Published in final edited form as:

Exp Eye Res. 2017 October ; 163: 58–63. doi:10.1016/j.exer.2017.04.006.

Role of EGF Receptor Signaling on Morphogenesis of Eyelid and Meibomian Glands

Fei Dong¹, Mindy Call¹, Ying Xia^{1,2}, and Winston W-Y Kao¹

¹Departments of Ophthalmology College of Medicine University of Cincinnati, Cincinnati, Ohio, USA

²Environmental Health College of Medicine University of Cincinnati, Cincinnati, Ohio, USA

Abstract

The epidermal growth factor receptor (EGFR) signaling has a pivotal role in the regulation of morphogenesis during development and maintenance of homeostasis in adult eyelid and its adnexa. Studies have demonstrated that during eyelid morphogenesis the EGFR signaling pathway is responsible for keratinocyte and mesenchymal cell proliferation and migration at the eyelid tip. For meibomian gland morphogenesis, EGFR signaling activation stimulates meibomian gland epithelial cell proliferation. EGFR signaling pathway functions through multiple downstream signals such as ERK, Rho/ROCK and integrin and is regulated by a variety of upstream signals including Adam17, GPR48 and FGFR signaling. Herein we review the literature that describe the role of EGFR and its related signaling pathways in eyelid and meibomian gland morphogenesis.

I. Introduction

The eyelid is a thin skin fold that consists of several distinct tissues—skin, palpebral conjunctiva, meibomian glands, orbital septum, tarsal plates and palpebral muscles. The eyelid serves as the first physical protection of the eye and crucial for the ocular surface morphogenesis and homeostasis. Eyelid abnormalities lead to ocular diseases, including eyelid coloboma, blepharophimosis, congenital eyelid imbrication syndrome (CEIS) and lagophthalmos. A normally-developed eyelid is crucial for the development of ocular adnexa, such as meibomian glands, lacrimal glands, extraocular muscles, as well as other ocular surface tissues such as the cornea and lens (Meng et al., 2014a, 2014b; J. Wang, Call, Mongan, Kao, & Xia, 2017)The meibomian glands are a group of holocrine glands, pivotal for maintaining ocular surface health. They secrete meibum, which forms a lipid barrier that prevents aqueous evaporation and maintains normal tear film composition. Increasing evidence implicates meibomian gland dysfunction (MGD) as a main cause of dry eye syndrome, leading to increased tear evaporation, friction and the onset of inflammation at the ocular surface (Foulks & Bron, 2003; Nelson et al., 2011). Therefore, the formation and

maintenance of eyelid and functional meibomian glands is of paramount importance for maintaining a healthy ocular surface.

To date, many studies have focused on elucidating the inter- and intracellular communications which govern eyelid and meibomian gland morphogenesis (Rubinstein, Weber, & Traboulsi, 2016). The epidermal growth factor receptor (EGFR) signaling pathway is one of the most important signaling pathways that regulates morphogenesis of eyelid and its adnexa. In mice, the absence of EGFR signaling during development results in a failure of embryonic eyelid closure (EOB, eye open at birth, phenotype) and malformation of eyelids and its adnexa (Luetke et al., 1993, 1994; Mann et al., 1993; Miettinen et al., 1995). Interestingly, excess EGFR signaling in the epidermis and eyelid stroma also results in eyelid anomalies such as precocious postnatal eye opening and meibomian gland malformation (Dominey et al., 1993; Vassar & Fuchs, 1991). Studies have demonstrated that the EGFR signaling pathway accounts for epithelial cell proliferation and migration, two major cellular events in the leading edge during eyelid fusion, through autocrine and paracrine mechanisms. During meibomian gland morphogenesis EGFR signaling regulates the proliferation of meibomian gland epithelial cells and mesenchymal cells surround the glands, and consequently affect meibomian gland morphogenesis.

Herein we review the literature that describes the role of EGFR and its related signaling pathways in eyelid and meibomian gland morphogenesis during development.

II. Eyelid and Meibomian Gland Morphogenesis in Mouse

In mammals, morphogenesis of the eyelid involves two major cellular events. 1). Differentiation of surface ectoderm gives rise to epithelia of epidermis, conjunctiva and eyelid glands (lacrimal and meibomian glands), 2). Periocular mesenchymal cells of neural crest and mesoderm origin lead to the formation of the eyelid stroma, extrinsic muscle and tarsal plate of the eyelids. Eyelid fusion and subsequent reopening is a well-conserved process during the development of most terrestrial vertebrates including human (Gage, Rhoades, Prucka, & Hjalt, 2005; Tawfik, Abdulhafez, Fouad, & Dutton, 2016). In mice, the eyelids start to form around embryonic day 11.5 (E11.5), when the periocular epithelium of surface ectoderm origin forms an eyelid fold at the junction of the future conjunctiva and cornea surface after the lens detaches from surface ectoderm. The ocular surface epithelia migrate out of the eyelid tip forming an eyelid protrusion at E12.0. As development progresses, the tip epithelia migrate, resulting in fusion of the upper and lower eyelids by E16.5 (<http://www.emouseatlas.org/emap/home.html>). Accompanying the fusion process, mesenchymal cells of the mesoderm and neural crest origins migrate to fill the space between the epidermal and conjunctival epithelium and form the eyelid stroma. The eyelids remain closed for approximately 2 weeks. Eyelid reopening occurs at around postnatal day 12 (P12) as a result of apoptosis and keratinization of the epithelial cells at the lid fusion junction (Mohamed, Gong, & Amemiya, 2003).

During the time when the eyelids are closed, the formation of eyelid adnexal structures, such as tarsal plate, periocular muscles, orbicularis oculi, and meibomian gland, is completed (Findlater, McDougall, & Kaufman, 1993; Gage et al., 2005). Meibomian gland

morphogenesis commences at E18.5. The first sign of gland development is detected at the lid junction where the mesenchyme of neural crest and mesoderm origin underneath the future gland primordium starts to condense (Dong et al., 2015). The overlaying epithelial cells of ectoderm origin form the epithelium placode. The placodes then invaginate into the eyelid mesenchyme at P0 and elongate. Meibomian glands branch from P5 and finally differentiate into a mature meibomian gland at about 2 weeks of age concurrent with eye opening (Nien et al., 2010).

III. EGFR Signaling in Eyelid Morphogenesis

EGFR is a cell-surface tyrosine kinase receptor. Upon binding to its extracellular ligands, including epidermal growth factor (EGF), heparin-binding EGF-like growth factor (HB-EGF), transforming growth factor- α (TGF- α), amphiregulin (AR), epiregulin (EPR), epigen, betacellulin (BTC) and neuregulins (NRGs), EGFR undergoes phosphorylation and dimerization and activates a number of intracellular signaling pathways. Activation of the EGFR pathway ultimately leads to the regulation of cell proliferation and differentiation in a tissue-, cell type- and biological process-specific manner (Singh, Carpenter, & Coffey, 2016). During eyelid morphogenesis, EGFR signaling plays a pivotal role in eyelid fusion by promoting eyelid epithelial cell proliferation and sheet migration. EGFR signaling is also responsible for ocular adnexa morphogenesis. Based mostly on cell culture, EGFR signaling could promote proliferation and inhibit differentiation of meibomian gland epithelial cells. At the same time, EGFR regulates eyelid mesenchymal cells proliferation and ECM secretion, and affect the meibomian gland morphogenesis through secondary effects. In lacrimal glands, EGFR ligands are found to regulate aqueous secretion. In Part III, we will discuss EGFR signaling on the regulations of actin stress fiber formation and gene expression, and intricate signaling network via epithelial-mesenchymal interactions during eyelid morphogenesis.

A. EGF/HB-EGF/TGF α /EGFR signaling in eyelid epithelium proliferation and leading edge migration

As mentioned above, eyelid morphogenesis involves eyelid fusion and reopening. EGFR signaling regulates eyelid fusion, during which proliferation and migration are the two major cellular events. The EGFR ligands are temporal-spatially expressed in the developing eyelids. By *in situ* hybridization, Berkowitz et al. found that expression of *Tgfa* is restricted to the leading edge of the developing mouse eyelid at E15.5-E16.5 (Berkowitz et al., 1996). By measurement of the *lacZ* transgene under the control of the *Hb-egf* promoter, Mine et al., detected activation of the *Hb-egf* promoter at the leading edge of the embryonic eyelid from E15-E16.5 (Mine, Iwamoto, & Mekada, 2005). Although the EGFR is ubiquitously detected in the eyelid epithelial cells, the phosphorylated EGFR is predominantly abundant in the eyelid leading edge where HB-EGF and TGF α are expressed. Immunohistochemistry data suggest that TGF α and HB-EGF may be responsible for activation of the EGFR signaling pathway specifically in cells at the leading edge. Ablation of EGFR ligand, *Tgfa* or *Hb-egf* in mice causes delayed eyelid closure (Berkowitz et al., 1996; Mine et al., 2005).

EGF and TGF α have the ability of promoting keratinocyte proliferation and migration *in vitro and in vivo* (Barrandon & Green, 1987; Hormi & Lehy, 1996). *K14-TGF α* transgenic mice exhibited long filiform projections at the eyelid leading edge, suggesting an increase in cell proliferation and migration capabilities in the induction of TGF α (Vassar & Fuchs, 1991). Intriguingly HB-EGF does not show the proliferative effect on eyelid keratinocytes (Mine et al., 2005) though it does promote proliferation of many cell types such as corneal epithelial cells, pterygium epithelial cells and fibroblasts and mesangial cells (Krampera et al., 2005; Nolan, Di Girolamo, Coroneo, & Wakefield, 2004; Takemura et al., 1999). Mine et al. demonstrated that impeded eyelid fusion in *Hb-egf* mutant mice is independent of cell proliferation but owing to slower migration and reduced actin bundle formation.

B. EGFR signaling in epithelial-mesenchymal interactions of the eyelid

The fast fusion of eyelid relies on epithelial cell proliferation and migration as well as the interactions between the epithelium and underlying mesenchyme. As a mitogen, TGF α can stimulate the proliferation of eyelid mesenchymal cells. In *KR/TGF α* transgenic mice, excess TGF α secreted by keratocytes stimulates hyper proliferation of eyelid tenocytes in eyelid tendon and tarsal plate (Dong et al., 2015). Reneker, et al., using *α A-crystallin-TGF α* transgenic mouse showed that as early as E15, TGF α could act as a chemoattractant to induce periocular mesenchymal cell migration towards the lens from both an anterior and posterior direction (Reneker, Silversides, Patel, & Overbeek, 1995). In situ hybridization revealed *TGF α* expression in the advancing margins of the eyelid epithelium while EGFR mRNA is highly expressed in the periocular mesenchyme, suggesting that the migratory response of eyelid mesenchyme is mediated by receptor activation (Berkowitz et al., 1996). Extracellular matrix (ECM) component, such as biglycan, a proteoglycan, can sequester TGF α and abolish TGF α -induced periocular mesenchymal cell migration in *Kera-Bgn* transgenic mice (Hayashi et al., 2005). In conclusion, EGFR signaling is involved in periocular mesenchyme proliferation, migration and epithelial-mesenchymal interaction.

C. Regulation of EGFR signaling during eyelid morphogenesis

There is an intricate network of signaling pathways controlling cell behavior during development and maintaining homeostasis in the adult. Researchers have found several regulating systems of EGFR signaling pathways for eyelid morphogenesis.

C1. Regulation of ligand expression

ADAM17, EGFR ligand ectodomain shedding enzyme: ADAM17 is an important sheddase of HB-EGF, TGF α , amphiregulin and epiregulin (Blobel, 2005). Similar to these EGFR ligands, ADAM17 expression is at the leading edge of eyelid epithelium and is essential for epithelial cell migration by transactivating EGFR signaling. ADAM17 mutant mice, both the dominant negative *Adam17^{Zn/Zn}* and Adam17 hypomorphic mutation *woe*, manifest EOB phenotype resembling the mice lacking TGF α (or EGFR) (Hassemer et al., 2010; Peschon et al., 1998). The *woe* EOB phenotype is rescued by *Egfr^{Dsk5}* mice, which carry a point mutation in *Egfr*, resulting in constitutively active EGFR signaling.

FGF10/FGFR2 signaling pathway: It is known that FGF10 promotes keratinocyte proliferation at the early stage (~E11.5) of eyelid development and regulate keratinocyte

migration at the late stage (~E15). *Fgf10*-null mice exhibit EOB phenotype and down regulated activin and TGF α expression. Exogenous FGF10 rescued eyelid fusion as well as activin, TGF α and SHH expression in tissue culture suggesting that FGF10 promotes eyelid closure through activating activin and TGF α -EGFR signaling (H. Tao et al., 2005; H. Tao et., 2006).

Biglycan: As described above, biglycan is an ECM component that can sequester TGF α to perturb EGFR activation in eyelid keratinocytes. The defects of EGFR signaling by excess biglycan were further augmented by the interruption of HB-EGF expression elicited by the EGFR signaling pathway (Hayashi et al., 2005).

Other molecules: such as G-protein coupled receptor 48 (GPR48) and the mammalian Grainyhead-like Epithelial Transactivator 1 (Get1) are implicated in eyelid keratinocyte proliferation and migration by regulating EGFR ligands. They are both highly expressed in the eyelid leading edge. Mice lacking *Gpr48* or *Get1* display EOB phenotype and decreased phosphorylation of EGFR (Jin et al., 2008; Yu et al., 2008). In cultured keratinocytes, GPR48-mediated cell proliferation is blocked by EGFR tyrosine kinase inhibitor or HB-EGF inhibitor (Z. Wang et al., 2010). In an organ culture model, exogenous TGF α can increase levels of phospho-EGFR and promote cell shape changes as well as leading edge formation in *Get1*^{-/-} eyelids. Together these data indicate that in eyelid closure GPR48 and Get1 act upstream of EGFR signaling.

C2. EGFR signal transduction and downstream targets of EGFR EGFR/ERK signal transduction—There are three major kinase cascades of the mitogen-activated protein kinases (MAPKs), including the extracellular signal regulated kinases (ERKs), the c-Jun N-terminal kinases (JNKs), and the p38. It has been shown that ERK and MAP3K1 are activated via EGFR signaling. Administration of EGF to HEK293 cells results in pronounced induction of the activated form of MAPK, however it remains to be clarified, which MAPK is activated (Du, 2004). Other studies have demonstrated that HB-EGF and TGF α secreted from the tip of the leading edge activate EGFR signaling, leading to the induction of ERK activation and eyelid epithelial cell migration (Mine et al., 2005).

Activation of the ERK/MAPKinase cascade by EGFR is mediated in part via secondary transactivation of sphingosine-1-phosphate (S1P) receptors (S1PRs). The proposed mechanism involves EGFR-dependent activation of sphingosine kinase (SphK), resulting in the production of S1P, the ligand of the S1PRs. Ligand binding to the receptors leads to activation of ERK and induction of epithelial sheet extension. Ablation of S1PR2 and 3 reduces EGF/ERK signaling and delays epithelial sheet extension (Herr et al., 2013).

EGFR/RhoA/ROCK/MAPK signal transduction: It has been well studied that Rho/ROCK/MAP3K1/JNK signal transduction is responsible for actin cytoskeleton formation and gene expression stimulated by activin B during mouse eyelid morphogenesis (L. Zhang et al., 2005; L. Zhang et al., 2003). Recent studies show TGF α /EGFR signaling could also activate RhoA/ROCK signaling at the eyelid leading edge. Rho-associated kinase 1 (ROCK-1) is an essential regulator of the actin cytoskeleton through stress fiber formation and myosin light chain (MLC) phosphorylation, also causes the EOB phenotype (Shimizu et

al., 2005). Meanwhile, RhoA/ROCK activation upregulates AP-1 activation and downstream gene expression (Geh et al., 2011).

EGFR/Integrin interaction: Evidence available shows that the TGF α effects on cell adhesion and migration may depend on the upregulation of $\alpha 5\beta 1$ integrin and its ligand fibronectin at the eyelid tip as cell motility depends on an optimal level of integrin expression: at lower or higher levels motility is inhibited (Huttenlocher & Horwitz, 2011). Among the large family of integrins, $\alpha 5$ and/or $\beta 1$ integrin are involved in eyelid epithelium migration for mice overexpressing $\alpha 5$ and/or $\beta 1$ integrin subunits are born with open eyes, whereas overexpression of the $\alpha 2$, $\alpha 3$ or $\alpha 6$ subunits are not (J M Carroll, Romero, & Watt, 1995; Joseph M. Carroll, Luetkeke, Lee, & Watt, 1998). Consistent with this observation, in wild type mice, $\alpha 5\beta 1$ integrin and its ligand fibronectin were found exclusively expressed in the migrating eyelid tip, in contrast other integrins, e.g., $\alpha 2\beta 1$, $\alpha 3\beta 1$, $\alpha 6\beta 4$, were not. In *Tgfa* null mouse embryos, expression of $\alpha 5\beta 1$ integrin and fibronectin decreased dramatically at the eyelid tip suggesting that the Egfr signaling pathway regulates integrin expression during eyelid fusion (Joseph M. Carroll et al., 1998).

Downstream targets of EGFR: Activation of EGFR in the eyelid tip ultimately leads to three events: proliferation, migration and downstream gene expression. As described above, EGFR ligands are mitogens t promote proliferation of both keratinocytes and mesenchymal cells in the eyelid tip during eyelid morphogenesis. EGFR signaling promotes migration through F-actin polymerization mediated actin stress fiber formation at the leading edge. The ligand-EGFR mediated mesenchymal-epithelial interaction induces eyelid mesenchymal cell migration. Upon signal transduction, EGFR signaling can lead to ERK or Rho/ROCK mediated AP-1 activation which causes an increase in gene expression including *Hb-egf*, *c-Jun*, *Map3k1*. Increased expression of these molecules provides a regulatory loop, which in turn augments EGFR and related signaling (Geh et al., 2011; Zenz et al., 2003).

IV. EGFR Signaling in Meibomian Gland Morphogenesis

The meibomian gland, consisting of acini and ducts, derive from surface ectoderm. So far, little is known about the molecular and cellular mechanisms of meibomian gland morphogenesis. Events occurring after eyelid closure that direct MG development are for the most part unknown. A few molecules, e.g., sex steroid, all-trans retinoic acid and several growth factors, play regulatory roles on meibomian gland formation during development and maintenance of homeostasis in adult. Based on evidence from in vitro cell culture, EGF may regulate meibomian gland morphogenesis. Adding EGF to cultured, immortalized human meibomian gland epithelial cells results in significant, time-dependent cell proliferation by upregulating genes of the cell cycle, DNA replication, ribosomes, translation and a significant decrease in those related to cell differentiation, tissue development, lipid metabolic processes and peroxisome proliferator-activated receptor signaling (Maskin & Tseng, 1992).

Our previous study shows overexpression of TGF α in postnatal eyelid stromal cells also leads to meibomian gland malformation. Excess TGF α stimulates proliferation but inhibits differentiation of tarsal plate cells, which is one of the eyelid mesenchymal cells and forms

the tarsal plate by their secretion of extracellular matrix. Tarsal plate offers a scaffold for meibomian gland morphogenesis. Hyper proliferative tarsal plate cells triggered by TGF α accumulate around the meibomian glands, therefore perturbing meibomian gland morphogenesis due to improper tarsal plate formation and altered mesenchymal–epithelial interactions (Dong et al., 2015).

Interestingly, Pax6 was found transiently expressed in the postnatal P4-P11 meibomian gland when the epithelial cells begin to differentiate (Call, Fischesser, Lunn, & Kao, 2016). Pax6 previously was found to express in many ocular tissues including the lens, retina, cornea, conjunctiva and lacrimal gland at early stages and regulates their development (Grindley, Davidson, & Hill, 1995). Pax6 is required for the initiation of eyelid formation and for differential development of the keratinized cells in the eyelid (Li Lu, 2005; Shi et al., 2013). It is possible that Pax6 is also involved in meibomian gland epithelial cell differentiation. Further studies are needed to define the role of Pax6 on meibomian morphogenesis during development.

We have recently examined the meibomian morphogenesis in a cohort of genetically modified mouse lines exhibiting various severities of EOB phenotype. Our data suggest that meibomian gland formation requires eyelid closure and fusion during development (J. Wang, et al., 2017, *The Ocular Surface*, in press 2017). The nature of such a large number of mutant genes of various pathways, which manifest EOB phenotypes, remains largely elusive. However, the eyelid fusion requires cell proliferation and migration, thus, any genetic perturbations compromising such cellular activities have adverse effects on eyelid fusion and malformation of meibomian glands as exemplified by the effects of perturbed EGFR signaling on meibomian formation resulting from the failure of eyelid fusion. Future studies should focus on determining the molecular and cellular mechanisms of meibomian formation during development.

V. Conclusion

The EGFR signaling pathway is responsible for keratinocyte and mesenchymal cell proliferation and migration at the eyelid tip during eyelid morphogenesis. For meibomian gland morphogenesis, EGFR signaling is activated to stimulate meibomian gland epithelial cell proliferation. There is an intricate regulatory network and signal transduction of EGFR signaling (Figure 1). 1) EGFR ligands are regulated by multiple molecules and signals from both epithelial and mesenchymal cells including growth factor FGF10 and extracellular matrix component biglycan secreted from stromal cells, sheddase ADAM17 and G-protein coupled receptor GPR48 located in both stromal cells and epithelial cells and transcriptional factor Get1 from epithelial cells. 2) EGFR signaling pathway functions through multiple downstream signals such as EGFR/ERK, EGFR/RhoA/ROCK/MAPK and EGFR regulated integrin expression. 3) The activation of EGFR signaling finally leads to cell proliferation, actin cytoskeleton formation and nuclear transactivation of related genes to regulate eyelid morphogenesis.

Acknowledgement

The study was in part supported by grants NIH/NEI R01-EY011845 (WK) and R01-EY015227 (YX), Research to Prevent Blindness, Ohio Lions Eye Research Foundation.

References

- Barrandon Y, & Green H (1987). Cell migration is essential for sustained growth of keratinocyte colonies: the roles of transforming growth factor-alpha and epidermal growth factor. *Cell*, 50(7), 1131–7. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/3497724> [PubMed: 3497724]
- Berkowitz E. a, Seroogy KB, Schroeder J. a, Russell WE, Evans EP, Riedel RF, ... Luetetke NC (1996). Characterization of the mouse transforming growth factor alpha gene: its expression during eyelid development and in waved 1 tissues. *Cell Growth & Differentiation : The Molecular Biology Journal of the American Association for Cancer Research*, 7(9), 1271–82. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/8877107> [PubMed: 8877107]
- Blobel CP (2005). ADAMs: key components in EGFR signalling and development. *Nature Reviews Molecular Cell Biology*, 6(1), 32–43. <https://doi.org/10.1038/nrm1548> [PubMed: 15688065]
- Call M, Fischesser K, Lunn MO, & Kao WW-Y (2016). A unique lineage gives rise to the meibomian gland. *Molecular Vision*, 22, 168–76. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/26957900> [PubMed: 26957900]
- Carroll JM, Luetetke NC, Lee DC, & Watt FM (1998). Role of integrins in mouse eyelid development: studies in normal embryos and embryos in which there is a failure of eyelid fusion. *Mechanisms of Development*, 78(1), 37–45. [https://doi.org/10.1016/S0925-4773\(98\)00145-2](https://doi.org/10.1016/S0925-4773(98)00145-2) [PubMed: 9858678]
- Carroll JM, Romero MR, & Watt FM (1995). Suprabasal integrin expression in the epidermis of transgenic mice results in developmental defects and a phenotype resembling psoriasis. *Cell*, 83(6), 957–68. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/8521519> [PubMed: 8521519]
- Dominey AM, Wang XJ, King LE, Nanney LB, Gagne TA, Sellheyer K, ... Greenhalgh DA (1993). Targeted overexpression of transforming growth factor alpha in the epidermis of transgenic mice elicits hyperplasia, hyperkeratosis, and spontaneous, squamous papillomas. *Cell Growth & Differentiation : The Molecular Biology Journal of the American Association for Cancer Research*, 4, 1071–1082. [PubMed: 8117621]
- Dong F, Liu C-Y, Yuan Y, Zhang Y, Li W, Call M, ... Kao WWY (2015). Perturbed meibomian gland and tarsal plate morphogenesis by excess TGFα in eyelid stroma. *Developmental Biology*, 406(2), 147–157. <https://doi.org/10.1016/j.ydbio.2015.09.003> [PubMed: 26363126]
- Du X (2004). Velvet, a Dominant Egfr Mutation That Causes Wavy Hair and Defective Eyelid Development in Mice. *Genetics*, 166(1), 331–340. <https://doi.org/10.1534/genetics.166.1.331> [PubMed: 15020428]
- Findlater GS, McDougall RD, & Kaufman MH (1993). Eyelid development, fusion and subsequent reopening in the mouse. *Journal of Anatomy*, 183 (Pt 1), 121–9. Retrieved from <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1259860&tool=pmcentrez&rendertype=abstract> [PubMed: 8270467]
- Foulks GN, & Bron AJ (2003). Meibomian Gland Dysfunction: A Clinical Scheme for Description, Diagnosis, Classification, and Grading. *The Ocular Surface*, 1(3), 107–126. [https://doi.org/10.1016/S1542-0124\(12\)70139-8](https://doi.org/10.1016/S1542-0124(12)70139-8) [PubMed: 17075643]
- Gage PJ, Rhoades W, Prucka SK, & Hjalt T (2005). Fate maps of neural crest and mesoderm in the mammalian eye. *Investigative Ophthalmology & Visual Science*, 46(11), 4200–8. <https://doi.org/10.1167/iovs.05-0691> [PubMed: 16249499]
- Geh E, Meng Q, Mongan M, Wang J, Takatori A, Zheng Y, ... Xia Y (2011). Mitogen-activated protein kinase kinase kinase 1 (MAP3K1) integrates developmental signals for eyelid closure. *Proceedings of the National Academy of Sciences of the United States of America*, 108(42), 17349–54. <https://doi.org/10.1073/pnas.1102297108> [PubMed: 21969564]
- Goto E (2003). Tear Evaporation Dynamics in Normal Subjects and Subjects with Obstructive Meibomian Gland Dysfunction. *Investigative Ophthalmology & Visual Science*, 44(2), 533–539. <https://doi.org/10.1167/iovs.02-0170> [PubMed: 12556380]

- Grindley JC, Davidson DR, & Hill RE (1995). The role of Pax-6 in eye and nasal development Development (Cambridge, England), 121(5), 1433–42. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/7789273> [PubMed: 7789273]
- Hassemer EL, Le Gall SM, Liegel R, McNally M, Chang B, Zeiss CJ, ... Sidjanin DJ (2010). The waved with open eyelids (woe) Locus Is a Hypomorphic Mouse Mutation in Adam17. Genetics, 185(1), 245–255. <https://doi.org/10.1534/genetics.109.113167> [PubMed: 20194968]
- Hayashi Y, Liu C-Y, Jester JJ, Hayashi M, Wang I-J, Funderburgh JL, ... Kao WW-Y (2005). Excess biglycan causes eyelid malformation by perturbing muscle development and TGF-alpha signaling. Developmental Biology, 277(1), 222–34. <https://doi.org/10.1016/j.ydbio.2004.09.022> [PubMed: 15572151]
- Herr DR, Lee C-W, Wang W, Ware A, Rivera R, & Chun J (2013). Sphingosine 1-phosphate receptors are essential mediators of eyelid closure during embryonic development. The Journal of Biological Chemistry, 288(41), 29882–9. <https://doi.org/10.1074/jbc.M113.510099> [PubMed: 24003216]
- Hormi K, & Lehy T (1996). Transforming growth factor-alpha in vivo stimulates epithelial cell proliferation in digestive tissues of suckling rats. Gut, 39(4), 532–8. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/8944561> [PubMed: 8944561]
- Huttenlocher A, & Horwitz AR (2011). Integrins in cell migration. Cold Spring Harbor Perspectives in Biology, 3(9), a005074 <https://doi.org/10.1101/cshperspect.a005074> [PubMed: 21885598]
- Jin C, Yin F, Lin M, Li H, Wang Z, Weng J, ... Tu L (2008). GPR48 Regulates Epithelial Cell Proliferation and Migration by Activating EGFR during Eyelid Development. Investigative Ophthalmology & Visual Science, 49(10), 4245 <https://doi.org/10.1167/iovs.08-1860>
- Krampera M, Pasini A, Rigo A, Scupoli MT, Tecchio C, Malpeli G, ... Vinante F (2005). HB-EGF/HER-1 signaling in bone marrow mesenchymal stem cells: inducing cell expansion and reversibly preventing multilineage differentiation. Blood, 106(1).
- Lemp MA, Crews LA, Bron AJ, Foulks GN, & Sullivan BD (2012). Distribution of aqueous-deficient and evaporative dry eye in a clinic-based patient cohort: a retrospective study. Cornea, 31(5), 472–8. <https://doi.org/10.1097/ICO.0b013e318225415a> [PubMed: 22378109]
- Li T, & Lu L (2005). Epidermal Growth Factor-induced Proliferation Requires Down-regulation of Pax6 in Corneal Epithelial Cells. Journal of Biological Chemistry, 280(13), 12988–12995. <https://doi.org/10.1074/jbc.M412458200> [PubMed: 15659382]
- Luetteke NC, Phillips HK, Qiu TH, Copeland NG, Earp HS, Jenkins NA, & Lee DC (1994). The mouse waved-2 phenotype results from a point mutation in the EGF receptor tyrosine kinase. Genes & Development, 8(4), 399–413. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/8125255> [PubMed: 8125255]
- Luetteke NC, Ting Hu Qiu, Peiffer RL, Oliver P, Smithies O, & Lee DC (1993). TGFα deficiency results in hair follicle and eye abnormalities in targeted and waved-1 mice. Cell, 73, 263–278. [https://doi.org/10.1016/0092-8674\(93\)90228-I](https://doi.org/10.1016/0092-8674(93)90228-I) [PubMed: 8477445]
- Mann GB, Fowler KJ, Gabriel A, Nice EC, Williams RL, & Dunn AR (1993). Mice with a null mutation of the TGFα gene have abnormal skin architecture, wavy hair, and curly whiskers and often develop corneal inflammation. Cell, 73(2), 249–261. [https://doi.org/10.1016/0092-8674\(93\)90227-H](https://doi.org/10.1016/0092-8674(93)90227-H) [PubMed: 8477444]
- Maskin SL, & Tseng SC (1992). Clonal growth and differentiation of rabbit meibomian gland epithelium in serum-free culture: differential modulation by EGF and FGF. Investigative Ophthalmology & Visual Science, 33(1), 205–17. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/1370439> [PubMed: 1370439]
- Meng Q, Mongan M, Carreira V, Kurita H, Liu C, Kao WW-Y, & Xia Y (2014a). Eyelid Closure in Embryogenesis Is Required for Ocular Adnexa Development. Investigative Ophthalmology & Visual Science, 55(11), 7652 <https://doi.org/10.1167/iovs.14-15155>
- Meng Q, Mongan M, Carreira V, Kurita H, Liu C-Y, Kao WW-Y, & Xia Y (2014b). Eyelid closure in embryogenesis is required for ocular adnexa development. Investigative Ophthalmology Visual Science, 55(11), 7652–61. <https://doi.org/10.1167/iovs.14-15155> [PubMed: 25377219]
- Miettinen PJ, Berger JE, Meneses J, Phung Y, Pedersen RA, Werb Z, & Derynck R (1995). Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. Nature, 376(6538), 337–41. <https://doi.org/10.1038/376337a0> [PubMed: 7630400]

- Mine N, Iwamoto R, & Mekada E (2005). HB-EGF promotes epithelial cell migration in eyelid development *Development* (Cambridge, England), 132(19), 4317–26. <https://doi.org/10.1242/dev.02030> [PubMed: 16141218]
- Mohamed YH, Gong H, & Amemiya T (2003). Role of apoptosis in eyelid development. *Experimental Eye Research*, 76(1), 115–23. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/12589781> [PubMed: 12589781]
- Nelson JD, Shimazaki J, Benitez-del-Castillo JM, Craig JP, McCulley JP, Den S, & Foulks GN (2011). The international workshop on meibomian gland dysfunction: report of the definition and classification subcommittee. *Investigative Ophthalmology & Visual Science*, 52(4), 1930–7. <https://doi.org/10.1167/iovs.10-6997b> [PubMed: 21450914]
- Nien CJ, Massei S, Lin G, Liu H, Paugh JR, Liu C-Y, ... Jester JV (2010). The development of meibomian glands in mice. *Molecular Vision*, 16, 1132–40. Retrieved from <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2901193&tool=pmcentrez&rendertype=abstract> [PubMed: 20664693]
- Nolan TM, Di Girolamo N, Coroneo MT, & Wakefield D (2004). Proliferative Effects of Heparin-Binding Epidermal Growth Factor-like Growth Factor on Pterygium Epithelial Cells and Fibroblasts. *Investigative Ophthalmology & Visual Science*, 45(1), 110 <https://doi.org/10.1167/iovs.03-0046>
- Peschon JJ, Slack JL, Reddy P, Stocking KL, Sunnarborg SW, Lee DC, ... Black RA. (1998). An essential role for ectodomain shedding in mammalian development *Science* (New York, N.Y.), 282(5392), 1281–4. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/9812885> [PubMed: 9812885]
- Reneker LW, Silversides DW, Patel K, & Overbeek PA (1995). TGF alpha can act as a chemoattractant to peri optic mesenchymal cells in developing mouse eyes *Development* (Cambridge, England), 121, 1669–1680. [PubMed: 7600984]
- Rubinstein TJ, Weber AC, & Traboulsi EI (2016). Molecular biology and genetics of embryonic eyelid development *Ophthalmic Genetics*, 37(3), 252–259. <https://doi.org/10.3109/13816810.2015.1071409> [PubMed: 26863902]
- Shi F, Fan Y, Zhang L, Meng L, Zhi H, Hu H, ... Cvekl A (2013). The Expression of Pax6 Variants Is Subject to Posttranscriptional Regulation in the Developing Mouse Eyelid. *PLoS ONE*, 8(1), e53919 <https://doi.org/10.1371/journal.pone.0053919> [PubMed: 23326536]
- Shimizu Y, Thumkeo D, Keel J, Ishizaki T, Oshima H, Oshima M, ... Narumiya S (2005). ROCK-I regulates closure of the eyelids and ventral body wall by inducing assembly of actomyosin bundles. *The Journal of Cell Biology*, 168(6), 941–953. <https://doi.org/10.1083/jcb.200411179> [PubMed: 15753128]
- Singh B, Carpenter G, & Coffey RJ (2016). EGF receptor ligands: recent advances. *F1000Research*, 5, 2270 <https://doi.org/10.12688/f1000research.9025.1>
- Takemura T, Murata Y, Hino S, Okada M, Yanagida H, Ikeda M, & Yoshioka K (1999). Heparin-binding EGF-like growth factor is expressed by mesangial cells and is involved in mesangial proliferation in glomerulonephritis. *The Journal of Pathology*, 189(3), 431–438. [https://doi.org/10.1002/\(SICI\)1096-9896\(199911\)189:3<431::AID-PATH460>3.0.CO;2-I](https://doi.org/10.1002/(SICI)1096-9896(199911)189:3<431::AID-PATH460>3.0.CO;2-I) [PubMed: 10547607]
- Tao H, Ono K, Kurose H, Noji S, & Ohuchi H (2006). Exogenous FGF10 can rescue an eye-open at birth phenotype of Fgf10-null mice by activating activin and TGFalpha-EGFR signaling. *Development, Growth and Differentiation*, 48(5), 339–346. <https://doi.org/10.1111/j.1440-169X.2006.00869.x>
- Tao H, Shimizu M, Kusumoto R, Ono K, Noji S, & Ohuchi H (2005). A dual role of FGF10 in proliferation and coordinated migration of epithelial leading edge cells during mouse eyelid development. *Development*, 132(14), 3217–3230. <https://doi.org/10.1242/dev.01892> [PubMed: 15958512]
- Tawfik HA, Abdulhafez MH, Fouad YA, & Dutton JJ (2016). Embryologic and Fetal Development of the Human Eyelid. *Ophthalmic Plastic and Reconstructive Surgery*, 32(6), 407–414. <https://doi.org/10.1097/IOP.0000000000000702> [PubMed: 27124372]
- Vassar R, & Fuchs E (1991a). Transgenic mice provide new insights into the role of TGF-alpha during epidermal development and differentiation. *Genes & Development*, 5, 714–727. <https://doi.org/10.1101/gad.5.5.714> [PubMed: 1709129]

- Vassar R, & Fuchs E (1991b). Transgenic mice provide new insights into the role of TGF- α during epidermal development and differentiation. *Genes & Development*, 5(5), 714–727. <https://doi.org/10.1101/gad.5.5.714> [PubMed: 1709129]
- Wang J, Call M, Mongan M, Kao WW-Y, & Xia Y (2017). Meibomian gland morphogenesis requires developmental eyelid closure and lid fusion. *The Ocular Surface*. <https://doi.org/10.1016/j.jtos.2017.03.002>
- Wang Z, Jin C, Li H, Li C, Hou Q, Liu M, ... Tu L (2010). GPR48-Induced keratinocyte proliferation occurs through HB-EGF mediated EGFR transactivation. *FEBS Letters*, 584(18), 4057–4062. <https://doi.org/10.1016/j.febslet.2010.08.028> [PubMed: 20732323]
- Yu Z, Bhandari A, Mannik J, Pham T, Xu X, & Andersen B (2008). Grainyhead-like factor Get1/Grhl3 regulates formation of the epidermal leading edge during eyelid closure. *Developmental Biology*, 319(1), 56–67. <https://doi.org/10.1016/j.ydbio.2008.04.001> [PubMed: 18485343]
- Zenz R, Scheuch H, Martin P, Frank C, Eferl R, Kenner L, ... Wagner EF (2003). c-Jun regulates eyelid closure and skin tumor development through EGFR signaling. *Developmental Cell*, 4(6), 879–89. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/12791272> [PubMed: 12791272]
- Zhang L, Deng M, Parthasarathy R, Wang L, Mongan M, Molkentin JD, ... Xia Y (2005). MEKK1 Transduces Activin Signals in Keratinocytes To Induce Actin Stress Fiber Formation and Migration. *Molecular and Cellular Biology*, 25(1), 60–65. <https://doi.org/10.1128/MCB.25.1.60-65.2005> [PubMed: 15601830]
- Zhang L, Wang W, Hayashi Y, Jester JV, Birk DE, Gao M, ... Xia Y (2003). A role for MEK kinase 1 in TGF- β /activin-induced epithelium movement and embryonic eyelid closure. *The EMBO Journal*, 22(17), 4443–54. <https://doi.org/10.1093/emboj/cdg440> [PubMed: 12941696]

Highlights

EGFR signaling plays a pivotal role in eyelid morphogenesis and homeostasis

Signaling cascades of cell proliferation and migration regulate eyelid morphogenesis

Eyelid fusion is a prerequisite for meibomian gland formation

Cross talks of multiple signaling cascades regulate eyelid formation

FIGURE 1. Summary of EGFR signaling on eyelid and meibomian gland morphogenesis.

The EGFR signaling pathway is responsible for keratinocyte and mesenchymal cell proliferation and migration at the eyelid tip and meibomian gland epithelial cell proliferation. Ligands binding EGFR activates EGFR/ERK and EGFR/RhoA/ROCK/MAPK cascades and results in epithelial cell and eyelid stromal cell proliferation and migration at the tip of eyelid. The activation of EGFR upon ligand binding can activate α5β1 integrin and sphingosine-1-phosphate receptors S1PR, which mediates epithelial cell binding to ECM and cell proliferation via ERK signaling cascades (black arrows). Perturbations of the signaling cascades due to genetic mutations manifest Eye Open at Birth (EOB) phenotype and malformation of meibomian glands. EGFR ligands are regulated by multiple molecules and signals from both epithelial and mesenchymal cells. Adam 17, the sheddase, releases the membrane-bound EGFR ligands such as TGFα and Hb-EGF that serve as paracrine and autocrine to epithelial cells. Growth factor FGF10 and extracellular matrix component biglycan secreted from stromal cells modulate the expression of ligands through mesenchyme/epithelium interaction which contributes to eyelid fusion and formation of meibomian glands (blue arrows). The activation of EGFR signaling finally leads to cell proliferation, actin cytoskeleton formation and nuclear transactivation of related genes such as c-Jun, MAP3K1 and HB-EGF to regulate eyelid morphogenesis.