Mutating E-cadherin in Rats to Model Lobular Breast Cancer

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Abstract

Invasive lobular cancer (ILC) is the most common special histological subtype of breast cancer, accounting for 8-14% of all breast cancer cases. 95% of ILCs are ER+ and treated with endocrine therapy. Optimal rodent models of ILC are needed to investigate ILC evolution and new treatment strategies. Mouse models of ILC have been made by mutating CDH1 (which encodes E-cadherin), a hallmark of ILC, but the resulting tumors have not been well characterized for ER and estrogen dependence. None of the hundreds of mouse models of breast cancer have been reported to exhibit estrogen dependence with the exception of perhaps STAT1 knockout mice. Rats, however, have mammary tissue more similar to humans, and easily generate ER+, estrogen-dependent mammary tumors. Our lab has successfully modelled ER+, estrogen-dependent ductal breast tumors in rats using intraductal injection of lentiviral oncogene or CRISPR/Cas9 genome editing, but a rat model of ILC is still lacking. In this study, we aim to model ILC in rats by intraductal injection of virus to CRISPR-edit the CDH1 locus. We will first examine the effect on rat mammary gland development, proliferation and apoptosis. Next, we will combine somatic deletion of CDH1 with activation of PIK3CA, the most commonly mutated protooncogene in human breast cancer. We will characterize the resulting tumors for histopathology, ER and PR expression, estrogen dependence, transcriptome and metastasis to distant organs, and compare the data with human ILCs. In summary, this study will develop a clinically relevant rat model of ER+ ILC, and will help understand ILC evolution and discover new treatment strategies.

Methods

To model ILC in rats, we will use intraductal injection-directed adeno-associated virus (AAV) to introduce CRISPR/Cas9 genome editing in the rat mammary gland to induce somatic deletion of CDH1 and activation of oncogenes. We will combine immunohistochemistry, immunofluorescence, transcriptomic analysis and PET-CT to characterize the resulting tumor and distant metastases, and compare the data with human ILCs.

Results and Strategies

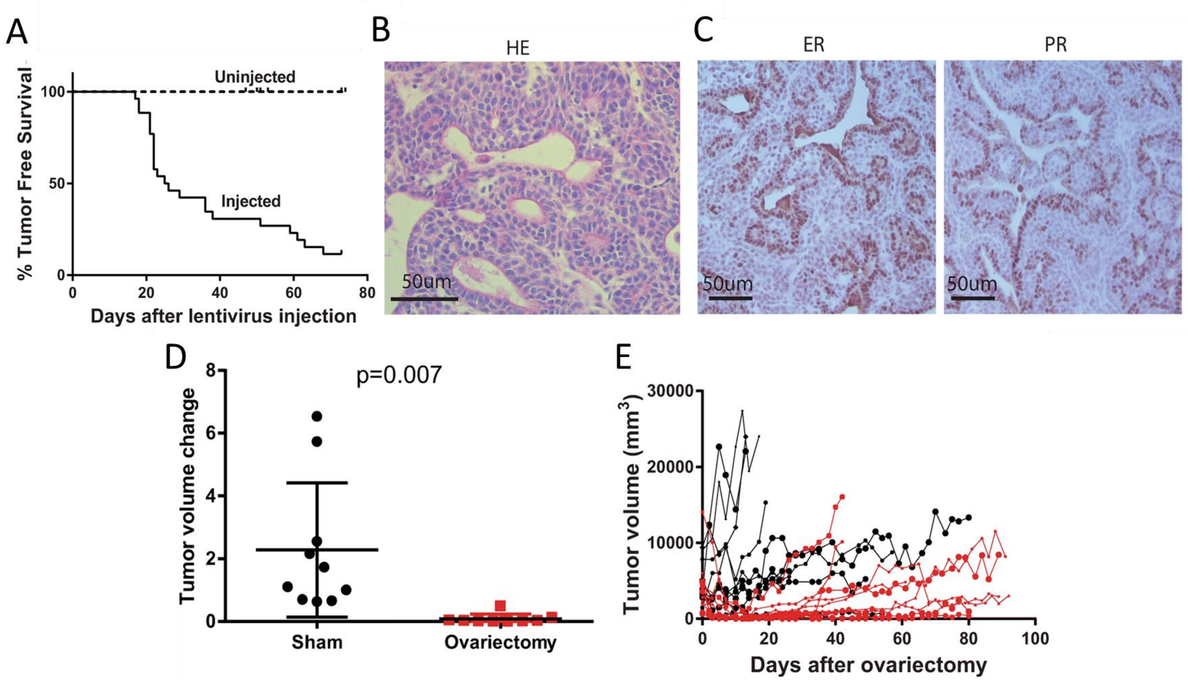

Fig 1. Development of a rat somatic gene expression technology: intraductal injection of lentiviral *HrasQ61L* induces hormone receptor-positive and hormone-dependent mammary tumors. (A) Kaplan-Meier curve of tumor-free survival in Sprague Dawley rats intraductally injected with lentiviral *HrasQ61L* (7×10^7 IU/gland/rat, n = 26). Contralateral uninjected glands were used as negative controls. (B-C) Representative images of H&E staining (B) and immunohistochemistry staining (C) for ER (left) and PR (right) of tumors induced by lentiviral *HrasQ61L.* (D-E) Tumor volume changes at two weeks and over time in rats after ovariectomy (n = 10) or sham surgery (n = 10). Plots were modified from Bu et al., 2020.

Results and Strategies

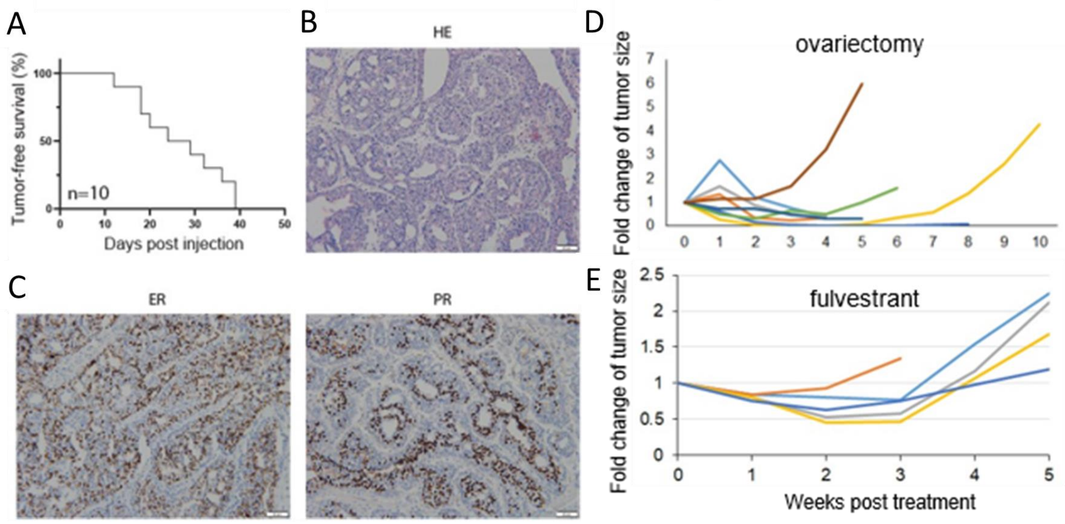

Fig 2. Development of a rat somatic knockout technology: NF1/Tp53 CRISPR KO leads to ER+/PR+ mammary tumors. (A) Kaplan-Meier curve of tumor-free survival in Cas9 transgenic rats intraductally injected with AAV carrying gRNA to delete NF1 and Tp53. (B-C) Representative images of H&E staining (B) and immunohistochemistry staining (C) for ER (left) and PR (right) of tumors induced by NF1/Tp53 CRISPR KO. (D-E) Tumor volume changes over time in rats after ovariectomy or treated with fulvestrant (125 mg/kg/week, s.c.). Each line represents one tumor.

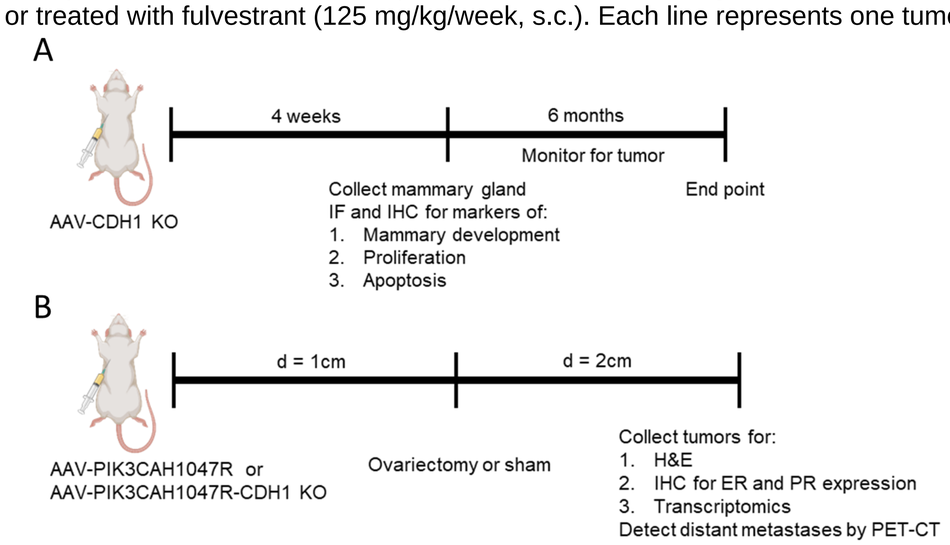

Fig 3. Strategies to knock out CDH1 to model ILC in rats. (A) Cas9 rats will be intraductally injected with AAV carrying gRNA to delete CDH1. Four weeks later, mammary glands will be collected to perform IF and IHC for markers of mammary gland development, proliferation and apoptosis. (B) Cas9 rats will be intraductally injected with AAV for generating PIK3CAH1047R or AAV for producing both PIK3CAH1047R and CDH1 KO. At d = 1.0 cm, we will perform ovariectomy or sham surgery. At d = 2.0 cm, tumors will be characterized for histopathology, ER and PR expression and transcriptomics, and rats will be examined for distant metastasis by PET-CT.

Conclusions

- CRISPR editing generates hormone receptor-positive and hormone-dependent mammary tumors in rats.
- CRISPR editing is a promising strategy to model ILC in rats.

References

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Acknowledgments

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