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Genetically engineered mouse models for skin research: taking the next step

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Abstract

Genetically engineered mouse models are invaluable to investigators in nearly all areas of biomedical research. The use of genetically engineered mice has allowed researchers to explore fundamental functions of genes in a mammal that shares substantial similarities with human physiology and pathology. Genetically engineered mice are often used as animal models of human diseases that are vital tools in investigating disease development and in developing and testing novel therapies. Gene targeting in embryonic stem cells allows endogenous genes to be specifically altered. As knowledge regarding precise genetic abnormalities underlying a variety of dermatological conditions continues to emerge, the ability to introduce corresponding alterations in endogenous gene loci in mice, often at a single base pair level, has become essential for detailed studies of these genetic diseases. In this review, we provide examples of mouse models harboring modified endogenous gene(s), generated using the technique commonly referred to as the “knock-in” approach, to exemplify the important and sometimes superior role of this methodology in dermatological research.

Keywords

knock-in; knockout; gene targeting; ES cell; targeted mutation; homologous recombination; mouse model; skin

Introduction

Mouse (*Mus musculus*) is a widely used mammalian model organism for functional genomic and reverse genetic studies to address questions relating to human health and welfare in nearly every biomedical field. In many respects, murine skin shares striking similarities with human skin and mouse models have been extremely useful in examining skin-related gene functions and in modeling skin diseases.

Mouse models harboring genetic abnormalities can be classified as either having a spontaneous mutation or as being genetically engineered by man. Spontaneous mutations affecting the skin typically result in overt skin and coat phenotypes and are often readily identified. Unfortunately, many human skin diseases do not naturally occur in mice.

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Therefore, most skin phenotypes must be induced by manipulation of environmental conditions or via modification of the mouse genome by, for example, gene targeting.

Gene targeting

A variety of techniques are available for creating genetically engineered mouse models (GEMMs), including the use of transgenes, targeted endogenous gene alterations, random gene inactivation, and random mutagenesis (Table 1). Targeted endogenous gene alterations allow for very specific gene modification, *i.e.*, a pre-selected gene locus can be disrupted or altered at its natural location to generate targeted mutant mice. Targeted disruption or deletion of a gene ("knockout") via homologous recombination in ES cells is a very powerful method for studying events associated with the absence of a gene product. The knockout technology has had a tremendous impact on gene function studies, drug discovery, and many other areas of biomedical research. Not surprisingly, shortly after the completion of the human and mouse genome projects, public and private entities began strategically generating knockout mouse models across the entire genome [1, 2]. The ultimate acknowledgement of the importance of gene targeting occurred when the 2007 Nobel Prize in Medicine and Physiology was awarded to three investigators (Capecchi, Smithies, and Evans) "for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells."

Another form of targeted gene alteration is commonly referred to as the "knock-in" approach. In the knock-in approach, target gene expression is normally not completely disrupted by the introduced modification. Instead, the modification creates a subtle point mutation or introduces additional genetic material. The knock-in approach has also been used to partially or completely replace an endogenous gene locus with a foreign gene, to generate human-mouse chimeric genes, or to replace an entire mouse gene with its human analog (Fig. 1). This technique has evolved to such a robust degree that researchers are now able to use the knock-in approach to correct genetic defects in mouse ES cells and use these corrected cells to generate phenotypically normal mice [3].

Key events associated with the generation of targeted mutant mice, including knock-in mice, via homologous recombination in ES cells are outlined in Figure 2. Efficient ES cell targeting is highly dependent on the targeting vector. Compared to the generation of knockout targeting vectors, the construction of a knock-in targeting vector is substantially restricted by the location of the intended modification within the endogenous gene. In many cases, it is practically impossible to introduce a subtle mutation or insertion using conventional restriction and ligation methods. Advancement of technologies such as cre-recombinase mediated site-specific recombination, the use of bacterial artificial chromosome (BAC) vectors and bacterial homologous recombination has allowed for greater flexibility and success in creating vectors for targeted gene modification [4]. Figure 3 illustrates the use of BAC vectors and bacterial homologous recombination in creating ES cell targeting vectors. A recently developed high-throughput VelociGene system which takes the advantage of high targeting efficiency of large BAC vectors that can carry virtually any genetic modification up to 70 kb and "loss-of-native-allele" assays, further streamlined ES cell gene targeting by circumventing the need of positive-negative selection and the use of isogenic DNA sequences [5].

GEMMs in skin research and the significance of the targeted gene modification approach

Transgenic mouse models have been remarkable tools for gene function studies and modeling of a variety of skin conditions [6]. The knockout approach is also pervasive in skin

research as illustrated by the fact that almost every gene known to be important in skin development, homeostasis, and disease has been examined using knockout mouse models. Despite the obvious strengths of transgenic and knockout mouse models, the forced over-expression of a transgene or complete ablation of an endogenous gene imposes certain limitations on gene function studies and human disease modeling. One overt limitation is the possibility of interference with the normal expression patterns of genes surrounding the often randomly inserted transgene or near the inactivated gene locus. Even though the evolution of inducible and conditional approaches have provided greater flexibility in utilizing these technologies in skin research, transgenic and knockout mice often cannot adequately recapitulate the genetic alterations observed in certain skin conditions.

To circumvent such limitations, the knock-in approach can be utilized to specifically modify an endogenous gene locus while maintaining its expression profile. A few examples of knock-in mouse models used in skin research are listed in Table 2. The combination of the knock-in strategy with inducible approaches also provides unique opportunities to mimic certain skin pathologies resulted from persistent or spontaneous mutant gene expression. The following examples demonstrate the critical and, sometimes, superior roles of knock-in mouse models as compared with transgenic and knockout mouse models in skin gene function studies, skin disease modeling, and skin cancer studies.

1. GEMMs for studying keratin gene function and keratin disorders

All epithelial cells contain keratin intermediate filaments (IFs) which are polymers of keratin protein heterodimers. Keratin IFs provide epithelial cells with resilient strength to withstand mechanical damage. Many keratin genes exist and expression of these genes exhibits a certain degree of redundancy. Expression of a particular keratin may be restricted to a specific tissue type and/or to specific differentiation and homeostatic stages. In humans, defects in keratin genes result in a wide spectrum of skin diseases with tremendous variation in clinical presentation [28].

Many GEMMs have been utilized for studying keratin genes. For example, several mouse models have been generated to study the genes encoding the keratin 6 (*Krt6*) [29–35]. In one study, the transgenic approach was used to express mutant *Krt6a* and the resulting mice displayed dramatic skin and hair phenotypes [29]. This study demonstrated the dominant-negative effects of *Krt6a* mutations and provided evidence for the involvement of *Krt6a* in skin blistering and hair loss. However, the constitutive expression of mutant *Krt6a* resulted in devastating damage to the skin which prevented further investigation of these *Krt6a* mutations.

Another GEMM, in which the *Krt6a* gene was ablated, exhibited re-epithelialization defects in wound healing [30]. Interestingly, the lack of *Krt6a* did not prevent the normal development or general function of epithelial tissues and ectodermal appendages. This result suggested that a closely related *Krt6b* gene might compensate in the absence of *Krt6a*. When both *Krt6a* and *Krt6b* were deleted from the *Krt6* locus, double null mice developed hyperplastic lesions in the oral cavity which were associated with mechanical stress from food intake [31, 33]. Surprisingly, aside from fatal starvation resulting from upper digestive tract constriction, the absence of *Krt6a* and *Krt6b* did not produce a defect in the skin or its appendages. While these knockout models were useful in furthering our understanding of keratin genes (*e.g.,* data from these GEMMs suggested redundancy of keratin genes in epidermal keratinocytes and indicated that some of these genes may be dispensable), no correlation between *Krt6* gene function and any human skin disease was identified.

Pachyonychia congenita (PC) is the only known disease associated with *KRT6A* and *KRT6B*. PC-1 and PC-2 are caused by dominant mis-sense mutations in *KRT6A* or *KRT16*

(PC-1) and *KRT6B* or *KRT17* (PC-2), respectively. It is clinically characterized by distally progressive hypertrophic onychodystrophy and focal hyperkeratosis of the palms and soles [36]. A number of transgenic and knockout mouse models have been made with PC-causing genes but none fully reproduced the pachyonychia congenita phenotypes [37]. We recently generated a knock-in mouse model carrying a dominant mutation in mouse *Krt75* (previously known as *K6hf*), which corresponds to a frequently observed point mutation in *KRT6A* in PC patients [16]. Mice carrying this dominant mutation developed congenital hair phenotypes and partially mimicked the hypertrophic nail dystrophy of PC [16]. While this mouse model does not recapitulate every feature of pachyonychia congenita, it clearly demonstrated the role of *Krt75* in hair and nail formation, which might not have been revealed using transgenic or knockout approaches.

2. Mouse models of epidermolytic hyperkeratosis

Epidermolytic hyperkeratosis (EHK), also termed bullous congenital ichthyosiform erythroderma (BCIE), is an autosomal dominant form of ichthyosis caused by mutations in the *KRT1* and *KRT10* genes which are expressed in suprabasal keratinocytes of the epidermis [38, 39]. Clinical features of EHK include blistering, erythroderma, and peeling of skin at birth, followed by the development of acanthosis and hyperkeratosis, predominantly over areas prone to pressure and mechanical stress [40]. Infants with EHK can develop large areas of skin erosion resulting in an increased risk of neonatal mortality. Histologically, EHK blisters exhibit keratinocyte cytolysis within the suprabasal layers of the epidermis with damaged cells exhibiting peri-nuclear clumps of keratin IFs.

A number of GEMMs were developed for EHK [17, 41–44]. The first mouse model expressed a mutant *Krt10* transgene [42] and was reported shortly before the genetic basis of EHK was published [38, 45, 46]. This model demonstrated that *Krt10* mutation could result in EHK phenotypes and that the presence of mutant *Krt10* molecules caused disruption of the keratin IF network. The utility of this transgenic model for studying EHK was limited in that the transgene was a truncated *Krt10*, which was in contrast to the commonly observed *Krt10* point mutations in EHK patients, and that the transgene was only expressed at 2–10% of the level of endogenous *Krt10*.

Krt10 knockout mice were also generated and surprisingly, *Krt10* null mice displayed a well-developed and functional epidermis that did not exhibit tissue fragility or epithelial cell IF aggregates [41]. This model provided insight into keratin gene redundancy and the ability of other keratin filaments to compensate in the absence of *Krt10*, but did not recapitulate EHK phenotypes.

In another GEMM, an HPRT mini gene was inserted between exon 2 and exon 8 of *Krt10* to interrupt transcription of the *Krt10* allele [44]. Transcriptional activity of the targeted *Krt10* allele was substantially reduced; however, a truncated K10 polypeptide (K10T) was produced. The K10T molecule exerted a dominant interference effect, resulting in the presence of keratin aggregates, suprabasal cytolysis of keratinocytes, and severe hyperkeratosis in heterozygous mice. Homozygosity resulted in fatal barrier defects [41]. To a certain extent, this mouse model resembled a knock-in mouse model because the targeted allele produced a mutant gene product. The full spectrum of EHK phenotypes could be exhibited in homo- and heterozygous mutant mice, such that fragile skin with small blisters and large areas of skin loss were observed in the homozygous and new born mice, whereas hyperkeratosis developed in heterozygous adults. The segregation of phenotypes in these mutant mice may be explained by the fact that the mutation in K10T mice is different from the mutations discovered in humans with EHK, and that the amount of mutant Krt10 protein in the epidermis was drastically less than that of endogenous normal K10 protein [41]

The most successful EHK mouse model developed thus far was created by modifying the endogenous *Krt10* locus to introduce a frequently observed single-nucleotide mis-sense mutation [17]. This mouse model additionally utilized a *cre*-mediated inducible system (Fig. 4) to allow the activation of a conditional mutant *Krt10* allele in a restricted area of the skin to circumvent neonatal fatalities commonly associated with congenital skin fragility syndromes. Topical application of an inducer, RU486, "activated" the mutant allele in the genome of epidermal keratinocytes, including epidermal stem cells resulting in continual production of progeny cells harboring the mutant allele, even after the inducer is no longer applied. These mice developed blisters that gradually resulted in hyperkeratotic lesions which persisted for life [17]. Moreover, this inducible knock-in model allowed for the mosaic form of EHK to be precisely reproduced, a result that had not been obtained using other GEMM techniques.

3. Oncogene activation and the development of nonmelanoma skin cancer

Tumor studies are best carried out *in vivo* since tumors are often highly heterogeneous and are dependent upon interactions with the stroma, the host immune system, and the tissue environment. During tumor development, cells typically undergo multiple genetic events, such as the activation of oncogenes or mutation of tumor suppressor genes. Mutations in *ras* genes are commonly found in human skin cancers [47, 48] and are frequently induced by the chemical tumor initiator, dimethylbenz[a]anthracene (DMBA), in mouse skin chemical carcinogenesis experiments [49–51]. The role of activated *ras* in skin tumor initiation has been well-established using a variety of GEMMs [52].

Mutations in the *p53* gene, the most studied and highly characterized of the known tumor suppressor genes, are found in about 50% of all cancers [53]. Interestingly, *p53*-null mice rarely develop skin tumors prior to succumbing to internal tumor burden [54, 55]. However, more than 50% of human squamous cell carcinomas (SCCs) exhibit *p53* mutations. For example, a mis-sense mutation resulting in the substitution of a histidine for arginine at amino acid 175 is frequently observed in human skin cancer [56, 57]. A transgenic mouse model in which an equivalent mutation in the murine *p53* gene ($p53^{R172H}$) was overexpressed in the epidermis provided early evidence of a gain-of-function property of *p53* mutation in skin cancer [58]. In contrast to wildtype and *p53*-null mice, the $p53^{R172H}$ transgenic mice exhibited increased susceptibility to skin chemical carcinogenesis, tumor progression, and metastasis. However, the use of this model was limited in that the transgene was not expressed at levels comparable to endogenous *p53*, thus leaving room to question whether this mutant *p53* molecule truly represented the effect of the same mutation in the endogenous *p53* gene.

To effectively mimic the consequence of endogenous gene mutations during tumorigenesis, a knock-in approach is needed. Knock-in mouse models expressing mutant endogenous *p53* genes that are analogous to those found in clinical tumor studies recapitulated distinctive features of Li-Fraumeni Syndrome [26, 27]. These models also provided evidence for gain-of-function properties of mutant *p53* in cancer development. However, this study did not examine the potential gain-of-function property of mutant *p53* in skin tumorigenesis since, much like the *p53* knock-out mice, these mice succumbed to internal tumors without having developed skin tumors. To overcome this limitation, an inducible, tissue-specific knock-in mouse model was created in which mutant endogenous *p53* ($p53^{R172H}$) allele and endogenous *K-ras* ($K\text{-}ras^{G12D}$) allele could be focally and conditionally activated in epidermal stem cells as illustrated in Figure 4 [20]. In the same study, conditional knock-in mutant *K-ras* mice were also mated with mice harboring a floxed *p53* allele. This study was the first to directly compare, in a genetically equivalent manner, the role of *p53* loss-of-function and gain-of-function in skin tumorigenesis with a common initiation event (activation of $K\text{-}ras^{G12D}$). This study confirmed the gain-of-function capabilities of

p53^{R172H}. Cooperation between this mutant *p53*, but not loss of *p53*, and activated *ras* in skin tumor initiation was observed and a role for this *p53* mutant in skin tumor progression and metastasis was confirmed [20]. This *K-ras*^{G12D}/*p53*^{R172H} model is the first skin cancer model capable of recapitulating multiple endogenous genetic events in a focal, inducible, and cell type-specific manner, and emphasizes the importance of the conditional, tissue-specific knock-in technique in skin cancer research.

Perspectives on future developments of mouse models for dermatological research using endogenous gene targeting

The utility of endogenous gene modifications in skin research reaches beyond merely studying skin gene functions and modeling of genodermatoses and cancer which have a clear genetic etiology. As an example, bullous pemphigoid (BP) is the most common antibody-mediated blistering autoimmune disease. It is caused by the cross-reaction of autoantibodies with the BP180 epitopes in the NC16A domain of *COL17*. However, the endogenous mouse *Col17* does not cross-react with the disease causing IgG of humans. Therefore, it is not possible to establish *in vivo* models of BP by passive transfer of patient IgG into mice. In order to build a mouse model of this disease, Nishie and colleagues used strategic breeding of *Col17* knockout mice with transgenic mice expressing human *COL17* [59]. Transgenic mice expressing human *COL17* rescued the lethal phenotype in *Col17*-deficient mice. Consequently, this strategy achieved complete humanization of the mouse BP antigen in the offspring of these mice, which permitted the cross-reaction of pathogenic human IgG with the human epitope and recapitulated the corresponding BP phenotypes [59]. This mouse model also became a successful *in vivo* model for testing potential recombinant peptide therapies for BP [59]. Utilizing a knock-in approach, a novel GEMM was recently established in which the endogenous *Col17* locus was partially humanized by replacing the mouse *BP180NC14A* genomic locus with the corresponding immunogenic human *BP180NC16A* locus [60]. This strategy allowed the endogenously expressed mouse-human hybrid COL17 to cross-react with the pathogenic human autoantibody after passive transfer. Furthermore, this mouse model can be used directly in the mapping of the pathogenic epitope of BP180 *in vivo* [60].

The ability to modify the endogenous mouse genome within non-coding regions raises the possibility of building more complex genetic disease models, such as models for skin carcinoma that result from chromosomal translocations and inversions. In conjunction with other mutant mouse models and various inbred strains and the incorporation of a broad range of new technologies, such as reporter genes and *in vivo* imaging systems, protein tags, tissue-, organ-, or temporal-specific approaches, and RNA interference, the knock-in approach enables, but is not limited to, the engineering of mouse models that are capable of: studying modifier genes in epistasis and eventually recapitulating sophisticated polygenic or non-Mendelian skin conditions; accurately track endogenous gene expression patterns in the skin; overcome infertility or premature death caused by certain essential genetic modifications; properly recapitulate spontaneous genetic events in tumorigenesis; and to mimic the exact genetic and phenotypic changes of certain skin disease conditions, and be used unbiasedly in drug target validation, preclinical testing of novel therapeutic approaches, and biomarker discovery.

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Abbreviations

BAC	bacterial artificial chromosome
EHK	epidermolytic hyperkeratosis
ES cell	embryonic stem cell
GEM	genetically engineered mouse
GEMM	genetically engineered mouse model
IF	intermediate filament
HSV-TK	herpes simplex virus thymidine kinase

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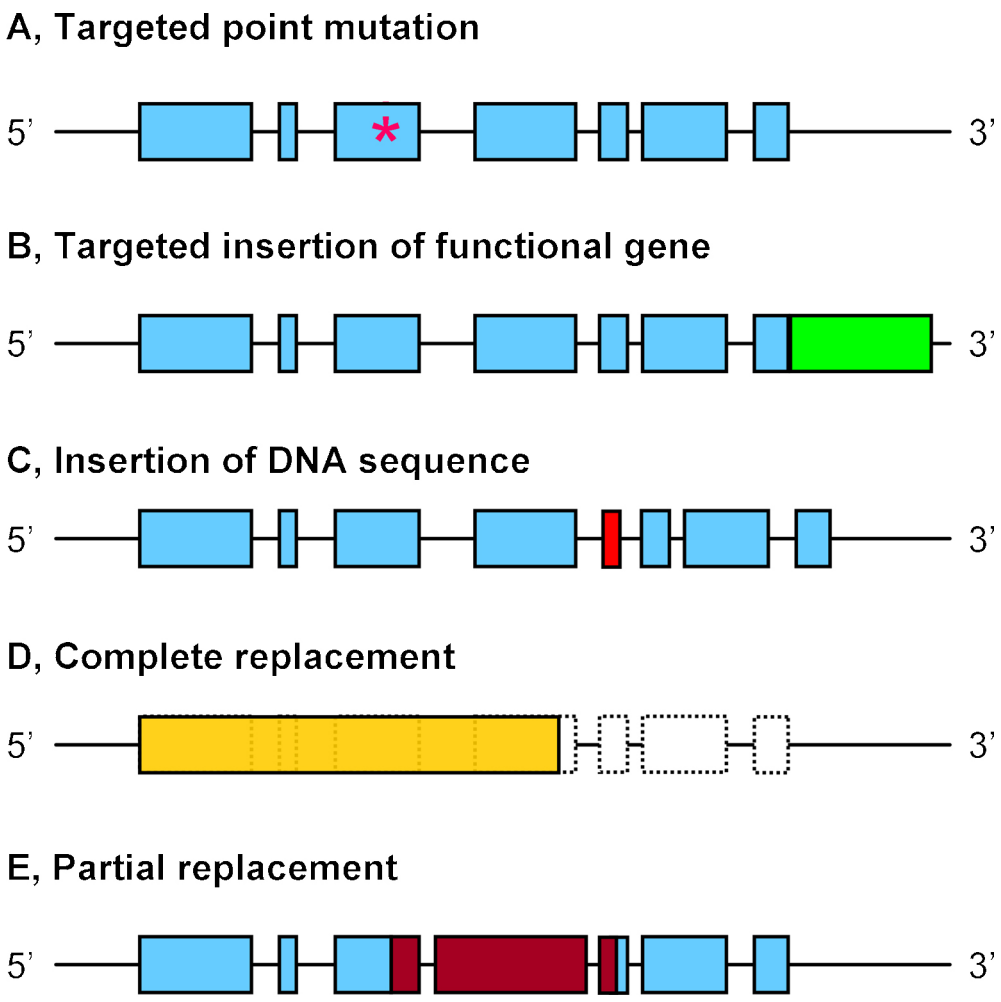

Fig. 1.

Various applications of genetically engineered knock-in mouse models. (A) Placing a subtle point mutation (asterisk) within a coding sequence is the most common use of the knock-in technology. (B) Insertion of a functional gene or sequence (*e.g.*, *lacZ*, nuclear localization signal, *etc.*) expressed in tandem or as a fusion protein (green box) with the endogenous gene. (C) Targeted insertion of a DNA sequence (red box) that is not expressed but functions at the genomic or RNA level (*e.g.*, loxP sites, siRNA). (D) Complete replacement of an endogenous gene locus (shaded box) with another related or unrelated functional gene (yellow box), such as a human homolog, *cre* recombinase, *etc.* (E) Partial replacement of a DNA sequence that has critical functions with a corresponding region of a related gene (red boxes).

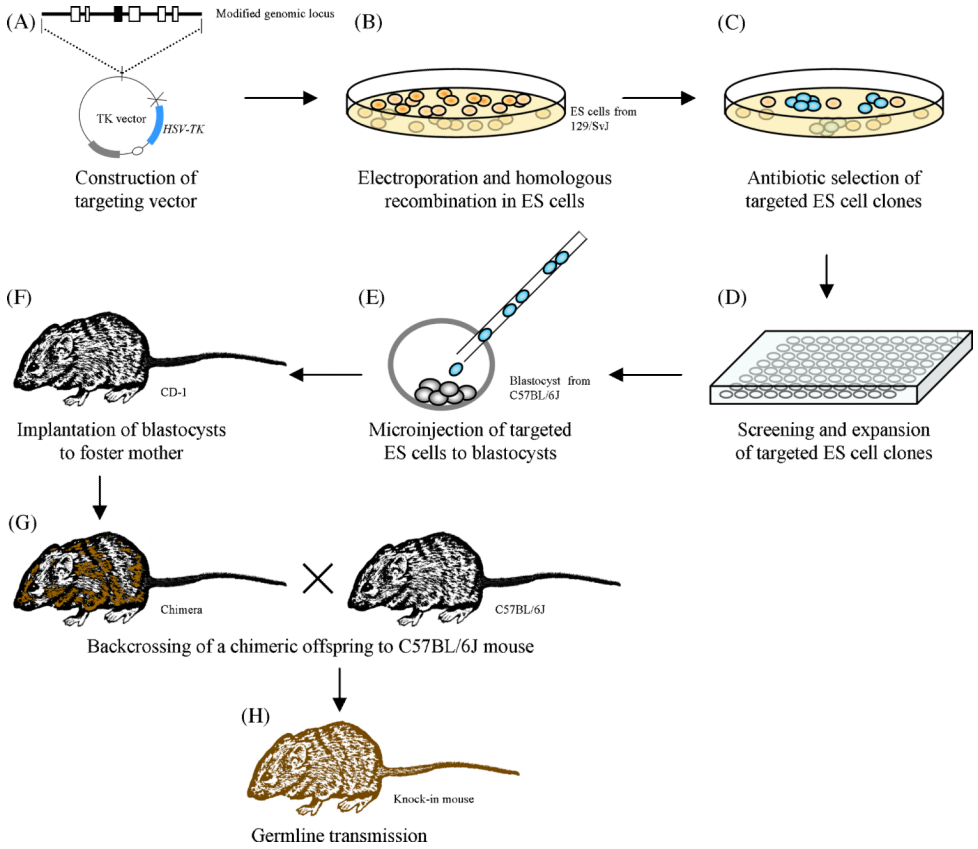

Fig. 2.

Procedure of gene targeting through homologous recombination in mouse embryonic stem cells. (A) Construction of a targeting vector. The targeting vector contains the modified gene locus (filled box), and typically harbors a *neo*-cassette for positive selection (not shown) and the *HSV-TK* gene for negative selection of correctly targeted ES cells. (B) The targeting vector is linearized and introduced into ES cells (typically from 129/SvJ background) by electroporation. (C) ES cells that incorporated the targeting vector form colonies (blue) after selection with appropriate antibiotics. (D) Selected colonies are characterized by southern blotting to identify cells in which the desired gene targeting event occurred. (E) Correctly targeted ES cells are expanded and injected into C57BL/6J host embryos. (F) Blastocysts harboring a mixture of C57BL/6J embryonic cells and the targeted 129/SvJ ES cells are transferred to the oviducts of pseudo-pregnant CD-1 foster mothers. (G) Chimeric newborns are back-crossed with C57BL/6J mice. (H) Breeding of chimeric progeny carrying the targeted mutation to achieve germline transmission.

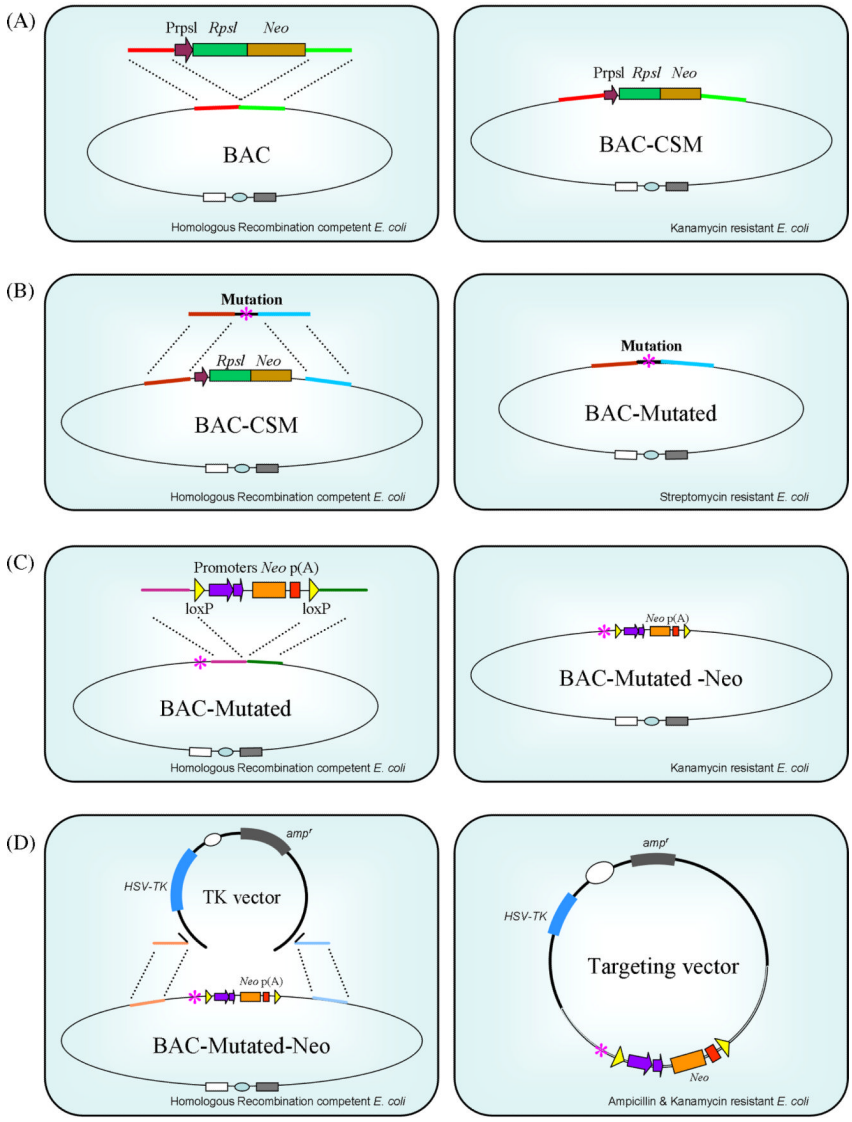

Fig. 3.

Bacterial homologous recombination in the construction of targeting vectors. (A) Left, a homologous recombination-competent bacterial strain harboring a BAC vector is shown. An *RpsL-Neo* cassette, as selection marker and counter selection marker (CSM) genes, is introduced via electroporation. The *RpsL-Neo* cassette is flanked with short DNA sequences (red and green) that are homologous to the target gene locus in the BAC vector. Right, selection for kanamycin resistant bacterial clones (conferred by *Neo*) identifies BAC vectors that have the *RpsL-Neo* cassette inserted into the designated gene locus through homologous recombination (BAC-CSM). (B) Left, a DNA fragment containing a subtle mutation (asterisk) and DNA sequences (brown and blue) that are homologous to the target gene locus in the BAC-CSM vector replaces the *RpsL-neo* cassette. Right, streptomycin selection for mutant BAC clones (resistance is conferred by the absence of *RpsL*) (BAC-Mutated). (C) Insertion of a *neo*-cassette as in (A) and (B). The *neo*-cassette is driven by dual prokaryotic and eukaryotic promoters and is flanked by *loxP* sites. This configuration enables the *neo* gene to be used to select for antibiotic resistance in *E. coli* (kanamycin) as well as in ES cells (geneticin). (D) Left, the modified gene locus in the BAC vector (BAC-Mutated-Neo) is subcloned into a gene targeting vector (*TK* Vector) through homologous

recombination. The backbone of the targeting vector contains *HSV-TK* and ampicillin resistant (*amp*^{r}) genes. Right, ampicillin and kanamycin selection identifies targeting vectors that contain both the plasmid backbone and the modified gene locus.

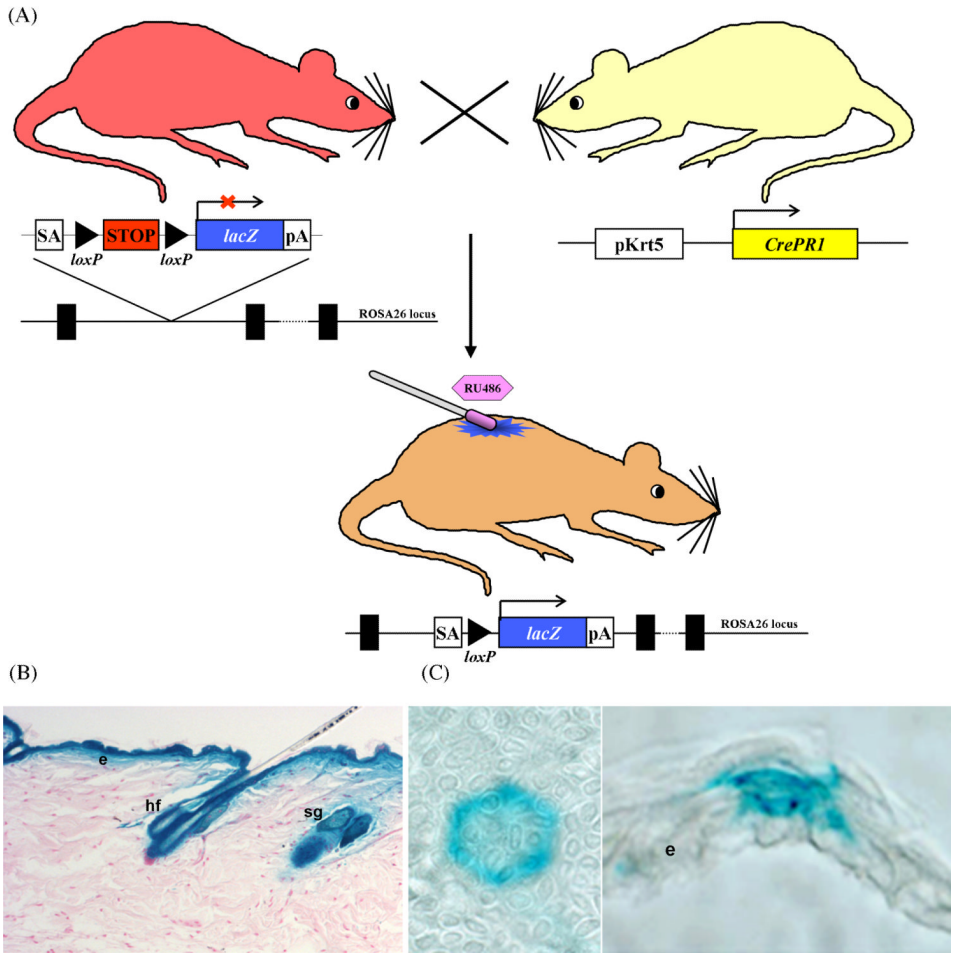

Fig. 4.

Cre recombinase-mediated tissue-specific inducible system. (A) The generation of an inducible *lacZ* mouse. A "target" mouse, where a splice-acceptor (SA) sequence, a STOP sequence (*PGK-Neo-pA*) flanked by *loxP* sites, and a *lacZ* gene were targeted into the ROSA26 locus, is shown at upper left. Transcription of the *lacZ* gene is blocked by the STOP sequence [61]. An "activator" mouse, expressing a fusion protein consisting of *Cre*-recombinase with a mutated ligand-binding domain of a progesterone receptor (*CrePR1*) under the control of skin-specific *Krt5* promoter (*pKrt5*), is shown at upper right [62]. A bigenic inducible mouse obtained by crossing the above "target" (*lacZ*) and "activator" (*Cre*) mice is shown below. Topical application of an inducer (RU486) on the back skin of the bigenic mouse was able to activate *Cre* to excise the STOP signal placed between the two *loxP* sites, therefore, inducing expression of the *lacZ* gene in the skin. (B-C) X-gal staining of skin sections after induction of the conditional *lacZ* allele. Note the uniform expression of *lacZ* in the epidermis, hair follicles and sebaceous glands 6 months following activation of CrePR1 *in utero* in (B), confirming that K5CrePR1 is expressed in multipotent stem cells in *B* (Zhou et al., 2002). Topical application of RU486 to adult epidermis results in activation of K5Cre*PR1, and thus expression of *lacZ* in individual stem cells and their progeny is shown in (C) (Caulin et. al., 2007). Shown are both an *en face* (left) and cross (right) view of an epidermal proliferative unit expressing *lacZ.* Abbreviations: e, epidermis; hf, hair follicle; sg, sebaceous gland.

Table 1

Genetically Engineered Mouse Models

	Transgenic	Targeted mutation		Random gene inactivation	Random mutagenesis
		Knockout	Knock-in		
Application	Expression of normal, modified, or exogenous gene(s)	Deletion or disruption of gene expression	Genomic modification by targeted point mutation, addition, or deletion	Random disruption of gene expression	Generation of random point mutations across the genome
Procedure	Microinjection of recombinant DNA into pronucleus of oocyte and implantation into oviducts of foster female	Homologous recombination in ES cells, injection of targeted ES cells into blastocytes, and implantation into oviducts of foster females		Gene trapping in ES cells	Chemicals or radiation Male mice are exposed to
Characterization	Transgene(s) is inserted randomly in the mouse genome. Protein(s) encoded by the transgene(s) is expressed.	Targeted gene(s) is inactivated. Phenotype is due to the absence of targeted gene product.	Targeted gene(s) is modified. Phenotype is due to the expression of modified gene product.	Gene-trap vector randomly inserts into a gene locus and interrupts transcription at the site of integration. Phenotype is generally due to loss-of-function.	mutagen and their progeny are screened for phenotypes (forward genetics). Multiple randomly mutated genes need to be mapped.

Table 2

Examples of knock-in mouse models used in skin research

	Gene	Modification	Application	Reference
Gene Function	*Runx1*	Targeted replacement of *Runx1* (exon 7–8) with *lacZ*	Expression pattern of *Runx1* during skin embryogenesis	[7, 8]
	Gata-3	Targeted replacement of *Gata-3* (exon 1–2) with *lacZ*	Regulation of hair follicle morphogenesis	[9, 10]
	Ccd6	Targeted insertion of *EGFP* to *Ccr6* locus	Expression of *Ccr6* in langerhans cells	[11]
	Apc	Targeted mutation of *Apc* (*Apc* ^{1638T})	Nipple-associated cutaneous cysts	[12]
	Col5a2	Targeted substitution of *Col5a2* (exon 6) with *neo* - cassette	Regulatory role of *Col5a2* during matrix assembly	[13]
	Egfr	Targeted replacement of mouse *Egfr* with human *EGFR* cDNA	Hair follicle and hair cycle defects	[14]
	Dsc1	Targeted deletion of exon 17 of *Dsc1* (*Dsc1* $^{\Delta E17}$)	Cytoplasmic domain of *Dsc1* in epidermal development	[15]
	Krt75	Targeted point mutation of *Krt75* (*Krt75* ^{N159del})	Dominant effects of mutant *Krt75* in hair and nail formation	[16]
Disease Models	*Krt10*	Targeted mutation of *Krt10* (*Krt10* ^{R154C})	Inducible mouse model of EHK	[17]
	Krt14	Targeted point mutation of *Krt14* (*Krt14* ^{R131C})	Inducible mouse model of EBS	[18]
Skin Cancer	*k-Ras*	Targeted point mutation of *k-ras* (*k-ras* ^{G12D})	Study of spontaneous oncogenic *k-ras* activation in cancer	[19, 20]
	Krt10 - *Krt14*	Targeted replacement of head and tail domains *Krt14* with corresponding domains of *Krt10*	Role of intermediate filaments in skin cancer susceptibility	[21]
	Kit	Targeted point mutation of *Kit* (*Kit* ^{V558Del})	Role of gain-of-function mutation of *Kit* in skin cancer	[22]
	Cdk4	Targeted point mutation of *Cdk4* (*Cdk4* ^{R24C})	*Cdk4* activation in skin carcinogenesis	[23]
	p53	Targeted humanization of core domains (exon 4–9) of *p53*	Recapitulation of frequent DNA damage to human *p53* mutation	[24, 25]
	p53	Targeted point mutation of *p53* (*p53* ^{R171A,} *p53* ^{R172H,} *p53* ^{R270H})	Recapitulation of Li-Fraumeni Syndrome by expressing gain- of-function mutant *p53* at its endogenous gene locus	[26, 27]