PI3K signaling and adherens junctions in invasive lobular breast cancer

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Appendices

ENGLISH SUMMARY

Invasive lobular carcinoma (ILC) is the second most common histological type of breast cancer, after invasive ductal carcinoma (IDC), accounting for 8-14% of breast cancers. The incidence of ILC is rising and, compared with IDC, ILC is more likely to be detected at an advanced stage of the disease, with a larger tumor size. Disruption of intercellular adherens junctions is a hallmark of ILC, resulting in a characteristic histologically observed pattern, with single files of cancer cells and noncohesive individually dispersed cells. Classic ILCs often express receptors for estrogen and progesterone, while ILCs of the pleomorphic subtype more frequently exhibit HER2 amplification and/or p53 expression.

A substantial fraction of breast cancers, including ILC, have acquired mutations that lead to activation of the phosphoinositide 3-kinase (PI3K) signaling pathway, which plays a central role in cellular processes that are essential in cancer, such as cell survival, growth, division and motility. Oncogenic mutations in the PI3K pathway generally involve either activating mutation of the gene encoding PI3K (*PIK3CA*) or AKT(*AKT1*), or loss or reduced expression of PTEN. A general introduction to this thesis is provided in **chapter 1**, giving an overview of ILC and the role of adherens junctions, the role of PI3K signaling, and preclinical research models. In **chapter 2**, we review mouse models of PI3K signaling in breast cancer. We discuss the role of PI3K pathway mutations in human breast cancer and relevant genetically engineered mouse models (GEMMs), with special attention to the role of PI3K signaling in oncogenesis, in therapeutic response, and in resistance to therapy.

Treatment of primary tumors is an obvious and important goal in oncology, but one of the main clinical challenges is to prevent metastatic spread and to effectively treat metastases. Metastatic disease, rather than direct effects of the primary tumor, accounts for more than 90% of cancer-related deaths. The development of effective antimetastatic agents has been hampered by the paucity of relevant preclinical models of human metastatic disease. In **chapter 3**, we present a preclinical mouse model of invasive lobular breast cancer metastasis. Using the conditional *K14cre;Cdh1Flox/Flox;Trp53Flox/Flox* (KEP) mouse model of *de novo* mammary tumor formation, we mimicked the clinical course of treatment by conducting a mastectomy, after which the mice developed widespread overt metastatic disease in lymph nodes, lungs, and gastrointestinal tract.

Effective treatment of ILC is hampered by poor response to chemotherapy. Phosphoinositide 3-kinase (PI3K) signaling, one of the major druggable oncogenic

signaling networks, is frequently activated in ILC. Mammalian target of rapamycin (mTOR) is one of the main kinases in this network. In **chapter 4**, we investigated treatment response and resistance to the mTOR inhibitor AZD8055 in the KEP mouse model of metastatic ILC. Inhibition of mTOR signaling blocked the growth of primary KEP tumors as well as the progression of metastatic disease. This response was associated with activation of antigen presentation and increased numbers of tumor-infiltrating major histocompatibility complex class II-positive (MHCII+) immune cells. After two months, primary tumors and distant metastases eventually acquired resistance during long-term AZD8055 treatment, despite continued effective suppression of mTOR signaling in the tumor tissue. Resistance to treatment was associated with loss of MHCII+ cells and reduced expression of genes related to the adaptive immune system. By demonstrating that mTOR inhibition is less effective in treating KEP tumors in *Rag1-/-* mice lacking mature T and B lymphocytes, and that resistant tumors regain part of their sensitivity to treatment after transplantation in a treatment-naive host, we show that part of the therapeutic effect of mTOR inhibition is mediated by the adaptive immune system.

Activation of the PI3K pathway, by either activating *PIK3CA* mutations or homozygous deletions or inactivating mutations in *PTEN*, is frequently observed in ILC. In **chapter 5**, we show that PTEN loss in E-cadherin-deficient mouse mammary epithelial results in development of classic invasive lobular carcinomas. While loss of E-cadherin induced cell dissemination and apoptosis, additional PTEN inactivation promoted cell survival and rapid formation of invasive mammary tumors that recapitulate the histological and molecular features, estrogen receptor (ER) status, growth kinetics, metastatic behavior, and tumor microenvironment of human classic invasive lobular carcinoma. These tumors were sensitive to pharmacological inhibition of PI3K. PTEN activation alone did not induce ILC formation, but resulted in squamous metaplastic mammary tumors. With this study, we provide evidence for the causal role of combined E-cadherin loss and activation of PI3K signaling in ILC, suggesting that pharmacological inhibition of PI3K may be a promising therapeutic strategy.

In **chapters 6 and 7**, we present our findings regarding the role of p120-catenin (p120), another important molecule in the adherens junction complex. Cell-cell adhesion between epithelial cells is maintained through homophilic interaction of the cell surface glycoprotein E-cadherin. p120 stabilizes E- cadherin by binding to its intracellular juxtamembrane domain. In addition, p120 mediates several important intracellular signaling pathways, including Wnt and Rho-ROCK. It is known that loss of E- cadherin leads to dismantling of the adherens junction and subsequent translocation of p120 to

the cytosol and nucleus. In **chapter 6**, we show that somatic loss of p120 in a conditional mouse model of noninvasive mammary carcinoma results in formation of stromal-dense tumors that resemble human metaplastic breast cancer and metastasize to lungs and lymph nodes. Loss of p120 in anchorage- dependent breast cancer cell lines strongly promoted anoikis resistance through hypersensitization of growth factor receptor (GFR) signaling. Interestingly, p120 deletion also induced secretion of inflammatory cytokines, a feature that likely underlies the formation of the prometastatic microenvironment in p120-negative mammary carcinomas. Using mouse models with mammary gland-specific inactivation of E-cadherin, p120 and p53, we demonstrate in **chapter 7** that ILC formation induced by E-cadherin and p53 loss is impaired upon concomitant inactivation of p120. Tumors that developed in the triple-knockout mice were mostly basal-like tumors, with an epithelial-to- mesenchymal-transition (EMT) phenotype. We show that loss of p120 in the context of the p53- deficient mouse models is dominant over E-cadherin inactivation and its inactivation promotes the development of basal, epithelial-to-mesenchymal-transition (EMT)-type invasive mammary tumors. The thesis is concluded with a general discussion and future perspectives in **chapter 8**.