Mapping of EGFR treatment effects and uncovering DNA repair mechanisms using quantitative proteomics

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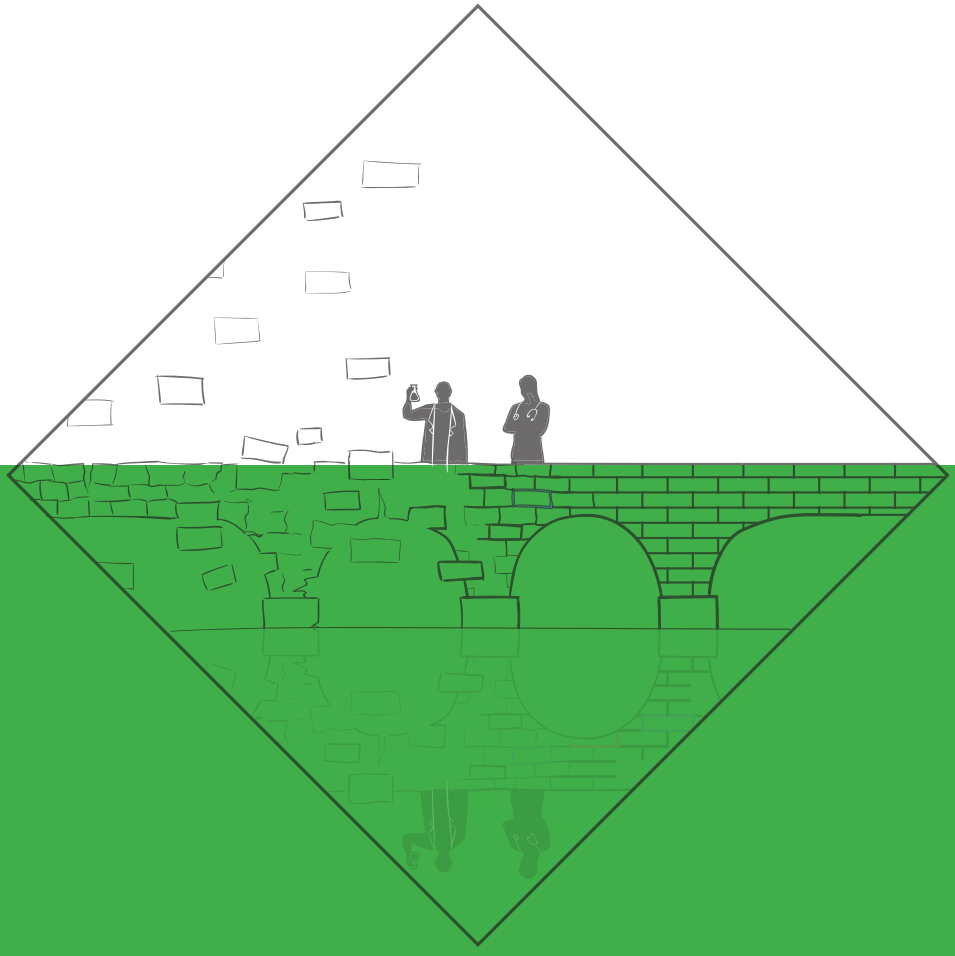
Summary and discussion



Chapter 8

Summary

Cancer cells depend highly on deregulation of biological processes to acquire and maintain oncogenic properties, known as the hallmarks of cancer (1). These features include sustained proliferation and genomic instability. Sustained proliferation is a fundamental hallmark in many cancers that is frequently achieved through alterations in growth factor signaling pathways. This may occur through elevated levels of growth factors, or more commonly, through mutation or amplification of key components in growth factor pathways, resulting in continuous signaling flux and increased cell division rates. Tumorigenesis can be accelerated by defects in the processes that control genome integrity, allowing increased mutation rates, resulting in genomic instability (1). Genomic instability has therefore been coined 'an enabling cancer hallmark' that drives and maintains tumorigenesis, and can promote the acquisition of additional malignant features (1).

Interestingly, acquisition of cancer hallmarks may result in so-called 'oncogene addiction', of which the disruption forms the basis of many molecularly-targeted therapeutics in cancer therapy (2). Furthermore, cancer cells increasingly rely on supportive processes ('enabling cancer hallmarks') for their survival. This feature has been coined 'non-oncogene addiction, and interfering with such supportive processes offers additional possibilities to therapeutically target tumor cells. The rationale behind this latter approach is that disrupting supportive processes can shift the balance from maintaining an oncogenic state towards failure of tumor cells to remain viable (3,4).

Over the last decade, genetic changes that frequently occur in tumors have been mapped to great detail, for instance by The Cancer Genome Atlas (TCGA) consortia (5). Additionally, drugs that target oncogenic and non-oncogenic pathways increasingly become available. In order to optimally employ these molecularly targeted agents, it is crucially important to identify actionable weaknesses of specific tumors. However, for many targeted therapies, despite promising preclinical efficacy as single agent, is has become clear that combination therapies are required to prevent emerging resistance and reach durable treatment response (6). This not only requires insight in the wiring of the targeted mechanisms, but also elucidation of how and when to apply individual agents in combination therapies (6). This could be guided by novel methods, which accurately detect the effects of individual components of a therapy on tumor cells.

In this thesis, we aimed to determine at the proteomic level how cancer cells deal with therapeutic targeting of oncogenic pathways or supporting non-oncogenic pathways. To this end, we combined quantitative proteomics with a range of cell biological and genetic techniques to uncover mechanisms and markers of treatment efficacy.

In **Chapter 1**, a general introduction and an outline of this theses was provided on how we addressed this aim.

In **Chapters 2 – 4,** we addressed the first aim of this thesis, which was to discover drug-specific protein responses to cancer therapeutics, and to subsequently apply specific proteins to monitor effective treatment response as 'effect sensors'. Therefore, in **Chapter 2** we reviewed the involvement of the human epidermal growth factor receptor (HER) family in human cancers. Proper patient selection is essential for effective EGFR-family targeted treatment, while existing or acquired resistance mechanisms circumvent or hinder drug effectiveness. Focusing on (pre)clinical studies, we described how molecular imaging can be used to expand upon conventional patient selection. Measurement of target abundance, target dynamics in response to treatment, the binding differences between mutational

variants, and effect sensors can be achieved using a wide range of imaging tracers. Lastly, we discussed how an integrative omics discovery platform can guide selection of tracer targets. Since 'omics' platforms are increasingly used in (pre)clinical studies, these large datasets are attractive to identify markers that differentiate between tumor subtypes or treatment outcome. In addition, with integrative omics analyses of pre- and post-treatment samples could be used to identify effect sensors for drug specific responses, which can then be applied by serial molecular imaging.

In **Chapter 3**, we applied quantitative proteomics to discover effect sensors of the EGFR inhibitor erlotinib, by measuring differential expression of membrane proteins using SILAC proteomics. From this analysis, we identified Mucin-1 (MUC1) in three erlotinib-sensitive breast cancer cell lines, and validated this response in non-small cell lung cancers (NSCLC) cell lines. Mechanistic studies unraveling the processes underlying MUC1 upregulation uncovered a role for rewiring of the AKT pathway rewiring, and involvement of the STAT family of transcription factors, as both mechanisms drove MUC1 expression in response to EGFR inhibition. To validate the effect sensor concept, we next tested whether MUC1 dynamics could be assessed in vivo by molecular imaging and by shedding of MUC1 in plasma. PET-imaging of erlotinib-induced MUC1 in SUM149 xenografts using an 89Zr-labeled MUC1-targeting antibody did not yet result in adequate contrast between MUC1-targeted tumor uptake and tumor uptake of an IgG-control antibody to visualize MUC1 expression induced by erlotinib. However, and most importantly, increased levels of shed MUC1 were detected in plasma of erlotinib-treated mice compared to control treated mice. These results suggest a potential role for shed MUC1 measurement in plasma of erlotinib-treated cancer patients as a new non-invasive method to monitor effect EGFR-treatment.

The discovery and application of MUC1 as an effect sensor of EGFR treatment in breast cancer cell lines motivated us to expand this concept to other cancer models and treatments. In **Chapter 4**, we analyzed proteomic changes in response to EGFR-targeted treatment of NSCLC and colorectal cancer (CRC) models, and proteomic changes to DNA damaging agents in breast cancer cell lines. Pathway analysis of the treatment-induced proteomic responses confirmed effective EGFR targeting in NSCLC and CRC models, and showed effective cell cycle inhibition in the DNA damage-induced breast cancer models. For each treatment entity, we identified a number of potential effect sensors for follow-up validation.

The second aim of this thesis was to study proteomic changes during the cell cycle in relation to the response to DNA damage. We focused on the transition between cell cycle phases, as these are accompanied by differential usage of DNA repair pathways. Repair of double stranded breaks (DSBs) in G1 phase cells is performed by non-homologous end-joining (NHEJ). In contrast, during and after DNA replication in S-phase, DSBs can be repaired by homologous recombination (HR), as sister chromatids become available, which can serve as templates for repair. Strikingly, although DNA breaks are recognized during mitosis, repair of DSBs by either NHEJ or HR is inactivated during this cell cycle phase.

In **Chapter 5**, we described based on the current literature how the anaphase-promoting complex/cyclosome (APC/C) influences DNA repair within different cell cycle phases. The APC/C is an E3 ligase that binds target proteins through so-called 'KEN-box' or 'D-box' motifs, and subsequently marks target proteins for proteasomal degradation by addition of ubiquitin residues. Thus far, the APC/C is best known for regulating the timely onset of anaphase during mitosis, after binding of co-activators Cdc20 and Cdh1. We here described recent data showing that the APC/CCdh1 is also

activated in response to DNA damage in G2-phase. DNA damage in G2-phase activates the G2-M cell cycle checkpoint, and the APC/CCdh1 ensures that this checkpoint is propagated until the damage is resolved by degradation of cell cycle promoting factors. Furthermore, by regulating the abundance of specific proteins involved in the DNA damage response, the APC/CCdh1 controls DNA damage dynamics.

In **Chapter 6**, we searched for novel targets of the APC/CCdh1 that are involved in DNA damage repair. To this end, we used SILAC-MS analysis of proteins that are down-regulated during the transition from mitosis to G1-phase. Amongst many known mitotic targets of the APC/CCdh1, we found a number of novel targets involved in DNA damage repair, including CtIP and Rif1. Further bioinformatic analysis of CtIP predicted an interaction with APC/CCdh1 through a conserved KEN box, resulting in the selection of CtIP for further validation. CtIP is an endonuclease that is required for the initiation of HR repair in G2 phase (7). Mutation of the KEN-box in CtIP prevented ubiquitination and impeded CtIP down-regulation both during G1 and after DNA damage in G2. Blocking the CtIP-Cdh1 interaction resulted in reduced HR efficiency, and delayed clearance of CtIP foci after DNA damage. Combined, our results showed that APC/CCdh1 safeguards genome integrity by controlling CtIP stability in a cell cycle- and DNA damage-dependent manner.

In **Chapter 7**, we studied Rif1 during mitosis. Rif1 was analyzed as part of validation experiments on DNA damage targets of the APC/C in chapter 6. Unexpectedly, fluorescence microscopy analysis showed that Rif1 localizes to so-called ultra-fine DNA bridges (UFBs) in anaphases. This finding did not match previously described functions of Rif1, were it was described as a downstream effector of 53BP1 in DSB repair (8). We discovered that Rif1 localization to UFBs depends on the DNA translocase PICH, which is known to resolve UFBs together with the helicase BLM (9,10). Similarly, Rif1 promoted the resolution of UFBs, as Rif1 depletion increased the frequency of nucleoplasmic bridges and RPA70-positive UFBs in late anaphase. Moreover, in the absence of either component of Rif1, PICH or its associated protein BLM, more nuclear bodies with damaged DNA were present in G1 daughter cells as a result from unfaithful chromosome segregation. Our data revealed a novel role for Rif1 in the resolution of UFBs during anaphase to protect genomic integrity.

Discussion and future perspectives

Proteomic approaches to study cancer biology

In this thesis, proteomics analysis was used to identify changes in protein expression induced by EGFR inhibition, chemotherapeutic agents or modulation of cell cycle phase. Although large sets of peptides were measured quantitatively, in our assays we typically identified ~2,000-4,000 unique peptides/proteins, leaving multiple proteins undetected. Ongoing efforts in proteomics aim to reach full proteome coverage, comparable to whole genome sequencing techniques. However, current liquid chromatography-mass spectrometry (LC-MS) proteomic techniques do not yet capture complete proteomes (11). In many proteomic approaches this is caused by peptides from highly abundant proteins that take up relatively more elution time during LC separation, resulting in a higher probability for those peptides to be selected for mass analysis compared to low abundant peptides. Whereas RNA sequencing techniques can amplify lowly transcribed RNAs to a level of minimal detection and reach high coverage of the transcriptome, proteomic samples however often require sample enrichment to reduce the complexity of a protein samples, or use multiple measurements per sample. In the latter approach, previously detected –highly abundant– peptides can be excluded to improve the detection of less

abundant peptides. Furthermore, SILAC proteomics requires measurement of both light and heavy isotope-labeled peptides for accurate quantification, which further limits the number of identified proteins included in final analyses. These limitations have a significant impact on the amounts of analyzable peptides between multiple samples in SILAC-MS. However, as these limitations inherently skew towards analysis of highly abundant proteins, this can be seen as advantageous for the identification of robust protein biomarkers that require sufficient contrast of detection in validation and application studies.

In chapter 3 and 4, we set out to discover effect sensors of EGFR targeting and DNA damaging cancer therapeutics using SILAC-based proteomics, in a confined panel of cell lines. Our primary SILAC-MS of erlotinib treatment in three breast cancer cell lines resulted in 1,787 – 2,131 proteins identified per cell line with a 61% – 73% overlap across all three cell lines using an Orbitrap mass analyzer, with similar results for analysis of the two NSCLC cell lines. For subsequent analyses of a cetuximab-treated CRC cell line and breast cancer cell lines treated with DNA-damaging agents, we made use of the more sensitive Q-Exactive Pro mass analyzer. These analyses resulted in 3,186 – 4,111 identified proteins per cell line, greatly increasing the detection of low abundant proteins. Combining the SILAC-MS data of breast cancer, NSCLC, and CRC cell lines resulted in only 818 overlapping proteins identified. Thus, with a restricted number of cell lines used, a dataset with relatively small overlap is reached, consisting of abundantly expressed proteins. Increasing the number of models should ideally increase the confidence for the selection of proteins for further validation. However, by focusing only on overlapping proteins, many informative proteins are neglected that could be used as tissue specific markers, or increase the strength of pathway analysis. Future experiments, including higher numbers of samples representing more models, should focus at integrating proteomic data while permitting a certain level of incomplete overlap, to maximize the total number of proteins identified, at the expense of higher false discovery rates (12).

Our SILAC-MS analyses in chapter 3, 4, and 6 were focused on the quantification of changes in abundance of proteins, which resulted in the novel insight into the regulation of MUC1, Rif1, and CtIP. However, biological processes are dynamic, and protein abundance is only one aspect of what determines the activity and efficiency of the process. Post-translational modification (PTM) of proteins at specific residues is essential for controlling many proteins, as PTMs control subcellular localization, activity, abundance and complex formation with other proteins. One example of PTMs, is the extensive glycosylation of tandem-repeat domain in the extracellular part of MUC1, which in tumor-associated MUC1 is actually severely reduced, due to changes in the type of glycosylation. Of note, this specific PTM is currently being explored as diagnostic and therapeutic target (13). Similarly, although we identified CtIP as a target of APC/CCdh1 due to a change in abundance, this process appeared to be regulated by specific ubiquitination of CtIP. Mass spectrometry (MS), including SILAC-MS, is very well suited for the discovery and quantification of PTMs, as the presence of PTMs is captured in the m/z-values measured by MS. This does require enrichment of the PTM of interest to capture sufficient numbers of peptides containing PTMs and perform accurate quantification. An experimental approach focused on ubiquitinated peptides in the SILAC-MS of cells exiting mitosis might have identified the specific ubiquitin PTM of CtIP on lysine 467, as well many other APC/CCdh1 binding sites and regulated proteins. Ideally, both the abundance and PTMs on a protein should be detected in proteomic studies to increase depth of information gained from these experiments. For example, knowing both the expression and activation state of an identified protein

might improve the selection for further validation and provide a more focused direction for follow-up analysis towards biological function or application. This was nicely illustrated in a large MS proteomic study performed with genomically annotated breast cancers of the TCGA cohort. In this study, the amplification of the genomic locus containing HER2 was accompanied with increased protein expression and phosphorylation of the neighboring gene CDK12, identifying a potential novel drugable kinase as a therapeutic target in HER2-amplified breast cancers (14). In chapter 2, we further discussed how PTMs, such as phosphorylation, acetylation, and ubiquitination, are increasingly studied in biomarker studies and might serve to monitor the activation of drug-targeted processes. Thus, there is an opportunity to exploring protein modifications in future proteomic studies to expand the number and type of effect sensors that can be discovered.

Effect sensor discovery and application

Using an explorative, unbiased SILAC-MS approach, we identified and validated MUC1 as an effect sensor for EGFR-targeting therapeutics in breast cancer cells, and identified a number of potential effect sensors for EGFR-targeting therapeutics in NSCLC and CRC. Previously, a SILAC-proteomic approach similar to ours was used to study proteomic changes induced by EGFR inhibitor gefitinib in a single EGFR inhibitor sensitive epidermal cancer cell line, specifically A431 (15). In this analysis, MUC1 was not detected. In line with this report, using Western blot analysis of A431 cells, we were not able to detect MUC1, nor upregulation of MUC1 expression in response to erlotinib. This suggests that tissue background may be important for erlotinib-induced MUC1 expression, and underscores a need to further test for heterogeneity between tumor cell lines and tissue types that are known to be sensitive to EGFR treatment.

Concerning the mechanism behind MUC1 upregulation, our results showed that AKT/mTOR signaling and transcription factor STAT3 were required for MUC1 expression in breast cancer cell lines. AKT/mTOR and STAT3 involvement in MUC1 regulation was predicted from pathway analysis of changes in expression of all discovered proteins. Although we focused mainly on validation of MUC1 as downstream consequence of these two pathways, it is likely that other downstream targets of this compensatory regulation could also function as effect sensors of EGFR targeting. In a panel of 4 sensitive NSCLC cell lines, we showed erlotinib-induced MUC1 elevation, similar to breast cancer cell lines, however involvement of AKT/MTOR or STAT3 was not explored. It will need to be assessed if these or other mechanisms are responsible for MUC1 upregulation after erlotinib in the NSCLC cell line panel.

In vivo detection of MUC1 showed that shedding of MUC1 in plasma of mice could be used as a proxy for elevated MUC1 expression in SUM149 xenograft tumors, whereas molecular imaging of MUC1 on tumor cells using radiolabeled antibodies did not show sufficient dynamic range (Chapter 3). These results warrant further study to analyze whether monitoring of shed MUC1 levels in erlotinib-treated patients by serial blood sampling predicts treatment response. MUC1 plasma levels have previously been used as a biomarker of tumor load, known as the cancer antigen (CA) 15-3 (16). Specifically, plasma MUC1 levels have been used to monitor recurrence of disease after therapy in patients with high plasma MUC1 levels before treatment or surgery (16). Interestingly, in a study of 70 erlotinib-treated advanced NSCLC patients, increased shed MUC1 levels after 2 and 4 weeks of treatment with EGFR inhibitor gefitinib was only observed in patients with progressive disease (17). These data suggest that MUC1 levels increase as a measure of tumor burden, and that measurements prior

to treatment might be required to measure EGFR inhibitor-induced MUC1 expression. Additionally, the majority of the patients with progressive diseases in this analysis had wt-EGFR tumors. As EGFR treatment of wt-EGFR NSCLC is considered ineffective, this may have impaired the detection of MUC1 changes in effectively treated patients. This further suggests to reassess their results in a patient group selected according to current guidelines for EGFR inhibitors, to validate whether increased shed MUC1 levels after EGFR treatment is indeed predictive of progressive disease.

Many targeted therapies are not used as single agent, but are used in combination with other drugs or radiotherapy. For example, HER2-positive breast cancers are treated with HER2-targeting antibody trastuzumab after a regimen of doxorubicin, cyclophosphamide and paclitaxel. Our studies to discover effect sensors of chemotherapeutic agents, including doxorubicin, showed a remarkably different proteomic profile when compared to those induced by EGFR-targeted therapy in breast cancer cell lines. These differences are not unexpected when the different mechanisms-of-actions of these agents are taken into consideration. Future studies aiming to discover, validate or implement the use of effect sensors could focus on the actually-used treatment regimens in the clinic, by monitoring multiple effect sensors simultaneously. This could ultimately lead to the development of multiple tests consisting of single or combinations of effect sensors to monitor treatments effects of combinational therapies.

Safeguarding genome integrity

Genomic instability was previously coined as an enabling hallmark of cancer that describes an increased acquisition of mutations and other genomic alterations in cancer cells (1). This further accelerates the selection of cancer cells with alterations in oncogenes and tumor-suppressor genes that promote tumorigenesis. By deregulation of DNA damage repair (DDR) pathways, or acquiring mutations therein, cancer cells can compromise the control on genome integrity. However, this also places a dependency on the remaining DDR pathways for tumor cell viability. This dependency has become a novel therapeutic targeting strategy, in which inactivation of compensating DDR components results in catastrophic amounts of DNA damage, leading to cell killing. For instance, genomic instability of tumors with homologous recombination (HR) defects can be targeted by PARP1 inhibitors, which leads to DNA lesions that require HR for repair (18). For the second aim of this thesis we used proteomics to study cell cycle-dependent regulation of DNA damage pathways, to find novel insights in how cancer cells can depend on supportive pathways that safeguard genomic integrity.

By analyzing the changes in protein expression of cells exiting from mitosis into G1-phase, we identified two proteins involved in DNA damage repair, CtIP and Rif1, to be decreased after mitotic exit. Of these, we could validate CtIP to be degraded by the APC/CCdh1, an E3 ligase that marks substrate proteins for proteasomal degradation through ubiquitination. DNA end-resection of DSBs by CtIP is crucial for initiation of HR repair, that can occur after DNA has been replicated in S-phase (7). CtIP degradation in G1 cells therefore is in line with a limited ability to perform HR after mitotic exit. Normally, the APC/CCdh1 is inhibited by cyclin-dependent kinases (CDKs) in S and G2 phase of the cell cycle. However, in the presence of DNA damage, activation of DNA damage checkpoint leads to inactivation of CDKs, which allows for APC/CCdh1 activation (19). Indeed, we found that APC/CCdh1 activation in G2 limits the abundance of CtIP, and failure to degrade CtIP in G2 resulted in high levels of DNA-end resection, leading to impaired resolution of DNA damage foci by HR. Recently, a role for CtIP in G1 was identified in facilitating resection-dependent NHEJ repair of irradiation-induced or

topoisomerase inhibitor-induced complex DSBs (20). It is possible that, similar as to HR repair in G2, APC/C-mediated degradation of CtIP in G1 is required to limit the extend of end-resection for this type of repair, which can be tested by modification of the phosphorylation sites in CtIP required for resection-dependent NHEJ repair. Furthermore, pharmacological inhibition of APC/C was shown to be feasible in a research setting, and prevents the interaction of APC/C with both Cdc20 and Cdh1 resulting in mitotic arrest similar to microtubule targeting cancer drugs (21,22). Interestingly, synthetic lethality was shown for these APC/C inhibitors in cancers models with DNA cohesion deficiencies, a deficiency that occurs in various tumor types (23). Whether there is additional therapeutic benefit of targeting APC/C regulation of DNA repair in G2 needs to be further explored in pre-clinical models.

Although we could not conclusively confirm regulation of Rif1 by the APC/C-Cdh1, we did discover a novel function of Rif1 during our validation studies. Specifically, in these studies we found Rif1 present in mitosis on thread-like DNA structures during the separation of sister chromatids in anaphase. These thread-like DNA structures occurred as a result of unresolved DNA replication during S-phase. If these structures are not resolved before cells reach metaphase in mitosis, they become apparent as ultra-fine bridges during anaphase (UFBs), and need to be resolved for proper chromosomal distribution in to daughter cells (24). We showed that Rif1 was required, similar to other UFB-localizing proteins PICH and BLM, to resolve UFBs in anaphase, especially after treatment with topoisomerase II inhibitors. Failure to resolve UFBs due to absence of Rif1, resulted in elevated numbers of nuclear bodies with DNA damage in G1 daughter cells, which are recognized as sites of DNA damage and markers of genome instability (25). It can be expected that the clinically-used topoisomerase II inhibitors etoposide and doxorubicin will elicit UFBs as a consequence of inhibiting DNA replication, and topoisomerase II inhibitor treated cancer cells may thus depend on UFB resolution to limit genome instability. As there seems to be a repair mechanism in place to repair UFBs, it could be interesting to test if inhibition of this repair mechanism synergizes with topoisomerase inhibition. This notion could be tested using a recently described chemical BLM inhibitor, although the effects of inhibiting BLM in mitosis will be difficult to separate from BLM function in DNA repair during interphase (26). Contrary to BLM, PICH function is restricted exclusively to mitosis and requires the ATPase domain in PICH for its localization to chromatin (27). Since no chemical inhibitors have yet been developed, genetic inactivation of PICH could be used to explore cancer mutations or drug treatments that are synthetic lethal with persistence of UFBs. Furthermore, it is still not fully understood how the UFB repair complex is actively recruited to UFBs. Elucidation of other components that are required for UFB resolution will further explain the organization and regulation of DNA repair machineries at UFBs, and might offer novel opportunities to target this type of DNA repair.

References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell. 2011;144:646-674.
2. Weinstein IB. Cancer. Addiction to oncogenes-the achilles heal of cancer. Science. 2002;297:63–64.
3. Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction. Cell. 2009;136:823–837.
4. Solimini NL, Luo J, Elledge SJ. Non-oncogene addiction and the stress phenotype of cancer cells. Cell. 2007;130:986–988.
5. Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, et al. Mutational landscape and significance across 12 major

cancer types. Nature. 2013;502:333–339.
6. Lopez JS, Banerji U. Combine and conquer: challenges for targeted therapy combinations in early phase trials. Nat Rev Clin Oncol. 2017;14:57–66.
7. Sartori AA, Lukas C, Coates J, Mistrik M, Fu S, Bartek J, et al. Human CtIP promotes DNA end resection. Nature. 2007;450:509–514.
8. Silverman J, Takai H, Buonomo SBC. Human Rif1, ortholog of a yeast telomeric protein, is regulated by ATM and 53BP1 and functions in the S-phase checkpoint. Genes Dev 2004;18:2018-2119.
9. Chan KL, North PS, Hickson ID. BLM is required for faithful chromosome segregation and its localization defines a class of ultrafine anaphase bridges. EMBO J. 2007;26:3397-3409.
10. Baumann C, Körner R, Hofmann K, Nigg EA. PICH, a centromere-associated SNF2 family ATPase, is regulated by Plk1 and required for the spindle checkpoint. Cell. 2007;128:101–114.
11. Aebersold R, Mann M. Mass-spectrometric exploration of proteome structure and function. Nature Research;. 2016;537:347–355.
12. Zhang B, Wang J, Wang X, Zhu J, Liu Q, Shi Z, et al. Proteogenomic characterization of human colon and rectal cancer. Nature. 2014;513:382-387.
13. Danielczyk A, Stahn R, Faulstich D, Löffler A, Märten A, Karsten U, et al. PankoMab: A potent new generation anti-tumour MUC1 antibody. Cancer Immunol Immunother. 2006;55:1337–1347.
14. Mertins P, Mani DR, Ruggles K V., Gillette MA, Clauser KR, Wang P, et al. Proteogenomics connects somatic mutations to signaling in breast cancer. Nature. 2016;534:55–62.
15. Kani K, Faca VM, Hughes LD, Zhang W, Fang Q, Shahbaba B, et al. Quantitative proteomic profiling identifies protein correlates to EGFR kinase inhibition. Mol Cancer Ther. 2012;11:1071–1081.
16. Duffy MJ. Serum tumor markers in breast cancer: Are they of clinical value? Clin. Chem. 2006;52:345–351.
17. Ishikawa N, Hattori N, Yokoyama A, Tanaka S, Nishino R, Yoshioka K, et al. Usefulness of monitoring the circulating Krebs von den Lungen-6 levels to predict the clinical outcome of patients with advanced nonsmall cell lung cancer treated with epidermal growth factor receptor tyrosine kinase inhibitors. Int J Cancer. 2008;122:2612–2620.
18. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med. 2009;361:123–134.
19. Bassermann F, Frescas D, Guardavaccaro D, Busino L, Peschiaroli A, Pagano M. The Cdc14B-Cdh1-Plk1 axis controls the G2 DNA-damage-response checkpoint. Cell. 2008;134:256–267.
20. Biehs R, Steinlage M, Barton O, Juhász S, Künzel J, Spies J, et al. DNA double-strand break resection occurs during non-homologous end joining in G1 but Is distinct from resection during homologous recombination. Mol Cell. 2017;65:671–684.
21. Sackton KL, Dimova N, Zeng X, Tian W, Zhang M, Sackton TB, et al. Synergistic blockade of mitotic exit by two chemical inhibitors of the APC/C. Nature. 2014;514:646–649.
22. Zeng X, Sigoillot F, Gaur S, Choi S, Pfaff KL, Oh D-C, et al. Pharmacologic inhibition of the anaphase-promoting complex induces a spindle checkpoint-dependent mitotic arrest in the absence of spindle damage. Cancer Cell. 2010;18:382–395.
23. de Lange J, Faramarz A, Oostra AB, de Menezes RX, van der Meulen IH, Rooimans MA, et al. Defective sister chromatid cohesion is synthetically lethal with impaired APC/C function. Nat Commun. 2015;6:8399.
24. Mankouri HW, Huttner D, Hickson ID. How unfinished business from S-phase affects mitosis and beyond. EMBO J. 2013;32:2661–2671.
25. Lukas C, Savic V, Bekker-Jensen S, Doil C, Neumann B, Sølvhøj Pedersen R, et al. 53BP1 nuclear bodies form around DNA lesions generated by mitotic transmission of chromosomes under replication stress. Nat Cell Biol. 2011;13:243–253.
26. Nguyen GH, Dexheimer TS, Rosenthal AS, Chu WK, Singh DK, Mosedale G, et al. A small molecule inhibitor of the BLM helicase modulates chromosome stability in human cells. Chem Biol. 2013;20:55–62.
27. Kaulich M, Cubizolles F, Nigg EA. On the regulation, function, and localization of the DNA-dependent ATPase PICH. Chromosoma. 2012;121:395–408.