Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 315 (2004) 73–78
BBRC
www.elsevier.com/locate/ybbrc

The ectodermal dysplasia receptor represses the Lef-1/β-catenin-dependent transcription independent of NF-κB activation☆

Masahisa Shindo[a,b] and Preet M. Chaudhary[a,*]

[a] *The Hamon Center for Therapeutic Oncology Research and Division of Hematology-Oncology, UT Southwestern Medical Center, Dallas, TX 75390-8593, USA*

[b] *Department of Dermatology, Faculty of Medicine, Tottori University, 86 Nishi-machi, Yonago, Tottori 683-8504, Japan*

Received 5 December 2003

Abstract

EDAR plays a key role in the process of ectodermal differentiation via activation of the NF-κB pathway. We present evidence that EDAR also represses Lef-1/β-catenin-dependent transcription and this ability is defective in EDAR mutants associated with anhidrotic ectodermal dysplasia. While IKK1/IKKα and IKK2/IKKβ are required for EDAR-induced NF-κB activation, they are dispensable for its ability to repress Lef-1/β-catenin-dependent transcription. In contrast, NIK is not involved in EDAR-induced NF-κB activation or Lef-1/β-catenin transcriptional repression. As Lef-1/β-catenin pathway controls the expression of EDAR ligand, ectodysplasin-A (EDA), our results point to a negative feedback regulation of EDA–EDAR axis.

Keywords: EDAR; EDA; NF-κB; β-Catenin; Lef-1; Ectodermal dysplasia

Ectodermal dysplasia receptor (EDAR) is a recently isolated member of the TNFR family which plays a critical role in the development of several ectodermal organs in humans and mice [1–3]. Mutations in EDAR have been found in several patients with anhidrotic ectodermal dysplasia and in *downless* mice [1,2]. We and others have previously demonstrated that EDAR is capable of activating the NF-κB pathway and this activity is defective in EDAR mutants found in patients with anhidrotic ectodermal dysplasia and *downless* mice [4–6]. An important role of the NF-κB pathway in ectodermal differentiation and hair-follicle morphogenesis is also suggested by the discovery of mutations in NEMO/IKKγ, a key regulatory protein of the NF-κB pathway in patients with X-linked ectodermal dysplasia and immune deficiency [7–9].

β-Catenin is the mammalian homologue of the *Drosophila armadillo* protein. The β-catenin/Lef-1 signal transduction pathway plays a central role in normal embryonic development and in malignant transformation of many types of human cells [10]. Under normal conditions of growth, β-catenin is found associated with adenomatous polyposis coli tumor suppressor protein (APC), axin or conductin, protein phosphatase 2A, dishevelled and glycogen synthase kinase (GSK3) [10]. In this complex, GSK3 constitutively phosphorylates β-catenin at a set of regulatory amino-terminal Ser/Thr residues, thereby marking it for ubiquitination and for subsequent proteasomal-mediated degradation [11,12]. The β-catenin pathway is activated by Wnt. The binding of the Wnt proteins to their receptor, frizzled, stabilizes β-catenin by inhibiting the activity of a serine/threonine kinase GSK-3β [13,14]. An accumulation of free β-catenin results in its translocation into the nucleus where it binds to lymphoid enhancer factor 1 (Lef-1) and T cell factor (TCF) transcription factor and activates Lef-1/TCF-dependent transcription [15].

☆ *Abbreviations:* EDAR, ectodermal dysplasia receptor; TNF, tumor necrosis factor; TNFR, TNF receptor; NF-κB, nuclear factor-κB; EDA, ectodysplasin A; NIK, NF-κB-inducing kinase; IKK, IκB kinase; TRAF, TNFR-associated factor; EDARADD, Edar-associated death domain; LEF, lymphoid enhancer factor; TCF, T cell factor; MEF, murine embryonic fibroblast.

* Corresponding author. Fax: 1-214-648-4940.
E-mail address: preet.chaudhary@utsouthwestern.edu (P.M. Chaudhary).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.01.025

The importance of β-catenin and Lef-1 in hair follicle organogenesis is suggested by several lines of evidence. Lef-1 is expressed in epithelial and mesenchymal compartments as early as the ectodermal placode stage [16]. *Lef-1* null animals display a marked reduction of hair follicles and complete lack of whiskers, suggesting that Lef-1 may be an essential factor in generation of secondary hair follicles [17]. Transgenic mice overexpressing Lef-1 or a constitutively active form of β-catenin in the ectoderm display signs of de novo hair follicle morphogenesis [16,18]. Binding sites for Lef-1/TCF are found in a group of hair-specific promoters active in the cortex and cuticle of postnatal hair follicles [16]. Finally, Lef-1 was recently shown to control the expression of ectodysplasin A, the EDAR ligand [19,20].

Given the fact that both EDAR and Lef-1/β-catenin signaling pathways play important roles in ectodermal differentiation and hair follicle morphogenesis, we have examined the cross-talk between these two pathways. We report that EDAR signaling represses Lef-1/β-catenin-dependent transcriptional activity independent of its effect on the NF-κB pathway and this repression is defective in EDAR mutants associated with anhidrotic ectodermal dysplasias.

Materials and methods

Cell lines and plasmids. 293T cells were obtained from Dr. David Han (University of Washington, Seattle, WA). MEF cells deficient in NIK, IKK1, and IKK2 have been described previously and were obtained from Drs. Robert D. Schreiber, Richard Gaynor, and Inder Verma, respectively [21–23]. Constructs encoding EDAR, its deletion, and point mutants and super-repressor form of IκBα have been described previously [4]. TOPFLASH and FOPFLASH reporter plasmids were obtained from Dr. Richard Gaynor.

Luciferase reporter assays. 293T cells were transfected in duplicate in each well of a 24-well plate with 500 ng of empty vector or an EDAR-encoding plasmid along with plasmids encoding either Lef-1 (25 ng), β-catenin (250 ng) or both. TOPFLASH (50 ng/well) and RSV-lacZ (40 ng/well) reporter plasmids were transfected as well and the total amount of transfected DNA was kept constant by adding empty vector. Twenty-four to thirty hours later, cells were lysed and extracts were used for the measurement of luciferase and β-galactosidase activities, respectively. Luciferase activity was normalized relative to β-galactosidase activity to control for the difference in the transfection efficiency. Essentially a similar procedure was used for transfection of MEF cells except Lipofectamine2000 (Invitrogen) was used for transfection and a plasmid encoding *Renilla* luciferase (phRG-TK; Promega) was used for control of transfection efficiency. NF-κB luciferase reporter assay was performed as described previously [4].

Results and discussion

EDAR represses the Lef-1/β-catenin-dependent transcription

Members of the TNFR family can be activated in a ligand-independent fashion upon transient-transfection based over-expression [24,25]. In order to study the cross-talk between the EDAR and Lef-1/β-catenin signaling pathways we used the TOPFLASH luciferase reporter plasmid to assay for the transcriptional activation of the Lef-1/β-catenin pathway. This plasmid contains four consensus Lef-1/Tcf binding motifs and a minimal Fos promoter upstream of the luciferase reporter gene [26]. As shown in Fig. 1A, transfection of an EDAR plasmid along with β-catenin and Lef-1 expression constructs in 293T cells led to significant inhibition of the TOPFLASH reporter activity. However, transfection of TOPFLASH reporter plasmid without β-catenin and Lef-1 failed to significantly increase the luciferase reporter activity, which was not blocked by EDAR, thereby arguing against the possibility that EDAR leads to non-specific transcriptional suppression (Fig. 1A). As another control, we also transfected a control reporter plasmid (FOPFLASH) which lacks the Lef-1 binding sites [26]. Transfection of this reporter led to an extremely low level of luciferase activity which was not inhibited by EDAR, suggesting that the observed effect was due to inhibition of Lef-1-mediated transcription (data not shown).

Defect in Lef-1/β-catenin transcriptional repression by EDAR mutants associated with anhidrotic ectodermal dysplasia

Next we tested whether two EDAR mutations seen in association with anhidrotic ectodermal dysplasia could affect the ability of EDAR to represss the Lef-1/β-catenin pathway. The E379K mutation is an autosomal-recessive mutation in the death domain of murine EDAR and is responsible for the spontaneous *downless*^{Jackson} phenotype, whereas the R420Q mutation has been detected in the death domain of human EDAR in a family with autosomal-dominant anhidrotic ectodermal dysplasia [1,2]. As shown in Fig. 1B, whereas the E379K mutant retained significant residual ability to repress the Lef-1/β-catenin activity, the R420Q mutant demonstrated a significant loss of this inhibitory activity. Consistent with the published reports [4–6], the E379Kmutant also retained significant ability to activate the NF-κB pathway while the R420Q mutant demonstrated a significant loss of this activity (Fig. 1C). Thus, EDAR mutants seen in association with anhidrotic ectodermal dysplasia have defect in both NF-κB activation and Lef-1/β-catenin transcriptional repression.

Role of death domain in EDAR-induced Lef-1/β-catenin repression

EDAR possesses a region in its cytoplasm with weak homology to the classical death domain present in the death receptors [1,2]. The death domain of EDAR has been shown to play a crucial role in NF-κB activation by

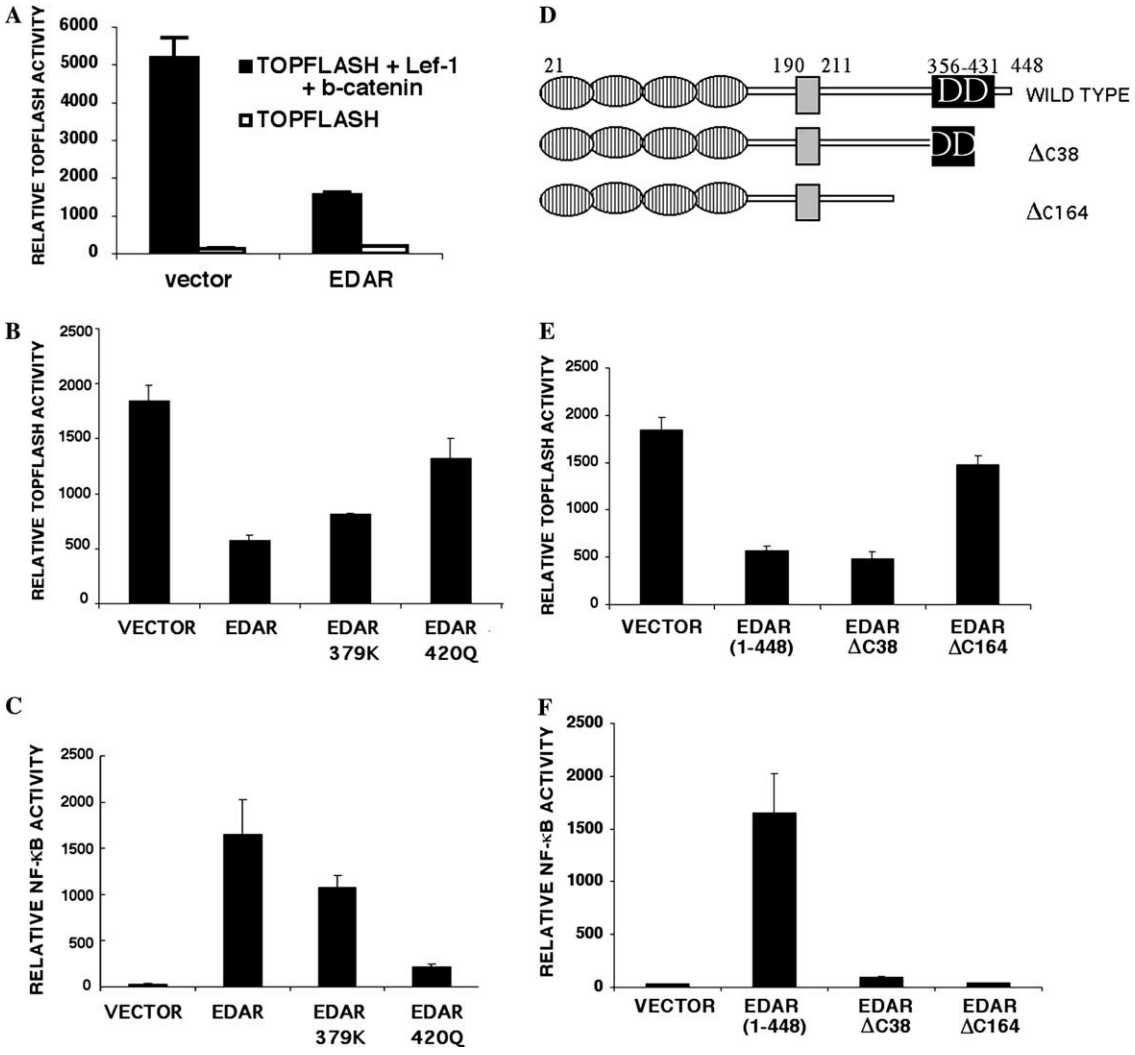

Fig. 1. EDAR represses the Lef-1/β-catenin-dependent transcription. (A) 293T cells were transfected in duplicate with 500 ng empty vector or an EDAR-encoding plasmid and TOPFLASH assay performed as described in "Materials and methods." The values shown are averages (means ± SEM) of a representative of two independent experiments in which each transfection was performed in duplicate. (B) Repression of Lef-1/β-catenin-dependent transcription (TOPFLASH) by EDAR mutants associated with anhidrotic ectodermal dysplasia. (C) Activation of NF-κB reporter activity by EDAR mutants associated with anhidrotic ectodermal dysplasia. (D) Schematic representation of the wild-type and mutant EDAR constructs. The ligand-binding domain is shown in stripes, the transmembrane domain in gray, and the death domain (DD) in black. (E) Lef-1/β-catenin transcriptional repression by wild-type and deletion mutants of EDAR. (F) Activation of NF-κB reporter activity by wild-type and deletion mutants of EDAR.

this receptor [4,6]. We used C-terminal deletion mutagenesis to study the contribution of death domain in EDAR-induced Lef-1/β-catenin transcriptional repression (Fig. 1D). As shown in Fig. 1E, deletion mutant EDARΔC38 which is missing the C-terminal 38 amino acids and possesses a partial death domain could effectively repress the Lef-1/β-catenin-dependent transcription. Consistent with the published results, this mutant demonstrated a significant loss of the ability to activate the NF-κB pathway as compared to the wild-type receptor (Fig. 1F). In contrast, deletion mutant EDARΔC164, which is missing 164 amino acids from the C-terminal and lacks a death domain, demonstrated only a weak ability to repress the Lef-1/β-catenin pathway and activate the NF-κB pathway (Figs. 1E and F). Collectively, the above results suggest that the C-terminal 164 amino acids of EDAR are important for both Lef-1/β-catenin repression and NF-κB activation.

Role of EDARADD in Lef-1/β-catenin transcriptional repression

EDAR-associated death domain (*EDARADD*, also known as crinkled) is a death domain-containing cytosolic adaptor protein that is recruited to the death domain of EDAR and has been shown to play a key role in NF-κB activation by this receptor [27,28]. Mutations in EDARADD have been seen in *crinkled* mice and in

some patients with anhidrotic ectodermal dysplasia [27,28]. EDARADD is believed to have a modular structure in which the C-terminal death domain is believed to interact with the death domain of EDAR and the N-terminal region is responsible for signal transduction [27,28]. In order to investigate the possibility that EDARADD is also involved in Lef-1/β-catenin repression by EDAR, we tested the ability of wild-type EDARADD and its N-terminal deletion mutant (EdaraddΔN70) to activate the NF-κB and repress Lef-1/β-catenin transcriptional activity upon transient transfection in 293T cells. As shown in Figs. 2A and B, we observed significant repression of Lef-1/β-catenin reporter activity and significant stimulation of NF-κB reporter activity by wild-type EDARADD but not by its N-terminal deletion mutant. Taken together, the above results suggest a role of EDARADD in both NF-κB activation and Lef-1/β-catenin repression by EDARADD.

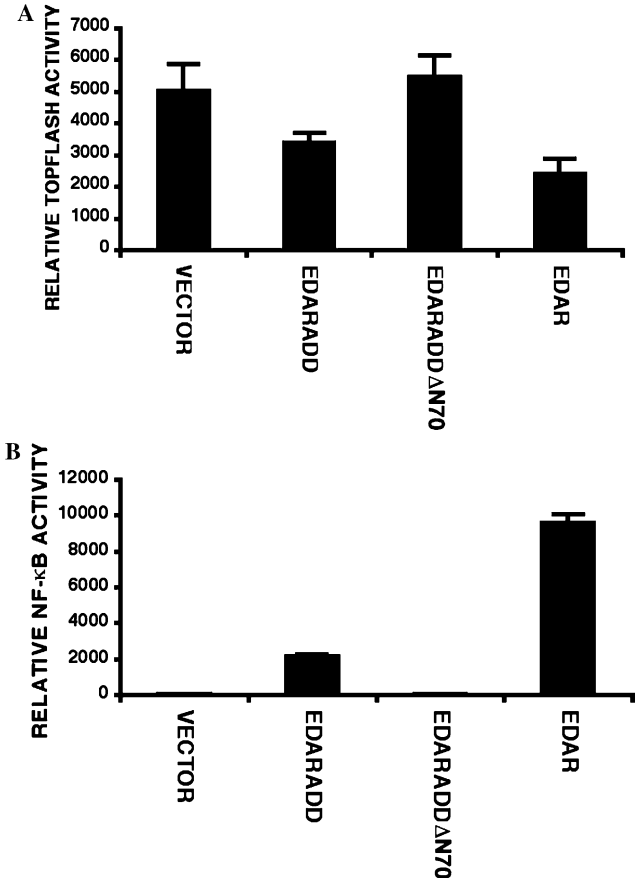

Fig. 2. EDARADD-induced Lef-1/β-catenin transcriptional repression and NF-κB activation. 293T cells were transfected with 500 ng of an empty vector or expression vector encoding EDARADD, EdaraddΔN70, and EDAR and TOPLASH and NF-κB reporter activities were measured as described in "Materials and methods." The values shown are averages (means ± SEM) of a representative of two independent experiments in which each transfection was performed in duplicate. (A) Lef-1/β-catenin transcriptional repression by wild-type and deletion mutants of EDARADD. (B) Activation of NF-κB reporter activity by EDARADD and its deletion mutant.

Role of NIK in EDAR-induced β-catenin repression and NF-κB activation

NIK is a serine threonine kinase of the MAP kinase pathway which is believed to play a key role in NF-κB activation by the lymphotoxin β receptor [21]. A role of NIK in EDAR signaling has been suspected based on the ability of dominant-negative mutants of N1K to block EDAR-induced NF-κB in a luciferase reporter assay. However, the role of NIK in NF-κB activation is enigmatic. For example, although a dominant-negative mutant of NIK can block NF-κB activation via TNFR1, subsequent studies using NIK−/− mouse embryonic fibroblast (MEF) cells have argued against the involvement of this kinase in TNFR1-induced NF-κB [21]. In order to re-examine the involvement of NIK in EDAR-induced NF-κB activation and Lef-1/β-catenin repression in a more physiological setting, we took advantage of mouse embryonic fibroblast cells (MEFs) from wild-type (wt) and NIK−/− animals [21]. As shown in Figs. 3A and B, EDAR was found to activate NF-κB and repress Lef-1/β-catenin in both the wild-type and NIK−/− MEFs. Collectively, the above results argue against a major role of NIK in EDAR-induced NF-κB activation or Lef-1/β-catenin transcriptional repression.

Role of IKK1/IKKα and IKK2/IKKβ in EDAR-induced NF-κB activation and Lef-1/β-catenin transcriptional repression

Biochemical and genetic characterization of cytokine-inducible NF-κB activation has led to the identification of a multisubunit I κB kinase (IKK) complex consisting of two catalytic subunits, IKK1/IKKα and IKK2/IKKβ, respectively, and a regulatory subunit, NEMO/IKKγ [29,30]. We have previously reported that dominant-negative mutants of IKK1 and IKK2 can partially block EDAR-induced NF-κB activation, suggesting a role of these kinases in EDAR-induced NF-κB activation [4]. However, a number of recent studies argue against a major role of IKK1 in cytokine-induced activation of the canonical NF-κB pathway [31–33]. In the light of this controversy, we have re-examined the contribution of IKK1/IKKα and IKK2/IKKβ in EDAR-induced NF-κB activation and Lef-1/β-catenin transcriptional repression using MEFs derived from wild-type, IKK1−/− and IKK2−/− animals. As shown in Fig. 3C, transient transfection of EDAR led to significant NF-κB activation in the wild-type MEFs but failed to do so in IKK1−/− and IKK2 −/− MEFs. Although a small increase in EDAR-induced NF-κB was observed in IKK1−/− MEFs, both the basal and induced NF-κB activity in these cells was significantly lower as

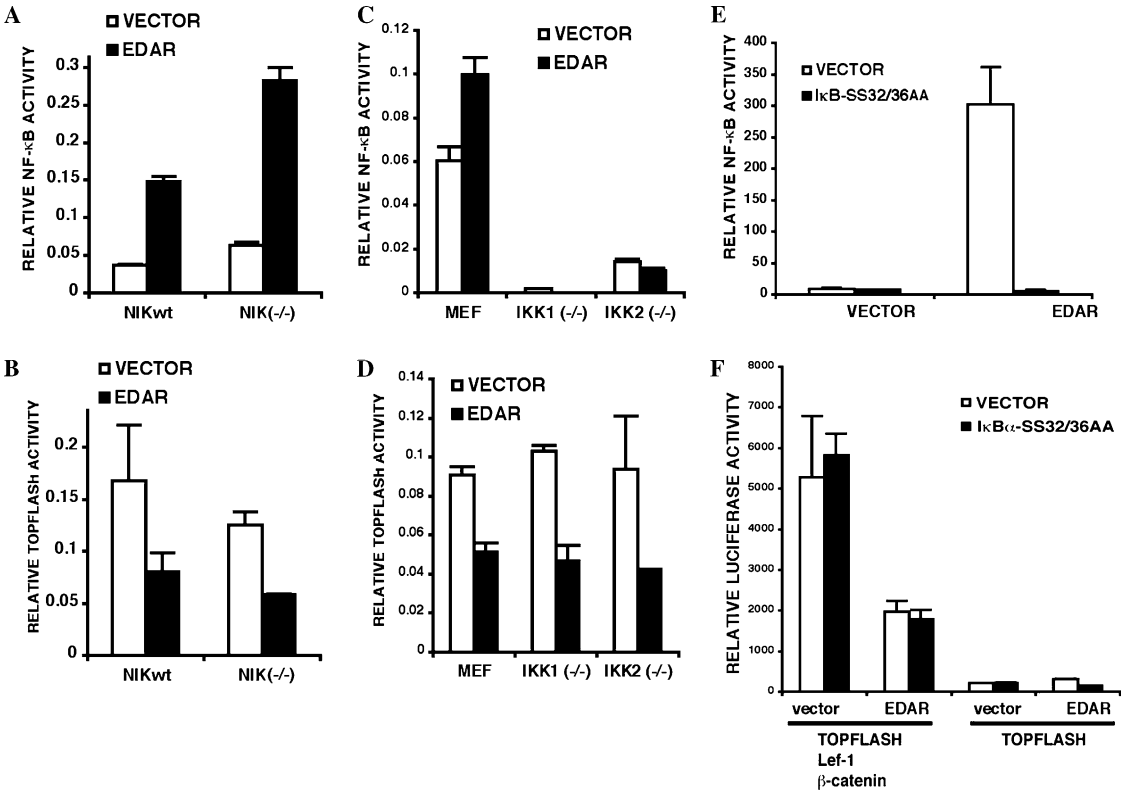

Fig. 3. Role of NIK, IKK1, IKK2, and IκBα in EDAR-induced Lef-1/β-catenin transcriptional repression and NF-κB activation. (A–D) Murine embryonic fibroblast cells derived from wild-type, NIK−/−, IKK1−/−, and IKK2−/− embryos were transfected with 500 ng of empty vector or EDAR expression plasmid along with NF-κB (A,C) or TOPFLASH (B,D) reporter plasmids using Lipofectamine2000 (Invitrogen). A *Renilla* luciferase (phRG-TK) construct (75 ng/well) was co-transfected to serve as a normalization control. Cells were lysed 30 h after transfection and cell lysates were used for reporter assays. TOPFLASH- and NF-κB-driven firefly luciferase activities were normalized relative to *Renilla* luciferase activity to control for the difference in transfection efficiency. The values shown are averages (means ± SEM) of a representative of at least two independent experiments in which each transfection was performed in duplicate. (E–F) 293T cells were transfected with the indicated plasmids and the experiment was performed as described under "Materials and methods." The amount of IκBα-SS32/36AA plasmid (750 ng/well) was 7.5 times the amount of receptor plasmids (100 ng/well) and the total amount of DNA transfected was kept constant by adding empty vector. The values shown are averages (means ± SE) of a representative of at least two independent experiments in which each transfection was performed in duplicate. IκBα-SS32/36AA completely blocks EDAR-induced NF-κB activation (A) but fails to block Lef-1/β-catenin-dependent transcriptional repression (B).

compared to the wild-type MEFs. We next examined the role of IKK1 and IKK2 in EDAR-induced Lef-1/β-catenin transcriptional repression. As shown in Fig. 3D, we observed equivalent repression of Lef-1/β-catenin transcriptional activity in the wild-type, IKK1−/−, and IKK2−/− MEF cells. Collectively, the above results confirm major roles of IKK1 and IKK2 in EDAR-induced NF-κB activation but argue against a role of these kinases in EDAR-induced Lef-1/β-catenin repression. Moreover, the above results suggest that EDAR represses the Lef-1/β-catenin transcriptional activity independent of its ability to activate the NF-κB pathway.

Role of IκBα phosphorylation and degradation in EDAR-induced Lef-1/β-catenin transcriptional repression

The IKKs activate the NF-κB pathway by mediating inducible phosphorylation and degradation of IκB proteins. In order to confirm that EDAR represses the β-catenin activity independent of its ability to activate the NF-κB pathway, we used a phosphorylation-resistant dominant-negative form of IκBα (IκBα super-repressor). This mutant contains serine-to-alanine substitutions at amino acids 32 and 36, respectively, is resistant to phosphorylation-induced degradation of IκBα, and is known to block NF-κB activation via diverse stimuli [34]. As shown in Figs. 3A and B, IκBα-SS32/36AA mutant completely blocked EDAR-induced NF-κB activation but failed to block β-catenin transcriptional repression. Taken together with the studies using IKK1- and IKK2-deficient MEFs, the above results suggest that EDAR represses the Lef-1/β-catenin transcriptional activity independent of its ability to activate the NF-κB pathway.

In summary, our results suggest that EDAR signaling has opposing effects on two major pathways involved in ectodermal differentiation and hair follicle morphogenesis—it stimulates NF-κB activity and represses the Lef-1/β-catenin-dependent transcription. While EDARADD appears to be the proximal adaptor involved in both

these activities, the signaling intermediates involved in the regulation of NF-κB and β-catenin pathways appear to diverge distally. A recent study suggests that β-catenin cellular localization and transcriptional activity are differentially regulated by IKK1 and IKK2 [26]. While we have confirmed the role of these kinases in EDAR-induced NF-κB activation, our results argue against their major role in the Lef-1/β-catenin-dependent transcriptional repression by EDAR. Consistent with our results, TNFα has been reported to repress β-catenin-dependent gene expression and this effect is only partially blocked by an IKKβ dominant-negative mutant [26]. While the signaling intermediates involved in EDAR-mediated repression of Lef-1/β-catenin transcriptional activity remain to be identified, it is interesting to note that EDAR ligand, ectodysplasin A (EDA-A1), is one of the target genes of Lef-1/β-catenin pathway [19]. Thus, EDA-A1 may downregulate its expression via a negative feedback-loop involving EDAR-mediated repression of Lef-1/β-catenin transcription. Future studies should address the biological significance of this negative-feedback regulation of EDAR–EDA axis during ectodermal differentiation and hair follicle morphogenesis.

Acknowledgments

We thank Drs. Robert D. Schreiber, Richard Gaynor, and Inder Verma for providing the NIK-, IKK1-, and IKK2-deficient MEF cells. This work was supported by a Grant (R01 DE 15189) from the National Institute of Dental and Craniofacial Research.

References

[1] D.J. Headon, P.A. Overbeek, Nat. Genet. 22 (1999) 370–374.
[2] A.W. Monreal, B.M. Ferguson, D.J. Headon, S.L. Street, P.A. Overbeek, J. Zonana, Nat. Genet. 22 (1999) 366–369.
[3] S.A. WiSniewski, A. Kobielak, W.H. Trzeciak, K. Kobielak, J. Appl. Genet. 43 (2002) 97–107.
[4] A. Kumar, M.T. Eby, S. Sinha, A. Jasmin, P.M. Chaudhary, J. Biol. Chem. 276 (2001) 2668–2677.
[5] M. Yan, L.C. Wang, S.G. Hymowitz, S. Schilbach, J. Lee, A. Goddard, A.M. de Vos, W.Q. Gao, V.M. Dixit, Science 290 (2000) 523–527.
[6] P. Koppinen, J. Pispa, J. Laurikkala, I. Thesleff, M.L. Mikkola, Exp. Cell Res. 269 (2001) 180–192.
[7] J. Zonana, M.E. Elder, L.C. Schneider, S.J. Orlow, C. Moss, M. Golabi, S.K. Shapira, P.A. Farndon, D.W. Wara, S.A. Emmal, B.M. Ferguson, Am. J. Hum. Genet. 67 (2000) 1555–1562.
[8] R. Doffinger, A. Smahi, C. Bessia, F. Geissmann, J. Feinberg, A. Durandy, C. Bodemer, S. Kenwrick, S. Dupuis-Girod, S. Blanche, P. Wood, S.H. Rabia, D.J. Headon, P.A. Overbeek, F. Le Deist, S.M. Holland, K. Belani, D.S. Kumararatne, A. Fischer, R. Shapiro, M.E. Conley, E. Reimund, H. Kalhoff, M. Abinun, A. Munnich, A. Israel, G. Courtois, J.L. Casanova, Nat. Genet. 27 (2001) 277–285.
[9] S. Dupuis-Girod, N. Corradini, S. Hadj-Rabia, J.C. Fournet, L. Faivre, F. Le Deist, P. Durand, R. Doffinger, A. Smahi, A. Israel, G. Courtois, N. Brousse, S. Blanche, A. Munnich, A. Fischer, J.L. Casanova, C. Bodemer, Pediatrics 109 (2002) e97.
[10] K. Willert, R. Nusse, Curr. Opin. Genet. Dev. 8 (1998) 95–102.
[11] J.D. Laney, M. Hochstrasser, Cell 97 (1999) 427–430.
[12] T. Maniatis, Genes Dev. 13 (1999) 505–510.
[13] K. Willert, S. Shibamoto, R. Nusse, Genes Dev. 13 (1999) 1768–1773.
[14] P. Polakis, Genes Dev. 14 (2000) 1837–1851.
[15] Q. Eastman, R. Grosschedl, Curr. Opin. Cell Biol. 11 (1999) 233–240.
[16] P. Zhou, C. Byrne, J. Jacobs, E. Fuchs, Genes Dev. 9 (1995) 700–713.
[17] C. van Genderen, R.M. Okamura, I. Farinas, R.G. Quo, T.G. Parslow, L. Bruhn, R. Grosschedl, Genes Dev. 8 (1994) 2691–2703 (the above report in format).
[18] U. Gat, R. DasGupta, L. Degenstein, E. Fuchs, Cell 95 (1998) 605–614.
[19] M.C. Durmowicz, C.Y. Cui, D. Schlessinger, Gene 285 (2002) 203–211.
[20] G. Pengue, A.K. Srivastava, J. Kere, D. Schlessinger, M.C. Durmowicz, J. Biol. Chem. 274 (1999) 26477–26484.
[21] L. Yin, L. Wu, H. Wesche, C.D. Arthur, J.M. White, D.V. Goeddel, R.D. Schreiber, Science 291 (2001) 2162–2165.
[22] Q. Li, D. Van Antwerp, F. Mercurio, K.F. Lee, I.M. Verma, Science 284 (1999) 321–325.
[23] Q. Li, Q. Lu, J.Y. Hwang, D. Buscher, K.F. Lee, J.C. Izpisua-Belmonte, I.M. Verma, Genes Dev. 13 (1999) 1322–1328.
[24] A.M. Chinnaiyan, K. O'Rourke, G.L. Yu, R.H. Lyons, M. Garg, D.R. Duan, L. Xing, R. Gentz, J. Ni, V.M. Dixit, Science 274 (1996) 990–992.
[25] P.M. Chaudhary, M. Eby, A. Jasmin, A. Bookwalter, J. Murray, L. Hood, Immunity 7 (1997) 821–830.
[26] C. Lamberti, K.M. Lin, Y. Yamamoto, U. Verma, I.M. Verma, S. Byers, R.B. Gaynor, J. Biol. Chem. 276 (2001) 42276–42286.
[27] D.J. Headon, S.A. Emmal, B.M. Ferguson, A.S. Tucker, M.J. Justice, P.T. Sharpe, J. Zonana, P.A. Overbeek, Nature 414 (2001) 913–916.
[28] M. Yan, Z. Zhang, J.R. Brady, S. Schilbach, W.J. Fairbrother, V.M. Dixit, Curr. Biol. 12 (2002) 409–413.
[29] M. Karin, A. Lin, Nat. Immunol. 3 (2002) 221–227.
[30] S. Ghosh, M. Karin, Cell 109 (Suppl) (2002) S81–S96.
[31] K. Takeda, O. Takeuchi, T. Tsujimura, S. Itami, O. Adachi, T. Kawai, H. Sanjo, K. Yoshikawa, N. Terada, S. Akira, Science 284 (1999) 313–316.
[32] Y. Hu, V. Baud, M. Delhase, P. Zhang, T. Deerinck, M. Ellisman, R. Johnson, M. Karin, Science 284 (1999) 316–320.
[33] Q. Li, G. Estepa, S. Memet, A. Israel, I.M. Verma, Genes Dev. 14 (2000) 1729–1733.
[34] A.S. Baldwin Jr., Annu. Rev. Immunol. 14 (1996) 649–683.