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The signaling pathways in tissue morphogenesis: a lesson from mice with eye-open at birth phenotype

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Abstract

Tissue morphogenesis during development is regulated by growth factors and cytokines, and is characterized by constant remodeling of extracellular matrix in response to signaling molecules. MEK kinase 1 (MEKK1) is a mitogen-activated protein kinase (MAPK) kinase kinase originally identified as an upstream activator for several MAPK pathways. During mouse embryogenesis, MEKK1 controls cell shape changes and formation of actin stress fibers that are required for sealing epidermis in the embryos in a process known as eyelid closure. MEKK1-null mice display eye-open at birth (EOB), a phenotype found also in mice impaired in activin, a subgroup of the transforming growth factor β (TGFβ) family, or in epidermal growth factor receptor (EGFR) or its ligand TGFα, or in transcription factor c-Jun. Molecular analyses have revealed at least two signaling mechanisms in the control of eyelid closure. One is originated from the activins and is transduced through MEKK1, leading to transcription-independent actin stress fiber formation and transcription-dependent keratinocyte migration. Another is the TGFα/EGFR signal that is transduced through a MEKK1-independent pathway to the activation of the ERK MAPK, which also leads to keratinocyte migration. c-Jun might serve as a connection between the two pathways. As embryonic eyelid closure is a specific morphogenetic process that is easily detectable, genetic mutant mice with EOB will be ideal models to understand the signaling mechanisms in the control of epithelial cell migration and the morphogenetic process of epithelial sheet movement.

Keywords: TGFβ; Activin; TGFα; EGFR; MEKK1; JNK; p38; ERK; c-Jun; Smad; Transcription; Eyelid closure; Epithelial cell migration; Epithelial sheet movement; Actin stress fiber

1. Introduction

In mammals, there are three major groups of the MAPKs, including the extracellular signal regulated kinases (ERKs), the c-Jun N-terminal kinases (JNKs), and the p38 [1]. The MAPK activities are controlled by a three-component signal transduction cascade, composed of a MAPKKK, a MAPKK and a MAPK. The MAPKKK receives activation signals from upstream cues and in turn phosphorylates and activates the MAPKK and thereafter the MAPK. The specificity of a MAPK module is mostly provided at the level of the MAPKKKs, consisting of more than 20 protein kinases that share conserved kinase domains but divergent regulatory regions. This property allows individual MAPKKKs to receive designated signals through their regulatory domains interacting with upstream factors [2–6] and to activate the downstream MAPKK–MAPK through their kinase domain [7–10]. The activated MAPKs phosphorylate effector molecules in cytoplasm and nucleus that become ultimately responsible for changes of cell functions [11].

Although in vitro studies failed to unequivocally define a role for each MAPKKK in MAPK signaling [12–14], genetic knockout of the MAPKKKs in mice have revealed their specific and limited functions. The MEKK3-null fetuses die at early embryonic stages because of impairment in placentation and blood vessel formation [15]. MEKK2 ablation in mice does not affect embryonic development, but it reduces JNK activation in response to T cell receptor engagement, thus impairing T cell function [16]. $Ask^{-/-}$ mice also appear normal; however, fibroblasts

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derived from them are partially resistant to H_2O_2-induced apoptosis [17]. These findings strongly suggest that each MAPKKK confers specificity to MAPK signaling and subsequent biological function.

2. Molecular factors involved in mouse eyelid development and closure

MEKK1 was originally isolated as a mammalian homolog of the yeast MAPKKK STE11 [18]. Although overexpression studies have connected MEKK1 to many cellular activities [13], the endogenous MEKK1 exhibits rather specific functions. MEKK1-null cells are only slightly affected in survival under stress conditions, but are obviously impaired in cell migration [19,20]. Mice lacking either the kinase domain (*Mekk1*$^{\Delta KD/\Delta KD}$) or the entire MEKK1 polypeptide (*Mekk1*$^{-/-}$) complete embryonic development, are born with relatively normal appearance and are fertile, but display an EOB phenotype [19,21].

The development of the eyelid initiates at E13.5 with eyelid folds that extend over the cornea and move toward the center of the eye, with eyelid closure accomplished at E15.5–16.5. This process is followed by fusion of the eyelid epidermis to form a closed eyelid that covers the ocular surface, which serves as a protective barrier crucial for normal eye development [22,23]. Mouse embryonic eyelid closure requires morphogenetic changes of the epithelium. The involvement of MEKK1 in this process is suggested by its abundant expression in the developing tip of the eyelid epithelium, with very little if any MEKK1 protein being detected in the eyelid stroma. Interestingly, MEKK1-null fetuses also display a complete abolishment in the N-terminal phosphorylation of c-Jun, a well-known phosphorylation target of the JNK MAPK, suggesting the requirement for MEKK1 in JNK activation and the latter might also be critical for eyelid development and closure [21].

The mammalian JNK family consists of several polypeptides encoded by three genes; two of them, JNK1 and JNK2, are ubiquitously expressed, whereas the third, JNK3, is restricted to the brain, heart and testis [24]. A role for JNK in ocular tissue morphogenesis was first suggested in mice with compound ablation of JNK1 and JNK2. Mice lacking individual *Jnk* genes or missing one *Jnk1* and both *Jnk2* alleles (*Jnk1*$^{+/-}$ *Jnk2*$^{-/-}$) appear to develop normally [25]; however, mice with a single *Jnk2* allele and no *Jnk1* (*Jnk1*$^{-/-}$ *Jnk2*$^{+/-}$) exhibit a number of developmental defects of the eye, including open eye, small lenses, and retinal coloboma, and only 20% of them survive to adulthood [26]. As far as the eyelid closure is concerned, JNK1 plays a more crucial role than JNK2.

One possible downstream target of JNK is the nuclear c-Jun, the transcriptional activity of which is markedly enhanced upon phosphorylation by JNK [27]. c-Jun phosphorylation alone, however, does not seem to be essential for eyelid closure, because mice harboring a mutant allele of c-Jun, coding for a variant in which JNK phosphoacceptor serines were changed to alanines (JunAA), display normal eyelid development [28]. Possibly, c-Jun phosphorylation by JNK provides a permissive state, but is not essential for the eyelid closure to take place. The critical control of eyelid development by JNK might be attributed to phosphorylation of other cellular targets.

Nevertheless, the presence of c-Jun protein in the epidermis is essential, as its skin-specific ablation in mouse results in the EOB phenotype [29,30]. This role of c-Jun might be accomplished through controlling the expression of several essential genes, for without c-Jun, there are reduced levels of epidermal growth factor receptor (EGFR) and its ligand HB-EGF in the epidermis [29]. Excess expression of biglycan, a proteoglycan-type extracellular matrix, also leads to defective eyelid morphogenesis, likely resulted from biglycan binding and sequestering TGFα, thus interrupting the EGFR signaling pathways. It is well known that the integrity of the EGFR signaling is important for eyelid closure, because its deficiency in *wa-1*, TGFα-null and EGFR-null mice is associated with EOB [31–34]. An impaired EGFR signaling is probably responsible, at least in part, for the defects in eyelid development of the c-Jun knockout and biglycan transgenic mice.

Several lines of evidence suggest that embryonic eyelid closure requires also the activin of the TGFβ family. Mice with impaired activin signaling, including knocking out activin βB, transgenically overexpressing follistatin, a ubiquitous antagonist of activin, or expressing Smad 7, a cytoplasmic inhibitor of the TGFβ signaling, all develop EOB [35–37]. It is noteworthy, however, that not all EOB in mice are caused by defect in eyelid closure. Mice with truncational mutation of fibroblast growth factor receptor 2 (FGFR2) although exhibit EOB, they completely lack the eyelids. It turns out that the FGFR2 is effective at E11.5, a very early stage of eyelid development, and its mutation perturbs epithelial cell proliferation and completely prevents eyelid bud formation [38]. Hence, despite of the similarity in the EOB phenotype, the FGFR2 mutants are defective in eyelid development distinct from the mice that are impaired in eyelid closure.

3. Signaling mechanisms in the control of keratinocyte migration and embryonic eyelid closure

As genetic defects in various signaling factors all lead to a specific impairment in eyelid closure, it is of interest to find how these factors coordinate in action and what cellular activity they control that is critical for the morphogenetic development of the eyelid. Recent studies of the MEKK1- and keratinocyte-specific c-Jun-deficient mice [21,30] have uncovered some cell activities that are instrumental for eyelid closure. Eyelid closure is

associated with cell shape changes, and formation of actin stress fiber and filopodia in the developing eyelid epithelium, all key features for epithelial cell migration. MEKK1 ablation prevents cell shape change and F-actin formation, while c-Jun ablation in the epidermis reduces the filopodia in the developing eyelid. These observations suggest that eyelid closure needs the movement of epithelial cells, the impairment of which is likely responsible for EOB. If the problems with eyelid closure were primarily due to impaired epithelial cell migration, such defects would be found in keratinocytes isolated from the EOB mice. Indeed, both c-Jun- and MEKK1-knockout keratinocytes show defects in migration in response to specific stimuli, while c-Jun-deficient keratinocytes also exhibit altered proliferation and differentiation programs, similar to what is observed with EGFR-knockout cells [21,29,30,39].

Examination of the molecular mechanisms of keratinocyte migration has revealed the involvement of at least two signaling pathways (Fig. 1). One of these is the activin/TGFβ signals, transduced through RhoA to MEKK1 and JNK, leading to the transcription-independent induction of actin stress fiber formation and, through a yet to be defined RhoA-independent mechanism of MEKK1 activation, to the activation of the p38 MAPKs. The latter, although not required for F-actin formation, is essential for the transcription-dependent induction of epithelial cell migration. Importantly, activin/TGFβ signals transduced by MEKK1

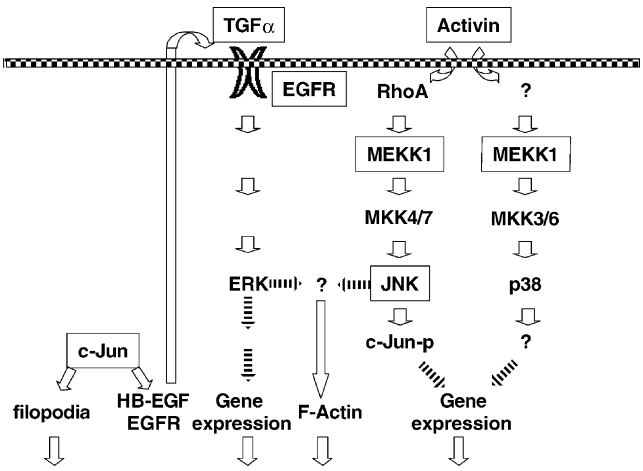

Fig. 1. The molecular pathways in the control of mouse epithelial sheet movement and eyelid closure. In the eyelid epithelium, at least two pathways are critical for actin polymerization and epithelial sheet movement: (1) the c-Jun-controlled expression of HB-EGF and EGFR, leading to activation of the ERK pathway; (2) the TGFβ/activin-induced MEKK1-JNK pathway, which induces actin polymerization and c-Jun phosphorylation. Both pathways are essential for eyelid closure and they may be connected through transcription factor c-Jun. c-Jun regulated filopodia might be of importance for eyelid fusion. The signaling factors whose ablation or perturbation lead to EOB in mice are boxed, the unknown factors in these pathways are denoted as question marks, the open arrows indicate the pathways or cell functions demonstrated by experimental data and the shaded arrows represent hypothetic pathways that are yet to be established.

do not involve the classical pathway that leads to the activation of the SMAD proteins, but is needed for c-Jun phosphorylation [21] [Zhang et al., unpublished results]. As inhibition of Smad activation by overexpressing Smad 7 also causes EOB, it is likely that as far as the regulation of eyelid closure is concerned, the TGFβ/activin signals are mediated through both a Smad-dependent pathway and a Smad-independent but MEKK1-dependent pathway.

The other pathway appears to be originated from the TGFα-induced EGFR activation, the signal of which is transduced via an MEKK1-independent mechanism to activate the ERK MAPK, leading to also the induction of actin stress fiber formation and cell migration [21]. Initiation of the TGFα/EGFR-ERK pathway might depend on c-Jun needed for the expression of an EGFR ligand in the eyelid epithelium. By phosphorylation of the c-Jun activation domain in the leading edge of the eyelid epithelium, the MEKK1-JNK cascade might contribute to up-regulation of the TGFα/EGFR-ERK pathway in this particular location, despite that c-Jun N-terminal phosphorylation itself may not be sufficient for initiation of this pathway [21].

In cultured keratinocytes, both the TGFβ/activin-MEKK1-JNK/p38 and the TGFα/EGFR-ERK pathways are fully functional and activation of either leads to actin stress fiber formation and cell migration [21]; however, in vivo both pathways are required, because knocking out a component of either pathway results in an EOB phenotype. How these pathways are coordinated in vivo during the morphogenetic process of eyelid closure is still a mystery. It is possible that in the developing mouse eyelid, one pathway may be activated in a cell-type-specific fashion to generate a signal that initiates epithelial movement. This signal may later be enhanced by activation of yet other signaling pathways through autocrine or paracrine mechanisms.

4. Conclusion

We learn from studies of the EOB mice that embryonic eyelid closure requires at least two signaling pathways, involving TGFβ/activin-MEKK1-JNK/p38 and TGFα/EGFR-ERK (Fig. 1). The known end point of the former pathway is the actin stress fiber formation and the phosphorylation of nuclear factor c-Jun, the expression or activity of which might be of importance for the induction of EGFR and the activation of the second pathway. Both pathways are involved in eyelid closure, likely through the regulation of migration, although some factors in these pathways might also function through altering proliferation and differentiation programs of the epithelial cells.

Mouse embryonic eyelid closure, involving the movement of epithelial sheets, is a tissue morphogenetic process that takes place also in many physiological or pathological

procedures. The signaling pathways controlling eyelid closure might also be effective in epidermal wound healing and tumorigenesis, both requiring epithelial cell migration and morphogenesis. It is therefore not surprising to find that impairment in activin signaling causes delayed healing of a skin wound [36] and that c-Jun-null mice show reduced skin wound healing and tumor formation, similar to mice deficient in TGFα or EGFR signaling [29,30,40]. Hence, molecular factors important for eyelid closure might serve as potential targets for pharmaceutical intervention for diseases that result from deregulation of epithelial cell migration and morphogenesis. It is noteworthy that there are many additional contributory factors, including cytokines and chemotractants, to a complex pathophysiological condition such as tumorigenesis. The next challenge is to identify molecular targets, the perturbation of which would alter specific epithelial cell functions with minimal side effects. In this regard, the EOB mice with known gene defects would serve as an ideal model to provide a wealth of information about specificity of the gene function, or the lack thereof.

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References

[1] Chang L, Karin M. Mammalian MAP kinase signalling cascades. Nature 2001;410:37–40.
[2] Russell M, Lange-Carter CA, Johnson GL. Direct interaction between Ras and the kinase domain of mitogen-activated protein kinase kinase kinase (MEKK1). J Biol Chem 1995;270:11757–60.
[3] Christerson LB, Gallagher E, Vanderbilt CA, Whitehurst AW, Wells C, Kazempour R, et al. p115 Rho GTPase activating protein interacts with MEKK1. J Cell Physiol 2002;192:200–8.
[4] Fanger GR, Johnson NL, Johnson GL. MEK kinases are regulated by EGF and selectively interact with Rac/Cdc42. EMBO J 1997;16: 4961–72.
[5] Su YC, Han J, Xu S, Cobb M, Skolnik EY. NIK is a new Ste20-related kinase that binds NCK and MEKK1 and activates the SAPK/JNK cascade via a conserved regulatory domain. EMBO J 1997;16: 1279–90.
[6] Pomerance M, Multon MC, Parker F, Venot C, Blondeau JP, Tocque B, et al. Grb2 interaction with MEK-kinase 1 is involved in regulation of Jun-kinase activities in response to epidermal growth factor. J Biol Chem 1998;273:24301–4.
[7] Xia Y, Wu Z, Su B, Murray B, Karin M. JNKK1 organizes a MAP kinase module through specific and sequential interactions with upstream and downstream components mediated by its amino-terminal extension. Genes Dev 1998;12:3369–81.
[8] Cheng J, Yang J, Xia Y, Karin M, Su B. Synergistic interaction of MEK kinase2, c-Jun N-terminal kinase (JNK) kinase 2, and JNK1 results in efficient and specific JNK1 activation. Mol Cell Biol 2000;20: 2334–42.
[9] Nihalani D, Meyer D, Pajni S, Holzman LB. Mixed lineage kinase-dependent JNK activation is governed by interactions of scaffold protein JIP with MAPK module components. EMBO J 2001;20:3447–58.
[10] Nakamura K, Johnson GL. PB1 domains of MEKK2 and MEKK3 interact with the MEK5 PB1 domain for activation of the ERK5 pathway. J Biol Chem 2003;278:36989–92.
[11] Cobb MH, Goldsmith EJ. How MAP kinases are regulated. J Biol Chem 1995;270:14843–6.
[12] Minden A, Karin M. Regulation and function of the JNK subgroup of MAP kinases. Biochim Biophys Acta 1997;1333:85–104.
[13] Schlesinger TK, Fanger GR, Yujiri T, Johnson GL. The TAO of MEKK. Front Biosci 1998;3:1181–6.
[14] Hagemann C, Blank JL. The ups and downs of MEK kinase interactions. Cell Signal 2001;13:863–75.
[15] Yang J, Boerm M, McCarty M, Bucana C, Fidler IJ, Zhuang Y, et al. Mekk3 is essential for early embryonic cardiovascular development. Nat Genet 2000;24:309–13.
[16] Su B, Cheng J, Yang J, Guo Z. MEKK2 is required for T-cell receptor signals in JNK activation and interleukin-2 gene expression. J Biol Chem 2001;276:14784–90.
[17] Tobiume K, Matsuzawa A, Takahashi T, Nishitoh H, Morita K, Takeda K, et al. ASK1 is required for sustained activations of JNK/p38 MAP kinases and apoptosis. EMBO Rep 2001;2:222–8.
[18] Lange-Carter CA, Pleiman CM, Gardner AM, Blumer KJ, Johnson GL. A divergence in the MAP kinase regulatory network defined by MEK kinase and Raf. Science 1993;260:315–9.
[19] Yujiri T, Ware M, Widmann C, Oyer R, Russell D, Chan E, et al. MEK kinase 1 gene disruption alters cell migration and c-Jun NH2-terminal kinase regulation but does not cause a measurable defect in NF-kappa B activation. Proc Natl Acad Sci USA 2000;97:7272–7.
[20] Xia Y, Makris C, Su B, Li E, Yang J, Nemerow GR, et al. MEK kinase 1 is critically required for c-Jun N-terminal kinase activation by proinflammatory stimuli and growth factor-induced cell migration. Proc Natl Acad Sci USA 2000;97:5243–8.
[21] Zhang L, Wang W, Hayashi Y, Jester JV, Birk DE, Gao M, et al. A role for MEK kinase 1 in TGF-beta/activin-induced epithelium movement and embryonic eyelid closure. EMBO J 2003;22:4443–54.
[22] Harris MJ, Juriloff DM. Eyelid development and fusion induced by cortisone treatment in mutant, lidgap-Miller, foetal mice. A scanning electron microscope study. J Embryol Exp Morphol 1986;91:1–18.
[23] Findlater GS, McDougall RD, Kaufman MH. Eyelid development, fusion and subsequent reopening in the mouse. J Anat 1993;183:121–9.
[24] Davis RJ. Signal transduction by the JNK group of MAP kinases. Cell 2000;103:239–52.
[25] Sabapathy K, Kallunki T, David JP, Graef I, Karin M, Wagner EF. c-Jun NH_2-terminal kinase (JNK)1 and JNK2 have similar and stage-dependent roles in regulating T cell apoptosis and proliferation. J Exp Med 2001;193:317–28.
[26] Weston CR, Wong A, Hall JP, Goad ME, Flavell RA, Davis RJ. JNK initiates a cytokine cascade that causes Pax2 expression and closure of the optic fissure. Genes Dev 2003;17:1271–80.
[27] Hunter T, Karin M. The regulation of transcription by phosphorylation. Cell 1992;70:375–87.
[28] Behrens A, Sibilia M, Wagner EF. Amino-terminal phosphorylation of c-Jun regulates stress-induced apoptosis and cellular proliferation. Nat Genet 1999;21:326–9.
[29] Li G, Gustafson-Brown C, Hanks SK, Nason K, Arbeit JM, Pogliano K, et al. c-Jun is essential for organization of the epidermal leading edge. Dev Cell 2003;4:865–77.
[30] Zenz R, Scheuch H, Martin P, Frank C, Eferl R, Kenner L, et al. c-Jun regulates eyelid closure and skin tumor development through EGFR signaling. Dev Cell 2003;4:879–89.
[31] Luetteke NC, Qiu TH, Peiffer RL, Oliver P, Smithies O, Lee DC. TGF alpha deficiency results in hair follicle and eye abnormalities in targeted and waved-1 mice. Cell 1993;73:263–78.

[32] Mann GB, Fowler KJ, Gabriel A, Nice EC, Williams RL, Dunn AR. Mice with a null mutation of the TGF alpha gene have abnormal skin architecture, wavy hair, and curly whiskers and often develop corneal inflammation. Cell 1993;73:249–61.
[33] Sibilia M, Wagner EF. Strain-dependent epithelial defects in mice lacking the EGF receptor. Science 1995;269:234–8.
[34] Threadgill DW, Dlugosz AA, Hansen LA, Tennenbaum T, Lichti U, Yee D, et al. Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. Science 1995;269:230–4.
[35] Vassalli A, Matzuk MM, Gardner HA, Lee KF, Jaenisch R. Activin/inhibin beta B subunit gene disruption leads to defects in eyelid development and female reproduction. Genes Dev 1994;8:414–27.
[36] Wankell M, Munz B, Hubner G, Hans W, Wolf E, Goppelt A, et al. Impaired wound healing in transgenic mice overexpressing the activin antagonist follistatin in the epidermis. EMBO J 2001;20:5361–72.
[37] He T, Stepulak A, Holmstrom TH, Omary MB, Eriksson JE. The intermediate filament protein keratin 8 is a novel cytoplasmic substrate for c-Jun N-terminal kinase. J Biol Chem 2002;277:10767–74.
[38] Li C, Guo H, Xu X, Weinberg W, Deng CX. Fibroblast growth factor receptor 2 (Fgfr2) plays an important role in eyelid and skin formation and patterning. Dev Dyn 2001;222:471–83.
[39] Sibilia M, Steinbach JP, Stingl L, Aguzzi A, Wagner EF. A strain-independent postnatal neurodegeneration in mice lacking the EGF receptor. EMBO J 1998;17:719–31.
[40] Sibilia M, Fleischmann A, Behrens A, Stingl L, Carroll J, Watt FM, et al. The EGF receptor provides an essential survival signal for SOS-dependent skin tumor development. Cell 2000;102:211–20.