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The ER Fusion System in Mouse Models: A Reversible Switch

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Abstract

Reversible regulatory mouse models have significantly contributed to our understanding of normal tissue and cancer biology, providing the opportunity to temporally control initiation, progression, and evolution of physiological and pathological events. The tamoxifen inducible system, one of the best-characterized “reversible switch” models, has a number of beneficial features. In this system, the hormone-binding domain of the mammalian estrogen receptor is used as a heterologous regulatory domain. Upon ligand binding, the receptor is released from its inhibitory complex and the fusion protein becomes functional. In this article we summarize the advantages and drawbacks of the system, describe several mouse models that rely on it, and discuss potential improvements that could render it even more useful and versatile.

Background

Genetically engineered mouse models have proven extremely valuable in validating gene functions, identifying novel oncogenes and tumor suppressors, as well as tumor biomarkers. Their use has provided insight into the molecular and cellular mechanisms underlying tumorigenesis, and supported the development of preclinical models in which to test novel therapeutic strategies. In particular, the use of models that allows a gene or gene function to be turned off as well as on, thereby providing a reversible regulatory switch, has given the opportunity to temporally control tumor initiation and follow tumor evolution over time, in a reproducible and reliable manner. One of the best-characterized animal models is the tamoxifen/ER regulatable system that provides this level of reversible control.

In previous in vitro studies, several intracellular proteins were rendered functionally hormone dependent by fusion with the hormone-binding domain (HBD) of steroid receptors (reviewed in Mattioni et al. [1994] and Picard [1993]). This strategy has been used successfully to generate conditional forms of several viral and cellular transcription factors as well as protein kinases. Thus, the HBD fusion appeared a versatile approach, generally applicable to different functional types of protein. The most common interpretation of its mechanism of action is that HBD-fusion proteins are inactive in the absence of ligand because they are complexed with a variety of intracellular polypeptides, of which Hsp90 is

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the prototype (Pratt 1990; Smith and Toft 1993) (Fig. 1). Upon ligand binding, the receptors are released from the inhibitory complexes and become functional. Indeed, five vertebrate steroid receptors—the glucocorticoid, mineralocorticoid, androgen, progesterone, and estrogen receptors—are known to be associated with, and inactive in, such complexes. Of these five receptors, the HBD of the human estrogen receptor (ER) has been most widely used as a heterologous regulatory domain. Its natural ligand is 17 β -estradiol (E2), which is relatively cheap and easy to obtain. One advantage of such a system is that many cell types lack an endogenous estrogen receptor. However, the HBD of ER has significant practical drawbacks as a switch. First, it possesses an inherent ligand-dependent transactivation activity (TAF-2 or AF-2), which may, therefore, contribute to the total transcriptional activity of the fusion protein. As a consequence, interpretation of results is practically impossible when the heterologous partner has weak transcriptional activity or is a transcriptional repressor. Second, the high levels of estrogens present in plasma preclude the use of ER-based switches in in vivo transgenic systems. In order to overcome these drawbacks, a G525R mutant murine estrogen receptor (ER^{TAM}) was generated and adopted for in vivo studies (Danielian et al. 1993). This mutant has 1000-fold lower affinity for estrogen than the wild-type HBD, possesses no TAF-2 transactivation activity, yet remains responsive to activation by the synthetic steroid 4-hydroxytamoxifen (4-OHT) (Danielian et al. 1993). 4-OHT is quite expensive, but animals can be treated with the cheaper precursor tamoxifen, which is converted into 4-OHT by the liver enzyme CYP2D6. Methods for delivering tamoxifen or 4-hydroxytamoxifen into mice are described in the accompanying protocol (**Tamoxifen Administration to Mice** [Whitfield et al. 2015]).

HBD fusion proteins were further improved to create two human ER HBD variants, each having a set of triple mutations: one contains the G400V/M543A/L544A and the other contains the G400V/L539A/L540A triple mutation (Feil et al. 1997). The first mutant, known as ER^{T2}, is induced by 4-OHT four times more efficiently, compared with the response observed for ER^{TAM}. The second mutant responds to the synthetic anti-estrogen ICI 182,780 (ICI), but, like ER^{TAM} or ER^{T2}, this mutant is insensitive to 17 β -estradiol (E2).

We describe here some of the mouse models that were created during the past two decades, and show how the ER fusion system has contributed to our knowledge of physiological and pathological events. We will also discuss the possibility of further improving the system.

The ER System in Gene Targeting Technology

The first application of the ER inducible system in vivo was revolutionary for the mouse gene targeting community. In the mid-1990s, it was still not possible to introduce somatic mutations in a tissue-specific manner at a chosen time during the lifespan of the mouse. In a striking breakthrough, Feil et al. (1997) made use of a conditional site-specific recombination system, based on a new version of the Cre/lox system. In their approach, the fusion of Cre recombinase to a mutant form of the ligand-binding domain of the human estrogen receptor (ER^{TAM}) resulted in a tamoxifen-dependent Cre recombinase (Cre-ER^{TAM}) that could be specifically activated by tamoxifen, but not by estradiol. Transgenic mice were then generated expressing Cre-ER^{TAM} under the control of a cytomegalovirus promoter. For the first time, excision of a chromosomally integrated gene flanked by *loxP*

sites was induced by administration of tamoxifen to these transgenic mice, in a temporally controlled manner (Fig. 2).

The Cre-ER^{TAM} system was then used by several other groups. Danielian et al. (1998) used the system to modulate gene activity in developing mouse embryos. This raised an important issue: tamoxifen causes strong abortogenic side effects and cannot be regularly administered during pregnancy. However, by using the enhancer of the Wnt1 gene to restrict the expression of Cre-ER^{TAM} to the embryonic neural tube, Danielian et al. showed that a single injection of tamoxifen into pregnant mice induced Cre-mediated recombination within the embryonic central nervous system, thereby activating expression of a reporter gene. Moreover, whereas a single 2.0 mg injection of tamoxifen at 8.5 d post-coitum led to rapid loss of the embryos, a single 1.0 mg injection was not lethal and, critically, was functional in activating the Cre-ER^{TAM} protein. These data showed that ligand-inducible recombination could be used to modify gene activity in the mouse embryo in utero (Danielian et al. 1998).

A second generation of Cre-ER^{TAM} transgenic mice was created by the same group a few years later. In this mouse line, Cre-ER^{TAM} was ubiquitously expressed downstream of the *actin* promoter to permit temporally regulated Cre-mediated recombination in diverse tissues of the mouse at embryonic and adult stages (Hayashi and McMahon 2002). In this model, a single, intraperitoneal injection of tamoxifen into a pregnant mouse at 8.5 d post-coitum leads to detectable recombination in the developing embryo within 6 h of injection and efficient recombination of a reporter gene within 24 h. In addition, the authors showed for the first time that, by varying the dose of tamoxifen injected, the percentage of cells undergoing a recombination event in the embryo could be controlled (Hayashi and McMahon 2002).

The Cre-ER^{TAM} system was also used in a tissue-specific manner. Zhu et al. (2003), for instance, established transgenic mice expressing the *Cre-ER^{TAM}* gene specifically in the liver, using the *Albumin* promoter to drive the transgene. This innovative tool was the first of its kind for studying and establishing the role of a gene function in the pathologies of the liver.

Dor et al. (2004) instead developed a method for genetic lineage tracing based on a switchable model to determine the stem cell contribution to the development of pancreatic islets of Langerhans. The *Rat Insulin Promoter (RIP)* was used to drive expression of tamoxifen-inducible Cre recombinase (*RIP-Cre-ER^{TAM}*), and a ‘pulse’ of tamoxifen was used to label β -cells by triggering expression from an alkaline phosphatase transgene preceded by a *lox-stop-lox* cassette. With this method, fully differentiated β -cells were heritably labeled in transgenic mice. After a long period, during which cellular turnover (“chase”) occurred, β -cells were examined for the presence of the label. Cells generated after the pulse were labeled only if they were the progeny of pre-existing (labeled) β -cells, while β -cells derived from any other source, such as stem cells, were not labeled. This label analysis showed that pre-existing β -cells, rather than stem cells, are the major source of new β -cells during adult life and after pancreatectomy in mice (Dor et al. 2004).

In other experiments by two separate groups, Cre-ER^{TAM} was placed under the control of the Col2a1 promoter to achieve selective and temporally regulated recombination in tissues expressing Type II collagen transcripts (Chen et al. 2007; Nakamura et al. 2006).

The newer generation of ER variants included the “triple mutant” ER^{T2} (Danielian et al. 1993; Feil et al. 1997) that was initially tested in animal models as a fusion with Cre recombinase. The first comparison between Cre-ER^{TAM} and Cre-ER^{T2} was made by Indra et al. (1999). They engineered transgenic mice expressing an LoxP-flanked transgene reporter and placed either Cre-ER^{TAM} or Cre-ER^{T2} under the control of the bovine *keratin 5* promoter that is specifically active in the epidermis basal cell layer. A dose–response study showed that Cre-ER^{T2} was ~10-fold more sensitive to 4-OHT activation compared with Cre-ER^{TAM} (Indra et al. 1999).

More recently, Monvoisin et al. (2006) established a mouse line expressing Cre-ER^{T2} under the regulation of the vascular endothelial cadherin promoter (VECad). Specificity and efficiency of Cre activity was documented by crossing VECad-Cre-ER^{T2} with the ROSA26R reporter mouse, in which a floxed-stop cassette has been placed upstream of the β -galactosidase reporter gene. The VECad-Cre-ER^{T2} mouse can be used as a valuable tool to study the function of genes involved in vascular development, homeostasis, and neoangiogenesis (Monvoisin et al. 2006).

The ER System and Transgenic Technology

The importance of the reversible aspect of the ER fusion inducible system—its ability to turn off as well as turn on the activity of a protein of interest—is underscored by its use in studies of tumorigenesis. In this section we describe experiments involving two aspects of tumor development: the first in the study of oncogenes and the second in the analysis of tumor suppressors.

ER-Fused Oncogenes

Classical transgenic technology was dramatically affected by the development of the ER inducible system. Typical models had previously suffered from the profound limitation that the transgene of interest was placed under the control of a tissue-specific regulatory element, which was generally expressed throughout the ontogeny of the organism and tissue. This expression pattern led to a phenotype which was potentially the net result of a protracted and complex web of compensatory processes. Moreover, standard transgenic technology allowed a gene of interest to be turned on but not off; yet this reversible facility, to turn off as well as on, is essential in determining whether sustained activation of the gene is also required to maintain the observed phenotype. This paradigm was changed by the work of Pelengaris et al. who targeted expression of a switchable form of the proto-oncogene c-Myc protein, c-MycER^{TAM}, to the skin epidermis, a well-characterized homeostatic tissue, by placing it under the control of the *Involucrin* promoter (Pelengaris et al. 1999). They showed that activation of c-MycER^{TAM} in adult suprabasal epidermis rapidly triggers proliferation and disrupts differentiation of postmitotic keratinocytes, leading to papillomatosis and premalignant changes that resemble hyperplastic actinic keratosis, a commonly observed human precancerous epithelial lesion. Taking advantage of the reversibility of the model for

the first time, the authors also showed that these premalignant changes spontaneously regressed upon deactivation of c-MycERTAM (Pelengaris et al. 1999).

Soon after, Blyth et al. used c-MycER^{TAM} in a mouse model of T-cell tumorigenesis, making use of the tissue-specific CD2 promoter (Blyth et al. 2000). They showed that induction of Myc activity in thymic tissue can result in both proliferation and apoptosis, and that abrogating Myc-induced apoptosis is not an essential requirement for tumor formation in vivo.

This was not the case in another tissue-specific c-MycER^{TAM} mouse model, described by Pelengaris et al. (2002). Here, activation of c-Myc driven by the *Insulin* promoter in adult mature β -cells induces uniform β -cell proliferation but is accompanied by overwhelming apoptosis that rapidly reduces β -cell number. Thus, the oncogenic potential of c-Myc in β -cells is masked by apoptosis. However, upon suppression of c-Myc-induced β -cell apoptosis by coexpression of Bcl-xL, c-Myc triggers rapid and uniform progression into angiogenic, invasive tumors. Again, subsequent c-Myc deactivation induced rapid regression of tumors (Pelengaris et al. 2002).

Myc was also recently used as a fusion with the ER^{T2} system to understand how activation of intrinsic tumor suppression must be triggered only when Myc signaling is oncogenic, since Myc is required for normal cell proliferation. Murphy et al. (2008) have developed an Myc transgenic mouse in which expression of the reversible-switch variant MycER^{T2} was driven by the constitutive and ubiquitously-active *Rosa26* promoter. Overt MycER^{T2} expression was then triggered in any target tissue by the hit-and-run action of Cre recombinase. Due to the relative weakness of the *Rosa26* promoter, the level of MycER^{T2} expressed in tissues of such animals is low and close to the physiological level of Myc following normal mitogenic stimulation. This model defined for the first time the oncogenic properties of Myc in vivo when its production is deregulated but not significantly overexpressed (Murphy et al. 2008). These studies showed that distinct threshold levels of Myc govern its output in vivo: low levels of deregulated Myc are competent to drive ectopic proliferation of somatic cells and oncogenesis, but activation of the apoptotic and ARF/p53 intrinsic tumor surveillance pathways requires elevated Myc expression (Murphy et al. 2008).

In addition to extensive use in MycER fusion models, the ER system has also been applied to other oncogenes. For instance, E2AER was created by inserting a tamoxifen-responsive region of the estrogen receptor (ER) at the carboxyl end of the *tcf2a* gene (Jones et al. 2009). E2A is the best-characterized E-protein family member in mammals, and has been shown to have stage-specific roles in cell differentiation, lineage commitment, proliferation, and survival. Jones et al. characterized and analyzed the efficiency and kinetics of this inducible system in the context of B-cell development. In the absence of ligand, E2AER mice showed a block in B-cell development, and E2AER DNA-binding activity was not detected. Rapid induction of E2AER DNA-binding activity was observed upon tamoxifen treatment but, disappointingly, was not sufficient to rescue B-cell development in live animals. Only direct exposure of bone marrow cells to tamoxifen in an ex vivo culture could rescue and support early B-cell development from the pre-proB cell stage (Jones et al. 2009).

ER-Fused Tumor Suppressors

The ER-inducible system *in vivo* has also proved extremely valuable in the study of tumor suppressor genes. One of these genes, *Trp53*, encodes the tumor suppressor protein known as p53 which, when activated by various intracellular signaling networks, mediates the suppression of tumor development. Christophorou et al. were the first to use the ER system to investigate whether the absence of p53 function was crucial only at specific, transient stages of tumor evolution—such as when cells sustain acute genotoxic injury or a genome-destabilizing crisis—or whether tumor cells carry persistent p53-activating signals that necessitate sustained loss of p53 function throughout a tumor's natural history (Christophorou et al. 2005). A constitutive knockout could not be used to address this issue, because subsequent reversal of p53 status is a prerequisite for understanding the temporal relationship between genotoxic injury, tumor evolution and the role of p53 in mediating the DNA damage response and suppressing cancer. In the model of Christophorou et al. the endogenous *Trp53* gene was modified to express an ectopically regulatable form of the p53 protein, p53ER^{TAM}. Of note, in contrast to the MycER^{TAM} model, administration of tamoxifen to this mouse does not activate the target ER-fusion protein, but merely renders it to a state that can be activated by pre-existing cellular signals. Therefore, administration of tamoxifen makes these mice functionally wild-type for p53, that is, still susceptible to its natural activating signals such as oncogenic and/or cytotoxic stress. These mice can be rapidly, reversibly, and repeatedly toggled between the wild-type and p53-deficient states (Christophorou et al. 2005) (Fig. 3).

This model was further used to show that the p53-mediated pathological response to whole-body irradiation, a prototypical genotoxic carcinogen, is irrelevant for suppression of radiation-induced lymphoma (Christophorou et al. 2006). In contrast, delaying the restoration of p53 function until the acute radiation response has subsided abrogates all of the radiation-induced pathology, yet preserves much of the protection from lymphoma (Christophorou et al. 2006).

The p53ER^{TAM} model was also used to explore the possibility of restoring p53 activity as a tumor therapy. Martins et al. used the well-characterized *Eμ-myc* lymphoma model to show that p53 is spontaneously activated when restored in established *Eμ-myc* lymphomas *in vivo*, triggering rapid apoptosis and conferring a significant increase in survival (Martins et al. 2006). Nonetheless, restoration of p53 function potently selected for the emergence of p53-resistant tumors through inactivation of p19ARF or p53 (Martins et al. 2006).

In addition to activation networks, p53 activity is also subject to negative regulation by various interacting proteins; one of these is the oncogene protein MDM2 (in humans HDM2). In a therapeutic approach similar to that used by Christophorou, the p53ER^{TAM} model was used to explore the possibility of restoring p53 activity in tumor cells by inhibiting MDM2/HDM2 and to determine whether this inhibition would also activate p53 in normal cells (Ringshausen et al. 2006). Ringshausen et al. showed that p53 was spontaneously active in all tested tissues of MDM2-deficient mice, triggering fatal pathologies that included ablation of classically radiosensitive tissues. In apoptosis-resistant tissues, spontaneous unbuffered p53 activity triggered profound inhibition of cell proliferation (Ringshausen et al. 2006).

The same therapeutic opportunity of restoring p53 function in the absence of MDMX (another negative regulator of p53) was also recently explored by Garcia et al. (2011). They showed that MDMX is continuously required to buffer p53 activity in adult normal tissues and their stem cells. Importantly, although, the effects of transient p53 restoration in the absence of MDMX are nonlethal and reversible, unlike transient p53 restoration in the absence of MDM2. Moreover, the therapeutic impact of restoring p53 is enhanced in the absence of MDMX, affording a significant extension of life span over p53 restoration in the presence of MDMX. Hence, systemic inhibition of MDMX is both a feasible therapeutic strategy for restoring p53 function in tumors that retain wild-type p53, and likely to be significantly safer than inhibition of MDM2 (Garcia et al. 2011).

Finally, Junttila et al. (2010) used the p53ER^{TAM} mouse to model the likely therapeutic impact of p53 restoration in a spontaneously evolving mouse model of non-small-cell lung cancer (NSCLC) that is initiated by sporadic oncogenic activation of endogenous KRas. The rationale for such a study was that p53 is frequently inactivated in NSCLC and an oncogenic form of Ras, which occurs very commonly in NSCLC, can be a potent trigger of p53. Hence, pharmacological restoration of p53 seemed an appealing therapeutic strategy for treating this disease. Surprisingly, they showed that p53 restoration failed to induce significant regression of established tumors although it did result in a significant decrease in the relative proportion of tumors classed as high grade (Junttila et al. 2010). This was due to selective activation of p53 only in the more aggressive tumors cells within each tumor. Such selective activation of p53 correlated with marked up-regulation in Ras signal intensity and induction of the oncogenic signaling sensor p19^{ARF}. These data revealed inherent limits in the capacity of p53 to restrain early tumor evolution and to the efficacy of therapeutic p53 restoration to eradicate cancers (Junttila et al. 2010).

Future Prospects

The ER^{Tam} and ER^{T2} tamoxifen-dependent domains have been extensively and successfully used to reversibly toggle target protein function in animal models; however, there remain a number of drawbacks. First, tamoxifen is a partial nonsteroidal estrogen agonist acting as a type II competitive inhibitor of estradiol at its receptor (Danielian et al. 1993), giving rise to the possibility that tamoxifen itself contributes to the observed phenotype in vivo. Several pure estrogen antagonists are available, such as Fulvestrant (Johnston and Cheung 2010), but these have not been evaluated in ER^{Tam} fusion systems in mice. In addition to the estrogen receptor, other steroid hormone receptors (such as the glucocorticoid and progesterone receptors) have been used. As with tamoxifen, the systemic effects of their ligands may obscure specific effects on the heterologous fusion protein. Second, prolonged administration of tamoxifen to experimental animals is not without problems. Because of its poor solubility in aqueous media, tamoxifen is generally prepared as a suspension in sterile peanut oil before administration by intraperitoneal injection or oral gavage (see **Tamoxifen Administration to Mice** [Whitfield et al. 2015]). Repeated intraperitoneal injection results in the buildup of oil in the peritoneal cavity and sterile peritonitis limiting this route of administration. Although mice can be maintained on a tamoxifen diet, the kinetics of fusion protein activation are likely to be slower than with intraperitoneal injection and the mice suffer a temporary loss of weight immediately following transfer to the new diet.

One avenue to improving the utility of ligand-dependent switching of protein activity *in vivo* is to use non-mammalian receptors with ligands that are readily available and that are expected to have little impact on mammalian cells. The insect ecdysone receptor, for example, is a potential candidate, and the insect steroid hormone ponasterone A has been used in mammalian inducible expression system. However, the ecdysone receptor is a noncovalent heterodimer of two proteins—the ecdysone receptor (EcR), the insect orthologue of the mammalian farnesoid X receptor (FXR) and the ultra-spiracle protein, the insect orthologue of the mammalian retinoid X receptor (RXR). Clearly, this arrangement does not easily allow fusion to a heterologous protein. Given the structural similarity of 17 β -estradiol and ponasterone A, an alternative approach is to modify the ligand-binding domain of the mammalian estrogen receptor such that it binds ponasterone A with high affinity but is unable to bind 17 β -estradiol. Indeed, directed evolution of estrogen receptors to novel ligands has already been achieved (Chockalingam et al. 2005).

Rapid and reversible switching between a protein's functional and nonfunctional state has yielded a remarkable dividend in improved mouse models. The potential for non-mammalian or synthetic ligands and their engineered receptors holds great promise for refining this technology. Moreover, these engineered receptors could be used to independently regulate the activity of co-expressed proteins in a temporal fashion in response to distinct ligands.

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References

- Blyth K, Stewart M, Bell M, James C, Evan G, Neil JC, Cameron ER. Sensitivity to myc-induced apoptosis is retained in spontaneous and transplanted lymphomas of CD2-mycER mice. *Oncogene*. 2000; 19:773–782. [PubMed: 10698495]
- Chen M, Lichtler AC, Sheu TJ, Xie C, Zhang X, O'Keefe RJ, Chen D. Generation of a transgenic mouse model with chondrocyte-specific and tamoxifen-inducible expression of Cre recombinase. *Genesis*. 2007; 45:44–50. [PubMed: 17211877]
- Chockalingam K, Chen Z, Katzenellenbogen JA, Zhao H. Directed evolution of specific receptor-ligand pairs for use in the creation of gene switches. *Proc Natl Acad Sci*. 2005; 102:5691–5696. [PubMed: 15811944]
- Christophorou MA, Martin-Zanca D, Soucek L, Lawlor ER, Brown-Swigart L, Verschuren EW, Evan GI. Temporal dissection of p53 function *in vitro* and *in vivo*. *Nat Genet*. 2005; 37:718–726. [PubMed: 15924142]
- Christophorou MA, Ringshausen I, Finch AJ, Swigart LB, Evan GI. The pathological response to DNA damage does not contribute to p53-mediated tumour suppression. *Nature*. 2006; 443:214–217. [PubMed: 16957739]
- Danielian PS, Muccino D, Rowitch DH, Michael SK, McMahon AP. Modification of gene activity in mouse embryos *in utero* by a tamoxifen-inducible form of Cre recombinase. *Curr Biol*. 1998; 8:1323–1326. [PubMed: 9843687]
- Danielian PS, White R, Hoare SA, Fawell SE, Parker MG. Identification of residues in the estrogen receptor that confer differential sensitivity to estrogen and hydroxytamoxifen. *Mol Endocrinol*. 1993; 7:232–240. [PubMed: 8469236]

- Desta Z, Ward BA, Soukhova NV, Flockhart DA. Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6. *J Pharmacol Exp Ther*. 2004; 310:1062–1075. [PubMed: 15159443]
- Dor Y, Brown J, Martinez OI, Melton DA. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature*. 2004; 429:41–46. [PubMed: 15129273]
- Feil R, Wagner J, Metzger D, Chambon P. Regulation of Cre recombinase activity by mutated estrogen receptor ligand-binding domains. *Biochem Biophys Res Commun*. 1997; 237:752–757. [PubMed: 9299439]
- Garcia D, Warr MR, Martins CP, Brown Swigart L, Passegue E, Evan GI. Validation of MdmX as a therapeutic target for reactivating p53 in tumors. *Genes Dev*. 2011; 25:1746–1757. [PubMed: 21852537]
- Hayashi S, McMahon AP. Efficient recombination in diverse tissues by a tamoxifen-inducible form of Cre: A tool for temporally regulated gene activation/inactivation in the mouse. *Dev Biol*. 2002; 244:305–318. [PubMed: 11944939]
- Indra AK, Warot X, Brocard J, Bornert JM, Xiao JH, Chambon P, Metzger D. Temporally-controlled site-specific mutagenesis in the basal layer of the epidermis: Comparison of the recombinase activity of the tamoxifen-inducible Cre-ER(T) and Cre-ER(T2) recombinases. *Nucleic Acids Res*. 1999; 27:4324–4327. [PubMed: 10536138]
- Johnston SJ, Cheung KL. Fulvestrant—A novel endocrine therapy for breast cancer. *Curr Med Chem*. 2010; 17:902–914. [PubMed: 20156170]
- Jones ME, Kondo M, Zhuang Y. A tamoxifen inducible knock-in allele for investigation of E2A function. *BMC Dev Biol*. 2009; 9:51. [PubMed: 19822014]
- Junttila MR, Karnezis AN, Garcia D, Madriles F, Kortlever RM, Rostker F, Brown Swigart L, Pham DM, Seo Y, Evan GI, et al. Selective activation of p53-mediated tumour suppression in high-grade tumours. *Nature*. 2010; 468:567–571. [PubMed: 21107427]
- Martins CP, Brown-Swigart L, Evan GI. Modeling the therapeutic efficacy of p53 restoration in tumors. *Cell*. 2006; 127:1323–1334. [PubMed: 17182091]
- Mattioni T, Louvion JF, Picard D. Regulation of protein activities by fusion to steroid binding domains. *Methods Cell Biol*. 1994; 43:335–352. [PubMed: 7823870]
- Monvoisin A, Alva JA, Hofmann JJ, Zovein AC, Lane TF, Iruela-Arispe ML. VE-cadherin-CreERT2 transgenic mouse: A model for inducible recombination in the endothelium. *Dev Dyn*. 2006; 235:3413–3422. [PubMed: 17072878]
- Murphy DJ, Junttila MR, Pouyet L, Karnezis A, Shchors K, Bui DA, Brown-Swigart L, Johnson L, Evan GI. Distinct thresholds govern Myc's biological output in vivo. *Cancer Cell*. 2008; 14:447–457. [PubMed: 19061836]
- Nakamura E, Nguyen MT, Mackem S. Kinetics of tamoxifen-regulated Cre activity in mice using a cartilage-specific CreER(T) to assay temporal activity windows along the proximodistal limb skeleton. *Dev Dyn*. 2006; 235:2603–2612. [PubMed: 16894608]
- Pelengaris S, Khan M, Evan GI. Suppression of Myc-induced apoptosis in beta cells exposes multiple oncogenic properties of Myc and triggers carcinogenic progression. *Cell*. 2002; 109:321–334. [PubMed: 12015982]
- Pelengaris S, Littlewood T, Khan M, Elia G, Evan G. Reversible activation of c-Myc in skin: Induction of a complex neoplastic phenotype by a single oncogenic lesion. *Mol Cell*. 1999; 3:565–577. [PubMed: 10360173]
- Picard D. Steroid-binding domains for regulating the functions of heterologous proteins in cis. *Trends Cell Biol*. 1993; 3:278–280. [PubMed: 14731747]
- Pratt WB. Interaction of hsp90 with steroid receptors: Organizing some diverse observations and presenting the newest concepts. *Mol Cell Endocrinol*. 1990; 74:C69–C76. [PubMed: 2178103]
- Ringshausen I, O'Shea CC, Finch AJ, Swigart LB, Evan GI. Mdm2 is critically and continuously required to suppress lethal p53 activity *in vivo*. *Cancer Cell*. 2006; 10:501–514. [PubMed: 17157790]
- Smith DF, Toft DO. Steroid receptors and their associated proteins. *Mol Endocrinol*. 1993; 7:4–11. [PubMed: 8446107]

- Whitfield J, Littlewood T, Evan GI, Soucek L. Tamoxifen administration to mice. Cold Spring Harb Protoc. 2015; doi: 10.1101/pdb.prot077966
- Zhu HZ, Chen JQ, Cheng GX, Xue JL. Generation and characterization of transgenic mice expressing tamoxifen-inducible cre-fusion protein specifically in mouse liver. World J Gastroenterol. 2003; 9:1844–1847. [PubMed: 12918135]

Figure 1.

The HBD fusion system. The sequence shows the mechanism for switching the activity of the HBD-fusion protein. In the absence of the ligand, the chimeric protein, consisting of the HBD fused to a protein of interest (POI), is in complex with a variety of intracellular polypeptides, such as Hsp90, which inhibit its activity. Upon ligand binding, the receptor is released from the inhibitory complexes and the protein of interest becomes functional (POI*).

Figure 2.

Gene targeting by CreER. The CreER system allows excision of a chromosomally integrated gene of interest (GOI) flanked by *loxP* sites. Activation of the Cre recombinase (Cre*) occurs following administration of tamoxifen (4-OHT).

Figure 3.

The p53ER^{TAM} knock-in switchable system. In this model, endogenous *Tip53* gene was modified to express the ectopically regulatable form of the p53 protein, p53ER^{TAM}. Administration of tamoxifen to this mouse renders the ER-fusion protein activatable (p53*) by various stress signals, such as those produced in response to oncogenic and/or cytotoxic stress. Therefore, administration of tamoxifen renders these mice functionally wild-type for p53, which remains susceptible to its natural activating signals. Of note, the control of p53 function is reversible.