Regulation of actomyosin contraction as a driving force of invasive lobular breast cancer

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Appendices

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English Summary

Cancer is described as a disease where cells grow uncontrollably and are capable of spreading to other tissues. The origin of this disease lies in the disruption of cellular processes that determine how our cells function, especially processes that are involved in stimulating cell division and cell survival. These disruptions are caused by (epi)genetic alterations in so-called driver genes that cause malignant transformation when altered. It is important to know which driver genes are altered in a tumor as these could serve as targets for therapeutic intervention. The driver genes that are responsible for malignant transformation can differ greatly from one tumor (sub)type to another and even within a specific subtype there are differences between individual tumors. The chapters in this thesis investigate the driver genes and cellular processes required for the development and treatment resistance of invasive lobular carcinoma (ILC), a subtype of breast cancer that accounts for 8-14% of all breast cancer cases. The most frequently altered driver gene in ILC is *CDH1* (also known as E-cadherin) which is inactivated in the majority of ILC cases. Mouse models with mammary gland-specific inactivation of E-cadherin have taught us that the loss of E-cadherin by itself is not enough to induce cancer. This indicates that additional driver genes need to be altered in order for ILC to develop. With the research presented in this thesis we set out to increase our understanding of the drivers and cellular processes underlying ILC development and resistance to therapy.

In **Chapter 2** of this thesis we performed a genetic screening method to identify new driver genes that play a role in ILC development. For this screen we used mice in which we combined E-cadherin loss with *Sleeping Beauty* transposon based Insertional mutagenesis in the mammary gland. Insertional mutagenesis is a method that randomly alters genes. If these altered genes are driver genes they will induce tumor formation and the more potent these alterations are the more frequently we will find them. The tumors that arose by combined loss of E-cadherin and insertional mutagenesis resembled ILCs from human patients indicating that the driver genes responsible for tumor induction are relevant. Four of the most frequently altered genes (*Mypt1/2*, *Aspp2* and *Myh9*) were found in a mutually exclusive manner hinting at a shared underlying mechanism. Of these four genes, *Mypt2*, *Aspp2* and *Myh9* are also frequently altered in human ILCs. We determined that for *Mypt1/2* and *Aspp2*, the insertional mutations caused the expression of truncated proteins while insertions in

Myh9 caused a reduction in protein expression. We next validated that expression of these truncation variants of MYPT1 and ASPP2 or reduction in MYH9 protein levels is sufficient to induce ILC formation when combined with E-cadherin loss.

What remained unclear from the experiments described in **Chapter 2** was how the identified driver genes collaborate with E-cadherin loss in ILC development. We addressed this question in **Chapter 3**. To investigate which cellular process need to be altered for tumor initiation upon loss of E-cadherin, we created a mouse model where E-cadherin loss was coupled to expression of a reporter, allowing us to follow the fate of E-cadherin deficient cells *in vivo*. We observed that mammary epithelial cells (MECs) which loose E-cadherin not only extrude into the lumen where they die by apoptosis but also extrude in the other direction towards the basement membrane. In contrast to the luminally extruded MECs the basally extruded MECs do not die but are capable of persisting in the stroma directly surrounding the mammary ducts. The basally extruded E-cadherin deficient MECs persist but do not proliferate, explaining why E-cadherin loss alone is insufficient to induce ILC. Analysis of these basally extruded MECs showed that they often have increased actomyosin contractility and cannot adhere and survive on fibrillar collagen which is a prevalent extracellular matrix component of ILC. Instead, they rely on adhesion to the basement membrane component laminin to survive. We then showed that reduction of actomyosin contractility allows basally extruded MECs to adhere and survive on fibrillar collagen. Additionally, we show that expression of truncated MYPT1 results in actomyosin relaxation which is required for tumor formation.

In **Chapter 4** we show that truncated ASPP2, similar to truncated MYPT1, also induces actomyosin relaxation that enables the survival and growth of E-cadherin deficient MECs on stiff matrices. We then wondered whether actomyosin relaxation also cooperates with the PI3 kinase pathway, which is frequently activated in ILCs. We therefore generated mice with mammary gland-specific overexpression of truncated MYPT1/ASPP2 and combined loss of E-cadherin and PTEN, a negative regulator of the PI3K pathway. We found that combined activation of the PI3K pathway and actomyosin relaxation in E-cadherin deficient MECs results in faster ILC formation and larger tumors at the end of the experiment. We next assessed how ILCs driven by actomyosin relaxation progress over time. We observed that ILCs driven by truncated MYPT1 progress very slowly while ILCs driven by

truncated ASPP2 progress significantly faster. We then went on to show that this increase in progression was due to another function of truncated ASPP2, namely dephosphorylation of the transcription factor YAP, which is important for organ growth and often deregulated in cancer. We found that activation of YAP by truncated ASPP2 is not required for ILC initiation but rather drives tumor growth and progression.

The most frequently altered gene in our insertional mutagenesis screen described in **Chapter 2** was Fibroblast Growth Factor Receptor (FGFR) 2. FGFR inhibitors have been developed as anti-cancer therapeutics, but tumors invariably become resistant to these inhibitors over time. In **Chapter 5**, we used insertional mutagenesis to investigate how tumors driven by FGFR signaling become resistant to FGFR inhibitors. To this end, we orthotopically transplanted mouse ILCs with insertional mutations in *Fgfr2* and treated these tumors with the potent FGFR inhibitor (FGFRi) AZD4547. Tumors responded well initially and showed regression but all tumors eventually became resistant to FGFR inhibition. To identify how these tumors had become resistant we performed transcriptomic analysis and determined which transposon insertions were unique or enriched in the resistant tumors. This two-pronged approach yielded multiple known and novel mechanisms of FGFRi resistance. Most resistance mechanisms resulted in the reactivation of the MAPK-ERK pathway which is known to stimulate proliferation and survival. Of the discovered resistance mechanisms two were identified uniquely by *de novo* transposon insertions highlighting the utility of insertional mutagenesis for identifying novel mechanisms of anti-cancer therapy resistance.

In the final **Chapter 6,** I discuss the main findings of the work described in this thesis, how these findings expand on previous studies, and how they challenge certain concepts in the field of ILC.