Chromatin modifiers in DNA repair and human disease

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6 PERSPECTIVES

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Introduction

Since the genetic information in our cells is constantly threatened by a large variety of DNA damage-inducing agents, the detection and accurate repair of DNA lesions is vital to preserve genome stability. Among the most devastating types of DNA damage are DNA double strand breaks (DSBs). DSBs can be generated endogenously by physiological processes, for instance upon replication stress or during meiotic recombination. Additionally, DSBs can be inflicted exogenously by physical agents such as ionizing radiation (IR) or by chemicals such as chemotherapeutic drugs. Cells respond to DSBs by sensing the DNA damage and initiating a cascade of signaling events that are capable to activate DNA repair and cell cycle checkpoints (Smeenk and van Attikum, 2013). This intricate network of defense mechanisms towards DNA damage is termed the DNA damage response (DDR). The signaling of DSBs is driven by posttranslational modifications (PTMs) (primarily phosphorylation and ubiquitylation) of proteins that function as DNA damage sensors or signal transducers. Ultimately this cascade of events regulates effector proteins that facilitate DNA damage repair and control cell cycle progression. Since chromatin often forms a barrier for DNA repair proteins to access the damaged DNA, the cellular response to DNA damage demands accurate and timely changes in chromatin structure to allow efficient protection against DNA damage. Chromatin modifiers and remodelers are capable to level this barrier by changing nucleosomal organization in the vicinity of DSBs and modulating PTMs on for example histones. This leads to a temporal increase in the accessibility of the chromatin surrounding the lesion (Smeenk and van Attikum, 2013). Hence chromatin modifiers and remodelers are considered to be key players in the DSB response and their loss can have severe effects on genome stability and consequently the development and health of an organism. Perhaps not surprisingly, genetic defects in these chromatin factors are frequently found in human disorders. Interestingly, such disorders have a number of common clinical characteristics like developmental defects, neurological degeneration, immunodeficiency and cancer predisposition. In order to identify the molecular origin of these diseases, it is essential to determine the function of chromatin factors involved in development and maintenance of genome stability.

In this study we characterized and deciphered the function of three chromatin factors EHMT1, RSF1 and ZBTB24 in the cellular response to DSBs. The histone methyltransferase EHMT1 was identified as a possible negative regulator of 53BP1 recruitment to DSBs that promotes DSB repair via non-homologous end-joining (NHEJ) and homologous recombination (HR) (Helfricht et al., 2013) (chapter 2). Remodeling and Spacing Factor 1 (RSF1), on the other hand, deposits centromeric proteins at DSBs. These proteins appeared to be critical for the RSF1-dependent recruitment of the important NHEJ-factor XRCC4 to DSBs. Interestingly besides NHEJ, RSF1 is also involved in the efficient repair of DSBs via HR (Helfricht et al., 2013) (chapter 3) and the function of RSF1 during both DSB repair pathways might be dependent on SUMOylation (chapter IV). Moreover, in **chapter 5** we discovered a role for ZBTB24 during classical NHEJ by means of promoting PARP1 activity and stabilizing PARP1-associated PAR-chains, thereby facilitating the PARP1/PARylation-dependent assembly of NHEJ complexes at DSBs. Moreover, we found ZBTB24's role in NHEJ to be critical for class-switch recombination (CSR), providing an explanation for the immunological phenotype of ZBTB24-deficient ICF2 patients (chapter 5). In conclusion, these findings contribute to our current understanding of the chromatin alterations taking place during the signaling and

repair of DSBs, and raise several questions regarding their link to human diseases, which are discussed in the following sections.

EHMT1 involved in intellectual disability syndrome and the DDR

Epigenetic processes such as DNA methylation are fundamental for (neuronal) development and cognitive functioning (Day and Sweatt, 2011). Consequently, the disruption of the methylation machinery can cause cognitive disorders (Miller et al., 2010) such as Kleefstra syndrome (KS) (OMIM #610253). KS is caused by haploinsufficiency of the histone methyltransferase EHMT1 due to loss-of-function mutations or deletions in the encoding gene at chromosome 9q34.3. The clinical core features of KS patients are developmental delay/intellectual disability, (childhood) hypotonia and characteristic facial features such as disproportional shortness of the head, synophrys, midface hypoplasia, unusual shape of the lips, protruding tongue and prognathism (Willemsen et al., 2012). Defective learning and memory phenotypes were also observed in an EHMT mutant in Drosophila melanogaster. Interestingly, these phenotypes were rescued upon restoration of EHMT expression in adult flies, indicating that cognitive defects are reversible in EHMT mutants (Kramer et al., 2011). Moreover, since homozygous Ehmt1 deficiency leads to embryonic lethality between E9.5 and E12.5 in mice, heterozygous Ehmt1+/- mouse models were employed. Ehmt1 protein levels were strongly reduced in heterozygous Ehmt1+/- cells, indicative of haploinsufficiency of Ehmt1 (Balemans et al., 2013). In line with these findings, Ehmt1+/- mice phenocopied the KS core features observed in the Drosophila EHMT mutant and haploinsufficient KS patients (Balemans et al., 2010; Balemans et al., 2014). Hence Ehmt1+/- mice can be used as a model for KS to investigate whether learning and memory formation can also be restored by the expression of functional Ehmt1. In addition, since mice and humans show 95% similarity in their genes, Ehmt1+/- mice provide a model for KS that is more closely related to the human situation compared to the Drosophila EHMT mutant. The Ehmt1+/- mice can also be used to define the exact role of EHMT1 in cellular processes, most notably in transcription and the DDR.

Gene expression analysis of heterozygous Ehmt1+/- mice already revealed a significant upregulation of bone tissue related genes, which likely results from decreased Ehmt1-induced H3K9me2 levels in the promotor region of these genes. This altered gene expression most likely contributes to the cranial dysmorphic features of KS (Balemans et al., 2014). In addition, our functional studies on the role of EHMT1 suggests that EHMT1 is a factor involved in the DDR that may act as a negative regulator of 53BP1 accrual at DSBs. EHMT1 also functions in DSB repair: in **chapter 2** we showed that EHMT1 promotes DSB repair via both NHEJ and HR. Whether EHMT1 functions directly in DSB repair or mediates DSB repair via promoting the recruitment of DDR signaling proteins such as 53BP1 requires further investigation. To this end, it would be interesting to further study EHMT1's interactors as these could be potential substrates for methylation. Substrates of EHMT1 and EHMT2 have already been identified using SILAC combined with quantitative MS on proteins captured with an engineered mono- or dimethylation-binding domain from normal and EHMT1/2 inhibitor treated cells (Moore et al., 2013). 23 proteins were appointed as EHMT1/2 substrates amongst which are known EHMT1/2 methylation targets like WIZ, the adaptor protein that stabilizes EHMT1/EHMT2 complex formation. Other potentially relevant substrates are DNA ligase 1 (LIG1), the chromatin remodeler SMARCA5 and the NHEJ factor DNA-PKcs (Moore et al., 2013). SMARCA5 and DNA-PKcs are both involved in DSB repair and could potentially provide a causal link for the observed decrease in DSB repair efficiency

upon EHMT1-depletion in cells containing the NHEJ or HR reporter (chapter 2). It would be relevant to map the methylation site(s) in these proteins and generate non-methylatable mutants. By using complementation studies the effect of their expression on DSB repair could be determined in order to assess the role of EHMT1/2-mediated methylation of these proteins in DSB repair.

Studies with mouse or human cells may reveal the relevance of results from genetic interaction studies in Drosophila that investigated changes in vein formation in the Drosophila wing upon modulating the expression of EHMT alone or with other factors simultaneously. This study described a functional link between EHMT1 and several epigenetic regulators including the histone H3K4 methyltransferase KMT2C, the heterochromatin binding protein MBD5 and the nuclear receptor NR1I3. Mutations in these genes and the core-component of the hSWI/SNF chromatin remodeling complex SMARCB1 were identified in human individuals with severe intellectual disability that comprise features closely resembling those of KS patients. KMT2C, MBD5 and NR1I3 cooperate with EHMT1, whereas SMARCB1 directly interacts with KMT2C. These findings lead to the proposal of a putative conserved epigenetic network that underlies cognitive disorders and as such a tight epigenetic control of higher brain function (Kleefstra et al., 2012). Whether this network of chromatin modifiers is equally relevant for human cells or if EHMT1 is the only factor of this network that participates in regulating the DDR remains to be investigated. Ultimately, examination of protein levels and recruitment of relevant DDR factors to DNA damage is required to shed light on the mechanisms by which EHMT1 regulates DSB repair.

Dissecting the role of RSF1 in DNA repair

RSF1 protects cells from the harmful effects of genotoxic agents such as IR (Helfricht et al., 2013; Min et al., 2014), most likely by contributing to the repair of IR-induced DSBs via HR and NHEJ (chapter 3). RSF1 is recruited to laser-induced DNA damage and site-specific DSBs in an ATM-dependent manner (Min et al., 2014) and deposits the centromere proteins CENP-S and CENP-X at DSBs (Helfricht et al., 2013) (chapter 3). This role of RSF1 may require its DNA damage-induced SUMOylation (chapter 4), but surprisingly does not rely on the presence of its binding partner SMARCA5 (Helfricht et al., 2013) (chapter 3). Remarkably, we found that CENP-S and CENP-X exclusively stimulate DSB repair through NHEJ by promoting the recruitment of XRCC4, a factor critical for the final ligation step of this repair process (Helfricht et al., 2013) (chapter 3). However, the exact role(s) of these centromere proteins in NHEJ have yet to be determined.

The assessment of a putative role of RSF1 in the signaling of DSBs revealed that RSF1, in contrast to its binding partner SMARCA5 (Helfricht et al., 2013; Smeenk et al., 2013), is dispensable for the RNF8/RNF168-mediated ubiquitin signaling cascade (Helfricht et al., 2013) (chapter 3). In contrast to our findings, however, another report showed the analysis of nuclear foci (γH2AX, MDC1 and 53BP1) induced by the radiomimetic agent phleomycin and revealed a reduction in foci formation in RSF1-depleted U2OS cells (Min et al., 2014) favoring a role of RSF1 in the signaling of DSBs. Whether these contradictory results reflect the nature of the DNA damaging agent, the acute versus chronic genotoxic exposure or the timing of foci analysis after DNA damage induction remains elusive and requires further investigation.

Another important function of RSF1 is the maintenance of centromeric chromatin. This function involves the incorporation of the histone H3 variant centromere protein A (CENP-A) and its positioning along the centromeric chromatin (Perpelescu et al., 2009). Similar to RSF1,

CENP-A was shown to be recruited to DSBs (Zeitlin et al., 2009). However, unexpectedly only CENP-S and CENP-X were recruited to sites of laser-induced DNA damage in our experimental set-up in a manner strictly dependent on RSF1 (Helfricht et al., 2013) (chapter 3). Moreover, CENP-S and CENP-X have been shown to form an evolutionary conserved complex with the Fanconi anaemia (FA) complementation group M (FANCM) protein that is required for the repair of DNA interstrand crosslinks (ICLs) and genome stability maintenance (Singh et al., 2010; Yan et al., 2010). FA is a rare genetic disease that affects 1 in 160.000 individuals worldwide. It is characterized by physical abnormalities, bone marrow failure as well as cancer predisposition and is caused by a genetic defect in one of the FA group proteins. RSF1 could possibly facilitate ICL repair through the loading of the CENP-S and CENP-X proteins at sites of ICLs. This subsequently promotes or coordinates the accrual of other FA proteins and might implicate RSF1 as a yet unknown FA gene. It is evident that more work is required to unravel the exact role of RSF1 in ICL repair and other cellular processes. For instance, its contribution to ICL repair, recruitment to ICLs and functional interplay with known FA proteins should be studied using a combination of cell biology, biochemistry and microscopy approaches.

CENP-N, CENP-U and CENP-T have also been shown to be recruited to sites of laser-induced DNA damage (Zeitlin et al., 2009). However, whether these CENP proteins, similar to CENP-S and CENP-X, rely on RSF1 for their recruitment is unclear. Moreover, their recruitment to sites of DNA damage raises the question as to whether RSF1 is involved in the formation of a CENP complex at DSBs. Particularly, is this complex if present at DSBs comparable to the one that is formed at kinetochores (Perpelescu and Fukagawa, 2011)? On the other hand, we also lack understanding of how RSF1 recruits CENP proteins and to what extent the accrual of RSF1 and CENP proteins induces structural changes in DSB-flanking chromatin that makes it amenable to DNA repair. RSF1-induced chromatin structural changes should therefore be studied in response to DNA damage, for instance by examining nucleosome occupancy and compaction at site-specific DSBs by ChIP-seq and MNase-based assays. Alternatively, the effect of recombinant CENP proteins on the compaction of reconstituted nucleosomal arrays could be studied by biophysical approaches in vitro.

In addition, recombinant CENP proteins could be investigated for their effect on chromatin folding in vitro by monitoring chromatin fiber composition in biophysical experiments. Finally, it would be interesting to know whether CENP proteins undergo PTMs upon DNA damage induction. Interestingly, CENP-S was recently shown to be ubiquitylated upon exposure to IR (Elia et al., 2015), but whether this PTM is important for its function at DSBs remains elusive.

Currently, the mechanism by which RSF1 executes its role in DSB repair is vague. Intriguingly, RSF1 itself does not display any enzymatic activity, yet it is able to load CENP proteins at sites of DNA damage (Helfricht et al., 2013) (chapter 3). A step towards understanding the mechanistic role of RSF1 in DSB repair is to elucidate whether RSF1 acts individually, with SMARCA5 as part of the RSF complex or even as a member of a another complex. One approach to address this key question is to perform DSB repair experiments in RSF1- and/or SMARCA5-depleted cells and monitor whether RSF1 and SMARCA5 act epistatically or synergistically. Additionally, interactors of RSF1 could be identified by SILAC-based MS analysis following DNA damage induction and their interplay with RSF1 in DSB repair should be studied.

ICF1-4 ... is there a common mechanism?

ICF patients have been categorized into four subgroups (ICF1, 2, 3 and 4; causally linked to mutations in DNMT3b, ZBTB24, CDCA7 and HELLS, respectively) dependent on their genotype. Interestingly, a few ICF cases do not have mutations in one of the four ICF genes, which means that at least one additional gene can be identified as ICF-disease gene. In spite of this remarkable genetic heterogeneity of the ICF syndrome, the clinical phenotypes of ICF patients are substantially overlapping. This raises the question whether analogously to ZBTB24, the ICF-causing genes DNMT3B, CDCA7 and HELLS also play a role during NHEJ and CSR. This is an intriguing question as to our knowledge DNMT3B, ZBTB24, CDCA7 and HELLS do not share enzymatic activities, whereas all four genes affect CpG methylation. ZBTB24 and CDCA7 were described to maintain CpG methylation whereas DNMT3B has a role in establishing methylated CpGs (Okano et al., 1999). HELLS on the other hand functions in both processes (Thijssen et al., 2015; Zhu et al., 2006). A key goal of future research is to reach mechanistic understanding of how the four hitherto identified ICF genes DNMT3B, ZBTB24, CDCA7 and HELLS cause ICF syndrome. A variety of assays focusing on DSB repair, immunoglobulin serum levels and CSR in control and patient material of all ICF subtypes could shed light on the above-mentioned question. DNMT3B and HELLS have already been implicated to function in DSB repair, but their precise roles in NHEJ and/or CSR still remain to be resolved (Burrage et al., 2012; O'Hagan et al., 2008).

One of the phenotypes of ICF patients is DNA hypomethylation especially at centromeric repeats. DNA methyltransferase 1 (DNMT1) maintains DNA methylation during DNA replication and has been shown to bind non-covalently to PARylated PARP1, which leads to DNMT1 inactivation and subsequently to DNA hypomethylation (Reale et al., 2005). Whether DNMT3B also binds to (PARylated) PARP1 to become inactivated, requires further investigation. One possibility is that DNMT3B and ZBTB24 compete for the binding of PAR chains. In the case of ICF2 patients, the established PAR chains might become available for DNMT3B binding due to ZBTB24 loss, leading to the observed DNA hypomethylation phenotype. However, there is currently no obvious mechanism that could explain the DNA hypomethylation phenotype of ICF3 and ICF4 patients carrying mutations in CDCA7 or HELLS, respectively. No function has yet been described for CDCA7, while mouse Hells/Lsh has been reported to associate with Dnmt3a or Dnmt3b, but not with Dnmt1, and to aid in the establishment of de novo methylation (Zhu et al., 2006). To investigate the possible roles of CDCA7 and HELLS particularly in relation the DDR, cell biology, microscopy and mass spectrometry based approaches should be employed. These will help to unravel whether these proteins localize to sites of DNA damage, what their mode of action is in which biochemical context they operate at DNA lesions is.

Chromatin modifiers in cancer

Recent studies have indicated that human cancers exhibit global epigenetic abnormalities as well as genetic alterations (Jones and Baylin, 2007). In contrast to the latter, epigenetic changes are reversible and can be enzymatically restored to their non-disease state. Therefore, more and more studies focus on understanding chromatin modifiers and the PTMs they induce in various pathways to identify novel targets for cancer therapy.

Somatic mutations in many of the histone modifying and chromatin remodeling genes are associated with cancer development (Shih et al., 2012) (chapter 1, Table1). In addition, the overexpression of chromatin remodeling proteins is often linked to a poor prognosis for cancer patients and can therefore serve as a prognostic tumor marker (Guan et al., 2014; Lee

et al., 2014; Li et al., 2014; Xie et al., 2014). The chromatin modifying proteins EHMT1, RSF1 and ZBTB24 studied in this thesis, have been linked to cancer and are therefore discussed in the following sections.

While reduced EHMT1 activity leads to KS, the overexpression of EHMT1 seems to promote cancer development, for instance in the case of esophageal squamous cell carcinomas (Guan et al., 2014). The overexpression of EHMT1 leads to an increase in the repressive H3K9me1/2 chromatin marks in general and more specifically at promoter regions of genes frequently silenced in cancer (Yoo and Jones, 2006). As a conceivable hypothesis, increased EHMT1 expression might also alter the DDR and might lead to impaired DSB repair. The proposed hypothesis could straightforwardly be addressed using DSB repair assays in cells transiently overexpressing EHMT1.

Also RSF1 has been linked to tumorigenesis and as much as 191 unique somatic mutations have been identified in various cancers listed in the catalogues of somatic mutations in cancer (COSMIC). Whether these mutations affect RSF1 expression and/or function and influence DNA repair levels in cancer is an important question. Intriguingly, RSF1 was also found to be overexpressed in various types of cancer with a frequency of 55% in ovarian carcinomas, 50% in colon cancer tissues and 45% in prostate cancer specimens, and this phenotype correlates with a poor prognosis for the length of patient survival (Davidson et al., 2006; Liu et al., 2012; Shih et al., 2005). Interestingly, siRNA-mediated knockdown of RSF1 in cells with high endogenous RSF1 expression remarkably decreased cell proliferation and colony formation (Li et al., 2014). Furthermore, the overexpression of RSF1 is likely to increase DNA damage levels as evidenced by increased γH2AX levels and chromosomal aberrations in ovarian cancer cells (Sheu et al., 2010). Hence it is tempting to speculate that increased RSF1 expression negatively impacts on DNA damage repair and ultimately leads to chromosomal instability in tumor cells. Accordingly, the question raises as to what extend the equilibrium of SMARCA5-containing complexes might be disturbed through RSF1 overexpression. One way to discover an imbalance in SMARCA5-containing complexes and their putative impact on DSB repair is to assess their composition by mass spectrometry and perform quantitative DSB repair assays in cells transiently overexpressing RSF1. The latter should also clarify whether increased levels of RSF1 in cancer cells affect the equilibrium between DSB repair via HR and NHEJ. A change in the balance between these two repair pathways is important and critical for the choice of therapy as this might sensitize cancer cells to certain drugs. For instance PARP inhibitors could be applied during therapy in the case that altered expression of RSF1 renders cells HR deficient (see also section on PARP inhibitor-based cancer therapy). In conclusion, given RSF1's critical role in DSB repair and its link with carcinogenesis, it may serve as an important marker and/or therapeutic target in personalized cancer therapy.

We discovered that ICF2 patients with mutations in ZBTB24 display defects in CSR, which is the immunoglobulin (Ig) gene-diversification process occurring in B-cells (chapter 5), explaining the immunodeficiency phenotype of these patients. During CSR, recombination events between different switch (S) regions within the heavy chain Ig (IgH) locus occur upon DSB induction by the cytidine deaminase (AID) (chapter 1, Fig. 5). Under normal conditions CSR mediates the removal of a DNA segment between switch regions on one chromosome, whereas defects in CSR can also lead to NHEJ-mediated translocations between two different chromosomes. Several chromosomal breakpoints have been found in the IgH switch regions in a number of different translocations in lymphoma, leukemia and myeloma. The common location of these chromosomal translocation breakpoints strongly suggests their occurrence

to originate from mistakes in CSR, which links CSR to tumorigenesis (Bergsagel et al., 1996; Janz, 2006; Kuppers and Dalla-Favera, 2001). Unfortunately, ICF patients die at a young age usually in the first or second decade of life mostly from the disastrous consequences of severe, opportunistic and recurrent infections (Weemaes et al., 2013). Hence, it is rather difficult to assess the effect of ZBTB24 on IgH translocations and cancer development in these patients. ZBTB24 knockout mice would therefore be extremely helpful to investigate the role of ZBTB24 in translocation formation and cancer development. However, attempts to generate ZBTB24 knockout mice indicated that complete loss of ZBTB24 leads to embryonic lethality (unpublished data). Thus, a conditional ZBTB24 knock-out mouse would be desired now, which could for instance allow the study of ZBTB24 loss on translocation formation in B-cells specifically.

Interestingly, already 78 unique somatic mutations have been identified in ZBTB24 in various cancers listed within the COSMIC database. Despite the young age of 4 up to 19 years, a few ICF patients have been diagnosed with different cancers such as myelodysplastic syndrome, classical Hodgkin lymphoma (Hagleitner et al., 2008; Schuetz et al., 2007) and adrenocortical adenoma (Kubota et al., 2004). The Hodgkin lymphoma was diagnosed in a 4 year old ICF2 patient (Weemaes et al., 2013), while the other detected cancers not certainly originated from ICF2 patients. Another case reported on the death of a 21 year old ICF1 patient from complications of a metastatic angiosarcoma of the liver (van den Brand et al., 2011). Since angiosarcoma is utterly rare at such a young age, this could suggest a link between tumorigenesis and defective DNA methylation caused by a mutation in DNMT3B in this ICF1 patient. However, so far we can only speculate about what exactly leads to tumorigenesis in those four described ICF patients and whether ICF patients in general are predisposed to develop cancer.

Chromatin modifier-defects and therapy options

Cancer is a disease that is driven by genomic instability, a feature that can arise from a defective DDR. Currently, the established approach to treat cancer is to kill tumors cells through the induction of DNA damage via chemotherapy or radiation, but this strategy also targets healthy cells for cell death. Thus, alternative therapy methodologies that specifically target cancer cells are to be found. One promising approach to enhance the efficacy of cancer therapy is the use of specific inhibitors that target DDR factors in cancer cells to disable certain DNA repair pathways (Jackson and Bartek, 2009). The DDR is therefore intensely investigated to identify novel (chromatin-modifying) factors that are suitable anti-drug targets in anti-cancer regimes.

PARP inhibitors for instance are effective in cells comprising a defect in HR; HR-deficient BRCA1/2 tumors therefore display high sensitivity towards PARP inhibitors, providing an example of a synthetic lethal relation (Bryant et al., 2005; Farmer et al., 2005). Remarkably, the treatment of siRSF1-depleted U2OS cells with the PARP inhibitor Olaparib resulted in reduced cell survival (Pessina and Lowndes, 2014). This suggests that tumors with decreased expression of RSF1 are likely to be sensitive to PARP inhibitors. Whether the latter can also provide an efficient therapy for malignancies that comprise altered expression levels of EHMT1 or ZBTB24 is not known and will require further investigations. However, it is promising that our research implicates all three factors in the repair of DSBs via HR (chapter 2, 3, 5), a requisite for an effective PARP inhibitor treatment. However, EHMT1, RSF1 and ZBTB24 also promote NHEJ (chapter 2, 3, 5) and hence, NHEJ might also be defective in cancer cells missing functional EHMT1, RSF1 or ZBTB24. This could be a disadvantage for

a PARP inhibitor-based therapy, since loss of the NHEJ-promoting factor 53BP1 or REV7 (a factor acting downstream of 53BP1 in blocking HR), has been shown to diminish the PARP inhibitor cytotoxicity in HR-deficient cells (Bouwman et al., 2010; Bunting et al., 2010; Xu et al., 2015). PARP1 inhibition induces the formation of lethal radial chromosomes in HR-deficient cells that likely result from mis-rejoined DSBs. This is prevented by 53BP1 deletion (Lottersberger et al., 2013), suggesting that combined loss of HR and NHEJ may compromise an effective PARP inhibitor treatment. Surprisingly, however, despite the role of RSF1 in NHEJ, RSF1-depleted cells were sensitive to PARP inhibition (Pessina and Lowndes, 2014). In order to obtain direct proof for a possible sensitivity towards PARP inhibitors, cell killing (e.g. measured by clonogenic survival) of EHMT1- or ZBTB24-knockdown cells and EHMT1-, RSF1- or ZBTB24-overexpressing cells should be assessed. In addition, further genetic screening for other synthetic lethality combinations in cells containing a defect in DDR factors will be of great importance for the development of additional therapy opportunities for personalized cancer treatments in the future. Administering chemical compounds in the framework of personalized medicine that are tailored to the (epi)genetic defects of a tumor will possibly lead to an increase in treatment success rates for patients with genetic alterations in chromatin factors, as is the case for the majority of tumors comprising mutations in BRCA1 or BRCA2 (Bao et al., 2015).

Also the development of specific inhibitors that restrain the activity of overexpressed chromatin factors in cancer cells might lead back to a non-disease state. For instance reversing the epigenetic changes induced by aberrant EHMT1 activity due to its overexpression in certain cancers by means of EHMT1/2 inhibition, might lead to the re-expression of genes that had been silenced through an increase in EHMT1/2-mediated H3K9me1/2 marks. Efforts have been made to develop small-molecule inhibitors for EHMT1 and EHMT2. A few of these inhibitors have recently been proven to provide a way to counteract EHMT1 activity in breast cancer, esophageal squamous carcinoma and leukemia cells (Liu et al., 2013). Thus, EHMT inhibitors may ultimately improve the poor survival prognosis of patients with aberrant EHMT1 expression in the future (Curry et al., 2015; Guan et al., 2014; Pappano et al., 2015).

ICF patients suffer from severe respiratory and opportunistic infections caused by their immunodeficiency. Current therapeutic opportunities for ICF patients mainly concentrate on counteracting these severe infections. In 4 out of 5 ICF patients hematopoietic stem cell (HSC) transplantations have been successfully performed to restore their immunity. Interestingly, HSC transplantations have so far never been performed in ICF2 patients (Weemaes et al., 2013), which could be linked to the generally more pronounced humoral immunodeficiency in ICF1 patients. In any case, an early diagnose of ICF syndrome is of great importance, since early immunoglobulin supplementation can improve the course of the disease. A drawback of this method is however the availability of a compatible donor. Therefore, gene therapy might form a potent alternative and employs the transfer of a transgene via for instance viral infection to patient-derived HSCs. These cells are subsequently transplanted back into the patient. Notably, this form of gene therapy already became available for patients with specific types of severe combined immunodeficiency (Mukherjee and Thrasher, 2013). Another approach to restore gene function could be gene correction, where the mutated DNA sequence is replaced by a wildtype DNA sequence using for instance CRISPR/Cas9-based genome editing. Such an experimental approach might not only be beneficial for ICF patients but could also provide an interesting strategy for the development of therapies for KS patients if applicable in humans in the future.

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