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Upregulation of chemokine (C–C motif) ligand 20 in adult epidermal keratinocytes in direct current electric fields

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Abstract

Electric fields (EFs) of around 100 mV/mm are present in normal healing wounds and induce the directional migration of epithelial cells. Reepithelialization during wound healing thus may be controlled in part by this electrical signal. In this study, the early transcriptional response of human epidermal keratinocytes to EFs is examined using microarrays. Increased expression of various chemokines, interleukins, and other inflammatory response genes indicates that EFs stimulate keratinocyte activation and immune stimulatory activity. Gene expression activity further suggests that interleukin 1 is either released or activated in EFs. Expression of the chemokine CCL20 steadily increases at 100 mV/mm over time until around 8 h after exposure. This chemokine is also expressed at field strengths of 300 mV/mm—above the level of endogenous wound fields. The early effects of EFs on epithelial gene expression activity identified in these studies suggest the importance of naturally occurring EFs both in repair mechanisms and for the possibility of controlling these responses therapeutically.

Keywords

Electrical stimulation; Wound healing; Keratinocyte; Chemokine; Inflammatory response

Introduction

As part of their role providing barriers to environmental insults and maintaining homeostasis, epidermal cells maintain a potential difference between epidermal and dermal layers through the action of sodium and potassium pumps. Injuries to the skin cause this potential difference to “short circuit”, leading to electric fields (EFs) measured at 40–100 mV/mm [1, 9].

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Within hours after injury, wound edge keratinocytes weaken intercellular attachments and begin migrating laterally across the wound bed [5]. As with other cells, keratinocyte activation is influenced by gradients of growth factors and contact with provisional matrix components, such as collagen and fibronectin that comprise the wound microenvironment [12, 30, 50]. Endogenous wound EFs, however, may also contribute to keratinocyte activation during healing. In electric fields of physiological strength, keratinocytes have been shown to migrate toward the negative pole in a process termed galvanotaxis [28]. Galvanotaxis of keratinocytes is promoted by both collagen and fibronectin substrates, but not laminin, an inhibitor of keratinocyte migration [42, 49]. The galvanotactic response in keratinocytes has also been shown to be partially dependent on protein kinase A, tyrosine kinases, and calcium influx; but not on voltage-dependent calcium channels [7, 37, 46]. The absence of growth factors, particularly epidermal growth factor (EGF) and transforming growth factor beta (TGF-β), can diminish the galvanotactic response of epithelial cells [35, 52]. Intensity and directionality of galvanotaxis is also affected by activity of beta-adrenerginic receptors and EGFRs [8, 36, 53].

Although particular growth factors, receptors, extracellular matrix proteins, and soluble factors influence galvanotaxis in epithelial cells, the exact mechanisms underpinning cellular effects of EFs remain unclear. Because studies have shown that multiple growth factors are capable of influencing the response, there may be multiple different pathways activated in EFs. The fact that directed migration still occurs when there are no growth factors present, which suggests that epidermal cells may produce their own growth factors to mediate this response when stimulated with EFs. Another possible mechanism for galvanotaxis and other effects of electrical fields is receptor redistribution. Receptors that are mobile within the membrane, particularly EGFR, have been shown to redistribute toward the negative pole [7], establishing a receptor gradient that may lead to increased signal transduction on the side facing the negative pole [52].

The EF stimulus is present immediately upon wounding and persists until the epidermal layer is reestablished, capable of exerting influence beyond the initial inflammatory phase of healing through the proliferative and migratory events of keratinocytes that occur within hours after injury [6]. However, very little is known about the effects of electric fields on keratinocytes beyond those related to the migratory response. This study examines the changes in gene expression and biochemical pathways activated in keratinocytes upon exposure to physiological strength EFs using microarrays. It is anticipated that EFs trigger regulation of genes associated with reepithelialization and wound repair. The expression of particular genes over time and at varying field strengths is also considered.

Materials and methods

Cell culture

Adult human epidermal keratinocytes (HEKa) (Cascade Biologiecs, Portland, OR, USA) were plated at passage 4 onto 22 mm square coverslips coated with a thin layer of type I bovine collagen (BD Biosciences, Bedford, MA, USA) in a six-well plate in EpiLife® Medium supplemented with Human Keratinocyte Growth Supplement (Cascade Biologics). After reaching approximately 60% confluence, cells were exposed to electric fields in a humidified incubator at 37°C and 5% CO_2.

Electric field exposure

Cells on coverslips were placed cell side down into a stimulation apparatus constructed of Delrin® and assembled with vacuum grease, which minimized the cross-sectional area of the media exposed to current to guard against heat production as described in previous

studies [16]. Glass tubes containing a gel consisting of 2% agar in saline served as bridges between stimulation chambers and silver/silver chloride electrodes in saline solution, thereby minimizing contamination of media with electrode products. Current through electrodes produced by a power supply was adjusted until the voltage drop across the media wells was measured to produce desired field strengths. Since the commonly reported average field strength occurring in wounds is 100 mV/mm and galvanotaxis response of keratinocytes begins within the first 15 min, microarray experiments were carried out at 100 mV/mm for 1 h [1, 28]. RT–PCR expression measurement of selected transcripts was performed for shorter and longer durations (30 min, 1, 4, 8, and 12 h), and for 1 h at varying field strengths ranging from 50–300 mV/ mm.

RNA isolation

Total RNA was obtained from coverslips using Trizol® (Invitrogen, Carlsbad, CA) and chloroform extraction. A pool of six samples used for each GeneChip®. Total RNA from one sample per experimental condition was used for RT–PCR analysis.

Microarray

RNA was checked for quality and quantity before labeling and hybridization to Affymetrix HG-133 Plus Genechips®. Three microarrays from three experimental replicates were compared to three microarrays for control replicates.

Data analysis

Affymetrix raw data were collected and analyzed using GeneChip® Operating System (GCOS, Affymetrix, Santa Clara, CA, USA). Raw data (.cel and .chp files) were uploaded onto a web-based software program, GeneTraffic™ (Iobion, Inc., La Jolla, CA, USA), for normalization using GC-Robust Multichip Analysis (GC-RMA) with controls as a baseline. p values were obtained using a two-class unpaired unequal variance t test. Transcripts identified as regulated compared to controls were imported into Netaffx™ (Affymetrix), and into Pathway Architect® (Stratagene, La Jolla, CA, USA) software to assist with interpretation of pathway analysis.

RT–PCR

Two transcripts identified as upregulated in microarrays were selected to confirm results using real time RT–PCR primers were designed based on Affymetrix target sequences for upregulated transcripts (Table 1). Total RNA was isolated as described previously; with the exception that experimental material was not pooled and contained total RNA from one experimental condition. First strand cDNA was transcribed from total RNA using Reverse-iT™ MAX first strand synthesis kit (ABGene, Rochester, NY, USA). Real-time PCR using ABsolute™ QPCR SYBR green mix with Fluorescein (ABGene) was performed using an i-Cycler (Biorad, Hercules, CA, USA). Expression levels normalized to an internal control housekeeping gene (GAPDH) were determined using the standard curve method.

Results

Statistical analysis of multiple microarray experiments identified 161 gene transcripts that were significantly increased over controls, and 245 transcripts significantly decreased after 1 h of exposure to 100 mV/mm EFs. Of these, 17 transcripts were significantly increased and 67 were decreased by more than 1.4-fold (average signal log ratio >0.05 or <−0.05). Above a twofold regulation (average signal log ratio >1 or <−1), 1 transcript was significantly increased (NFKBIZ) and 3 were significantly decreased (ATRX, ZBTB34, and EIF4G3).

Analysis by function

Affymetrix's NetAffx™ web-based program was used to identify associated biological functions of regulated transcripts. Table 2 lists select genes that are significantly upregulated above 1.4-fold that can be classified according to biological function. Table 3 lists genes identified as downregulated at greater than 1.4-fold and their associated biological functions. Genes that fell into multiple categories of biological functions were classified under functions most relevant to wound healing, and redundant expression results have been omitted. Since relatively few genes were regulated at statistically significant levels, all genes identified as having a greater than twofold average change and their p values are listed in Table 4.

Among genes with increased expression in electric fields, several fell into the category of inflammatory response functions, including interleukins and chemokines. Signal transduction proteins were also expressed at increased levels. Transcripts that were decreased fell into categories of growth and differentiation, adhesion and motility, apoptosis, and transport, among others.

Analysis by pathway

Filtered gene lists imported into Pathway Assist software identified biochemical pathways with multiple genes regulated. Based on the presence of multiple regulated transcripts within certain biological pathways, software suggested that possibly active pathways included chemokine, apoptosis, JAK-STAT, Wnt, and G-protein MAPK activation signaling pathways. Although it is not confirmed that these pathways or receptors were activated, expression of transcripts downstream in these pathways was regulated.

Real-time RT–PCR

Although nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta (NFKBIZ) was the only gene identified in the microarray study as statistically increased over twofold, increased expression of this transcript was not confirmed by RT–PCR for any of the experimental conditions (Fig. 1).

Expression of chemokine (C–C motif) ligand 20 (CCL20) was confirmed to be increased by RT–PCR after 1 h of stimulation with 100 mV/mm. Expression was increased further at 4 and 8 h of continuous stimulation (Fig. 2a). At all field strengths tested, expression of CCL20 was increased over controls and was increased by 23-fold after 1 h of stimulation with 300 mV/mm (Fig. 2b).

Discussion

EF exposure induces changes in the expression of crucial wound response genes in keratinocytes. Thirty-eight transcripts were identified as statistically different above a 1.4-fold level; and although several transcripts were regulated above a twofold level, only one was detected to be statistically significant in the microarray study, NFKBIZ. This small number of detected gene expression changes contrasts with the pronounced change in the activity of keratinocytes during galvanotaxis. Most galvanotaxis studies, however, report the effects on neonatal or corneal epidermal cells, whereas in this study adult dermal keratinocytes were used. It is possible that responsiveness of cells to electric fields may be dependent on age or tissue location. It has been shown that keratinocytes expressing involucrin, an indicator of differentiation, display diminished galvanotactic responses [31] and subpopulations of neonatal keratinocytes do not respond to EFs or migrate toward the anode [36]. Differentiation of keratinocytes in these culture conditions with low calcium medium is suppressed, and differentiation marker expression has been found to be similar

between adult and neonatal keratinocytes in similar culture conditions [25]. The response of adult keratinocytes to electric fields may be reduced compared to neonatal cells or only present in smaller subsets of cells, making the data more variable and decreasing the likelihood of detecting significant differences.

Zhao et al. have linked galvanotaxis to increases in phosphoinositol-3-OH-kinase-γ (PI3K-γ) signaling and decreases in phosphatase and tensin homolog (PTEN) signaling [55]. Microarray analysis identified PTEN expression was decreased significantly by 1.2-fold in keratinocytes exposed to EFs. Although there were no detectable changes in expression of PI3K-γ, another phosphoinositol kinase protein, phosphoinositide-3-kinase, class 2, alpha polypeptide (PIK3C2A) was increased by 1.5-fold. The activity of this newly identified protein in the migratory response remains unknown; however, this protein has a known role in clathrin-mediated membrane trafficking [10]. This protein may be involved in the asymmetric redistribution of membrane proteins through an alternative mechanism to electrophoretic movement [34]. Figure 4 shows the direct interactions between significantly upregulated transcripts—PIK3C2A, protein tyrosine phosphatase, non-receptor type 11 (PTPN11), and NFKBIZ—and EGFRs, which are also active in the galvanotaxis response.

Keratinocyte migration in vivo requires the elimination of intercellular attachments and degradation of surrounding matrix. Expression of plasminogen activated urokinase receptor (PLAUR) was increased over twofold in this study. Binding of urokinase-type plasminogen activator (uPA) to PLAUR converts plasminogen to plasmin, activating growth latent growth factors such as TGF-β1, activating MMPs to digest ECM, and cleaving extracellular components leading to the breakdown of desmosomes [2, 3, 18]. Increased expression of PLAUR in epidermal cells induced by EFs indicates EFs not only increase direct migratory responses of detached keratinocytes but also increase their ability to manipulate intercellular and ECM attachments.

The role of increased chemokine expression in keratinocytes as found in the present study on the galvanotactic response of keratinocytes is unknown but would likely enhance galvanotactic activity. Studies by Pullar et al. indicate that beta 2-adrenergic receptor (B2-AR) antagonists increase galvanotaxis and B2-AR agonists suppress these responses [36]. IL-1 has been shown to attenuate activity within the B-AR adenylyl cyclase system and thus is antagonistic to B2-ARs [17]. Furthermore, inhibition of interleukin and cytokine production by B2-AR agonists may explain their negative regulation of galvanotaxis [15, 47].

The majority of transcripts increased by more than twofold after electric field exposure is associated with inflammatory response after injury and includes chemokines, interleukins, and growth factors. Only NFKBIZ was increased at a statistically significant level, however. Transcription of NFKBIZ, a nuclear protein that interacts with NFkB and the IL-6 promoter, is induced sharply by IL-1, lipopolysaccharide, and toll-like receptor ligands [19, 51]. As it is an inhibitor of the inflammatory protein NFkB, it may be classified as an anti-inflammatory agent, preventing extreme inflammatory reactions during wound healing [26]. Upregulation of NFKBIZ in EFs may be mediated by IL-1 release in keratinocytes, and may be required for the accompanying increase in IL-6 expression. Although the location of receptors that trigger induction of NFKBIZ (toll-like receptors and IL-1 receptors) in EFs are unknown, asymmetric redistribution due to EFs similar to that seen for EGFRs may also enhance this effect [8, 53].

In addition to migrating across the wound bed to recover barrier function, keratinocytes synthesize and secrete factors that signal macrophage and neutrophil migration and promote angiogenesis. Keratinocytes produce and store interleukin 1a (IL-1a), which is quickly

released upon exposure to environmental stresses [21–23, 33]. IL-1a secretion signals the activation of endothelial cells, fibroblasts, and other keratinocytes [4, 24, 39]. IL-1 secretion also attracts circulating leukocytes and may induce expression of IL-8, TNF, and other chemokines induced in this study [29, 43]. Results of this study suggest that keratinocytes release IL-1 after exposure to EFs during the process of normal wound healing, which then mediates changes in gene expression during keratinocyte activation (Fig 3.) With increased sample size, these expression results may have reached statistical significance.

IL-8 and IL-6, both with increased expression over controls on exposure to EFs, can enhance the healing response by promoting migration, proliferation, and angiogenesis [11, 20, 38, 44]. There were also increases in expression of CXCL2 and CXCL3 (GRO-β and GRO-γ), which are structurally related to IL-8 and share activity in promoting neutrophil infiltration and angiogenesis [40, 45]. TNF-α acts to promote the production of other signaling molecules and their receptors, including EGFR [10, 32]. Upregulation of EGFRs has been observed in corneal epithelial cells exposed to similar EFs, an effect possibly mediated by TNF-α [54]. TNF-α may also be responsible for increased expression of CCL20, which functions to recruit inflammatory cells to tissue and has been found to be expressed in epidermal cells with disrupted barrier function and therefore in the presence of EFs [13, 27, 41]. Increased expression of cytokines and chemokines may also result in the upregulation of leukemia inhibitory factor (LIF), which then may reinforce upregulation of cytokines, particularly interleukins [14, 48].

RT–PCR measurement shows that the expression of the chemokine CCL20 in a 100 mV/mm field increases steadily over time until sometime after 8 h, and then declines. CCL20 expression is also increased dramatically at 300 mV/mm, which is greater than the strength of EFs in normal skin wounds. Although an essential early step in the progression of normal wound healing, expression of chemokines at extremely high levels or over prolonged periods of time interferes with the timely resolution of the inflammatory response during the normal progression of healing. These results suggest that EFs of strengths similar to those present in normal healing wounds induce a moderate expression of chemokines in keratinocytes, which diminishes within hours.

In this study, keratinocytes were stimulated in medium designed to support expansion of keratinocytes and suppress differentiation. This media contained supplements of a defined concentration of epidermal growth factor but an undefined concentration of other growth factors contained within a bovine pituitary extract. EGF is particularly involved in keratinocyte galvanotaxis, but other growth factors such as *TGF-*β also influence EF effects in epidermal cells [8, 53, 54]. Since the concentration of specific growth factors in bovine pituitary extract is unknown and high concentrations of EGF were supplied in the media, these results may be selective for the effects of EFs that are EGF-dependent. It should be noted that the concentration of ions and growth factors in wound fluid after the collapse of the trans-epithelial potential and cell signaling differs from the medium used in this study. This model system also differs from the in vivo situation in that specific interactions between keratinocytes and matrix as well as between other cell types are absent. Cross talk between keratinocytes and other cells influenced by exposure to EFs, such as fibroblasts and endothelial cells, may play a major role in the coordination of EF-induced effects in vivo.

Statistical analysis of multiple microarray experiments and data mining tools helped to narrow the list of candidate genes for further studies and confirmation of microarray results with RT–PCR. However, expression of NFKBIZ measured by RT–PCR did not agree with microarray data. Additionally, multiple microarray experiments may have revealed induction of different genes at later time points or at different fields. Pathway analysis also identified possible activation of particular cytokine signaling pathways such as JAK-STAT,

although specific phosphorylation status or pathway activation was not confirmed in this study. Since gene expression analysis is used to indicate changes in protein synthesis, proteomic analysis of transcripts identified in this study may provide more reliable results. Furthermore, the role that electric field-induced gene expression plays in the processes of migration, cell signaling, and wound healing could be further elucidated by blocking receptors, such as chemokine receptors, or by gene silencing experiments. Ultimately, confirmation of changes in gene expression and cellular activity within an in vivo model is necessary to fully understand the role of EFs in keratinocyte activation. Moreover, the impact of EF-induced effects on keratinocytes in the clinical progression of wound healing deserves further study.

EFs of similar strength to those generated in normal wounds induce gene expression in keratinocytes important to the wound healing response. In addition to promoting migration, increased expression of chemokines induced upon EF exposure may profoundly affect the inflammatory phase of wound healing. Further studies are needed to confirm the production and release of these proteins in EFs and their contribution to galvanotaxis, reepithelialization, and wound healing in general. Visualization of protein location in EFs using labeled antibodies may also identify asymmetries in receptor location or protein secretion capable of controlling the directionality of keratinocyte migration needed for reepithelialization. Overall, this study substantiates the importance of endogenous EFs in reepithelialization and suggests strategies to control keratinocyte response during the inflammatory and proliferative phases of wound healing.

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Abbreviations

CCL20	Chemokine (C–C motif) ligand 20
EF	Electric field
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
HEKa	Human epidermal keratinocytes
IL	Interleukin
LIF	Leukemia inhibitory factor
NFKBIZ	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor zeta
PTEN	Phosphatase and tensin homolog
PIK3C2A	Phosphoinositide-3-kinase class 2 alpha Polypeptide
PI3K-γ	Phosphoinositol-3-OH-kinase-γ
PLAUR	Plasminogen activated urokinase receptor
TGF-β	Transforming growth factor beta
TNF	Tumor necrosis factor
uPA	Urokinase-type plasminogen activator

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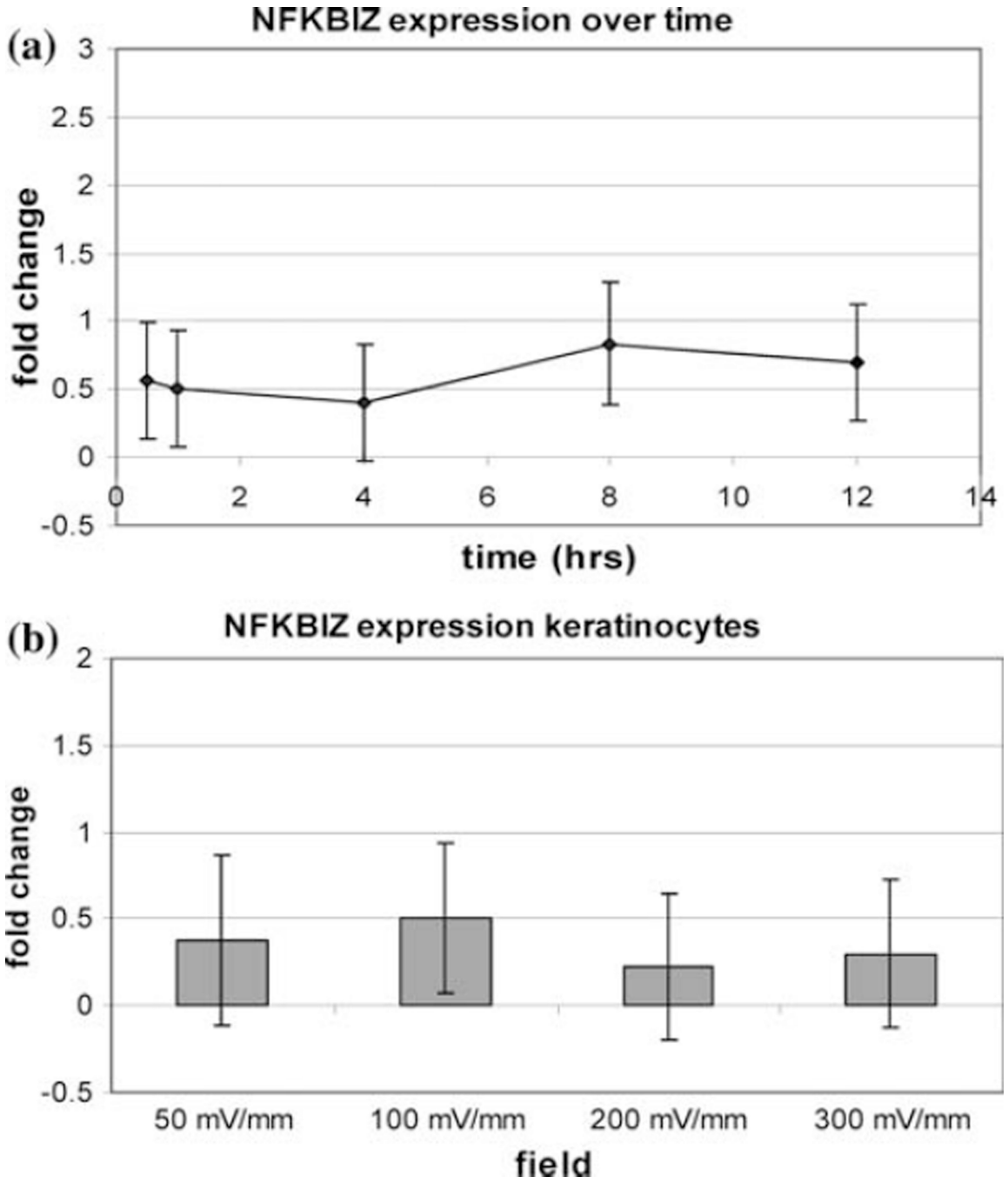

Fig 1.

NFKBIZ expression in keratinocytes (**a**) over time at 100 mV/ mm and (**b**) at different field strengths for 1 h

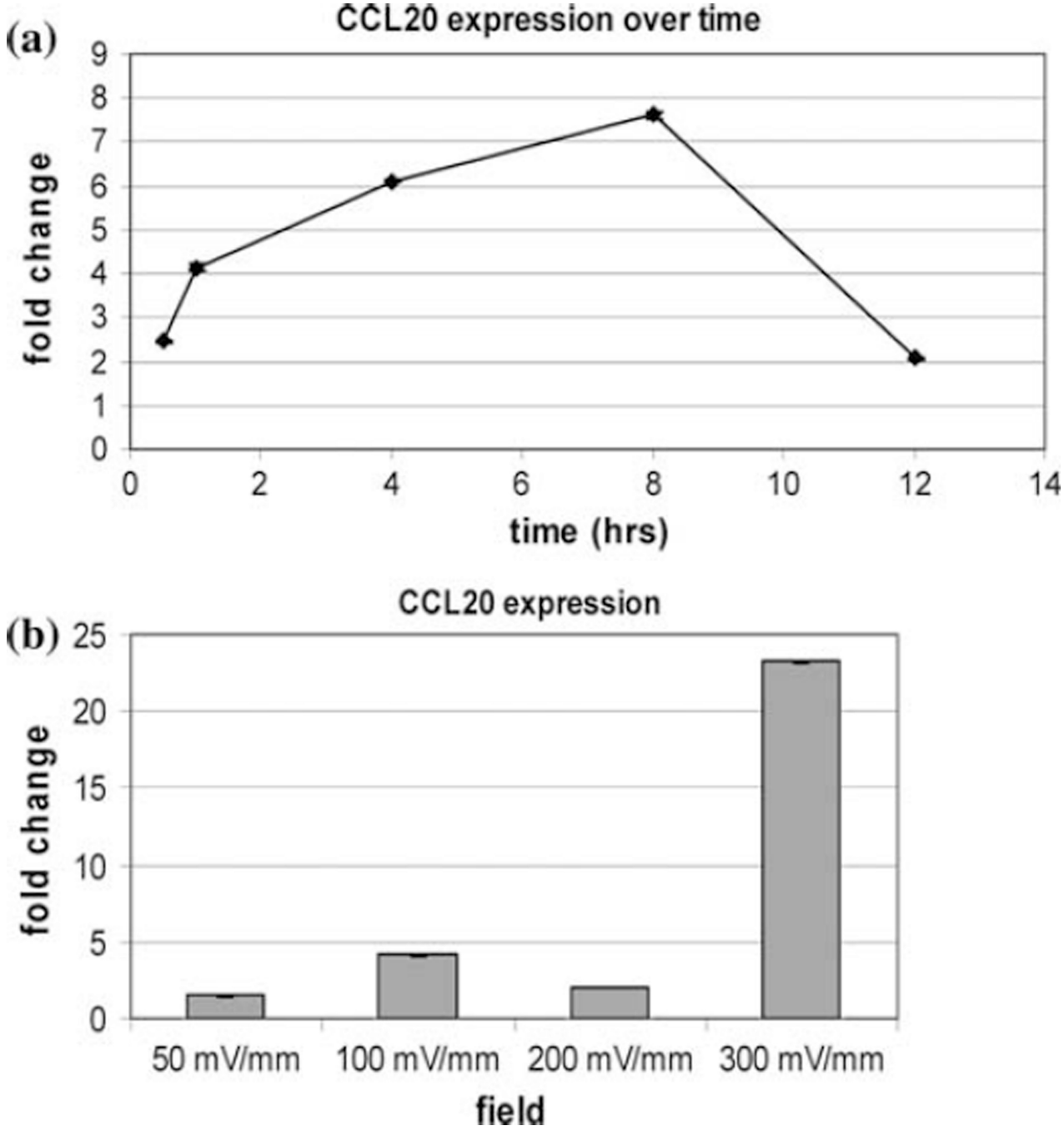

Fig 2.
CCL20 expression in keratinocytes (**a**) over time at 100 mV/ mm and (**b**) at different field strengths for 1 h

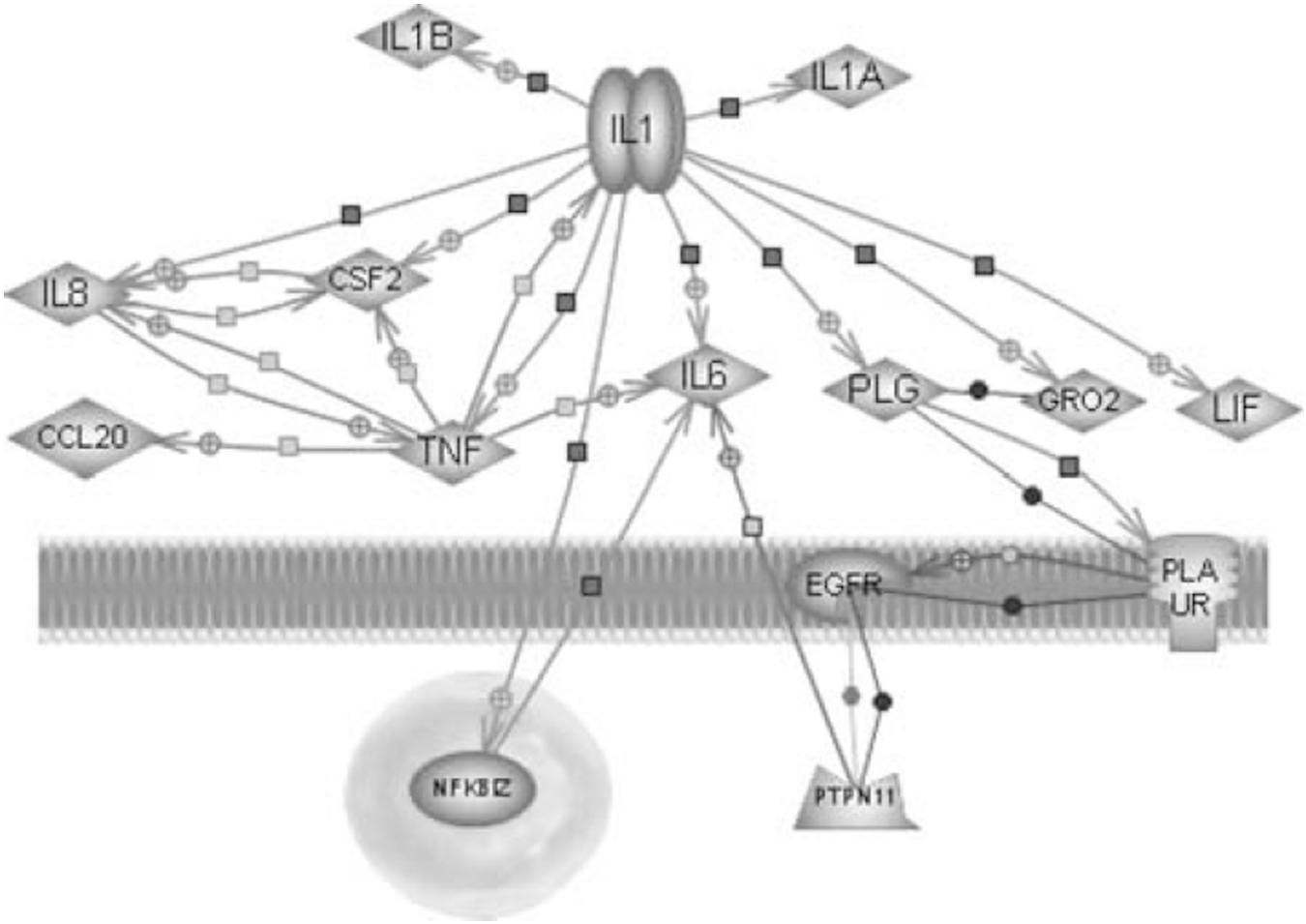

Fig 3.

Gene expression in keratinocytes related to IL-1 signaling. Pathway Architect® diagram showing increases in expression of CCL20, CSF2, GRO-beta, IL-6, IL-8, LIF, NFKBIZ, PTPN11, and TNF indicate activity stimulated by IL-1, which is released by keratinocytes upon injury

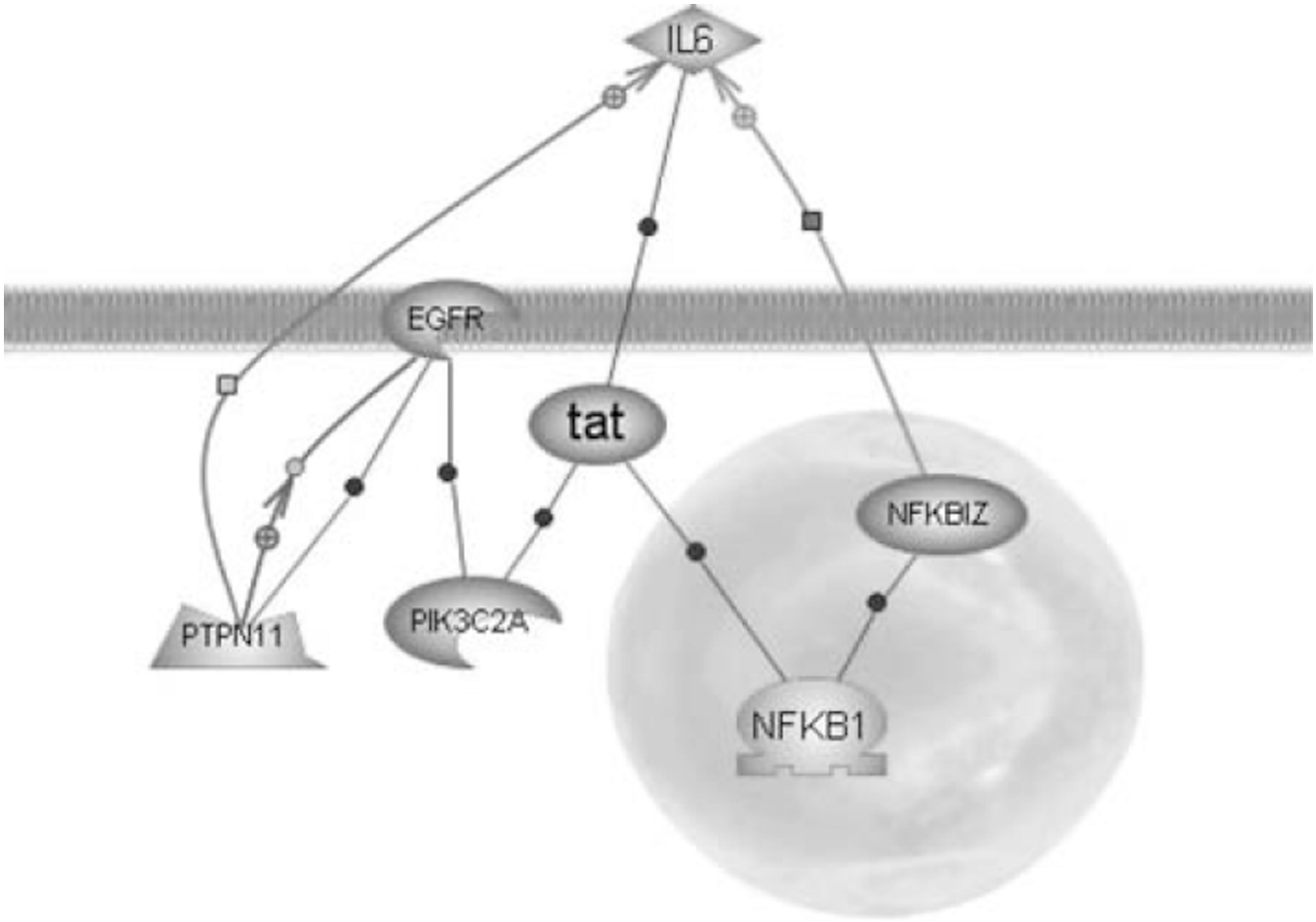

Fig 4.
Statistically significant interactions. Pathway Architect® diagram of relations between the significantly upregulated transcripts NFKBIZ, PIK3C2A, and PTPN11 in EFs and IL-6 whose upregulation was not statistically significant and EGFR which has been shown to be upregulated in other studies of EF effects on epithelial cells

Table 1

Primers

Gene	Sequences
NFKBIZ	
Forward	5′ GATGCTGTCCGCCTGTTGA 3′
Reverse	5′ CTGGCTGTTCGTTCTCCAAGT 3′
CCL20	
Forward	5′ CTGGCTGCTTTGATGTCAGTGCT 3′
Reverse	5′ GCAGTCAAAGTTGCTTGCTGCTTC 3′
GAPDH	
Forward	5′ ATGGGGAAGATAAAGGTCG 3′
Reverse	5′ TAAAAGCAGCCCTGGTGACC 3′

Table 2

Transcripts significantly increased in HEKa

Affymetrix Probeset ID	Gene symbol	Gene name	Fold change
Inflammation and immune response			
223217_s_at	NFKBIZ	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta	2.5
Signal transduction			
207993_s_at	CHP	Calcium binding protein P22	1.7
1569022_a_at	PIK3C2A	Phosphoinositide-3-kinase, class 2, alpha polypeptide	1.5
243452_at	B4GALT6	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 6	1.5
205868_s_at	PTPN11	Protein tyrosine phosphatase, non-receptor type 11 (Noonan syndrome 1)	1.4
Lipid metabolism			
215891_s_at	GM2A	GM2 ganglioside activator	1.4
Ubiquitin cycle			
222567_s_at	RKHD2	Ring finger and KH domain containing 2	1.5

Transcripts increased above 1.4-fold relative to controls with $p < 0.05$ were classified using NetAffx™ and listed according to associated biological functions

Table 3

Transcripts significantly decreased in HEKa

Affymetrix ID	Gene symbol	Gene name	Fold change
Growth and differentiation			
241681_at	MBNL1	Muscleblind-like (Drosophila)	1.6
229422_at	NRD1	Nardilysin (N-arginine dibasic convertase)	1.4
225318_at	WHSC1L1	Wolf-Hirschhorn syndrome candidate 1-like 1	1.4
Adhesion and motility			
236752_at	PKP4	Plakophilin 4	1.4
Apoptosis			
232565_at	RAB6IP2	RAB6 interacting protein 2	1.7
216621_at	ROCK1	Rho-associated, coiled-coil containing protein kinase 1	1.6
Signal transduction			
228779_at	LOC440456	Similar to pleckstrin homology domain containing, family M (with RUN domain) member 1; adapter protein 162	1.8
236007_at	AKAP10	A kinase (PRKA) anchor protein 10	1.7
240758_at	CENTG2	Centaurin, gamma 2	1.6
216621_at	ROCK1	Rho-associated, coiled-coil containing protein kinase 1	1.6
Transport			
202940_at	WNK1	WNK lysine deficient protein kinase 1	1.8
232565_at	RAB6IP2	RAB6 interacting protein 2	1.7
239274_at	PICALM	Phosphatidylinositol binding clathrin assembly protein	1.7
1554557_at	ATP11B	ATPase, Class VI, type 11B	1.7
240758_at	CENTG2	Centaurin, gamma 2	1.6
156920 l_a_at	SEC15L2	SEC15-like 2 (S. cerevisiae)	1.6
240600_at	AP3B1	Adaptor-related protein complex 3, beta 1 subunit	1.5
214244_s_at	ATP6V0E	ATPase, H^+ transporting, lysosomal 9 kDa, V0 subunit e	1.5
222906_at	FLVCR	Feline leukemia virus subgroup C cellular receptor	1.5
Cell cycle arrest			
1553407_at	MACF1	Microtubule-actin crosslinking factor 1	1.5
Transcription			
236778_at	ATRX	Alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae)	2.0
227111_at	ZBTB34	Zinc finger and BTB domain containing 34	2.0
232716_at	EDG2	Endothelial differentiation, lysophosphatidic acid G- protein-coupled receptor, 2	1.9
232565_at	RAB6IP2	RAB6 interacting protein 2	1.7
239238_at	SMARCC1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 1	1.7
238883_at	THRAP2	Thyroid hormone receptor associated protein 2	1.6
213659_at	ZNF75	Zinc finger protein 75 (D8C6)	1.6
233239_at	ZNF407	Zinc finger protein 407	1.6
235862_at	RAI1	Smith-Magenis syndrome chromosome region, candidate 3	1.5
Protein modification or biosynthesis			
243149_at	EIF4G3	Eukaryotic translation initiation factor 4 gamma, 3	2.1

Affymetrix ID	Gene symbol	Gene name	Fold change
240240_at	UBE2J2	Ubiquitin-conjugating enzyme E2, J2 (UBC6 homolog, yeast)	1.4

Transcripts decreased below 1.4-fold relative to controls with $p < 0.05$ were classified using NetAffx™ and listed according to associated biological functions

Table 4

Transcripts regulated at a greater than twofold level

Affymetrix ID	Gene symbol	Gene name	Fold change	*p* value
211506_s_at	IL8	Interleukin 8	4.3	0.12
205476_at	CCL20	Chemokine (C–C motif) ligand 20	4.1	0.08
220322_at	IL1F9	Interleukin 1 family, member 9	3.5	0.15
209774_x_at	CXCL2	Chemokine (C–X–C motif) ligand 2	3.2	0.05
207850_at	CXCL3	Chemokine (C–X–C motif) ligand 3	3.0	0.28
205207_at	IL6	Interleukin 6 (interferon, beta 2)	2.9	0.24
207113_s_at	TNF	Tumor necrosis factor (TNF superfamily, member 2)	2.8	0.26
210229_s_at	CSF2	Colony stimulating factor 2 (granulocyte–macrophage)	2.5	0.44
223217_s_at	NFKBIZ	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta	2.5	0.03
211924_s_at	PLAUR	Plasminogen activator, urokinase receptor /// plasminogen activator, urokinase receptor	2.3	0.36
208539_x_at	SPRR2B	Small proline-rich protein 2B	2.3	0.14
1555673_at	KRTAP2-1	Keratin associated protein 2-1	2.2	0.10
205266_at	LIF	Leukemia inhibitory factor (cholinergic differentiation factor)	2.1	0.16
205220_at	GPR109B	G protein-coupled receptor 109B///G protein-coupled receptor 109B	2.1	0.15
22407 l_at	IL20	Interleukin 20	2.1	0.23
228964_at	PRDM1	PR domain containing 1, with ZNF domain	2.0	0.41
234079_at	RGNEF	Rho-guanine nucleotide exchange factor	−2.0	0.08
244010_at	ABP1	Amiloride binding protein 1 [amine oxidase (copper-containing)]	−2.2	0.12