

Transgenic dry eye mouse models: powerful tools to study dry eye disease

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Abstract

• Dry eye disease (DED) is one of the most common chronic multifactorial ocular surface diseases with high prevalence and complex pathogenesis. DED results in several ocular discomforts, vision fluctuation, and even potential damage of the ocular surface, bringing heavy burdens both on individuals and the society. The pathology of DED consists of tear film hyperosmolarity and immune responses on the ocular surface. Mice are widely used for developing models that simulate human DED features for investigating its pathogenesis and treatment. DED can be classified into aqueous-deficiency dry eye (ADDE) and evaporative dry eye (EDE). ADDE can be further divided into Sjögren syndrome dry eye (SSDE) and non-Sjögren syndrome dry eye (NSSDE). SSDE mouse models include natural strains, typified by non-obese diabetic (NOD) mice, and genetically engineered ones, like *Aire*^{-/-} and *Id3* knockout mice. Intrinsic EDE mainly refers to meibomian gland dysfunction (MGD). *Eda*^{-/-} Tabby, *Sod1*^{-/-}, *Elovl1*^{-/-} are the most common transgenic MGD mouse models. Transgenic mouse models provide useful tools for studying the pathogenesis of DED and evaluating its novel therapies. This review compares the major transgenic dry eye mouse models and discusses their applications in DED research.

• **KEYWORDS:** dry eye disease; transgenic; mouse models; ocular surface; pathology

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INTRODUCTION

Dry eye disease (DED) is one of the most common chronic multifactorial ocular surface diseases in the department of ophthalmology^[1]. The prevalence of DED is 5%-50% on a global scale, which is expected to rise with the aging of the population^[2-3]. Besides, the widespread use of digital products makes DED a much more common disorder in young generations^[4]. DED results in several ocular discomforts, vision fluctuation, and even potential damage to the ocular surface^[1]. Long term interventions will be needed once DED is diagnosed, which may substantially affect patients' quality of life^[5]. In the meantime, the decline of the workplace productivity will have a great negative impact on the economy^[6]. Overall, DED brings heavy burdens both on individuals and the society, which becomes an increasingly critical health issue.

The Tear Film and Ocular Surface Dry Eye Workshop (TFOS DEWS) II classified DED into two categories: aqueous-deficiency dry eye (ADDE) and evaporative dry eye (EDE). ADDE results from lacrimal diseases or dysfunction, which can be further divided into Sjögren syndrome dry eye (SSDE) and non-Sjögren syndrome dry eye (NSSDE). EDE can be divided into intrinsic and extrinsic EDE according to the etiology, and the former one mainly refers to meibomian gland dysfunction (MGD)^[1]. The pathogenesis of DED is complicated and remains elusive. The fundamental mechanism of DED in all cases is the vicious circle caused by tear film hyperosmolarity and ocular surface inflammation^[1]. Tear film hyperosmolarity activates mitogen-activated protein kinase (MAPK) and NF-κB signal pathway on the ocular surface and induces innate and adaptive immune response. The elevation of interleukin-1 (IL-1), tumor necrosis factor α (TNF-α) and matrix metalloproteinase 9 (MMP9) leads to the loss of conjunctival goblet cells and subsequent decline of mucins on the conjunctiva and in the tear film. This compromises the tear film stability, and tear film instability, in turn, amplifies tear film hyperosmolarity. Henceforth, the vicious circle is generated^[7]. With the study of new pathological pathways, novel drugs that specifically target certain aspects of DED are under exploration.

At present, the treatment for DED is nonspecific, including lifestyle improvement, artificial tears, physical devices,

and topical anti-inflammatory agents^[8]. The translations of new therapies first rely on suitable animal models to obtain primary efficacy and safety data. There have been many methods to establish dry eye animal models by far, including lacrimal gland excision (LGE), desiccating environmental stress (DES), genetic modification and so on. The dry eye animal models established by different methods simulate different mechanisms of dry eye subtypes and have their own advantages and limitations. Specifically, transgenic and gene knockout models are very suitable to study the mechanism of a particular pathway in the development of DED.

Mice have been greatly preferred and widely used in basic research not only for their small body size, easy operation, and relatively low cost, but also genetic and pathophysiological similarities to humans. Most transgenic mouse models are developed by promoting, suppressing, or changing the expression of a certain gene. These manipulations help to study the structure, function, and expression regulation of this gene. The etiology and pathogenesis of DED are still not fully understood. Transgenic mouse models will certainly be of much help in exploring the mechanisms of DED. Besides, they can also be reliable workhorses of novel drug screening. On one hand, their inherent features similar to human DED leave out the difficult and laborious modeling process. On the other, the identified pathogenesis of a certain model helps to investigate the specific pharmacodynamics and action mechanism of the drug. Therefore, transgenic dry eye mouse models can be very powerful tools in DED research, and it's necessary to look into and make the best of them.

This review aims to summarize existing common transgenic dry eye mouse models, and evaluate their characteristics, advantages, and limitations, trying to provide a reference for the establishment of reliable transgenic dry eye mouse models for future research work.

SJÖGREN SYNDROME DRY EYE TRANSGENIC MOUSE MODELS

Sjögren syndrome (SS) is an autoimmune disorder characterized by dry eye (keratoconjunctivitis sicca) and a dry mouth (xerostomia), which is usually accompanied by rheumatoid arthritis, lupus and other autoimmune disorders. Many autoimmune animal models resembling SS have been developed so far. They have similar characteristics of autoimmune exocrinopathy, secretory dysfunction, female predominance and presence of autoantibodies, along with other unique features.

Natural Mice Strains with Features of Dry Eye Disease

Non-obese diabetic-based strains Non-obese diabetic (NOD) mouse is a spontaneous type I diabetes (T1D) mouse model^[9]. NOD mouse has been commonly utilized as a secondary SS mouse model due to its spontaneous SS-like symptoms^[10].

Although diabetes and sialoadenitis are more prevalent in females, the onset of dacryoadenitis in male NOD mice is surprisingly higher^[11].

A great number of studies about DED drug development have been conducted with NOD mice. The efficacy of cyclosporine A (CsA) and the drug carrier development to improve its bioavailability have been studied using NOD mice^[12-13]. In addition, Cathepsin S (CTSS) inhibitors and rapamycin could mitigate the ocular manifestations of SS in NOD mice^[14-15].

However, the coexisting T1D in NOD mice requires long-term insulin injections, which not only add the feed cost, but also potentially affect the study of SS. To overcome these problems, several NOD strains have emerged. They exhibit aqueous tear deficiency equivalent to NOD mice without T1D manifestations.

1) NOD.B10-H2^b mice

Major histocompatibility complex (MHC) I-A^{g7} is essential for the development of diabetes and insulinitis in NOD mice. NOD.B10-H2^b mice are generated by the replacement of MH CI-A^{g7} fragment of NOD strain with MHC I-A^b of B10 strain. NOD.B10-H2^b mice are absent from autoimmune diabetes but still retain symptoms of secondary SS^[16].

NOD.B10-H2^b mice exhibited saliva and tear secretory loss. Focal lymphocytic infiltration was presented in the lacrimal glands (LG) of NOD.B10-H2^b mice. The expression levels of pro-inflammatory cytokines in the conjunctiva and LG increased with age^[17]. Additionally, novel SS autoantibodies like anti-CA6, anti-SP1 (but not anti-PSP) increased in the pre-clinical phase before the presence of antinuclear antibody (ANA)^[16].

Many novel formulations have been tested on this strain, such as high-mobility group box 1 and IRT5 probiotics^[18-19]. Besides, the efficacy tests of poly(ethylene glycol) and catechin nanocomplex, sulglycotide, RGN-259, and cevimelines were conducted in NOD.B10-H2^b mice treated with desiccation stress^[20-23].

2) C57BL/6.NOD-*Aec1Aec2* mice

Idd3 and *Idd5* are two chromosomal intervals closely correlated with the spontaneous development of SS in NOD mice. C57BL/6.NOD-*Aec1Aec2* mice are descended from C57BL/6.NODc3 mice carrying *Idd3* (*Aec1*) and C57BL/6.NODc1 mice carrying *Idd5* (*Aec2*).

C57BL/6.NOD-*Aec1Aec2* mice showed increased expression level of IL-1 β , TNF- α , and declined goblet cell density in the conjunctiva at 12wk. They also exhibited lymphocytic infiltration consisting predominantly of CD4⁺ T cells in the LG at 20wk. Notably, the LG infiltration did not result in loss of tear production^[24].

C57BL/6.NOD-*Aec1Aec2* mice were widely used to explore potential therapeutic targets of SS. Interestingly, they were

more frequently seen in SS studies focused on sialadenitis^[25-26]. More studies focused on dacryoadenitis would be needed to testify their applicability to study DED.

3) NOD.*IL4*^{-/-} mice

Transgenic animal models make it possible to identify the impacts of a particular molecule or pathway in the pathogenesis of SS. The advent of several cytokine gene knockout mice with NOD background contributes to the study of the mechanism of relevant cytokines in the development of SS.

The importance of IL-4-STAT6 signal transduction pathway was investigated using NOD.*IL4*^{-/-}, NOD.B10-H2^b.*IL4*^{-/-}, and NOD.B10-H2^b.*C-Stat6*^{-/-} mouse model. IgG antibodies against muscarinic receptor 3 (M3R) were detected in NOD.B10-H2^b.*C-Stat6*^{+/+} mice but not in NOD.B10-H2^b.*C-Stat6*^{-/-} mice^[27]. This suggested that IL-4-STAT6 pathway might be a potential therapeutic target for SS treatment.

4) NOD.*IFN-γ*^{-/-} mice

Interferon- γ (IFN- γ) is indispensable in both the early and later immune phase of DED. The high expression of IFN- γ in the conjunctiva is correlated with goblet cell loss and mucin decrease^[28].

IFN- γ increased markedly both in the LG and in tears of male NOD mice, who spontaneously developed dacryoadenitis but not sialoadenitis^[11]. However, neither the NOD.*IFN-γ*^{-/-} nor the NOD.*IFN-γR*^{-/-} mice exhibited leucocytic infiltration or secretory dysfunction in the LG and salivary glands (SG). Detectable antibodies were also absent. These findings suggested that IFN- γ was indispensable in the development of SS^[29].

MRL/+ and MRL/lpr mice MRL/+ and MRL/lpr are two congenic substrains of MRL/MpJ mice. Both MRL/+ and MRL/lpr mice develop lacrimal inflammation, but it's earlier, more severe and extensive in MRL/lpr mice^[30].

Apart from systemic lupus erythematosus (SLE) symptoms, the phenotype of MRL/lpr mice encompasses the key features of SS, including female sex predilection, decreased saliva and tear secretion, lymphocytic infiltration in the SG and LG, and anti-SSA and anti-SSB antibodies production^[31]. Sex-related differences in gene expression contributed to the development of dacryoadenitis^[32], and could be influenced by testosterone treatment^[33]. Increased levels of pro-inflammatory cytokines were observed in the LG^[34]. And the gene expression levels of MMP2 and MMP9 also increased^[35]. Besides, injection of p38-MAPK inhibitor, SB203580, into the LG significantly improved tear production. This indicated that the activation of p38-MAPK pathway played an important role in the development of DED of the MRL/lpr mouse model^[36].

However, MRL/lpr mice spontaneously develop SLE-like autoimmune injuries. They're likely to die of renal failure with an average life span of 6mo. MRL/+ mice usually live up to 2y,

but they are rarely used due to the late onset and slight degree of the DED^[30].

D3Tx IQI/Jic mice IQI/Jic mice strain is an inbred established from JCL-ICR mice, characterized by the lacrimal and salivary lymphocytic infiltration. The LG and SG lesions progressed with age. The dominant lymphocytes in small foci were CD4⁺ T cells, while B cells (B220⁺) were more common in larger lesions. ANA, but not anti-SSA and anti-SSB, was present in IQI/Jic mice. The onset of sialoadenitis and dacryoadenitis in IQI/Jic mice happened late in life, and the increasingly serious autoimmune injuries in other organs restrict their application^[37].

Interestingly, IQI/Jic mice with thymectomy on day 3 after birth (D3Tx) developed more severe LG lesions since as early as 16wk. Namely, D3Tx selectively accelerated the progression of dacryoadenitis with little impact on the SG^[38]. Early thymectomy helped to eliminate lacrimal-specific Treg cells, which contributed to the rapid development of autoimmune dacryoadenitis in IQI/Jic mice.

D3Tx NFS/sld mice NFS/sld mice strain is a mutant phenotype derived from NFS/N strain. D3Tx NFS/sld mice spontaneously developed significant lacrimal and salivary infiltration at about 6-8wk^[39]. Compared with age-matched control mice, tear secretion of D3Tx NFS/sld mice was greatly decreased, and the gene expression levels of pro-inflammatory cytokines in the cornea and LG were significantly increased. Fluorescein staining confirmed the corneal epithelial injury resulting from dry eye. The majority of inflammatory infiltration in the LG were CD4⁺ T cells^[40].

The chemokine CCL22 produced by tissue-resident macrophages might impair the local immune tolerance in D3Tx NFS/sld mice, and administration of anti-CCL22 antibody could suppress the autoimmune lesions, suggesting a novel therapeutic or diagnostic target for SS^[39].

***Aly/aly* mice** The principal property of homozygous *aly/aly* mice is the alymphoplasia caused by an autosomal recessive mutation, represented by the lack of lymph nodes and Peyer's patches. Chronic inflammatory changes could be observed in the exocrine glands of homogenous *aly/aly* mice, but not in the heterogeneous (*aly/+*) ones^[41]. Salivary and lacrimal inflammation in homogenous *aly/aly* mice occurred at 14wk and increased with age. The CD4⁺ T cell infiltrations in the LG were more severe than those in the SG. Autoantibodies were not detected presumably due to the humoral immunity defects^[41].

In the pathological development of SS of *aly/aly* mice, T cell migration to autoimmune targets was verified to be regulated by RelB/NF- κ B2 pathway through TGF β /TGF β R-dependent regulation of CXCL12/CXCR4 signaling. This might suggest a potential therapeutic target for SS treatment^[42].

Table 1 Features of common mice strains which spontaneously develop SSDE

Mice strain	Sex difference	Involvement of other organs	Composition of lymphocytic infiltration	Loss of tear production	Pro-inflammatory cytokines	Autoantibodies
NOD	Dacryoadenitis M>F; sialoadenitis F>M	Yes	Mainly CD4+ T cells	Yes	Yes	Yes
NOD.B10-H2 ^b	Dacryoadenitis M>F; sialoadenitis F>M	ND	Mainly CD4+ T cells	Yes	Yes	Yes
C57BL/6.NOD- <i>Aec1Aec2</i>	Dacryoadenitis M>F (F=0); sialoadenitis F>M	ND	Mainly CD4+ T cells	Yes	Yes	Yes
NOR	ND	ND	ND	Yes	Yes	ND
NOD.H-2 ^{h4}	F>M	Yes	Mainly CD4+ T cells	Yes	Yes	Yes
MRL/lpr	Dacryoadenitis F>M; sialoadenitis F=M	Yes	Mainly CD4+ T cells	Yes	Yes	Yes
D3Tx IQI/Jic	F>M	Yes	CD4+ T and B220+ cells	ND	Yes	Yes
D3Tx NFS/sld	F>M	No	Mainly CD4+ T cells	Yes	Yes	Yes
<i>Aly/aly</i>	F=M	Yes	Mainly CD4+ T cells	ND	Yes	No
NZB/W F1	F>M	Yes	Mainly CD4+ T cells	Yes	Yes	Yes
BXSB/MpJ-Yaa	F>M	Yes	Mainly B220+ cells	No	Yes	Yes

F: Female; M: Male; ND: Not determined.

NZB/W F1 mice NZB/W F1 mice are recognized as SLE models, and they also spontaneously develop SS-like symptoms. The lymphocytic infiltration composed predominantly of CD4+ T cells in the SG and LG occurred at 4 months old, and increased with age. The saliva secretion of female NZB/W F1 mice significantly declined at 6-6.5mo. However, the presence of LG inflammation in NZB/W F1 mice did not correlate with tear flow impairment. Multiple autoantibodies and circulating immune complexes (CIC) could be detected in serum at 2-4mo and 4-6mo, respectively^[43]. Furthermore, the development of SS in NZB/W F1 mice could be retarded by low-fat diet or androgen administration and aggravated by high fat diet or inflammatory stimuli of incomplete Freund's adjuvant^[44-46].

BXSB/MpJ-Yaa mice BXSB/MpJ-Yaa mice are more extensively defined as SLE models, but they also spontaneously develop mild or early-stage DED from 8wk or earlier. They showed increased serum levels of anti-dsDNA antibody and S/B in 8-20wk. The lymphocytic infiltrations in the LG of BXSB/MpJ-Yaa mice were primarily comprised of B220+ cells, and the goblet cell density in the conjunctiva declined after 20wk^[47].

Unlike other SS models, male BXSB/MpJ-Yaa have more severe autoimmune symptoms than females because of the Y-linked autoimmune acceleration (Yaa) mutation on the Y chromosome^[48] (Table 1).

Genetically Engineered Mice with Features of Sjögren Syndrome Dry Eye

RbAp48 transgenic mice Retinoblastoma-associated protein 48 (RbAp48) is a multifunctional protein that regulates cell growth and apoptosis through the MAPK pathway^[49]. Estrogen deficiency initiates p53-mediated apoptosis in the exocrine gland cells through RbAp48 overexpression. RbAp48

transgenic mice are marked by RbAp48 overexpression. Almost all RbAp48-Tg mice spontaneously developed autoimmune exocrinopathy resembling SS. IL-18 and IFN- γ were increased in the SG and LG, and a high titer of autoantibodies against SSA, SSB, and 120-kD α -fodrin was detected in the serum. These manifestations increased with the progression of the disease. Similarly, the incidence was higher in females of all ages^[50].

Overall, RbAp48 transgenic mouse seems to be an ideal animal model to study autoimmune DED induced by estrogen deficiency.

***Aire*^{-/-} mice** Autoimmune polyendocrinopathy syndrome, type 1 (APS1) is an autosomal recessive disease caused by *Aire* gene mutation. In addition to the triad of adrenal insufficiency, hypoparathyroidism and chronic mucosal skin infection, APS1 also manifests with many ocular complications^[51].

Aire^{-/-} mice spontaneously developed SS-like symptoms and pathologic changes in the exocrine organs. *Aire*^{-/-} mice from 6-7 weeks of age exhibited poor tear secretion, extensive lymphocytic infiltration, reduced innervation, and increased vascularization in the cornea and LG^[52-53]. Th1 polarized CD4+ T cells were the major contributors to the autoimmune response^[54]. Notably, BALB/c *Aire*^{-/-} mice showed a milder autoimmune phenotype than NOD ones, while *Aire*^{-/-} mice in C57BL/6 background failed to develop SS^[24].

Despite their infertility and organ-specific autoimmunity, *Aire*^{-/-} mice have an equivalent life expectancy to their wild type littermates^[55]. *Aire*^{-/-} mouse is a useful model to study the mechanism and treatment of SSDE. Keratinizing squamous metaplasia (SQM) of the ocular surface is a blinding consequence of severe DED. The functional roles of IL-1/IL-1 receptors in the initiation and progression of SQM were

investigated with *Aire*^{-/-} mice, providing new therapeutic approaches for keratinizing ocular surface diseases^[56-57]. Alterations in corneal biomechanics in the early phase of DED also suggested new potential targets^[58]. In addition, topical administration of lacritin, a tear glycoprotein, could promote tear secretion and maintain ocular surface integrity in *Aire*^{-/-} mice^[59].

Id3 knockout mice Inhibitor of DNA binding 3 (Id3) deficiency in *Id3* knockout (KO) mice induces activation of follicular helper T (T_{fh}) cells and exacerbates SS by causing B cell hyperactivity^[60]. *Id3* KO mice developed SG and LG lymphocytic infiltration predominantly of CD4⁺ T cells at 8wk as they aged. Tear and saliva secretion decreased at 2-4mo with no significant difference between females and males. Additionally, anti-SSA and anti-SSB could be detected after 12mo^[61].

Increased IL-13 produced by T cells in the early life played an essential role in the exocrinopathy in *Id3* KO mice^[62]. CD20 monoclonal antibody treatment led to a significant reduction of serum IgG3 and improvement of disease symptoms in 6-month *Id3* KO mice^[63].

Class-IA PI3K knockout (*r1AT/r2n*) mice Phosphatidylinositol 3 kinases (PI3K) plays a critical role in thymocyte development^[64]. Both class IA and IB PI3K contribute to the immune function in T cells, and class-IA PI3K is the dominant subgroup in B cells. Thus, class-IA PI3K deficiency will cause profound defects in antigen responsiveness^[65].

Class-IA PI3K KO (*r1f/r2n*) mice spontaneously developed a relatively inclusive organ-specific SS phenotype in the LG as early as 2mo but more commonly between 4mo and 1y. The infiltration was mainly composed of CD4⁺ T cells as well as a few CD8⁺ T cells and B220⁺ cells. ANA, anti-SSA, and anti-SSB autoantibodies increased with age and were more frequently detected after 1 year of age. Unlike in the LG, there was little difference in the SG between PI3K KO and control mice^[66].

Act-1^{-/-} mice The adaptor molecule Act-1 is a negative regulator of CD40- and B-cell activation factor (BAFF)-mediated B cell survival^[67]. It's also an essential molecule in IL-17 receptor (IL-17R) signaling and IL-17-dependent immune responses^[68]. *Act-1*^{-/-} mice exhibit a general increase of peripheral B cells, leading to multi-organ inflammation and autoantibody production^[69].

Balb/c.*Act-1*^{-/-} mice developed systemic autoimmune diseases with histological and serological features of SS. Histological analyses revealed profound lymphocytic infiltration in the SG and LG at 4-6mo. The infiltration consisted mostly of B220⁺ cells, and also a significant number of CD4⁺ and CD8⁺ T cells. High titers of anti-SSA and anti-SSB could be detected in the serum^[69]. Interestingly, B6.*Act-1*^{-/-} mice developed SLE-

like disease but failed to develop SS-like symptoms^[70].

STAT3 knockout and *IκB-ζ* deficient mice IκB-ζ interacts with the NF-κB subunit in the nucleus, and positively and negatively regulates various transcriptional activities^[71]. *IκB-ζ*-deficient mice manifested chronic inflammation on the ocular surface and perioral skin from 4-6wk. The inflammatory infiltration in the subconjunctival tissue of the eyelids was composed primarily of CD45R/B220⁺ and CD4⁺ T cells^[72]. Two months old *IκB-ζ*-deficient mice exhibited periductal lymphocytic infiltration in the LG, along with reduced tear secretion. High titers of ANA, anti-SSA, and anti-SSB could be detected in the serum^[73].

STAT3 is a key transcription factor of the Janus kinase/signal transducer and activator of transcription (JAK-STAT) pathway that regulates cell proliferation and apoptosis, and it's negatively regulated by *IκB-ζ*^[74]. Epithelial cell-specific *STAT3* KO mice developed periocular inflammation similar to *IκB-ζ*-deficient mice both in time of onset and severity^[73].

IL-2Rα/CD25 knockout mice The IL-2/IL-2R signaling pathway plays an important role in immunity. *CD25* KO mice developed CD4⁺ infiltration in the LG at 8wk and peaked at 12wk, accompanied by secretory dysfunction, without gender differences. Both Th1 and Th17-associated cytokines in the LG were significantly increased in *IL-2Rα* KO mice of 8-16 weeks old. Lacrimal acinar atrophy, loss, and fibrosis were presented at 16wk^[75]. Besides, corneal innervation reduced as early as 4wk after birth^[76].

Remarkably, the deletion of IFN-γ and the existence of commensal bacteria could protect LG from morphologic and functional lesions in *CD25* KO mice^[77-78].

TSP-1 knockout mice *TSP-1* KO mice were created by removing the *TSP-1* gene in C57BL/6 mice based on the pathogenic effect of TGF-β exerted on SS^[79]. Increased apoptosis and deterioration, elevated expression levels of pro-inflammatory cytokines, and evident CD4⁺ T cells inflammatory infiltration could be observed in the LG of *TSP-1* KO mice at 8, 12, and 12-24wk, respectively. They also showed secretory dysfunction of LG. Besides, anti-SSA and anti-SSB antibodies were detected at 12-16-week-old *TSP-1* KO mice^[24].

TSP-1 KO mice only resemble dry eye symptoms but have better health conditions and less severe pathological abnormalities, compared with *TGF-β1* KO mice^[24]. Thrombospondin-derived peptide and TGF-β activating peptide, KRFBK, were confirmed to attenuate the ocular surface inflammation of *TSP-1* KO mice, suggesting novel therapeutic options for SSDE^[80-81] (Table 2).

GENETICALLY ENGINEERED MICE WITH FEATURES OF MEIBOMIAN GLAND DYSFUNCTION

MGD is a chronic, diffuse abnormality of the meibomian glands (MG), commonly characterized by terminal duct

Table 2 Genetically engineered mice with features of SSDE

Mice strain	Sex difference	Involvement of other organs	Composition of lymphocytic infiltration	Loss of tear production	Pro-inflammatory cytokines	Autoantibodies
<i>RbAp48</i>	F>M	Yes	Mainly CD4+ T cells	ND	Yes	Yes
<i>Aire</i> ^{-/-}	F=M	Yes	Mainly CD4+ T cells	Yes	Yes	Yes
<i>Id3</i> KO	F=M	Yes	Mainly CD4+ T cells	Yes	Yes	Yes
Class-IA <i>PI3K</i> KO	ND	Yes	ND	ND	Yes	Yes
<i>Act-1</i> KO	ND	Yes	Mainly B220+ cells	ND	Yes	Yes
<i>STAT3</i> KO/ <i>IκB-ζ</i> KO	F=M	Yes	Mainly CD4+ T cells	Yes	Yes	Yes
<i>IL-12Rα/CD25</i> KO mice	F=M	Yes	Mainly CD4+ T cells	Yes	Yes	ND
<i>TGF-β1</i> KO	ND	Yes	Mainly CD4+ T cells	Yes	Yes	Yes
<i>TSP-1</i> KO	ND	Yes	Mainly CD4+ T cells	No	Yes	Yes

F: Female; M: Male; ND: Not determined.

Table 3 Genetically engineered mice with features of MGD

Mice strain	MGD characteristics	Involvement of other organs	Loss of tear production	Pro-inflammatory cytokines
<i>Eda</i> ^{-/-} <i>Tabby</i>	Meibomian gland loss, corneal damage, ocular surface inflammation	Yes	No	Yes
<i>Sod1</i> ^{-/-}	Decreased acinar density, increased fibrosis, inflammatory infiltration	Yes	Yes	Yes
<i>Elovl1</i> ^{-/-}	Decreased chain lengths of meibum lipids, increased blink rate, corneal damage	Yes	No	ND

MGD: Meibomian gland dysfunction; ND: Not determined.

obstruction, qualitative or quantitative changes in the glandular secretion^[82]. MGD is recognized as one of the major causes of DED. Different transgenic mouse models for MGD have been developed with similar features to MGD in humans. These models provide powerful tools to study different aspects of the MG, especially certain cell types and molecular pathways in MG morphogenesis and homeostasis.

***Eda*^{-/-} Tabby Mice** *Eda*^{-/-} Tabby mice simulate the *Eda* gene mutation in humans. Their ocular characteristics include MG loss, corneal damage and ocular surface inflammation. Tabby mice progressively developed severe vision-threatening lesions like corneal defects and neovascularization 8 to 16wk after birth^[83]. Tabby mice showed dry eye symptoms, including poor tear secretion and high blinking rate. The LG of Tabby mice also existed pathophysiological defects^[84].

Eda signaling plays a crucial role in the cornea-lacrimal gland feedback loop^[84]. Exogenous *Eda* protein could promote corneal epithelial cell proliferation to maintain corneal epithelial homeostasis of Tabby mice^[85].

***Sod1*^{-/-} Mice** *Sod1*^{-/-} mice exhibited dry eye symptoms at 10wk and aggravated at 50wk^[86]. Inflammatory cells in the LG were predominantly composed of CD4+ T cells. Pro-inflammatory cytokines in tear and serum significantly increased from 10 to 50wk^[87]. Moreover, the MG showed similar alterations^[88].

Sod1^{-/-} mouse provides a promising model to study reactive oxygen species associated with DED and has already been used to assess the efficacy of 2% rebamipide and 3% diquafosol sodium eye drops for DED^[89-90].

***Elovl1*^{-/-} Mice** *Elovl1*^{-/-} mice showed decreased chain lengths of meibum lipids and increased blink rate, leading to evaporative dry eye phenotype. Corneal damage could be observed at 5 months old^[91]. Mineral oil-containing ophthalmic solution (MO) could reduce the plugging of meibomian gland orifices, inhibit lipid aggregate production in the MG, suppress corneal damage, and improve tear film stability^[92] (Table 3).

SUMMARY AND FUTURE DIRECTIONS

DED is a very common ocular surface disease with high prevalence and complex pathogenesis. A safe, effective, and convenient therapeutic method is a research focus in today's DED studies, especially with recent advances in the area of new therapeutic targets and biological drug candidates for DED. Thus, a mature model system for DED is a demanding task for further research. Various methods to establish dry eye animal models have been developed. There are pros and cons to each choice. To be specific, LGE is relatively easy and low-cost, but it cannot always induce significant dry eye symptoms due to incomplete excision or compensatory tear secretion of accessory LG^[93-94]. Besides, it may cause ocular pain and anxiety-like behaviors in mice^[95]. Systemic administration of acetylcholine receptor blockers, such as scopolamine, simulates chronic DED pathogenesis well, but can be time-consuming, laborious, short-lasting, and self-limited^[96]. Immunity induction is able to provoke relatively exclusive autoimmune responses in the LG, but the procedure is so tedious and complicated that place great demands on experimental skills^[97].

Transgenic dry eye mouse models are widely used in the basic research for DED. A general understating of transgenic dry

eye mouse models will guide and improve future research. Despite the differences between mice and humans, the study of DED in mice brings significant insights into the disease in humans. Mice are very amenable to genetic manipulation, making it possible to explore more specific and sophisticated mechanisms at the molecular level, as well as safer, more effective and convenient therapeutic options. Many successful therapies for human disease arise from corresponding mouse models. The usage of CsA, diquafosol tetrasodium, or essential fatty acids for DED is based on their efficacy in experimental mouse models^[98-99].

Transgenic dry eye mouse models possess some unique advantages over induced models. For example, the inherent features of transgenic dry eye mouse models have omitted the difficult and laborious modeling procedures, so a great deal of time and manpower can be saved. Besides, the DED manifestations of transgenic dry eye mouse models are more balanced and consistent among different litters or in different experiments. What's more, the identified pathogenesis of transgenic dry eye mouse models clarifies the biological functions of a certain gene, protein, cell type, or signal pathway. There are also many limitations of transgenic dry eye mouse models. First, they are usually expensive to purchase and maintain, since genetic manipulation could be very difficult and challenging. And some transgenic mice strains co-exist systemic comorbidities and difficulties in reproduction. Second, the genetic engineering techniques confine the most experimental animals to small animals, potentially resulting in limited ocular findings and requiring more delicate operation skills due to their small body sizes. In addition, the different biological characteristics between mice and humans, and the different DED pathogenesis of transgenic dry eye mouse models and major clinical DED patients, have demanded more translations from bench to bedside.

The possibility of the potential application for personalized DED therapy is evolving with increasing knowledge and utilization of transgenic dry eye mouse models. Many biologic treatments have been developed whose targeting pathways or molecules are involved in the pathogenesis^[100]. For instance, based on the discovery that the overexpression of bone morphogenetic protein 6 (BMP6) locally in the SG and LG led to secretory dysfunction and pathological changes^[101], BMP signaling inhibitors LDN-212854 and LDN-193189 were found to recover SG function and decrease inflammatory markers in C57BL/6.NOD-*Aec1Aec2* mice^[102]. Furthermore, the elevated inflammatory biomarkers in tear proteomics play an important role in the development of DED^[103]. Drug targets for those biomarkers could possibly be verified by transgenic dry eye mouse models. Based on the discovery of increased CTSS in the tear of SS patients, CTSS inhibitors were found to

mitigate the ocular manifestations in NOD mice^[14,104].

In summary, transgenic dry eye mouse models can be very powerful tools in DED research. With greater access to the data from basic research, scientists and ophthalmologists will be able to further understand the pathophysiology of DED and develop better therapies based on clinical and scientific insights.

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