSBs, an increase of genomic instability, and subsequently cell death.

Similarly, other research suggests that ssDNA gaps that occur prior to replication become only problematic upon conversion into DSBs, as the mutational signature of BRCA1-deficient tumours corresponds to one-ended DSBs and faulty repair of these lesions (14). Additionally, PARPi has been shown to activate the DNA damage response (DDR) more in the second cell cycle, when ssDNA gaps have been converted into DSBs, compared to the first cell cycle (15). All together, these results (discussed in more detail in chapter 2 (16)) suggest that a DSB repair defect ultimately causes the genomic instability in BRCA1-deficient cells.

Additional research is required to give final proof, but this is complex due to the close connection of the different functions of BRCA1 that are not mutually exclusive. For example, when the replication fork encounters a ssDNA gap or nick, replication stress occurs. Replication stress leads to stalled replication forks which requires the need for replication fork protection by BRCA1. Persistent replication stress can be repaired with alternative repair mechanisms, often leading to ssDNA gaps to be repaired post-replicatively. Alternatively, when replication stress persists, the replication fork can be converted into a one-ended DSB, which requires HR to be repaired (17). This highlights how ssDNA gaps can lead to replication stress and one-ended DSBs, and similarly replication stress can lead to ssDNA gaps. As all BRCA1 roles are closely intertwined, better separation-of-function-mutations for BRCA1 are required to definitively solve the ongoing debate.

TREATMENT OF BRCA1-MUTATED TUMOURS

PARP inhibitor treatment

BRCA1 mutated tumours are currently treated in the clinic with PARP inhibitors (PARPi) such as Olaparib. As described in Chapter 1, originally it was conceived that PARPi block the activity of PARP1 and trap the PARP1 protein onto the DNA (18,19), stalling replication forks which subsequently leads to an increase of DSBs. Cells lacking functional BRCA1 will be unable to repair the accumulating DSBs, ultimately killing specifically the BRCA1-deficient or mutated cells. This view was disputed when increased ssDNA gaps were described as the mechanism behind PARPi toxicity (11,12,20). PARPi increase replication fork speed, which is accompanied by accumulation of post-replicative gaps (21,22). Additionally, PARPi were found to cause toxicity unrelated to trapping, but due to an increase of transcription-replication conflicts (23). Collectively, these findings do not exclude that accumulation of DSBs underly chemosensitivity, as discussed in more detail in Chapter 2. However, these recent findings highlight the need for additional mechanistic studies on PARPi.

Resistance to PARP inhibitors

A pressing problem concerning PARPi is that 40% of ovarian cancer patients with BRCA mutations fail to respond to PARPi (24-26). In addition, prolonged PARPi treatment of HR deficient tumours induces acquired resistance in a substantial proportion of patients. As discussed in Chapter 1, the currently known mechanisms of PARPi resistance are the increased efflux of PARPi, decreased PARP trapping, restored HR or stabilization of stalled replication forks (27,28). Cells can restore HR via reversion mutations in BRCA which restore protein function (29-32). Alternatively, cells can become PARPi resistant by acquiring mutations in 53BP1, RIF1 or the Shieldin complex, shifting the balance by resection inhibition, allowing cells to perform HR in the absence of BRCA1 (33-43). Loss of EZH2 and PTIP, normally recruiting nucleases MUS81 and MRE11 to stalled replication forks, increase PARPi resistance through stabilization of stalled replication forks (44,45). Recently, a new mechanism to stabilize stalled replication forks and thereby acquiring chemoresistance was described. Loss of histone H2AX restores replication fork stability, as normally yH2AX drives replication fork degradation by suppressing CTIP-mediated fork protection (46). The above examples highlight the ability of tumours to develop a wide variety of chemoresistance mechanisms to avoid sensitivity to PARPi. As PARPi treatment is the sole treatment currently approved and available in the clinic to specifically treat HR-deficient tumours, additional therapeutic targets to treat HR-deficient tumours are a necessity to prevent these high numbers of resistance.

EXO1 loss is synthetically lethal with BRCA1-deficiency

The search for synthetic lethal (SL) interactions has become popular with the rise of CRISPR/Cas9-mediated genome-wide synthetic lethality screens. This increased interest in synthetic lethal interactions resulted in the identification of many SL interactions with BRCA1 besides PARP1 , namely RAD52, POLQ, APEX2, FEN1, USP1, and BLM (47-52), and led to a novel treatment option as the first clinical trials for POLQ inhibitors are now ongoing. Chapter 3 of this thesis describes a new SL interaction with BRCA1-deficiency: the loss of EXO1 (13). BRCA1/EXO1 double-deficient cells accumulate DSBs due to lack of DSB repair by SSA in addition to their HR defect. Not only BRCA1-deficient cells, also BRCA1-mutated tumours depend on EXO1-mediated SSA as these tumours show an increase in EXO1 expression as well as increased SSA-associated genomic scars. All together, these findings show that EXO1 is a promising novel target in the treatment of BRCA1-mutated tumours.

The potential and risks of EXO1 inhibitors as treatment for BRCA1-deficient tumours

Our research shows the importance of the catalytic activity of EXO1 for the survival of BRCA1-deficient cells. In BRCA1/EXO1 double-deficient cells, re-expression of the catalytic dead EXO1^{D173A} mutant does not avert the synthetic lethality (13). A small molecule inhibiting the catalytic activity of EXO1 could therefore be a viable option to treat BRCA1-deficient tumours. Although, some considerations should be taken in account.

Besides a role in DNA end-resection and SSA, EXO1 also plays a role in mismatch repair (MMR) and an EXO1 inhibitor could therefore affect MMR as well as SSA. The 5' to 3' exonuclease activity of EXO1 in MMR is however redundant to the 5' to 3' exonuclease activity of RECJ and EXOVII and substantial MMR activity remains after EXO1 loss (53-55). This correlates with the low levels of toxicity exhibited by different BRCA1-proficient cell models upon EXO1 loss and the low essentiality (both based on the Bayes factor (56) and the Z-score (57)) of EXO1 (13,58). Cells lacking EXO1 do however show increased PARylation and γH2AX levels, indicating that long-term treatment with an EXO1 inhibitor should be avoided.

A more concerning drawback of an EXO1 inhibitor is acquired resistance. Reversion mutations in BRCA1 and mutations in 53BP1, RIF1, or the Shieldin complex, all reactivate HR. There is clinical evidence for these resistance mechanisms to PARPi treatment (27) and these mechanisms would cause EXO1 inhibitor resistance as well. Therefore, EXO1 inhibitors would be a good candidate for combinational therapy, to increase therapy response and thereby reducing the time tumour cells have to acquire resistance. Preliminary results in our lab indicate increased toxicity in BRCA1-deficient cells when depleting EXO1 and PARP1 or POLQ simultaneously. Further research into the therapeutic window to optimally kill BRCA1-mutated tumour cells by targeting both EXO1, PARP1 and POLQ is required.

MECHANISMS BEHIND SYNTHETIC LETHALITY WITH BRCA1

Synthetic lethality with POLQ and RAD52

Both alt-EJ and SSA, mediated by respectively POLQ and EXO1 or RAD52 are suggested to act as a back-up mechanism to HR (13,47,49,50), thereby explaining the synthetic lethal interaction between these proteins and BRCA1. However, not the role of POLQ in Alt-EJ, but in post-replicative gap filling has recently been implied to explain the synthetic lethal interaction between POLQ and BRCA (59-61). These findings, discussed in more detail in chapter 2, were obtained in both *Xenopus laevis* egg extracts and *in vitro*, therefore requiring additional research in cell models. In parallel, evidence for a role of POLQ in DSB repair specifically during mitosis has been published. RHINO and PLK1 were identified to mediate recruitment of POLQ to mitotic breaks by two distinct mechanisms (62,63). The role of POLQ in mitotic DSB repair is a compelling reason for the synthetic lethality between POLQ and BRCA1 as PARPi induced DNA lesions in BRCA-deficient cells persist into mitosis and POLQ inhibition has been described to enhance PARPi toxicity in BRCA-deficient tumour cells (64,65). Whether the role of POLQ in mitotic DSB repair also explains the success of POLQ inhibition in PARPi resistant cells, e.g. *BRCA1*$^{\Delta 11}$ *TP53BP1*$^{-/-}$, remains unproven.

Similarly, for RAD52 alternative mechanisms explaining the synthetic lethal interaction with BRCA1 have been published. Depletion of RAD52 has been shown to further decrease RAD51 foci formation and HR, determined by a DR-GFP recombination reporter assay in BRCA2-deficient cells (66,67). Comparable results were found in BRCA1-deficient cells (47). Additionally, the function of RAD52 was independent of BRCA1/2 status in these cells. All together, these findings suggest a role for RAD52 in a BRCA-independent RAD51-mediated homology directed repair pathway. More recently, the role of RAD52 in resolving R-loops has been suggested to contribute to the synthetic lethal interaction between RAD52 and BRCA (68).

The mechanistic understanding of both Alt-EJ and SSA is far from complete and the factors currently known to play a role in Alt-EJ and SSA, as exemplified above by POLQ and RAD52, are not unique to Alt-EJ or SSA but are involved in multiple processes important for genome maintenance. Better understanding of Alt-EJ, mitotic DSB repair, and SSA would allow the research field to more definitively pinpoint the exact mechanism behind the synthetic lethal interaction with BRCA1.

Defective ATR checkpoint activation described as toxicity upon EXO1 loss

Chapter 3 of this thesis describes the synthetic lethal interaction between BRCA1 and EXO1. The essentiality of EXO1 in BRCA1-, but not BRCA2-deficient cells was later confirmed by other researchers (69). Similar to our findings, they discuss the increased EXO1 expression in BRCA1-deficient tumours, suggesting dependency of BRCA1-deficient tumours on EXO1. In contrast to the findings in chapter 3, they attribute the synthetic lethal interaction to the role of both BRCA1 and EXO1 in ATR activation through ssDNA gap extension. They describe an increase in RPA signal upon EXO1 loss, resulting from an increase in ssDNA gaps. Subsequently, García-Rodríguez *et al*. checked CHK1 phosphorylation upon MMS treatment as a readout for ATR checkpoint activation and noticed a significant decrease upon EXO1 loss. This decrease was most prominent 40-60 minutes with MMS treatment and after a 2 hour MMS treatment cells show wildtype levels of CHK1 phosphorylation (69). Further research is needed to assess whether the 1 hour delay in ATR checkpoint activation upon EXO1 loss is sufficient to cause lethality in BRCA1-deficient cells. Additionally, further research is required to determine whether the ATR checkpoint activation defect or defective SSA upon EXO1 loss is more toxic to BRCA1-deficient cells. Ideally, an EXO1 separation of function mutant would be used to answer this question. Yet, the EXO1 catalytic activity is required for both ATR activation and DSB-repair (69,70). Similarly, the EXO1-PIP-box-mutant, defective in PCNA binding and ATR checkpoint activation has been described defective for recruitment of EXO1 to DSBs as well (69,71).

In addition to the synthetic lethal interaction between EXO1 and BRCA1, García-Rodríguez et al. also describe the synthetic lethal interaction between BRCA1 and two other long-range end-resection factors, DNA2 and BLM. Previously, DNA2 has been found essential in BRCA1-proficient cells as well as BRCA1-deficient cells (72-74). Recently, the synthetic lethal interaction between BLM and BRCA1 has been attributed to their complementing roles in dissolving double Holliday junctions (48). The lethality upon the loss of BLM in BRCA1-deficient cells remains lethal after depletion of 53BP1, whereas the loss of 53BP1 in *BRCA1*$^{-/-}$ *EXO1*$^{-/-}$ cells rescues the lethality (13,48). This dissimilarity stipulates two distinct mechanisms causing the toxicity of either EXO1 or BLM loss in BRCA1-deficient cells as is indeed described by van de Kooij et al. and Tsukada et al. (13,48). In contrast, the ATR activation defect as the mechanism behind the synthetic lethality described by García-Rodríguez et al. cannot explain this distinction between EXO1 and BLM. In conclusion, defective ATR checkpoint activation is unlikely the sole reason EXO1 loss is toxic to BRCA1-deficient cells.

Open questions in the synthetic lethality between BRCA1 and EXO1

The findings in chapter 3 show that EXO1 loss is synthetic lethal with BRCA1-deficiency and not BRCA2-deficiency (13). Even though this has been confirmed by other researchers (69), some questions still remain unanswered. Does PALB2 loss, an important binding partner of BRCA2, show a similar phenotype to BRCA2-deficient cells and thus no sensitivity to EXO1 loss? BARD1, the obligatory binding partner of BRCA1, does show a similar phenotype to BRCA1, as BARD1-deficient cells are sensitive to EXO1 loss (13).

Secondly, BRCA1-deficient cells show reduced SSA, which is further abrogated upon EXO1 loss. In contrast, BRCA2-deficient cells have already elevated levels of SSA which are not significantly affected by EXO1 loss. Elevated SSA in BRCA2-deficient cells has been described previously (75,76) and might be explained by the enhanced recruitment of RAD52 instead of RAD51 to resected ends, subsequently increasing SSA. However, further mechanistic studies are necessary to proof that this occurs in BRCA2-deficient and not BRCA1-deficient cells, as both proteins play a role in RAD51 loading onto resected DNA. To further strengthen the results in chapter 3, additional assays analysing end-resection in either BRCA1- or BRCA2-deficient cells upon either EXO1 or DNA2 loss should be performed to confirm that BRCA1-deficient cells depend solely on EXO1 for long-range end-resection and indeed have defective DNA2 recruitment (77).

The importance of short-range end-resection in HR is clear. However, the importance of long-range end-resection preceding HR remains an ongoing discussion in the field (78-80). Long-range end-resection factors have been shown to stimulate HR, contrary to research showing that long-range end-resection factors are redundant for successful HR (81-88). The results in chapter 3 are in line with the previous findings that show long-range end-resection factors are redundant for HR. We show that EXO1 loss does not significantly decrease RAD51 foci formation and HR in the reporter assay is even slightly increased upon EXO1 loss. However, additional research is required as the importance of long-range end-resection factors in HR could depend on the chromatin context of the lesion and distance to the homologous sequence. For example, previous work in yeast shows Exo1 is dispensable for HR between closely linked sequences but required for HR between interchromosomal sequences (88).

EXO1 loss increases S-phase specific PAR-decorated DNA lesions independent of BRCA-status. Previously, unprocessed Okazaki fragments have been shown as the main source of S-phase PARylation (89). EXO1 and FEN1, the primary endonuclease in Okazaki fragment maturation (OFM), show structural similarities, both possess flap-endonuclease activity and are both synthetic lethal with BRCA1-deficiency (52,90,91). Additionally, EXO1 has already been suggested to play a role in Okazaki fragment maturation (OFM) in yeast (92,93). As the evidence thus far is mainly circumstantial, the role of EXO1 in DNA maturation should be further investigated using the recently developed EdU alkaline comet assay (94,95). Even though both EXO1 and FEN1 loss are synthetic lethal with BRCA1-deficiency, evidence suggests both proteins do not share a common mechanism causing this synthetic lethality. First, FEN1

loss, unlike EXO1 loss, is also toxic to BRCA2-deficient cells (13,52). Secondly, when EXO1 indeed plays a role in OFM, the deleterious effect of EXO1 loss on OFM is inferior to the deleterious effect upon FEN1 loss as FEN1 is a key player in OFM (96). The above findings suggest that when EXO1 plays a role in OFM, it is important only under specific circumstances or in the absence of FEN1.

HYPOMORPHIC BRCA1-MUTATIONS

Many diseases including cancer show a large heterogeneity in missense mutations. In many instances, these missense mutations do not lead to a complete loss-of-function of the protein. Therefore, using models with full deficiency of a protein to identify novel vulnerabilities, is not necessarily the best approach. Chapter 4 of this thesis describes the genetic vulnerabilities of two previously described hypomorphic BRCA1 mutations, BRCA1^{I26A} and BRCA1^{R1669Q}. Our research shows that cells with hypomorphic BRCA1 mutations can have a different phenotype and unique genetic vulnerabilities compared to BRCA1-depleted cells. One example of such a unique vulnerability, discussed in more detail below, is the toxicity of NDE1 loss in BRCA1^{R1699Q} cells and not BRCA1-depleted cells.

Synthetic lethality with BRCA1-depletion

Chapter 4 describes a novel synthetic lethal interaction between BRCA1-depletion and CSA loss, a protein important for transcription-coupled nucleotide excision repair (TC-NER). This synthetic lethal interaction was thus far only validated in BRCA1-depleted RPE1 and BRCA1-mutated HCC1937 cells. Further research is required, using both clonogenic survival assays as well as multi-colour competition assays, to assess the essentiality of CSA in full BRCA1 knockout cells as well as other cell types. Interestingly, the HCC1937 cell line is both BRCA1 and SHLD2-mutated, rendering the cells both HR-proficient and PARPi resistant as SHLD2 loss has a synthetic survival interaction with BRCA1 loss (33). This suggests that CSA loss is toxic to PARPi resistant BRCA1-mutated tumours and therefore the lethality upon CSA loss in other BRCA1-mutated PARPi-resistant cell lines should be tested for validation. Previously, it has been suggested that BRCA1 and CSA can both polyubiquitinate CSB, another TC-NER protein (97). Additional research, analysing polyubiquitination of CSB and TC-NER upon either depletion of CSA, BRCA1 or both will further elucidate the possible auxiliary interaction between BRCA1 and CSA.

A second synthetic lethal interaction described in chapter 4 is the interaction between GPX4/PSTK loss and BRCA1-depletion. Both GPX4 and PSTK protect cells against ferroptosis and GPX4 inhibition has been described before to induce cell death in BRCA1-deficient cells (98-100). Both Lei *et al.* and we found BRCA1-status independent toxicity upon GPX4 loss, although Lei *et al.* show this unwanted toxicity is cell line dependent (98). Further characterising

which BRCA1-proficient cell lines do or do not show toxicity upon GPX4 loss might improve our understanding behind the mechanism of the toxicity and could improve the prospects for the already available GPX4 inhibitors as anti-cancer treatment.

Synthetic lethality with BRCA1^{R1699Q}

Loss of the mitotic spindle associated protein NDE1 is toxic to specifically BRCA1^{R1699Q} cells and not to BRCA1-depleted or BRCA1-proficient cells (chapter 4). NDE1 loss in a BRCA1^{R1699Q} background increases both micronuclei formation and the amount of anaphase bridges indicating a mitotic defect and increase in genomic instability. Further validation by complementation assays with exogenous NDE1 and mechanistic studies, including metaphase spreads to assess chromosomal aberrations are required. As both proteins play a role in the regulation of centrosome duplication, it would not be unlikely that this process is involved in the synthetic lethality between BRCA1 ^{R1699Q} and NDE1-loss (101-104). Analysing the recruitment of either BRCA1^{R1699Q} or wildtype BRCA1 to centrosomes in either an NDE1-deficient or -proficient background could help determine whether this is the correct direction for subsequent research on the mechanism behind this synthetic lethal interaction.

The aforementioned novel synthetic lethal interactions with BRCA1-depletion and BRCA1^{R1699Q} underline the value of CRISPR/Cas9-mediated genome-wide synthetic lethality screens. Novel synthetic lethal interactions are still found and each one uncovers a novel link between proteins or mechanisms in the cell. Furthermore, researching the genetic vulnerabilities of hypomorphic BRCA1 mutations compared to BRCA1 depletion, shows that cells with hypomorphic BRCA1 mutations can have unique vulnerabilities, such as BRCA1^{R1699Q} and NDE1. In contrast, cells with hypomorphic BRCA1 mutations can also exhibit less sensitivity to known vulnerabilities of BRCA1-deficient cells. For instance, BRCA1^{R1669Q} cells are less sensitive to loss of POLQ or PARP1 compared to BRCA1-depleted cells. These examples highlight the importance to study the vulnerabilities and *by proxy* the effective treatments for individual patient-derived mutations.

BRCA1-COMPLEXES

The multiple domains of BRCA1 are important for the formation of different BRCA1 multiprotein complexes, each with a different function. How each complex formation is regulated precisely is still largely unknown and for the tandem BRCT domains of BRCA1 it becomes even more puzzeling. The tandem BRCT domains of BRCA1 bind mutually exclusive to ABRAXAS1, BRIP1 or CTIP through the same binding mechanism. The binding is dependent on the phosphorylation of the serine in the SXXF motif in the binding proteins (105-107). How the choice to bind to either protein is made remains unknown as the binding affinity is fairly similar for all three proteins. This could indicate the binding is regulated by the abundance of either ABRAXAS1, BRIP1 and CTIP, or through an alternative mechanism.

The importance of ABRAXAS1

The role of the BRCA1-A complex (consisting of BRCA1, ABRAXAS1, BRCC36, MERIT40, BRE and RAP80 forming a dimeric complex (108,109)) has been point of discussion in the field already for a long time. The most common model is that the BRCA1-A complex sequesters BRCA1 away from the DSB, limiting excessive end-resection and thereby inhibiting HR (109-112). Chapter 5 of this thesis discusses our most recent findings featuring the BRCA1-A complex. Surprisingly, disrupted binding between BRCA1 and ABRAXAS1 decreases end-resection whereas, as shown previously, loss of ABRAXAS1 increases end-resection (110-112). All together these data, discussed in more detail in chapter 5, suggest the BRCA1-A complex plays an intricate role in safeguarding end-resection. I surmise that ABRAXAS1 limits end-resection specifically to prevent HR with non-homologous sequences.

Another observation made while studying ABRAXAS1 was the difficulty to make ABRAXAS1-knockout cells as well as the disappearance of the observed end-resection phenotype over time in either ABRAXAS1-knockout or ABRAXAS1-mutated cells. Sequencing of these cells confirmed the cells were still either ABRAXAS1-knockout or ABRAXAS1-mutated, indicating cells tend to compensate for dysfunctional ABRAXAS1. In conclusion, regulation of end-resection by ABRAXAS1 is indispensable to the cell and more research is required to fully understand the extend of the importance of ABRAXAS1 to the cell.

FINAL REMARKS

As exemplified by this chapter, many questions in BRCA1 biology remain unanswered. Moreover, answers to these questions will most likely again lead to novel questions. This chapter also underlines the amount of variables and interplay between different processes in the cell. Things are clearly not just black and white. For example, each DSB is very different, which also affects the repair mechanism used and subsequently the repair outcome. Proteins play a role in multiple processes, sometimes only under specific circumstances and keeping the balance is key. For example, end-resection seems good as it allows cells to repair DSBs using faithful HR, however extensive end-resection increases genomic instability. Researchers therefore have to remain critical and open-minded as some observations might only be made in certain situations and hence generalisation is a common threat.

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