

MOUSE MODELS OF BREAST CANCER

The focus of our research group is on the genetic dissection of human breast cancer through the use of genetically engineered mouse models. For this, we have developed models for p53-induced breast cancer, BRCA1- and BRCA2- associated hereditary breast cancer, and E-cadherin-mutated lobular breast cancer. We are using these models to (1) investigate genotype-phenotype relations in mammary tumorigenesis; (2) study therapy response and resistance of primary tumors and metastases; (3) identify genetic changes underlying breast tumorigenesis; (4) study the role of immunity in breast cancer development and metastasis.

Functional genetic screens and complementation assays in BRCA1-deficient ES cells We have generated ES cells and mice containing selectable conditional (SCo) knockout alleles of *Brca1* and *Brca2*. To identify factors rescuing cells from growth arrest induced by BRCA1 loss we have performed clonal survival screens in *R26cre-ERT2;Brca1^{SCo/D}* ES cells using PiggyBac (PB)-based insertional mutagenesis. We found that inactivating PB insertions in *53BP1* rescued ES cells from BRCA1 deficiency. Loss of *53BP1* partially restores the homologous-recombination defect of BRCA1-deficient cells and reverts their hypersensitivity to DNA-damaging agents. Notably, loss of *53BP1* expression is also found in subsets of sporadic triple-negative and BRCA-associated breast cancers.

We have also used our *R26cre-ERT2;Brca1^{SCo/D}* ES cells to perform functional complementation assays for testing human *BRCA1* variants of unknown clinical significance (VUS). For this, we have engineered our ES cells to enable rapid knock-in of human *BRCA1* cDNAs by FLP recombinase mediated cassette exchange (RMCE). Introduction of wild-type *hBRCA1* – but not pathogenic *hBRCA1* mutants – rescues the growth defect of switched *R26cre-ERT2;Brca1^{SCo/D}* cells. We have so far tested 60 defined *hBRCA1* VUSs in our functional complementation assay system.

Conditional mouse models for BRCA-associated breast cancer We have previously generated conditional mouse mutants with *K14cre-* or *WAPcre-* mediated tissue-specific loss of *Brca1/2* and *p53* to establish models for BRCA1- and BRCA2-associated breast cancer. The *Brca1^{-/-};p53^{-/-}* mammary tumors share histopathological and molecular features with BRCA1-deficient breast cancers in women: they are highly proliferative, poorly differentiated, hormone receptor and HER2 negative mammary adenocarcinomas with a high degree of genomic instability. Interestingly, we have found that mammary tumor formation in our BRCA1 model is still estrogen-dependent. We are currently investigating whether this estrogen dependence is due to autocrine or paracrine mechanisms.

The central role of BRCA1 and BRCA2 in the DNA damage response (DDR) implies that BRCA-deficient tumors are especially sensitive to therapeutics that directly or indirectly induce DNA double-strand breaks. In collaboration with Sven Rottenberg and Piet Borst we have used our BRCA1/2 models to test the anti-tumoral efficacy of the PARP inhibitor AZD2281 (olaparib) from KuDOS-AstraZeneca, which may be selectively toxic to BRCA-deficient cells because it suppresses DNA single-strand break repair. Administration of olaparib to mice with *Brca1^{-/-};p53^{-/-}* or *Brca2^{-/-};p53^{-/-}* mammary tumors induced tumor regression without signs of toxicity. However, long-term treatment with olaparib resulted in the development of drug resistance caused by upregulation of the *Mdr1* genes encoding P-glycoprotein (Pgp) drug efflux transporters. To study Pgp-independent mechanisms of olaparib resistance, we have crossed the BRCA1 mammary tumor model onto an *Mdr1^{-/-}* background and transplanted the resulting *Brca1^{-/-};p53^{-/-};Mdr1^{-/-}* tumors into wildtype recipients. Treatment of these mice with olaparib resulted in induction of resistance without loss of target (PARP) inhibition. Intriguingly, a fraction of olaparib-resistant *Brca1^{-/-};p53^{-/-};Mdr1^{-/-}* tumors showed loss of *53BP1* expression, highlighting the role of this DDR factor in therapy resistance.

In collaboration with Hein te Riele and Jo Morris (King's College London), we have generated *Brca1* mouse mutants that mimic three common human founder mutations, the *BRCA1-185delAG*, *BRCA1-5382insC* frameshift mutants and the



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Selected publications

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BRCA1-C61G missense variant. We have crossed these 3 mutant mouse strains into our BRCA1 mammary tumor model to study the impact of these defined mutations on tumor development, therapy response and resistance. All 3 mutants are clearly pathogenic: homozygous Brca1 mutant mice are embryonic lethal and mammary tumors develop faster in heterozygous Brca1 mutant mice which undergo K14cre-mediated tissue-specific loss of the conditional p53^F and Brca1^F alleles. Treatment of the resulting Brca1^{185delAG};⁻;p53⁻;⁻, Brca1^{5382insC};⁻;p53⁻;⁻ and Brca1^{C61G};⁻;p53⁻;⁻ mammary tumors revealed remarkable differences in sensitivity to cisplatin and the PARP inhibitor olaparib. Whereas Brca1⁻;⁻;p53⁻;⁻ tumors never develop resistance to cisplatin due to the large Brca1 deletion removing exons 5-13, the Brca1^{C61G};⁻;p53⁻;⁻ and Brca1^{185delAG};⁻;p53⁻;⁻ tumors readily become resistant to this drug while retaining the Brca1 founder mutation, suggesting that BRCA1 RING activity is required for tumor suppression but dispensable for therapy resistance.

Conditional mouse models for E-cadherin-deficient metastatic breast cancer

Loss of E-cadherin is associated with invasive lobular carcinoma (ILC), which accounts for 10-15% of all breast cancers. To study the causal role of E-cadherin in breast oncogenesis, we have generated a mouse model for invasive lobular carcinoma (ILC) based on epithelium-specific inactivation of E-cadherin and p53. Compared to p53⁻ mammary carcinomas, Ecad⁻;p53⁻ tumors show a significantly reduced latency, a morphological switch from ductal to lobular carcinoma, and a phenotypic change from non-invasive to highly invasive and metastatic tumors. We have used the E-cadherin mammary tumor model for intervention studies with mTOR and SRC inhibitors. Whereas mTOR inhibition leads to tumor stasis, SRC inhibition does not affect primary tumor growth. We are currently testing the effects of mTOR and SRC inhibition on tumor metastasis.

The inflammatory tumor-microenvironment and its impact on breast cancer development and therapy

Immune cells are one of the most abundant cell types recruited to the microenvironment of many tumors. Their role during tumorigenesis is, however, controversial, as both tumor-protective and tumor-promoting properties have been reported. The overall research goal of Karin de Visser is to address the role of the adaptive and innate immune system during spontaneous breast cancer progression and metastasis formation. In addition, the influence of the inflammatory tumor-microenvironment on response and resistance of tumors to chemotherapy is addressed. For these studies, the E-cadherin mammary tumor model is employed. Like human breast cancers, mammary carcinomas arising in this mouse model are characterized by abundant presence of immune cells, including degranulating mast cells and macrophages, T- and B-lymphocytes, antibody depositions and increased levels of pro-inflammatory mediators. By genetic elimination and pharmacological inhibition of specific subsets of the adaptive and innate immune system, we are currently investigating their functional significance in a tumor-stage specific manner. We have identified a critical role for adaptive immune cells in spontaneous metastasis formation, and we are currently investigating the underlying mechanisms. We are also studying the ability of the immune system to modulate chemotherapy response and resistance. These studies may shift therapeutic focus towards a combined cancer cell-intrinsic and -extrinsic viewpoint.

Genomic analysis of mouse mammary tumors We have performed array-based comparative genomic hybridization (aCGH) analysis of panels of mammary tumors derived from our mouse models. We have developed two novel algorithms for multi-experiment analysis of aCGH data: KC-SMART identifies regions with significantly recurrent DNA copy number alterations (CNAs) and comparative KC-SMART identifies significant differences in recurrent CNAs between sample groups. We have used both algorithms in cross-species comparisons to identify conserved BRCA1- or BRCA2-specific CNAs in mouse mammary tumors and human BRCA-associated breast cancers. Furthermore, we have developed a novel approach to find co-occurring or mutual exclusive CNAs. Using these methods, we identified several mutual exclusive amplifications in mouse mammary tumors that might represent redundant oncogenic pathways.