Investigating genetic events in the progression of ductal carcinoma *in situ*

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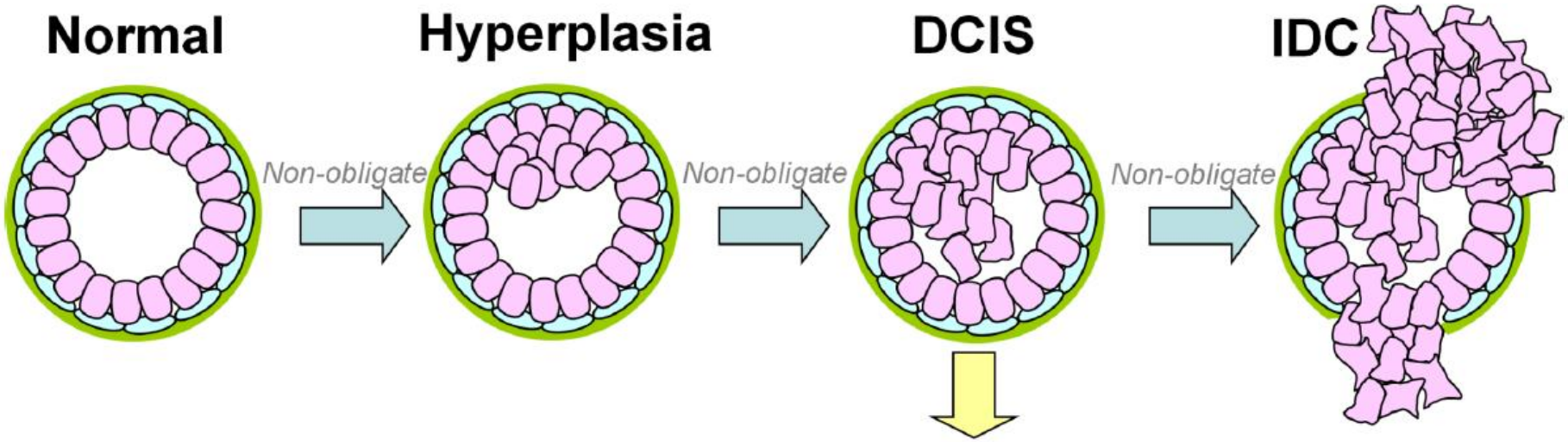
Mouse Cancer Genetics Program

National Cancer Institute

Outline

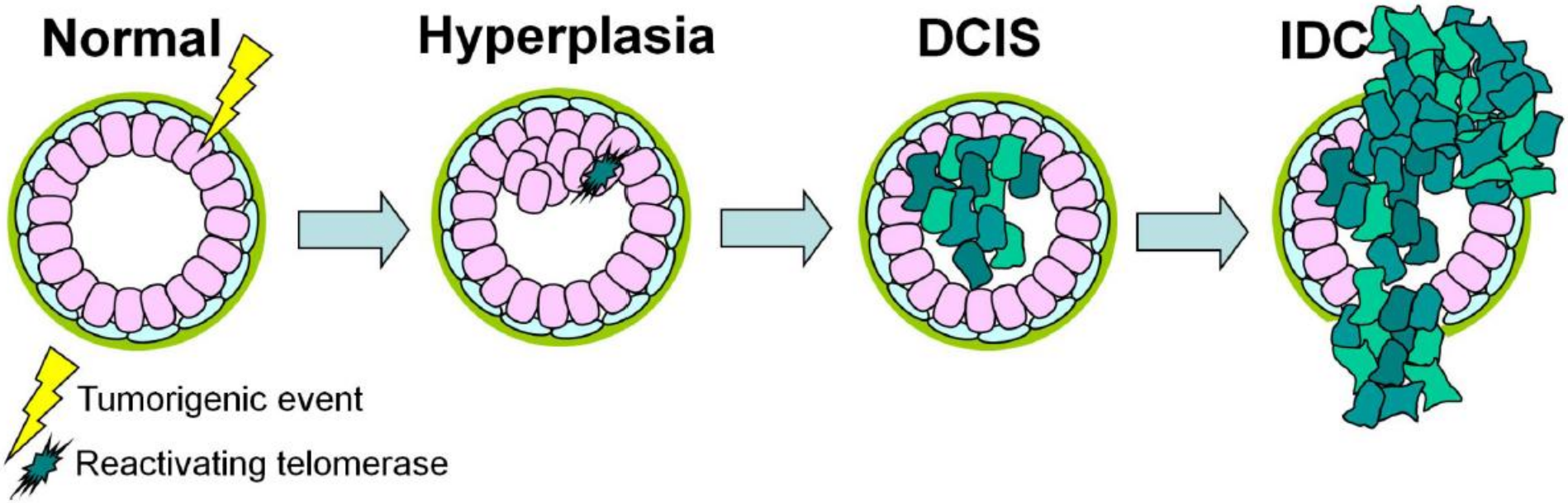
- Introduction to ductal carcinoma *in situ* (DCIS)
- Modeling DCIS progression in mice
- Techniques for noninvasively tracking preinvasive cancer progression in mice
- Summary

Genes and pathways in progression of ductal carcinoma *in situ* (DCIS)



Jansen SA 2015

Telomere crisis model of DCIS progression



- Implications:
  - DCIS and IDC are genetically similar

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Cancer stem cell model of DCIS progression

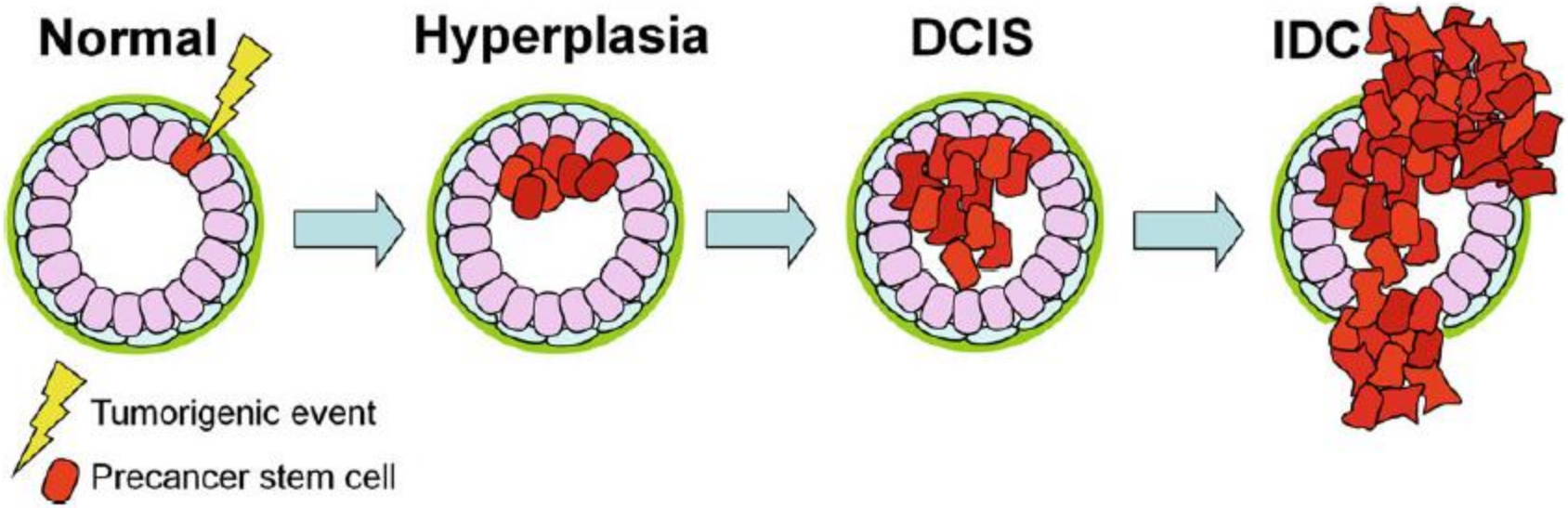
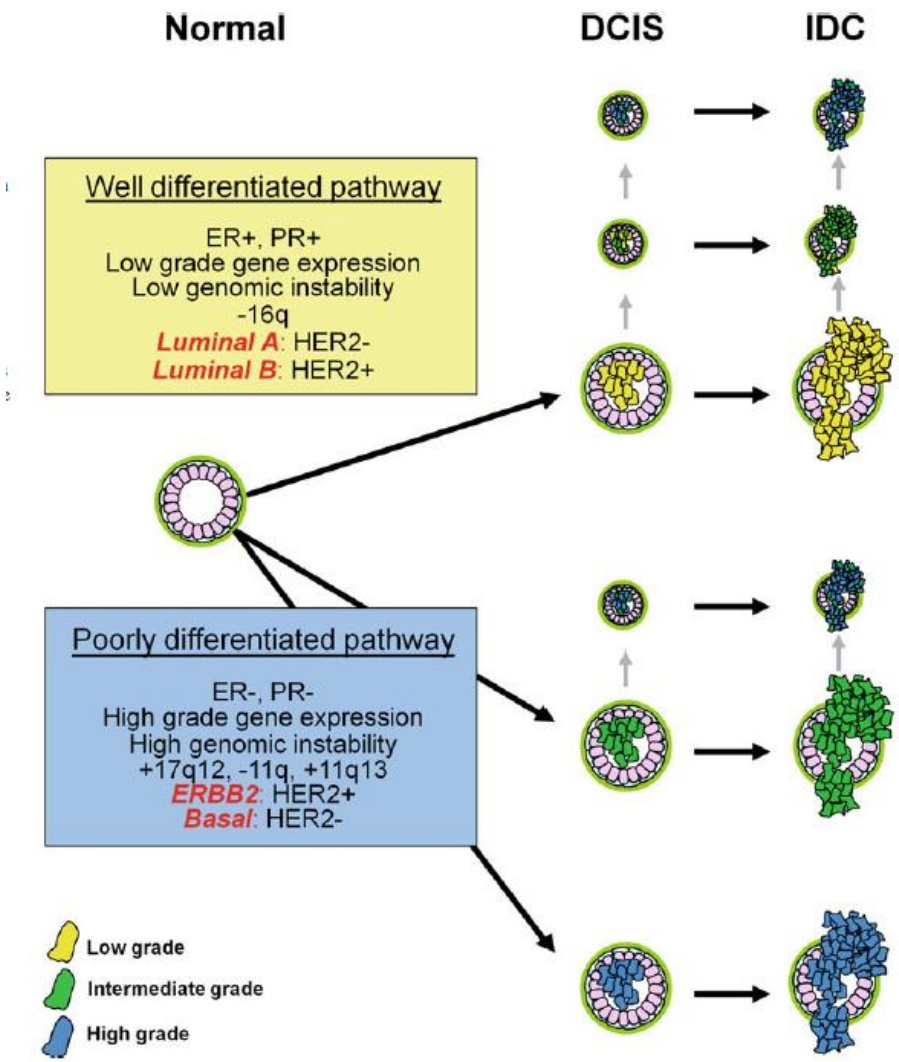


Fig. 3 The precancer stem cell model of DCIS progression. DCIS lesions arise as a result of tumorigenic events (e.g., oncogene activation, loss of tumor suppressor) occurring initially in a precancer stem cell. The molecular and biological properties of the ensuing DCIS lesion including its potential for progressing to invasive disease are pre-encoded within the initial target cell. In this way, the bulk of malignant transformation has occurred by the DCIS stage

Jansen SA 2015

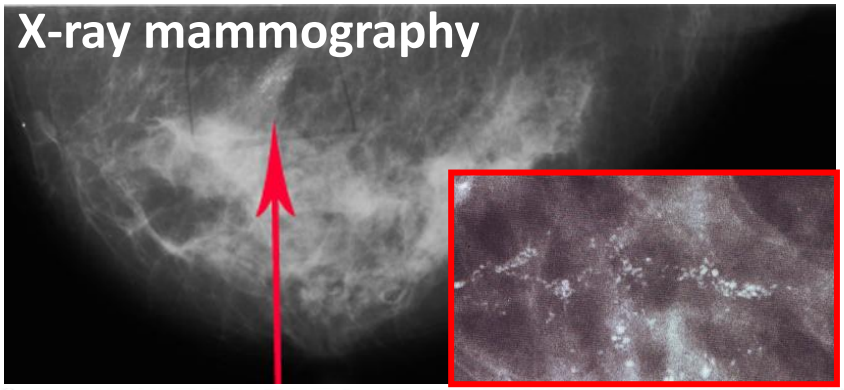
Breast cancers evolve along genetic pathways defined at or before DCIS stage

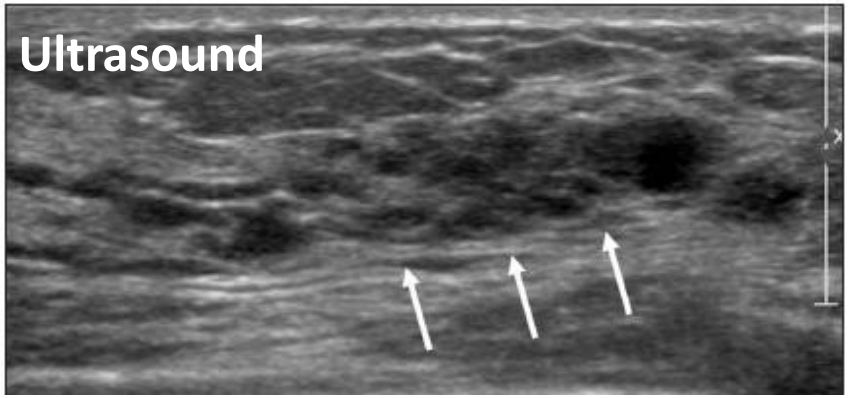


Jansen SA 2015

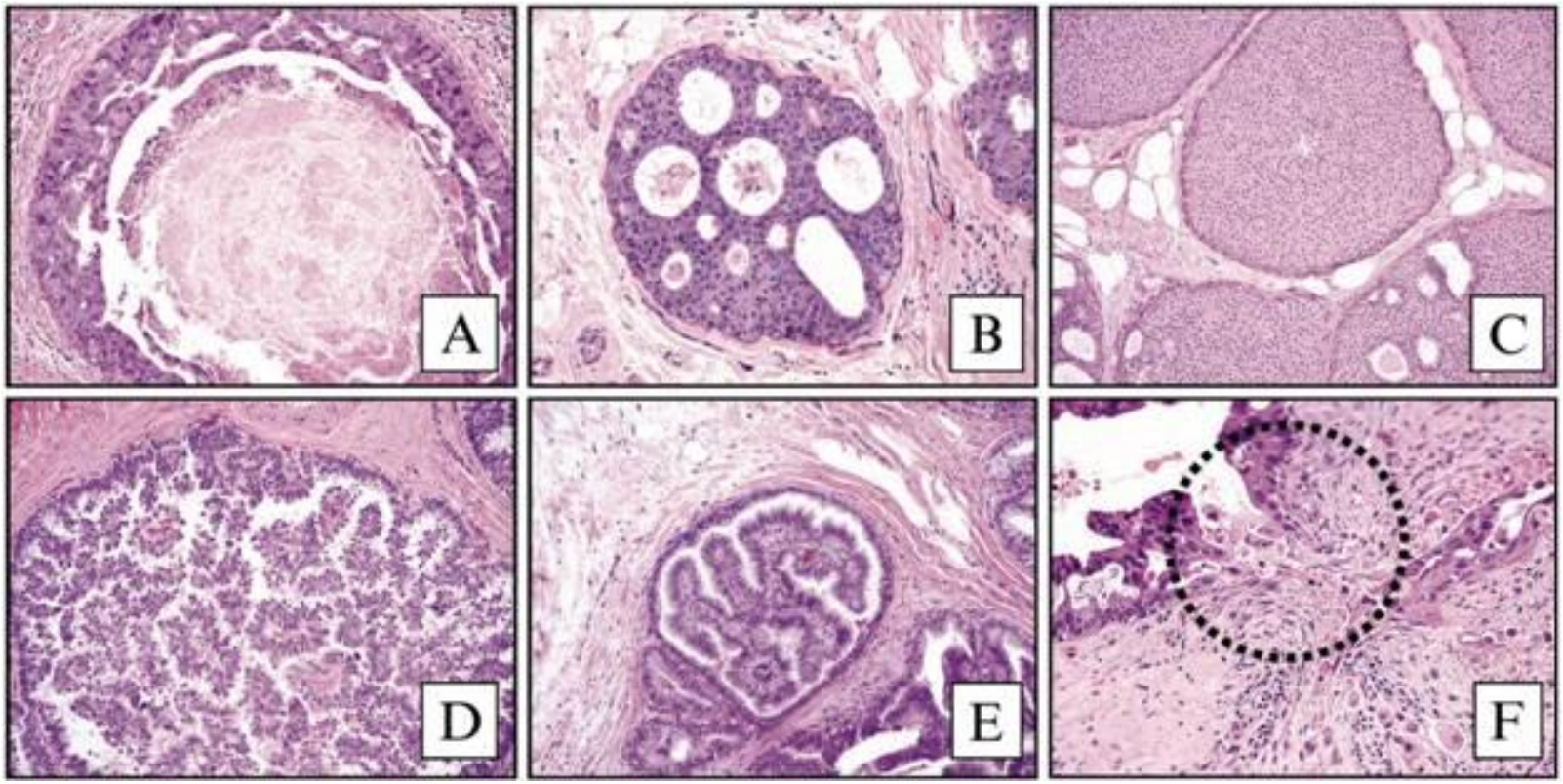
DCIS is a disease revealed by imaging

- Because of early detection, DCIS comprises 25-30% of all newly diagnosed breast cancers





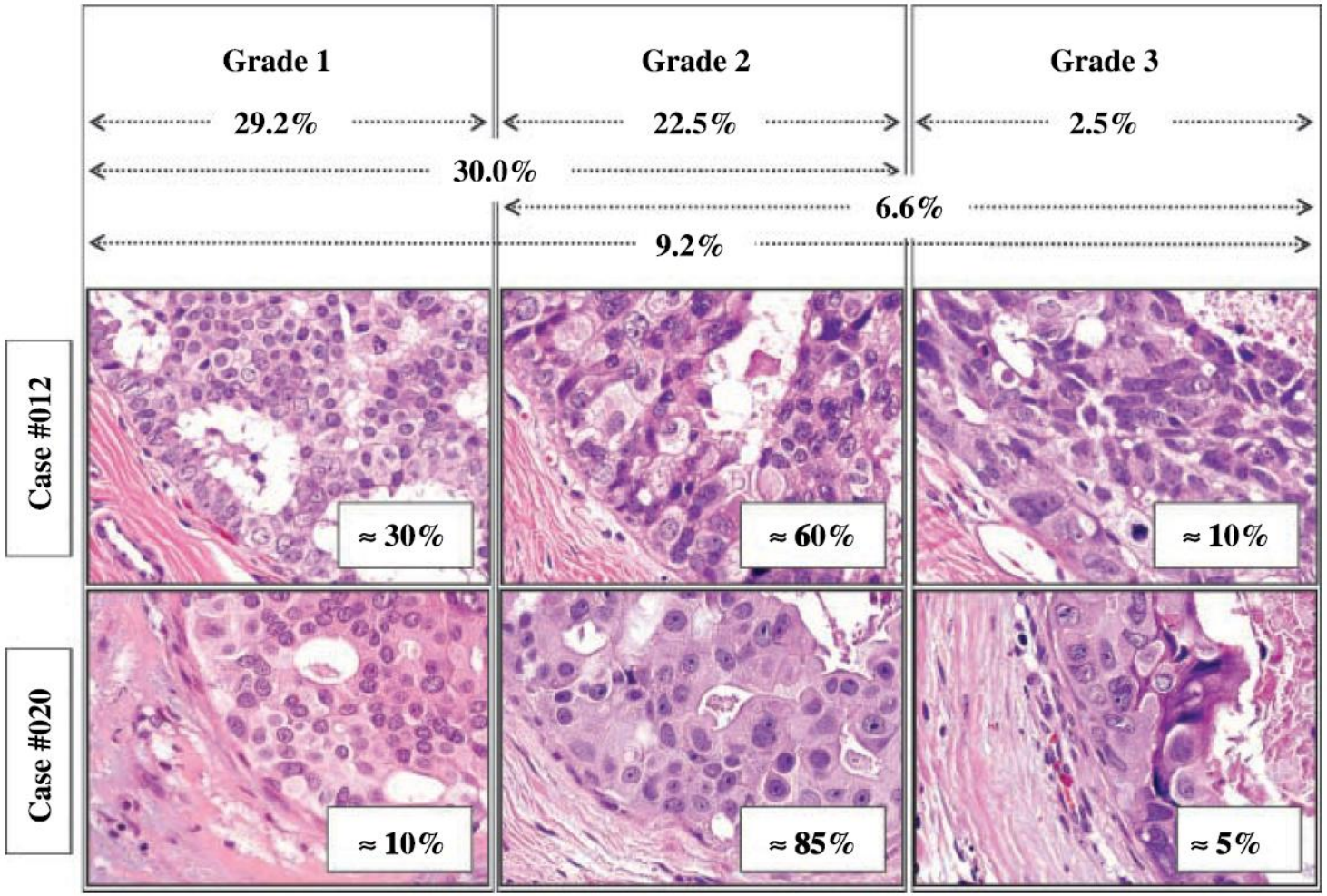
DCIS has *inter*lesion heterogeneity



- Subtypes based on growth pattern

Allred et al *JNCI* 2010

DCIS has *intra*lesion heterogeneity



Allred et al *Clin Can Res* 2008

Finding biomarkers for DCIS progression is critical

NIH State-of-the-Science Conference Statement on

Diagnosis and Management of Ductal Carcinoma In Situ (DCIS)

"The primary question for future research must focus on the accurate identification of patient subsets diagnosed with DCIS, including those persons who may be managed with less therapeutic intervention."

"#14: Are there definable properties of a non-malignant (in situ) lesion that predict the likelihood of progression to invasive or metastatic disease?"

But biomarkers for progression remain elusive

Table 1 Summary of the molecular markers used to characterize DCIS

Molecular markers	Functions	Molecular signatures correlating with increased risk of recurrence
ER, PR	Steroid receptors	ER− HER2+ ER−/HER2+ ER−/HER2 +/Ki-67+
HER2	Regulates proliferation and apoptosis	
p53	Regulates cell-cycle, apoptosis, and genomic stability; p53 is an important tumor suppressor	p53+
Rb/p16 pathway	Regulates cell-cycle; Rb is an important tumor suppressor	p16+ Ki-67+ COX-2+ p16+/COX-2-/Ki-67+ (DCIS recurrence) p16+/COX-2+/Ki-67+ (invasive recurrence)
Ki-67	Proliferation marker	
COX-2	Enzyme for prostaglandin synthesis; expressed during inflammatory response	
Akt/PTEN pathway	Regulates proliferation, survival and motility; PTEN is an important tumor suppressor	
BRCA1/2	DNA damage repair	
c-myc	Transcription factor that can activate proliferation; c-myc is a proto-oncogene	
VEGF, vascular patterns	Angiogenesis and vascular markers	
Cyclin A, cyclin E, p21, p27	Cell-cycle regulators	p21+
Bcl-2, Bax, Survivin	Apoptosis regulators	Bcl-2− Survivin+

Included are the molecular signatures that have been shown to correlate with an increased risk of subsequent recurrence in some reports

Jansen SA 2012

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Rb	Regulates cell-cycle; Rb is an important tumor suppressor	p16+
Ki-67	Proliferation marker	Ki-67+
COX-2	Enzyme for prostaglandin synthesis; expressed during inflammatory response	COX-2+ p16+/COX-2-/Ki-67+ (DCIS recurrence) p16+/COX-2+/Ki-67+ (invasive recurrence)
PTEN	Regulates proliferation, survival and motility; PTEN is an important tumor suppressor	
BRCA1	DNA damage repair	
	Transcription factor that can activate proliferation; c-myc is a proto-oncogene	
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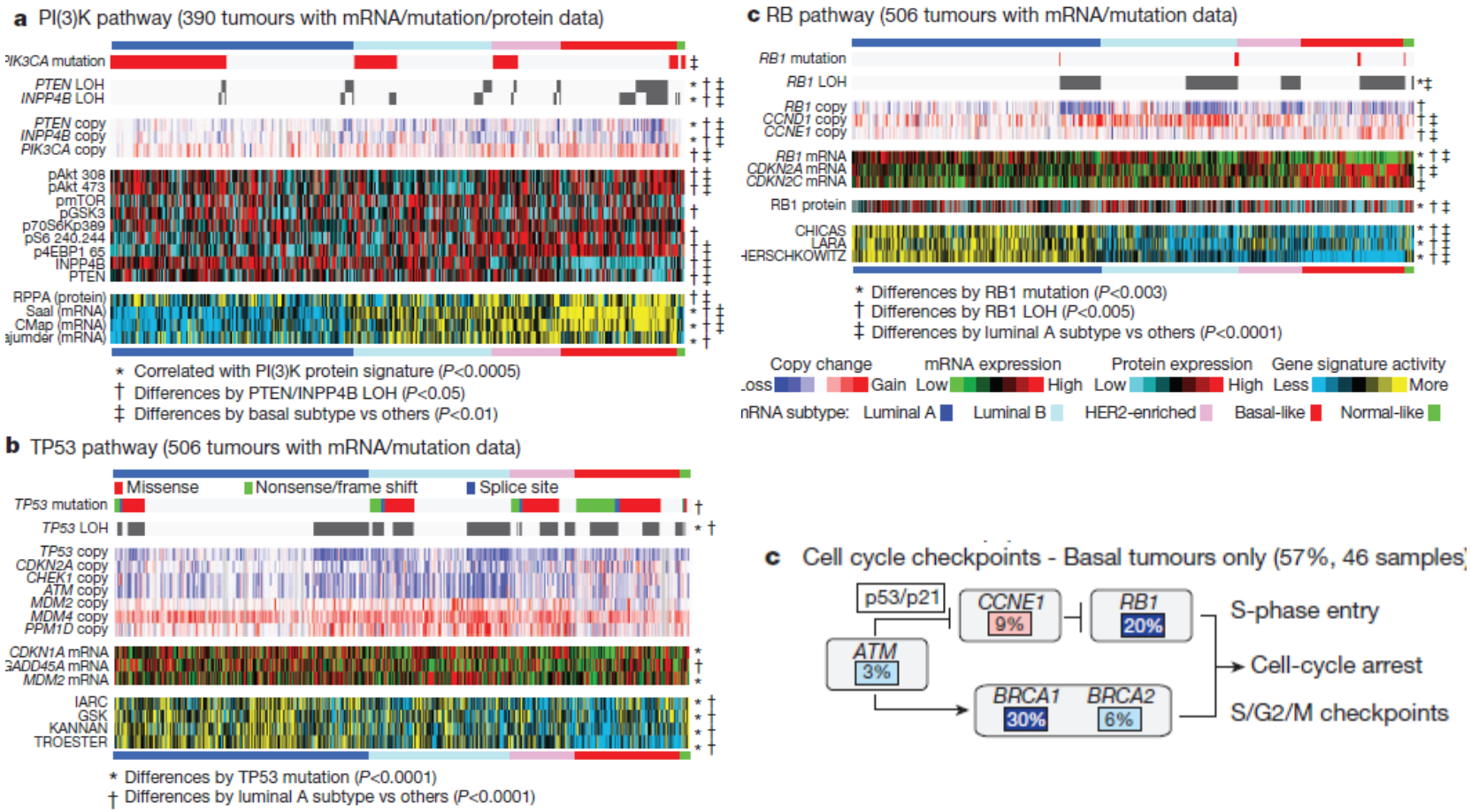
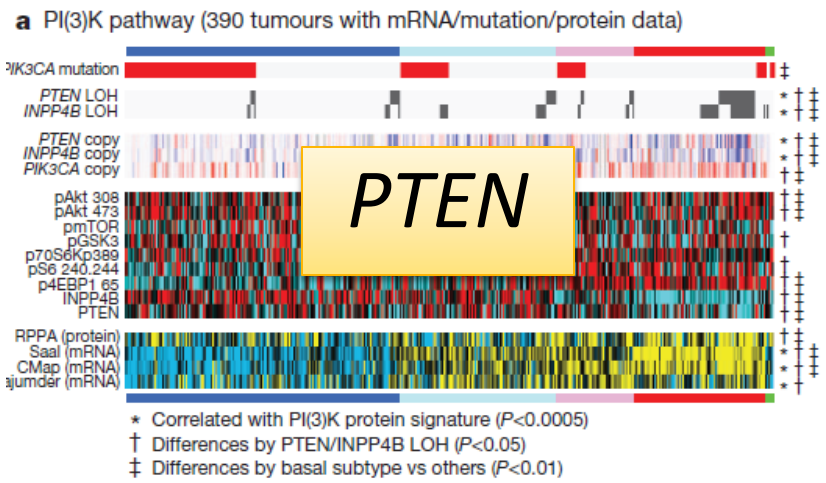
Jansen SA 2012

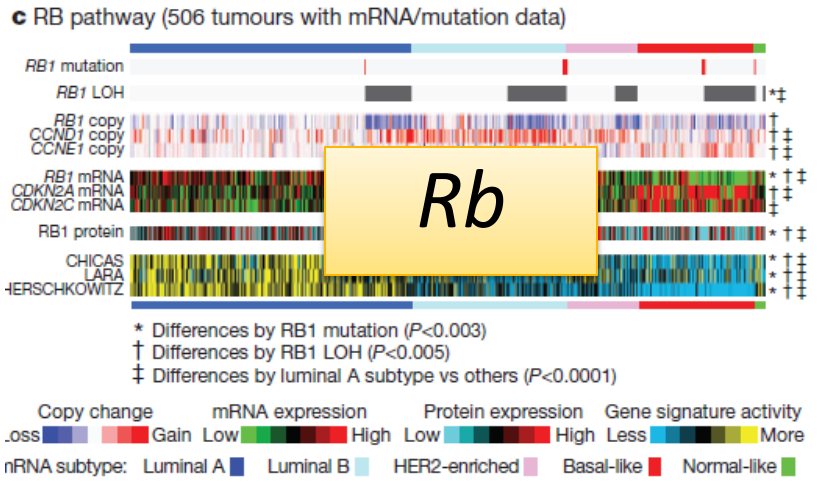
These tumor suppressor pathways are key in *invasive* breast cancer

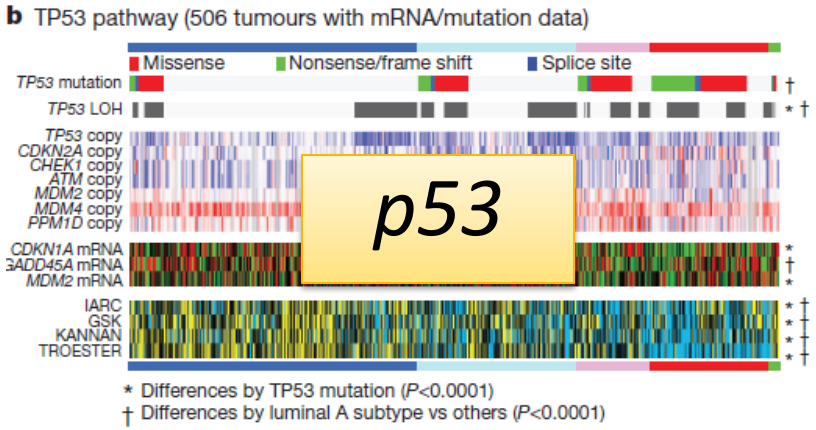


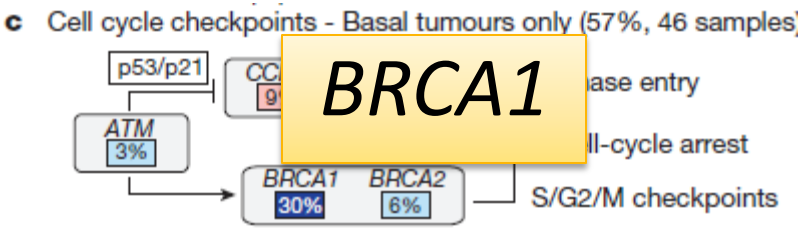
TCGA *Nature* 2012

These tumor suppressor pathways are key in *invasive* breast cancer



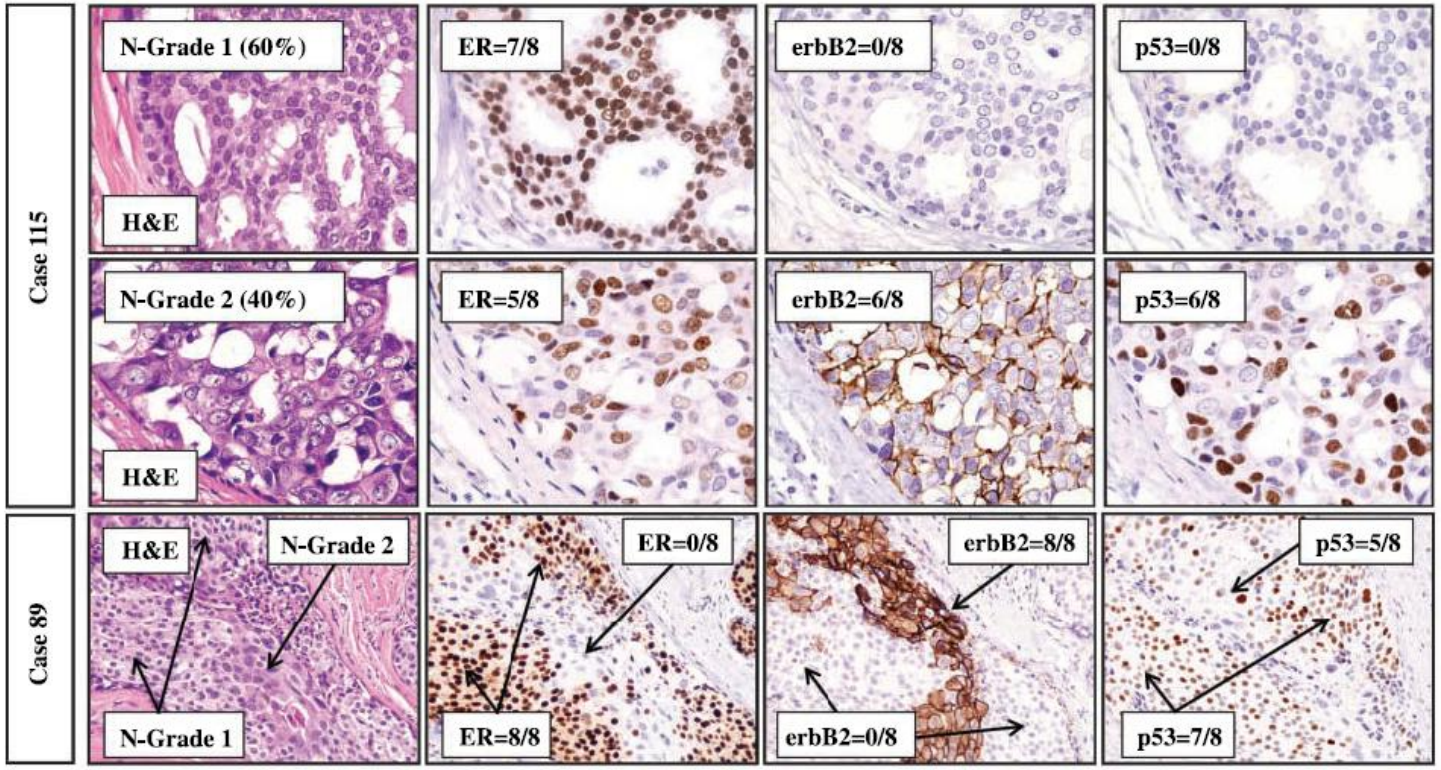






TCGA *Nature* 2012

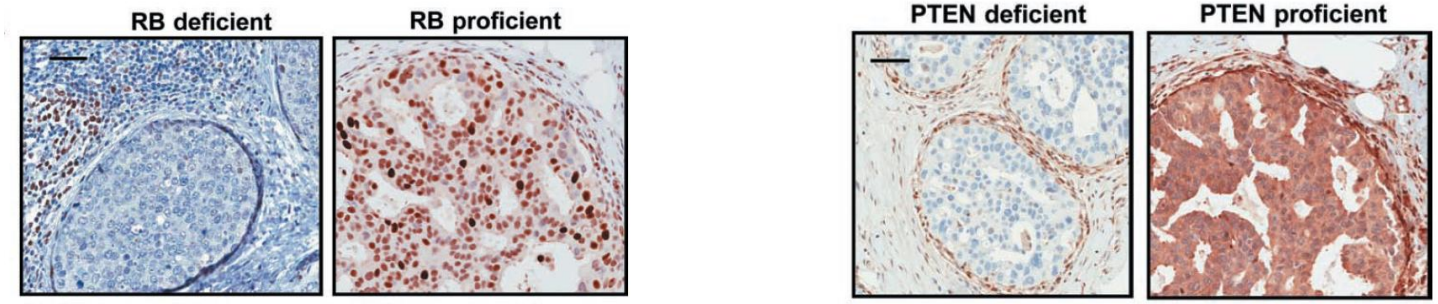
Accumulation of p53 correlates with increased heterogeneity



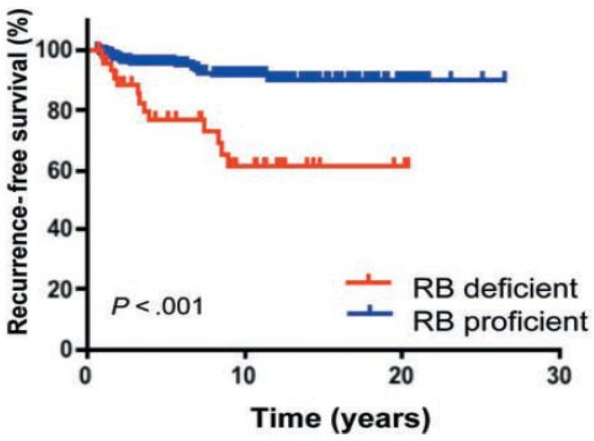
Role of p53 in DCIS heterogeneity and progression?

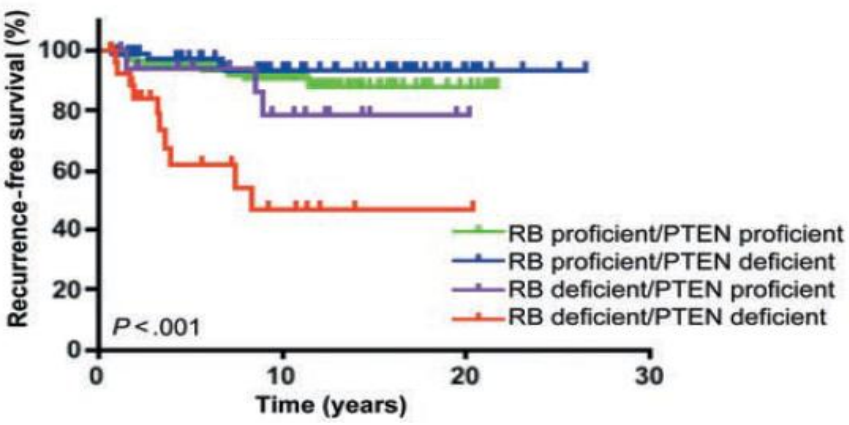
Allred et al *Clin Can Res* 2008

Increased recurrences in Rb and PTEN deficient DCIS



Invasive breast cancer recurrences





Role of PTEN and Rb in DCIS progression?

Knudsen et al *JNCI* 2012

Decreased incidence of DCIS in BRCA1 mutation carriers

Screening trial, Mammo+MRI	No. of tumors in BRCA MC	No. tumors that are DCIS
Warner et al, 2011	9	0/9
Sardanelli et al, 2010	21	2/10
Rjinsburger et al, 2010	21	2/21
Gilbert et al, 2009	15	0/15
Shah et al, 2009	11	2/11
Kaas et al, 2008	39	3/39
Schrading et al, 2008	23	0/14
Total	**139**	**9/139 (6%)**

Role of BRCA1 on DCIS imaging properties and progression?

Jansen SA *Sem. MR, CT, US* 2011

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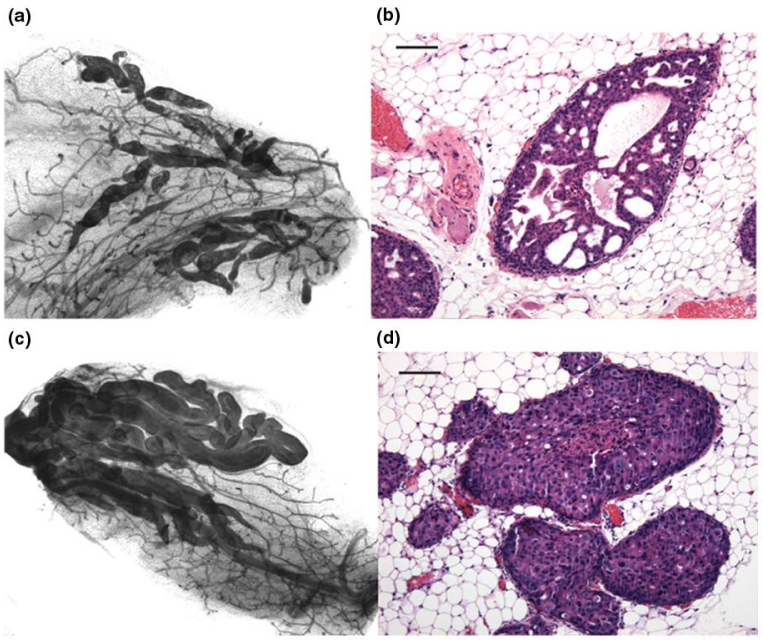
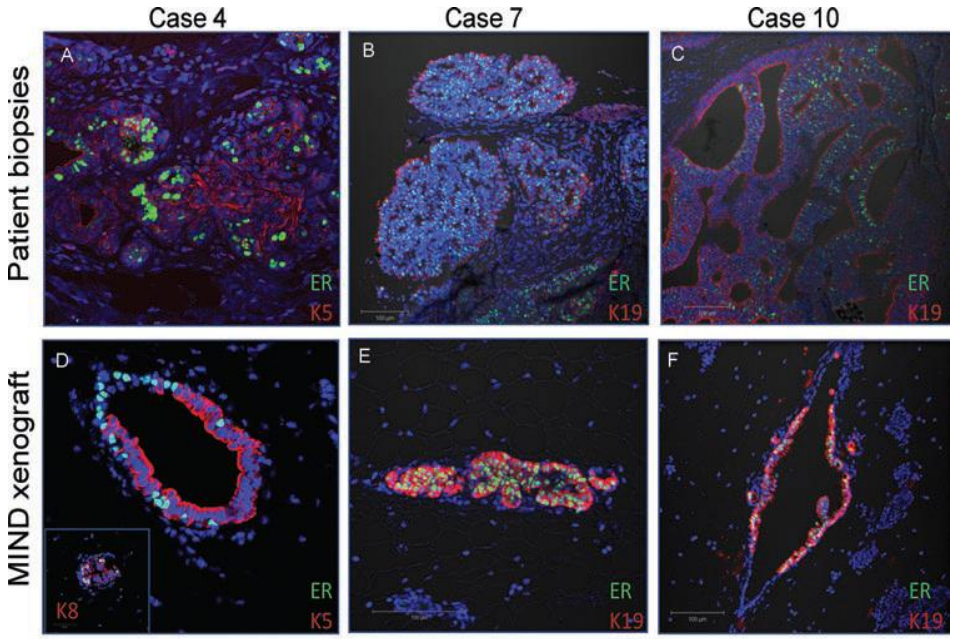
Modeling DCIS progression in mice

Xenograft vs. GEM models of DCIS



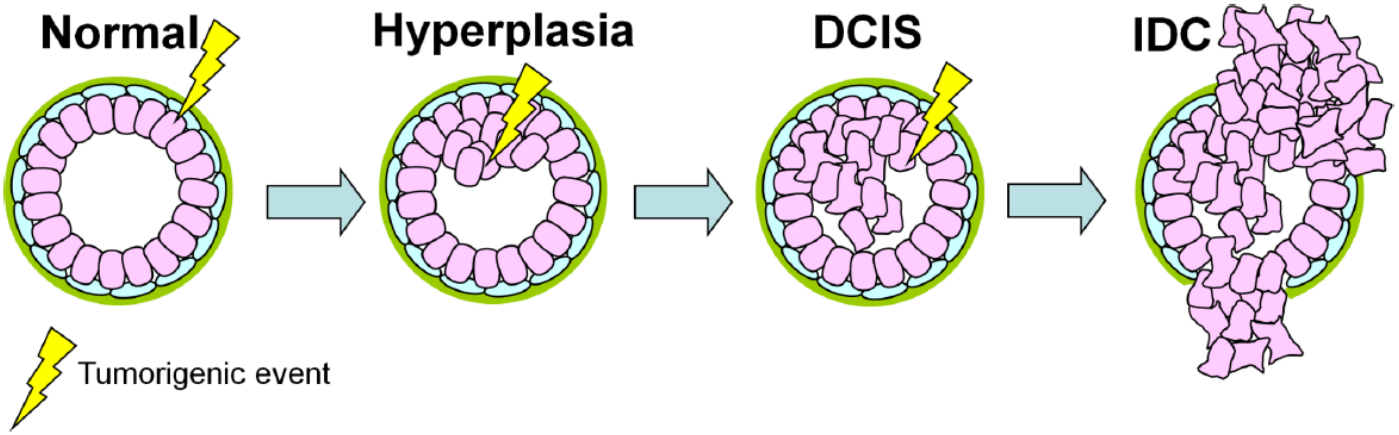


- Intraductal xenograft models of DCIS

Behbod et al 2009, Valdez et al 2011

Modeling DCIS progression in mice

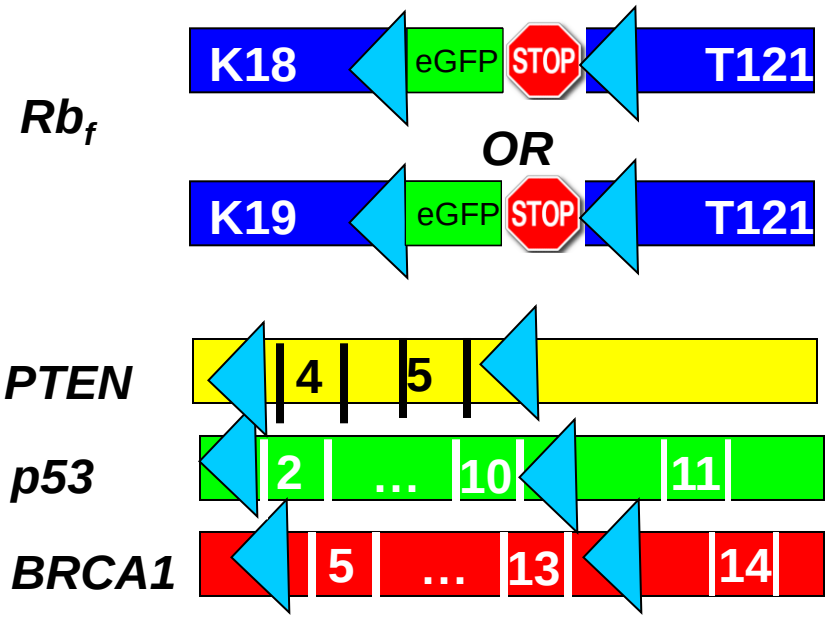
Genetic transformation may not be linear



- Bulk of genetic transformation has already occurred by DCIS stage (Ma et al PNAS 2003, Chin et al Nature Genetics 2004)

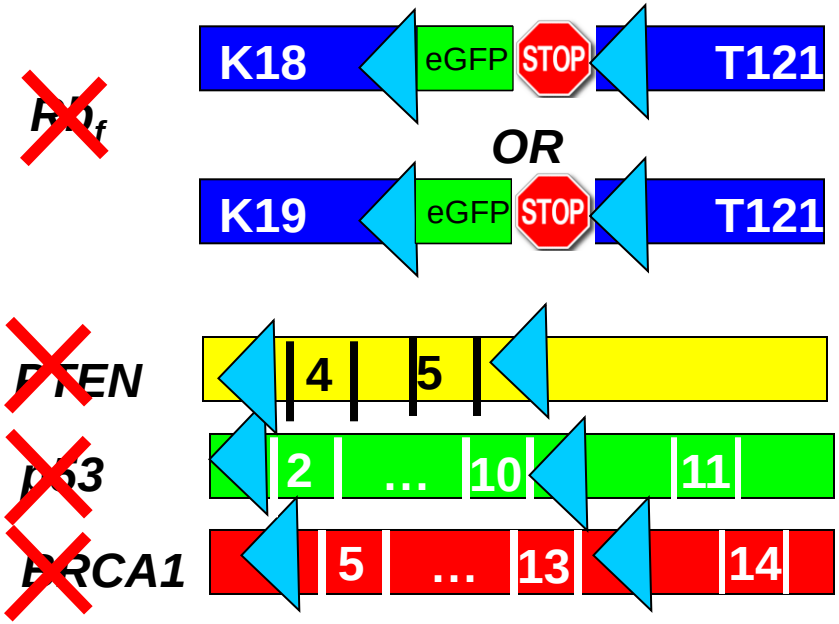
Modeling DCIS progression in mice

Keratin promoters

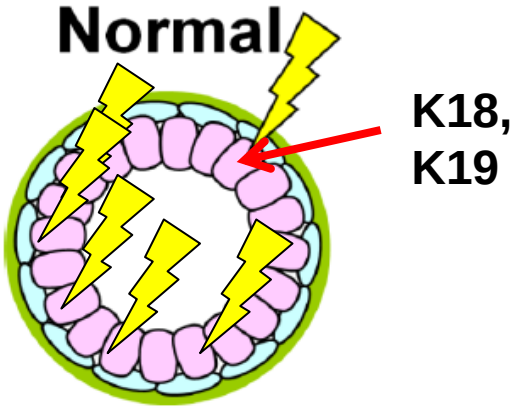


Modeling DCIS progression in mice

Keratin promoters



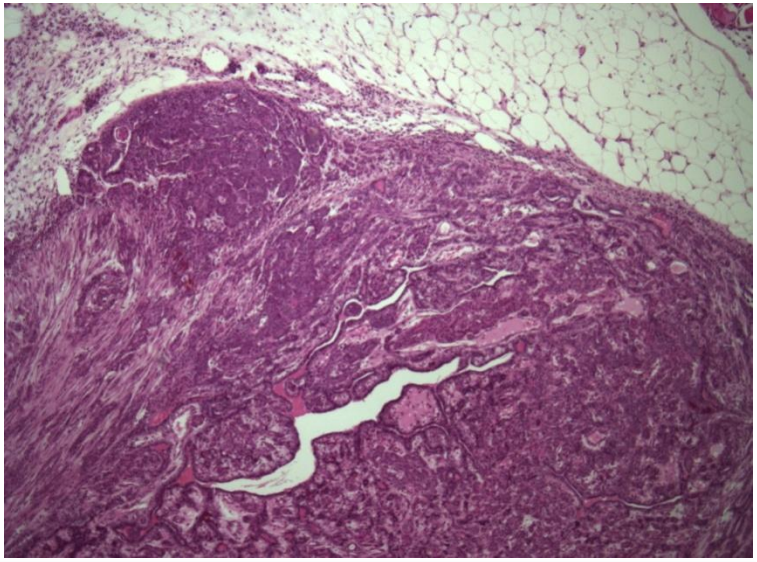
Pb-Cre
WAP-Cre



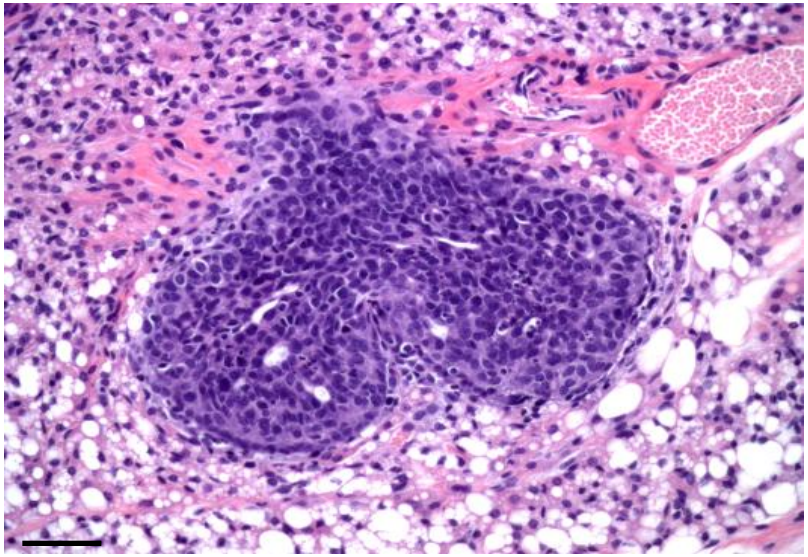
Modeling DCIS progression in mice

Keratin promoters: Rationale

- Evidence suggesting that these models can initiate mammary carcinomas



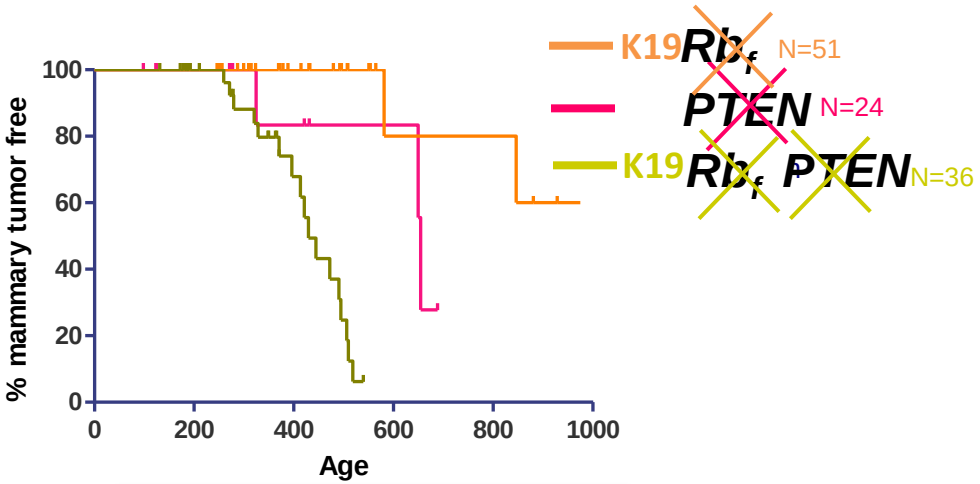
K19-T121tg/+; Pb Cre tg/+



K18-T121tg/+; B-actin Cre tg/+

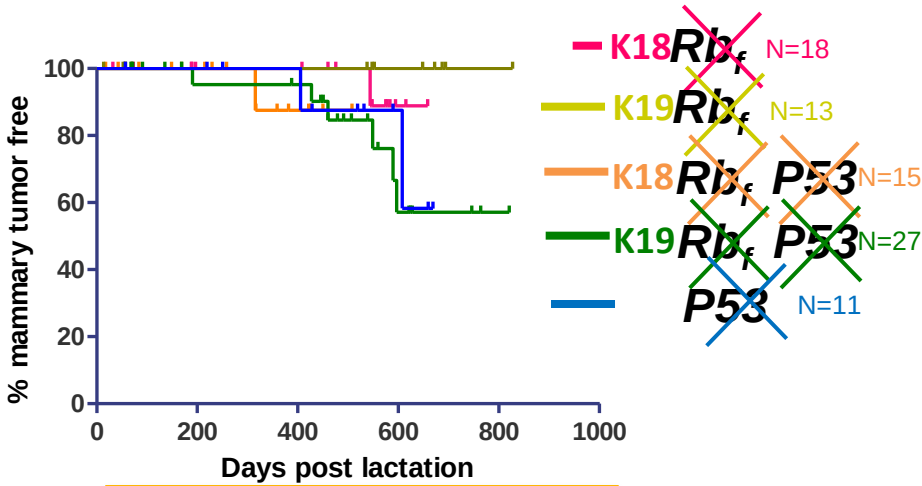
But these models have weak penetrance and long latency

Pb-Cre induction



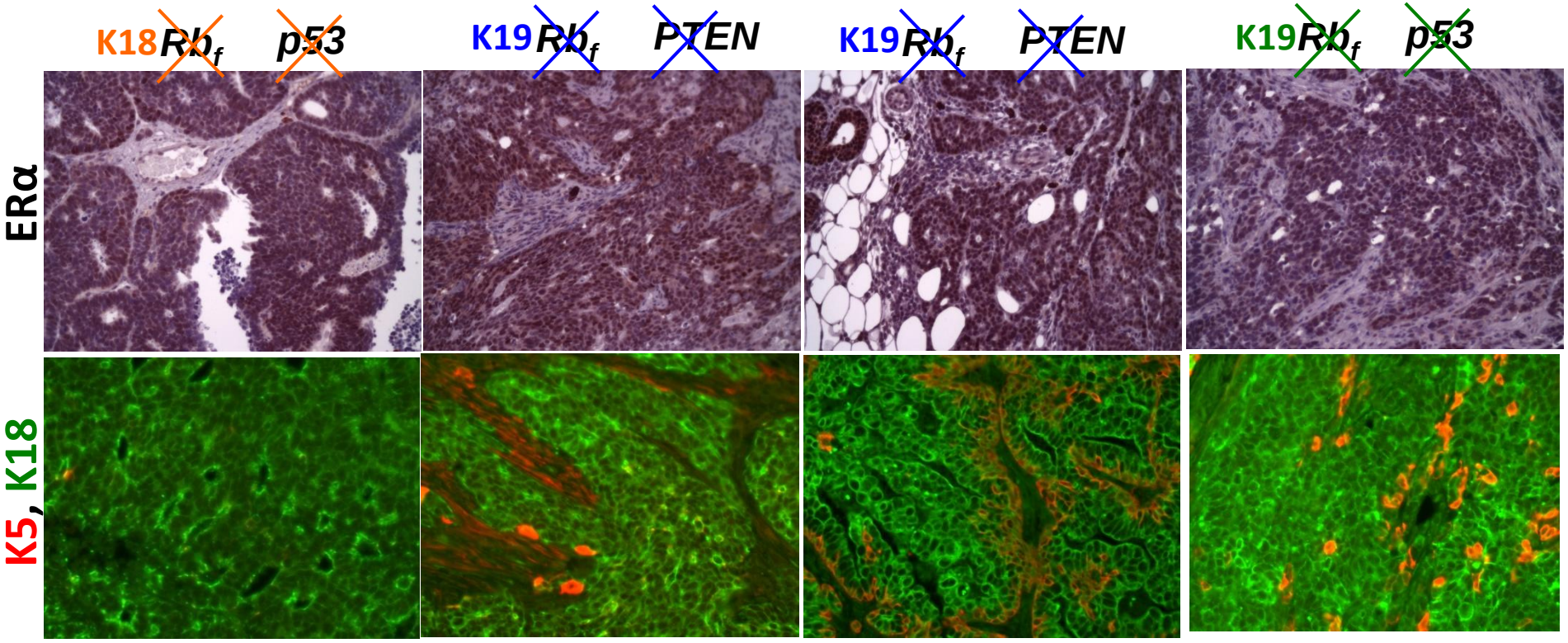
111 study mice
22 tumors
~20% penetrance
472 days median age at tumor onset

WAP-Cre induction



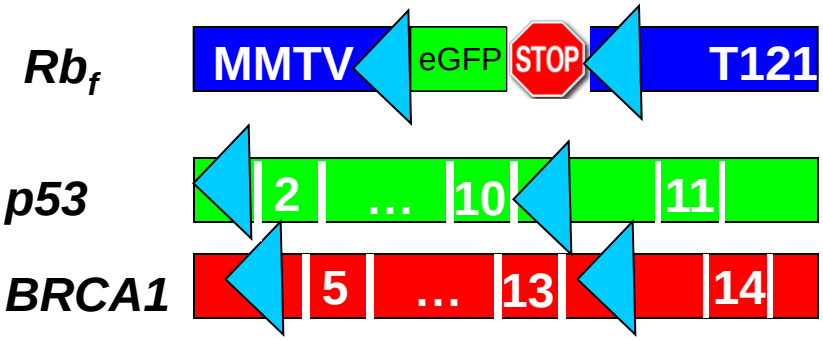
84 study mice
10 tumors
~12% penetrance
459 days median post lactation at tumor onset

Tumors have luminal characteristics



Modeling DCIS progression in mice

MMTV promoter



Modeling DCIS progression in mice

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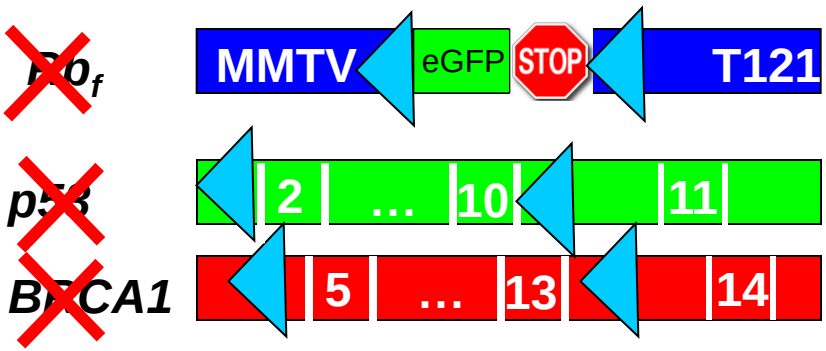
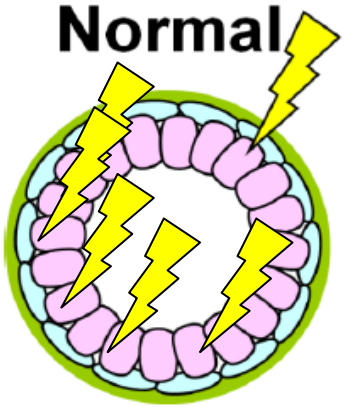


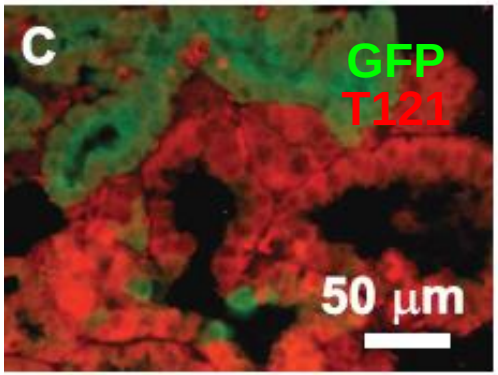
+

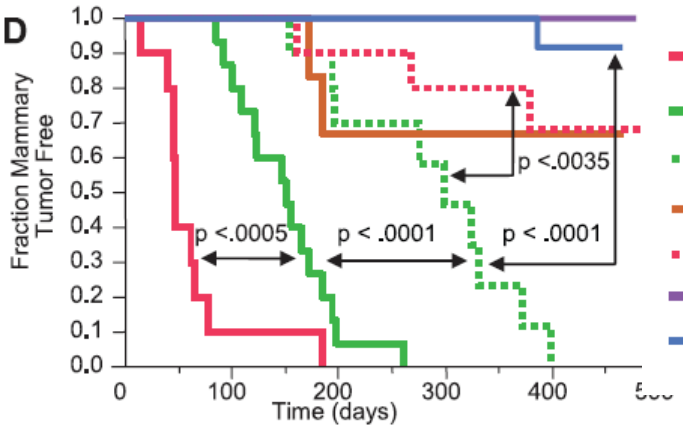
WAP-Cre



Modeling DCIS progression in mice

MMTV Promoter





Genotype	Acronym	n
MFT_{121}^{+}, $Brca1^{f/f}$, $p53^{f/f}$	TBP	10
MFT_{121}^{+}, $p53^{f/f}$	TP	15
MFT_{121}^{+}, $p53^{f/+}$		10
$Brca1^{f/f}$, $p53^{f/f}$	BP	8
MFT_{121}^{+}, $Brca1^{f/+}$, $p53^{f/+}$		10
MFT_{121}^{+}	T	14
MFT_{121}^{+}, $Brca1^{f/f}$	TB	13

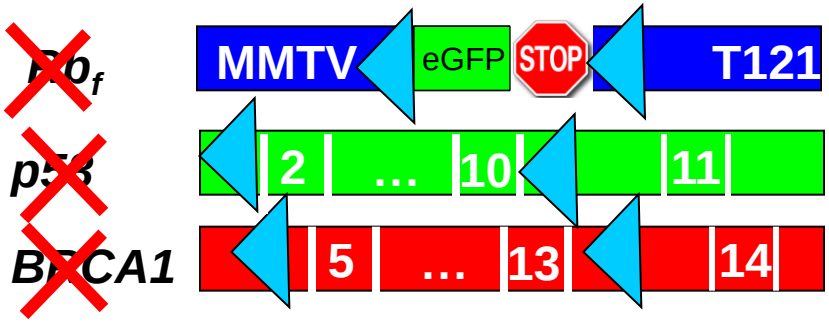
~~*Rb*$_f$~~ ~~*p53*~~ ~~*BRCA1*~~ Predominantly ***basal*** gene expression profile

~~*Rb*$_f$~~ ~~*p53*~~ Predominantly ***luminal B*** gene expression profile

Kumar et al *PLOS Genetics* 2012

Modeling DCIS progression in mice

Focal induction with lenti-Cre



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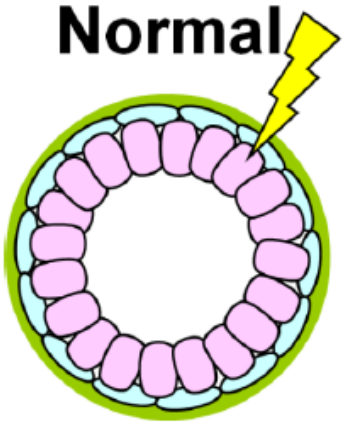
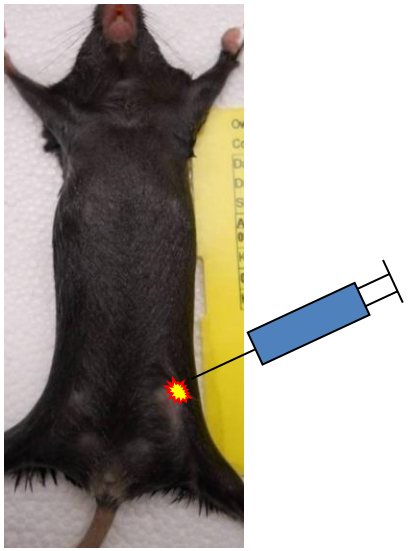
Lenti-Cre

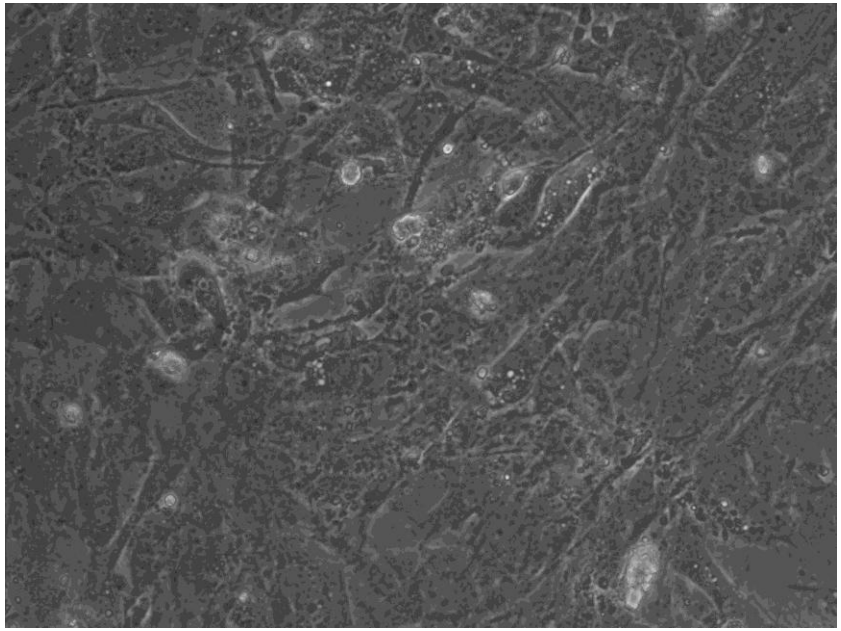


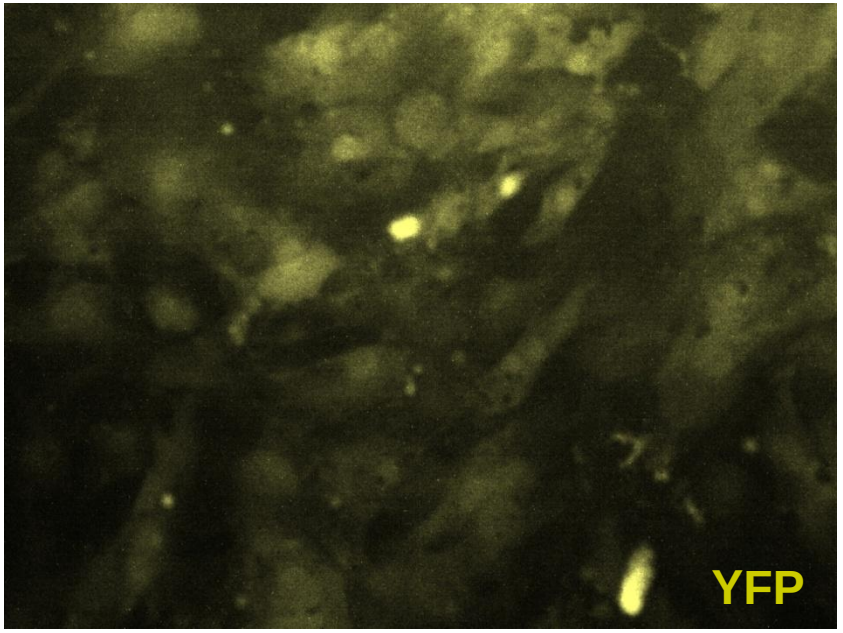


Modeling DCIS progression in mice

Focal induction with lenti-Cre

- Rosa-YFP MEFs treated with lenti-Cre at 200 MOI





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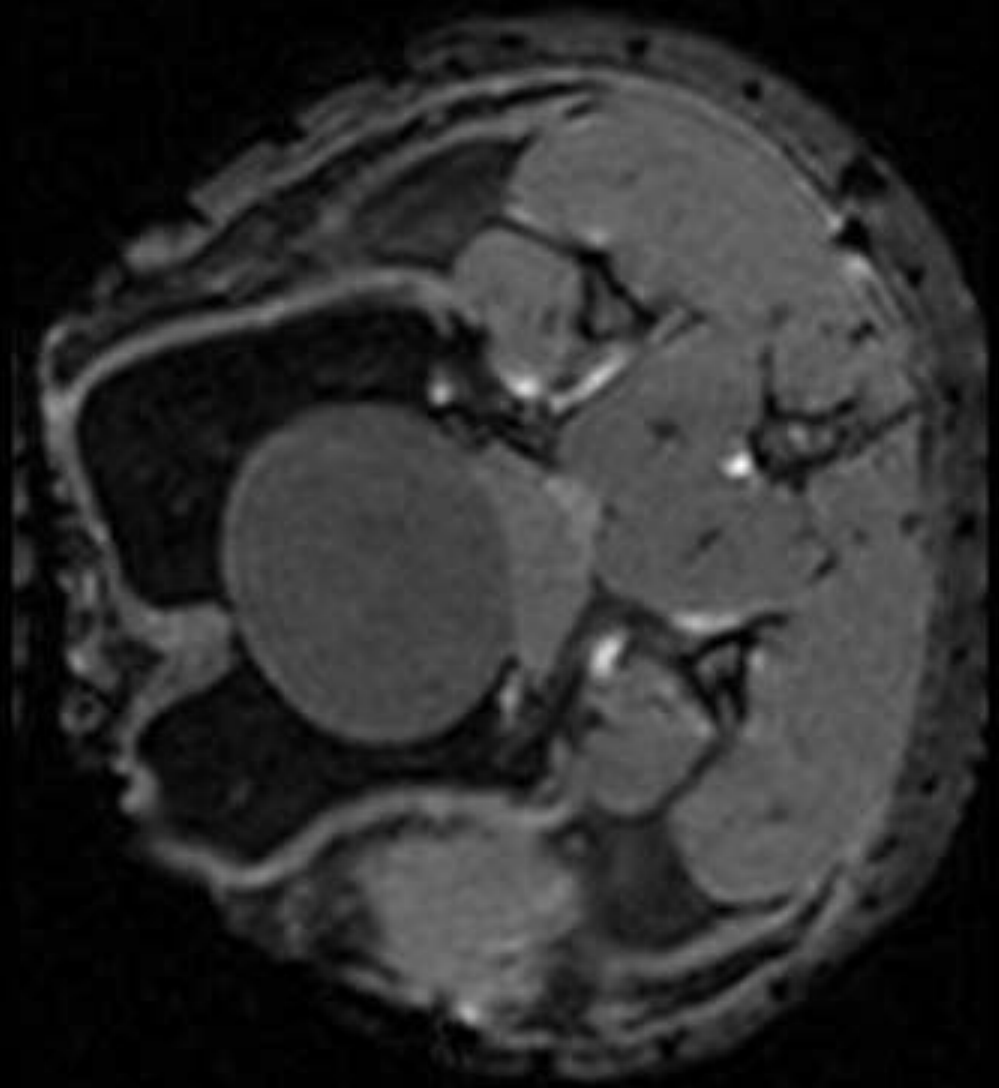
C3(1) Tag mice



Movie

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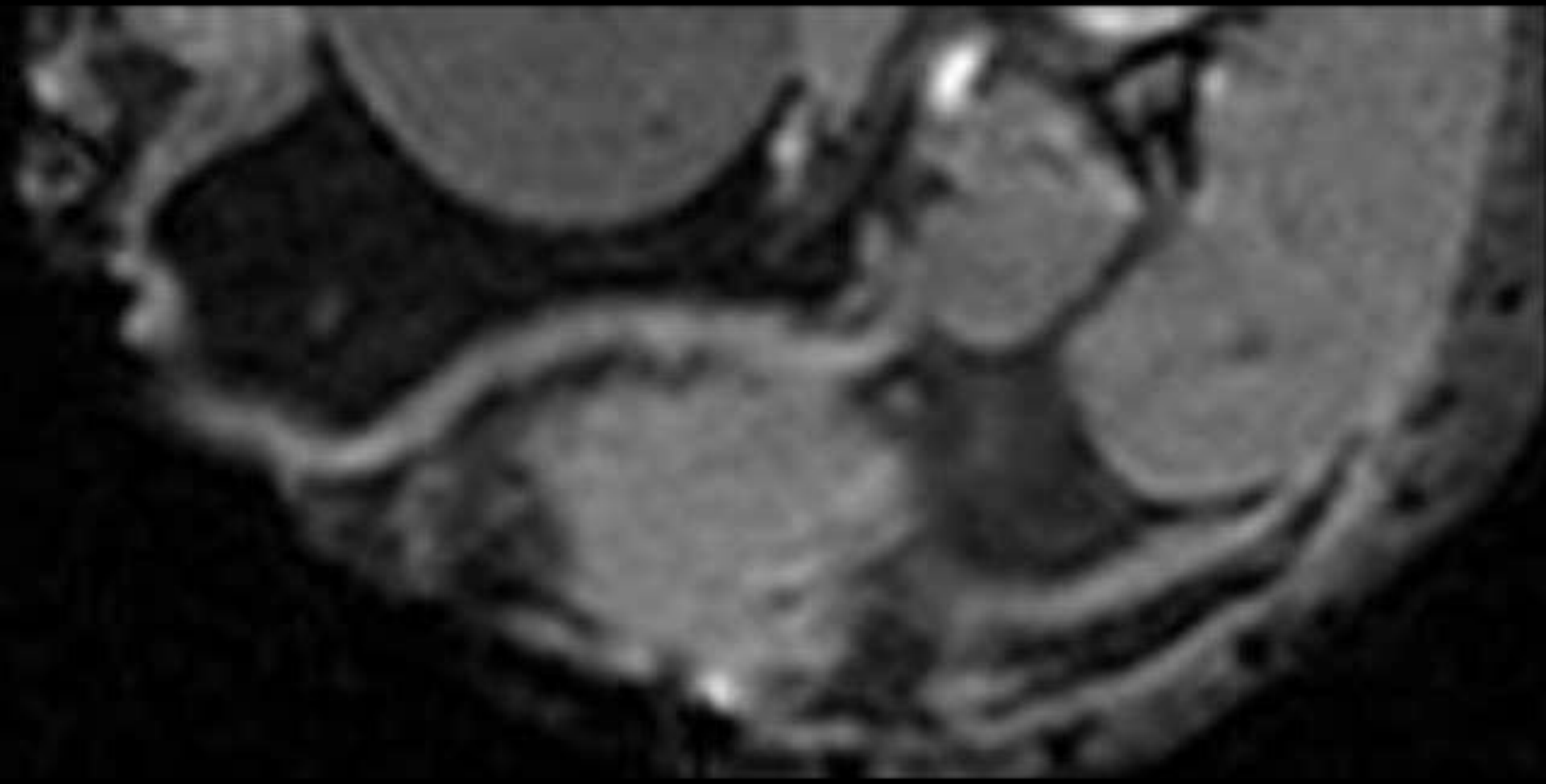

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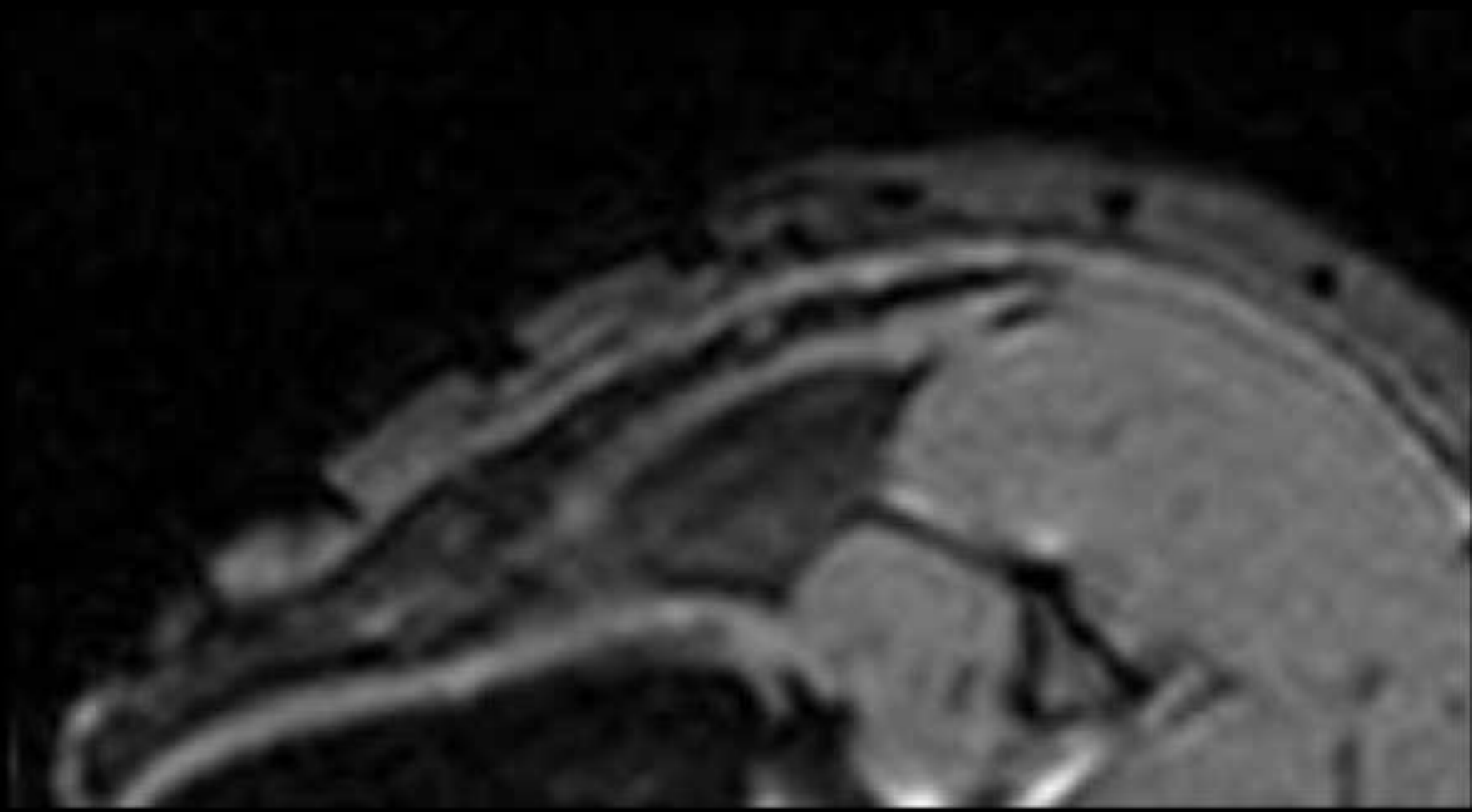

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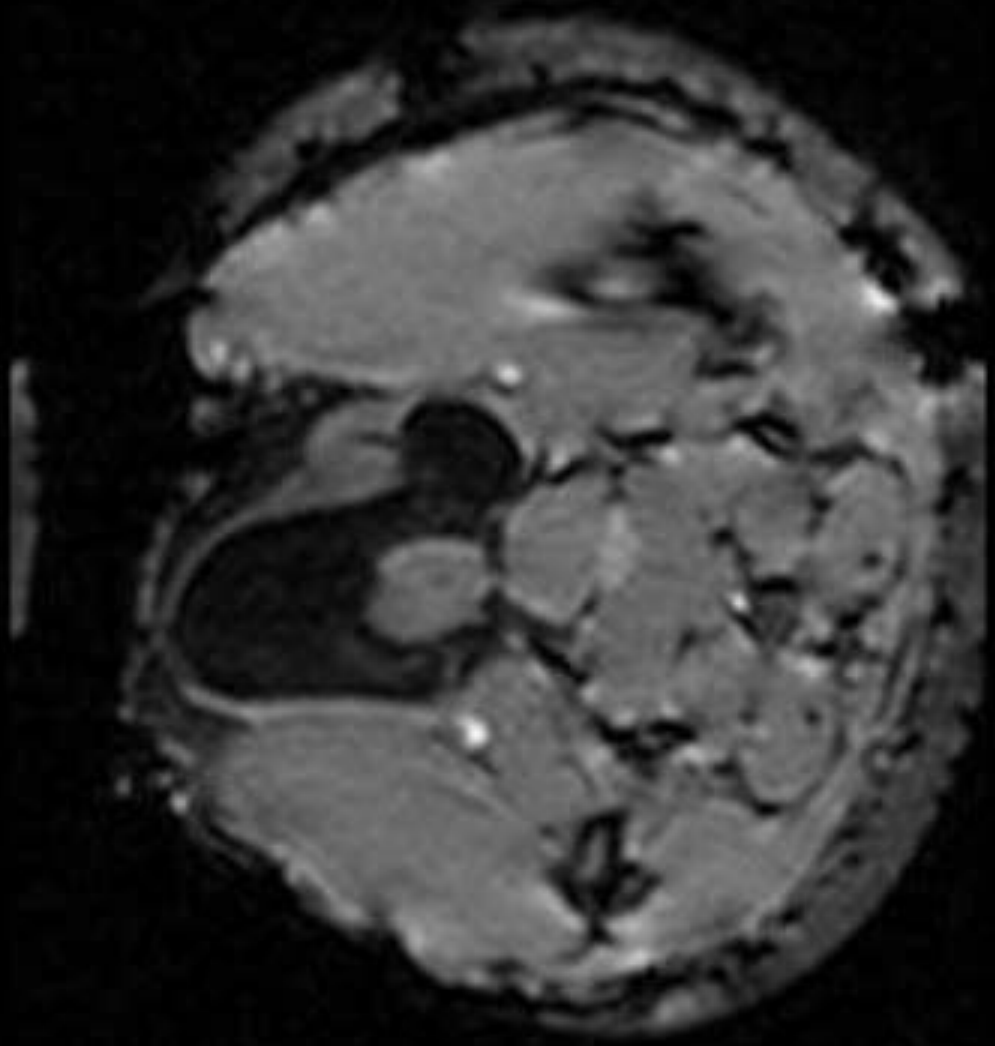

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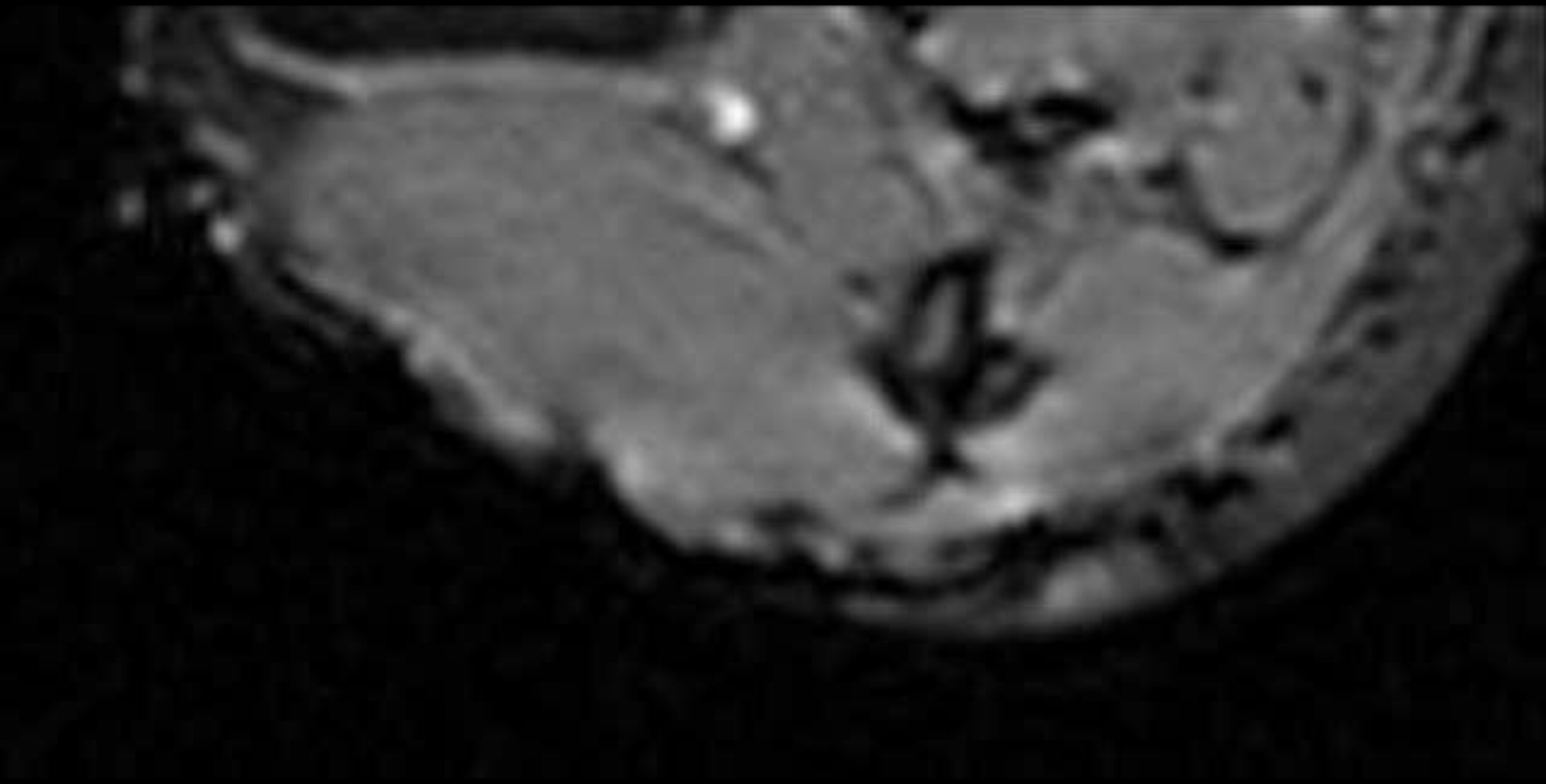
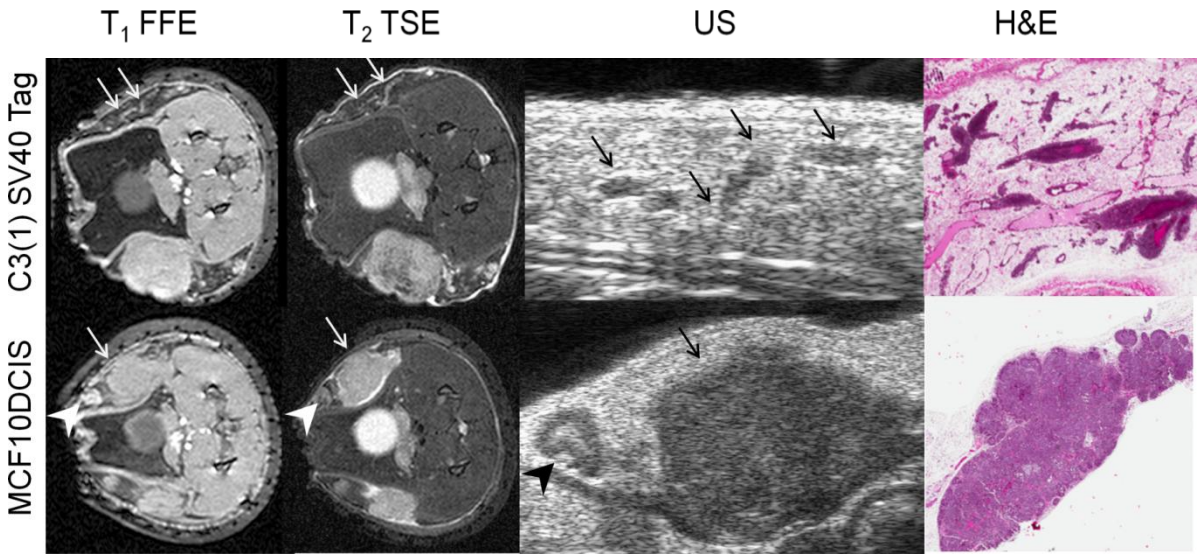


Movie

Accurate imaging methods to follow preinvasive cancer progression



	Number of DCIS	Sensitivity of MRI	Sensitivity of MRI-directed ultrasound
All inguinal glands	60	88% (53/60)	72% (38/53)
Posterior inguinal glands	33	94% (31/33)	97% (30/31)
Anterior inguinal glands	27	82% (22/27)	36%(8/22)

Jansen et al *submitted*

Rapid whole body MR screening for preinvasive cancer

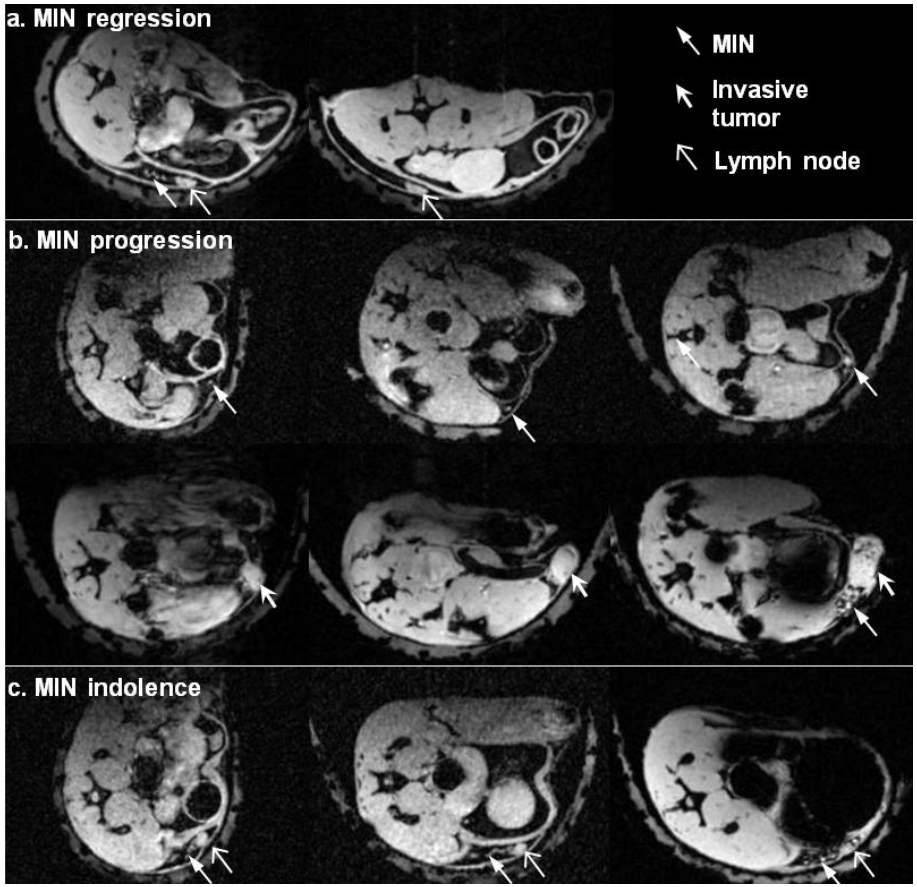
10-15 minutes per mouse to screen all mammary glands

- ***Mouse Mammary*** collection on The Cancer Imaging Archive
  https://wiki.cancerimagingarchive.net/display/Public/Mouse-Mammary

Movie

MRI can be used to follow progression of DCIS in mice

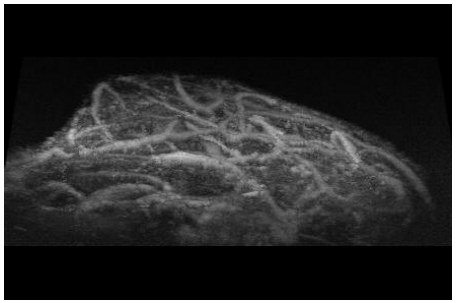
- Classify as progressing, regressing, indolent



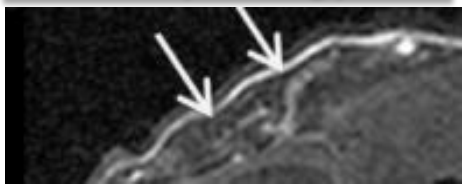
Jansen et al 2009

Other imaging techniques for DCIS

Contrast enhanced US

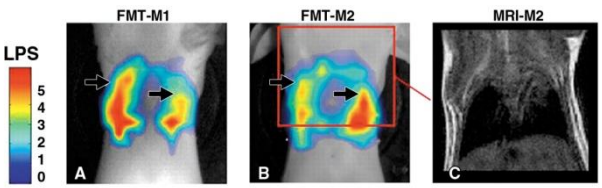


Quantitative MRI morphology

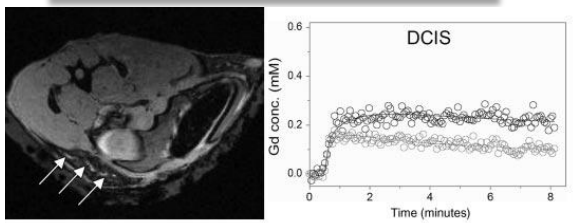


$$f_1 = \sum_{i=1}^{G}\sum_{j=1}^{G} p(i,j)^2$$

Fluorescence Molecular Tomography



Contrast enhanced MRI



Outline

- Introduction
- Modeling DCIS progression in mice
- Techniques for noninvasively tracking preinvasive cancer progression in mice
- Summary

Summary

- Genetic events in progression of DCIS are not well understood
- We have characterized mouse models to study genetics of DCIS progression and developed noninvasive imaging techniques for interogating these models

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