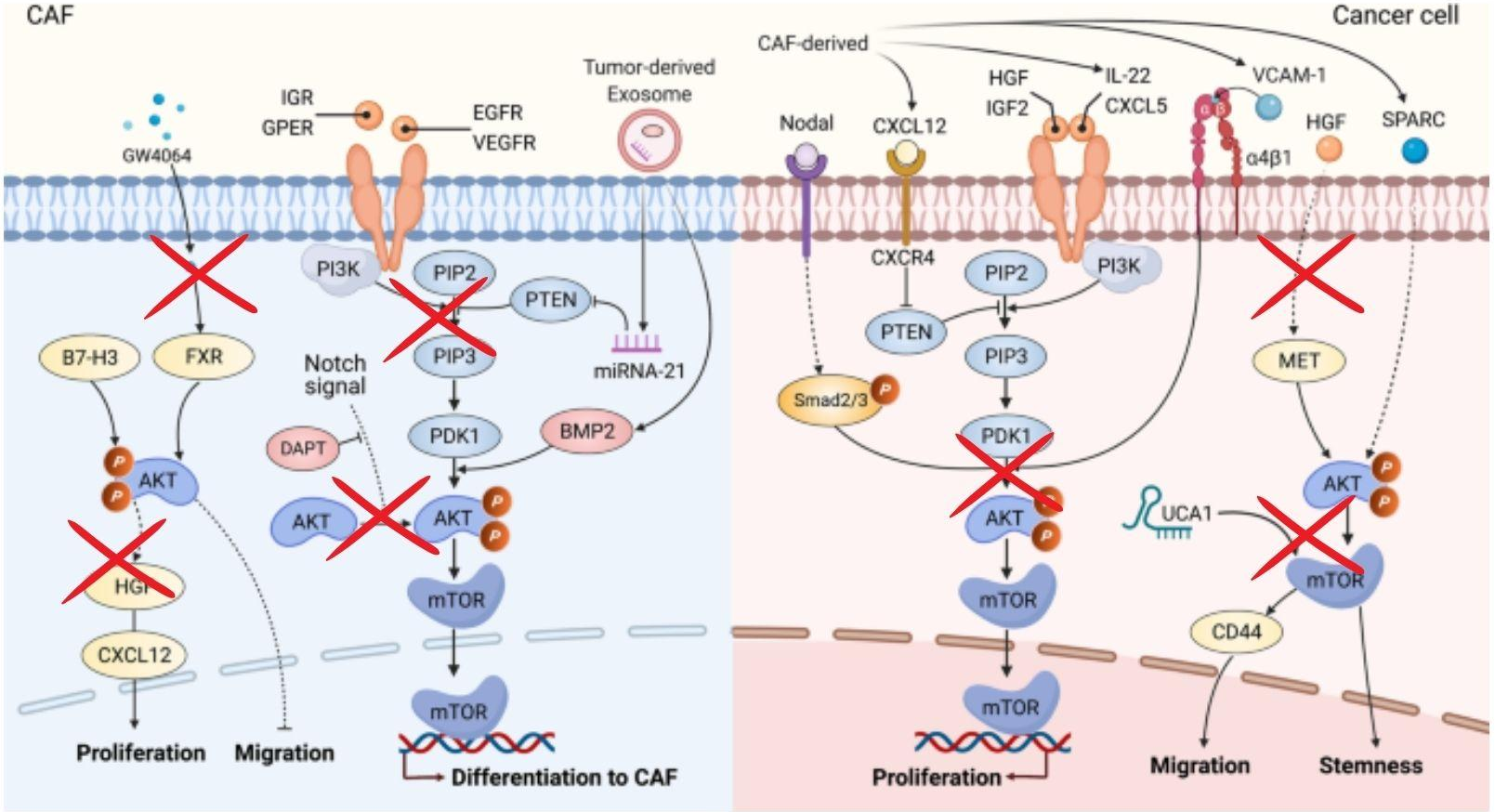
Molecular Targets and Validation

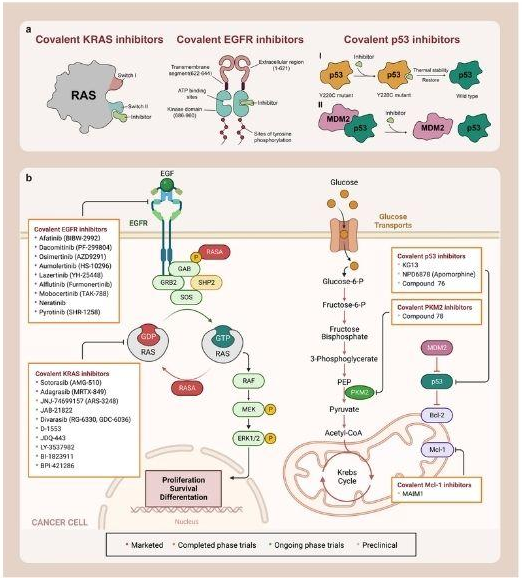
Group 1

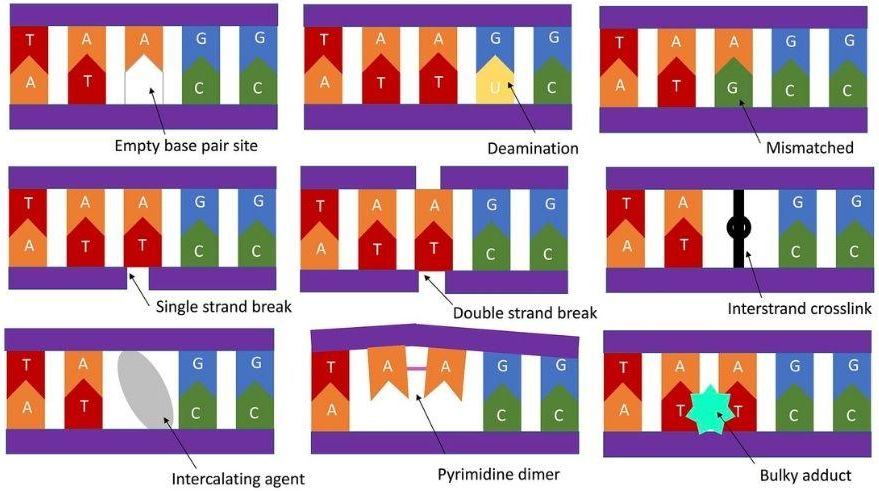
Molecular target For Cancer Drugs



Genetic lesions

- Oncogenic proteins
- Mutated tumor suppressor proteins.



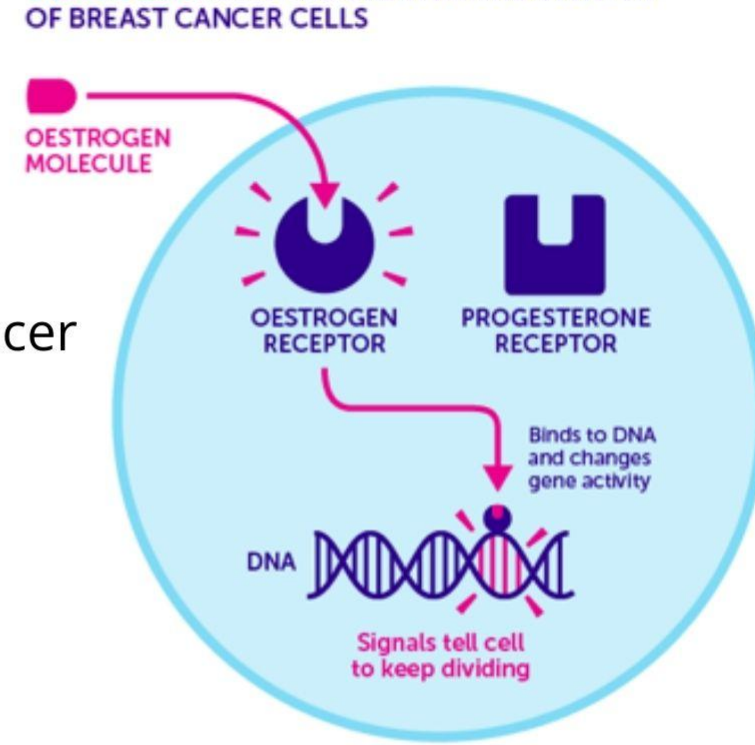


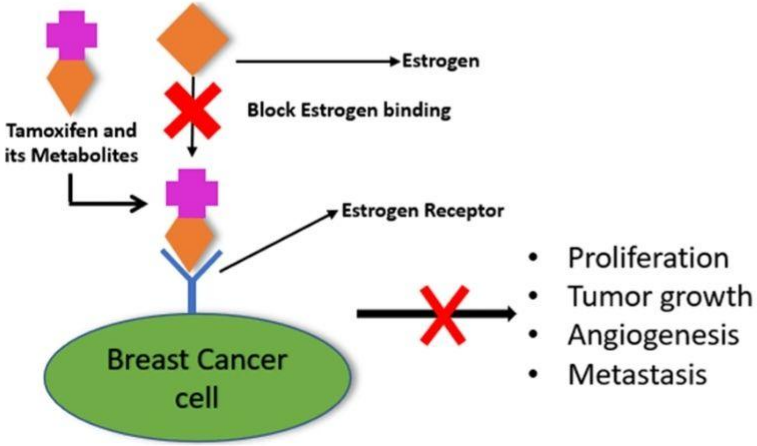
Genetic lesions

Target the aberrant protein itself OR components of the pathways they affect

or differentiation pathways

- Hormones or factor influence
- Estrogen acts as a mitogen in breast cancer
- Tamoxifenthe action of estrogen



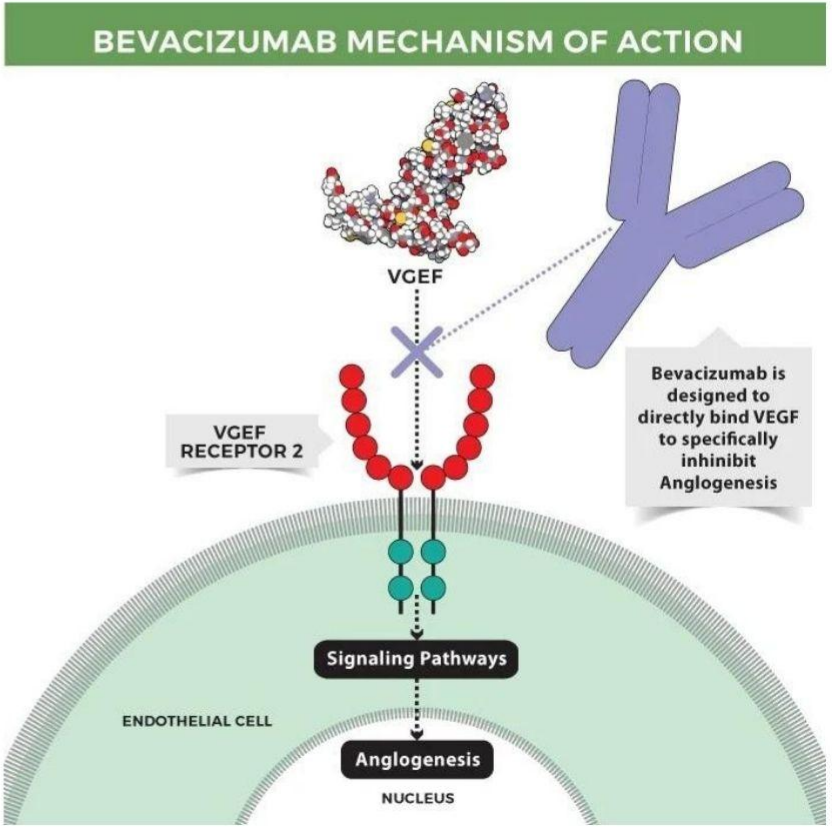


Target Host Processes

.g. Anti-angiogenic theraypy

Host Processes vs. Tumor Biology

Molecular regulators of angiogenesis as good therapeutic targets (e.g. VEGF,

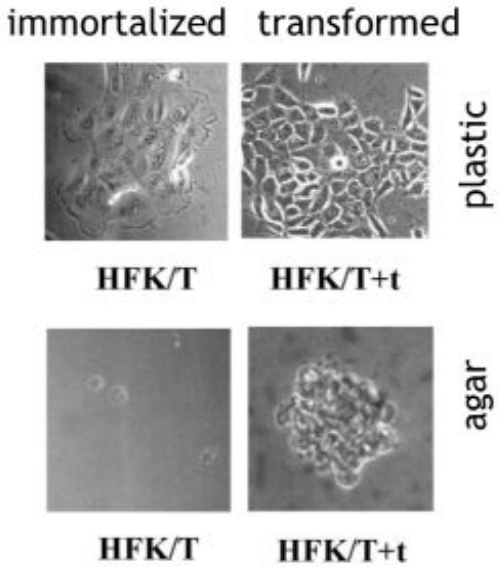


Target Validation in cell model

Characteristics of cancer cells

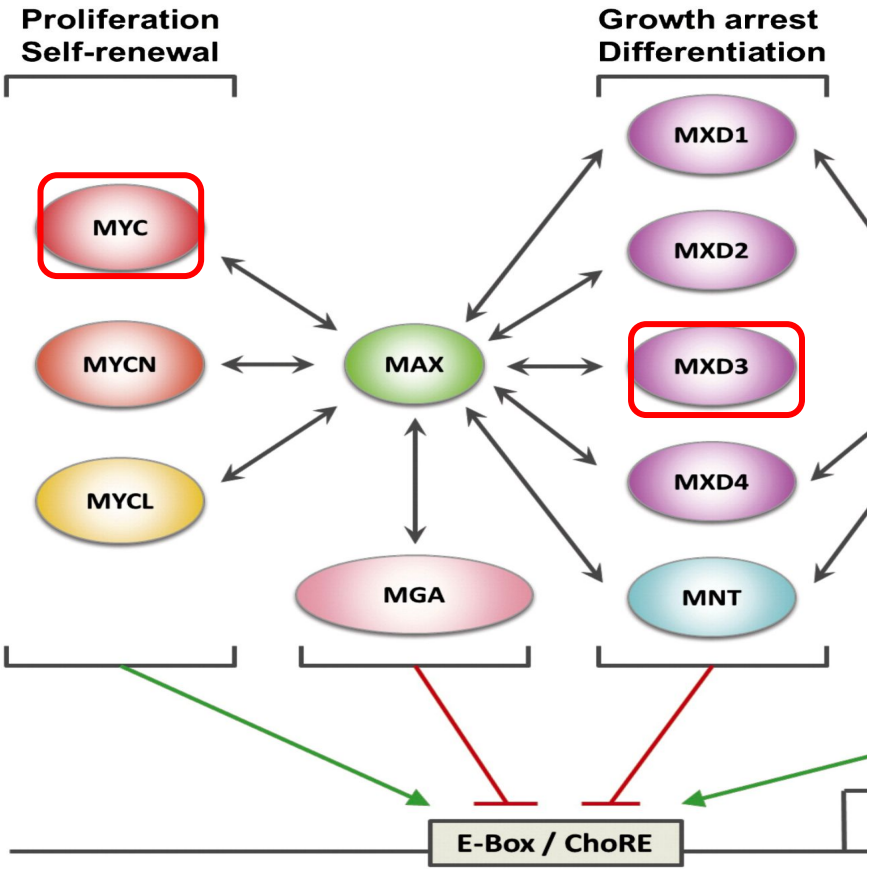
How can oncogenes be identified in a laboratory?

- Clone the gene from tumor cells into expression vector.
- Transfection of DNA into immortalized primary cells.
- Evaluation whether transfected cells obtain altered growth characteristics (so-called transformation assay).

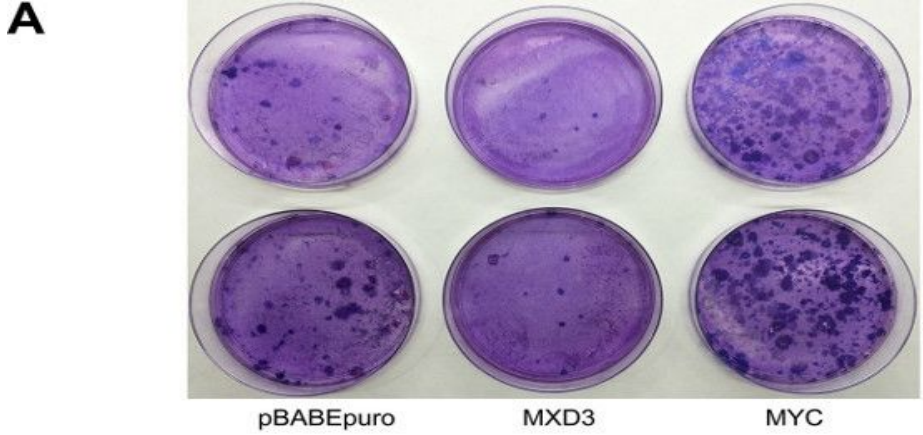


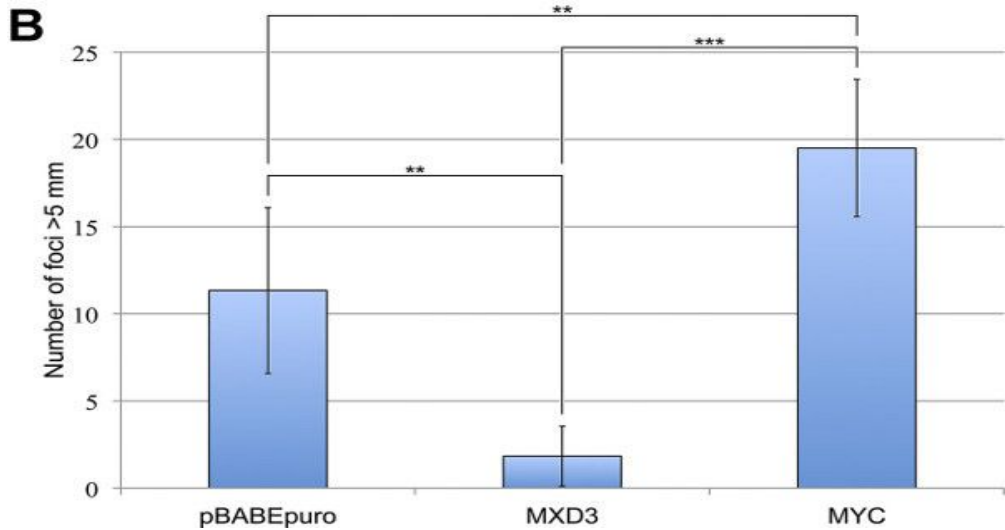
Yuan et al., J. Virol. (2002)

Focus Formation Assay



Diolaiti et al. 2015 Functional interactions among members of the MAX and MLX transcriptional network during oncogenesis





Alvarez, A., Barisone, G. A., & Diaz, E. (2014). Focus formation: a cell-based assay to determine the oncogenic potential of a gene

Target Validation in Animal model

Genetic Validation of Specific Genetic Lesion:

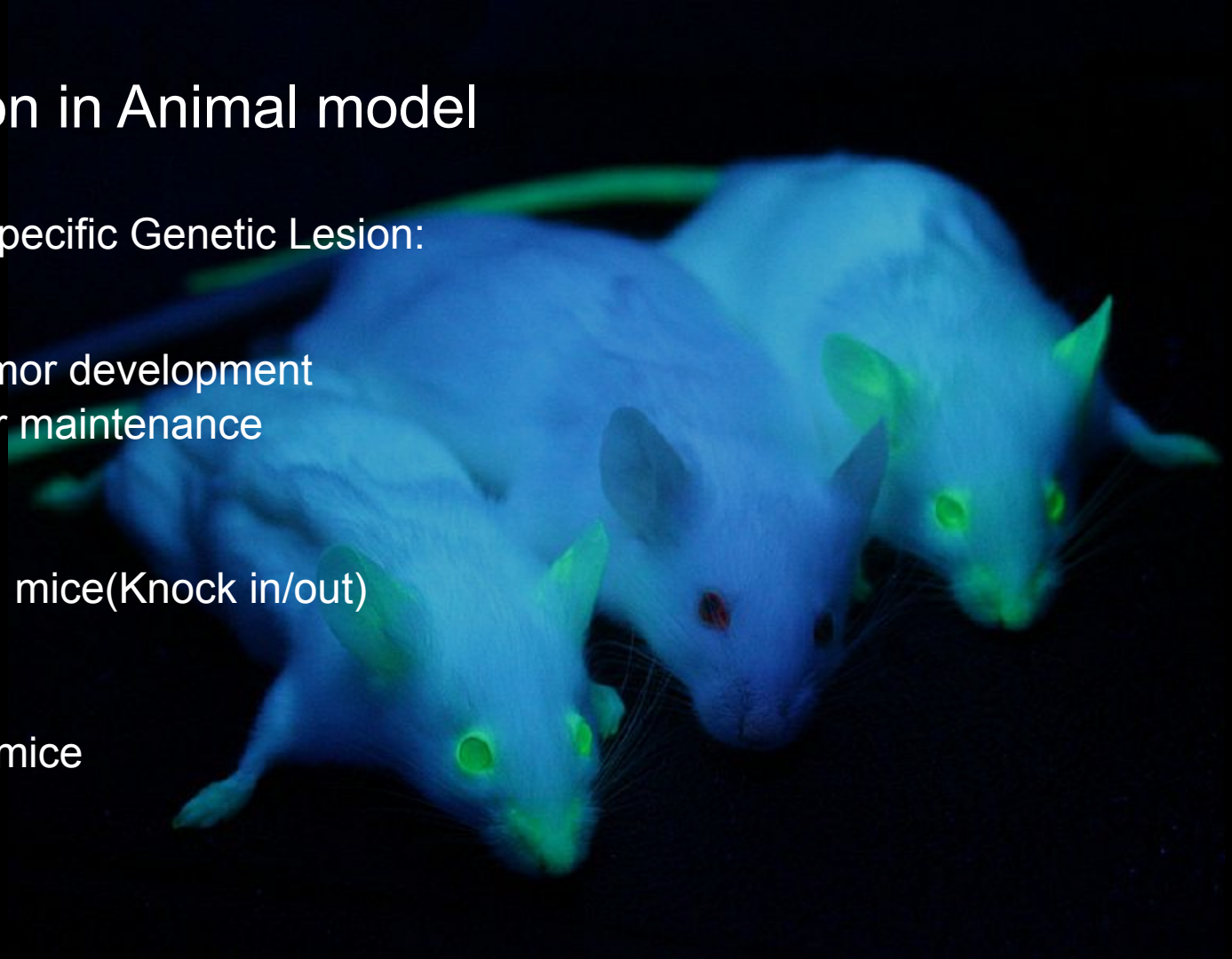
1. Tumor specific
2. Occurs in early tumor development
3. Required for tumor maintenance

In Mice model:

Genetically engineered mice(Knock in/out)

Tissue Specific

Inducible transgenetic mice



loxP - Cre homologous recombination

THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 259, No. 3, Issue of February 10, pp. 1509-1514, 1984
Printed in U.S.A.

Bacteriophage P1 Site-specific Recombination

PURIFICATION AND PROPERTIES OF THE Cre RECOMBINASE PROTEIN*

(Received for publication, July 14, 1983)

Ken Abremski and Ronald Hoess

From the Laboratory of Molecular Biology, Basic Research Program—LBI, Frederick Cancer Research Facility, Frederick, Maryland 21701

Bacteriophage P1 encodes a site-specific recombination system that consists of a site (*loxP*) at which recombination occurs and a gene, *cre*, whose protein product is essential for recombination. The *loxP*-Cre recombination event can be studied in greater detail by the use of an *in vitro* system that efficiently carries out recombination between two *loxP* sites. This paper

containing two 13-bp inverted repeats separated by an 8-bp spacer region (7). Only these 34 bp appear to be required for efficient recombination (6). An *in vitro* system to carry out *loxP* × *loxP* recombination has been established that uses a partially purified extract derived from a P1 lysogen of *E. coli* (6). This extract can efficiently carry out recombination between two *loxP* sites, with only Mg^{2+} or spermidine needed as

Validation of target using transgenic mice

PTEN and Trp53 tumor suppressor gene validation in mice:

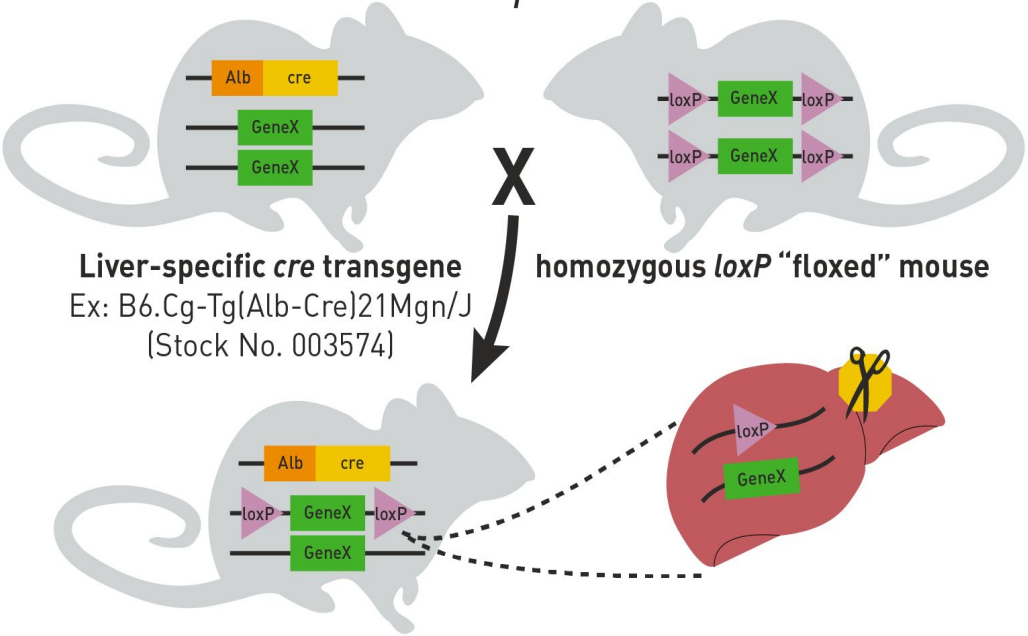
METHODS

Go to:

Pten and *Trp53* mutant mice

Pten$^{loxP/loxP}$ mice were generated as described previously[19], and *Trp53*$^{loxP/loxP}$ mice were generated with a similar strategy (Supplementary Fig. S1; details are available from the authors). Female *Pten*$^{loxP/loxP}$;*Trp53*$^{loxP/loxP}$ mice were crossed with male *PB-Cre4* transgenic mice[21] for the prostate-specific deletion of *Pten* and *Trp53*. For genotyping, tail DNA was subjected to

Chen, Z. et al. (2005). Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. *Nature*

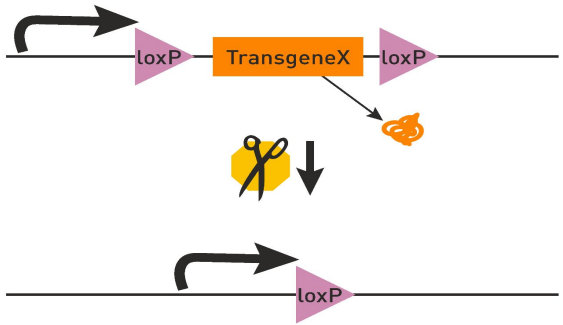


Liver-specific *cre* transgene
Ex: B6.Cg-Tg(Alb-Cre)21Mgn/J
(Stock No. 003574)

homozygous *loxP* "floxed" mouse

Cre-lox mouse:
heterozygous for GeneX conditional knockout after 1 generation

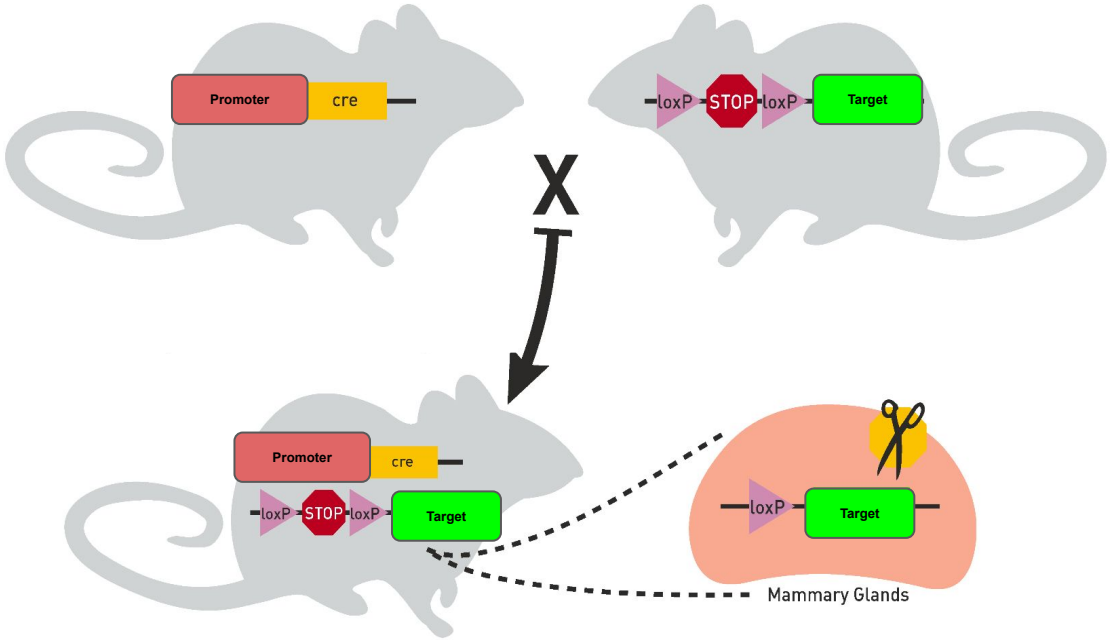
Tissue specific Knock out (validate tumor-suppressor gene)



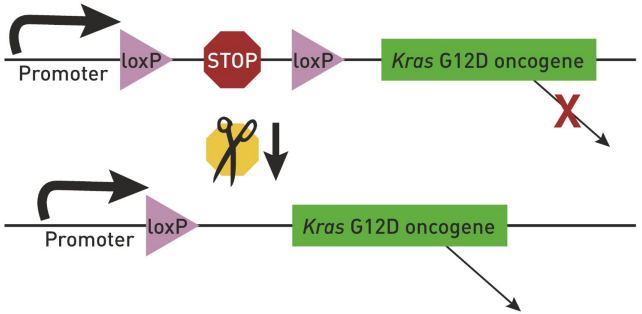
adopted from JAX.org, Cre Lox Breeding for Beginners

$Pten^{loxP/loxP}$ $Trp53^{loxP/loxP}$ ✖ *PB-Cre4*

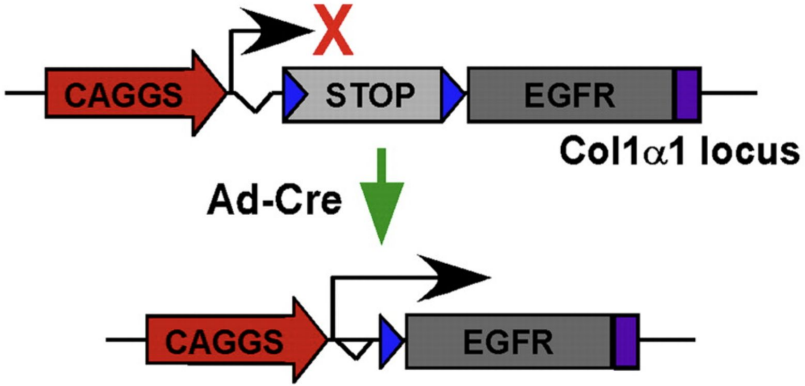
In cells with active **PB** promoter(prostate epithelium),
Cre recombines the two **LoxP** loci flanking **Pten** and **Trp53**.



Tissue specific activation (validate oncogene)



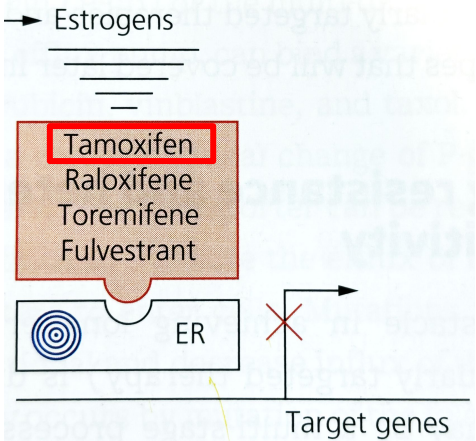
adopted from JAX.org, Cre Lox Breeding for Beginners



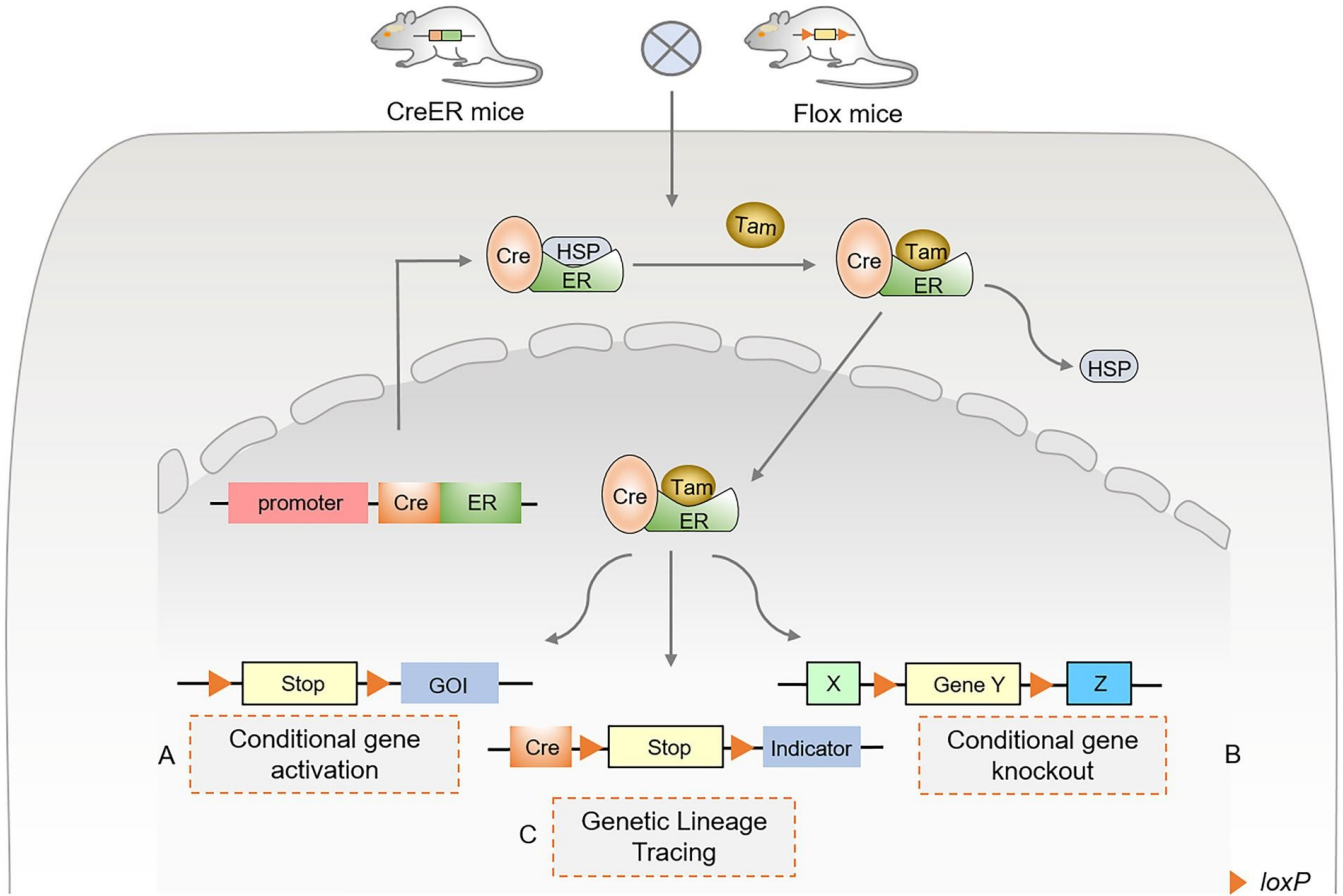
Oncogenic EGFR signaling cooperates with loss of tumor suppressor gene functions in gliomagenesis

Haihao Zhu[a], Jaime Acquaviva[a], Pranatartiharan Ramachandran[b], Abraham Boskovitz[b], Steve Woolfenden[a,e], Rolf Pfannl[b], Roderick T. Bronson[d], John W. Chen[c], Ralph Weissleder[c], David E. Housman[e,f,1], and Al Charest[a,b,e,f,1]

Inducible model Cre-ERT mice



Lauren Pecorino, Molecular Biology of Cancer, 5th edition, Figure 2.22



Wang et al. Cre-loxP-mediated genetic lineage tracing: Unraveling cell fate and origin in the developing heart Front. Cardiovasc. 2023

The use of AI in target validation.