Precision modeling of breast cancer in the CRISPR era

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Appendices

English summary

The molecular mechanisms that instigate a healthy cell to become malignant are fueled by (epi)genetic alterations in so-called driver genes (i.e. because they drive the transformation process). While the Holy Grail of precision medicine is to identify these genetic dependencies and to target them with specific compounds in a personalized fashion, this has proven a daunting task, as tumors are exquisitely characterized by genetic instability and a mutator phenotype. As deep-sequencing studies have shown, each single tumor is actually a heterogeneous mass in which actionable driver events are confounded by a plethora of additional passenger mutations that do not contribute to tumorigenesis. Deconvolution of this complexity requires not only powerful bioinformatics approaches, but also functional validation in model organisms to establish robust genotype-phenotype relationships. Genetically engineered mouse models (GEMMs) are uniquely suited for this purpose, as they enable *in vivo* assessment of *de novo* tumorigenesis in a mammalian organism with intact immune and stromal compartments upon perturbation of (combinations of) oncogenes and/or tumor suppressor genes (TSG). Moreover, GEMMs can be used for preclinical testing of novel (combinations of) anticancer therapeutics and to identify potential mechanisms of drug resistance.

The laboratory of Prof. Jos Jonkers has focused in the last 15 years on the genetic dissection of cancer development, therapy response and resistance in mouse models of breast cancer. **Chapter 2** provides an overview of all models generated and their applications, ranging from patient-derived xenografts (PDXs) to germline and somatic GEMMs of breast cancer.

The latter are based on the direct modification of adult mammary epithelium upon intraductal injection of viral vectors designed for genetic engineering purposes. **Chapter 3** describes how this somatic approach can be used to model invasive lobular carcinoma (ILC), a breast cancer subtype characterized by loss of E-cadherin. Lentiviral vectors were equipped to express the Cre recombinase, the CRISPR/Cas9 system or both, and injected in female mice carrying conditional alleles of the *Cdh1* gene, encoding E-cadherin. With this approach we were able to disrupt endogenous TSGs (e.g. *Pten*) in ILC-initiating cells and thereby evaluate their role in this breast cancer subtype. We also observed that injection of Cas9-encoding lentiviral vectors elicits strong Cas9-specific immune responses, which could be circumvented by intraductal injection of sgRNA-encoding vectors in mice with mammary gland-specific expression of Cas9.

Somatic modeling of cancer using CRISPR technology *in vivo* proved to be a true game-changing tool, allowing for rapid functional validation of candidate cancer genes enrolling from forward genetic screens and catalogs of alterations in human tumors.

For example, in **Chapter 4** we deployed Sleeping Beauty transposon-based insertional mutagenesis to identify genes that collaborate with E-cadherin loss in the development of ILC. Recurrent and mutually exclusive transposon insertions were identified in *Myh9, Ppp1r12a, Ppp1r12b* and *Trp53bp2*, which are all frequently mutated in human ILC and involved in regulation of the actin cytoskeleton. Using somatic approaches, we could rapidly validate all four genes as *bona fide* collaborators of E-cadherin loss in ILC development.

The following four chapters focus on triple negative breast cancer (TNBC), a mammary tumor subtype characterized by poor prognosis and limited treatment options. As these tumors are often defective in homologous recombination-mediated repair of DNA double-strand breaks, we used germline and somatic GEMMs with conditional *Brca1* and *Trp53* alleles, and we could recapitulate in the resulting tumors the genomic instability observed in patient samples. Strikingly, we observed that engineered overexpression of MYC in this model has profound impact on the DNA copy-number profile of the tumors. Comparative oncogenomics analysis of mouse and human tumors unveiled new culprits of tumorigenesis and MCL1 as a novel therapeutic vulnerability in this breast cancer subtype. The results of this study are presented in **Chapter 5**.

In **Chapter 6** we further expanded the possibilities of somatic engineering by implementing *in vivo* base editing in mammary gland epithelium. Indeed, while canonical CRISPR-based gene disruption offers a rapid way for inactivating candidate TSGs, it does not permit *in vivo* recapitulation of clinically relevant missense mutations, which represent the most common somatic alterations seen in human breast cancer. To this end, we generated knock-in mice with Cre-conditional expression of a cytidine base editor and tested their utility for precise somatic engineering of missense or nonsense mutations in key cancer drivers. Upon intraductal delivery of sgRNA-encoding vectors, we could install point mutations with high efficiency in one or multiple endogenous genes *in situ*, and assess the effect of defined allelic variants on mammary tumorigenesis.

In **Chapter 7** we used the organoid technology to efficiently derive three-dimensional lines from BRCA1/2-proficient and –deficient TNBCs from our GEMMs. These mouse mammary tumor organoids can be easily genetically modified *in vitro* (e.g with CRISPR-Cas9 methods) and upon orthotopic transplantation give rise to tumors that preserve the characteristics of the original GEMM tumors. The ease with which they can be modified makes mouse mammary tumor organoids powerful tools for studying tumor biology and drug resistance. Indeed, in **Chapter 8** we performed *in vitro* functional genetic screens and identified loss of the CST complex as a novel mechanism by which BRCA1-deficient tumor cells can escape from the synthetic lethal effects of PARP blockade. We could deplete the members of this complex *in vitro* in 3D organoids and validate their role in drug resistance upon treatment of organoid-derived tumors with PARP inhibitors.