The handle http://hdl.handle.net/1887/82703 holds various files of this Leiden University dissertation.

Author: Annunziato, S.
Title: Precision modeling of breast cancer in the CRISPR era
Issue Date: 2020-01-16
General introduction
Breast cancer represents the most common type of cancer in women worldwide (25% of cases), the second most common cancer overall (12% of cases) and the second most common cause of cancer-related deaths (Torre et al., 2016). Breast cancer encompasses a very diverse family of pathologies that can be classified into distinct subtypes with specific molecular features, phenotypic characteristics and clinical prognosis. Over the last decades, screening campaigns, novel therapeutics and improved treatment schemes have increased overall survival of breast cancer patients, but development of drug resistance and metastatic disease still pose a serious challenges to breast oncologists. Importantly, actionable molecular vulnerabilities are difficult to study in individual full-blown human tumors, as cancer genomes are exquisitely heterogeneous, each displaying distinctive spectra of driver events and passenger mutations of less clear significance. While sequencing studies have shed light on the compendium of somatic alterations in breast cancer (Nik Zainal et al., 2016), deconvoluting breast cancer’s complexity and molecular underpinnings requires tractable and uniform models in which robust genotype-phenotype relationships can be drawn. To elucidate the biology of breast cancer, several mouse models with different degrees of sophistication have been established over the years. While a comprehensive catalog of murine mammary tumor models is beyond the scope of this thesis (reviewed in Holen et al., 2017), they can broadly be classified into two main categories. The first category entails models based on orthotopic implantation of human or murine tumor material (cell lines, organoids, tumor fragments) in immunocompromised or syngeneic mice, whereas the second category involves genetically engineered mouse models (GEMMs), in which de novo mammary tumorigenesis is induced by perturbing the function of oncogenes and tumor suppressor genes in the mouse mammary gland. A third strategy, which is more rarely deployed or used in conjunction with GEMMs, relies on induction or acceleration of mammary tumors via carcinogens, ionizing radiation, slow-transforming retroviruses (e.g. MMTV) or transposons.

The simplest models are based on engraftment of human breast cancer cell lines to immunocompromised mice (‘xenografts’) or mouse mammary tumor cells in syngeneic immunocompetent mice (‘allografts’). Cell line-based xenografts are the most commonly used breast cancer models for preclinical testing of novel drugs. While allowing great manipulability, cell lines are usually derived from highly aggressive tumors or pleural effusions containing multiple mutations. Moreover, tumor cell lines undergo stringent selective pressures to adapt to in vitro culture conditions, resulting in limited genetic heterogeneity and genetic drift (Ben-David et al., 2018). Patient-derived xenografts (PDXs), on the contrary, have been shown to recapitulate the genetic and phenotypic complexity of their donors and, importantly, their response to therapy, although mouse-specific tumor evolution has been reported after prolonged in vivo passaging (Ben-David et al., 2017). Some groups have pioneered the concept of co-clinical trials using...
mouse PDX “avatars” for developing patient-tailored therapeutic schemes, while others are humanizing mouse recipients with immune components to test immunotherapy approaches in PDX models (Hidalgo et al., 2014; Byrne et al., 2017). While also PDXs suffer from selection towards aggressive malignancies, recent reports of successful propagation of more indolent subtypes (e.g. luminal ER-positive) or pre-cancerous samples (e.g. ductal carcinoma in situ, DCIS) via intraductal injection (rather than fat pad injection) of patient material herald hope for future developments (Behbod et al., 2009; Fiche et al., 2018).

GEMMs represent the most sophisticated models of human breast cancer (Kersten et al., 2017). They rely on the transgenic expression of an oncogene and/or the inactivation of a tumor suppressor gene (TSG) in the mammary gland. Tissue-specificity and sporadic de novo tumor formation is commonly achieved by cross-breeding mice with mammary gland-specific expression of the Cre recombinase together with mice with Cre-conditional alleles for cancer genes of interest. Although the genetic perturbation of the cancer gene is usually irreversible, systems based on doxycycline- or tamoxifen-inducible gene expression/repression permit spatio-temporal control of oncogenic drivers and in vivo assessment of oncogene addiction. A major advantage of GEMMs is the capability to simulate the step-wise progression of a healthy mammary cell to hyperplasia, DCIS and finally invasive disease in the context of a native stromal compartment and in the presence of a functional immune system to be evaded (Gil Del Alcazar et al., 2017) or hijacked (Coffelt et al., 2015). However, an important limitation of GEMMs is that the generation of novel models, which are often based on compound mutant mice carrying multiple engineered alleles, requires great amount of resources, money and time, thus limiting the experimental throughput of these models.

In this thesis, I will describe in Chapter 2 the different mouse models of breast cancer that have been developed in our laboratory during the past 15 years, how they have been exploited to investigate crucial aspects of tumor cell biology, including metastasis spreading, therapy resistance and tumor heterogeneity, and how they have been of paramount importance for translational cancer medicine in developing effective treatment schemes and in leading to the clinical approval of new drugs.

In recent years, a new technology, named CRISPR-Cas9 genome editing, has revolutionized gene function studies. The unprecedented ease at which endogenous loci can be perturbed with this game-changing technology has opened myriad possibilities in terms of in vivo modeling of alterations observed in human malignancies. In Chapter 3 I will show how in situ CRISPR-Cas9 genome editing was used as proof-of-concept for the fast-track development of new mouse models of invasive lobular breast cancer, by manipulating the adult mammary tissue via intraductal injection of sgRNA-encoding
lentiviruses in Cas9-transgenic mice, ultimately resulting in sporadic mammary tumor formation.

An important advantage of somatic gene editing is the possibility to rapidly test cancer-relevant hypotheses coming from human deep-sequencing studies or mouse forward genetic screens, without the need to generate complex GEMMs. In Chapter 4 I will describe an in vivo insertional mutagenesis screen that we performed with the Sleeping Beauty transposon system to identify genes that collaborate with E-cadherin loss in the genesis of invasive lobular carcinoma (ILC). Notably, our somatic engineering pipeline permitted immediate in vivo validation of the top candidates of the screen as bona fide ILC driver genes.

In Chapter 5 I will shift to triple-negative breast cancer (TNBC), an aggressive subtype that often displays dysfunctional DNA repair (e.g. mutations in BRCA1) and is therefore primarily driven by DNA copy-number alterations (CNAs) containing large numbers of candidate driver genes. By combining germline and somatic engineering of the mammary gland with comparative oncogenomics analysis of recurrent CNAs in human and mouse tumors we uncovered new culprits of tumorigenesis and identified MCL1 inhibition as a novel therapeutic vulnerability of these tumors.

In Chapter 6 we took somatic engineering of the mouse mammary gland to the next level by developing new mammary tumor models with the help of in vivo base editing. We used this new technology to install in situ oncogenic missense and nonsense variants in relevant oncogenes and TSG. With this fast-track approach, we could rapidly test their contribution to tumorigenesis in a mouse model of BRCA1-deficient TNBC.

The possibility to transplant GEMM-derived tumors into syngeneic mice has proven invaluable for studying drug response and resistance in vivo. In Chapter 7 I will describe how we applied the organoid culture technology to our well-defined genetic mouse models of BRCA1/2-mutated TNBC, in order to develop a powerful platform for testing mechanisms of drug resistance by deriving 3D cultures that can be genetically modified prior to orthotopic transplantation in recipient mice.

Finally, in Chapter 8 we went in search of novel mechanisms of PARP inhibitor resistance by deploying CRISPR-Cas9 in vitro forward genetic screens. We found restoration of homologous recombination (HR) due to loss of the CST complex to be a BRCA1-independent escape mechanism from the synthetic lethal effects of PARP blockade. Importantly, we could deploy the organoid technology described in Chapter 7 to genetically deplete members of this complex in vitro, and demonstrate their role in in vivo drug resistance upon transplantation of the modified organoids.
References


