Into the blue...Using mouse models to uncover genes driving tumorigenesis and therapy resistance in human breast cancer

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A.1 English summary

Cancer is a disease in which normal cells are deregulated by disruption of their cellular processes, resulting in increased proliferation, survival and invasion of surrounding tissues. This disruption is generally attributed to (epi)genetic alterations in so-called driver genes, which provide cells with a selective growth advantage and drive their malignant transformation. To improve cancer treatments, personalized medicine approaches have aimed to identify exactly which mutations are driving tumor development in a given patient and specifically target these mutations using precision therapies. However, one of the main challenges of this approach is identifying which mutations are true drivers, as tumors typically contain many additional passenger mutations that do not actually contribute to tumor development. Besides this, many patients often relapse after prolonged treatment due to the emergence of acquired resistance, limiting the clinical effectiveness of targeted treatments.

To address these issues, it is crucial to (i) determine exactly which mutations are driving the development of certain cancer types and (ii) identify potential resistance mechanisms to targeted treatments. However, identifying driver genes in human sequencing studies has been challenging, due to the large amounts of potential drivers that are typically identified in these studies. Similarly, the identification of resistance mechanisms is generally limited by lacking availability of pre- and post-treatment tumor samples from patients, complicating the discovery of all potential resistance mechanisms for a given treatment. Model systems such as mouse models provide several complementary approaches for studying tumor development, by enabling the development of renewable models resembling specific subtypes of human cancer, which can then be studied in more detail to identify candidate cancer genes and therapy resistance mechanisms. An overview of several of these approaches is provided in **Chapter 1**.

In this thesis, we mainly focus on using genetically engineered mouse models to identify candidate cancer genes and therapy resistance mechanisms in two different types of breast cancer: invasive lobular carcinoma (ILC) and triple-negative breast cancer (TNBC) (**Chapter 2**). ILC is a histological subtype of breast cancer that accounts for 10-15% of all breast cancer cases and is characterized by mutations in E-cadherin (encoded by the *Cdh1* gene). Although ILCs generally show increased expression of ERα, long-term outcomes of ILC are typically worse than other ER-positive breast cancers, indicating that biological differences are influencing therapy response. TNBCs account for another 10-17% of breast cancers and are characterized by low expression of HER2 and the steroid hormone receptors ERα and PR. As such, TNBCs cannot be treated with specialized hormone therapies, leaving chemotherapy

as standard-of-care. Combined with the aggressive nature of these tumors, this results in a relatively poor prognosis for patients with TNBC.

A common theme in both ILC and TNBC, is that they respond less favorably to existing therapies than other breast cancer subtypes. As such, patients would benefit from further research identifying which genes are driving the development of these breast cancers, allowing the development of novel therapies targeting vulnerabilities stemming from the identified drivers.

In **Chapter 3**, we aimed to identify potential driver genes of ILC by using *Sleeping Beauty* (SB) transposon-based insertional mutagenesis (IM) to screen for candidate cancer drivers in a mouse model with mammary-gland specific inactivation of *Cdh1*. This showed that mice with combined loss of *Cdh1* and activation of IM develop tumors resembling human ILC, demonstrating the relevance of our model. Analysis of the insertions in these tumors identified several known and novel drivers of ILC, providing leads for the development of future therapies. Most notably, we identified several recurrent and mutually exclusive SB transposon insertions in *Myh9*, *Ppp1r12a*, *Ppp1r12b* and *Trp53bp2*, whose products have been implicated in the regulation of the actin cytoskeleton. *MYH9*, *PPP1R12B* and *TP53BP2* were also found to be frequently aberrated in human tumors, indicating that this is a novel oncogenic pathway in (human) ILC that might be exploited in future therapies.

One of the main challenges in the analysis of IM screens is that they can identify many potential cancer genes, of which only a fraction is likely involved in tumorigenesis. Additionally, the DNA-based approaches used to identify transposon insertions and associated candidate genes do not provide much insight into how genes are affected by the insertions. In **Chapter 4**, we addressed these issues by developing an approach called IM-Fusion, which identifies transposon insertions from gene-transposon fusions in RNA-sequencing data. Moreover, by combining insertion detection with differential expression analysis, this approach is capable of providing detailed insight into the effects of insertions on gene expression. To demonstrate the utility of our approach, we applied IM-Fusion to two existing RNA-sequencing datasets with matched DNA-based data. This demonstrated that IM-Fusion detects transposon insertions and their true target genes more accurately than DNA-based analyses.

In **Chapter 5**, we aimed to explore how the mutational landscape of BRCA1-deficient TNBC is affected by the presence of several established driver genes (*Myc*, *Met* and *Rb1*), in order to identify additional genes that collaborate with these established drivers and may therefore be targetable in novel therapies. Our analyses showed that BRCA1-deficient TNBCs with MYC overexpression exhibit a dramatically different copy number landscape than TNBCs from other models, indicating that high MYC

levels strongly affect the evolution of these tumors. By comparing recurrently aberrated regions between mouse and human TNBCs, we identified *Mcl1* as the main driver gene in a locus that is specifically amplified in MYC-driven TNBC, suggesting that MCL1 collaborates with MYC in TNBC development. Supporting this, additional experiments in a BRCA1-deficient patient-derived xenograft (PDX) model showed that MCL1 inhibition increases the effectiveness of the poly(ADP-ribose) polymerase (PARP) inhibitor olaparib (which targets the BRCA-deficiency of these tumors), underscoring the therapeutic potential of this combination treatment for BRCA-deficient TNBC.

In the remaining chapters, we focused on identifying potential resistance mechanisms to targeted treatments in ILC and TNBC.

First, in **Chapter 6**, we sought to explore the effects of FGFR inhibition in ILC, as *Fgfr2* was frequently activated by transposon insertions in our insertional mutagenesis screen (Chapter 3). To this end, we transplanted fragments of an ILC with activated FGFR signaling into multiple recipient mice and treated these mice with the FGFR inhibitor AZD4547. This showed that tumors initially regress upon treatment with the inhibitor, but quickly become resistant to treatment. To identify potential resistance mechanisms, we exploited the ongoing insertional mutagenesis in these tumors to identify transposon insertions that were acquired during treatment and may explain the observed resistance. Combined with a transcriptomic analysis of these tumors, this approach identified several known and novel resistance mechanisms. Notably, two novel resistance mechanisms were only identified by insertional mutagenesis, demonstrating that IM is an effective tool for identifying resistance mechanisms to targeted treatments in mice.

Next, in **Chapter 7**, we aimed to identify potential resistance mechanisms in BRCA2-deficient breast cancer to PARP inhibition (PARPi), which specifically targets the HR-deficiency of BRCA-deficient tumors. To do so, we performed *in vitro* PARPi resistance screens in BRCA2-deficient mammary tumor cells. Besides this, we also performed an *in vivo* analysis by transplanting pieces of BRCA2-deficient mammary tumors into multiple recipient mice, treating these mice with PARPi until resistance and subsequently contrasting the sensitive and resistant tumors. Strikingly, both these analyses identified loss of poly(ADP-ribose) glycohydrolase (PARG) as a major resistance mechanism. Further characterization showed that depletion of PARG induces resistance by restoring PAR formation, rescuing controlled DNA replication fork progression and promoting the recruitment of downstream DNA repair factors. The potential relevance of PARG in clinical PARPi resistance is underscored by the presence of PARG-negative clones in a subset of human TNBC and serous ovarian cancers.

Finally, in **Chapter 8**, we reflected on the methods and results presented in this thesis and how they may be applied or extended in future work. Besides this, we also consider several technological advances and important challenges that remain to be addressed in the field.