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

Ruiter, J.R. de

2

Scope of this thesis

2.1 Introduction

Breast cancer is the most common malignancy affecting women in the Western world, with more than 17,000 cases being diagnosed in the Netherlands alone each year*. Overall treatment of breast cancer is relatively successful, however recurrence of the disease remains a significant problem in clinical practice[1]. Breast cancer is known to be a heterogeneous disease and has therefore been subdivided into different subtypes, based on histological characteristics[2], gene expression patterns[3] and expression of different markers such as estrogen receptor-α (ER-α), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2, aka ERBB2), which are known to drive expression of downstream signaling pathways and increase cellular proliferation[4–6]. Genetic analyses have identified several common genetic alterations associated with specific subtypes[7–9], indicating that biological differences between the subtypes strongly shape tumor development.

2.2 A tale of two breast cancer subtypes

In the remainder of this thesis, we focus on two particular subtypes of human breast cancer: invasive lobular carcinoma and triple-negative breast cancer.

Invasive lobular carcinoma (ILC) is a histological subtype of breast cancer that represents 8-14% of all breast cancer cases. The classical form of ILC is characterized by rows of small discohesive cells, which invade into the surrounding stroma in a single-file pattern[10]. This invasive phenotype is generally attributed to the functional loss of E-cadherin (encoded by the *CDH1* gene), a cell-cell adhesion molecule that forms a key component of adherens junctions and plays an important role in maintaining epithelial integrity[11]. Functional loss of E-cadherin occurs in 90% of all ILCs and is mainly due to mutational inactivation, loss of heterozygosity (LOH) or impaired integrity of the components of the E-cadherin–catenin complex[9,12–14]. Besides this, ILCs are generally ER- and PR-positive and rarely show amplification of HER2. However, long-term outcome of ILC is generally worse than stage-matched invasive ductal carcinoma (IDC)[15], suggesting that biological differences between the two subtypes may be influencing treatment efficacy.

Triple-negative breast cancer (TNBC) is a heterogeneous subtype of breast cancer that is characterized by low expression of ER, PR and HER2. Altogether, TNBC accounts for 10-17% of all breast cancer cases, depending on the methods and thresholds used to assess the status of the three receptors[16]. At the mutational level, TNBCs are enriched for mutations in *TP53*[7] and *BRCA1*[17], which plays a key role in the

*www.cijfersoverkanker.nl

repair of DNA double-strand breaks (DSBs) via homologous recombination (HR). As such, BRCA-deficient TNBCs generally show high levels of chromosomal instability, which is attributed to the HR deficiency of these tumors[18]. Compared to other subtypes, TNBCs occur more frequently in younger patients (<50 years) and are significantly more aggressive[19,20]. Moreover, due to the lack of specialized therapies, chemotherapy currently remains standard-of-care for patients with TNBC[21], resulting in a relatively poor prognosis.

2.3 Identifying drivers of human (breast) cancer

A common feature of ILC and TNBC, is that they respond more poorly to existing therapies than other breast cancer subtypes. As such, both breast cancer subtypes would be benefited by the development of novel therapies targeting specific vulnerabilities in these tumors. Efforts to identify such vulnerabilities have generally focused on identifying genes driving tumor development and determining if these drivers can be exploited to develop novel therapies, either by targeting the drivers themselves or by exploiting other vulnerabilities stemming from the drivers.

Recently, several human sequencing studies have been undertaken to identify drivers of human ILC besides functional loss of E-cadherin[9,22]. Together, these studies have shed light on additional genetic alterations that are thought to be driver events, including chromosomal gains of 1q and 16p[23], loss of 16q[24], activating mutations in *PIK3CA*[25,26] and inactivating mutations in *TP53*[27]. Further molecular characterization has identified multiple aberrations in other components of the PI3K-AKT pathway, indicating that PI3K-AKT signaling plays an important role in ILC development[9,22,28]. However, a large fraction of human ILCs cannot be explained by activated PI3K-AKT signaling and *TP53* mutations, indicating that other aberrations are likely to play additional roles in tumorigenesis. Therefore, to identify novel genes and pathways driving ILC development, we used the *Sleeping Beauty* (SB) transposon system to perform an insertional mutagenesis (IM) screen in female mice with mammary-gland specific inactivation of *Cdh1*. The results of this screen are described in **Chapter 3**.

One of the main challenges of identifying candidate cancer drivers using an IM-based forward genetic screen, is that these screens can detect many potential cancer genes, of which only a fraction is actually involved in driving tumorigenesis. Besides this, it can be challenging to identify how genes are affected by their transposon insertions, as the targeted DNA-sequencing approaches that are typically employed for detecting insertion sites[29–31] do not provide any evidence of how insertions affect gene expression. We reasoned RNA-sequencing-based insertion site detection approaches could

alleviate these issues by focusing on detecting insertions that are actually expressed (and therefore more likely to have an actual effect), whilst simultaneously providing insight into how the expression of candidate genes is affected. To demonstrate this, we developed a computational approach and accompanying software package called IM-Fusion, which identifies transposon insertions from gene-transposon fusions in RNA-sequencing data. Details of the approach, including a comparison with targeted DNA-sequencing-based approaches, are described in **Chapter 4**.

As a result of their chromosomal instability, BRCA-deficient TNBCs develop characteristic patterns of copy number aberrations[32], suggesting that these aberrations harbor additional genes driving tumorigenesis. Unfortunately, these aberrations generally harbor tens-to-hundreds of genes, complicating the search for the true driver genes in these regions. To address this issue, computational approaches (e.g. RUBIC, GISTIC) have been developed to identify minimal recurrently aberrated regions and thereby narrow down lists of potential drivers[33,34]. Besides this, comparative oncogenomics approaches have also been used to restrict lists of candidate driver genes, by focusing on genes that are recurrently aberrated in tumors from both mouse models and human patients[35]. In **Chapter 5**, we explore the copy number landscape of *BRCA1*-mutated TNBC using several mouse models containing previously identified drivers such as *Myc*, *Met* and *Rb1*. By applying RUBIC in a comparative analysis between both mouse and human tumors, we show that engineered MYC overexpression in BRCA1-deficient TNBC dramatically reshapes the copy number landscape and identify MCL1 as a druggable driver in these tumors.

2.4 Preventing therapy resistance

Besides identifying druggable target genes, a significant challenge in the development of targeted therapies is the emergence of (acquired) therapy resistance, which is unfortunately frequently observed in patients after prolonged treatment with several targeted therapies[36]. To prevent the development of therapy resistance, it is crucial to gain an understanding of how tumors become resistant to therapies and use these insights to develop new (combination) treatments that aim to prevent or overcome resistance. Besides this, it is important to identify which patients are likely to be intrinsically resistant to treatment, so that these patients can be treated accordingly.

As part of the insertional mutagenesis screen described in Chapter 3, we identified FGFR2 as a key driver of ILC, suggesting that FGFR inhibition would be a suitable therapeutic strategy for treating FGFR-driven ILC. Although no FGFR-targeting therapies are currently approved for the treatment of human cancers, several thera-

peutics are currently being evaluated in phase I/II clinical trials for different types of cancers[37,38]). Unfortunately, studies with some of these inhibitors have shown that tumors can develop resistance to treatment, mainly via secondary mutations in FGFRs[39–41] and activation of alternative RTKs[42–45]. In **Chapter 6**, we explore the effectiveness of FGFR inhibition in FGFR-driven ILCs by transplanting tumor fragments into multiple recipient mice and treating them with the FGFR inhibitor AZD4547. Besides this, we exploit the ongoing transposon mutagenesis in these tumors to identify potential resistance mechanisms to AZD4547, which may be used for developing novel (combination) therapies that prevent or overcome resistance.

In BRCA-deficient TNBC, the most promising targeted treatments have aimed to exploit vulnerabilities resulting from the HR-deficiency incurred by BRCA1/BRCA2 loss[18]. This has led to the development of several PARP inhibitors, which indirectly induce the accumulation of DSBs in the DNA[46,47]. These DSBs cannot be repaired in an error-free fashion without BRCA1/2, leading to extensive DNA damage and cell death in BRCA-mutant cells[48,49]. Unfortunately, the clinical effectiveness of PARP inhibitors is limited by the emergence of therapy resistance[50,51], typically due to restoration of HR function via secondary mutations in *BRCA1/2*[52,53] or mutations in the 53BP1-RIF1-REV7 pathway (reviewed by Annunziato *et al.*[54]). However, for *BRCA2*-mutant tumors, there is no evidence that HR can be restored in the absence of BRCA2, suggesting that other mechanisms must be driving therapy resistance. To identify these additional resistance mechanisms, we combined *in vitro* screens in BRCA2-deficient mammary tumor cells with multi-omics analysis of BRCA2-deficient mouse mammary tumors that acquired PARPi resistance *in vivo*. The results of these analyses are described in **Chapter 7**.

2.5 Future perspectives

Coming to the end of this thesis, in **Chapter 8** we reflect on the methods and results presented in this work and how they may be applied or extended in future endeavours. Besides this, we also consider several technological advances and important challenges that remain to be addressed in the field. Finally, we discuss several limitations of mouse models in the light of expanding human datasets and development of three-dimensional cell culture models, and what this means for the future role of mouse models in cancer research.

2.6 References

[1] Collin Kent, Janet Horton, Rachel Blitzblau, and Bridget F Koontz. "Whose disease will recur after mastectomy for early stage, node-negative breast cancer? A systematic review". In: *Clinical Breast Cancer* 15.6 (2015), pp. 403–412 (cit. on p. 34).

[2] Britta Weigelt, Felipe C Geyer, and Jorge S Reis-Filho. "Histological types of breast cancer: how special are they?" In: *Molecular Oncology* 4.3 (2010), pp. 192–208 (cit. on p. 34).

[3] Joel S Parker, Michael Mullins, Maggie C U Cheang, et al. "Supervised risk predictor of breast cancer based on intrinsic subtypes". In: *Journal of Clinical Oncology* 27.8 (2009), pp. 1160–1167 (cit. on p. 34).

[4] Julie M Hall, John F Couse, and Kenneth S Korach. "The multifaceted mechanisms of estradiol and estrogen receptor signaling". In: *Journal of Biological Chemistry* 276.40 (2001), pp. 36869–36872 (cit. on p. 34).

[5] Manfred Beleut, Renuga Devi Rajaram, Marian Caikovski, et al. "Two distinct mechanisms underlie progesterone-induced proliferation in the mammary gland". In: *Proceedings of the National Academy of Sciences* 107.7 (2010), pp. 2989–2994 (cit. on p. 34).

[6] Alejandro Wolf-Yadlin, Neil Kumar, Yi Zhang, et al. "Effects of HER2 overexpression on cell signaling networks governing proliferation and migration". In: *Molecular Systems Biology* 2.1 (2006), p. 54 (cit. on p. 34).

[7] Sohrab P Shah, Andrew Roth, Rodrigo Goya, et al. "The clonal and mutational evolution spectrum of primary triple-negative breast cancers". In: *Nature* 486.7403 (2012), p. 395 (cit. on p. 34).

[8] Cancer Genome Atlas Network et al. "Comprehensive molecular portraits of human breast tumours". In: *Nature* 490.7418 (2012), p. 61 (cit. on p. 34).

[9] Giovanni Ciriello, Michael L Gatza, Andrew H Beck, et al. "Comprehensive molecular portraits of invasive lobular breast cancer". In: *Cell* 163.2 (2015), pp. 506–519 (cit. on pp. 34, 35).

[10] Frank W Foote and Fred W Stewart. "A histologic classification of carcinoma of the breast". In: *Surgery* 19.1 (1946), pp. 74–99 (cit. on p. 34).

[11] Carien M Niessen and Cara J Gottardi. "Molecular components of the adherens junction". In: *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1778.3 (2008), pp. 562–571 (cit. on p. 34).

[12] CB Vos, AM Cleton-Jansen, Geert Berx, et al. "E-cadherin inactivation in lobular carcinoma in situ of the breast: an early event in tumorigenesis". In: *British Journal of Cancer* 76.9 (1997), p. 1131 (cit. on p. 34).

[13] Roland Moll, Margarete Mitze, Uwe H Frixen, and Walter Birchmeier. "Differential loss of E-cadherin expression in infiltrating ductal and lobular breast carcinomas." In: *The American Journal of Pathology* 143.6 (1993), p. 1731 (cit. on p. 34).

[14] Emad A Rakha, Arjun Patel, Des G Powe, et al. “Clinical and biological significance of E-cadherin protein expression in invasive lobular carcinoma of the breast”. In: *The American Journal of Surgical Pathology* 34.10 (2010), pp. 1472–1479 (cit. on p. 34).

[15] Bernhard C Pestalozzi, David Zahrieh, Elizabeth Mallon, et al. “Distinct clinical and prognostic features of infiltrating lobular carcinoma of the breast: combined results of 15 International Breast Cancer Study Group clinical trials”. In: *Journal of Clinical Oncology* 26.18 (2008), pp. 3006–3014 (cit. on p. 34).

[16] Sunil Badve, David J Dabbs, Stuart J Schnitt, et al. “Basal-like and triple-negative breast cancers: a critical review with an emphasis on the implications for pathologists and oncologists”. In: *Modern Pathology* 24.2 (2011), pp. 157–167 (cit. on p. 34).

[17] Beth N Peshkin, Michelle L Alabek, and Claudine Isaacs. “BRCA1/2 mutations and triple negative breast cancers”. In: *Breast Disease* 32.1-2 (2011), pp. 25–33 (cit. on p. 34).

[18] Christine S Walsh. “Two decades beyond BRCA1/2: homologous recombination, hereditary cancer risk and a target for ovarian cancer therapy”. In: *Gynecologic Oncology* 137.2 (2015), pp. 343–350 (cit. on pp. 35, 37).

[19] Rebecca Dent, Maureen Trudeau, Kathleen I Pritchard, et al. “Triple-negative breast cancer: clinical features and patterns of recurrence”. In: *Clinical Cancer Research* 13.15 (2007), pp. 4429–4434 (cit. on p. 35).

[20] Katrina R Bauer, Monica Brown, Rosemary D Cress, Carol A Parise, and Vincent Caggiano. “Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype”. In: *Cancer* 109.9 (2007), pp. 1721–1728 (cit. on p. 35).

[21] Hanan Ahmed Wahba and Hend Ahmed El-Hadaad. “Current approaches in treatment of triple-negative breast cancer”. In: *Cancer Biology & Medicine* 12.2 (2015), p. 106 (cit. on p. 35).

[22] Magali Michaut, Suet-Feung Chin, Ian Majewski, et al. “Integration of genomic, transcriptomic and proteomic data identifies two biologically distinct subtypes of invasive lobular breast cancer”. In: *Scientific Reports* 6 (2016), p. 18517 (cit. on p. 35).

[23] Daniel E Stange, Bernhard Radlwimmer, Falk Schubert, et al. “High-resolution genomic profiling reveals association of chromosomal aberrations on 1q and 16p with histologic and genetic subgroups of invasive breast cancer”. In: *Clinical Cancer Research* 12.2 (2006), pp. 345–352 (cit. on p. 35).

[24] P T Simpson, J S Reis-Filho, M B K Lambros, et al. “Molecular profiling pleomorphic lobular carcinomas of the breast: evidence for a common molecular genetic pathway with classic lobular carcinomas”. In: *The Journal of Pathology* 215.3 (2008), pp. 231–244 (cit. on p. 35).

[25] Fiamma Buttitta, Lara Felicioni, Fabio Barassi, et al. “PIK3CA mutation and histological type in breast carcinoma: high frequency of mutations in lobular carcinoma”. In: *The Journal of Pathology* 208.3 (2006), pp. 350–355 (cit. on p. 35).

[26] Matthias Christgen, Monika Noskowicz, Elisa Schipper, et al. “Oncogenic PIK3CA mutations in lobular breast cancer progression”. In: *Genes, Chromosomes and Cancer* 52.1 (2013), pp. 69–80 (cit. on p. 35).

[27] Cigdem Ercan, Paul J Van Diest, Bram Van Der Ende, et al. “p53 mutations in classic and pleomorphic invasive lobular carcinoma of the breast”. In: *Cellular Oncology* 35.2 (2012), pp. 111–118 (cit. on p. 35).

[28] Christine Desmedt, Gabriele Zoppoli, Gunes Gundem, et al. “Genomic characterization of primary invasive lobular breast cancer”. In: *Journal of Clinical Oncology* 34.16 (2016), pp. 1872–1881 (cit. on p. 35).

[29] Marco J Koudijs, Christiaan Klijn, Louise van der Weyden, et al. “High-throughput semiquantitative analysis of insertional mutations in heterogeneous tumors”. In: *Genome Research* 21.12 (2011), pp. 2181–2189 (cit. on p. 35).

[30] Aaron L Sarver, Jesse Erdman, Tim Starr, David A Largaespada, and Kevin AT Silverstein. “TAPDANCE: An automated tool to identify and annotate transposon insertion CISs and associations between CISs from next generation sequence data”. In: *BMC Bioinformatics* 13.1 (2012), p. 1 (cit. on p. 35).

[31] Karen M Mann, Justin Y Newberg, Michael A Black, et al. “Analyzing tumor heterogeneity and driver genes in single myeloid leukemia cells with SBCapSeq”. In: *Nature Biotechnology* 34.9 (2016), pp. 962–972 (cit. on p. 35).

[32] Tesa M Severson, Justine Peeters, Ian Majewski, et al. “BRCA1-like signature in triple negative breast cancer: Molecular and clinical characterization reveals subgroups with therapeutic potential”. In: *Molecular Oncology* 9.8 (2015), pp. 1528–1538 (cit. on p. 36).

[33] Ewald van Dyk, Marlous Hoogstraat, Jelle ten Hoeve, Marcel JT Reinders, and Lodewyk FA Wessels. “RUBIC identifies driver genes by detecting recurrent DNA copy number breaks”. In: *Nature Communications* 7 (2016), p. 12159 (cit. on p. 36).

[34] Craig H Mermel, Steven E Schumacher, Barbara Hill, et al. “GISTIC2.0 facilitates sensitive and confident localization of the targets of focal somatic copy number alteration in human cancers”. In: *Genome Biology* 12.4 (2011), R41 (cit. on p. 36).

[35] Daniel Peeper and Anton Berns. “Cross-species oncogenomics in cancer gene identification”. In: *Cell* 125.7 (2006), pp. 1230–1233 (cit. on p. 36).

[36] Caitriona Holohan, Sandra Van Schaeybroeck, Daniel B Longley, and Patrick G Johnston. “Cancer drug resistance: an evolving paradigm”. In: *Nature Reviews Cancer* 13.10 (2013), p. 714 (cit. on p. 36).

[37] Irina S Babina and Nicholas C Turner. “Advances and challenges in targeting FGFR signalling in cancer”. In: *Nature Reviews Cancer* 17.5 (2017), pp. 318–332 (cit. on p. 37).

[38] Rut Porta, Roberto Borea, Andreia Coelho, et al. “FGFR a promising druggable target in cancer: Molecular biology and new drugs”. In: *Critical reviews in oncology/hematology* 113 (2017), pp. 256–267 (cit. on p. 37).

[39] Lipika Goyal, Supriya K Saha, Leah Y Liu, et al. “Polyclonal Secondary FGFR2 Mutations Drive Acquired Resistance to FGFR Inhibition in Patients with FGFR2 Fusion–Positive Cholangiocarcinoma”. In: *Cancer Discovery* 7.3 (2017), pp. 252–263 (cit. on p. 37).

[40] V Chell, K Balmanno, AS Little, et al. “Tumour cell responses to new fibroblast growth factor receptor tyrosine kinase inhibitors and identification of a gatekeeper mutation in FGFR3 as a mechanism of acquired resistance”. In: *Oncogene* 32.25 (2013), pp. 3059–3070 (cit. on p. 37).

[41] Sara A Byron, Huaibin Chen, Andreas Wortmann, et al. “The N550K/H mutations in FGFR2 confer differential resistance to PD173074, dovitinib, and ponatinib ATP-competitive inhibitors”. In: *Neoplasia* 15.8 (2013), 975IN28–988IN30 (cit. on p. 37).

[42] Maria Teresa Herrera-Abreu, Alex Pearson, James Campbell, et al. “Parallel RNA interference screens identify EGFR activation as an escape mechanism in FGFR3-mutant cancer”. In: *Cancer Discovery* 3.9 (2013), pp. 1058–1071 (cit. on p. 37).

[43] SM Kim, H Kim, MR Yun, et al. “Activation of the Met kinase confers acquired drug resistance in FGFR-targeted lung cancer therapy”. In: *Oncogenesis* 5.7 (2016), e241 (cit. on p. 37).

[44] Jinjia Chang, Shanshan Wang, Zhe Zhang, et al. “Multiple receptor tyrosine kinase activation attenuates therapeutic efficacy of the fibroblast growth factor receptor 2 inhibitor AZD4547 in FGFR2 amplified gastric cancer”. In: *Oncotarget* 6.4 (2015), p. 2009 (cit. on p. 37).

[45] Jun Wang, Oliver Mikse, Rachel G Liao, et al. “Ligand-associated ERBB2/3 activation confers acquired resistance to FGFR inhibition in FGFR3-dependent cancer cells”. In: *Oncogene* 34.17 (2015), pp. 2167–2177 (cit. on p. 37).

[46] Guotai Xu, Jos Jonkers, and Sven Rottenberg. “PARP Inhibitor Resistance—What Is Beyond BRCA1 or BRCA2 Restoration?” In: *PARP Inhibitors for Cancer Therapy*. Springer, 2015, pp. 453–471 (cit. on p. 37).

[47] Junko Murai, N Huang Shar-yin, Benu Brata Das, et al. “Trapping of PARP1 and PARP2 by clinical PARP inhibitors”. In: *Cancer Research* 72.21 (2012), pp. 5588–5599 (cit. on p. 37).

[48] Helen E Bryant, Niklas Schultz, Huw D Thomas, et al. “Specific killing of BRCA2-deficient tumours with inhibitors of poly (ADP-ribose) polymerase”. In: *Nature* 434.7035 (2005), pp. 913–917 (cit. on p. 37).

[49] Hannah Farmer, Nuala McCabe, Christopher J Lord, et al. “Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy”. In: *Nature* 434.7035 (2005), pp. 917–921 (cit. on p. 37).

[50] M William Audeh, James Carmichael, Richard T Penson, et al. “Oral poly (ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial”. In: *The Lancet* 376.9737 (2010), pp. 245–251 (cit. on p. 37).

[51] Peter C Fong, David S Boss, Timothy A Yap, et al. “Inhibition of poly (ADP-ribose) polymerase in tumors from BRCA mutation carriers”. In: *New England Journal of Medicine* 361.2 (2009), pp. 123–134 (cit. on p. 37).

[52] Stacey L Edwards, Rachel Brough, Christopher J Lord, et al. “Resistance to therapy caused by intragenic deletion in BRCA2”. In: *Nature* 451.7182 (2008), pp. 1111–1115 (cit. on p. 37).

[53] Elizabeth M Swisher, Wataru Sakai, Beth Y Karlan, et al. “Secondary BRCA1 mutations in BRCA1-mutated ovarian carcinomas with platinum resistance”. In: *Cancer Research* 68.8 (2008), pp. 2581–2586 (cit. on p. 37).

[54] Stefano Annunziato, Marco Barazas, Sven Rottenberg, and Jos Jonkers. “Genetic dissection of cancer development, therapy response, and resistance in mouse models of breast cancer”. In: *Cold Spring Harbor Symposia on Quantitative Biology*. Vol. 81. Cold Spring Harbor Laboratory Press. 2016, pp. 141–150 (cit. on p. 37).