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#### Publications

Rottenberg S, Jonkers J. *Modeling therapy resistance in genetically engineered mouse cancer models. Drug Resist Updat.* 2008;11:51-60

Klijn C, Holstege H, de Ridder J, Liu X, Reinders M, Jonkers J\*, Wessels L\*. *Identification of cancer genes using a statistical framework for multi-experiment analysis of non-discretized array CGH data. Nucleic Acids Res.* 2008;36:e13 (\* joint corresponding authors).

Doumont G, de Visser KE, Derksen PW, Jonkers J. *Models for angiogenesis: from fundamental mechanisms to anticancer treatment research. Drug Discov Today: Disease Models* 2008;4:75-82

Borst P, Rottenberg S, Jonkers J. *How do real tumors become resistant to cisplatin? Cell Cycle* 2008;7:1353-1359

## 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-associated metastatic breast cancer. We are using these models to (1) investigate genotype-phenotype relations in mammary tumorigenesis; (2) perform therapeutic intervention studies; (3) identify genetic changes underlying breast tumorigenesis; (4) study the role of innate and adaptive immunity in breast cancer development.

**Conditional mouse models for BRCA-associated breast cancer** We have previously generated conditional mouse mutants with tissue-specific loss of *Brca1/2* and *p53* to establish models for BRCA1- and BRCA2-associated breast cancer. The *Brca1<sup>-/-</sup>;p53<sup>-/-</sup>* 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 pushing borders and 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.

**Tumor intervention studies in the BRCA mammary tumor models** The central role of BRCA1 and BRCA2 in DNA double-strand break (DSB) repair via homologous recombination (HR) implies that BRCA-deficient tumors are especially sensitive to DSB inducing chemotherapeutics. Indeed, in vitro cytotoxicity studies with BRCA2-deficient mammary tumor cell lines and in vivo tumor intervention studies in our BRCA1 mammary tumor model showed high sensitivity of BRCA-deficient tumors to DNA-damaging agents such as doxorubicin or platinum drugs. In collaboration with Sven Rottenberg, Piet Borst and KuDOS Pharmaceuticals, we have used our BRCA1/2 models to test the anti-tumor effects of PARP inhibition, which may be selectively toxic to HR-deficient cells because it suppresses DNA single-strand break repair. Indeed, in vitro studies showed selective toxicity of the PARP inhibitor AZD2281 in *Brca2<sup>-/-</sup>;p53<sup>-/-</sup>* mammary tumor cells, compared to *p53<sup>-/-</sup>* cells. Administration of AZD2281 to mice with *Brca1<sup>-/-</sup>;p53<sup>-/-</sup>* mammary tumors induced tumor regression without signs of toxicity, resulting in strongly increased survival. Long-term treatment with AZD2281, however, resulted in the development of drug resistance caused by upregulation of P-glycoprotein drug efflux pumps. Importantly, resistance could be reversed by co-administration of AZD2281 and the P-glycoprotein inhibitor tariquidar. Moreover, combination of AZD2281 with platinum drugs significantly prolonged recurrence-free survival, suggesting that AZD2281 potentiates the effect of these DNA-damaging agents. Together, these data demonstrate *in vivo* efficacy of AZD2281 against BRCA-deficient breast cancer, and illustrate how conditional mouse models of human cancer can be used for preclinical evaluation of novel therapeutics, for investigating drug resistance mechanisms and for testing ways to overcome therapy resistance.

**Conditional mouse models for E-cadherin-deficient metastatic breast cancer** While metastatic disease is the main cause of death in breast cancer patients, the underlying mechanisms are poorly understood. Loss of E-cadherin is strongly associated with tumor invasion and metastasis, as well as with invasive lobular carcinoma (ILC), which accounts for 10-15% of all breast cancers. To study the role of E-cadherin in breast oncogenesis, we have generated a mouse model for invasive lobular breast carcinoma (ILC) based on epithelium-specific inactivation of E-cadherin and p53. Compared to *p53<sup>-/-</sup>* mammary carcinomas, *Ecad<sup>-/-</sup>;p53<sup>-/-</sup>* mammary 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. Moreover, *Ecad<sup>-/-</sup>;p53<sup>-/-</sup>* mammary tumor cell lines – but not *p53<sup>-/-</sup>* cell lines – are resistant to detachment induced apoptosis, aka anoikis. We have performed kinome-wide siRNA screens to identify factors that modulate anoikis resistance of *Ecad<sup>-/-</sup>;p53<sup>-/-</sup>* tumor cells, which may serve as a surrogate assay

## Publications (continued)

for survival of circulating tumor cells. We have identified several kinases that are essential for survival of *Ecad*<sup>-/-</sup>; *p53*<sup>-/-</sup> cells under non-adherent culture conditions. In vivo validation studies will be performed using pharmacological inhibitors or inducible expression of shRNAs against these kinases in *Ecad*<sup>-/-</sup>; *p53*<sup>-/-</sup> mammary tumors in mice.

**Tumor intervention studies in the E-cadherin mammary tumor model** We are also using the E-cadherin mammary tumor model for preclinical testing of novel therapeutics. For this purpose we have derived panels of clonal, luciferase-marked mammary tumor cell lines from our mouse models. Together with the spontaneous models, these reagents permit validation of candidate drug targets and testing of (combinations of) anticancer drugs on four different levels: (1) in vitro, on the panels of tumor cell lines; (2) in vivo, on tumor outgrowths from orthotopically grafted cell lines; (3) in vivo, on tumor outgrowths from orthotopically grafted tumor fragments (allowing parallel treatments on genetically identical tumors); (4) in vivo, on the sporadic tumors that develop in the mouse mammary tumor models (allowing intervention at early stages of tumor development). We are currently using this multi-level platform to test a number of conventional and targeted anti-cancer drugs.

**Array-CGH analysis of mouse mammary tumors** We have performed array-based comparative genomic hybridization (aCGH) analysis of panels of mammary tumors derived from our conditional mouse models. To identify regions with significantly recurrent DNA copy number aberrations (CNAs), we have developed KC-SMART (Kernel Convolution – a Statistical Method for Aberrant Region deTectioN) for multi-experiment analysis of aCGH data. KC-SMART generates a Kernel Smoothed Estimate (KSE) of recurrent CNAs across the genome, aggregated over all tumors. The peaks in the KSE curves are tested against randomly permuted data to identify significantly recurrent CNAs. We have used this approach to significantly recurrent CNAs in mouse and human breast cancer panels and in human cancer cell line panels. We are using these data sets for (1) cross-species comparisons of CNAs in mouse and human tumors; (2) co-occurrence analysis of CNAs in human tumor cell lines; (3) integration of aCGH and gene expression data to identify candidate cancer genes in recurrent CNAs.

#### The role of innate and adaptive immunity in breast cancer development

Studies in a transgenic mouse model for skin carcinogenesis revealed an unexpected role for B lymphocytes and their soluble mediators in initiating chronic inflammation during premalignancy and subsequent progression to invasive skin cancer (De Visser *et al.* Cancer Cell 2005;7:411-23). We are currently investigating whether a similar link exists between innate/adaptive immunity and breast cancer development, progression and therapy resistance in our mouse model for E-cadherin deficient metastatic breast cancer. Similar to human breast cancer lesions, neoplastic mammary glands of these mice are characterized by leukocyte infiltration. To assess the roles of innate and adaptive immune systems in breast cancer development and progression, these mice have been crossed with various immune-deficient (*Rag1*<sup>-/-</sup>, *Il2rg*<sup>-/-</sup>) mice. In the resulting compound mutant mice, chronic inflammation, angiogenesis, premalignant progression, cancer development and metastasis formation are being analyzed. We will also perform adoptive transfer experiments with bone marrow cells from mice with macrophage-specific expression of the diphtheria toxin receptor to study the effects of diphtheria toxin-mediated macrophage ablation on mammary tumor development and progression.

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