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Disrupting the transcriptional machinery to combat triple-negative breast cancer

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Chapter 7

Summary, discussion and future perspectives

Triple-negative breast cancer (TNBC) is a subtype of breast cancer with very poor prognosis and limited treatment options[1]. In this thesis, our primary objective was to explore novel approaches for the treatment of TNBC. Our investigation began by assessing the responsiveness of TNBC cells to kinase inhibitors, revealing their relative resistance to several kinase inhibitors, including differential sensitivity to those targeting MEK and AKT pathways, while demonstrating notable sensitivity to pan-CDK inhibitors **(Chapter 2).** Building upon these findings, our research refined its focus to examine inhibitors targeting a more specific subset of CDKs, namely transcriptional CDKs, as promising and previously underexplored therapeutic targets for TNBC **(Chapter 3-6)**. In this section, we will discuss the main findings from this thesis and their implications for the research field. Moreover, we will discuss the used technology and highlight current advances that could enhance future research in this area.

Fundamental insights and their implications in advancing transcriptional cyclin-dependent kinases as targets for cancer therapy

Resilience of TNBC against targeting of single signaling pathways

In **Chapter 1**, we discuss frequently altered pathways in TNBC and the ongoing progress in targeting these pathways for treatment. Despite decades of effort, with the exceptions of PARP inhibitor therapy and immunotherapy, targeted treatment approaches have largely fallen short. Even PARP inhibitor treatment and immunotherapy, are effective only in a subset of patients[2,3]. Moreover, although they offer improved responses and fewer severe side effects compared to chemotherapy, they do not consistently lead to durable outcomes.

In **Chapter 2**, we conducted a comprehensive analysis of the sensitivity of 20 TNBC cell lines to a large kinase inhibitor library. This investigation revealed that the targeting of individual deregulated proteins typically fails to inhibit TNBC cell proliferation effectively. Even the inhibition of critically deregulated and mutated pathways, such as the PI3K and MAPK pathways, remained ineffective in most cell lines. This observation aligns with the limited patient benefits observed for these inhibitors in clinical trials[4]. Although we found a cell cycle gene expression signature that could distinguish these PI3K and MAPK pathway inhibitor-resistant cells from sensitive ones, we were not able to restore this sensitivity, highlighting the need for alternative therapies.

The general lack of success in clinical studies aimed at enhancing TNBC targeted therapy **(Chapter 1)** coupled with the overall resistance observed in our kinase inhibitor study **(Chapter 2)**, suggests that targeting one or two proteins within a pathway, involving numerous other mediators, to eventually regulate cell survival and proliferation, may not be the most effective approach for TNBC treatment. The intricate signaling network between the target and effectors of cell survival and proliferation may give too many opportunities for drug resistance. In **Chapter 2**, we also identified

a select number of kinase inhibitors to which most TNBC cell lines displayed relative vulnerability, particularly pan-CDK inhibitors such as flavopiridol and dinaciclib. CDKs have a more direct and central role in regulating critical cellular processes, including cell cycle progression and transcription, making them compelling targets for TNBC treatment[5].

The transcriptional machinery as core vulnerability underlying various TNBC dependencies

To address the challenge of targeting individual proteins within signaling pathways, our research therefore further focused on targeting transcriptional regulation through transcriptional CDKs in **Chapters 3-6**. As discussed in **Chapter 3**, these CDKs serve as critical hubs, integrating signals from numerous signaling pathways and converging them to regulate the expression of genes responsible for various cancer hallmarks. TNBC exhibits rapid proliferation, demanding efficient production of proteins, initially driven by gene transcription. Transcriptional addiction and oncogene-driven elevation of global transcription, or “hypertranscription”[6–8], underscore the substantial reliance of cancer cells on the activity of the transcriptional machinery.

Moreover, inhibitors targeting transcriptional CDKs have shown promise in disrupting specific genes important for cancer, as illustrated in **Chapter 3.** Here, we examined the current literature concerning the targeting of the transcriptional machinery in triple-negative breast cancer (TNBC), focusing on CDKs 7, 8, 9, 12, and 13, as well as BRD4, and their associations with various genomic abnormalities in TNBC. We describe that disrupting the transcriptional machinery deregulates genes involved in processes compromised by genomic aberrations in TNBC, such as the DNA damage response, the cell cycle machinery, super-enhancer transcriptional regulation, anti-cancer immunity, and various signaling pathways, including the MAPK and PI3K pathway. It is important to note that disrupting the transcriptional machinery can have diverse reported effects, depending on factors like the inhibitor type, cancer model, and focus of the specific study. This diversity and the lack of studies using TNBC models emphasizes the need for the systematic approach taken in our research on TNBC throughout the rest of this thesis. **Chapters 4 and 5** focus on the impact of CDK9 and CDK12/13 inhibition, respectively, while **Chapter 6** provides a systematic comparison of the consequences of targeting transcriptional CDKs 7, 8, 9, 12, and 13.

While CDK7 has previously been described as a critical vulnerability of TNBC[9], our research, including direct comparisons in **Chapter 4 and 6**, suggests that targeting CDK9 and CDK12/13 may be equally, if not more, effective. While CDK7 has been explored thoroughly as a target for TNBC [9–14], CDK9 and CDK12 have received comparatively less attention in TNBC research. Given that their targeting is relatively underexplored and their high impact on proliferation and gene transcription, the work in this thesis thus provides important new insights and a strong rationale for developing transcriptional CDK9 and CDK12-based treatment strategies.

Furthermore, our findings in **Chapters 4-6** supports that targeting various tran-

scriptional CDKs does not uniformly suppress global mRNA expression. Instead, it selectively suppresses genes associated with pathways that have potential for cancer treatment. Genes that were significantly and consistently downregulated following CDK9 inhibition and knockout, or after CDK12 (and CDK13) inhibition or knockout, in **Chapters 4-6**, were frequently associated to cell cycle progression and DNA damage responses. Importantly, their rapid downregulation suggests that they are not merely consequences but rather the causes of the inhibition of cell cycle progression discussed in **Chapters 4 and 5**. This may thus be an alternative approach to targeting various cell cycle proteins, which has gained substantial interest in the past decade[15,16]. Furthermore, a similar reduction of DNA damage response genes was previously identified with the non-selective CDK12 inhibitor dinaciclib, sensitizing TNBC cells and preventing drug resistance to PARP inhibition[17]. While selective downregulation of DNA damage genes has been widely reported after CDK12 inhibition or knockout[18–20], such observations of gene-specific control have been limited following CDK9 inhibition. Additionally, it is worth noting that the concurrent and specific downregulation of genes associated with cell cycle progression and DNA replication due to CDK12 inhibition has been acknowledged, but remains a relatively unexplored area of study, despite the clear interconnectedness of these processes with the DNA damage response[21].

Chapters 4-6 also show that targeting CDK9 and CDK12 reduces the activity of transcriptional regulators like transcription factors and histone modifiers, which could further mediate transcriptional reprogramming after CDK9 and CDK12 inhibition[22]. Epigenetic modifications and the aberrant expression of transcription factors are increasingly recognized as critical factors in cancer development and targets for therapy[23–25], hence their targeting through CDK9 and CDK12 may provide options for tackling them simultaneously and/or targeting of undruggable transcription factors. In **Chapter 4**, we demonstrate that the individual downregulation of several affected transcription factors, such as SOX9, EN1, PLAG1 and NR2C2, is sufficient to halt TNBC cell proliferation. While previous research has indicated that inhibition of CDK7 and BRD4 can impair expression of multiple transcription factors through regulation of super-enhancer activity[26–28], this has not yet been well established regarding inhibition of CDK9 or CDK12/13.

In addition, **Chapters 4 and 6** unveil the deregulation of genes involved in cancer stemness and differentiation following CDK9 and CDK12 inhibition or knockout. **Chapter 6** also shows that CDK8 and CDK13 knockout influence genes related to stemness and differentiation. Given the importance of cancer stem cells in chemoresistance, tumor recurrence, and metastasis[29], future research into the effects of CDK targeting on the cancer stem cell population is imperative to comprehend potential mechanisms of drug resistance and the possibility of selectively eliminating cancer stem cells. Moreover, the deregulation of genes related to immune system pathways after CDK7, CDK8, and CDK9 knockout underscores the significance of incorporating immune cell types into our research. Several important studies have indeed shown the promise of using transcriptional CDK inhibitors to unleash anti-cancer immunity in

other cancer types[30–32].

Additionally, our observations from **Chapters 4**, **5, and 6** include a notable upregulation of certain genes, particularly those involved in oxidative phosphorylation, after CDK9 and CDK12 inhibition and knockout. While the functional consequences of this upregulation remain unclear, it could be part of an adaptive stress response[33–35]. Tumors often experience hypoxia[36] or rely on aerobic glycolysis instead of oxidative phosphorylation[37]. Therefore, glycolysis is critical for their energy production, and they may become more susceptible to further metabolic changes as provoked by targeting CDK9 or CDK12. In **Chapter 6**, we demonstrate that CDK12 knockout could sensitize TNBC to a glycolysis inhibitor, PFK15. Similar to genes associated with oxidative phosphorylation, targeting of CDK9 and CDK12 consistently increased the expression of genes encoding ribosomal proteins in **Chapters 5 and 6**. Importantly, CDK12 was recently also associated with protein translation via 4E-BP1 phosphorylation[38] and could thus affect both transcription and translation. The increase in ribosomal proteins might represent an adaptive response, improving mRNA translation efficiency to maintain protein production despite reduced mRNA levels. Recent studies have indicated that increased ribosomal protein expression and ribosomal RNA expression can induce breast cancer metastasis[39,40], highlighting the need to further investigate the observed upregulation of these genes and the role of CDK9 and CDK12 therein. In summary, targeting transcriptional CDKs induces significant reprogramming of the transcriptome, altering the expression of specific genes, to which cancer cells may be particularly vulnerable. However, the functional consequences thereof and the specific mechanisms through which these CDKs precisely regulate the expression of these genes require further investigation. In addition to the fundamental insight into how transcriptional CDKs inhibit TNBC cell proliferation, the insights from this thesis could be used the explore potential combination therapies and biomarkers for response.

The critical role of ABC-transporters in pharmacological interactions and drug efficacy

In **Chapter 4 and 5**, we demonstrate a pronounced synergistic interaction between CDK inhibitors and various kinase inhibitors, including lapatinib. Intriguingly, our investigations in **Chapter 5** elucidated that this synergy is a consequence of the inhibition of ATP-binding cassette transporter G2 (ABCG2) by these synergistic kinase inhibitors, reducing the efflux of transcriptional CDK inhibitors. While prior studies have noted the existence of synergy between different CDK inhibitors and other kinase inhibitors[10,41–43], the potential involvement of ABC-transporters in mediating this interaction has largely been overlooked. This mechanistic insight likely extends to scenarios involving other (yet still unknown) ABCG2 inhibitors and their substrates beyond CDK inhibitors. However, the precise conditions under which this potent synergy occurs warrant further investigation. Furthermore, the elevated expression of ABC-transporters in (cancer) stem cells is noteworthy[44,45]. Given that our CDK inhibitors additionally influence the expression of genes related to stemness, this underscores the need for

further investigation of these treatments in this particular population. Nonetheless, the findings outlined in this thesis warrant a closer examination of these interactions in general and raise questions about the fundamental insights previously derived from synergistic interactions involving CDK inhibitors.

In **Chapter 4** we observed increased toxicity when combining I-73 with lapatinib in tumor xenograft mouse models. Given the interaction with ABCG2, this toxicity could be caused by higher levels of I-73 inside cells. As I-73 is not a completely selective inhibitor and also inhibits CDK1/2/4, among other kinases, these off-target effects may contribute to the observed toxicity[46]. Consequently, exploring combination treatments involving lapatinib with more selective CDK9 or CDK12/13 inhibitors remains a promising strategy that warrants further in vivo evaluation. Additionally, considering interspecies differences in expression levels and substrate affinities between murine and human ABC transporters[47–49], caution is needed when interpreting these models.

Technological framework of this thesis: current utilization and future advances

Selectivity of targeting of transcriptional CDKs

The selectivity of transcriptional CDK inhibitors and in vitro tools for selectively depleting targets, such as CRISPR, has strongly improved in the past decade. Before that, most of the research about transcriptional CDKs was based on non-selective CDK inhibitors, such as pan-CDK inhibitors dinaciclib and flavopiridol[50–52]. This complicated the understanding of the specific function of each transcriptional CDK and the safety and efficacy of targeting them. In **Chapter 4** we showed the efficacy of a panel of novel CDK9 inhibitors, including I-73[53], D10-81 and Y3-21, that are more selective and potent against CDK9 compared to other targets than the first described pan-CDK inhibitors. However, these inhibitors still also inhibited at least one or multiple other CDKs, including CDK1, 2, 4 and 7, at similar potency. Thus the effects observed in that study cannot be pinpointed to CDK9 with complete certainty. In **Chapter 5 and 6** we therefore aimed to use more, recently discovered, selective inhibitors and CRISPR-Cas9 knockouts to understand the role of each CDK and potency of targeting them. Nevertheless, given their high similarity in kinase domain, there are no selective kinase inhibitors available for CDK12 and CDK13 individually.

In addition to attributing the deregulated genes described in the previous section with more certainty to these CDKs, the use of CDK knockouts and selective inhibitors gave us additional insights. For instance, previous studies have shown great promise in inhibiting CDK7 using the CDK7/12/13 inhibitor THZ1. However, the use of more selective inhibitors such as BS-1814[54] and CT7001 (ICEC0942/Samuraciclib)[55], as well as selective CDK7 knockout, in **Chapter 6**, enabled us to reveal that, in our models, targeting CDK7 did not exert effects on gene transcription or proliferation as strongly as observed with selective CDK9 or CDK12/13 inhibition and knockout.

Although we did observe overlap in specific pathways and genes deregulated by knockout of CDK9 and CDK12 versus CDK9 inhibition and CDK12/13 inhibition in **Chapter 6**, the overall overlap was limited. For example, while pro-survival MCL1 and Bcl-xL levels were significantly downregulated after CDK9 or CDK12/13 inhibition, they were not after CDK9 or CDK12 knockout, despite their efficient proliferation inhibition. Moreover, while we observed strong intronic polyadenylation after CDK12/13 inhibition, we did not observe it after CDK12 knockout. Here, CDK13 inhibition may enhance the effect of CDK12 inhibition, which has been suggested previously[56,57]. This could be due to that, in addition to CDK12, also CDK13 can contribute to the phosphorylation of several RNA processing factors[58]. Other studies have shown intronic polyadenylation after CDK12 knockout, indicating potential differences in functionality and compensation by CDK13 in various cancer models[18,58]. Given these findings, and as specific cancers have CDK12 inactivating mutations[59,60], selective CDK13 inhibition could potentially be synthetically lethal, providing a rationale for the development of selective CDK13 inhibitors. As CDK12 knockout did strongly impact gene expression and proliferation without inducing intronic polyadenylation, this suggests that intronic polyadenylation is likely not the only mechanism behind gene-specific regulation after CDK12 (and CDK13) inhibition, urging for further research in this field.

Moreover, the differences between knockout and inhibition may also stem from the inherent distinction between gene knockout and protein inhibition. A gene knockout causes an eventual complete, but gradual depletion of the protein expression, enabling adaptation. Moreover, the complete absence of the protein could have different effects than the inhibition of kinase activity of a protein (e.g. remaining activity or function and binding not dependent on kinase activity). While CRISPR/Cas9 knockout technology[61] enabled precise investigation of individual transcriptional CDKs, its effects may therefore not directly mirror the consequences of selective kinase inhibitors that are most commonly used in clinical settings. Therefore, alternative techniques may offer advantages over creating knockouts, including utilizing kinase-dead mutant proteins[62,63], generating analogue-sensitive proteins[64,65], or employing recently refined CRISPR-based techniques including CRISPRi (transcriptional interference by deactivated Cas9)[66], CasTuner (analog tuning of gene expression through deactivated Cas9, fused to degron and repressor domains)[67] or CRISPR-Cas13d (interference and modulation of RNA)[68]. Additionally, target-specific effects of inhibitors can be validated through the generation of cells with inhibitor-refractory mutant protein expression[69]. Genetic approaches that deplete the protein, instead of inhibiting it, may have similar effects as molecular degraders, which have also recently been developed for CDK9 and CDK12[70,71]. Altogether, when assessing the relevance of targets, it is crucial to consider the method of targeting and its potential implications. Nevertheless, the combined use of highly selective CDK inhibitors and knockouts used in **Chapter 6** enabled us to identify high-confidence mechanisms and targets.

High-throughput technologies and transcriptomics to rapidly pinpoint key players of complex mechanisms

In this research, we utilized multiple high-throughput methods, which have the advantage of providing an unbiased and complete perspective on certain research questions. This led us to findings that would have otherwise required extensive research through low-throughput, hypothesis-based research. For example, in **Chapter 5** we screened large kinase inhibitor libraries using high-throughput and robust SRB proliferation assay and ABCG2 inhibitory activity pheophorbide A read-outs. These screening approaches led us to the unique and simultaneous discovery that an unexpected amount of kinase inhibitors could synergize with transcriptional CDK inhibitors by inhibiting ABCG2; this would have not been possible when testing single agents in a more narrow, hypothesis-based approach. However, it's important to note that these compound libraries primarily enable discoveries related to existing drugs, limiting their applicability to novel target identification, and complicating mechanistic insights due to potential off-target effects. To overcome these limitations, in **Chapter 5**, we employed a whole-genome CRISPR-Cas9 pooled knockout screen to find genes that sensitized cells to THZ531 treatment upon knockout. This led us to pinpoint ABCG2 as a critical contributor to THZ531 resistance. Similarly, other CRISPR-based screening technologies, such as CRISPR interference and activation screening[66], and arrayed CRISPR screening[72] enabling phenotypic read-outs, could provide additional insights into drug resistance of CDK inhibitors in the future.

In addition to these high-throughput interventions for target identification, we turned to transcriptomics to gain a comprehensive understanding of drug resistance. In **Chapters 2 and 5**, we identified gene expression patterns in untreated cell lines, associated with drug resistance against MEK and Akt inhibitors (elevated cell cycle signature), as well as transcriptional CDK inhibitors (increased ABCG2 expression), respectively. Furthermore, we extensively utilized RNA-sequencing in **Chapters 4, 5, and 6** to explore genes and associated pathways deregulated by transcriptional CDK inhibitors. These findings provided a wealth of insights into the (in)direct mechanisms of action, potential adaptation, drug resistance mechanisms, and potential biomarkers for response and combination treatment strategies. Moreover, in **Chapters 5 and 6** we describe a new computational method, utilizing publicly available data from the polyAatlas[73], to quantify intronic polyadenylation based on commonly used whole-transcript mRNA sequencing data. As this analysis can be applied to regular mRNA sequencing data, although preferably with higher sequencing depth (50M reads), it can also be utilized to analyze data generated for regular differential gene expression analysis or splicing analysis, which includes most sequencing data of clinical samples. However, as intronic polyadenylation could influence transcript stability and thus mRNA levels[74], nascent RNA-sequencing could give a more comprehensive view of this[75]. Moreover, sequencing near the 3'-ends only (3' sequencing), could quantify these events with more certainty, and could also quantify other alternative polyadenylation sites[76].

In addition, more advanced sequencing methods such as nascent RNA-sequencing and chromatin immunoprecipitation (ChIP) sequencing can give further insights into distinguishing direct from indirect responses and the mechanism of preferential gene control (e.g. binding motifs, distribution within gene body and/or distal regulatory elements)[75,77]. By investigating the stability of nascent synthesized RNA (e.g. Bru-Seq combined with Bru-Chase-Seq), CDK12 inhibition has recently been implicated in inhibiting transcriptional read-through and possibly and negatively influencing the stability of mRNA, which could be an additional mechanism of gene expression regulation to intronic polyandeylation[78]. Advanced sequencing techniques are thus essential for understanding the intricate gene regulation mechanisms by components of the transcriptional machinery. Moreover, investigating the direct interactions of (chromatin-bound) transcriptional CDKs with other proteins through (chromatin) immunoprecipitation pulldown assays followed by proteomics analysis (e.g. RIME[79], IP LC-MS[80]) and/or effect of their inhibition on the phosphorylation of proteins, using phospho-proteomics, can shed more light on their function. In addition, further research is essential to confirm if the genes and pathways described in this thesis lead to changes at the protein level and affect functionality. In conclusion, our study leveraged high-throughput technologies and transcriptomics to uncover the consequences of transcriptional CDK depletion and inhibition. While these approaches yielded valuable insights, the application of additional tools and techniques can further elucidate the intricate mechanisms underlying these consequences.

Models to study anti-cancer efficacy: limitations of cancer cell lines and technological advances

In our research, we primarily utilized immortalized cancer cell lines grown on plastic dishes as models to investigate transcriptional CDKs. These cell line models offer advantages such as cost-effectiveness, ease of handling, and nearly limitless supply, making them suitable for the high-throughput studies described in this work. However, these models have limitations in terms of their representation of human tumors. Over time, these cells have undergone changes and adaptations through numerous passages on plastic surfaces and cultivation in medium containing non-representative and undefined growth factors. Additionally, the in vitro environment of these cell lines lacks critical components of the tumor micro-environment, such as extracellular matrix interactions and interactions with others cells, such as immune cells or fibroblasts.

To improve the translational potential of our findings in cell lines, we explored the effects in Hs578T xenografted immunocompromised mice, as described **in Chapter 4**. This approach can improve clinical relevance by providing 3D tumor growth surrounded with extracellular matrix and a more physiologically relevant mixture of growth factors. However, these xenografts still originate from cell lines that do not represent human tumors well. We therefore also incorporated a TNBC patient-derived xenograft mouse model, in which tumor cells from a human TNBC tumor were propagated solely in mice. While patient-derived xenograft (PDX) in vivo models come closer to mimicking human tumors, they still lack certain components present in actual human tumors,

such as immune cells and human growth factors. In addition, these models are not suitable for the early and high-throughput target discovery described in this research.

To better reflect human tumor conditions at early stages of target validation and drug discovery, avoiding the high costs and ethical issues with in vivo studies, in vitro and ex vivo culture methods are currently being improved. For example, 3D cultures of immortalized cell lines using physiologically relevant extracellular matrix materials, such as basement membrane extract and collagen, can restore functionality and alter drug responses[81–83]. To avoid the use of cell lines, patient or patient-derived xenograft tumor cells can be used directly for drug research, for example as tumor slices, that maintain relevant extracellular matrix interactions[84], or isolated tumor cells[85]. More advanced models involve the culture and expansion of cells isolated from patients or patient-derived xenograft tumors as 3D organoids in matrices with physiologically relevant medium conditions[86,87]. In **Chapter 6**, we therefore demonstrated the efficacy of transcriptional CDK inhibitors in isolated tumor cells from pleural effusions and patient-derived xenograft organoids from two TNBC patients. This proof-of-concept further illustrates the potency of transcriptional CDK inhibitors and the utility of these models to investigate drug efficacy.

Furthermore, the adverse events resulting from I-73 and lapatinib combination treatment that we observed in the mouse models in **Chapter 4**, demonstrate that it is imperative to assess potential toxicity in healthy tissues in early drug and target discovery as well. Many potential drugs can efficiently kill tumor cells at certain concentrations, but the clinical therapeutic window is critical for their successful development as safe and effective anti-cancer treatments. Although mice models can provide insights into this balance of safety and efficacy, these are usually only used in later stages of research and significant differences exist between mice and humans and, in general, murine models are described as poor predictors of human toxicity and efficacy in clinical trials[88,89].

Therefore, it is crucial to evaluate the effects in vitro in human healthy tissue alongside clinically relevant tumor models during early drug and target discovery. Adverse events causing termination of clinical drug development or market withdrawal are mostly related to toxicity in the nervous system, liver, or heart[90]. Moreover, anti-cancer drugs frequently induce (acute) myelosuppression (e.g. neutropenia, leukopenia, anemia) and gastrointestinal toxicity (e.g. severe nausea, vomiting and diarrhea, likely caused through effects on nervous system [91]). Notably, the initial clinical trial of a selective CDK9 inhibitor also observed a high frequency of these adverse events, with myelosuppression being the primary cause of the most severe, high-grade events[92]. Modeling of these, and other, healthy tissue types, is therefore important to further steer the optimization of CDK inhibitors, alongside of testing in clinically relevant in vitro/ex vivo cancer models. While immortalized cell lines representing various healthy tissues are available, they are not ideal models for the same reasons mentioned for immortalized cancer cell lines[93]. Recent advances include the use of primary cells or differentiation of inducible pluripotent stem cells (iPSC) that resemble different cell types. Although

primary cells from organs like the liver[94], heart, bone marrow and nervous system are very limited due to their scarcity as left-over after surgical procedures, mammary tissue is more readily available from surgeries such as reduction mammoplasty or (prophylactic) lumpectomy[95]. Studying potential toxicity in mammary tissue can reveal whether a drug selectively kills breast cancer cells compared to healthy breast cells, shedding light on oncogenic transformation-induced vulnerabilities. Alternatively, iPSCs can be differentiated into cells and/or organoids resembling various tissues, such as iPSC-derived hepatocytes, cardiomyocytes and cells representing various neuronal lineages[93]. While these differentiated iPSC-derived cells are less mature than normal tissue cells, and the differentiation process can be laborious, challenging and costly, these models provide a valuable resource for understanding and avoiding potential toxicity of novel cancer therapeutics, such as CDK inhibitors.

Concluding remarks and future perspectives

In conclusion, this thesis highlights the potential of transcriptional CDK inhibitors as a promising avenue for treating TNBC. It systematically compares the efficacy of targeting various CDKs, with CDK9 and CDK12 emerging as highly potent targets for disrupting TNBC cell proliferation. These inhibitors induce transcriptional reprogramming rather than global shutdown, shedding light on their mechanisms of action and potential opportunities for combination treatments. Additionally, our research uncovers a crucial mechanism of drug resistance of transcriptional CDK inhibitors involving ABCG2 transporters and a means of targeting it. Future research should delve into understanding the precise mechanisms of gene-selective regulation by CDK9 and CDK12, as well as investigating resistance mechanisms. Evaluating the efficacy and safety of these inhibitors in more clinically relevant models, including primary cancer tissues, healthy tissues, and in vivo models, will be crucial for guiding further clinical development of these inhibitors. All together, the insights from this thesis, and the proposed future efforts, will help to further steer the (pre-)clinical development and strategy of using transcriptional CDK inhibitors for the treatment of TNBC.

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