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FROM ONCOGENIC REPLICATION STRESS TO DRUG RESISTANCE: F-BOX PROTEINS AS SIGNALLING HUBS IN CANCER

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From oncogenic replication stress to drug resistance: F-box proteins as signalling hubs in cancer

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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Daß ich erkenne, was die Welt
im Innersten zusammenhält

J.W. von Goethe

ABSTRACT

Cancer arises from cells that acquire genetic and epigenetic changes during the course of a, sometimes decades-long, somatic evolutionary process. These changes result in deregulation of a multitude of cellular processes leading to novel capabilities, often referred to as hallmarks of cancer, and a strong selective advantage for these cells albeit at a dramatic cost to the organism as a whole. Both, gene expression but also turn-over of gene products can become deregulated. The ubiquitin-proteasome system is responsible for the targeted degradation of proteins, and components of this system are altered during cancer development. Target specificity of this system is largely attained through E3 ubiquitin ligases that mediate the covalent attachment of ubiquitin to their substrates. The largest group of E3s are cullin-RING domain ligases (CRLs) with SKP1-cullin1-F-box protein (SCF) E3 ligases, or CRL1, representing one of the best-characterised subgroups of CRLs. These SCF ligases are multiprotein complexes containing one of, in human cells, 69 F-box proteins which function as substrate-adaptor subunits. Collectively, the family of F-box proteins has been found to be critically involved in virtually all the cancer hallmarks. However, despite their important role in cancer development, only a handful of SCFs has been molecularly and functionally well-characterised and detailed knowledge of how deregulation of specific SCF ligases and downstream substrate effectors impinges on cancer traits is lacking.

One of the main aims of the work presented in this thesis is to find cellular vulnerabilities resulting from deregulation of F-box proteins in cancer. FBXW7 is the most commonly mutated F-box protein in human cancers. Its inactivation leads to upregulation of its substrates including cyclin E, MYC or SOX9 (**paper IV**) resulting in deregulated proliferation, increased metastasis and drug resistance but also replication stress. Cancer cells undergoing replication stress become more dependent on signalling pathways detecting and repairing damaged DNA (**papers I** and **III**) and are consequently more sensitive to therapies targeting checkpoint and repair proteins such as WEE1, ATR or DNA-PK kinases (**paper II**).

In **paper I** we describe a novel function for the largely uncharacterised F-box protein FBXL12 in regulating the response to oncogene-induced replication stress. FBXL12 complements the Fanconi anaemia (FA) DNA repair pathway by targeting its central component FANCD2 for proteasomal degradation. The FA pathway not only plays a crucial role in resilience to endogenous sources of replication stress but also to drug-induced stress. FBXL12 and cyclin E are upregulated and correlated in human cancers and depletion of FBXL12 results in increased sensitivity to replication stress which posits FBXL12 as a potential cancer drug target. Ablation or pharmacological inhibition of FBXL12 prevents degradation of FANCD2 and breast cancer

cells are sensitised to the adverse effects of drug- as well as oncogenic cyclin E-induced replication stress.

In **paper II** we focus on exploring further ways of sensitising cancer cells to replication stress. We performed a screen to identify potential viability markers in response to replication stress induced by WEE1 inhibitor AZD1775 and discover novel synergistic combinations. Additionally, we determine a subset of basal-like breast cancer cells that responds to treatment initially but recovers after treatment cessation and identify PTEN as a novel predictive marker for such responses, with cells expressing low levels of PTEN being highly sensitive acutely and failing to recover. Furthermore, inactivation or genomic deletion of DNA-PK, an apical DNA damage kinase, attenuates recovery and sensitises basal-like breast cancer cells to AZD1775. Mechanistically, loss of PTEN or DNA-PK impair CHK1 activation and S-phase arrest in response to AZD1775 treatment, which finally ensues lethal replication stress and loss of survival.

In **paper III** we concentrate on FBXO28, another poorly-studied member of the F-box family, which we find to degrade ARHGEF6 and ARHGEF7 activators of the Rho-type GTPase RAC1, involved in cell motility. Surprisingly, we identify a novel function for FBXO28 and ARHGEF6/7 in promoting the repair of breaks in heterochromatin DNA. Following DNA damage, tightly chromatin-bound FBXO28 is released and promotes degradation of nucleoplasmic ARHGEF6/7 to modulate activation and inactivation cycles of nuclear RAC1 and allow for efficient resolution of H3K9me2/3-positive damaged sites.

In **paper IV** we add a key oncogenic transcription factor to the growing list of FBXW7 substrates; SOX9. FBXW7 ubiquitylates and degrades SOX9 upon phosphorylation by GSK3β. Mutation and inactivation of FBXW7 in medulloblastoma concurs with elevated SOX9 protein expression and poor patient outcome. In medulloblastoma cell line models we demonstrate increased cell motility, metastasis and increased resistance to cytostatic treatment after expression of a non-degradable SOX9 mutant. Conversely, inhibition of the PI3K/AKT/mTOR pathway promoted GSK3β-dependent SOX9 degradation and sensitised FBXW7-proficient medulloblastoma cells to cisplatin.

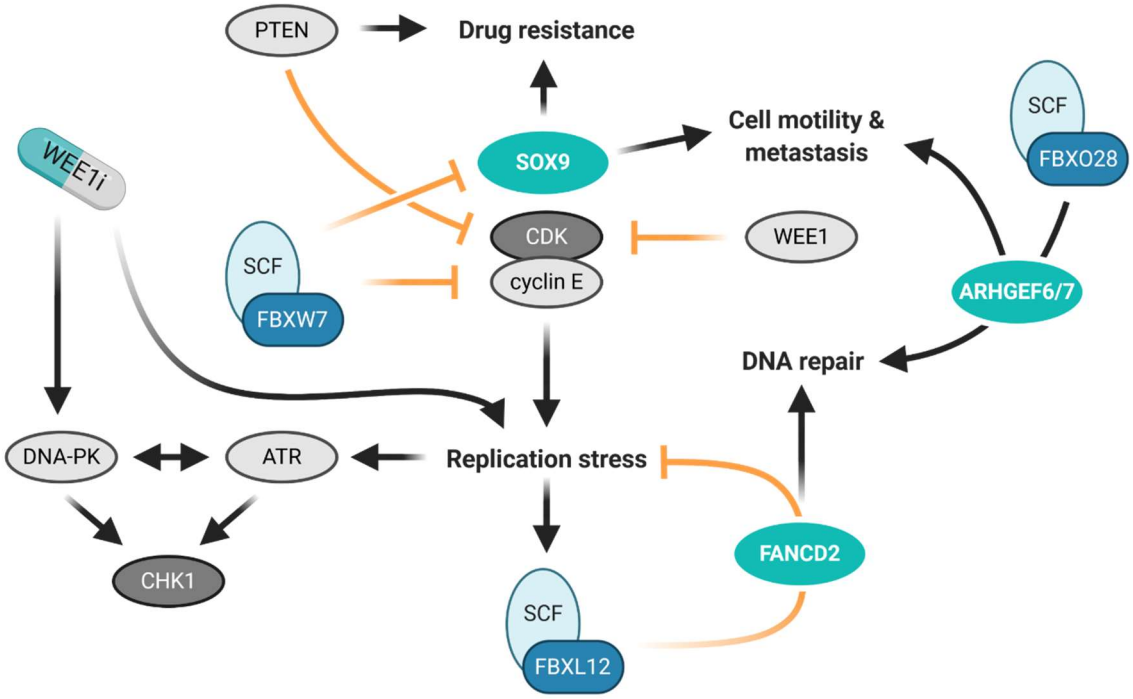

Fig. 1 Graphical abstract incorporating the main findings of the four constituent papers of this thesis. This figure and subsequent figures in this thesis have been generated using BioRender.

POPULAR SCIENCE SUMMARY

The human genome contains around 20 000 genes, which are the blueprint for the production of some 100 000 different protein species. These proteins perform the actions cells need to carry out to collectively form an entire organism. Some proteins are only needed for seconds while others have to be present for a lifetime. The so-called ubiquitin-proteasome system determines the fate of proteins. First, they are earmarked by the attachment of a small protein called ubiquitin, then run through a molecular shredder, the proteasome. Within this system, F-box proteins hold the power as they ultimately take the decision of which protein to mark for destruction. As one such F-box protein is responsible for controlling many different targets, which in turn may regulate additional proteins, the actions of F-box proteins can completely overturn the behaviour of a cell. This is why F-box proteins are frequently deregulated in cancer. The presence or absence of specific F-box proteins may, for example, decide if a cancer cell exposed to chemotherapy will repair the damage incurred from the drug and survive or fail to do so and die.

During my PhD research presented here, I focused on three F-box proteins, which go by the names FBXW7, FBXL12 and FBXO28, and their target proteins.

FBXW7 is among the infamous top 10 most frequently mutated cancer genes. It is a so-called tumour suppressor, which means losing it will promote the transition from a healthy cell to a cancer cell. Which molecular changes this entails in detail has been studied in a number of cancer types. We now focused on its role in the most common brain tumour in children, medulloblastoma. Most children survive this disease but have to live with severe long-term side effects of the treatment, such as reduced cognitive functions or secondary cancers later in life. Thus, therapy improvements are urgently needed. We show that FBXW7 destroys a stem cell protein called SOX9. If SOX9 cannot be degraded efficiently, cancer cells become less sensitive to chemotherapy and more prone to metastasise, forming new tumours elsewhere. Our study also identified drugs that enhance the destruction of SOX9 and thereby re-sensitised cancer cells with functional FBXW7 to chemotherapy, which may help to reduce chemotherapy drug concentrations and thereby the toxic side effects.

In contrast to FBXW7, much less is known about the F-box proteins FBXL12 and FBXO28. They also differ from FBXW7 in that they act like oncogenes, which means that high levels of FBXL12 or FBXO28 favour the development of cancer.

Oncogenes typically promote the rapid proliferation of cancer cells. However, at the same time, the fast-paced nature of most cancer cells means they are stressed and more likely to make

mistakes when copying their DNA. FBXL12, however, helps them cope with this particular stress known as replication stress. Healthy cells do not experience appreciable levels of replication stress which can thus be considered an Achilles' heel of cancer. Based on our results it may be possible to exploit this weakness by pharmacologically targeting FBXL12 while avoiding effects on non-cancer cells tantamount to side effects.

In addition, enhancing the level of replication stress through inhibition of control proteins, also known as checkpoint proteins, using compounds like AZD1775 could increase the dependence on FBXL12 further. Unfortunately, though, some cancer cells survive such treatment and grow back. Interestingly, we found that a highly frequent and, from the viewpoint of a cancer cell, highly beneficial mutation in a tumour suppressor gene known as PTEN renders aggressive cancer cells more sensitive to the replication stress-inducing drug AZD1775, potentially identifying patients whose cancers could be particularly sensitive to this agent.

Often F-box proteins not only regulate one single process related to cancer but several. In the case of FBXO28, we found that its deregulation promotes both cell migration as well as DNA repair. Intriguingly, chemotherapy treatment has been found to promote metastasis that is driven by migratory cells, hinting at FBXO28 being a molecular link between these two seemingly disparate processes.

Collectively, the results of this thesis emphasise the central role of F-box proteins and their targets in cancer-related pathways and pinpoint novel ways of intersecting them.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Det mänskliga genomet innehåller ungefär 20 000 gener som utgör ritningen för produktion av ca. 100 000 olika proteiner vilka tillsammans bidrar till uppbyggnaden av människokroppen. Vissa proteiner behövs endast under korta perioder medan andra måste vara närvarande under hela vår livstid. Mängden protein styrs av så kallade post-translationella modifieringar såsom fosforylering och ubiquitylering. Nedbrytning av proteiner sker när dessa öronmärkts med ett annat protein som kallas ubiquitin i cellens avfallskvarnar, proteasomerna. Det så kallade ubiquitin-proteasom-systemet styr således vilka proteiner som inte längre behövs. Inom detta system har F-box-proteiner en speciellt viktig funktion genom att i första hand, avgöra vilket protein som skall märkas in med ubiquitin och därmed brytas ner i proteasomerna. Eftersom F-box gener kontrollerar nedbrytningen av många olika proteiner, som i sin tur reglerar ytterligare proteiner, kan förändrad funktion av F-box proteiner få mycket stora effekter på cellernas beteende vilket är en orsak till att F-box proteiner ofta är muterade vid sjukdom såsom cancer. Närvaron eller frånvaron av ett specifikt F-box protein kan till exempel påverka hur en cancercell som utsätts för cytostatika kommer att reparera skador i DNA och överleva, eller inte och sålunda elimineras efter behandling.

Under mitt doktorandarbete som presenteras i denna avhandling har jag fokuserat på tre F-box-proteiner som kallas för FBXW7, FBXL12 och FBXO28.

FBXW7 är en av de gener som oftast är muterad i cancerceller. Det är en så kallad tumör suppressor vilket innebär att förlust av FBXW7 kan leda till att en normal cell omvandlas till en cancercell. Vilka molekylära förändringar detta innebär i detalj har studerats vid flera typer av cancer. I mitt arbete har jag fokuserat på dess roll i den vanligaste hjärntumören hos barn, medulloblastom. De flesta barn överlever denna sjukdom men måste leva med allvarliga och långvariga biverkningar efter behandlingen såsom minskade kognitiva funktioner eller sekundär cancer senare i livet. Således är terapiförbättringar mycket angelägna. Vi fann att FBXW7 förstör ett stamcellsprotein som heter SOX9. Om SOX9 inte kan förstöras av FBXW7 blir cancercellerna mindre känsliga för cytostatika vilket kan leda till spridning av cancerceller och utveckling av nya tumörer (metastaser). Våra studier visar även att specifika läkemedel som stimulerar nedbrytning av SOX9 i cancerceller med funktionellt FBXW7 protein kan göra cancerceller mer känsliga för kemoterapi. Detta arbete bidrar därmed till att öka vår kunskap kring hur cancerceller påverkas av cytostatikabehandling vilket i sin tur kan leda till minskade behandlingsbiverkningar hos patienter med medulloblastom.

Jämfört med FBXW7 är vår kunskap kring FBXL12 och FBXO28 mycket begränsad. Dessa F-box proteiner skiljer sig avsevärt från FBXW7 genom att de stimulerar cancercellernas tillväxt och överlevnad, i likhet med andra så kallade onkogener, vilket innebär att höga nivåer av FBXL12 och FBXO28 kan gynna utvecklingen av cancer.

Onkogener främjar snabb celldelning hos cancerceller. Detta innebär samtidigt att cancercellerna blir mer benägna att göra misstag vid kopiering av DNA (replikationsprocessen) och celldelning. Mitt arbete har visat att FBXL12 hjälper cancercellerna att hantera den ökade stressen vid replikation, så kallad replikationsstress, och övervinna de skador som uppstår i DNA hos snabbt växande cancerceller. Friska celler upplever inte denna typ av replikationsstress vilket kan betraktas som en akilleshäl hos cancercellen. Vårt arbete tyder på att det kan vara möjligt att utnyttja denna svaghet hos cancercellerna genom att inaktivera FBXL12 och/eller stimulera celldelning ytterligare med hjälp av andra läkemedel som driver celldelning, tex AZD1775, och därmed öka beroendet av FBXL12 än mer.

Tyvärr överlever och återväxer ofta cancerceller efter behandling med cancerläkemedel. Med hjälp av detaljerade molekylära analyser fann vi att en mycket frekvent och ur en cancercells synvinkel fördelaktig mutation i tumörsuppressorgenen PTEN visade sig påverka cancercellernas känslighet och förmåga att återväxa efter AZD1775 behandling. Detta arbete visar att cancerceller som inaktiverat PTEN är speciellt känsliga för AZD1775 vilket kan bidra till utveckling av mer individualiserad cancerbehandling.

Ofta reglerar F-box-proteiner flera olika processer som påverkar tumörutveckling. När det gäller FBXO28 har vi visat att detta protein styr både cellernas förmåga att förflytta sig samt reparera skador i DNA. Tidigare studier tyder på att cytostatikabehandling även kan främja cancercellers förmåga att förflytta sig och metastasera. En fascinerande möjlighet är följaktligen att FBXO28 skulle kunna utgöra en molekylär länk mellan dessa till synes olika processer, DNA reparation och metastasering.

Sammantaget understryker resultaten i denna avhandling F-box-proteiners centrala roll vid utveckling av cancer samt olika möjligheter att påverka cancercellers känslighet för cancerläkemedel.

POPULÄRWISSENSCHAFTLICHE ZUSAMMENFASSUNG

Das menschliche Genom umfasst ca. 20.000 Gene, Baupläne für die Produktion hunderttausender Proteine in unseren Zellen. Diese Proteine haben wiederum spezifische Aufgaben und sorgen gemeinsam dafür, dass Zellen individuell und als Teileinheiten eines Organismus funktionieren. Manche Proteine werden nur für wenige Sekunden benötigt, während andere ein Leben lang eine bestimmte Funktion erfüllen. Das sogenannte Ubiquitin-Proteasom-System kontrolliert dabei den Abbau von nicht mehr benötigten Proteinen. Zunächst werden sie durch einen Anhang in Form des kleinen Proteins Ubiquitin markiert, um daraufhin in einem molekularen Reißwolf, dem Proteasom, zerlegt zu werden. In diesem System kommt den F-Box-Proteinen eine besondere Rolle zu, da sie die Entscheidung treffen, welches Protein wann und wo markiert und damit abgebaut wird. Da ein solches F-Box-Protein viele weitere kontrolliert, die wiederum andere regulieren, können F-Box-Proteine das Verhalten einer Zelle ins Gegenteil verkehren. F-Box-Proteine wirken daher als Schaltzentralen und sind in Krebszellen häufig mutiert.

Diese Doktorarbeit befasst sich insbesondere mit drei F-Box-Proteinen: FBXW7, FBXL12 und FBXO28 sowie den von ihnen regulierten Proteinen.

FBXW7 ist unter jenen 10 Genen, die am häufigsten Mutationen in Tumoren aufweisen. Es ist ein sogenannter Tumorsuppressor, was bedeutet, dass sein Verlust die Tumorentwicklung fördert. Die molekularen Gründe dafür wurden in zahlreichen Krebsarten untersucht, aber diese Arbeit konzentriert sich diesbezüglich auf den bei Kindern häufigsten Gehirntumor, das Medulloblastom. Die meisten Patienten überleben diese Erkrankung, müssen allerdings mit lebenslangen Konsequenzen der Behandlung leben, unter anderem beeinträchtigte kognitive Funktionen oder sekundäre Tumore. Dementsprechend werden Behandlungsverbesserungen dringend gesucht. Wir zeigen hier, dass FBXW7 ein Stammzellprotein namens SOX9 abbaut. Funktioniert dieser Abbau nicht, werden Krebszellen weniger empfindlich gegenüber einer Chemotherapiebehandlung und metastasieren häufiger, formen also zusätzliche Tumoren an anderen Stellen im Körper. Allerdings konnten wir pharmakologische Inhibitoren identifizieren, die den FBXW7-abhängigen Abbau von SOX9 fördern und damit Medulloblastomzellen wieder für die Chemotherapie sensibilisieren. Daher könnte die Konzentration von Chemotherapiemedikamenten und damit Nebeneffekte basierend auf diesen Erkenntnissen reduziert werden.

Im Gegensatz zu FBXW7 waren die Funktionen von FBXL12 und FBXO28 zu Beginn dieser Arbeit kaum erforscht. Ferner haben sie auch den gegenteiligen Effekt auf Krebszellen: Hohe

Konzentrationen unterstützen die Entwicklung von Krebs, sie fungieren daher wie sogenannte Onkogene.

Onkogene fördern typischerweise die schnelle Proliferation von Krebszellen. Gleichzeitig bedeutet diese Geschwindigkeit jedoch einen Stressfaktor, der Fehler beim Replizieren der DNS bewirkt. FBXL12-abhängige Regulation von DNS-Reparaturfaktoren hilft mit diesem Replikationsstress umzugehen. Gesunde Zellen stehen hingegen kaum unter Replikationsstress. Basierend auf unseren Ergebnissen könnte es daher gelingen, durch Inhibition von FBXL12 diese spezifische Schwachstelle von Krebszellen auszunützen. Zugleich wären Auswirkungen auf gesunde Zellen, gleichbedeutend mit Nebenwirkungen, gering.

Ein neuartiges Medikament, AZD1775, das sich derzeit in klinischen Studien befindet, eliminiert einen Proliferationskontrollpunkt, sodass im Falle von Krebszellen eine kritische Schwelle der Proliferationsgeschwindigkeit überschritten wird, was mit irreparablen Fehlern in der DNS-Replikation und meist dem Zelltod einhergeht. Problematischerweise überleben einige wenige Krebszellen diese Therapie dennoch und beginnen danach wieder sich zu teilen. Der Verlust von FBXL12 erhöht die Empfindlichkeit gegen AZD1775. Weil FBXL12 aber von Krebszellen benötigt wird, um ihre grundsätzlich erhöhten Stressniveaus zu überleben, sind solche Mutationen sehr selten. Überraschenderweise konnten wir allerdings feststellen, dass eine sehr häufige und aus Sicht einer Krebszelle äußerst vorteilhafte Mutation in dem Tumorsuppressorgen PTEN dazu führt, dass sich solch mutierte Zellen nicht von einer AZD1775-Behandlung erholen können. Diese Erkenntnis könnte helfen, diejenigen Patienten im Vorhinein zu identifizieren, welche den größten Nutzen von einer solchen Behandlung hätten.

Häufig regulieren F-Box-Proteine nicht nur einen einzelnen, sondern mehrere ansonsten scheinbar unabhängige Prozesse, welche an der Entwicklung von Tumoren beteiligt sind. Im Falle von FBXO28 fanden wir heraus, dass dessen Deregulierung sowohl die Zellmigration als auch die DNS-Reparatur fördert. Chemotherapie, welche hauptsächlich die DNS von Krebszellen beschädigen und diese damit abtöten soll, kann in bestimmten Fällen auch die Metastasenbildung, bedingt durch migrierende Zellen, fördern. Eine faszinierende Möglichkeit wäre daher, dass FBXO28 eine molekulare Verbindung zwischen diesen beiden Prozessen und ein potenzielles Ziel für pharmakologische Intervention darstellt.

Insgesamt unterstreichen die Ergebnisse dieser Arbeit die zentrale Rolle von F-Box-Proteinen in der Krebsentstehung und zeigen neue Wege auf, diese Funktionen zu unterbinden.

LIST OF SCIENTIFIC PAPERS

THESIS PUBLICATIONS

I. **Brunner, A.**[§], Johansson, H., Kourtesakis, A., Viiliäinen, J., Widschwendtner, M., Wohlschlegel, J., Lehtiö, J,, Spruck, C., Orre, L.M., Rantala, J. K. and Sangfelt, O.[§] (manuscript) **Degradation of FANCD2 by SCF-FBXL12 alleviates cyclin E-driven replication stress and maintains genomic integrity**.

II. **Brunner, A.***, Suryo Rahmanto, A.*, Johansson, H.[±], Franco, M.[±], Viiliäinen, J., Gazi, M., Frings, O., Fredlund, E., Lehtiö, J., Rantala J. K., Larsson, L.-G. and Sangfelt, O.[§] (2020) **PTEN and DNA-PK as determinants of sensitivity and recovery in response to WEE1 inhibitor AZD1775 in human breast cancer**. *eLife* 2020;9:e57894.

III. Čermák, L.*[§], **Brunner, A.***, Baloghová, N., Ueberheide, B., Ng, H.-F., Wohlschlegel, J., Manser, E., Sangfelt, O.[±§] and Pagano, M.[±§] (manuscript) **FBXO28 controls nuclear RAC1 activity and safeguards efficient heterochromatin DNA repair by targeting ARHGEF6/7 for degradation.**

IV. Suryo Rahmanto, A.*, Savov, V.*, **Brunner, A.[±]**, Sara Bolin, S.[±], Weishaupt, H.[±], Malyukova, A., Rosén, G., Čančer, M., Hutter, S., Sundström, A., Kawauchi, D., Jones, D. T. W., Spruck, C., Taylor, M. D., Cho, Y.-J., Pfister, S.M., Kool, M., Korshunov, A., Swartling, F. J.[§] and Sangfelt, O.[§] (2016) **FBW7 suppression leads to SOX9 stabilization and increased malignancy in medulloblastoma**. *EMBO J.* 35(20): 2192–2212.

OTHER PUBLICATIONS BY THE AUTHOR

V. Engel, K., Rudelius, M., Slawska, J., Jacobs, L., Ahangarian Abhari, B., Altmann, B., Kurutz, J., Rathakrishnan, A., Fernández-Sáiz, V., **Brunner, A.**, Targosz, B.-S., Loewecke, F., Gloeckner, C. H. J., Ueffing, M., Fulda, S., Pfreundschuh, M., Trümper, L., Klapper, W., Keller, U., Jost, P. J., Rosenwald, A., Peschel, C. and Bassermann, F.[§] (2016) **USP9X stabilizes XIAP to regulate mitotic cell death and chemoresistance in aggressive B-cell lymphoma.** *EMBO Molecular Medicine* 8(8), 851–862.

VI. Luichtl, M., Fiesselmann, B. S., Matthes, M., Yang, X., Peis, O., **Brunner, A.**, and Torres-Ruiz, R. A.[§] (2013) **Mutations in the Arabidopsis RPK1 gene uncouple cotyledon anlagen and primordia by modulating epidermal cell shape and polarity.** *Biology Open* 2(11), 1093–1102.

* – equal contribution
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CONTENTS

LIST OF ABBREVIATIONS

Genes and proteins

AKT	Protein kinase B
ALDH3	Aldehyde dehydrogenase 3
APC/C	Anaphase-promoting complex/cyclososme
ARHGEF/PIX	Rho guanine nucleotide exchange factor
ARP2/3	Actin related protein 2/3
ATM	Ataxia telangiectasia-mutated
ATR	ATM- and Rad3-related
ATRIP	ATR-interacting protein
BLM	Bloom syndrome RecQ-like helicase
BRCA	Breast cancer type 1 susceptibility protein
BUB3	Budding uninhibited by benzimidazoles 3
BUBR1	Mitotic Checkpoint Serine/Threonine Kinase B
CCNE	Cyclin E
CDC	Cell division cycle
CDH1	Fizzy/cell division cycle 20 related 1
CDK	Cyclin-dependent kinase
CDT1	Chromatin licensing and DNA replication factor 1
CHK1/2	Checkpoint kinase 1/2
CIP/KIP	CDK interacting protein/Kinase inhibitory protein
CKI	CDK inhibitor
CKS1	Cyclin-dependent kinases regulatory subunit 1
CRL	Cullin-RING domain ligase
CUL1	Cullin 1
DDK	DBF4-dependent protein kinase
DUB	Deubiquitylase
DUSP2	Dual specificity phosphatase 2
EGF	Epidermal growth factor
EME1	Essential meiotic structure-specific endonuclease 1
EMI1	Early mitotic inhibitor 1/FBXO5
ER	Estrogen receptor
ERCC1	Excision repair complementing defective repair in Chinese hamster 1
ERK	Extracellular signal-regulated kinase
ESA	Epithelial specific antigen
EXO1	Exonuclease 1
FAAP	Fanconi anaemia associated protein
FAK	Focal adhesion kinase
FAN1	Fanconi-associated nuclease 1
FANCD2	Fanconi anaemia complementation group D2
FBH1	F-box DNA helicase 1/FBXO18

FBXL12	F-box and leucine rich repeat protein 12
FBXO28	F-box only protein 28
FBXW7	F-box and WD repeat domain containing protein 7
FGF	Fibroblast growth factor
GAP	GTPase-activating protein
GDI	Guanine-nucleotide dissociation inhibitor
GEF	Guanine exchange factor
GFP	Green fluorescent protein
GIT	G protein-coupled receptor kinase interactor
GLI	Glioma-associated oncogene
GSK3β	Glycogen synthase kinase 3β
HDAC	Histone deacetylase
HDM2	Human double minute 2 homolog
HECT	Homologous to the E6-AP carboxyl terminus
HER2	Human epidermal growth factor receptor 2
HP1	Heterochromatin protein 1
ID2	FANCI-FANCD2 heterodimer
INK4	Inhibitor of CDK4
KLF4	Krüppel-like factor
Ku80/XRCC5	X-ray repair complementing defective repair in chinese hamster 5
MAD2	Mitotic arrest deficient 2
MCL-1	Myeloid cell leukemia 1
MCM	Minichromosome maintenance
MHF/CENP	Centromere protein
MRE11	Meiotic recombination 11 homolog A
MRN	MRE11-RAD50-NBS1 complex
MRP1	Multi-drug resistance protein 1
mTOR	Mammalian target of Rapamycin
MYC	Avian myelocytomatosis viral oncogene homolog
MYT	Myelin transcription factor 1
NBS1	Nijmegen breakage syndrome 1
NEK11	NIMA-related kinase 11
NIMA	Never in mitosis gene A
ORC1	Origin recognition complex 1
PAK	p21 (RAC1)-activated kinase
PALB2	Partner and localiser of BRCA2
PARP	Poly(ADP-ribose) polymerase
PCH	Patched
PCNA	Proliferating cell nuclear antigen
PI3K	Phosphatidylinositol-4,5-bisphosphate 3-kinase
PIAS	Protein inhibitor of activated STAT
PICH	PLK1-interacting checkpoint helicase

PIN1	Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1
PLK1	Polo-like kinase 1
PTEN	Phosphatase and tensin homolog
RAC1	Rac family small GTPase 1
RAG2	Recombination activating gene 2
RB	Retinoblastoma-associated protein
RING	Really interesting new gene
RNF	RING finger protein
RRM2	Ribonucleotide reductase M2
RPA	Replication protein A
SCF	SKP1-cullin 1-F-box protein
SETDB1	SET domain bifurcated 1
SHH	Sonic hedgehog
SKP2	S-phase kinase associated protein 2
SLX4	Structure-specific endonuclease subunit
SMO	Smoothened
SOX9	SRY-box transcription factor 9
SUFU	Suppressor of fused
SUMO	Small ubiquitin-like modifier
SUV39H1	Suppressor of variegation 3–9 homolog 1
TCR	T-cell receptor
TGFβ1	Tranforming growth factor β1
TLK	Tousled-like kinase
TOPO2α	Topoisomerase 2α
UAF1	USP1-associated factor 1
UBCH10	Ubiquitin-conjugating enzyme E2C
UNC45	Unc-45 myosin chaperone
USP1	Ubiquitin-specific protease 1
WASP	Wiskott-Aldrich syndrome protein
WAVE	WASP family verprolin homologous protein
VCP/p97	Valosin containing protein
WNT	Wingless-related integration site
WRN	Werner syndrome RecQ-like helicase
XPF	Xeroderma pigmentosum group F-complementing protein
ZEB	Zinc finger E-box binding homeobox
p21/CDKN1A	Cyclin-dependent kinase inhibitor 1A
p27/CDKN1B	Cyclin-dependent kinase inhibitor 1B
p57/CDKN1C	Cyclin-dependent kinase inhibitor 1C
βTRCP	β-transducin repeat-containing protein

Other

ALL	Acute lymphoblastic leukaemia

AML	Acute myeloid leukaemia
bCHAM	Basic chromatin association motif
BER	Base-excision repair
BLBC	Basal-like breast cancer
CETSA	Cellular thermal shift assay
CFS	Common fragile site
CPD	CDC4 phospho-degron
CRISPR	Clustered regularly interspaced short palindromic repeats
CSC	Cancer stem or stem-like cell
DAPI	4′,6-diamidino-2-phenylindole
DDR	DNA damage response
DSB	Double strand break
EMT	Epithelial-mesenchymal transition
FA	Fanconi anaemia
FL	Full length
HA	Hemagglutinin
HR	Homologous recombination
HU	Hydroxyurea
ICL	Inter-strand crosslink
IDH	Isocitrate dehydrogenase
IR	X-ray irradiation
KO	Knockout
LRR	Leucine-rich repeat
MB	Medulloblastoma
MIC	MB-inducing cell
MMC	Mitomycin C
MMR	Mismatch repair
NCS	Neocarzinostatin
NER	Nucleotide excision repair
NHEJ	Non-homologous end joining
NRF1	Nuclear respiratory factor 1
NSCLC	Non-small-cell lung cancer
PIP3	Phosphatidylinositol-3,4,5-trisphosphate
PTM	Post-translational modification
SNP	Single-nucleotide polymorphism
TLS	Translesion synthesis
TNBC	Triple-negative breast cancer
UFB	Ultra-fine anaphase bridge
UPS	Ubiquitin-proteasome system
UV	Ultraviolet
WT	Wildtype

1 INTRODUCTION

1.1 THE UBIQUITIN-PROTEASOME SYSTEM

The human genome contains approximately 20 000 protein-coding genes which once transcribed can be differentially spliced and translated into a manifold higher number of protein isoforms. Additionally, post-translational modifications (PTMs) lead to further diversification adding another layer of proteome complexity. With the exception of some chromatin modifications, PTMs are typically of a more transient nature allowing for rapid adjustment to changes in the cell's environment or in response to physiological processes.

Ubiquitylation, the covalent attachment of the small 8.5kDa protein ubiquitin to the ε-amino group of lysines is one such PTM. Since its discovery, ubiquitylation has mostly been regarded as a regulatory mechanism in the context of proteasomal degradation, but in recent years, non-proteolytic functions have been demonstrated, highlighting its diverse effects on various biological processes. The ubiquitin-proteasome system (UPS) is a highly conserved ATP-driven process executed by a cascade of three key enzymes, E1, E2 and E3, that together catalyse ubiquitylation of specific target proteins. The E1 enzyme or ubiquitin-activating enzyme catalyses the first step of this multistep cascade. By hydrolysing ATP and adenylating the C-terminal glycine of ubiquitin, the E1 enzyme activates ubiquitin and allows it to bind to a cysteine within its active site. The activated ubiquitin is then passed on to an E2-conjugating enzyme which in a concerted reaction together with an E3 ubiquitin ligase catalyses the covalent attachment of ubiquitin through formation of an isopeptide bond between a lysine of the substrate protein and the glycine residue of ubiquitin. The respective E3 ligase involved largely conveys the high specificity of this system[1]. Ubiquitin is typically attached to a lysine via its carboxy terminus resulting in an initial monoubiquitylation, or if multiple ubiquitylation reactions occur at distinct sites, multi-monoubiquitylation. However, additional ubiquitin molecules can also be attached to the first one, yielding a polyubiquitin chain on substrates. Ubiquitin itself contains 7 lysines (K6, K11, K27, K29, K33, K48 and K63) which can be used to extend the polyubiquitin chain, resulting in structural diversity with multiple potential ubiquitin chain combinations (the "ubiquitin code"). The significance of most of these variations is not yet fully understood, but the attachment of for example K48-linked or mixed K48/K11 polyubiquitin chain has been shown to target proteins for degradation in the 26S proteasome, while K63 linkages typically are involved in autophagic protein quality control[2]. Proteins ubiquitylated and targeted for proteasomal degradation finally interact with the 26S proteasome which consists of a central barrel-shaped unit, the 20S core particle, and a cap, the 19S regulatory particle, on one or both ends of the barrel[3]. The latter is responsible for

selectively interacting with substrates, deubiquitylating and transferring them to the core[3]. The 20S unit comprises four ring-shaped heptamers stacked on top of each other with the central two rings containing subunits with proteolytic activity[3]. Much like phosphorylation which can be reversed by phosphatases, ubiquitylation is not an irreversible process and more than 100 proteases known as deubiquitylating enzymes (DUBs) can cleave off ubiquitin from substrates and replenish the pool of free ubiquitin molecules, thus introducing another layer of regulation of the UPS[4].

In general, the UPS is highly active in cancer cells, particularly in hematologic malignancies. Bortezomib (Velcade), the first approved drug targeting the 26S proteasome by reversibly binding one of the catalytic core subunits, is exploiting this circumstance and has proven efficient for the treatment of multiple myelomas and mantle cell lymphomas[5]. However, patients frequently relapse and bortezomib is less effective in solid tumours[5]. This has been suggested to be due to residual activity of the proteasome in the presence of bortezomib being sufficient in these tumours[6]. Although second-generation proteasome inhibitors are under development, targeting other components of the UPS such as E3 ligases or DUBs could prove more successful due to the greater specificity of such inhibitors as compared to general UPS shut-down[5]. However, potential redundancies may play a greater role for the occurrence of treatment resistance when targeting specific upstream UPS components which should be taken into account.

1.2 THE SCF UBIQUITIN LIGASE

In human cells more than 600 putative E3 ubiquitin ligases are carrying out the final step of the ubiquitylation cascade, while the number of E2 enzymes is much lower and there are even fewer, only two known, E1 enzymes[7, 8]. E3s can be classified into three families, HECT (homologous to E6-AP carboxyl terminus) domain ligases, really interesting new gene (RING) finger domain containing ligases and RING-in-between-RING (RBR) ligases with the RING-type ligases containing the largest group, Cullin-RING E3s (CRL). CRL ligases are multi-protein complexes consisting of a Cullin backbone subunit, a RING domain-containing protein responsible for binding the E2 enzyme, and an adaptor protein linking the core ligase to a substrate receptor subunit responsible for the specificity of the E3 ligase complex. Of eight such complexes, the CRL1 also known as the S-phase kinase-associated protein 1 (SKP1)-Cullin1-F-box protein (SCF) complex, is the best-characterised CRL E3 ligase[9]. The F-box protein, named after the first discovered family member cyclin F, is the variable component of the SCF ligase with an F-box domain binding SKP1 that links to the remainder of the complex.

There are at least 69 F-box genes in human cells, some with a well-defined set of substrates and functions, such as FBXW7 (also known as hCDC4), SKP2 or β-transducin repeat-containing protein 1 and 2 (βTRCP1/2), many, however, still without known functions or established substrates[1]. F-box proteins are themselves sub-divided into three classes according to their domain structure, one class containing all those with WD40 domains (FBXW), the second class containing those featuring leucine-rich repeat (LRR) domains (FBXL) and the last one containing all other F-box proteins with other domains or without any identified additional domains (FBXO) (**Fig. 2**)[10].

Due to their critical regulatory function of developmental transcription factors and master repair factors, SCF ligases can be viewed as molecular ¨hubs¨ of various biological processes and thus impact multiple hallmarks of cancer when deregulated[11–15].

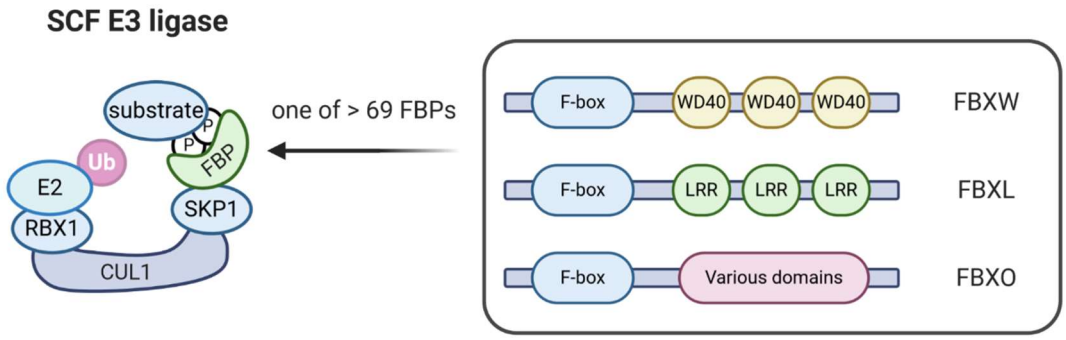

Fig. 2 Schematic representation of the components of the SCF E3 ligase complex interacting with a ubiquitin (Ub)-bound E2 enzyme and a phosphorylated substrate (left), as well as the three classes of F-box proteins (FBPs) containing either WD40, LRR or various other domains (right).

1.2.1 FBXW7 – master suppressor of oncoproteins

One of the best-characterised F-box proteins, FBXW7, is not co-incidentally encoded by one of the top 10 most frequently mutated genes across human cancers[16, 17]. An alternative name for this highly evolutionarily conserved F-box gene is human cell division control protein 4 (hCDC4), referring to its paralogue CDC4 in the yeast *S. cerevisiae*[11]. Hitherto identified substrates of FBXW7 include an array of transcription factors and potent oncoproteins such as cyclin E (CCNE), MYC, myeloid cell leukaemia 1 (MCL-1), NOTCH or SRY-box transcription factor 9 (SOX9) (**paper IV**), thus establishing FBXW7 as a general tumour-suppressor[11, 13, 26, 27, 18–25]. Typically, F-box proteins recognise their substrates after phosphorylation at specific recognition motifs known as phosphodegrons. In the case of FBXW7 the consensus recognition sequence is S/T-P-P-X-S/T/E/D, with X being any amino acid[28, 29]. This specific degron motif, denominated the CDC4 phospho-degron (CPD), is

typically phosphorylated by GSK3 at the first serine/threonine residue, after being primed by a phosphorylation at the "+4" position, and subsequently bound by FBXW7[30]. Many FBXW7 substrates contain more than one CPD, while not all match the consensus precisely, some being high- and others low-affinity degrons. The presence of degrons with different affinities may represent a mechanism to balance the threshold of kinase activities required for degradation of different substrates[29, 31]. Additionally, in order to efficiently degrade low-affinity substrates FBXW7 has been proposed to form homo-dimers and bind two low-affinity degrons at the same time[32]. Binding to the substrate is mediated by the eight WD40 domains in FBXW7 that form a β-propeller structure with arginines 465, 479 and 505 playing a central role in formation of a binding pocket for the negatively charged phosphodegron[29, 33]. Intriguingly, these arginines have been found to be mutation hot spots in many malignancies including T-ALL, ovarian or colorectal carcinoma highlighting substrate stabilisation as an important outcome of FBXW7 mutation or down-regulation in different cancers[17, 33, 34]. The FBXW7 gene encodes three isoforms α, β and γ with distinct sub-cellular localisations[33, 35]. FBXW7 isoforms only differ in the choice of the first exon and therefore all isoforms are affected by the arginine mutations mentioned above. Interestingly, the nucleoplasmic FBXW7α isoform appears to target most of the hitherto identified substrates, although both FBXW7α and FBXW7γ are for example required for efficient degradation of cyclin E[33, 35–37]. The FBXW7β isoform resides in the cytosol while FBXW7γ is enriched in the nucleoli[33].

In line with its extensive repertoire of substrates and involvement in a multitude of pathways the FBXW7 gene itself is regulated transcriptionally, translationally and post-translationally by a number of factors. For instance, p53 signalling results in increased expression of FBXW7 triggering degradation of cyclin E and MYC[38]. Recently, with the discovery of p53 as a novel target of FBXW7 degraded in response to DNA damage, another aspect was added to this regulatory network[39–41]. At the translational level miR-27a has been identified as a regulator of FBXW7 throughout the cell cycle up until the G1/S transition when repression is relieved to allow for timely degradation of cyclin E[42]. Additionally, like many F-box proteins, FBXW7 regulates its own stability through autoubiquitylation[43]. After phosphorylation of FBXW7 on threonine 205 by extracellular-regulated kinase (ERK), peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (PIN1) disrupts FBXW7 dimerization and enhances autoubiquitylation[43, 44]. Interestingly, PIN1 and FBW7α have also been reported to isomerize a noncanonical proline-proline bond in the CPD of cyclin E promoting efficient ubiquitylation of cyclin E by the SCF-FBW7γ complex[37]. More work is needed to understand how PTMs of FBW7 influence substrate degradation.

Ubiquitylation of its proteolytic substrates does not necessarily lead to proteasomal degradation. For instance, the DUB USP28 removes ubiquitin chains on specific FBXW7 substrates MYC, c-JUN, NOTCH1 as well as FBXW7 itself[45, 46], and K63-linked ubiquitylation of X-ray repair cross-complementing protein 4 (XRCC4) by FBXW7, rather than resulting in degradation, facilitates interaction with KU70/80 and promotes DNA damage repair[47].

1.2.2 FBXL12 – novel regulator of the replication stress response

Compared to well-characterised F-box proteins like FBXW7, βTRCP or SKP2 relatively little is known about other F-box proteins including FBXL12.

In human tissues while being ubiquitously expressed FBXL12 mRNA levels are highest in thymus, bone marrow and immune cells[48]. Accordingly, Zhao et al. showed that FBXL12 is crucial for proliferation of CD4-/CD8- double-negative (DN) thymocytes after T-cell receptor (TCR) β rearrangement and selection[49]. FBXL12 was shown to be up-regulated in response to pre-TCR activation and in collaboration with SKP2 ubiquitylate and promote the degradation of CDK inhibitor p27[49]. Conversely, in mouse lung epithelial cells an anti-proliferative function of FBXL12 and an indirect effect on p27 activity has been shown, as FBXL12 promotes degradation of calcium/calmodulin-dependent kinase 1 (CaMKI)[50]. CaMKI in turn regulates cyclin D1-CDK4 assembly but also phosphorylates p27 to allow translocation to the cytoplasm and cell cycle progression[50]. Interestingly, within the F-box protein family, FBXL12 is most closely related to SKP2 with 64% amino acid similarity (45% identity, as compared to 33% to the next-closely related leucine rich repeat F-box protein FBXL6) in their F-box domains and high similarity in the overall domain composition[10]. Even though, apart from a few cell-type specific exceptions, no SCF^{FBXL12} substrates have been firmly established, there is remarkable overlap between their (putative) targets besides p27, as both also mediate ubiquitylation of the CDK inhibitors p21 and p57 under certain conditions (**Fig. 3**) [51–55]. However, SKP2 appears to be the primary ligase for these shared substrates in most adult tissues, while regulation by FBXL12 has so far mainly been reported to be important during developmental stages or in specific cell lineages[49, 51–54]. Kim et al. showed that FBXL12 levels in osteoblasts are elevated in response to transforming growth factor (TGFβ1) signalling where it promotes proteasomal p57 degradation to maintain the osteoblast identity, while depletion of FBXL12 results in differentiation[52]. Despite

differential regulation of FBXL12 and SKP2, both require phosphorylation of Thr-310 in p57 by CDK2-cyclin E for efficient binding and ubiquitylation[51, 52].

Furthermore, homozygous FBXL12 knock-out (KO) mice have been generated which revealed embryonal growth retardation and intercrossing of $FBXL12^{+/-}$ mice resulted in a reduced ratio of homozygous KO mice as expected by Mendelian inheritance suggesting increased embryonal lethality[56]. While $FBXL12^{-/-}$ mice also had a lower rate of survival within 48h of birth and lower weights later on, adult mice neither exhibited any further abnormalities nor premature death[56]. The molecular basis of these phenotypes remains largely unclear.

In the same study, Nishiyama et al. also reported that cancer incidence was not increased in homozygous FBXL12 knock-out mice[55]. Apart from this notion, potential roles of FBXL12 in cancer have not been explored previously. However, embryonal lethality without defects in adult mice and no increased cancer incidence are well in line with a role of FBXL12 in alleviating replication stress in highly proliferative cells (see **paper I** of this thesis).

To date, one study implicates FBXL12 in the maintenance of genome integrity. Postow et al. employed a cell free frog egg extract system and showed binding of FBXL12 to linearized plasmid DNA, modelling double-strand breaks, but not circular DNA[57]. Furthermore, they demonstrated FBXL12-dependent polyubiquitylation of the non-homologous end joining (NHEJ) DNA repair factor Ku80 in response to DSB binding, proposing an involvement of FBXL12 in removal of Ku80 upon repair completion or prevention of excessive Ku80 binding to DSBs[57]. The NHEJ pathway repairs DSBs by re-ligating adjacent blunt DNA ends without the need for a homologous template[58]. A complex of Ku80/Ku70 rapidly binds to DSBs and guides repair towards NHEJ by recruiting downstream factors including DNA-PK and Artemis[58]. However, depending on factors such as cell cycle phase or chromatin context cells might employ the other prevalent DSB repair mechanism, homologous recombination (HR) repair, or choose alternative pathways[58]. It is tempting to speculate that FBXL12-induced degradation of DSB-bound Ku80 may shift repair towards other pathways such as HR repair, depending on cell cycle phase or chromatin context. In mammalian cells, RNF8, an E3 ubiquitin ligase recruited to DNA damage sites, has been shown to be required for K48-linked ubiquitylation and degradation of Ku80, while in its absence Ku80 is stabilised on chromatin[59]. The process of V(D)J recombination, whereby NHEJ is required to diversify the antigen receptor pool during thymocyte maturation, may be another potential link to FBXL12[60]. Upregulation of the *Rag1* gene involved in the initiating step of V(D)J recombination occurs at the DN2 stage and, as discussed above, FBXL12 is expressed in DN

thymocytes with expression levels peaking later at the β-selection stage in response to pre-TCR signalling[49, 61, 62].

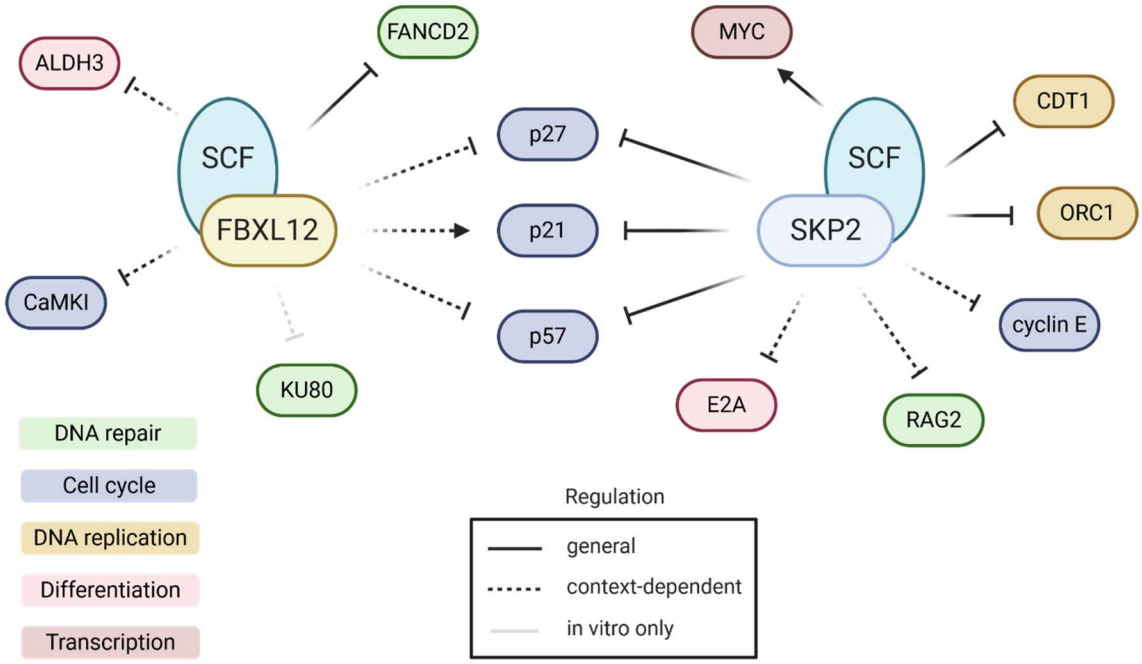

Fig. 3 Selected common and distinct ubiquitylation targets of FBXL12 and SKP2 and their respective functional affiliations. References: ALDH3[62]; CaMKI[50]; Chromatin licensing and DNA replication factor 1 - CDT1[63]; Cyclin E[64]; E2A[65]; FANCD2 (paper I); Ku80[57]; MYC[66]; Origin recognition complex 1 - ORC1[67]; p21[54, 55]; p27[49, 53, 68, 69]; p57[51, 52]; Recombination activating gene 2 - RAG2[70].

1.2.3 FBXO28 – emerging link between cancer hallmarks

FBXO28 is another poorly studied F-box protein, but in contrast to FBXL12 a link to cancer progression has previously been demonstrated[71–73]. Phosphorylation of FBXO28 on serine 344 by cyclin-dependent kinase 1/2 (CDK1/2) triggers non-proteolytic ubiquitylation of MYC, which increases MYC-regulated transcription[73]. Thus FBXO28 provides a link between CDK activity and cell cycle-regulated transcription of MYC target genes[73]. Interestingly, a SNP in close proximity to the FBXO28 promoter and highly correlated with its expression has been linked to poor outcome in ER-positive TP53-mutated breast cancers[74].

To date no proteolytic substrates of FBXO28 have been published. However, we and others have shown that FBXO28 is tightly bound to chromatin and interacts with topoisomerase 2α (TOPO2α), an essential enzyme which among other functions catalyses the separation of

replicated DNA strands (**Paper III**)[73, 75]. Depletion of FBXO28 promoted TOPO2α activity *in vitro*, delayed mitotic progression and resulted in formation of multinucleated cells[75]. The precise nature of this proposed regulatory mechanism remains unclear, however, as FBXO28 depletion did not alter TOPO2α stability or ubiquitylation patterns[75].

FBXO28 is highly conserved between different species and represented by two homologues in *Drosophila*, pallbearer (pall) and dampened (dpmd)[76, 77]. SCF^{pall} targets the ribosomal protein RpS6 for proteasomal degradation to regulate phagocytosis in *Drosophila* S2 cells[76]. As ablation of pall or RpS6 had opposing effects on RAC activity and cell motility, increased in the absence of RpS6 and decreased upon pall depletion, pall was proposed to regulate RAC via degradation of RpS6[76]. Consistently, in **paper III** of this thesis, we find that FBXO28 regulates RAC activity, however, by degrading the Rho guanine nucleotide exchange factors 6 and 7 (ARHGEF6/7), resulting in modulation of its downstream effectors.

Collectively, while we likely have an incomplete picture of its substrates to date, studies so far support an oncogenic role of FBXO28 whose functions may be linked to a multitude of cancer hallmarks including genomic instability, migration or the maintenance of proliferation.

1.3 THE HALLMARKS OF CANCER FROM AN F-BOX PERSPECTIVE

In 2000 Hanahan and Weinberg summarised the unifying features present in cancer in a landmark review which they updated 11 years later and expanded their initial set of hallmarks[15, 78]. The original six hallmarks are sustaining proliferative signalling, evading growth suppression, activating invasion and metastasis, enabling replicative immortality, inducing angiogenesis and resisting cell death[78]. The original hallmarks were subsequently joined by avoidance of immune destruction, deregulated cellular energetics, tumour-promoting inflammation and genomic instability[15]. All these hallmarks are intricately linked due to overlapping networks of regulatory proteins. Below, I will provide an overview of F-box proteins contributing to the development of selected cancer hallmarks in relation to the pathways immediately relevant to the studies presented in this thesis.

1.3.1 Deregulated proliferation

1.3.1.1 Cell cycle regulation

The two central processes of the cell cycle are DNA duplication and cell division. In order to maintain the cell's DNA content and genomic integrity, these processes need to be tightly regulated and carried out in a sequential order. Cyclin-CDK complexes, the drivers of the cell cycle and phase transitions orchestrate their own inactivation through proteasomal degradation of the cyclin component, which is essentially an irreversible process and thus crucial for the unidirectionality of the cell cycle[79]. Furthermore, the ubiquitin-proteasome system in general and the SCF ligase in particular are responsible for degradation of a multitude of additional cell cycle regulatory proteins [79]. These regulatory mechanisms often are part of feedback loops, positive or negative, to for example promote switching from one cell cycle phase to the next or to more rapidly approach a steady state[80].

The cell cycle of proliferating cells is subdivided into four distinct phases, G1, S-phase, G2 and mitosis. Cells in a resting, non-dividing phase outside of the cell cycle exist in a quiescent state termed G0. Commitment to cell division involves the activation of cyclins that drive the events of the cell cycle through binding to their partner CDKs. In G1 cyclin Ds activate CDK4/6 that in turn phosphorylate, among others, the retinoblastoma-associated protein (RB), which is instrumental for the commitment of cells to enter S-phase[81].
Once RB becomes hyperphosphorylated the cell passes the restriction point to commit to another round of the cell cycle and the binding partners of RB, E2F pocket proteins, are released to drive expression of genes promoting S-phase transition including *CCNE* (Cyclin E) and marking the onset of DNA replication[81]. However, recently the ubiquitin ligase APC/C has been proposed to be the ultimate point of no return since cells start replicating only minutes after APC/C^{Cdh1} inactivation while they can return to a quiescent state even after RB hyperphosphorylation but not once APC/C^{Cdh1} has been inactivated[82].
Cyclin E-CDK2 activity starts to rise before the restriction point resulting in inactivation of APC/C and continues to do so after this point to drive DNA replication[82, 83]. In addition to cyclins, CDK activity is regulated by binding of CDK inhibitors (CKI) and through phosphorylation[83]. CKIs are classed into INK4 and CIP families with INK4 members (p15, p15, p18, p19) inhibiting CDK4/6 by preventing their association with cyclin D while the CIP/KIP (p21, p27, p57) family inhibits G1/S and S-phase CDK-cyclin complexes and activates CDK4/6-complexes [81]. As mentioned earlier all members of the CIP/KIP family are targeted by the SCF ligase containing SKP2[49, 51, 54, 68, 84].

Cyclin E-CDK2 complexes also phosphorylate the transcription factor MYC, a central promoter of cell growth and proliferation which is frequently deregulated in human cancers[85]. Importantly, MYC drives the expression of critical cell cycle factors including cyclins, E2F proteins but also CUL1 or SKP2[85]. MYC is a very short-lived protein targeted by several E3 ubiquitin ligases. While its ubiquitylation by FBXW7 results in proteasomal degradation, FBXO28-mediated ubiquitylation supports MYC transcriptional activity, as mentioned earlier[13, 21, 73]. Interestingly, SKP2 also mediates destruction of MYC protein but at the same time stimulates MYC-induced G1/S transition[66, 86].

As mentioned, APC/C, the other major cell-cycle regulatory ubiquitin ligase besides the SCF ligase, has to be inactivated at the restriction point. APC/C forms a complex with either one of two substrate receptors, CDH1 or CDC20. The APC/C^{CDH1} complex is crucial for the establishment of G1 or G0 phases by degrading mitotic cyclins A and B along with mitotic kinases Aurora A and B, and PLK1 on the one hand, but also targeting proteins, such as SKP2 and CDC25A, responsible for G1/S-transition on the other[87–92]. One mechanism of APC/C^{CDH1} inactivation is based on the binding of FBXO5/EMI1 which acts as a pseudosubstrate for APC/C^{CDH1}, rather than forming an SCF complex[93, 94]. Other inactivation modes include a feedback loop based on autoregulation of CDH1 and its E2 UBCH10 through ubiquitylation and degradation[95, 96], phosphorylation of CDH1 by CDKs[97], as well as CDH1 ubiquitylation and degradation by $SCF^{\beta TRCP}$[98].

Similarly to RB, the prototype of a tumour suppressor, phosphatase and tensin homolog (PTEN), another antagonist to mitogenic signals, is one of the most frequently lost tumour suppressors[99]. PTEN is a negative regulator of phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signalling and is mutated, deleted or post-translationally deregulated in many human cancers[100, 101]. Besides dephosphorylating phosphatidylinositol-3,4,5-trisphosphate (PIP3) to counteract PI3K at the membrane, PTEN also has functions in the nucleus where it exerts its antitumorigenic effects phosphatase-independently as a scaffold protein by promoting association between APC/C and CDH1 ensuring establishment of G1 or G0[102]. To relieve PTEN-mediated inhibition its nuclear pool is targeted for proteasomal degradation by SCF^{FBXO22}[103].

The actual start of DNA replication forks from origins of replication, termed origin firing, is strictly separated from the preceding process: origin licensing. Licensing is the binding of the replicative helicase minichromosome maintenance 2-7 (MCM) complex along with CDC6,

CDT1 and ORC1-6 co-factors to form the pre-replicative complex (pre-RC) at several thousand sites along the genome[104, 105]. This step only occurs in G1 phase to prevent re-licensing of the same origins and thus re-replication within the S-phase of the same cycle [83]. To achieve this clear separation, several components of the pre-RC are degraded outside of late G1 phase. While ORC1 and CDT1 are targeted by SCF^{SKP2}, CDC6 is ubiquitylated by the actions of $SCF^{FBXO1/cyclin\ F}$ and APC^{CDH1}[67, 106–108]. Deregulation resulting in overexpression of CDT1 or CDC6 results in re-replication, activated DNA damage signalling and promotes malignant transformation[109]. In S-phase, cyclin D, too, is degraded by $SCF^{FBXO4\text{-}\alpha B\ Crystallin}$ after phosphorylation by glycogen synthase kinase 3β (GSK3β)[110]. Replication starts with the activation of MCM helicase by CDK and DBF4-dependent kinase (DDK) which is accompanied by the association of CDC45 and the GINS complex to form bidirectional replisomes at a subset of the previously licensed origins[111, 112]. Analogously to cyclin D, cyclin E is degraded during S-phase by the SCF^{FBXW7} ligase upon phosphorylation by GSK3β, and autophosphorylation by CDK2 on threonine 380 in a negative feedback loop[11, 19, 20].

In order to suppress early mitotic events in G2, WEE1 and MYT1 kinases prevent activation of CDK1 through inhibitory phosphorylations on tyrosine 15 and threonine 14, respectively[113–115]. WEE1 also phosphorylates tyrosine 15 of CDK2 at the G1/S border and early in S-phase to prevent excessive dormant origin firing and pre-mature replication onset[116, 117]. Upon cyclins reaching a critical level, cyclin E-CDK2 or cyclin A-CDK1 promote activation of the CDC25 phosphatases which in turn inactivate WEE1 and MYT1 to promote cell cycle progression[118]. Fine-tuning and correct timing of WEE1 activity are achieved by gradual increases of CDC25 activities, while WEE1 activity remains largely unaltered until a threshold is reached at which WEE1 activity is completely overridden[116]. Additionally, two SCF ubiquitin ligases target WEE1 for proteasomal degradation, $SCF^{\beta TRCP}$ and $SCF^{TOME\text{-}1}$, the latter one containing an F-box like protein[119, 120]. Phosphorylation of degron motifs in WEE1 by CDK1 and polo-like kinase 1 (PLK1), another crucial mitotic kinase, are a prerequisite for ubiquitylation by $SCF^{\beta TRCP}$, and likely $SCF^{TOME\text{-}1}$, thus creating a positive feedback loop to guarantee timely and complete activation of CDK1[119, 120].

Once the bulk of the genome has been replicated successfully these regulatory mechanisms result in activation of CDK1 and PLK1 to orchestrate processes related to cell division[83].
In early mitosis these two kinases phosphorylate APC/C inhibitor EMI1 which in turn results in its by recognition by $SCF^{\beta TRCP}$, ubiquitylation and proteasomal degradation[121, 122]. This allows for APC/C reactivation which regulates a multitude of mitotic processes[121, 122].

However, to delay APC/C activation from the onset of mitosis until attachment of all kinetochores, its substrate receptor subunit CDC20 is sequestered within the mitotic checkpoint complex consisting of BUBR1, BUB3, MAD2 and CDC20[123].

While APC/C is the central E3 ligase throughout mitosis, $SCF^{\beta TRCP}$ targets Bora, yet another mitotic kinase that regulates microtubule polymerisation and kinetochores, for proteasomal degradation in response to PLK1-dependent phosphorylation[124].

Once cyclin B is degraded by APC^{CDC20} and CDK1 activity drops upon metaphase to anaphase transition, CDH1 becomes dephosphorylated enabling it to gradually replace CDC20 in the complex with APC/C, promote degradation of CDC20, mitotic exit and establishment of G1 phase once again[125].

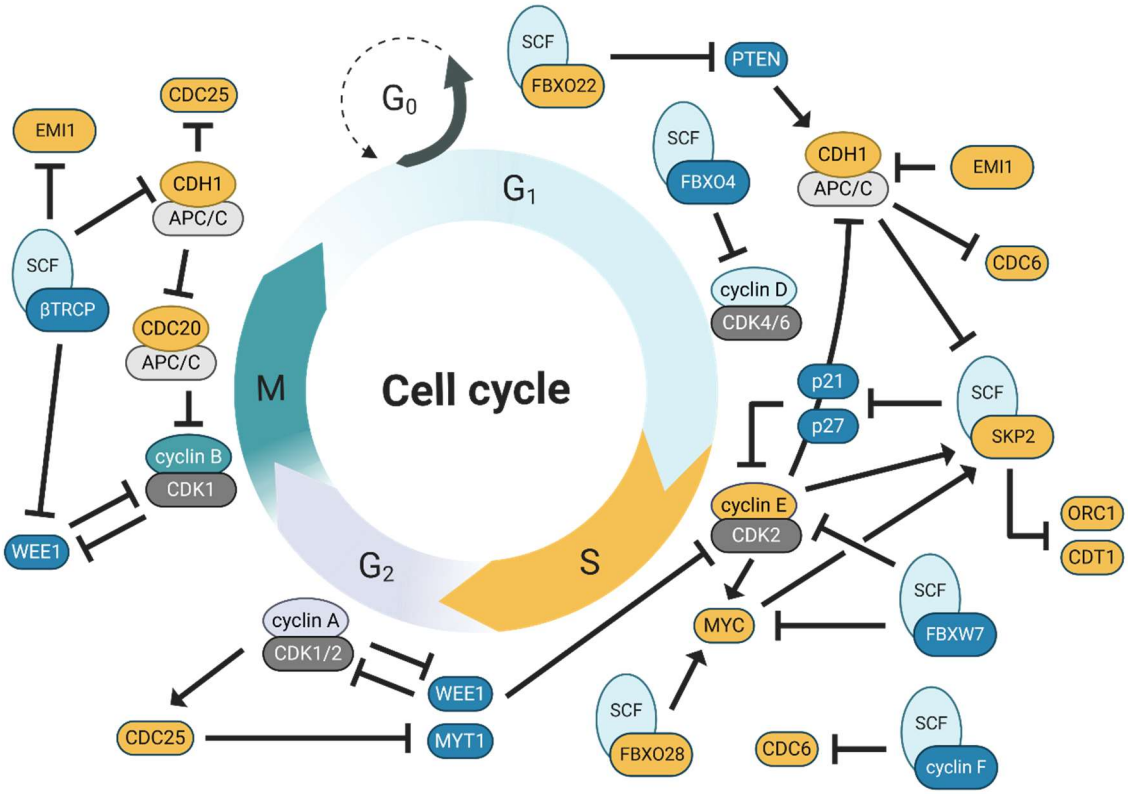

Fig. 4 Schematic representation of the SCF ligases and other central cell cycle regulators mentioned in the text and their regulatory functions throughout the cell cycle. Apart from cyclin-CDK complexes, proteins promoting cell cycle progression are depicted in orange, while proteins predominantly delaying progression are represented by dark blue symbols.

As alluded to throughout this section, deregulation of cell cycle regulators is a common theme in cancers resulting in uncurbed proliferation. Inactivation or loss of F-box genes like FBXW7 critically contributes to this process, while others such as FBXL12 (**paper I**) or FBXO28 (**paper III**) are involved in the aftermath, coping with excessive DNA damage and maintaining genomic integrity.

1.3.2 Maintaining genomic integrity

Tumorigenesis is regarded as a multi-step process in which, due to genetic or epigenetic changes, cells may acquire a selective advantage at every step of the way, resulting in their clonal expansion. Accordingly, genomic instability has been described as an enabling characteristic of cancer that provides the initial pool of different mutant genotypes[15]. Despite the actions of cell cycle-associated DNA damage checkpoints to safeguard genomic integrity of healthy cells, the vast majority of genomic changes is introduced through endogenous processes, mostly due to DNA repair defects, telomere erosion and/or replication stress[126]. The genome is constantly surveyed for alterations and depending on cell cycle context and type of damage specialised DNA repair pathways, including homologous recombination (HR) repair, non-homologous end joining (NHEJ) repair, base-excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), break-induced replication (BIR) and inter-strand crosslink (ICL) repair, are employed to correct lesions[127, 128]. If damage is beyond repair, cells will be purged from the cell pool by committing to programmed cell death or senescence[129]. Cancer cells need to circumvent these mechanisms to survive and continue to proliferate, while still maintaining a functionally required level of genome integrity to avoid more severe consequences such as replication or mitotic catastrophe where the genome may be "pulverised"[130].

1.3.2.1 Oncogene-induced replication stress

Very early on the path to becoming a cancer cell, in precancerous lesions, markers of an active DNA damage response (DDR) are visible, such as H2AX phosphorylated on serine 139 (γH2AX), CHK2 phosphorylated on threonine 68 or p53 phosphorylated on serines 15 and 20 [131–133]. This has been demonstrated to be a consequence of deregulation of oncogenes involved in cell proliferation such as cyclin E, RAS, MYC or MOS[132, 134, 135]. Overexpression of oncogenes induces replication fork slowing and stalling, phenomena collectively referred to as "replication stress". Crucially, replication stress leads to failure to duplicate DNA in a timely manner, under-replicated genomic regions persisting all the way into mitosis and ultimately resulting in double-stranded DNA breaks (DSBs).

However, differences in the landscapes of fragile sites induced by oncogenes (e.g. cyclin E, MYC or RAS) or treatment with aphidicolin, a DNA polymerase α and δ inhibitor, suggest that different oncogenes induce replication stress through distinct mechanisms[136].

Arguably, one of the most thoroughly studied oncogenes in the context of oncogene-induced replication stress is cyclin E which, as a crucial regulator of G1/S transition, derails various aspects of the replication programme upon its overexpression. On the one hand, pre-mature entry into S-phase results in a shortened time window for the licensing of replication origins, while overly active CDK2 at the same time results in firing of a greater proportion of licensed origins with two major consequences: The available pool of dormant origins is dramatically decreased and due to an increased number of active replication forks conflicts with the transcription machinery, either through direct collisions or increased torsional stress, occur more frequently[137]. Thus, the possibility to rescue stalled replication forks by dormant origins in their vicinity is drastically reduced[137]. Additionally, the increased number of active replication forks results in depletion of available dNTP thereby contributing to reduced replication fork speed[138]. While these mechanisms have been elucidated largely using a U2OS cell line model featuring inducible overexpression of cyclin E[132, 137], frequent amplification or overexpression of cyclin E in human cancers, in triple-negative breast cancers in particular, indeed results in high levels of replication stress[139].

RAS overexpression or expression of the oncogenic $HRAS^{V12}$ mutant potently induces replication stress in cell line models[140]. While RAS indirectly induces cyclin E expression through its regulatory role in the cell cycle and on D-type cyclins, RAS also promotes replication stress through upregulation of overall transcription[126]. Kotsantis et al. showed that this is mainly due to increased expression of the general transcription factor TATA-box binding protein (TBP) and ensuing elevated formation of R-loops, hybrids between RNA and single-stranded DNA, a considerable roadblock for replication forks[140].

In contrast to RAS overexpression which initially results in a burst of cell proliferation and increased replication fork progression to reduce fork speed only later on, elevated MYC levels rapidly trigger replication stress[141]. In both cases metabolic rewiring has been attributed to partially account for the induction of replication stress[141], notwithstanding mechanisms mentioned above. In addition, MYC has been demonstrated to induce replication stress through increased origin density and premature origin firing[142, 143].

While amplification or transcriptional deregulation of oncogenes inducing replication stress are frequent events across a variety of human malignancies, perturbances of their post-translational control likewise are frequently encountered. As mentioned above, one such mechanism is the mutation or loss of the tumour suppressor gene FBXW7 which negatively regulates both cyclin E and MYC and which is deleted in over 30% of human cancers[34, 35]. Another F-box protein, βTRCP with both oncogenic and tumour suppressive properties attributed to its diverse

range of substrates, regulates among many others CDC25A shown to induce replication stress[144, 145]. Accordingly, βTRCP somatic mutations observed in certain human cancers including gastric cancer may also contribute to replication stress due to aberrantly increased CDK activity[146]. A third example of an F-box protein impinging on replication stress through regulation of an oncogene with potential implications to human cancers is cyclin F/FBXO1 which is downregulated in hepatocellular carcinoma and whose low expression associated with poor patient survival and advanced clinical stage[147]. Importantly, $SCF^{Cyclin\ F}$ targets ribonucleotide reductase M2 (RRM2) on the one hand involved in the production of dNTPs to alleviate replication stress and the origin licensing factor CDC6 on the other whose deregulation contributes to replication stress[108, 148, 149].

Increased origin density and ensuing reduced replication fork processivity results in long stretches of single-stranded DNA (ssDNA), due to uncoupling of MCM helicase and polymerases[150], which renders these stretches prone to formation of secondary structures and processing by endonucleases[104]. In order to prevent these events, the heterotrimeric replication protein A (RPA) complex binds ssDNA and subsequently recruits the apical DDR kinase ATM- and Rad3-related (ATR) through direct binding of its coactivator ATR-interacting protein (ATRIP)[151, 152]. ATR activates CHK1 through phosphorylation of serines 317 and 345, and jointly a multitude of factors to globally install a replication checkpoint, also referred to as S-phase checkpoint, and prevent cell cycle progression and locally to prevent fork collapse and promote stalled fork restart.

Globally, the ATR/ATRIP-CHK1 axis suppresses CDK activity by promoting proteasomal degradation of CDC25A and consequential upregulated CDK phosphorylation by WEE1. While APC/C^{CDH1} targets CDC25A in mitosis and G1[89], $SCF^{\beta TRCP}$ mediates CDC25A degradation after a concerted CHK1 and NIMA (never in mitosis gene A)-related kinase 11 (NEK11) phosphorylation signal[153]. Moreover, during recovery from the S-phase checkpoint, Claspin, a large scaffold protein binding to stalled replication forks and promoting CHK1 activation[154], is degraded by the proteasome after being ubiquitylated by $SCF^{\beta TRCP}$[155, 156].

Locally, molecular targets of ATR and CHK1 signalling include fork remodellers, DNA repair factors, helicases, nucleases and proteins involved in chromatin assembly[157]. Suppression of ATR-mediated phosphorylation of the helicase Werner syndrome RecQ-like helicase (WRN), which is involved in stabilising stalled forks and resolving replication intermediates[158], impairs WRN foci formation at stalled forks resulting in fork breakage[159]. Furthermore, MCM components are phosphorylated by ATR to modulate its

interaction with members of the Fanconi anaemia (FA) pathway[160, 161], which will be described in detail below. Several FA proteins are themselves direct phosphorylation targets of either ATR or CHK1, or both [162]. Additionally, the HR repair pathway is tightly linked to the activity of ATR which phosphorylates BRCA1 and PALB2 to modulate their interaction[163, 164], while CHK1 phosphorylates the ssDNA-binding and central HR protein RAD51[165] to mediate its interaction with BRCA2[166] which is required to protect stalled forks from aberrant resection by exonucleases EXO1 and DNA2[167], themselves targets of S-phase checkpoint signalling involved in preventing fork reversal[168, 169]. RAD51 is kept in check by EMI1/FBXO5-mediated degradation[170]. Upon DNA damage, CHK1 phosphorylation of RAD51 on Thr309 counteracts this event, allowing RAD51 accumulation and HR repair[170].

1.3.2.2 The Fanconi anaemia DNA repair pathway

The FA pathway is a crucial DNA repair pathway that promotes recovery of stalled replication forks and balances dormant origin firing in response to replication stress, mainly through its central proteins FANCD2 and FANCI (Fanconi anaemia complementation group D2 and I)[171]. However, the classical, best-understood function of the FA pathway is removal of DNA ICLs. Until now, 31 proteins have been identified to be involved in this repair pathway, 22 of which carry causative mutations in patients with FA and are considered *bona fide* FA genes (**Tab. 1)**[172]. Characteristics of this rare disease, affecting 1/100 000 births, are bone marrow failure and a predisposition to develop cancer, both solid cancers and acute myeloid leukaemia (AML)[173]. Activation of ATR in response to genotoxic stress or during normal S phase leads to multiple phosphorylations of FANCI at S/TQ sites proximal to a lysine in position 523[174, 175]. FANCI forms a heterodimer with FANCD2, the ID2 complex, which too becomes phosphorylated in response to ATR activation[176]. As a result of these phosphorylations, the FA core complex, consisting of the E3 ubiquitin ligase FANCL, eight additional FA proteins and five accessory proteins (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCM, FANCT, FAAP100, MHF1, MHF2, FAAP20 and FAAP24), monoubiquitylates both FANCI and FANCD2 at K523 and K561, respectively[171]. The FA core component FANCM along with MHF1, MHF2 and FAAP24 ensures general anchoring of the entire complex to chromatin during S-phase while ID2 monoubiquitylation leads to its dissociation into monomers and binding to DNA adjacent to ICLs or stalled replication forks[171, 175, 177]. Interestingly, components of the FA core complex are also required for efficient ATR-CHK1 activation in response to replication stress and depletion of FANCM, itself a phosphorylation target of ATR at serine 1045[178], has been shown to impair ATR

signalling and ensuing replication fork restart[179]. The monoubiquitylated ID2 complex is believed to serve as binding platform for DNA repair nucleases SLX4, also known as FANCP, and FA-associated nuclease 1 (FAN1)[180, 181]. Additional structure-specific nuclease complexes MUS81-EME1 and XPF-ERCC1 as well as SLX1 bind and become activated by SLX4 and finally mediate incision and unhooking of the DNA lesion[171, 175]. At stalled replication forks this step results in a DSB in one of the newly synthesised double-strands and an oligonucleotide crosslinked to the other one[175]. At this stage three different DNA repair pathways need to be coordinated to resolve the situation: Firstly, the translesion synthesis (TLS) polymerase ζ incorporates nucleotides opposite of the crosslinked nucleotide, secondly HR repair uses this template to repair the DSB, and thirdly, NER removes the remaining crosslink adduct[175]. After the remaining gap has been filled by polymerases, ubiquitin-specific protease 1 (USP1) together with its co-factor USP1-associated factor 1 (UAF1) deubiquitylates ID2 which has been proposed to result in its release and deactivation of the pathway[182]. Indeed, monoubiquitylation of FANCD2 has been shown to be crucial for binding to and stabilisation of ID2 on dsDNA but intriguingly not to any of its binding partners *in vitro*[183]. In the free ID2 complex, monoubiquitylation sites are buried at the surface between FANCD2 and FANCI and are likely not readily accessible to ubiquitylation or deubiquitylation[184]. However, upon dsDNA binding the ID2 dimer forms a clamp around DNA and the FANCD2 monoubiquitylation site becomes exposed and can be ubiquitylated by FANCL to stabilise ID2:dsDNA interaction[185].

Additionally, the ID2 dimer has been shown to undergo sumoylation, the post-translational covalent attachment of small ubiquitin-like modifier (SUMO) proteins, similar to ubiquitylation, upon replication fork stalling[186]. This modification is mediated by protein inhibitor of activated STAT 1 (PIAS1) and PIAS4 and thereafter leads to polyubiquitylation by RING finger protein 4 (RNF4)[186]. In this case polyubiquitylation triggers extraction from chromatin via VCP/p97 segregase rather than proteasomal degradation[186]. Sumoylation has been proposed to balance recruitment and release of ID2 at repair sites[186].

Extensive resection at DSBs is a prerequisite for HR repair to occur. FANCD2 in complex with BRCA1 mediates recruitment of CtBP-interacting protein (CtIP), an end resection factor, to ICLs[187]. Additionally, the MRN (MRE11-RAD50-NBS1) complex which also processes DSBs interacts with and stabilises FANCD2 on ssDNA repair intermediates[188]. Subsequently, the BRCA2-PALB2 complex catalyses binding of recombinase RAD51 to resected ssDNA which in turn mediates strand invasion of the homologous template. Unscheduled RAD51 loading is prevented by F-box DNA helicase (FBH1/FBXO18) through

both direct displacement of RAD51 and polyubiquitylation followed by degradation[189]. However, correctly timed RAD51 association and strand invasion are finally followed by repair synthesis, D-loop resolution and ligation yielding a complete dsDNA molecule[171].

	Gene	Other designations	Molecular function
FA core complex	*FANCA*		Core subcomplex with FANCG and FAAP20
	FANCB		Core subcomplex with FANCL and FAAP100
	FANCC		FANCC, FANCE and FANCF form a subcomplex of the core complex and promote FANCL activity
	FANCE		
	FANCF		
	FANCG	*XRCC9*	Core subcomplex with FANCA and FAAP20
	FANCL		RING-type E3 ubiquitin ligase carrying out monoubiquitylations of ID2 complex
	FANCM		Recruitment of the core complex to damage sites
	FANCT	*UBE2T*	E2 ubquitin-conjugating enzyme
	FAAP10	*STRA13/CENPX/MHF2*	Stabilisation of FANCA; interaction with ubiquitylated TLS polymerase REV1
	FAAP16	*APITD1/CENPS/MHF1*	Interaction with FANCM to promote its localisation to chromatin
	FAAP20	*C1orf86*	Core subcomplex with FANCA and FANCG
	FAAP24	*C19orf40*	Interaction with FANCM to promote its localisation to chromatin
	FAAP100	*C17orf70*	Forms subcomplex with FANCB and FANCL
ID2	*FANCD2*		Recruitment of down-stream effectors; additional FA core-independent functions
	FANCI		Recruitment of down-stream effectors; additional FA core-independent functions
Downstream effectors/HR	*FANCD1*	*BRCA2*	Stabilisation of stalled replication forks; promoting RAD51 recruitment and activity
	FANCJ	*BRIP1*	Promoting HR through interaction with BRCA1; inhibiting TLS
	FANCN	*PALB2*	Interactor of BRCA2 and BRCA1; stabilisation of stalled forks
	FANCO	*RAD51C*	HR repair
	FANCP	*SLX4*	Scaffold nuclease regulating ERCC1-XPF, MUS81-EME1/2 and SLX1 nucleases
	FANCQ	*ERCC4, XPF*	DNA incision and NER
	FANCR	*RAD51*	Strand invasion in HR; stabilisation of stalled forks in conjunction with FANCD2
	FANCS	*BRCA1*	Release of CMG replicative complex at stalled forks; HR
	FANCU	*XRCC2*	HR repair
	FANCV	*REV7/MAD2L2*	Promoting end joining while inhibiting end resection
	FANCW	*RFWD3*	RING-type E3 ubiquitin ligase ubiquitylating RPA; involved in fork restart
Other FA-associated genes	*FAN1*		Restart of stalled forks; probably not involved in ICL repair
	UAF1		Deubiquitylation of ID2
	UHRF1		Lesion recognition and recruitment of FANCD2
	USP1		Deubiquitylation of ID2
	FANCX	Putative additional unidentified FA gene(s); expected based on FA patients without mutations in known FA genes	

Tab. 1 Components of the FA pathway and their respective functions.

As mentioned, the FA pathway gets activated and the ID2 dimer monoubiquitylated not only in response to genotoxic stress but also during an unperturbed S-phase[175]. Interestingly, FAN1 recruitment by monoubiquitylated ID2 is crucial to prevent chromosomal abnormalities upon hydroxyurea (HU) treatment but not treatment with ICL-inducing agent MMC[190]. Furthermore, FANCD2 is associated to nascent DNA and binds to the DNA replication clamp proliferating cell nuclear antigen (PCNA), as well as to the replicative helicase MCM2-7 in response to replication stress, consistent with a role in the response to replication stress[160, 191]. The association between FANCD2 and MCM2-7 is promoted by ATR but is independent

of ubiquitylation by the FA core complex[160]. Furthermore, FANCI has been demonstrated to colocalise with MCM2-7 at dormant origins and promote dormant origin firing in a monoubiquitylation-independent manner[161]. This has been suggested to occur mainly under conditions of mild replication stress which can be overcome by firing of additional origins while more severe stress triggers phosphorylation of FANCI by ATR which inhibits firing and promotes stabilisation and re-start of stalled replication forks, thus retaining potential back-up origins[161]. While FANCI depletion at low concentrations of HU leads to reduced dormant origin firing, FANCD2 depletion increases firing of origins in a FANCI-dependent fashion, suggesting a mechanism where FANCD2 directly inhibits or balances FANCI-mediated dormant origin firing[161].

One of the consequences of replication stress is the persistence of under-replicated regions in mitotic cells. Upon chromosome condensation and cytokinesis these under-replicated stretches can be visualised as ultra-fine anaphase bridges (UFBs) which are nearly devoid of proteins and cannot be detected with conventional DNA dyes such as DAPI[192]. However, depending on their origin, UFBs are associated with specific factors. Under unperturbed conditions UFBs can be found mostly in centromeric regions due to catenated DNA[192]. However, replication stress promotes the occurrence of UFBs associated with regions with inherent replication difficulties prone to being under-replicated, in particular common fragile sites (CFSs) [192]. Intriguingly, upon replication stress CFSs also become sites of DNA synthesis in mitosis which is likely a form of BIR acting to rescue under-replicated regions[193, 194]. Such sites colocalise with UFBs which are typically marked by twin foci of FANCD2 at either end of the bridge and associated with PLK1-interacting checkpoint helicase (PICH) as well as RPA along their length but also with the RECQ family helicase BLM, defective in Bloom's syndrome[192, 195]. FANCD2 is involved in the stability of CFSs and both FANCD2 and FANCI have been demonstrated to localise to the two CFSs most frequently associated with chromosome breaks, FRA3B and FRA16D[195, 196]. Accordingly, FA pathway proteins have been demonstrated to collaborate with BLM and promote efficient chromosome segregation after aphidicolin-induced DNA damage[195, 197]. Intriguingly, FANCM is degraded by $SCF^{\beta TRCP}$ in mitosis thereby releasing the FA core complex from chromatin and preventing its erroneous activation which at the same time indicates that mitotic functions of FANCD2 and FANCI are monoubiquitylation-independent[198].

Finally, FA proteins, in particular FANCM through its DNA translocase activity, also resolve DNA:RNA hybrids impairing replication fork progression, such as those occurring as a

consequence of collisions between transcription and replication machineries due to cyclin E overexpression[199].

1.3.2.3 Faithful heterochromatin repair and damage site motility

The choice of DNA repair pathways deployed depends on the type of detected lesion as well as on cell cycle phase. On top of those factors genomic context is decisive not only for sensitivity to damaging agents, such as in the case of replication stress and CFSs mentioned above, but also for repair pathway choice[58]. While many DNA damaging agents are biased towards inducing damage within highly transcribed parts of the genome, heterochromatic regions present exquisite challenges to faithful repair. As soon as a homologous template becomes available, typically on the sister chromatid as a result of replication, HR repair is often the pathway of choice in response to DSBs as opposed to NHEJ, the other major DSB repair pathway which is more error-prone but likely faster and independent of extensive homologous templates[58]. While the HR repair process itself is well understood, much of the research around this crucial pathway has been focused solely on euchromatic regions. However, heterochromatic DNA is highly repetitive and thus, identification of the correct template is difficult with potentially catastrophic consequences such as translocations, insertions or deletions[200]. Additionally, access of repair proteins to heterochromatic DSBs can be compromised due to densely packed chromatin[200]. Collectively, these properties might be the underlying cause for significantly higher mutation rates of cancer cells observed in heterochromatic regions, particularly those marked by H3K9me3[201].

Heterochromatin contains both constitutively silenced regions, comprising centromeres, pericentromeric regions, telomeres and highly repetitive sequences, but also facultative regions, transiently silenced or developmental genes[200]. Histones within the former are generally deacetylated and hypermethylated, specifically histone H3 lysine 9 (H3K9me2/3) and lysine 27 (H3K27me3), as well as histone H4 lysine 20 (H4K20me2/3)[200]. Factors maintaining the heterochromatin state and establishing these modifications include histone deacetylases (HDACs), histone modifier SET domain bifurcated 1 (SETDB1) and methyltransferase suppressor of variegation 3–9 (SUV39)[202]. F-box proteins regulate this chromatin architecture directly and indirectly. FBXL10 (KDM2B) and FBXL11 (KDM2A) assemble functional SCF complexes but also act as histone demethylases on methyl groups from lysines 4, 9, 27, 36 and 79 of histone H3[203]. Another histone demethylase, lysine-specific demethylase 4A (KDM4A), which specifically removes di- and trimethylations from histone H3 lysines 9 and 36, is targeted for proteasomal degradation by SCFFBXL4 and

SCFFBXO22[204, 205]. Additionally, FBXO44 was recently shown to, selectively in cancer cells, function as an essential repressor of repetitive elements, which are transcriptionally silenced and compose about 50% of the human genome[206]. Mechanistically, FBXO44 binds H3K9me3-modified nucleosomes at the replication fork and recruits SUV39H1, the CRL4 ubiquitin ligase, and the Mi-2ß/NuRD complex to maintain repetitive element silencing after DNA replication[206].

Interestingly, heterochromatin protein 1 (HP1) which is also associated with constitutive heterochromatin and interacts with H3K9me3, rapidly accumulates at sites of laser- or UV-induced damage in both hetero- and euchromatin, and promotes HR repair[207, 208]. This may prevent further DNA damage by restricting access and processing by nucleases or through stabilisation of DNA ends and thereby keeping sister chromatids within close proximity[200, 209]. Furthermore, a complex containing SUV39 is recruited to DSBs upon DNA damage where its activity results in transient peaks of H3K9me3 which in turn promotes ataxia telangiectasia-mutated (ATM) kinase DNA repair signalling[210]. KDM4B counteracts SUV39 to prevent aberrant H3K9-hypermethylation of regions surrounding breaks[211]. Surprisingly, accumulation of oncometabolites, 2-hydroxy-glutarate, succinate and fumarate which accumulate in human malignancies due to mutations of isocitrate dehydrogenase-1 or -2 (IDH1/2), act as inhibitors of KDM4B which subsequently results in masking of local methylation signals and accordingly hindrance of efficient DSB repair[211].

In *Drosophila* cells, unlike human cells, heterochromatin domains can be visualised as brightly-stained areas using DNA dyes[212]. Chiolo et al. used this property to demonstrate that DSBs within these domains in fact move to areas outside the heterochromatin domains to be repaired by HR only after their translocation, while the molecular mechanism behind this DNA break mobility remained largely unclear[213]. Recently, however, it was revealed that nuclear actin filaments and myosins mediate nucleation of actin at DNA damage sites involving the actin related protein 2/3 (ARP2/3) complex[214, 215]. ARP2/3 and its activator Wiskott-Aldrich syndrome protein (WASP) specifically participate in the repair of DSBs via HR but not NHEJ supporting the necessity of repair site mobility to promote faithful homology-dependent repair[215].

1.3.3 Cell migration and metastasis

Metastasis accounts for more than 90% of cancer-related deaths in patients with solid tumours[216]. For cancer cells to dissociate from the original tumour, invade surrounding

tissues, eventually enter the bloodstream, exit at a distinct location with different microenvironmental characteristics and continue to proliferate to finally establish another tumour, many obstacles need to be overcome. Molecularly, the initial release of cancer cells from the primary tumour site is associated with a developmental programme known as the epithelial-to-mesenchymal transition (EMT) which is frequently hijacked by cancer cells to loosen contact with surrounding cells and adopt migratory characteristics[216]. In animal models for melanoma metastasis less than 0.1% of cells injected in the blood stream subsequently had the potential to form metastasis[217]. These rare tumour-initiating cells exhibit many of the characteristics of normal stem cells including the ability to divide asymmetrically and give rise to the heterogeneity present in the initial tumour and are thus referred to as cancer stem cells[218]. Due to their low proliferation rate as compared to the bulk tumour cells and ability to enter dormancy, they are less sensitive to standard chemotherapies and represent a major driver of drug resistance requiring targeted therapy approaches[218].

1.3.3.1 Epithelial-to-mesenchymal transition

The loss of cell polarity within epithelial tissues and subsequent migration is required at several stages during normal development, perhaps most prominently during neural crest emigration[219]. Pleiotropically acting transcription factors SNAIL, SLUG, TWIST or ZEB1/2 orchestrate the required broad expression changes impinging on cell motility, cytoskeleton regulation, contact with neighbouring cells and the extracellular matrix as well as the release of proteases[220]. These transcriptional regulators are themselves regulated by a multitude of factors, including F-box proteins, underpinning their significance. SNAIL is phosphorylated by protein kinase D1 (PKD1) and subsequently targeted for proteasomal destruction by SCF^{FBXO11}[221]. Additionally, SNAIL is phosphorylated by GSK3β, a downstream kinase within the PI3K/AKT signalling pathway which is a predominantly positive regulator of EMT and inhibitor of TGFβ-dependent apoptosis, with TGFβ being a potent inducer of EMT[220, 222]. However, in a negative feedback loop phosphorylation by GSK3β leads to recognition and ubiquitylation by $SCF^{\beta TRCP}$, SNAIL degradation and inhibition of EMT[222]. Yet another F-box protein FBXL5 has been demonstrated to mediate SNAIL turnover by ubiquitylation and inhibition of DNA binding[223]. Intriguingly, FBXL5, originally identified as an iron-sensing protein and substrate receptor responsible for degradation of iron regulatory proteins[224], is downregulated in response to radiation-induced DNA damage which has been suggested to provide a link between radiotherapy and increased EMT[223]. In addition, FBXL14 acts as a key EMT repressor both during development and in human cancer cells by regulating protein stability of SLUG and TWIST1[225, 226], as well as

SNAIL under hypoxic conditions[227]. Another master transcription factor, SOX9, promotes EMT in gastric, lung and breast cancer and together with SLUG de-differentiates mammary epithelial cells to mammary stem cells while enhancing metastasis-forming capabilities[228–230]. We and others demonstrated that SCF^{FBXW7} targets SOX9 for proteasomal degradation upon phosphorylation of a degron motif by GSK3β (**paper IV**)[26, 27]. Expression of a non-degradable SOX9 mutant resulted in upregulation of the EMT signature[26]. Among others the ensuing transcriptional changes involve repression of E-cadherin which results in reduction of cell-cell contacts[228, 229]. Cancer cells activate the EMT programme in response to receptor tyrosine ligands activating TGFβ, SHH, NFκB or WNT/βCatenin signalling pathways which originate from tumour stroma and ultimately and crucially upregulate expression of key EMT transcription factors[220].

1.3.3.2 Cell migration and invasion

Gene expression changes in response to EMT encompass a multitude of factors directly involved in switching to a more migratory cell phenotype, most prominently the Ras homolog (Rho) GTPase[220]. Rho is the founding member of a family of small GTPases which are shifting between a GTP-bound active state and, through hydrolysis of GTP and corresponding conformational changes, an inactive GDP-bound state[231]. They are activated by receptor tyrosine kinase signalling, such as through TGFβ or EGF receptors, and their activity is modulated positively by guanine exchange factors (GEFs) and negatively by GTPase-activating proteins (GAPs)[231, 232]. Additionally, guanine-nucleotide dissociation inhibitors (GDIs) prevent release of GDP and sequester Rho-type GTPases in their inactive state in the cytosol[233]. Upon GTP binding Rho-type GTPases undergo conformational changes allowing them to bind to membranes and associate with their downstream effectors[231]. Post-translational modifications by SCF^{FBXL19} provide another layer of regulation by mediating the proteasomal destruction of Rac1 following its phosphorylation by AKT, although the physiological significance of this mechanisms is less clear[234].

At the plasma membrane activities of Rho-type GTPases Rho and Rac1 promote the formation of protrusions called lamellipodia, while CDC42 is needed for forming filopodia. Crucially, Rho-GTPases Rac1 and CDC42 activate p21-activated kinases (PAKs) through direct binding and relieving an autoinhibitory mechanism resulting in PAK1, PAK2 and PAK3 autophosphorylation and full activation[235]. Among the substrates of PAK kinases are ARHGEF6 also called αPIX (Pax-interacting exchange factor alpha) and ARHGEF7/βPIX, GEFs and thus activators of Rac1 and CDC42, mediating a positive feedback loop[236]. These

GEFs form pentameric complexes consisting out of ARHGEF trimers and G protein-coupled receptor kinase interactor (GIT) proteins, GAPs of Rac1 and CDC42, providing large regulatory units which themselves need to be tightly regulated[237]. In **paper 3** (this thesis) we show that SCF^{FBXO28} targets ARHGEF6/7 to modulate the activity of both cell-membrane associated but also nuclear Rac1 impacting on cell migration as well as on a novel function in HR repair. ARHGEF and GIT proteins are recruited to the sites of interaction between the cytoskeleton and the extracellular matrix, so-called focal adhesions, by the scaffold protein paxillin which is binding with and regulated by the focal adhesion kinase (FAK), in turn another target of PAK phosphorylation highlighting the interconnected nature of Rho GTPase signalling[238]. Importantly, Rac1 and CDC42 also activate WASP and WASP family verprolin homologous protein (WAVE) which promote formation of actin filaments through the action of ARP2/3 complexes, as mentioned, and thereby modulate nuclear cytoskeleton remodelling[239].

Apart from the cell membrane, Rho-type GTPases also localise to other membrane-enclosed structures such as the Golgi apparatus or mitochondria to regulate vesicle trafficking, as well as to the nucleus, where Rac1 has previously been suggested to be involved in regulating nuclear shape and thereby promotes cancer cell invasion[231, 240]. Accordingly, nuclear Rac1 levels are elevated in high grade human cancers[240].

1.3.3.3 The cancer stem cell concept and drug resistance

In analogy to somatic stem cells which have the capacity to form entire tissues, human tumours contain a small proportion of cells that when transferred to a different site can give rise to another tumour and replicate the heterogeneity detected at the original site[218]. Additionally, these cells, like their normal counterparts, divide asymmetrically always maintaining a pool of stem-like cells[218]. Based on these properties they are referred to as cancer stem or stem-like cells (CSCs) [218]. However, whole-genome sequencing studies revealed that human tumours contain cell populations derived from several genetically distinct CSC clones which has profound implications for targeted treatments[241]. Consistent with a Darwinian model of cancer evolution, different cancer cell clones do not exhibit the same sensitivity to targeted treatments, which results in a therapy-induced selection process of resistant clones[242]. Additionally, CSCs can persist in a quiescent state and thereby be insensitive to conventional chemo- and radiotherapy regimens targeting predominantly highly proliferative cells[241].

While it is still controversially debated whether the cell of origin for many human cancers is in fact a stem cell, some of the common markers and signalling pathways identified in CSCs are

also required for pluripotency of embryonal stem cells. These also encompass the Yamanaka reprogramming factors c-Myc, Klf4, Oct3/4 and Sox2 which are sufficient to obtain induced pluripotent stem (iPS) cells in mice[243, 244]. Due to their highly oncogenic potential these master transcription factors are heavily regulated, not least by SCF ubiquitin ligases. MYC is ubiquitylated by at least 16 E3s with SCF^{FBXW7} mediating its proteasomal degradation and $SCF^{\beta TRCP}$ counteracting FBXW7-mediated ubiquitylation[13, 21, 66, 73, 86, 245, 246]. As mentioned, FBXO28-dependent ubiquitylation promotes MYC transcriptional activity in response to phosphorylation by CDK2[73]. Moreover, KLF4 which is believed to counteract the pro-apoptotic effects of MYC expression has been demonstrated to be targeted by SCF^{FBXO22}[247].

Additional pathways that have been found to be specifically activated in CSCs in a range of human cancers include developmentally important NOTCH, WNT, Hedgehog, FGF and TGF-β signalling[244]. Again, it is SCF^{FBXW7} that regulates the turn-over of NOTCH1 and whose mutation is instrumental in allowing cancer development as a result of NOTCH deregulation in T-cell acute lymphoblastic leukaemia (T-ALL)[248]. A model of brain tumour induction, based on expressing a mutant version of MYC resistant to FBXW7-mediated degradation in mouse neural stem cells, revealed that orthotopically engrafted mice developed either gliomas, medulloblastomas or primitive neuroectodermal tumours[249]. One of the key factors determining brain tumour type was SOX9 which we and others demonstrated to be a target substrate of SCF^{FBXW7} (**paper IV**), further highlighting the critical function of FBXW7 during cancerogenesis[26, 249].

As mentioned, therapeutical interventions may drive the evolution of CSC clones and thereby development of treatment resistance[242]. Increased efflux of drugs through upregulation of ATP-Binding Cassette (ABC) transporters represents one mechanism of drug resistance encountered in CSCs[241]. In glioma stem-like cells elevated expression of ABCG2 promoted resistance to temozolomide, a conventional drug used to treat gliomas[250]. ABCG2 upregulation was associated with overly active PI3K/AKT signalling, particularly elevated AKT activity or PTEN loss[250]. Intriguingly, PI3K/AKT signalling also counteracts GSK3β-dependent phosphorylation of SOX9 and recognition by FBXW7, thereby promoting resistance to cisplatin in medulloblastoma which we found to be associated with upregulation of the copper transporter ATP7A (**paper IV**) [26]. Similarly, depletion of SKP2 and ensuing stabilisation of p27 promoted overexpression of the drug efflux pump multi-drug resistance protein 1 (MRP1) and was linked to drug resistance in an AML cell line[251].

Another common mechanism of drug resistance is upregulation of DDR components to increase the capacity for repairing DNA breaks in cancer cells[241]. For instance, the $CD24^-$/epithelial specific antigen $(ESA)^+$ stem-like population of human triple-negative breast cancer MDA-MB-231 cells is enriched in S-phase and exhibits increased expression of RAD51 associated with reduced DNA damage in response to irradiation[252]. Interestingly, while MDA-MB-231 cells respond to WEE1 inhibition through induction of replication stress in S-phase and massive DNA damage, cells re-proliferate after drug removal, but fail to do so in the absence of a functional HR repair signalling pathway as demonstrated in **paper II**[253]. If this is linked to survival of a stem-like population is not known. Further, development of radioresistance is a common phenomenon in gliomas and precludes curative treatment[254]. In glioma mouse models, cells expressing the common stem cell marker CD133 survive radiation to a higher extent then $CD133^-$ cells which could be overcome by targeting DNA damage checkpoints via inhibition of CHK1 and CHK2[254]. Similarly, a report in non-small-cell lung cancer (NSCLC) based on NSCLC stem cells derived from patients concluded that CHK1 is activated and promotes resistance to chemotherapy but renders cells sensitive to combination therapy with CHK1 inhibitors which resulted in premature cell cycle progression and mitotic catastrophe[255]. Intriguingly, high levels of FBXO6, which negatively regulates CHK1 through proteasomal degradation[256], are associated with favourable NSCLC patient outcome[257]. Furthermore, in cell lines FBXO6 depletion confers resistance to genotoxic cisplatin treatment[257]. In line with the significance of the DDR in CSC resistance, Zhang et al. characterised stem cells derived from mouse p53-null mammary gland tumours and detected elevated levels of genes involved in DNA repair including Brca1, Chk1, Ku80 or Rpa1[258].

Resistance mechanisms such as these, which distinguish CSCs and bulk tumour cells within the same clone and in the case of increased checkpoint activation would limit proliferation of bulk cells, may rely on epigenetic differences rather than genetic ones. Accordingly, negative regulators of DNA damage checkpoint signalling such as FBXO6, FBXO18, involved in degradation of RAD51, or βTRCP with a number of targets involved in DNA damage signalling have been found to be epigenetically silenced in different malignancies[14, 259–261].

Of note, however, while several additional resistance mechanisms including upregulation of developmental pathways, autophagy or stimuli from the microenvironment have been proposed, not all CSCs are necessarily resistant to standard therapies and CSC populations have been shown to be eradicated by chemo- or radiotherapy[241].

1.4 TARGETING THE SCF REGULATORY MACHINERY FOR CANCER THERAPY

General inhibition of the UPS by bortezomib and subsequently developed proteasome inhibitors carfilzomib and ixazomib has proven highly effective in treating some haematological malignancies[6]. Unfortunately, however, these initial successes could not be replicated in solid tumours and to date no proteasome inhibitor is approved for the treatment of cancers other than multiple myeloma and mantle cell lymphoma[5, 6]. While it is unclear why patients with solid cancers do not benefit from proteasome inhibitors, it is intuitively less surprising that generally inhibiting the plethora of cellular pathways regulated by the UPS may result in different outcomes based on disease context. For instance, Weyburne et al. demonstrated that therapeutically achievable concentrations of bortezomib or carfilzomib which mostly inhibit the β5 subunit of the 26S proteasome did not result in any appreciable cytotoxicity in xenografted triple-negative breast cancer cells which they attributed to residual proteasome activity through other subunits and an ensuing rebound effect by upregulation of proteasomal genes via the transcription factor NRF1[262]. Encouragingly, combination of bortezomib or carfilzomib and specific inhibitors targeting the β2 proteasome subunit resulted in re-sensitisation of xenografted cells and exhibited synergy in vitro[262].

Another feasible approach to overcoming resistance to inhibitors of the proteasome itself would be to target pathway components such as E3 ubiquitin ligases or DUBs with a select set of substrates. Accordingly, such inhibitors may be more tailored to specific disease context, limiting off-target effects. Indeed, more recently additional classes of putative pharmaceutical targets within the UPS have been investigated and specific inhibitors developed[263]. Due to their involvement in almost all aspects of cancer, their potential accessibility to pharmaceutical inhibition and their high substrate specificity the family of F-box proteins represents one such class of novel drug targets[264]. To facilitate rational drug development, detailed knowledge of the complete set of target substrates for distinct SCF ligases will be crucial.

Below I will provide a summary of treatment options and some considerations in regard to F-box proteins as therapy targets, focusing on the cancer types most relevant to the papers included in this thesis.

1.4.1 Targeting basal-like breast cancer

With 355 000 newly diagnosed cases in the EU-27 breast cancer is the most common cancer overall (13.3% of all cancers) and predictions expect 95 800 women to have succumbed to it in the EU-27 during 2020[265, 266]. These numbers are based on trends from previous years, however, which may be inaccurate due to reduced screening activity in the wake of the COVID-19 pandemic[267]. In turn, delays in diagnosis and treatment are expected to contribute to additional cancer mortality in following years[267].

There are different systems to define breast cancer sub-groups, but commonly they are classified based on the expression of specific markers, including the estrogen receptor (ER), progesterone receptor (PR), and HER-2 (ERBB2), which can be detected by immunohistochemistry and inform choices of targeted treatments[268]. Tumours which express ER and PR at low levels or not at all and do not overexpress HER-2 receptor are classified as triple-negative breast cancer (TNBC) [268]. These cancers are clinically aggressive and cannot be treated by endocrine therapy or targeting of HER-2[268]. Additionally, based on microarray profiling and recapitulated by proteome analysis at least 5 types of breast cancers with robust distinct molecular signatures are recognised today, basal-like, luminal A, luminal B, HER-2 and normal-like[269, 270]. The basal-like breast cancer (BLBC) group largely overlaps with TNBCs, containing approximately 80% of TNBCs. Due to the lack of targeted therapies these cancers are systemically treated with chemotherapy which has improved the outcome of BLBC/TNBC patients more greatly as compared to other breast cancers, despite the overall outcome after treatment being worse, nonetheless[268]. There is no consensus on chemotherapy used in this group but typically a combination of cyclophosphamide, methotrexate, and fluorouracil, sometimes with the addition of anthracyclines which has been reported to improve outcome [268, 271]. Additionally, cisplatin has been used in BRCA1-mutated breast cancers, molecularly mostly belonging to the BLBC group, Nonetheless, due to high treatment resistance and recurrence, options are limited and the development of targeted therapies for BLBC/TNBC is highly warranted.

Overexpression of cyclin E, e.g. epigenetically or through gene amplifications, is a frequently observed predictor of poor outcome in BLBC[272]. Furthermore, mutations of its negative regulator FBXW7, representing another mechanism of cyclin E upregulation, have been detected in breast cancer[17]. As discussed earlier, cyclin E overexpression causes severe replication stress which may represent a targetable vulnerability. Indeed, BLBC cell lines and patient-derived xenografts featuring cyclin E overexpression are highly sensitive to treatment with WEE1 inhibitor AZD1775[273]. We found that BLBC cell lines with low or no expression

of PTEN were hypersensitive and failed to recover following AZD1775 treatment [253]. Interestingly, treatment with AZD1775 downregulated cyclin E more effectively in PTEN-proficient BLBC cells, which was also associated with re-proliferation after treatment[253]. Currently, a phase II clinical trial is assessing the potential of AZD1775 in advanced solid tumours with cyclin E overexpression[273]. Furthermore, we and others found that the cytotoxic effects of AZD1775 could be potentiated by combination with ATR inhibitor AZD6738 regardless of PTEN levels, precluding regrowth of BLBC cell lines and xenografts and thereby broadening potential applications of AZD1775[253, 274–277].

Mutations in HR repair genes represent another Achilles' heel of some breast cancers. Olaparib and talazoparib, inhibitors of the BER regulator poly(ADP-ribose) polymerases (PARPs), act synergistically with HR defects and have been approved as therapeutical agents for breast cancer patients with germline mutations in BRCA1/2[278–280]. The great majority of cancers with mutations, both hereditary and somatic, in these critical FA/HR and cancer susceptibility genes molecularly belong to the BLBC subgroup thus representing another avenue for targeted treatments.

SCF^{EMI} ligase modulates sensitivity of BRCA mutated cells to PARPi by targeting RAD51 for proteasomal degradation[170]. In response to DSBs, CHK1 is activated, phosphorylates RAD51 to promote interaction with BRCA2, which in turn results in stabilisation of RAD51[170]. In the absence of BRCA2 RAD51 is thus depleted below a critical threshold required for HR repair[170]. BRCA-mutated cancers develop resistance in many instances and downregulation of EMI1 might represent one such mechanism[170].

Furthermore, BLBCs typically express higher levels of SKP2 and accordingly low levels of its primary proteolytic target p27 than do other breast cancer subtypes[268]. These changes are associated with an increase of the S-phase population, accelerated proliferation and dampened apoptosis[281]. Due to its unambiguously oncogenic effects small molecules inhibitors of SKP2 have been developed which have shown promise in preclinical models. One such inhibitor, compound #25, was identified using an *in silico* screen and was reported to prevent interaction between SKP2 and SKP1 and thus formation of a functional SCF complex[282]. Inhibition of SKP2 resulted in accumulation of p27 and triggered p53-independent apoptosis in cell lines[282]. Furthermore, #25 exhibited synergistic cytotoxicity when combined with cyclophosphamide or doxorubicin[282]. Additional SKP2 inhibitors were developed using a similar *in silico* approach based on the crystal structure of the ternary SKP2-CKS1-p27 complex, but in this case targeting a pocket at the interface between SKP2-CKS1 and p27[283].

Similarly to #25, these inhibitors potently prevented degradation of p27 accompanied by G1 arrest[283].

While a small number of other inhibitors targeting F-box proteins such as βTRCP have been reported with promising results *in vitro*, none have entered clinical trials so far[264, 284]. However, general inhibition of the SCF ligase by the neddylation inhibitor MLN4924/Pevonedistat, with neddylation of cullins being a prerequisite to SCF activation, has so far shown therapeutic potential in five completed phase I clinical trials of haematological and solid tumours and encouraged phase II and III trials currently recruiting patients[285].

1.4.2 Therapeutic options for medulloblastoma

Medulloblastoma (MB) is the most common childhood brain tumour with the median age of diagnosis at around 6 years[286]. MBs do occur in adults but the incidence is about 10-fold lower[286]. It is a tumour of the cerebellum suspected to arise from neural stem or progenitor cell populations already during foetal development[286]. Based on molecular profiles 4 groups of MB with clinical relevance are being recognised: Wingless-related integration site (WNT)-MB, sonic hedgehog (SHH)-MB, group 3 and group 4[287]. While WNT-MB has the most favourable prognosis and is typically non-metastatic at diagnosis, group 3 is the most aggressive with a <60% 5-year overall survival[286]. SHH-MB which is the most common type in infants and adults mostly has an intermediate prognosis[286]. Equally, Group 4 MB which is the most common type overall is associated with an intermediate prognosis[286]. Importantly, however, SHH-MB and groups 3 and 4 have recently been subdivided into additional groups with distinct clinical characteristics including patient survival[288].

MBs of all groups are treated by surgical resection of the tumour, removing a safe maximum of the tumour sparing healthy tissue[286]. This is in most cases followed by cranio-spinal irradiation, whose introduction resulted in great improvements in patient survival but also prompted serious side effects including permanent neurological damage associated with cognitive disabilities as well as secondary cancers[286]. Additionally, MB is treated with adjuvant multi-agent chemotherapy using cisplatin, carboplatin, vincristine, cyclophosphamide or lomustine[286]. Due to the severe and permanent side effects of current gold-standard MB treatment, development of targeted approaches is of utter importance.

The SHH pathway overactive in SHH-MB consists of the patched (PCH) receptor which inhibits its cognate receptor smoothened (SMO) but releases this inhibition upon binding of its

ligand SHH[289]. This in turn activates a downstream cascade through inactivation of suppressor of fused (SUFU) and activation of MYCN[289]. A mechanism of SUFU inactivation involves FBXL17 which mediates proteasomal destruction of SUFU[290]. Inhibition or degradation of SUFU drives gene expression changes via release of glioma-associated oncogene 1, 2 and 3 (GLI1/2/3) transcription factors[289]. All of these pathway components have been found to be mutated in SHH-MB with most activating mutations occurring upstream in PCH[286]. Interestingly mutations of S352 in SUFU present in MB patients result in increased proteasomal turn-over by SCF^{FBXL17} and accordingly pathway overactivation[290]. Such MB patients and others with an overly active SHH pathway could benefit from pharmacological inhibition of FBXL17. There are no such inhibitors but agents targeting SMO are available and are being investigated in clinical trials with promising first results indicating responses but at the same time revealing side effects in children pertaining to bone development associated with the physiological role of SHH signalling[291]. Downstream, another F-box protein, βTRCP, regulates the levels of GLI transcription factors after cAMP-dependent protein kinase (PKA) and GSK3β-dependent phosphorylation[292, 293].

β-TRCP also plays an important role in WNT-MB through degradation of β-catenin, the central transcription factor coordinating downstream WNT signalling which carries activating mutations in 86% of WNT-MB[286, 294]. Additionally, β-catenin protein turn-over is dependent on the tumour suppressor adenomatous polyposis coli (APC) which forms a complex with the scaffolding protein Axin, casein kinase 1 (CK1) and GSK3β to promote β-catenin phosphorylation and subsequent association with β-TRCP[295]. Intriguingly, a high proportion of β-catenin mutations in MB occur within these GSK3β phosphorylation sites and stabilise the protein[296, 297].

MYC is one of the most frequently altered oncogenes in MB and amplifications are associated with poor patient survival[286]. High-level amplifications are frequent in group 3 MB but less so in other groups, while amplifications of another MYC family member MYCN also commonly occur in SHH-MB and group 4 MB[298]. In line with its function as a crucial negative regulator of MYC protein stability, FBXW7 has been found to deleted, harbour recurrent mutations in its substrate interaction domains or be transcriptionally downregulated, particularly in SHH-MB but also group 3 MB (**paper IV**) [26]. Expression of CPD-mutated T58A n-myc, but not wildtype n-myc, in murine neural stem cells was sufficient to induce formation of different brain tumours upon orthotopic transplant[249]. Tumour type depended on the origin of the neural stem cell and activation of additional factors, whereby the FBXW7 target SOX9 was specifically associated with the formation of SHH-MB[26, 249]. As

mentioned earlier, increased expression of SOX9 resulted in resistance to cisplatin in MB cells[26]. This could be rescued by concurrent inhibition of PI3K/AKT/mTOR signalling which increased turn-over of SOX9 and may provide a strategy for the treatment of wildtype FBXW7-expressing MB (**paper IV**)[26].

General UPS inhibition by bortezomib has been shown to inhibit MB cells *in vitro* and in xenografted mice due to potent induction of caspase cleavage and apoptosis[299, 300]. Intriguingly, MB cells exhibited reduced levels of phosphorylated mTOR and AKT upon bortezomib treatment[300], which may represent a feedback mechanism to rescue GSK3β-dependent degradation. Despite its potency *in vitro* and in *in vivo* models, the clinical usability of bortezomib is limited as it does not readily cross the blood-brain barrier, a major obstacle for the development of targeted therapeutics for brain tumours[301, 302].

Analogously to other cancers G1/S transition is deregulated in MB. In some MBs, particularly group 4, CDK6 amplifications contribute to this phenotype[286]. The CDK4/6/cyclin D/RB pathway, however, is overly active also in the other non-WNT-MB and the application of CDK4/6 inhibitors aims at exploiting these alterations[303]. CDK4/6 inhibitors have shown great promise in patient-derived MB xenograft models and are now being investigated in clinical trials[286]. Besides CDKs, the critical S-phase onset promoter SKP2 is upregulated in MB coinciding with downregulation of its substrate p27[298, 304]. Deregulation of G1/S transition almost invariably results in replication stress. Indeed, MB have been demonstrated to be sensitive to WEE1 inhibition analogously to other cancers such as BLBC suffering from oncogene-induced replication stress, opening up yet another avenue for the development of novel targeted treatments[305].

2 DOCTORAL THESIS

2.1 AIMS

Following the discovery of the SCF ubiquitin ligase and the first member of the F-box family FBXO1/cyclin F, the significance of this complex for literally all cellular functions has gradually been revealed. The implications of deregulation of these regulatory nodes for the development and progression of human cancers have subsequently sparked great interest and efforts to reveal and disentangle the complete, highly interwoven substrate network of at least 69 human F-box proteins. Still, as of today only a handful of F-box proteins is well established as oncogenes or tumour suppressors, while the great majority remains minimally understood or incompletely characterised.

This thesis aimed to functionally characterise specific SCF ligases that contribute to oncogenic processes and define potential cancer vulnerabilities, ultimately guiding the development of novel treatment strategies.

Paper I aimed to identify F-box proteins regulating the response to oncogene-induced replication stress, an Achilles heel of cancer cells, and potential ways of targeting them.

Paper II aimed to determine potential markers of response and pathways of resistance using the clinically relevant WEE1 inhibitor AZD1775.

FBXO28 is among the most frequently altered F-box proteins across human cancers, yet no proteolytic targets had been identified until now. **Paper III** therefore aimed to discover unknown substrates of FBXO28 with implications for cancer development.

Paper IV aimed to functionally explore novel and clinically relevant FBXW7 substrates in MB which may be amenable to pharmacological modulation to improve patient outcome.

2.2 SUMMARY OF RESEARCH PAPERS

2.2.1 Paper I

Cancer cells experience high levels of DNA replication stress as a result of deregulated proliferation. Accordingly, activation of pathways mitigating these adverse effects in human malignancies may represent a vulnerability amenable to therapeutical intervention.

In paper I we initially focused on the FBXW7 target substrate cyclin E, which is a potent driver of replication stress and genetic instability when deregulated. To identify potential SCF ligases that support survival after cyclin E over-expression, we performed a loss-of-function screen individually depleting the majority of F-box genes by siRNAs and assessing their impact on the recovery from replication stress. Besides known regulators of linked processes such as FBXO18, which precludes unscheduled HR repair in response to hydroxyurea (HU) treatment, we identified FBXL12 as a novel putative regulator of replication re-start. Analysing the effect of FBXL12 knockdown at the level of individual replication forks by DNA fibre assays revealed that in the absence of FBXL12 a higher proportion of forks was stalling after release from an aphidicolin-induced replication block, which was accompanied by over-activation of dormant origins. Furthermore, induction of cyclin E over-expression in U2OS cells resulted in significantly reduced replication fork progression in the absence of FBXL12 while there was no clear difference at physiological levels of cyclin E, which supported the conclusion that FBXL12 is most relevant in response to oncogene-induced replication stress while it is less important in unchallenged cells. Intriguingly, cyclin E induction in this cell line model coincided with transcriptional upregulation of FBXL12. This relationship was corroborated by correlation of FBXL12 and cyclin E protein expression levels in breast cancer cell lines as well as enrichment of E2F pathway components in cell lines with FBXL12.

In line with an effect on replication fork speed, ablation of FBXL12 reduced S-phase progression and resulted in diminished proliferation of BLBC cells but not in untransformed cells. Markers of replication stress including pan-γH2AX or CHK1 phosphorylation on serine 345 were elevated in the absence of FBXL12, particularly in BLBCs with high cyclin E levels and already high levels of replication stress. Accordingly, we observed a high proportion of senescence markers and micronuclei indicative of mitotic problems in FBXL12-KO MDA-MB-231 cells.

To investigate the mechanism by which FBXL12 carries out its function, we immunopurified FBXL12 and interrogated its associated proteome of putative interactors. This resulted in the identification of interaction with FANCD2, as well as with its binding partner FANCI, which

was subsequently determined to occur primarily in the chromatin-associated cellular fraction. While binding of FANCI was dependent on the presence of FANCD2, that was not the case the other way around, suggesting that FANCD2 is the primary binding partner of FBXL12 in this complex. Importantly, we could show that this interaction results in polyubiquitylation and ensuing proteasomal degradation of chromatin-associated FANCD2. To our knowledge, this is the first demonstration of a ubiquitin ligase targeting FANCD2 for degradation. Intriguingly, activation of FA signalling by DNA damage-inducing agents such as mitomycin C (MMC) further increased the interaction and polyubiquitylation of FANCD2. Strikingly, we showed that FANCD2 could not be released from chromatin efficiently in the absence of FBXL12. Consequently, depletion of FBXL12 and trapping of FANCD2 on chromatin was accompanied by altered dynamics of the downstream FA effector RAD51. Furthermore, we tested whether FBXL12 and FANCD2 acted in the same pathway by combining siRNAs targeting both as compared to individual knock-down. Surprisingly, while FBXL12 or FANCD2 knockdown alone resulted in reduced replication fork speed accompanied by increased fork stalling and DNA damage signalling, combined knockdown restored these molecular phenotypes. The restored fork speed in FBXL12-FANCD2 co-depleted cells likely reflects residual FANCD2 activity as a result of incomplete depletion of FANCD2 expression by siRNAs. However, we cannot rule out that other pathways may contribute to the resolution of replication stress resulting from residual FANCD2 trapped on chromatin.

Mapping the FBXL12-interacting site of FANCD2 suggested that interaction occurs within the N-terminal region. Since SCF substrates are typically recognised and bound upon phosphorylation, and the N-terminus of FANCD2 contains predicted ATR, CHK1 and DNA-PK motifs, we tested whether one of these three major DDR kinases is involved in FBXL12-FANCD2 binding. Indeed, inhibition of both ATR as well as CHK1/CHK2 and subsequent mutagenesis of two potential CHK1 phosphorylation sites at serines 8 and 10 reduced FBXL12-FANCD2 interaction. Furthermore, FBXL12 efficiently promoted ubiquitylation of FANCD2 *in vitro* but failed to ubiquitylate a mutant in which serines 8 and 10 had been replaced by alanine, indicating that these sites are critical for interaction as well as ubiquitylation.

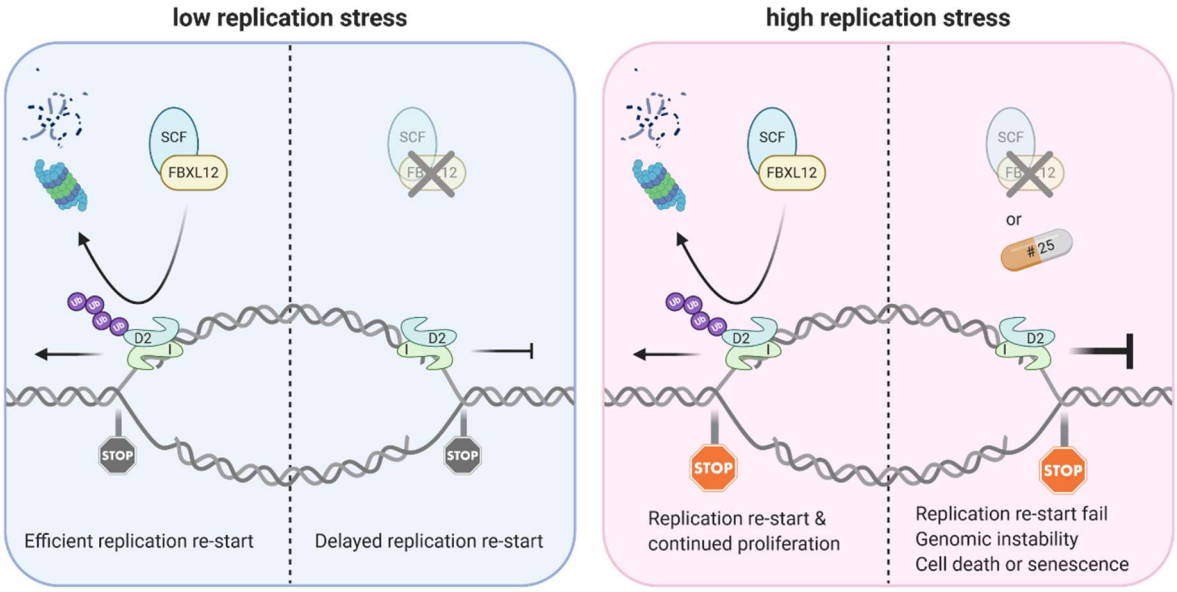

Fig. 5 Model summarising findings from paper I. SCF^{FBXL12} polyubiquitylates FANCD2 located at stalled replication forks resulting in its degradation, allowing access for downstream repair factors and efficient fork re-start. In the absence of FBXL12 under conditions of low replication stress in untransformed cells FANCD2 remains bound to chromatin for an extended period which favours dormant origin firing to rescue stalled replication forks (left box). On the other hand, under conditions of severe replication stress, e.g. due to cyclin E over-expression, FBXL12 promotes timely replication fork re-start while its absence leads to failure to re-start forks, exhaustion of dormant origins and either replication catastrophe or onset of mitosis with extensive under-replicated regions and ultimately cell death or senescence.

Based on the role of FBXL12 responding to oncogene (cyclin E)-induced replication stress through regulation of FANCD2, we wondered if FBXL12 also protects against pharmacologically induced replication stress. To this end, we utilised the WEE1 inhibitor AZD1775 which induces overactivity of CDKs and thus mechanistically exhibits parallels to overexpression of cyclin E. Expectedly, knockout of FBXL12 sensitised MDA-MB-231 cells to AZD1775 and prevented recovery after drug washout. Moreover, clearance of replication stress-associated DNA damage as marked by pan-γH2AX was significantly delayed in FBXL12-KO cells.

Based on sequence alignments of the F-box domain SKP2 is the most closely related paralogue of FBXL12. Due to its established role as an oncogene, small molecule compounds have been developed aiming to specifically inhibit SKP2. Chan et al. previously reported an inhibitor of SKP2, compound #25, that binds to its F-box domain and prevents formation of the SCF^{SKP2} complex resulting in upregulation of its substrate p27[282]. Despite a lack of structure data for FBXL12, its similarity to SKP2, particularly within the F-box, prompted us to explore whether this inhibitor might also bind FBXL12. Using cellular thermal shift assays (CETSA), which utilise the fact that protein thermostability is increased upon ligand binding, we found that #25 binds not only to SKP2 but also to FBXL12. Additionally, treatment with #25 increased

interaction between FBXL12 and FANCD2 suggesting reduced FBXL12-mediated FANCD2 turn-over. Finally, #25 synergised with AZD1775 in MDA-MB-231 cells, collectively indicating that this compound acts as inhibitor not only for SKP2 but also its most similar paralogue FBXL12.

Underlining the potential significance of the cyclin E-FBXL12 axis in cancer both proteins were correlated in human breast tumours and were significantly associated with poor breast cancer patient survival. Intriguingly, combined high versus low cyclin E and FBXL12 expression separated patient outcome more clearly than high and low expression of the cyclin E pathway alone. A trend which was also observed in other malignancies.

2.2.2 Paper II

AZD1775 potently induces replication stress resulting in DNA damage and cell death. Additionally, AZD1775 has shown great promise in clinical trials for the treatment of malignancies including breast cancer[273, 306] and as shown in **paper I**, inactivation of FBXL12 sensitises cells to AZD1775. While mutations or deletions of FBXL12 are rare in cancer, FBXL12 tends to be amplified and/or overexpressed in several malignancies. Although FBXL12 expression was found to correlate with cyclin E expression and high proliferation activity (**paper I**), a direct association between total FBXL12 expression levels and acute response to AZD1775 treatment was not evident in a panel of breast cancer cell lines profiled by transcriptomics and proteomics in the second paper.

Initially, we performed an image-based high-content screen of 16 breast cancer cell lines to assess acute response to AZD1775 mono-therapy. This revealed that BLBC cell lines were exquisitely sensitive to WEE1 inhibition as compared to luminal breast cancer cell lines. However, we also identified a sub-set of cell lines with reduced sensitivity to AZD1775 within the group of BLBC cell lines that also recovered post AZD1775 treatment.

Intriguingly, we found that AZD1775-sensitive cells continued to replicate DNA despite the presence of AZD1775-induced DNA damage, as revealed by EdU incorporation in γH2AX-positive cells. To investigate the molecular mechanism behind this phenotype and divergent sensitivities we carried out whole-proteome analysis and identified low PTEN expression as a major determinant of AZD1775 sensitivity. CRISPR-Cas9-mediated knock-out of PTEN sensitised resistant MDA-MB-231 cells to AZD1775 and prohibited recovery. Conversely,

expression of PTEN in PTEN-negative HCC38 resulted in reduced sensitivity to AZD1775 acutely and also allowed for recovery upon treatment wash-out.

This prompted us to combine AZD1775 with a panel of additional targeted drugs aiming to overcome re-proliferation and achieve more long-lasting effects. Supported by other studies, [274–277], we found that the ATR inhibitor AZD6738 exhibits potent synergy with AZD1775 while the PTEN low BLBCs, hypersensitive to AZD1775 did not exhibit any potentiating effect of combined treatment. AZD1775-resistant cells swiftly started to proliferate again and recovered following drug treatment and wash-out but completely failed to re-start upon AZD1775-AZD6738 combination treatment. This was supported by *in vivo* experiments which showed clear delay of tumour growth upon AZD1775-AZD6738 treatment as compared to either agent individually. Detailed analysis of the replicating cell population revealed severe replication stress resulting in an inability to clear DNA damage upon drug washout following combination treatment.

In an effort to identify additional components of the DDR that may regulate the ability to recover following AZD1775-induced replication arrest, we performed an siRNA-based viability screen of 300 DDR genes. In line with synergy upon ATR inhibition, HR repair pathway genes were significantly enriched among genes affecting sensitivity to AZD1775. Intriguingly, however, we found that DNA-PK depletion, an important regulator of NHEJ repair, significantly decreased viability in response to AZD1775. Furthermore, DNA-PK inhibitor NU7441 exhibited synergy with AZD1775 in a panel of BLBC cell lines. Mechanistically, we demonstrated that DNA-PK becomes phosphorylated and activated upon AZD1775 treatment and in the absence of ATR activity promotes phosphorylation of central ATR substrates, including RPA-serine 33 and CHK1-serine 345. Similar to PTEN-KO cells, DNA-PK depleted MDA-MB-231 cells did not recover following AZD1775 treatment which was linked to a lack of CHK1 activation and increased DNA damage in the EdU-positive replicating cell population.

Next, to investigate a possible link between AZD1775 hypersensitivity in PTEN- and DNA-PK-deficient cells, we interrogated DNA-PK activity and CHK1-seine 345 phosphorylation in PTEN-negative HCC38 cells which revealed increased DNA-PK activation but markedly alleviated CHK1 phosphorylation upon AZD1775 single agent treatment. These results indicate that DNA-PK-dependent sensitivity is likely not directly linked to PTEN while downstream CHK1 activation may play a role in both contexts. While PTEN was expressed at low levels in the sensitive BLBC sub-group, notably, cyclin E was highly expressed. Strikingly, in resistant MDA-MB-231 cells cyclin E was potently and rapidly down-regulated in response

to AZD1775 treatment. In stark contrast, both DNA-PK-KO and PTEN-KO exhibited failure or delay in reducing cyclin E protein levels, which provides another putative connection between these two determinants and AZD1775 sensitivity.

2.2.3 Paper III

Our group previously demonstrated a link between FBXO28 and increased MYC transcriptional activity and provided evidence for an oncogenic function of FBXO28 in breast cancer[73]. However, to date the existence of proteolytic SCF^{FBXO28} substrates in mammalian cells remain unknown. The work presented in paper III of this thesis reveals a novel regulatory mechanism involving the release of chromatin-bound FBXO28 and targeted destruction of ARHGEF6 and ARHGEF7.

Using affinity-purified FBXO28 and proteomics-based identification of interactors, we could show that while the majority of FBXO28 protein is restricted to chromatin, a small proportion of FBXO28 was found to interact with proteins not associated with chromatin. FBXO28 was also found to localise at distinct foci in the cell membrane where it colocalises with focal adhesion protein paxillin. Mass spectrometry analysis revealed interaction with several key cytoskeleton modulators including ARHGEF6 and ARHGEF7 as well as their interaction partners GIT1/2 and PAK1/2 proteins.

In line with nuclear localisation of the majority of FBXO28 protein, we found that FBXO28 binds tightly to chromatin, specifically chromatin enriched for histones with heterochromatin-associated modifications such as H3K9me2/3 or H4K20me1/2 as well as HP1. To explore this heterochromatin connection, we generated deletion mutants and identified a specific motif in the C-terminus of FBXO28 mediating chromatin association, RKSKR. Deletion or mutagenesis of this motif resulted in loss of chromatin binding while nuclear localisation was maintained. Curiously, this mutated version of FBXO28, subsequently referred to as KRmt, exhibited markedly increased interaction with ARHGEF6/7 proteins as shown by additional mass spectrometry assays comparing interactomes of FBXO28-WT and KRmt.

Next, we mapped the FBXO28 interaction sites of ARHGEF6/7 to their coiled-coil domains which are also responsible for binding to GIT proteins. Deletion of a short stretch within the coiled-coil domain, amino acids 768 to 776 in ARHGEF6, resulted in a complete loss of interaction with FBXO28.

Immunoprecipitation of FBXO28 revealed increased interaction with ARHGEF6/7 in the presence of MG-132 or MLN4924, specifically in the nucleoplasm but not on chromatin. In line with this observation, ARHGEF6/7 protein levels were almost completely abolished upon expression of the KRmt. While ARHGEF6/7 proteins were not degraded in the nucleoplasm of FBXO28-KO NIH3T3 cells, and minimally degraded after FBXO28-WT reconstitution, they were rapidly degraded upon FBXO28-KRmt expression. Accordingly, re-expression of FBXO28 in a HeLa FBXO28-KO model curtailed RAC1 activity in the nucleus and at the cell membrane through reduction of ARHGEF6/7 protein expression. While the major pool of ARHGEF6/7 proteins is located in the cytoplasm and at the cell membrane where they promote RAC1 and CDC42 activity, regulating cell motility and migration, the function of ARHGEF6/7-RAC1 in the nucleus is unknown. Collectively, our findings support a mechanism whereby FBXO28 bound to chromatin is unable to degrade ARHGEF6/7, while nucleoplasmic and membrane-bound ARHGEF6/7 is degraded when FBXO28 is released from chromatin.

In the search for factors releasing FBXO28 from chromatin, we noticed that induction of DNA damage by agents including neocarzinostatin (NCS), doxorubicin and etoposide increased FBXO28 interaction with ARHGEF6/7 in cells co-treated with MG-132. Indeed, FBXO28 was released upon doxorubicin treatment in a dose-dependent manner. These intriguing observations prompted us to focus on nuclear ARHGEF6/7 and a putative function related to the DDR. Recently, the RAC1 effector ARP2/3 complex has been reported to mediate HR repair by nucleating formation of actin filaments adjacent to DSBs resulting in movement of these sites outside of heterochromatin domains[214, 215]. This mechanism precluded erroneous recombination within repetitive heterochromatic regions and promoted faithful HR repair[214, 215]. Using high-content imaging-based quantification of γH2AX foci within heterochromatin domains, we could demonstrate that depletion of FBXO28 caused a delay in the clearance of heterochromatic DNA damage which could be rescued by the additional pharmacological attenuation of RAC1 activity. Furthermore, both overexpression of FBXO28 as well as siRNA-mediated depletion of ARHGEF7 resulted in reduced HR repair efficiency as assessed using the DR-GFP HR reporter assay. Equally, RAC1 and ARP2/3 inhibitors NSC23766 and CK-666, respectively, prohibited efficient HR repair. Finally, upon irradiation FBXO28-KO NIH3T3 cells exhibited an elevated number of 53BP1 foci, an NHEJ-promoting repair protein, as compared to FBXO28-WT reconstituted cells but still lower numbers than FBXO28-KRmt-expressing cells, which argued for a shift towards NHEJ in the absence of a functional FBXO28-ARHGEF6/7-RAC1-ARP2/3 axis. This interpretation was further

supported by the virtual absence of RAD51 foci in FBXO28-KRmt cells in response to irradiation.

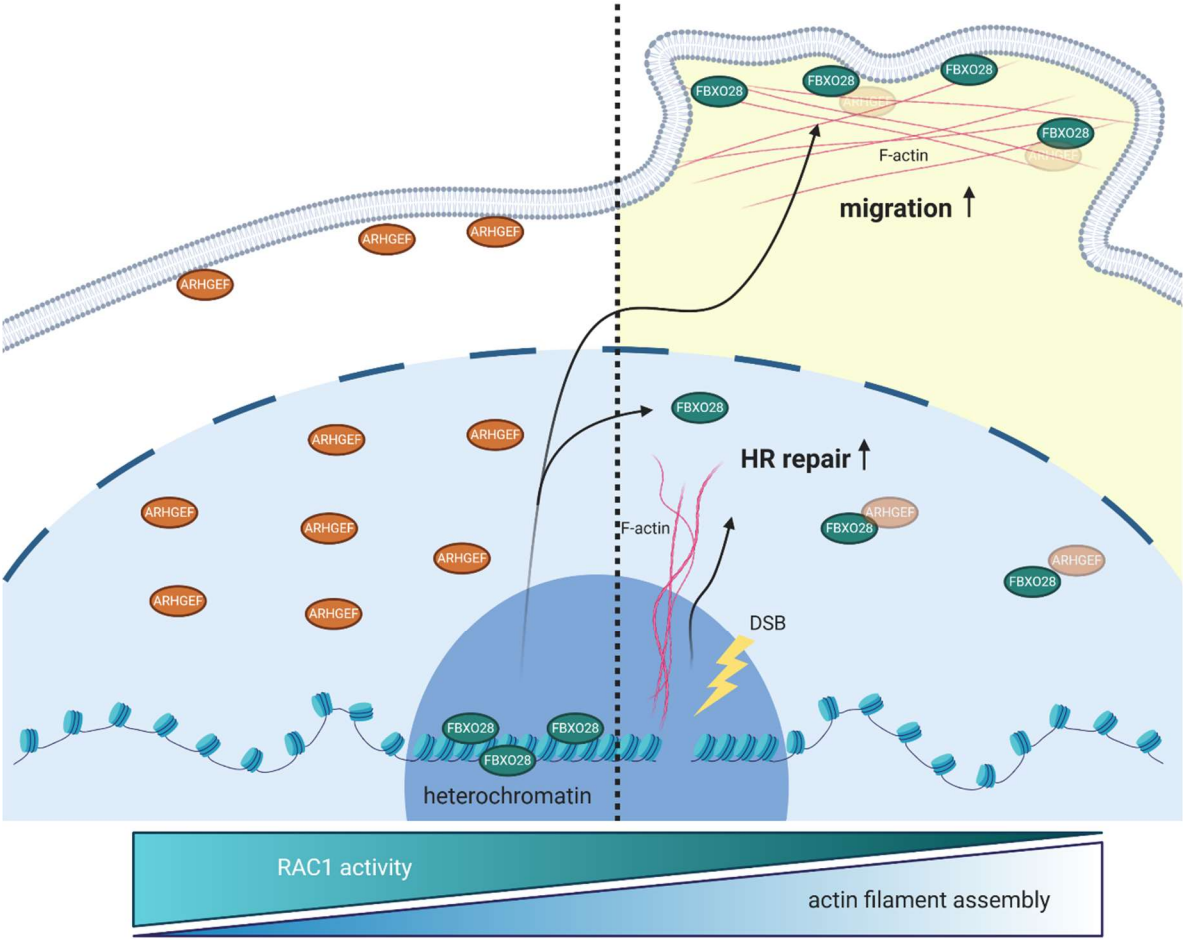

Fig. 6 Model summarising findings and conclusions from paper III. FBXO28 is tightly bound to heterochromatin where it cannot bind to its substrates. Upon release e.g. in response to induction of DSBs by IR or chemotherapy drugs, FBXO28 moves to the nucleoplasm and the membrane to ubiquitylate and degrade local ARHGEF6/7 pools. This in turn, results in reduced ARHGEF6/7-mediated RAC1 activation and allows for RAC1 inactivation-activation cycles that drive the dynamic assembly of actin filaments promoting DSB site relocation followed by HR repair in the nucleus and lamellipodia formation increasing migration at the cell membrane.

2.2.4 Paper IV

Despite the indisputable role of FBXW7 in carcinogenesis, detailed knowledge of how the FBXW7 pathway is altered in specific malignancies and in response to therapy is limited.

A combined approach to identify novel substrates of FBXW7, based on protein expression in FBXW7-WT and KO HCT116 cells and prediction of GSK3β phosphorylation and CPD motifs, shortlisted SOX9 as a putative substrate[28]. Using immunoprecipitation assays, we confirmed that FBXW7 and SOX9 interact and that this interaction depends on an evolutionarily conserved CPD motif phosphorylated by GSK3β. Consistently, mutagenesis of

this CPD motif, resulted in loss of interaction with FBXW7 and stabilisation of SOX9. Furthermore, depletion of FBXW7α but not the other isoforms resulted in marked stabilisation of SOX9 protein while over-expression of FBXW7α promoted turn-over of SOX9 in a proteasome-dependent manner. Accordingly, FBXW7 ubiquitylated SOX9 in vitro and cell lysate-based ubiquitylation assays. In contrast, mutagenesis of the FBXW7 substrate recognition domain, in this case R465A, or deletion of the F-box abolished SOX9 ubiquitylation. Regulation of SOX9 by FBXW7 was found in both untransformed cells and various cancer cell lines, including colorectal carcinoma, breast cancer, glioma and MB cell lines.

As SOX9 has previously been shown to determine MB sub-type, promote neural stem cell renewal and tumour development[249], we wondered if deregulation of FBXW7-mediated degradation affects these processes in MB. Therefore, we re-analysed whole-exome sequencing data from 133 SHH-MB tumours and identified FBXW7 mutations in about 11% of this cohort[307]. Many of these mutations were situated within the WD40 domains affecting three arginines crucial for substrate interaction, which suggests deregulation of substrates as the mechanistic basis for the presence of FBXW7 mutations in MB. Additionally, FBXW7 mRNA levels are low in MB, particularly in SHH-MB, as compared to normal cerebellum, and low expression levels are associated with poorer MB patient outcome. Although higher FBXW7 expression was linked to a significant survival advantage in group 3 MB, separation of low and high-risk groups based on FBXW7 expression was greatest in SHH-MB. In line with FBXW7-mediated SOX9 degradation as a putative determinant of MB malignancy, increased SOX9 protein levels were observed in tumours with lower FBXW7 mRNA expression and vice versa.

To determine if indeed SOX9 was the decisive target for FBXW7 in MB, presumably explaining its effect on patient survival, we orthotopically transplanted the human MB cell line DAOY overexpressing SOX9 either alone or together with FBXW7α into athymic nude Foxn1^{nu} mice and found that FBXW7α expression significantly prolonged survival and, strikingly, completely abolished metastasis formation. FBXW7 expression also reduced ability of DAOY cells to migrate in trans-well migration assays *in vitro*. The *in vivo* metastasis phenotype was reproduced using MB-inducing cells (MICs) isolated from the MYCN^{T58A} model. In this case, overexpression of SOX9 resulted in markedly increased metastatic spread as compared to MIC control cells. Furthermore, MB patients were significantly more likely to present with metastasis when tumours expressed high SOX9 protein, further supporting a clinical significance of the discovered mechanism.

To explore differential gene regulation as a result of increased SOX9 stabilisation in greater detail, we stably expressed SOX9-WT or the SOX9-CPDmt (T236/240A) mutant in MB002 cells, a human MB cell line, and profiled these cells by RNA sequencing. Among the gene sets most clearly upregulated in SOX9-WT as compared to control cells and further elevated upon expression of SOX9-CPDmt was the EMT gene set, encompassing upregulation of genes such as SLUG or vimentin. Gene set enrichment analysis further revealed that metastasis signatures were enriched upon induction of SOX9 overexpression, in line with clinical and *in vitro* migration and metastasis data above.

Based on the link between EMT, metastasis and drug resistance, we next asked whether stabilisation of SOX9 confers resistance to cisplatin, commonly used for the treatment of MB. Overexpression of SOX9 in MB002, DAOY and MICs increased cell viability upon cisplatin exposure which could be reverted by additional FBXW7α induction. Moreover, mice engrafted with DAOY-SOX9-WT or SOX9-CPDmt did not respond to cisplatin therapy, in contrast to mice transplanted with DAOY cells featuring endogenous SOX9 expression.

In the light of increased cisplatin resistance due to SOX9 upregulation, we reassessed our RNA sequencing datasets. We found DUSP2 and ATP7A, pointed out in previous reports as determinants of cisplatin resistance[308, 309], among the most differentially expressed genes between empty vector control and SOX9-CPDmt. Thus, these genes may underly increased cisplatin resistance in response to SOX9 overexpression.

These findings prompted us to explore ways to sensitise MBs exhibiting high SOX9 levels. Intriguingly, hyperactivation of PI3K/AKT/mTOR signalling in human MB is associated with poor survival[307] and AKT phosphorylates and inhibits GSK3[310], which in turn may mitigate SOX9 degradation. We hypothesized that pharmacological inhibition of PI3K/AKT/mTOR signalling might promote SOX9 turn-over. Indeed, treatment with AZD2014, AZD5363 or AZD8186 inhibiting mTOR, AKT and PI3K, respectively, enhanced FBXW7α-dependent turn-over of SOX9. Furthermore, AZD2014 and cisplatin synergistically reduced viability in MB cell lines but not upon expression of SOX9-CPDmt, which supports the idea that targeting SOX9 through pharmacological upregulation of GSK3β-FBXW7α-dependent degradation sensitises MBs to cisplatin chemotherapy.

2.3 DISCUSSION AND IMPLICATIONS

The papers presented in this thesis uncover novel ways of how the SCF ubiquitin ligase regulates pathways central to cancer hallmarks through its F-box protein subunits FBXL12, FBXO28 and FBXW7. Among these, FBXW7 is most frequently altered in human malignancies, either through deletions, mutations or epigenetic silencing, which via deregulation of its key proteolytic substrates cyclin E and MYC leads to hyperproliferation and the simultaneous onset of replication stress[34, 311–313].

In paper I we identified FBXL12-mediated degradation of FANCD2 as a crucial mechanism involved in counteracting such oncogene-induced replication stress. Depletion of FBXL12 resulted in failure to efficiently re-start replication under these conditions. This may be explained either by direct effects on factors involved in replication fork protection and re-start and/or more indirectly through deregulation of G1/S progression. The latter would be in line with previous studies implicating FBXL12 in the regulation of CDK inhibitors p21, p27 and p57[49, 52, 55]. However, FBXL12-dependent inhibition of p27 and p57 was shown to occur during thymocyte and osteoblast development, respectively, and their upregulation as a result of FBXL12 depletion would rather prevent G1/S transition[49, 52]. On the other hand, FBXL12 has been suggested to promote expression of p21 via the proteasome activator PA28γ[55]. In stark contrast, while we did observe interaction with PA28γ as well, we found p21 to rather be upregulated in the absence of FBXL12 which was associated with increased cellular senescence. Additionally, while progression within S-phase was markedly delayed, overall cell cycle profiles were only moderately affected by FBXL12 depletion, exhibiting a moderate increase in S-phase cells. Thus, CDK inhibitor-mediated deregulation of G1/S transition likely does not account for replication re-start problems.

Instead, we found that FBXL12 mediates degradation of the chromatin-associated, active pool of FANCD2. The FA pathway has previously been implicated in tolerance to replication stress, specifically the ID2 complex, exhibiting both FA core-dependent and -independent functions at stalled replication forks[171].

While the FA core complex encompassing the E3 ligase FANCL is responsible for monoubiquitylating the ID2 complex, USP1/UAF1 mediates the removal of the monoubiquitin moiety on FANCD2 which is required for the repair of ICL-induced lesions[182, 314]. Accordingly, ablation of USP1 results in an increase of chromatin-associated FANCD2-Ub concomitant with reduced FANCD2 foci formation and MMC hypersensitivity[314]. However, it is unclear if elevated FANCD2-Ub levels on chromatin are toxic, for example by

precluding access of down-stream repair factors, or if deubiquitylation is a prerequisite for subsequent repair steps. Depletion of both USP1 and FANCD2 did not rescue the MMC sensitivity which argues against the former mechanism[314]. Conversely, in the case of oncogene-induced replication stress, combined FBXL12 and FANCD2 knock-down improved replication fork speed and alleviated DNA damage as compared to FBXL12 depletion alone, indicating that high levels of chromatin-bound FANCD2 are indeed toxic under these circumstances.

How non-monoubiquitylated FANCD2 is being regulated has remained unclear and in the light of FA core-independent functions has become a more pressing question. To our knowledge, no E3 ligase targeting FANCD2 for proteasomal destruction has been reported to date. Intriguingly, non-ubiquitylated FANCD2 interacts with the MCM helicase to restrict replication fork progression in the presence of HU-induced replication stress[160]. Accordingly, we found that depletion of FBXL12 and concomitantly elevated FANCD2 levels lead to reduced replication fork speed. FANCI, on the other hand, prevents MCM activation at dormant origins during mild replication stress, which is counteracted by non-ubiquitylated FANCD2[161]. Of note, reduction of FANCD2 negatively affects FANCI levels and vice versa, which complicates interpretation of such data. Additionally, FANCD2 in concert with BRCA1 stabilises RAD51 filaments at stalled replication forks to protect ssDNA from excessive MRE11-dependent degradation[315, 316]. In line with this mechanism, increased FANCD2 due to loss of FBXL12 stabilised RAD51 foci, which were only temporally disrupted in response to induction of ICLs by MMC, possibly due to redistribution of RAD51 upon induction of additional DNA lesions.

Based on our findings, FBXL12 might be a potential novel target in cancer cells exhibiting high levels of replication stress, but to date no specific inhibitors for FBXL12 are available. However, by taking advantage of the wealth of knowledge surrounding the most closely related F-box protein, SKP2, we were able to pinpoint a SKP2 inhibitor that acts on FBXL12 and likely binds to the F-box domain of both SKP2 and FBXL12, thereby precluding ubiquitylation of their substrates. Interestingly, FBXL12-KO cells were hypersensitive to AZD1775 and, accordingly, this compound potently synergised with AZD1775. While we cannot exclude the possibility that synergy was due to stabilisation of SKP2 substrates, it is unlikely as increasing p27, the main SKP2 target, hinders G1/S transition which rather counteract the effects of WEE1 inhibition.

Collectively, our data are in line with a model whereby loss or pharmacological inhibition of FBXL12 results in stabilisation and trapping of FANCD2 at stalled replication forks, analogous

to PARP trapping by inhibitors[317], preventing efficient recruitment of down-stream factors or the switch to alternative pathways, effectively blocking repair. Throughout paper I we focused on cyclin E-induced replication stress. Despite this focus, we also found MYC signalling factors to be enriched together with FBXL12 and while FBXL12 was correlated with cyclin E in breast cancer cell lines and patient tumour samples, not all tumours highly expressing FBXL12 did express high levels of cyclin E. Thus, it is likely that FBXL12-dependent regulation of FANCD2 also impacts on the response to replication stress caused by additional oncogenes.

In paper II we found cyclin E downregulation to be associated with resilience to AZD1775 and recovery of BLBC cell lines. Accordingly, a recent report highlights high cyclin E levels in TNBCs as a determinant for AZD1775 sensitivity[273]. Although we did not investigate the mechanism of this downregulation in detail, given its rapid timing and our finding that deletion or inactivation of PTEN prevented this decrease, it is tempting to speculate that FBXW7 mediates cyclin E degradation in this context. Loss of PTEN results in overactivation of PI3K/AKT/mTOR signalling which in turn inhibits GSK3β activity and precludes recognition and degradation of many FBXW7 substrates including cyclin E[318, 319].

We sub-grouped BLBC cell lines into resistant and sensitive cell lines based on acute response to AZD1775. Global proteome and transcriptome analysis revealed low PTEN expression as one of the strongest predictors of AZD1775 sensitivity which was validated by PTEN knock-out in breast cancer cell lines. Furthermore, PTEN loss prevented recovery following AZD1775 wash-out, while re-expression in PTEN null cells promoted regrowth. Accordingly, these data support AZD1775 treatment as a potential strategy of targeting tumours with PTEN-inactivating mutations, which are exceedingly frequent in human malignancies and, importantly, endow cancers with increased resistance to other treatments including to targeted drugs such as PD-1 inhibitors or trastuzumab[320, 321]. Intriguingly, PTEN depletion results in increased levels of endogenous replication stress but promotes S-phase exit with under-replicated DNA which is visible as FANCD2-associated UFBs in mitosis[322]. Additionally, PTEN physically interacts with RAD51 and CHK1 which was proposed to result in stabilisation of RAD51 filaments at stalled forks[322]. Accordingly, we found that low PTEN expressing BLBCs fail to activate CHK1 and shut down replication in the presence of AZD1775-induced DNA damage.

Besides the dependency on PTEN, AZD1775 treatment potently induced replication stress in BLBCs and triggered activation of DNA-PK. DNA-PK was in turn required to activate CHK1 and install the replication checkpoint in the absence of ATR activity, which was in line with a

previous report demonstrating a DNA-PK-dependent backup mechanism to prevent excessive dormant origin firing under conditions of replication stress which could be abrogated by CHK1 inhibition[149]. Furthermore, we found that DNA-PK-dependent checkpoint activation also allowed for subsequent recovery and re-proliferation upon drug washout. Lastly, knock-out of DNA-PK prevented downregulation of cyclin E in response to AZD1775, analogously to PTEN ablation.

DNA-PK is mostly known for its role in NHEJ repair by mediating synapsis of DNA ends at a break, which are first recognised by the KU70/80 heterodimer and subsequently joined by DNA-PK catalytic subunit (DNA-PKcs) to form the entire DNA-PK complex[323]. NHEJ is believed to be the first-choice pathway for the repair of DSBs, while HR repair is employed only if a homologous template becomes available, typically the sister chromatid from S-phase and onwards[324]. Replication fork collapse as a consequence of severe replication stress results in the formation of one-ended DSBs which are an ideal substrate for homology-directed repair but cannot be faithfully repaired by NHEJ[324]. On the other hand, DSBs in regions with densely packed or highly repetitive sequences, namely heterochromatin, present challenges to HR repair[325].

In paper III we discovered a surprising link between FBXO28, RAC1 signalling and DSB repair pathway choice. FBXO28 is a mostly nuclear F-box protein with the capacity to bind chromatin regulating MYC transcriptional activity through non-proteolytic ubiquitylation[57, 73]. Querying the interactome of FBXO28 by mass spectrometry, we identified its first proteolytic substrates ARHGEF6 and ARHGEF7. These GEFs modulate RAC1 activity at the cell membrane to regulate cytoskeleton assembly and cell migration. Interestingly, we found FBXO28 to also be present outside of the nucleus and to colocalise with ARHGEFs at focal adhesions. In line with regulation of ARHGEF pools at the membrane, FBXO28 depletion impaired turn-over of focal adhesions and blocked migration.

In the nucleus we found FBXO28 to be enriched within heterochromatic regions which was dependent on a basic chromatin association motif (bCHAM) in its C-terminus. Moreover, a bCHAM mutant, referred to as KRmt above, unable to interact with heterochromatin was released, yet remained mostly nuclear and efficiently degraded nuclear ARHGEF6/7. A similar motif is present in the tail region of histone 3 and participates in the interaction with HP1 further supporting a function in tethering to heterochromatin[326].

Recently, ARP2/3 activity, a downstream mediator of RAC1 signalling, has been shown to be required for efficient HR-mediated repair of DSBs occurring in heterochromatin[214, 215].

ARP2/3 is recruited to DSBs formed within heterochromatin and destined to be repaired by HR and mediates F-actin nucleation at these sites[214, 215]. In mouse and *Drosophila* cells such DSBs are then rapidly moved outside of the heterochromatin domain and only there allowed to bind RAD51 and undergo the subsequent steps of HR[213–215]. In this context, the Smc5/6 complex is involved in preventing premature RAD51 association through an unknown mechanism[213]. Additionally, Smc5/6 recruits the myosin activator UNC45 which is equally required for break mobility and thus efficient repair[214]. So far, any regulatory mechanisms and factors upstream of the ARP2/3 complex involved in the mobility of DSBs have not been addressed. However, interestingly, RAC1 inhibition has been found to reduce H2AX phosphorylation and damage in response to topoisomerase inhibitors[327]. This may be due either to more efficient actin polymerisation or to a switch to NHEJ repair which exhibits faster kinetics but may be error-prone depending on the nature of the specific damage site[324]. Furthermore, GIT2 is phosphorylated by ATM and relocalises to IR-induced DSBs where it is required for repair by an unidentified mechanism[328]. GIT1/2 proteins form multimeric complexes with ARHGEF6/7 and together modulate RAC1 activity to determine cell polarity and motility[237]. However, if and how this might be mechanistically linked to DSB repair was not known by the time the present work described in this thesis started. By complementing the current picture of heterochromatin DSB repair upstream of ARP2/3 with the FBXO28-ARHGEF6/7-RAC1 axis, our data shed light on the underlying mechanisms behind these effects.

Finally, revisiting FBXW7, which regulates substrates including cyclin E, MYC or XRCC4[11, 13, 19–21, 47], an NHEJ protein, and thus impacts on all the cellular phenotypes investigated in the first three papers, we reported SOX9 as a novel substrate targeted for proteasomal degradation in paper IV. FBXW7 recognised SOX9 through a conserved CPD motif which required phosphorylation by GSK3β. Importantly, FBXW7 was mutated or downregulated in human MB which resulted in stabilisation of SOX9 and was associated with dismal patient prognosis.

The clinical significance of additional FBXW7 targets in MB, MYC and MYCN, raises the question of the relative importance of SOX9 deregulation. In this regard, Swartling et al. demonstrated that while expression of a stable non-degradable N-myc mutant, but not its wildtype version, was sufficient for the development of MBs in an orthotopic xenograft mouse model, additional high SOX9 expression was critical for the development of the SHH-MB subtype[249]. Furthermore, we found that SOX9 protein levels were high concurrently with low FBXW7 mRNA expression across all MB subtypes. Importantly, SOX9 expression

correlated with poor MB outcome and metastasis at diagnosis and we demonstrated that overexpression of the SOX9-CPDmt not targeted by FBXW7 resulted in extensive gene expression changes consistent with an enhanced capacity to migrate and metastasise. Increased metastasis and a more aggressive phenotype of high SOX9 expressing MBs could also be recapitulated in the orthotopically engrafted mouse models mentioned above.

Confirming our findings just weeks after paper IV was published, Hong et al. equally reported that SOX9 is targeted for degradation by FBXW7 in dependency of GSK3-mediated phosphorylation[27]. However, this study linked increased SOX9 turn-over to DNA damage through a mechanism surprisingly independent of the three apical DDR kinases ATM, ATR and DNA-PK or p53 status[27]. Appropriately, we showed that SOX9 degradation could be enhanced by pharmacological inhibition of the PI3K/AKT/mTOR signalling pathway, which in turn inhibits GSK3β, and thereby sensitise MB cells to cisplatin treatment. Collectively, this indicates that SOX9 mediates resistance to DNA-damaging agents that enhance its own FBXW7-dependent degradation. FBXW7 mutations have been implicated in both chemotherapy drug sensitivity and resistance depending on the type of agent employed[329]. The results above would be well in line with a treatment-induced strong selective pressure favouring the development of chemotherapy resistance based on mutations of FBXW7.

2.4 CONCLUDING REMARKS AND PERSPECTIVES

The SCF ubiquitin ligase and its substrates collectively impinge on every cellular function. The work presented in this thesis aimed to complement the picture of this intricate regulatory network and its implications in cancer with the ultimate goal of guiding the development of novel targeted cancer therapies.

We identified FBXL12-mediated degradation of FANCD2 as a targetable mechanism indispensable for cancer cells exhibiting high levels of oncogene-induced replication stress. While we used amplification and over-expression of cyclin E as a model oncogene to assess the replication stress response, the fact that high expression of FBXL12 and particularly FANCD2 correlates with markers of proliferation based on pan-cancer analysis and that high FBXL12 protein expressing tumours are also enriched for MYC signalling markers imply a more general involvement in oncogene-induced replication stress not restricted to activation of one particular oncogene.

Trapping of FANCD2 through pharmacological inactivation of FBXL12 may be a strategy to selectively kill these cancer cells or to sensitise to chemotherapy treatment in the case of cancers without endogenously high replication stress. However, compound #25, used here to inhibit FBXL12, needs to be used at micromolar concentrations and has first and foremost been identified as a SKP2 inhibitor, which triggers the accumulation of p27 resulting in a G1/S block[282, 330]. Thereby, it is rather expected to counteract the occurrence of replication stress. Accordingly, it will be important to develop additional FBXL12-selective inhibitors with improved pharmacological properties.

Intriguingly, an Fbxl12$^{-/-}$ mouse model has been reported and exhibits a similar phenotype as the Skp2$^{-/-}$ mouse in that both KO mice are born at a lower ratio than otherwise expected, indicating developmental problems, while mice that survive to term are smaller than their wildtype littermates but otherwise healthy[56, 331]. Furthermore, neither develop cancers spontaneously and Skp2 ablation in fact inhibited cancer development when crossed with Pten$^{+/-}$ mutants which is consistent with the oncogenic properties of Skp2[56, 331, 332]. Based on our findings, embryonal lethality of Fbxl12 deletion might be attributable to an inability to tackle replication stress in highly proliferative cells which remains to be determined *in vivo*.

After completion of embryonal development replication stress is mostly restricted to malignant cells which can be exacerbated by certain drug treatments[333, 334]. In paper II we aimed to identify determinants of the response to such an agent, the WEE1 inhibitor AZD1775 which has shown great promise in some clinical trials both as monotherapy and in combination with

chemotherapy drugs despite a lack of predictive biomarkers[306]. We showed that AZD1775-induced replication stress can be employed to target PTEN-null tumours which have proven to be clinically particularly challenging as they exhibit resistance to various other treatment strategies[320, 321]. BLBC cell lines expressing low levels of PTEN not only more strongly responded to AZD1775 treatment acutely but also failed to recover upon drug removal which was in contrast to PTEN-high BLBCs. Accordingly, these findings proffer PTEN as a predictive biomarker for AZD1775 treatment which could be incorporated into future clinical trials of BLBCs. Furthermore, remaining BLBCs, predicted to recur in response to AZD1775 monotherapy based on our data and reports from another group, may benefit from combined AZD1775 and ATR inhibitor treatment[253, 274]. In the absence of ATR activity and functional HR repair, the central NHEJ protein DNA-PK promoted activation of a CHK1-dependent replication checkpoint and, similarly to PTEN, DNA-PK status predicted sensitivity to AZD1775 alone.

In most contexts, DSB repair pathway choice depends on the availability of a homologous template that may enable HR-mediated repair as opposed to NHEJ[324]. Surprisingly, depletion of FBXO28 resulted in a shift towards NHEJ repair. Moreover, in paper III we identified ARHGEF6/7 as the first proteolytic substrates for human FBXO28 and demonstrated that this regulatory mechanism impinges on RAC1/ARP2/3-dependent heterochromatin DSB mobility which is required for their repair by HR. DSB formation resulted in release of FBXO28 from heterochromatin and subsequent targeting of nuclear but also membrane-associated ARHGEF6/7 which are implicated in cell migration[237]. Intriguingly, FBXO28 thus provides a mechanistic link between DNA damage formation and cell migration. This connection may be highly relevant for the treatment of cancers as standard chemotherapy has been linked to increased metastasis formation[335]. If and how this might be linked to FBXO28-mediated degradation of ARHGEF6/7 remains to be investigated.

Through its multitude of substrates FBXW7 links all the disparate functions discussed above and illustrates the significance of F-box genes in the regulation of cancer hallmarks. Adding to this complex network, we now identified SOX9 as a novel proteolytic FBXW7 target which is involved in MB malignancy promoting metastasis and drug resistance. Enhancing SOX9 turn-over using PI3K/AKT/mTOR inhibitors may provide an avenue for improved MB treatment to mitigate detrimental side effects seen with current standard therapies.

3 ACKNOWLEDGEMENTS

Allegedly, the acknowledgements receive more of readers' attention than any other section of a thesis, so I want to start it by mentioning some of the key findings once again:

- FBXL12 alleviates oncogenic replication stress by degrading FANCD2 and may be a cancer-specific target.
- WEE1 inhibition is a promising strategy to target basal-like breast cancers with low levels of PTEN or DNA-PK.
- FBXO28 degrades ARHGEF6/7 to promote cell migration as well as DNA repair, providing a direct link between the two.
- FBXW7 targets SOX9 for destruction which can be pharmacologically promoted to sensitise medulloblastoma to chemotherapy.

Mostly though, I want to use this opportunity to thank all the fantastic people who have supported me in one way or another during my time as a PhD student:

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4 REFERENCES

1. Wang Z, Liu P, Inuzuka H, Wei W (2014) Roles of F-box proteins in cancer. Nat Rev Cancer 14:233–247

2. Kwon YT, Ciechanover A (2017) The Ubiquitin Code in the Ubiquitin-Proteasome System and Autophagy. Trends Biochem Sci 42:873–886

3. Park J, Cho J, Song EJ (2020) Ubiquitin–proteasome system (UPS) as a target for anticancer treatment. Arch Pharm Res 43:1144–1161

4. Komander D, Clague MJ, Urbé S (2009) Breaking the chains: structure and function of the deubiquitinases. Nat Rev Mol Cell Biol 10:550–63

5. Dou QP, Zonder JA (2014) Overview of proteasome inhibitor-based anti-cancer therapies: perspective on bortezomib and second generation proteasome inhibitors versus future generation inhibitors of ubiquitin-proteasome system. Curr Cancer Drug Targets 14:517–36

6. Rolfe M (2017) The Holy Grail: Solid Tumor Efficacy by Proteasome Inhibition. Cell Chem Biol 24:125–126

7. Li W, Bengtson MH, Ulbrich A, Matsuda A, Reddy VA, Orth A, Chanda SK, Batalov S, Joazeiro CAP (2008) Genome-wide and functional annotation of human E3 ubiquitin ligases identifies MULAN, a mitochondrial E3 that regulates the organelle's dynamics and signaling. PLoS One 3:e1487

8. Uddin S, Bhat AA, Krishnankutty R, Mir F, Kulinski M, Mohammad RM (2016) Involvement of F-BOX proteins in progression and development of human malignancies. Semin Cancer Biol 36:18–32

9. Deshaies RJ, Joazeiro CAP (2009) RING domain E3 ubiquitin ligases. Annu Rev Biochem 78:399–434

10. Jin J (2004) Systematic analysis and nomenclature of mammalian F-box proteins. Genes Dev 18:2573–2580

11. Strohmaier H, Spruck CH, Kaiser P, Won K-A a, Sangfelt O, Reed SI (2001) Human F-box protein hCdc4 targets cyclin E for proteolysis and is mutated in a breast cancer cell line. Nature 413:316–322

12. Baumann U, Fernández-Sáiz V, Rudelius M, et al (2014) Disruption of the PRKCD-FBXO25-HAX-1 axis attenuates the apoptotic response and drives lymphomagenesis. Nat Med 20:1401–9

13. Welcker M, Orian A, Jin J, et al (2004) The Fbw7 tumor suppressor regulates glycogen synthase kinase 3 phosphorylation-dependent c-Myc protein degradation. Proc Natl Acad Sci U S A 101:9085–9090

14. Jeong Y-T, Rossi M, Cermak L, Saraf A, Florens L, Washburn MP, Sung P, Schildkraut CL, Schildkraut CL, Pagano M (2013) FBH1 promotes DNA double-strand breakage and apoptosis in response to DNA replication stress. J Cell Biol 200:141–9

15. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: The next generation. Cell

144:646–674

16. Lawrence MS, Stojanov P, Mermel CH, Robinson JT, Garraway LA, Golub TR, Meyerson M, Gabriel SB, Lander ES, Getz G (2014) Discovery and saturation analysis of cancer genes across 21 tumour types. Nature 505:495–501

17. Akhoondi S, Sun D, von der Lehr N, et al (2007) FBXW7/hCDC4 Is a General Tumor Suppressor in Human Cancer. Cancer Res 67:9006–9012

18. Yumimoto K, Nakayama KI (2020) Recent insight into the role of FBXW7 as a tumor suppressor. Semin Cancer Biol 67:1–15

19. Moberg KH, Bell DW, Wahrer DCR, Haber DA, Hariharan IK (2001) Archipelago regulates Cyclin E levels in Drosophila and is mutated in human cancer cell lines. Nature 413:311–316

20. Koepp DM, Schaefer LK, Ye X, Keyomarsi K, Chu C, Harper JW, Elledge SJ (2001) Phosphorylation-dependent ubiquitination of cyclin E by the SCFFbw7 ubiquitin ligase. Science 294:173–7

21. Yada M, Hatakeyama S, Kamura T, Nishiyama M, Tsunematsu R, Imaki H, Ishida N, Okumura F, Nakayama KIK, Nakayama KIK (2004) Phosphorylation-dependent degradation of c-Myc is mediated by the F-box protein Fbw7. EMBO J 23:2116–2125

22. Inuzuka H, Shaik S, Onoyama I, et al (2011) SCFFBW7 regulates cellular apoptosis by targeting MCL1 for ubiquitylation and destruction. Nature 471:104–109

23. Wertz IE, Kusam S, Lam C, et al (2011) Sensitivity to antitubulin chemotherapeutics is regulated by MCL1 and FBW7. Nature 471:110–4

24. Oberg C, Li J, Pauley A, Wolf E, Gurney M, Lendahl U (2001) The Notch intracellular domain is ubiquitinated and negatively regulated by the mammalian Sel-10 homolog. J Biol Chem 276:35847–53

25. Gupta-Rossi N, Le Bail O, Gonen H, Brou C, Logeat F, Six E, Ciechanover A, Israël A (2001) Functional Interaction between SEL-10, an F-box Protein, and the Nuclear Form of Activated Notch1 Receptor. J Biol Chem 276:34371–34378

26. Suryo Rahmanto A, Savov V, Brunner A, et al (2016) FBW7 suppression leads to SOX9 stabilization and increased malignancy in medulloblastoma. EMBO J 35:2192–2212

27. Hong X, Liu W, Song R, et al (2016) SOX9 is targeted for proteasomal degradation by the E3 ligase FBW7 in response to DNA damage. Nucleic Acids Res 44:8855–8869

28. Arabi A, Ullah K, Branca RMM, et al (2012) Proteomic screen reveals Fbw7 as a modulator of the NF-κB pathway. Nat Commun 3:976

29. Orlicky S, Tang X, Willems A, Tyers M, Sicheri F (2003) Structural basis for phosphodependent substrate selection and orientation by the SCFCdc4 ubiquitin ligase. Cell 112:243–56

30. Welcker M, Larimore EA, Frappier L, Clurman BE (2011) Nucleolar targeting of the fbw7 ubiquitin ligase by a pseudosubstrate and glycogen synthase kinase 3. Mol Cell Biol 31:1214–24

31. Nash P, Tang X, Orlicky S, Chen Q, Gertler FB, Mendenhall MD, Sicheri F, Pawson

T, Tyers M (2001) Multisite phosphorylation of a CDK inhibitor sets a threshold for the onset of DNA replication. Nature 414:514–21

32. Welcker M, Clurman BE (2007) Fbw7/hCDC4 dimerization regulates its substrate interactions. Cell Div 2:7

33. Davis RJ, Welcker M, Clurman BE (2014) Tumor suppression by the Fbw7 ubiquitin ligase: mechanisms and opportunities. Cancer Cell 26:455–64

34. Malyukova A, Dohda T, von der Lehr N, et al (2007) The tumor suppressor gene hCDC4 is frequently mutated in human T-cell acute lymphoblastic leukemia with functional consequences for Notch signaling. Cancer Res 67:5611–6

35. Spruck CH, Strohmaier H, Sangfelt O, Muller HM, Hubalek M, Muller-Holzner E, Marth C, Widschwendter M, Reed SI (2002) hCDC4 Gene Mutations in Endometrial Cancer. Cancer Res 62:4535–4539

36. Sangfelt O, Cepeda D, Malyukova A, van Drogen F, Reed SI (2008) Both SCF Cdc4α and SCF Cdc4γ are required for cyclin E turnover in cell lines that don't overexpress cyclin E. Cell Cycle 7:1075–1082

37. van Drogen F, Sangfelt O, Malyukova A, Matskova L, Yeh E, Means AR, Reed SI (2006) Ubiquitylation of cyclin E requires the sequential function of SCF complexes containing distinct hCdc4 isoforms. Mol Cell 23:37–48

38. Grinkevich V V., Nikulenkov F, Shi Y, Enge M, Bao W, Maljukova A, Gluch A, Kel A, Sangfelt O, Selivanova G (2009) Ablation of Key Oncogenic Pathways by RITA-Reactivated p53 Is Required for Efficient Apoptosis. Cancer Cell 15:441–453

39. Galindo-Moreno M, Giráldez S, Limón-Mortés MC, Belmonte-Fernández A, Reed SI, Sáez C, Japón M, Tortolero M, Romero F (2019) SCF(FBXW7)-mediated degradation of p53 promotes cell recovery after UV-induced DNA damage. FASEB J 33:11420–11430

40. Tripathi V, Kaur E, Kharat SS, Hussain M, Damodaran AP, Kulshrestha S, Sengupta S (2019) Abrogation of FBW7α-dependent p53 degradation enhances p53's function as a tumor suppressor. J Biol Chem 294:13224–13232

41. Cui D, Xiong X, Shu J, Dai X, Sun Y, Zhao Y (2020) FBXW7 Confers Radiation Survival by Targeting p53 for Degradation. Cell Rep 30:497-509.e4

42. Lerner M, Lundgren J, Akhoondi S, et al (2011) MiRNA-27a controls FBW7/hCDC4-dependent cyclin E degradation and cell cycle progression. Cell Cycle 10:2172–2183

43. Min SH, Lau AW, Lee TH, et al (2012) Negative Regulation of the Stability and Tumor Suppressor Function of Fbw7 by the Pin1 Prolyl Isomerase. Mol Cell 46:771–783

44. Ji S, Qin Y, Shi S, et al (2015) ERK kinase phosphorylates and destabilizes the tumor suppressor FBW7 in pancreatic cancer. Cell Res 25:561–573

45. Diefenbacher ME, Popov N, Blake SM, Schülein-Völk C, Nye E, Spencer-Dene B, Jaenicke LA, Eilers M, Behrens A (2014) The deubiquitinase USP28 controls intestinal homeostasis and promotes Colorectal cancer. J Clin Invest 124:3407–3418

46. Schülein-Völk C, Wolf E, Zhu J, et al (2014) Dual regulation of Fbw7 function and

oncogenic transformation by Usp28. Cell Rep 9:1099–1109

47. Zhang Q, Karnak D, Tan M, Lawrence TS, Morgan MA, Sun Y (2016) FBXW7 Facilitates Nonhomologous End-Joining via K63-Linked Polyubiquitylation of XRCC4. Mol Cell 61:419–433

48. Uhlén M, Fagerberg L, Hallström BM, et al (2015) Proteomics. Tissue-based map of the human proteome. Science 347:1260419

49. Zhao B, Yoganathan K, Li L, Lee JY, Zúñiga-Pflücker JC, Love PE (2019) Notch and the pre-TCR coordinate thymocyte proliferation by induction of the SCF subunits Fbxl1 and Fbxl12. Nat Immunol 20:1381–1392

50. Mallampalli RK, Kaercher L, Snavely C, Pulijala R, Chen BB, Coon T, Zhao J, Agassandian M (2013) Fbxl12 triggers G1 arrest by mediating degradation of calmodulin kinase I. Cell Signal 25:2047–2059

51. Kamura T, Hara T, Kotoshiba S, Yada M, Ishida N, Imaki H, Hatakeyama S, Nakayama KKI, Nakayama KKI (2003) Degradation of p57Kip2 mediated by SCFSkp2-dependent ubiquitylation. Proc Natl Acad Sci U S A 100:10231–6

52. Kim M, Nakamoto T, Nishimori S, Tanaka K, Chiba T (2008) A new ubiquitin ligase involved in p57KIP2 proteolysis regulates osteoblast cell differentiation. EMBO Rep 9:878–884

53. Carrano AC, Eytan E, Hershko A, Pagano M (1999) SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. Nat Cell Biol 1:193–199

54. Yu Z-K, Gervais JLM, Zhang H (1998) Human CUL-1 associates with the SKP1/SKP2 complex and regulates p21CIP1/WAF1 and cyclin D proteins. Proc Natl Acad Sci 95:11324–11329

55. Tsuruta F, Takebe A, Haratake K, et al (2016) SCF Fbl12 Increases p21 Waf1/Cip1 Expression Level through Atypical Ubiquitin Chain Synthesis. Mol Cell Biol 36:2182–2194

56. Nishiyama M, Nita A, Yumimoto K, Nakayama KI (2015) FBXL12-Mediated Degradation of ALDH3 is Essential for Trophoblast Differentiation During Placental Development. Stem Cells 33:3327–40

57. Postow L, Funabiki H (2013) An SCF complex containing Fbxl12 mediates DNA damage-induced Ku80 ubiquitylation. Cell Cycle 12:587–595

58. Scully R, Panday A, Elango R, Willis NA (2019) DNA double-strand break repair-pathway choice in somatic mammalian cells. Nat Rev Mol Cell Biol 20:1–17

59. Feng L, Chen J (2012) The E3 ligase RNF8 regulates KU80 removal and NHEJ repair. Nat Struct Mol Biol 19:201–206

60. Malu S, Malshetty V, Francis D, Cortes P (2012) Role of non-homologous end joining in V(D)J recombination. Immunol Res 54:233–246

61. López-Rodríguez C, Aramburu J, Berga-Bolaños R (2015) Transcription factors and target genes of pre-TCR signaling. Cell Mol Life Sci 72:2305–2321

62. Nita A, Nishiyama M, Muto Y, Nakayama KI (2016) FBXL12 regulates T-cell differentiation in a cell-autonomous manner. Genes to Cells 21:517–524

63. Li X, Zhao Q, Liao R, Sun P, Wu X (2003) The SCFSkp2 ubiquitin ligase complex interacts with the human replication licensing factor Cdt1 and regulates Cdt1 degradation. J Biol Chem 278:30854–30858

64. Nakayama K (2000) Targeted disruption of Skp2 results in accumulation of cyclin E and p27Kip1, polyploidy and centrosome overduplication. EMBO J 19:2069–2081

65. Nie L, Xu M, Vladimirova A, Sun XH (2003) Notch-induced E2A ubiquitination and degradation are controlled by MAP kinase activities. EMBO J 22:5780–5792

66. Von Der Lehr N, Johansson S, Wu S, et al (2003) The F-box protein Skp2 participates in c-Myc proteosomal degradation and acts as a cofactor for c-Myc-regulated transcription. Mol Cell 11:1189–1200

67. Méndez J, Zou-Yang XH, Kim SY, Hidaka M, Tansey WP, Stillman B (2002) Human origin recognition complex large subunit is degraded by ubiquitin-mediated proteolysis after initiation of DNA replication. Mol Cell 9:481–491

68. Sutterlüty H, Chatelain E, Marti A, Wirbelauer C, Senften M, Müller U, Krek W (1999) p45SKP2 promotes p27Kip1 degradation and induces S phase in quiescent cells. Nat Cell Biol 1:207–14

69. Tsvetkov LM, Yeh K-H, Lee S-J, Sun H, Zhang H (1999) p27Kip1 ubiquitination and degradation is regulated by the SCFSkp2 complex through phosphorylated Thr187 in p27. Curr Biol 9:661-S2

70. Jiang H, Chang FC, Ross AE, Lee J, Nakayama K, Nakayama K, Desiderio S (2005) Ubiquitylation of RAG-2 by Skp2-SCF links destruction of the V(D)J recombinase to the cell cycle. Mol Cell 18:699–709

71. Cerami E, Gao J, Dogrusoz U, et al (2012) The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2:401–4

72. Gao J, Aksoy BA, Dogrusoz U, et al (2013) Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 6:pl1

73. Cepeda D, Ng H-F, Sharifi HR, et al (2013) CDK-mediated activation of the SCF FBXO 28 ubiquitin ligase promotes MYC-driven transcription and tumourigenesis and predicts poor survival in breast cancer. EMBO Mol Med 5:1067–1086

74. Fagerholm R, Khan S, Schmidt MK, et al (2017) TP53-based interaction analysis identifies cis-eQTL variants for TP53BP2, FBXO28, and FAM53A that associate with survival and treatment outcome in breast cancer. Oncotarget 8:18381–18398

75. Kratz A-S, Richter KT, Schlosser YT, Schmitt M, Shumilov A, Delecluse H-J, Hoffmann I (2016) Fbxo28 promotes mitotic progression and regulates topoisomerase IIα-dependent DNA decatenation. Cell Cycle 15:3419–3431

76. Xiao H, Wang H, Silva EA, Thompson J, Guillou A, Yates JR, Buchon N, Franc NC (2015) The pallbearer E3 ligase promotes actin remodeling via RAC in efferocytosis by degrading the ribosomal protein S6. Dev Cell 32:19–30

77. Liu J, Ma J (2013) Dampened regulates the activating potency of Bicoid and the embryonic patterning outcome in Drosophila. Nat Commun 4:2968

78. Hanahan D, Weinberg RA (2000) The Hallmarks of Cancer. Cell 100:57–70

79. Bassermann F, Eichner R, Pagano M (2014) The ubiquitin proteasome system — Implications for cell cycle control and the targeted treatment of cancer. Biochim Biophys Acta - Mol Cell Res 1843:150–162

80. Ferrell JE (2013) Feedback loops and reciprocal regulation: recurring motifs in the systems biology of the cell cycle. Curr Opin Cell Biol 25:676–86

81. Vermeulen K, Van Bockstaele DR, Berneman ZN (2003) The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. Cell Prolif 36:131–149

82. Cappell SD, Chung M, Jaimovich A, Spencer SL, Meyer T (2016) Irreversible APCCdh1 Inactivation Underlies the Point of No Return for Cell-Cycle Entry. Cell 166:167–180

83. Lemmens B, Lindqvist A (2019) DNA replication and mitotic entry: A brake model for cell cycle progression. J Cell Biol 218:3892–3902

84. Tsvetkov LM, Yeh KH, Lee SJ, Sun H, Zhang H (1999) p27(Kip1) ubiquitination and degradation is regulated by the SCF(Skp2) complex through phosphorylated Thr187 in p27. Curr Biol 9:661–664

85. Bretones G, Delgado MD, León J (2015) Myc and cell cycle control. Biochim Biophys Acta 1849:506–16

86. Kim SY, Herbst A, Tworkowski KA, Salghetti SE, Tansey WP (2003) Skp2 regulates Myc protein stability and activity. Mol Cell 11:1177–1188

87. Bashir T, Dorrello NV, Amador V, Guardavaccaro D, Pagano M (2004) Control of the SCFSkp2–Cks1 ubiquitin ligase by the APC/CCdh1 ubiquitin ligase. Nature 428:190–193

88. Wei W, Ayad NG, Wan Y, Zhang G-J, Kirschner MW, Kaelin WG (2004) Degradation of the SCF component Skp2 in cell-cycle phase G1 by the anaphase-promoting complex. Nature 428:194–198

89. Donzelli M (2002) Dual mode of degradation of Cdc25 A phosphatase. EMBO J 21:4875–4884

90. Stewart S, Fang G (2005) Destruction Box–Dependent Degradation of Aurora B Is Mediated by the Anaphase-Promoting Complex/Cyclosome and Cdh1. Cancer Res 65:8730–8735

91. Honda K, Mihara H, Kato Y, Yamaguchi A, Tanaka H, Yasuda H, Furukawa K, Urano T (2000) Degradation of human Aurora2 protein kinase by the anaphase-promoting complex-ubiquitin-proteasome pathway. Oncogene 19:2812–2819

92. Geley S, Kramer E, Gieffers C, Gannon J, Peters J-M, Hunt T (2001) Anaphase-Promoting Complex/Cyclosome–Dependent Proteolysis of Human Cyclin a Starts at the Beginning of Mitosis and Is Not Subject to the Spindle Assembly Checkpoint. J Cell Biol 153:137–148

93. Hsu JY, Reimann JDR, Sørensen CS, Lukas J, Jackson PK (2002) E2F-dependent accumulation of hEmi1 regulates S phase entry by inhibiting APCCdh1. Nat Cell Biol 4:358–366

94. Miller JJ, Summers MK, Hansen D V., Nachury M V., Lehman NL, Loktev A, Jackson PK (2006) Emi1 stably binds and inhibits the anaphase-promoting complex/cyclosome as a pseudosubstrate inhibitor. Genes Dev 20:2410–2420

95. Listovsky T, Oren YS, Yudkovsky Y, Mahbubani HM, Weiss AM, Lebendiker M, Brandeis M (2004) Mammalian Cdh1/Fzr mediates its own degradation. EMBO J 23:1619–1626

96. Rape M, Kirschner MW (2004) Autonomous regulation of the anaphase-promoting complex couples mitosis to S-phase entry. Nature 432:588–595

97. Lukas C, Sørensen CS, Kramer E, Santoni-Rugiu E, Lindeneg C, Peters J-M, Bartek J, Lukas J (1999) Accumulation of cyclin B1 requires E2F and cyclin-A-dependent rearrangement of the anaphase-promoting complex. Nature 401:815–818

98. Fukushima H, Ogura K, Wan L, et al (2013) SCF-mediated Cdh1 degradation defines a negative feedback system that coordinates cell-cycle progression. Cell Rep 4:803–816

99. Leslie NR, Downes CP (2004) PTEN function: how normal cells control it and tumour cells lose it. Biochem J 382:1–11

100. Lee Y-R, Chen M, Pandolfi PP (2018) The functions and regulation of the PTEN tumour suppressor: new modes and prospects. Nat Rev Mol Cell Biol 19:547–562

101. Li J, Yen C, Liaw D, et al (1997) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275:1943–7

102. Song MS, Carracedo A, Salmena L, Song SJ, Egia A, Malumbres M, Pandolfi PP (2011) Nuclear PTEN Regulates the APC-CDH1 Tumor-Suppressive Complex in a Phosphatase-Independent Manner. Cell 144:187–199

103. Ge M-K, Zhang N, Xia L, et al (2020) FBXO22 degrades nuclear PTEN to promote tumorigenesis. Nat Commun 11:1720

104. Hills SA, Diffley JFXX (2014) DNA replication and oncogene-induced replicative stress. Curr Biol 24:R435–R444

105. Macheret M, Halazonetis TD (2018) Intragenic origins due to short G1 phases underlie oncogene-induced DNA replication stress. Nature 555:112–116

106. Liu E, Li X, Yan F, Zhao Q, Wu X (2004) Cyclin-dependent Kinases Phosphorylate Human Cdt1 and Induce Its Degradation. J Biol Chem 279:17283–17288

107. Mailand N, Diffley JFX (2005) CDKs Promote DNA Replication Origin Licensing in Human Cells by Protecting Cdc6 from APC/C-Dependent Proteolysis. Cell 122:915–926

108. Walter D, Hoffmann S, Komseli E-S, Rappsilber J, Gorgoulis V, Sørensen CS (2016) SCFCyclin F-dependent degradation of CDC6 suppresses DNA re-replication. Nat Commun 7:10530

109. Liontos M, Koutsami M, Sideridou M, et al (2007) Deregulated overexpression of hCdt1 and hCdc6 promotes malignant behavior. Cancer Res 67:10899–909

110. Lin DI, Barbash O, Kumar KGS, Weber JD, Harper JW, Klein-Szanto AJP, Rustgi A, Fuchs SY, Diehl JA (2006) Phosphorylation-Dependent Ubiquitination of Cyclin D1

by the SCFFBX4-αB Crystallin Complex. Mol Cell 24:355–366

111. Ilves I, Petojevic T, Pesavento JJ, Botchan MR (2010) Activation of the MCM2-7 Helicase by Association with Cdc45 and GINS Proteins. Mol Cell 37:247–258

112. Woodfine K, Fiegler H, Beare DM, Collins JE, McCann OT, Young BD, Debernardi S, Mott R, Dunham I, Carter NP (2004) Replication timing of the human genome. Hum Mol Genet 13:191–202

113. Parker LL, Piwnica-Worms H (1992) Inactivation of the p34cdc2-cyclin B complex by the human WEE1 tyrosine kinase. Science 257:1955–7

114. McGowan CH, Russell P (1993) Human Wee1 kinase inhibits cell division by phosphorylating p34cdc2 exclusively on Tyr15. EMBO J 12:75–85

115. Liu F, Stanton JJ, Wu Z, Piwnica-Worms H (1997) The human Myt1 kinase preferentially phosphorylates Cdc2 on threonine 14 and localizes to the endoplasmic reticulum and Golgi complex. Mol Cell Biol 17:571–583

116. Elbæk CR, Petrosius V, Sørensen CS (2020) WEE1 kinase limits CDK activities to safeguard DNA replication and mitotic entry. Mutat Res Mol Mech Mutagen 819–820:111694

117. Gu Y, Rosenblatt J, Morgan DO (1992) Cell cycle regulation of CDK2 activity by phosphorylation of Thr160 and Tyr15. EMBO J 11:3995–4005

118. Donzelli M, Draetta GF (2003) Regulating mammalian checkpoints through Cdc25 inactivation. EMBO Rep 4:671–677

119. Smith A, Simanski S, Fallahi M, Ayad NG (2007) Redundant Ubiquitin Ligase Activities Regulate Wee1 Degradation and Mitotic Entry. Cell Cycle 6:2795–2799

120. Watanabe N, Arai H, Nishihara Y, Taniguchi M, Watanabe N, Hunter T, Osada H (2004) M-phase kinases induce phospho-dependent ubiquitination of somatic Wee1 by SCF -TrCP. Proc Natl Acad Sci 101:4419–4424

121. Margottin-Goguet F, Hsu JY, Loktev A, Hsieh H-M, Reimann JDR, Jackson PK (2003) Prophase Destruction of Emi1 by the SCFβTrCP/Slimb Ubiquitin Ligase Activates the Anaphase Promoting Complex to Allow Progression beyond Prometaphase. Dev Cell 4:813–826

122. Guardavaccaro D, Kudo Y, Boulaire J, Barchi M, Busino L, Donzelli M, Margottin-Goguet F, Jackson PK, Yamasaki L, Pagano M (2003) Control of Meiotic and Mitotic Progression by the F Box Protein β-Trcp1 In Vivo. Dev Cell 4:799–812

123. Sudakin V, Chan GKT, Yen TJ (2001) Checkpoint inhibition of the APC/C in HeLa cells is mediated by a complex of BUBR1, BUB3, CDC20, and MAD2. J Cell Biol 154:925–936

124. Seki A, Coppinger JA, Du H, Jang C-Y, Yates JR, Fang G (2008) Plk1- and β-TrCP–dependent degradation of Bora controls mitotic progression. J Cell Biol 181:65–78

125. Schrock MS, Stromberg BR, Scarberry L, Summers MK (2020) APC/C ubiquitin ligase: Functions and mechanisms in tumorigenesis. Semin Cancer Biol 67:80–91

126. Kotsantis P, Petermann E, Boulton SJ (2018) Mechanisms of Oncogene-Induced Replication Stress: Jigsaw Falling into Place. Cancer Discov 8:537–555

127. Chatterjee N, Walker GC (2017) Mechanisms of DNA damage, repair, and mutagenesis. Environ Mol Mutagen 58:235–263

128. Costantino L, Sotiriou SK, Rantala JK, Magin S, Mladenov E, Helleday T, Haber JE, Iliakis G, Kallioniemi OP, Halazonetis TD (2014) Break-induced replication repair of damaged forks induces genomic duplications in human cells. Science 343:88–91

129. Childs BG, Baker DJ, Kirkland JL, Campisi J, van Deursen JM, Deursen JM Van (2014) Senescence and apoptosis: dueling or complementary cell fates? EMBO Rep 15:1–15

130. Toledo LI, Altmeyer M, Rask M-B, Lukas C, Larsen DH, Povlsen LK, Bekker-Jensen S, Mailand N, Bartek J, Lukas J (2013) ATR Prohibits Replication Catastrophe by Preventing Global Exhaustion of RPA. Cell 155:1088–1103

131. Gorgoulis VG, Vassiliou L-VF, Karakaidos P, et al (2005) Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature 434:907–13

132. Bartkova J, Horejsí Z, Koed K, et al (2005) DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. Nature 434:864–70

133. Kruse J-P, Gu W (2009) Modes of p53 regulation. Cell 137:609–22

134. Bartkova J, Rezaei N, Liontos M, et al (2006) Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. Nature 444:633–637

135. Murga M, Campaner S, Lopez-Contreras AJ, et al (2011) Exploiting oncogene-induced replicative stress for the selective killing of Myc-driven tumors. Nat Struct Mol Biol 18:1331–1335

136. Miron K, Golan-Lev T, Dvir R, Ben-David E, Kerem B (2015) Oncogenes create a unique landscape of fragile sites. Nat Commun 6:7094

137. Jones RM, Mortusewicz O, Afzal I, Lorvellec M, García P, Helleday T, Petermann E (2013) Increased replication initiation and conflicts with transcription underlie Cyclin E-induced replication stress. Oncogene 32:3744–3753

138. Bester AC, Roniger M, Oren YS, Im MM, Sarni D, Chaoat M, Bensimon A, Zamir G, Shewach DS, Kerem B (2011) Nucleotide Deficiency Promotes Genomic Instability in Early Stages of Cancer Development. Cell 145:435–446

139. Guerrero Llobet S, van der Vegt B, Jongeneel E, Bense RD, Zwager MC, Schröder CP, Everts M, Fehrmann RSN, de Bock GH, van Vugt MATM (2020) Cyclin E expression is associated with high levels of replication stress in triple-negative breast cancer. npj Breast Cancer 6:40

140. Kotsantis P, Silva LM, Irmscher S, Jones RM, Folkes L, Gromak N, Petermann E (2016) Increased global transcription activity as a mechanism of replication stress in cancer. Nat Commun 7:13087

141. Maya-Mendoza A, Ostrakova J, Kosar M, et al (2015) Myc and Ras oncogenes engage different energy metabolism programs and evoke distinct patterns of oxidative and DNA replication stress. Mol Oncol 9:601–616

142. Srinivasan S V., Dominguez-Sola D, Wang LC, Hyrien O, Gautier J (2013) Cdc45 Is a

Critical Effector of Myc-Dependent DNA Replication Stress. Cell Rep 3:1629–39

143. Dominguez-Sola D, Ying CY, Grandori C, Ruggiero L, Chen B, Li M, Galloway DA, Gu W, Gautier J, Dalla-Favera R (2007) Non-transcriptional control of DNA replication by c-Myc. Nature 448:445–451

144. Frescas D, Pagano M (2008) Deregulated proteolysis by the F-box proteins SKP2 and β-TrCP: tipping the scales of cancer. Nat Rev Cancer 8:438–449

145. Kok YP, Guerrero Llobet S, Schoonen PM, Everts M, Bhattacharya A, Fehrmann RSN, van den Tempel N, van Vugt MATM (2020) Overexpression of Cyclin E1 or Cdc25A leads to replication stress, mitotic aberrancies, and increased sensitivity to replication checkpoint inhibitors. Oncogenesis 9:88

146. Kim CJ, Song JH, Cho YG, Kim YS, Kim SY, Nam SW, Yoo NJ, Lee JY, Park WS (2007) Somatic mutations of the beta-TrCP gene in gastric cancer. APMIS 115:127–33

147. Fu J, Qiu H, Cai M, Pan Y, Cao Y, Liu L, Yun J, Zhang CZ (2013) Low cyclin F expression in hepatocellular carcinoma associates with poor differentiation and unfavorable prognosis. Cancer Sci 104:508–515

148. D'Angiolella V, Donato V, Forrester FM, Jeong Y-T, Pellacani C, Kudo Y, Saraf A, Florens L, Washburn MP, Pagano M (2012) Cyclin F-Mediated Degradation of Ribonucleotide Reductase M2 Controls Genome Integrity and DNA Repair. Cell 149:1023–1034

149. Buisson R, Boisvert JL, Benes CH, Zou L (2015) Distinct but Concerted Roles of ATR, DNA-PK, and Chk1 in Countering Replication Stress during S Phase. Mol Cell 59:1011–1024

150. Byun TS (2005) Functional uncoupling of MCM helicase and DNA polymerase activities activates the ATR-dependent checkpoint. Genes Dev 19:1040–1052

151. Zou L, Elledge SJ (2003) Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science 300:1542–8

152. Cortez D, Guntuku S, Qin J, Elledge SJ (2001) ATR and ATRIP: partners in checkpoint signaling. Science 294:1713–6

153. Melixetian M, Klein DK, Sørensen CS, Helin K (2009) NEK11 regulates CDC25A degradation and the IR-induced G2/M checkpoint. Nat Cell Biol 11:1247–1253

154. Smits VAJ, Cabrera E, Freire R, Gillespie DA (2019) Claspin – checkpoint adaptor and DNA replication factor. FEBS J 286:441–455

155. Peschiaroli A, Dorrello NV, Guardavaccaro D, Venere M, Halazonetis T, Sherman NE, Pagano M (2006) SCFβTrCP-Mediated Degradation of Claspin Regulates Recovery from the DNA Replication Checkpoint Response. Mol Cell 23:319–329

156. Mailand N, Bekker-Jensen S, Bartek J, Lukas J (2006) Destruction of Claspin by SCFβTrCP Restrains Chk1 Activation and Facilitates Recovery from Genotoxic Stress. Mol Cell 23:307–318

157. Toledo L, Neelsen KJ, Lukas J (2017) Replication Catastrophe: When a Checkpoint Fails because of Exhaustion. Mol Cell 66:735–749

158. Rodriguez-Lopez AM, Jackson DA, Iborra F, Cox LS (2002) Asymmetry of DNA replication fork progression in Werner's syndrome. Aging Cell 1:30–39

159. Ammazzalorso F, Pirzio LM, Bignami M, Franchitto A, Pichierri P (2010) ATR and ATM differently regulate WRN to prevent DSBs at stalled replication forks and promote replication fork recovery. EMBO J 29:3156–3169

160. Lossaint G, Larroque M, Ribeyre C, Bec N, Larroque C, Décaillet C, Gari K, Constantinou A (2013) FANCD2 Binds MCM Proteins and Controls Replisome Function upon Activation of S Phase Checkpoint Signaling. Mol Cell 51:678–690

161. Chen YH, Jones MJK, Yin Y, Crist SB, Colnaghi L, Sims RJ, Rothenberg E, Jallepalli P V., Huang TT (2015) ATR-Mediated Phosphorylation of FANCI Regulates Dormant Origin Firing in Response to Replication Stress. Mol Cell 58:323–338

162. Kaddar T, Carreau M (2012) Fanconi Anemia Proteins and Their Interacting Partners: A Molecular Puzzle. Anemia 2012:1–11

163. Tibbetts RS (2000) Functional interactions between BRCA1 and the checkpoint kinase ATR during genotoxic stress. Genes Dev 14:2989–3002

164. Buisson R, Niraj J, Rodrigue A, et al (2017) Coupling of Homologous Recombination and the Checkpoint by ATR. Mol Cell 65:336–346

165. Sorensen CS, Syljuasen RG (2012) Safeguarding genome integrity: the checkpoint kinases ATR, CHK1 and WEE1 restrain CDK activity during normal DNA replication. Nucleic Acids Res 40:477–486

166. Bahassi EM, Ovesen JL, Riesenberg AL, Bernstein WZ, Hasty PE, Stambrook PJ (2008) The checkpoint kinases Chk1 and Chk2 regulate the functional associations between hBRCA2 and Rad51 in response to DNA damage. Oncogene 27:3977–3985

167. Rickman KA, Noonan RJ, Lach FP, Sridhar S, Wang AT, Abhyankar A, Huang A, Kelly M, Auerbach AD, Smogorzewska A (2020) Distinct roles of BRCA2 in replication fork protection in response to hydroxyurea and DNA interstrand cross-links. Genes Dev 34:832–846

168. El-Shemerly M, Hess D, Pyakurel AK, Moselhy S, Ferrari S (2008) ATR-dependent pathways control hEXO1 stability in response to stalled forks. Nucleic Acids Res 36:511–519

169. Hu J, Sun L, Shen F, et al (2012) The Intra-S Phase Checkpoint Targets Dna2 to Prevent Stalled Replication Forks from Reversing. Cell 149:1221–1232

170. Marzio A, Puccini J, Kwon Y, Maverakis NK, Arbini A, Sung P, Bar-Sagi D, Pagano M (2019) The F-Box Domain-Dependent Activity of EMI1 Regulates PARPi Sensitivity in Triple-Negative Breast Cancers. Mol Cell 73:224-237.e6

171. Ceccaldi R, Sarangi P, D'Andrea AD (2016) The Fanconi anaemia pathway: new players and new functions. Nat Rev Mol Cell Biol 17:337–349

172. Niraj J, Färkkilä A, D'Andrea AD (2019) The Fanconi Anemia Pathway in Cancer. Annu Rev Cancer Biol 3:457–478

173. Kottemann MC, Smogorzewska A (2013) Fanconi anaemia and the repair of Watson and Crick DNA crosslinks. Nature 493:356–63

174. Ishiai M, Kitao H, Smogorzewska A, et al (2008) FANCI phosphorylation functions as a molecular switch to turn on the Fanconi anemia pathway. Nat Struct Mol Biol 15:1138–46

175. Kim H, D'Andrea AD (2012) Regulation of DNA cross-link repair by the Fanconi anemia/BRCA pathway. Genes Dev 26:1393–1408

176. Andreassen PR, D'Andrea AD, Taniguchi T (2004) ATR couples FANCD2 monoubiquitination to the DNA-damage response. Genes Dev 18:1958–63

177. Sareen A, Chaudhury I, Adams N, Sobeck A (2012) Fanconi anemia proteins FANCD2 and FANCI exhibit different DNA damage responses during S-phase. Nucleic Acids Res 40:8425–8439

178. Singh TR, Ali AM, Paramasivam M, Pradhan A, Wahengbam K, Seidman MM, Meetei AR (2013) ATR-Dependent Phosphorylation of FANCM at Serine 1045 Is Essential for FANCM Functions. Cancer Res 73:4300–4310

179. Schwab RA, Blackford AN, Niedzwiedz W (2010) ATR activation and replication fork restart are defective in FANCM-deficient cells. EMBO J 29:806–18

180. Yamamoto KN, Kobayashi S, Tsuda M, Kurumizaka H, Takata M, Kono K, Jiricny J, Takeda S, Hirota K (2011) Involvement of SLX4 in interstrand cross-link repair is regulated by the Fanconi anemia pathway. Proc Natl Acad Sci U S A 108:6492–6

181. Huang M, D'Andrea AD (2010) A new nuclease member of the FAN club. Nat Struct Mol Biol 17:926–8

182. Nijman SMB, Huang TT, Dirac AMG, Brummelkamp TR, Kerkhoven RM, D'Andrea AD, Bernards R (2005) The deubiquitinating enzyme USP1 regulates the Fanconi anemia pathway. Mol Cell 17:331–9

183. Tan W, van Twest S, Leis A, Bythell-Douglas R, Murphy VJ, Sharp M, Parker MW, Crismani W, Deans AJ (2020) Monoubiquitination by the human Fanconi anemia core complex clamps FANCI:FANCD2 on DNA in filamentous arrays. Elife. https://doi.org/10.7554/eLife.54128

184. Joo W, Xu G, Persky NS, Smogorzewska A, Rudge DG, Buzovetsky O, Elledge SJ, Pavletich NP (2011) Structure of the FANCI-FANCD2 complex: insights into the Fanconi anemia DNA repair pathway. Science 333:312–6

185. Alcón P, Shakeel S, Chen ZA, Rappsilber J, Patel KJ, Passmore LA (2020) FANCD2–FANCI is a clamp stabilized on DNA by monoubiquitination of FANCD2 during DNA repair. Nat Struct Mol Biol 27:240–248

186. Gibbs-Seymour I, Oka Y, Rajendra E, Weinert BT, Passmore LA, Patel KJ, Olsen J V., Choudhary C, Bekker-Jensen S, Mailand N (2015) Ubiquitin-SUMO circuitry controls activated fanconi anemia ID complex dosage in response to DNA damage. Mol Cell 57:150–164

187. Unno J, Itaya A, Taoka M, et al (2014) FANCD2 binds CtIP and regulates DNA-end resection during DNA interstrand crosslink repair. Cell Rep 7:1039–1047

188. Roques C, Coulombe Y, Delannoy M, et al (2009) MRE11-RAD50-NBS1 is a critical regulator of FANCD2 stability and function during DNA double-strand break repair. EMBO J 28:2400–2413

189. Chu WK, Payne MJ, Beli P, Hanada K, Choudhary C, Hickson ID (2015) FBH1 influences DNA replication fork stability and homologous recombination through ubiquitylation of RAD51. Nat Commun 6:5931

190. Lachaud C, Moreno A, Marchesi F, Toth R, Blow JJ, Rouse J (2016) Ubiquitinated Fancd2 recruits Fan1 to stalled replication forks to prevent genome instability. Science 351:846–9

191. Howlett NG, Harney JA, Rego MA, Kolling FW, Glover TW (2009) Functional interaction between the Fanconi Anemia D2 protein and proliferating cell nuclear antigen (PCNA) via a conserved putative PCNA interaction motif. J Biol Chem 284:28935–42

192. Liu Y, Nielsen CF, Yao Q, Hickson ID (2014) The origins and processing of ultra fine anaphase DNA bridges. Curr Opin Genet Dev 26:1–5

193. Macheret M, Bhowmick R, Sobkowiak K, Padayachy L, Mailler J, Hickson ID, Halazonetis TD (2020) High-resolution mapping of mitotic DNA synthesis regions and common fragile sites in the human genome through direct sequencing. Cell Res 30:997–1008

194. Ji F, Liao H, Pan S, et al (2020) Genome-wide high-resolution mapping of mitotic DNA synthesis sites and common fragile sites by direct sequencing. Cell Res 30:1009–1023

195. Chan KL, Palmai-Pallag T, Ying S, Hickson ID (2009) Replication stress induces sister-chromatid bridging at fragile site loci in mitosis. Nat Cell Biol 11:753–760

196. Howlett NG, Taniguchi T, Durkin SG, D'Andrea AD, Glover TW (2005) The Fanconi anemia pathway is required for the DNA replication stress respone and for the regulation of common fragile site stability. Hum Mol Genet 14:693–701

197. Naim V, Rosselli F (2009) The FANC pathway and BLM collaborate during mitosis to prevent micro-nucleation and chromosome abnormalities. Nat Cell Biol 11:761–768

198. Kee Y, Kim JM, D'Andrea A (2009) Regulated degradation of FANCM in the Fanconi anemia pathway during mitosis. Genes Dev 23:555–560

199. Schwab RA, Nieminuszczy J, Shah F, Langton J, Lopez Martinez D, Liang CC, Cohn MA, Gibbons RJ, Deans AJ, Niedzwiedz W (2015) The Fanconi Anemia Pathway Maintains Genome Stability by Coordinating Replication and Transcription. Mol Cell 60:351–361

200. Watts FZ (2016) Repair of DNA double-strand breaks in heterochromatin. Biomolecules 6:47

201. Schuster-Böckler B, Lehner B (2012) Chromatin organization is a major influence on regional mutation rates in human cancer cells. Nature 488:504–507

202. Kouzarides T (2007) Chromatin Modifications and Their Function. Cell 128:693–705

203. Randle SJ, Laman H (2016) F-box protein interactions with the hallmark pathways in cancer. Semin Cancer Biol 36:3–17

204. Van Rechem C, Black JC, Abbas T, Allen A, Rinehart CA, Yuan GC, Dutta A, Whetstine JR (2011) The SKP1-Cul1-F-box and leucine-rich repeat protein 4 (SCF-

FbxL4) ubiquitin ligase regulates lysine demethylase 4A (KDM4A)/Jumonji domain-containing 2A (JMJD2A) protein. J Biol Chem 286:30462–30470

205. Tan M-KM, Lim H-J, Harper JW (2011) SCFFBXO22 Regulates Histone H3 Lysine 9 and 36 Methylation Levels by Targeting Histone Demethylase KDM4A for Ubiquitin-Mediated Proteasomal Degradation. Mol Cell Biol 31:3687–3699

206. Shen JZ, Qiu Z, Wu Q, et al (2020) FBXO44 promotes DNA replication-coupled repetitive element silencing in cancer cells. Cell. https://doi.org/10.1016/j.cell.2020.11.042

207. Ayoub N, Jeyasekharan AD, Bernal JA, Venkitaraman AR (2008) HP1-β mobilization promotes chromatin changes that initiate the DNA damage response. Nature 453:682–686

208. Luijsterburg MS, Dinant C, Lans H, et al (2009) Heterochromatin protein 1 is recruited to various types of DNA damage. J Cell Biol 185:577–586

209. Baldeyron C, Soria G, Roche D, Cook AJL, Almouzni G (2011) HP1α recruitment to DNA damage by p150CAF-1 promotes homologous recombination repair. J Cell Biol 193:81–95

210. Ayrapetov MK, Gursoy-Yuzugullu O, Xu C, Xu Y, Price BD (2014) DNA double-strand breaks promote methylation of histone H3 on lysine 9 and transient formation of repressive chromatin. Proc Natl Acad Sci U S A 111:9169–9174

211. Sulkowski PL, Oeck S, Dow J, et al (2020) Oncometabolites suppress DNA repair by disrupting local chromatin signalling. Nature 582:586–591

212. Ryu T, Spatola B, Delabaere L, Bowlin K, Hopp H, Kunitake R, Karpen GH, Chiolo I (2015) Heterochromatic breaks move to the nuclear periphery to continue recombinational repair. Nat Cell Biol 17:1401–1411

213. Chiolo I, Minoda A, Colmenares SU, Polyzos A, Costes S V., Karpen GH (2011) Double-strand breaks in heterochromatin move outside of a dynamic HP1a domain to complete recombinational repair. Cell 144:732–744

214. Caridi CP, D'Agostino C, Ryu T, et al (2018) Nuclear F-actin and myosins drive relocalization of heterochromatic breaks. Nature 559:54–60

215. Schrank BR, Aparicio T, Li Y, Chang W, Chait BT, Gundersen GG, Gottesman ME, Gautier J (2018) Nuclear ARP2/3 drives DNA break clustering for homology-directed repair. Nature 559:61–66

216. Fares J, Fares MY, Khachfe HH, Salhab HA, Fares Y (2020) Molecular principles of metastasis: a hallmark of cancer revisited. Signal Transduct Target Ther 5:28

217. Luzzi KJ, MacDonald IC, Schmidt EE, Kerkvliet N, Morris VL, Chambers AF, Groom AC (1998) Multistep nature of metastatic inefficiency: Dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. Am J Pathol 153:865–873

218. Peitzsch C, Tyutyunnykova A, Pantel K, Dubrovska A (2017) Cancer stem cells: The root of tumor recurrence and metastases. Semin Cancer Biol 44:10–24

219. Kalcheim C (2015) Epithelial–Mesenchymal Transitions during Neural Crest and

Somite Development. J Clin Med 5:1

220. Kalluri R, Weinberg RA (2009) The basics of epithelial-mesenchymal transition. J Clin Invest 119:1420–1428

221. Zheng H, Shen M, Zha YL, et al (2014) PKD1 Phosphorylation-Dependent Degradation of SNAIL by SCF-FBXO11 Regulates Epithelial-Mesenchymal Transition and Metastasis. Cancer Cell 26:358–373

222. Zhou BP, Deng J, Xia W, Xu J, Li YM, Gunduz M, Hung MC (2004) Dual regulation of Snail by GSK-3β-mediated phosphorylation in control of epithelial-mesenchymal transition. Nat Cell Biol 6:931–940

223. Viñas-Castells R, Frías Á, Robles-Lanuza E, Zhang K, Longmore GD, García De Herreros A, Díaz VM (2014) Nuclear ubiquitination by FBXL5 modulates Snail1 DNA binding and stability. Nucleic Acids Res 42:1079–1094

224. Vashisht AA, Zumbrennen KB, Huang X, et al (2009) Control of iron homeostasis by an iron-regulated ubiquitin ligase. Science 326:718–21

225. Vernon AE, LaBonne C (2006) Slug stability is dynamically regulated during neural crest development by the F-box protein Ppa. Development 133:3359–3370

226. Lander R, Nordin K, LaBonne C (2011) The F-box protein Ppa is a common regulator of core EMT factors Twist, Snail, Slug, and Sip1. J Cell Biol 194:17–25

227. Viñas-Castells R, Beltran M, Valls G, Gómez I, García JM, Montserrat-Sentís B, Baulida J, Bonilla F, De Herreros AG, Díaz VM (2010) The hypoxia-controlled FBXL14 ubiquitin ligase targets SNAIL1 for proteasome degradation. J Biol Chem 285:3794–3805

228. Guo W, Keckesova Z, Donaher JL, et al (2012) Slug and Sox9 Cooperatively Determine the Mammary Stem Cell State. Cell 148:1015–1028

229. Zhou H, Li G, Huang SHU, Feng Y, Zhou A (2019) SOX9 promotes epithelial-mesenchymal transition via the hippo-YAP signaling pathway in gastric carcinoma cells. Oncol Lett 18:599–608

230. Huang JQ, Wei FK, Xu XL, et al (2019) SOX9 drives the epithelial-mesenchymal transition in non-small-cell lung cancer through the Wnt/β-catenin pathway. J Transl Med 17:143

231. Phuyal S, Farhan H (2019) Multifaceted Rho GTPase Signaling at the Endomembranes. Front Cell Dev Biol 7:127

232. Dise RS, Frey MR, Whitehead RH, Polk DB (2008) Epidermal growth factor stimulates Rac activation through Src and phosphatidylinositol 3-kinase to promote colonic epithelial cell migration. Am J Physiol Gastrointest Liver Physiol 294:G276–G285

233. Clayton NS, Ridley AJ (2020) Targeting Rho GTPase Signaling Networks in Cancer. Front Cell Dev Biol 8:222

234. Zhao J, Mialki RK, Wei J, Coon TA, Zou C, Chen BB, Mallampalli RK, Zhao Y (2013) SCF E3 ligase F-box protein complex SCFFBXL19 regulates cell migration by mediating Rac1 ubiquitination and degradation. FASEB J 27:2611–2619

235. Sorrell FJ, Kilian LM, Elkins JM (2019) Solution structures and biophysical analysis of full-length group A PAKs reveal they are monomeric and auto-inhibited in cis. Biochem J 476:1037–1051

236. Manser E, Loo T-H, Koh C-G, Zhao Z-S, Chen X-Q, Tan L, Tan I, Leung T, Lim L (1998) PAK Kinases Are Directly Coupled to the PIX Family of Nucleotide Exchange Factors. Mol Cell 1:183–192

237. Frank SR, Hansen SH (2008) The PIX-GIT complex: a G protein signaling cassette in control of cell shape. Semin Cell Dev Biol 19:234–44

238. López-Colomé AM, Lee-Rivera I, Benavides-Hidalgo R, López E (2017) Paxillin: A crossroad in pathological cell migration. J Hematol Oncol 10:50

239. Lane J, Martin T, Weeks HP, Jiang WG (2014) Structure and role of WASP and WAVE in Rho GTPase signalling in cancer. Cancer Genomics and Proteomics 11:155–166

240. Navarro-Lérida I, Pellinen T, Sanchez SA, Guadamillas MC, Wang Y, Mirtti T, Calvo E, DelPozo MA, Del Pozo MA (2015) Rac1 nucleocytoplasmic shuttling drives nuclear shape changes and tumor invasion. Dev Cell 32:318–334

241. Cojoc M, Mäbert K, Muders MH, Dubrovska A (2015) A role for cancer stem cells in therapy resistance: Cellular and molecular mechanisms. Semin Cancer Biol 31:16–27

242. Greaves M, Maley CC (2012) Clonal evolution in cancer. Nature 481:306–313

243. Takahashi K, Yamanaka S (2006) Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Cell 126:663–676

244. Hadjimichael C, Chanoumidou K, Papadopoulou N, Arampatzi P, Papamatheakis J, Kretsovali A (2015) Common stemness regulators of embryonic and cancer stem cells. World J Stem Cells 7:1150–84

245. Popov N, Schülein C, Jaenicke LA, Eilers M (2010) Ubiquitylation of the amino terminus of Myc by SCFβ-TrCP antagonizes SCFFbw7-mediated turnover. Nat Cell Biol 12:973–981

246. Chen Y, Sun X-X, Sears RC, Dai M-S (2019) Writing and erasing MYC ubiquitination and SUMOylation. Genes Dis 6:359–371

247. Tian X, Dai S, Sun J, Jin G, Jiang S, Meng F, Li Y, Wu D, Jiang Y (2015) F-box protein FBXO22 mediates polyubiquitination and degradation of KLF4 to promote hepatocellular carcinoma progression. Oncotarget 6:22767–22775

248. Takeishi S, Nakayama KI (2014) Role of Fbxw7 in the maintenance of normal stem cells and cancer-initiating cells. Br J Cancer 111:1054–1059

249. Swartling FJ, Savov V, Persson AI, et al (2012) Distinct neural stem cell populations give rise to disparate brain tumors in response to N-MYC. Cancer Cell 21:601–13

250. Bleau AM, Hambardzumyan D, Ozawa T, Fomchenko EI, Huse JT, Brennan CW, Holland EC (2009) PTEN/PI3K/Akt Pathway Regulates the Side Population Phenotype and ABCG2 Activity in Glioma Tumor Stem-like Cells. Cell Stem Cell 4:226–235

251. Xiao J, Yin S, Li Y, Xie S, Nie D, Ma L, Wang X, Wu Y, Feng J (2009) SKP2 siRNA

inhibits the degradation of P27kip1 and down-regulates the expression of MRP in HL-60/A cells. Acta Biochim Biophys Sin (Shanghai) 41:699–708

252. Al-Assar O, Mantoni T, Lunardi S, Kingham G, Helleday T, Brunner TB (2011) Breast cancer stem-like cells show dominant homologous recombination due to a larger S-G2 fraction. Cancer Biol Ther 11:1028–1035

253. Brunner A, Suryo Rahmanto A, Johansson H, et al (2020) PTEN and DNA-PK determine sensitivity and recovery in response to WEE1 inhibition in human breast cancer. Elife. https://doi.org/10.7554/eLife.57894

254. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN (2006) Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 444:756–760

255. Bartucci M, Svensson S, Romania P, et al (2012) Therapeutic targeting of Chk1 in NSCLC stem cells during chemotherapy. Cell Death Differ 19:768–778

256. Zhang YW, Brognard J, Coughlin C, You Z, Dolled-Filhart M, Aslanian A, Manning G, Abraham RT, Hunter T (2009) The F Box Protein Fbx6 Regulates Chk1 Stability and Cellular Sensitivity to Replication Stress. Mol Cell 35:442–453

257. Cai L, Li J, Zhao J, et al (2019) Fbxo6 confers drug-sensitization to cisplatin via inhibiting the activation of Chk1 in non-small cell lung cancer. FEBS Lett 593:1827–1836

258. Zhang M, Behbod F, Atkinson RL, et al (2008) Identification of tumor-initiating cells in a p53-null mouse model of breast cancer. Cancer Res 68:4674–4682

259. Navarrete-Meneses M del P, Pérez-Vera P (2017) Epigenetic alterations in acute lymphoblastic leukemia. Bol Med Hosp Infant Mex 74:243–264

260. Tseng RC, Lin RK, Wen CK, Tseng C, Hsu HS, Hsu WH, Wang YC (2008) Epigenetic silencing of AXIN2/betaTrCP and deregulation of p53-mediated control lead to wild-type β-catenin nuclear accumulation in lung tumorigenesis. Oncogene 27:4488–4496

261. Heyn H, Sayols S, Moutinho C, et al (2014) Linkage of DNA Methylation Quantitative Trait Loci to Human Cancer Risk. Cell Rep 7:331–338

262. Weyburne ES, Wilkins OM, Sha Z, Williams DA, Pletnev AA, de Bruin G, Overkleeft HS, Goldberg AL, Cole MD, Kisselev AF (2017) Inhibition of the Proteasome β2 Site Sensitizes Triple-Negative Breast Cancer Cells to β5 Inhibitors and Suppresses Nrf1 Activation. Cell Chem Biol 24:218–230

263. Li X, Elmira E, Rohondia S, Wang J, Liu J, Dou QP (2018) A patent review of the ubiquitin ligase system: 2015–2018. Expert Opin Ther Pat 28:919–937

264. Skaar JR, Pagan JK, Pagano M (2014) SCF ubiquitin ligase-targeted therapies. Nat Rev Drug Discov 13:889–903

265. European Comission 2020 Cancer incidence and mortality in EU-27 countries | EU Science Hub. https://ec.europa.eu/jrc/en/news/2020-cancer-incidence-and-mortality-eu-27-countries. Accessed 26 Nov 2020

266. Carioli G, Bertuccio P, Boffetta P, Levi F, La Vecchia C, Negri E, Malvezzi M (2020)

European cancer mortality predictions for the year 2020 with a focus on prostate cancer. Ann Oncol 31:650–658

267. Greenwood E, Swanton C (2020) Consequences of COVID-19 for cancer care — a CRUK perspective. Nat Rev Clin Oncol 1–2

268. Foulkes WD, Smith IE, Reis-Filho JS (2010) Triple-Negative Breast Cancer. N Engl J Med 363:1938–1948

269. Perou CM, Sørile T, Eisen MB, et al (2000) Molecular portraits of human breast tumours. Nature 406:747–752

270. Johansson HJ, Socciarelli F, Vacanti NM, et al (2019) Breast cancer quantitative proteome and proteogenomic landscape. Nat Commun 10:1600

271. Di Leo A, Isola J, Piette F, et al (2009) A meta-analysis of phase III trials evaluating the predictive value of HER2 and topoisomerase II alpha in early breast cancer patients treated with CMF or anthracycline-based adjuvant therapy. In: Poster Discuss. Abstr. American Association for Cancer Research, p 705

272. Foulkes WD, Brunet J-S, Stefansson IM, et al (2004) The prognostic implication of the basal-like (cyclin E high/p27 low/p53+/glomeruloid-microvascular-proliferation+) phenotype of BRCA1-related breast cancer. Cancer Res 64:830–5

273. Chen X, Low K-H, Alexander A, et al (2018) Cyclin E Overexpression Sensitizes Triple-Negative Breast Cancer to Wee1 Kinase Inhibition. Clin Cancer Res 24:6594–6610

274. Jin J, Fang H, Yang F, Ji W, Guan N, Sun Z, Shi Y, Zhou G, Guan X (2018) Combined Inhibition of ATR and WEE1 as a Novel Therapeutic Strategy in Triple-Negative Breast Cancer. Neoplasia (United States) 20:478–488

275. Ruiz S, Mayor-Ruiz C, Lafarga V, Murga M, Vega-Sendino M, Ortega S, Fernandez-Capetillo O (2016) A Genome-wide CRISPR Screen Identifies CDC25A as a Determinant of Sensitivity to ATR Inhibitors. Mol Cell 62:307–313

276. Bukhari AB, Lewis CW, Pearce JJ, Luong D, Chan GK, Gamper AM (2019) Inhibiting Wee1 and ATR kinases produces tumor-selective synthetic lethality and suppresses metastasis. J Clin Invest 129:1329–1344

277. Young LA, O'Connor LO, de Renty C, et al (2019) Differential Activity of ATR and WEE1 Inhibitors in a Highly Sensitive Subpopulation of DLBCL Linked to Replication Stress. Cancer Res 79:3762–3775

278. Yap TA, Plummer R, Azad NS, Helleday T (2019) The DNA Damaging Revolution: PARP Inhibitors and Beyond. Am Soc Clin Oncol Educ B 185–195

279. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ, Helleday T (2005) Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 434:913–917

280. Farmer H, McCabe N, Lord CJ, et al (2005) Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434:917–21

281. Zhang W, Cao L, Sun Z, Xu J, Tang L, Chen W, Luo J, Yang F, Wang Y, Guan X (2016) Skp2 is over-expressed in breast cancer and promotes breast cancer cell

proliferation. Cell Cycle 15:1344–1351

282. Chan C-H, Morrow JK, Li C-F, et al (2013) Pharmacological inactivation of Skp2 SCF ubiquitin ligase restricts cancer stem cell traits and cancer progression. Cell 154:556–68

283. Wu L, Grigoryan A V., Li Y, Hao B, Pagano M, Cardozo TJ (2012) Specific small molecule inhibitors of skp2-mediated p27 degradation. Chem Biol 19:1515–1524

284. Blees JS, Bokesch HR, Rübsamen D, et al (2012) Erioflorin Stabilizes the Tumor Suppressor Pdcd4 by Inhibiting Its Interaction with the E3-ligase β-TrCP1. PLoS One 7:e46567

285. Zhou L, Jia L (2020) Targeting Protein Neddylation for Cancer Therapy. In: Adv. Exp. Med. Biol. Springer, pp 297–315

286. Northcott PA, Robinson GW, Kratz CP, et al (2019) Medulloblastoma. Nat Rev Dis Prim 5:11

287. Taylor MD, Northcott PA, Korshunov A, et al (2012) Molecular subgroups of medulloblastoma: the current consensus. Acta Neuropathol 123:465–72

288. Cavalli FMG, Remke M, Rampasek L, et al (2017) Intertumoral Heterogeneity within Medulloblastoma Subgroups. Cancer Cell 31:737-754.e6

289. Liu X, Ding C, Tan W, Zhang A (2020) Medulloblastoma: Molecular understanding, treatment evolution, and new developments. Pharmacol Ther 210:107516

290. Raducu M, Fung E, Serres S, et al (2016) SCF (Fbxl17) ubiquitylation of Sufu regulates Hedgehog signaling and medulloblastoma development. EMBO J 35:1400–1416

291. Khatua S, Song A, Sridhar DC, Mack SC (2018) Childhood Medulloblastoma: Current Therapies, Emerging Molecular Landscape and Newer Therapeutic Insights. Curr Neuropharmacol 16:1045–1058

292. Bhatia N, Thiyagarajan S, Elcheva I, Saleem M, Dlugosz A, Mukhtar H, Spiegelman VS (2006) Gli2 is targeted for ubiquitination and degradation by β-TrCP ubiquitin ligase. J Biol Chem 281:19320–19326

293. Wang B, Li Y (2006) Evidence for the direct involvement of TrCP in Gli3 protein processing. Proc Natl Acad Sci 103:33–38

294. Latres E, Chiaur DS, Pagano M (1999) The human F box protein β-Trcp associates with the Cul1/Skp1 complex and regulates the stability of β-catenin. Oncogene 18:849–854

295. Su YY, Fu C, Ishikawa S, Stella A, Kojima M, Shitoh K, Schreiber EM, Day BW, Liu B (2008) APC Is Essential for Targeting Phosphorylated β-Catenin to the SCFβ-TrCP Ubiquitin Ligase. Mol Cell 32:652–661

296. Zurawel RH, Chiappa SA, Allen C, Raffel C (1998) Sporadic medulloblastomas contain oncogenic β-catenin mutations. Cancer Res 58:896–899

297. Eberhart CG, Tihan T, Burger PC (2000) Nuclear localization and mutation of β-catenin in medulloblastomas. J Neuropathol Exp Neurol 59:333–337

298. Hede S-M, Savov V, Weishaupt H, Sangfelt O, Swartling FJ (2014) Oncoprotein stabilization in brain tumors. Oncogene 33:4709–21

299. Taniguchi E, Min JC, Arenkiel BR, et al (2009) Bortezomib reverses a post-translational mechanism of tumorigenesis for Patched1 haploinsufficiency in medulloblastoma. Pediatr Blood Cancer 53:136–144

300. Yang F, Jove V, Buettner R, et al (2012) Bortezomib induces apoptosis and growth suppression in human medulloblastoma cells, associated with inhibition of AKT and NF-κB signaling, and synergizes with an ERK inhibitor. Cancer Biol Ther 13:349–357

301. Vriend J, Ghavami S, Marzban H (2015) The role of the ubiquitin proteasome system in cerebellar development and medulloblastoma. Mol Brain 8:64

302. Arvanitis CD, Ferraro GB, Jain RK (2020) The blood–brain barrier and blood–tumour barrier in brain tumours and metastases. Nat Rev Cancer 20:26–41

303. Cook Sangar ML, Genovesi LA, Nakamoto MW, Davis MJ, Knobluagh SE, Ji P, Millar A, Wainwright BJ, Olson JM (2017) Inhibition of CDK4/6 by palbociclib significantly extends survival in medulloblastoma patient-derived xenograft mouse models. Clin Cancer Res 23:5802–5813

304. Ayrault O, Zindy F, Rehg J, Sherr CJ, Roussel MF (2009) Two Tumor Suppressors, p27 Kip1 and Patched-1, Collaborate to Prevent Medulloblastoma. Mol Cancer Res 7:33–40

305. Moreira DC, Venkataraman S, Subramanian A, et al (2020) Targeting MYC-driven replication stress in medulloblastoma with AZD1775 and gemcitabine. J Neurooncol 147:531–545

306. Fu S, Wang Y, Keyomarsi K, Meric-Bernstein F (2018) Strategic development of AZD1775, a Wee1 kinase inhibitor, for cancer therapy. Expert Opin Investig Drugs 27:741–751

307. Kool M, Jones DTW, Jäger N, et al (2014) Genome sequencing of SHH medulloblastoma predicts genotype-related response to smoothened inhibition. Cancer Cell 25:393–405

308. Lin SC, Chien CW, Lee JC, Yeh YC, Hsu KF, Lai YY, Lin SC, Tsai SJ (2011) Suppression of dual-specificity phosphatase-2 by hypoxia increases chemoresistance and malignancy in human cancer cells. J Clin Invest 121:1905–1916

309. Galluzzi L, Senovilla L, Vitale I, Michels J, Martins I, Kepp O, Castedo M, Kroemer G (2012) Molecular mechanisms of cisplatin resistance. Oncogene 31:1869–1883

310. Hermida MA, Dinesh Kumar J, Leslie NR (2017) GSK3 and its interactions with the PI3K/AKT/mTOR signalling network. Adv Biol Regul 65:5–15

311. Yeh C-H, Bellon M, Nicot C (2018) FBXW7: a critical tumor suppressor of human cancers. Mol Cancer 17:115

312. Teixeira LK, Reed SI (2017) Cyclin E Deregulation and Genomic Instability. Springer, Singapore, pp 527–547

313. Sato M, Rodriguez-Barrueco R, Yu J, Do C, Silva JM, Gautier J (2015) MYC is a critical target of FBXW7. Oncotarget 6:3292–3305

314. Kim JM, Parmar K, Huang M, Weinstock DM, Ruit CA, Kutok JL, D'Andrea AD (2009) Inactivation of Murine Usp1 Results in Genomic Instability and a Fanconi Anemia Phenotype. Dev Cell 16:314–320

315. Schlacher K, Wu H, Jasin M (2012) A Distinct Replication Fork Protection Pathway Connects Fanconi Anemia Tumor Suppressors to RAD51-BRCA1/2. Cancer Cell 22:106–116

316. Schlacher K, Christ N, Siaud N, Egashira A, Wu H, Jasin M (2011) Double-strand break repair-independent role for BRCA2 in blocking stalled replication fork degradation by MRE11. Cell 145:529–542

317. Rose M, Burgess JT, O'Byrne K, Richard DJ, Bolderson E (2020) PARP Inhibitors: Clinical Relevance, Mechanisms of Action and Tumor Resistance. Front Cell Dev Biol 8:879

318. Welcker M, Clurman BE (2008) FBW7 ubiquitin ligase: a tumour suppressor at the crossroads of cell division, growth and differentiation. Nat Rev Cancer 8:83–93

319. Song MS, Salmena L, Pandolfi PP (2012) The functions and regulation of the PTEN tumour suppressor. Nat Rev Mol Cell Biol 13:283–296

320. Nagata Y, Lan KH, Zhou X, et al (2004) PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. Cancer Cell 6:117–127

321. George S, Miao D, Demetri GD, et al (2017) Loss of PTEN Is Associated with Resistance to Anti-PD-1 Checkpoint Blockade Therapy in Metastatic Uterine Leiomyosarcoma. Immunity 46:197–204

322. He J, Kang X, Yin Y, Clifford Chao KS, Shen WH (2015) PTEN regulates DNA replication progression and stalled fork recovery. Nat Commun 6:7620

323. Blackford AN, Jackson SP (2017) ATM, ATR, and DNA-PK: The Trinity at the Heart of the DNA Damage Response. Mol Cell 66:801–817

324. Kakarougkas A, Jeggo PA (2014) DNA DSB repair pathway choice: An orchestrated handover mechanism. Br J Radiol 87:20130685

325. Peterson CL (2011) The Ins and Outs of Heterochromatic DNA Repair. Dev Cell 20:285–287

326. Papamokos G V., Tziatzos G, Papageorgiou DG, Georgatos SD, Politou AS, Kaxiras E (2012) Structural Role of RKS Motifs in Chromatin Interactions: A Molecular Dynamics Study of HP1 Bound to a Variably Modified Histone Tail. Biophys J 102:1926–1933

327. Huelsenbeck SC, Schorr A, Roos WP, Huelsenbeck J, Henninger C, Kaina B, Fritz G (2012) Rac1 protein signaling is required for DNA damage response stimulated by topoisomerase II poisons. J Biol Chem 287:38590–38599

328. Lu D, Cai H, Park S-S, et al (2015) Nuclear GIT2 Is an ATM Substrate and Promotes DNA Repair. Mol Cell Biol 35:1081–1096

329. Wang Z, Fukushima H, Gao D, Inuzuka H, Wan L, Lau AW, Liu P, Wei W (2011) The two faces of FBW7 in cancer drug resistance. BioEssays 33:851–859

330. Nakayama KI, Hatakeyama S, Nakayama K (2001) Regulation of the cell cycle at the G1-S transition by proteolysis of cyclin E and p27Kip1. Biochem Biophys Res Commun 282:853–860

331. Zhu L (2010) Skp2 knockout reduces cell proliferation and mouse body size: And prevents cancer? Cell Res 20:605–607

332. Lin HK, Chen Z, Wang G, et al (2010) Skp2 targeting suppresses tumorigenesis by Arf-p53-independent cellular senescence. Nature 464:374–379

333. Kermi C, Aze A, Maiorano D (2019) Preserving genome integrity during the early embryonic DNA replication cycles. Genes (Basel) 10:398

334. Macheret M, Halazonetis TD (2015) DNA replication stress as a hallmark of cancer. Annu Rev Pathol Mech Dis 10:425–448

335. Martin OA, Anderson RL, Narayan K, MacManus MP (2017) Does the mobilization of circulating tumour cells during cancer therapy cause metastasis? Nat Rev Clin Oncol 14:32–44