AMPK/HuR-Driven IL-20 Post-Transcriptional Regulation in Psoriatic Skin

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IL-20 is involved in the development of skin psoriasis. The molecular mechanisms underlying IL-20 overexpression in psoriatic epidermis remain to be elucidated. We showed that IL-20 was primarily upregulated in psoriatic skin at the post-transcriptional level. The RNA-binding protein HuR relocalized to the cytoplasm of keratinocytes (KCs) of psoriatic patients, suggesting that it stabilizes numerous transcripts, as observed in the human KC cell lines used to assess IL-20 mRNA. We characterized epidermal HuR RNA targets in psoriatic skin using ribonucleoprotein immunoprecipitation analyzed via high-throughput sequencing. Numerous transcripts that are upregulated in psoriasis were targeted by HuR, supporting the participation of HuR in pathogenic processes such as morphological changes, innate and adaptive immune responses, and metabolic inflammatory responses. Finally, we identified the metabolic sensor AMP-activated protein kinase (AMPK) as being responsible for HuR cytoplasmic relocalization because its activity was severely impaired in human psoriatic epidermis, and in vivo drug-mediated AMPK inhibition in mouse epidermis promoted HuR cytoplasmic localization, IL-20 overproduction, acanthosis, and hyperkeratosis. These results provide insights into the molecular links between metabolism and post-transcriptional networks during chronic inflammation.

INTRODUCTION

The IL-20 subfamily of cytokines (IL-19, IL-20, IL-22, IL-24, and IL-26) (Rutz et al., 2014) has an important role in the pathogenesis of psoriasis because of their pro-inflammatory proprieties and overexpression in this disease (Rutz et al., 2014). Indeed, IL-19 and IL-24 and, more markedly, IL-20 and IL-22 have been shown to mimic certain classical psoriatic features in reconstituted human epidermis models (Sa et al., 2007), and mouse models overexpressing IL-20, IL-22, or IL-24 exhibit a psoriasis-like appearance (Blumberg et al., 2001; He and Liang 2010). The primary source of IL-22 and IL-26 is restricted to the lymphoid lineage, certain specific activated T-cell subpopulations (T helper type 22, T helper type 1, and T helper type 17), and natural killer cell subsets, whereas both immune cells and tissue cells secrete IL-19, IL-20, and IL-24 (Sabat, 2010). IL-20 has an important role in the later effector phase of psoriasis pathogenesis, during which it inhibits the terminal differentiation of keratinocytes (KCs) and contributes to antimicrobial competence and epidermal infiltration by neutrophilic granulocytes (Sabat and Wolk, 2011). Patients with psoriasis exhibit an increased level of IL-20 in lesional skin (LS) and in the blood, where it correlates with the Psoriasis Area Severity Index score (Sabat and Wolk, 2011). IL-20 binds to receptor heterodimers that are found on KCs but not on immune cells. Despite their central role in psoriasis, the molecular mechanisms that lead to the overexpression of the IL-20 subfamily of cytokines have been rarely investigated.

In the present study, we show that IL-20 is primarily upregulated in psoriatic skin at the post-transcriptional level via mRNA stabilization. We found that the RNA-binding protein HuR (Srikantan and Gorospe, 2012) binds to and regulates IL-20 mRNA and relocalizes to the cytosol of psoriatic KCs. We further identify additional HuR targets in psoriatic skin using ribonucleoprotein immunoprecipitation analyzed by high-throughput sequencing (RIP-Seq).

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Abbreviations: 3′UTR, 3′ untranslated region; ActD, actinomycin D; AMPK, AMP-activated protein kinase; CompC, compound C; IHC, immunohistochemistry; IP, immunoprecipitation; KC, keratinocyte; LS, lesional skin; NLS, non-lesional skin; RIP-Seq, ribonucleoprotein immunoprecipitation analyzed by high-throughput sequencing

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Consistent with comorbidity (Armstrong *et al.*, 2013) and enzymatic (Halprin and Ohkawara, 1966) studies, we demonstrate metabolic alterations in psoriasis based on the apparent decrease in the activity of the metabolic sensor AMP-activated protein kinase (AMPK) (O'Neill and Hardie, 2013) in psoriatic KCs. Finally, a specific AMPK inhibitor causes HuR cytosolic localization and IL-20 mRNA stabilization in human KCs. Topical application of the AMPK inhibitor on mouse skin causes HuR cytosolic localization and IL-20 overproduction and initiates certain histological features of psoriasis. These findings suggest that metabolic disorders affecting AMPK activation in KCs are important in the pathophysiology of psoriasis, permitting HuR-mediated stabilization of numerous mRNAs, including IL-20 mRNA.

RESULTS

IL-20 mRNA is stabilized by cellular stresses

To study IL-20 induction in a cellular model, we treated the HaCaT KC cell line with different cellular stresses, such as anisomycin (protein synthesis inhibition and p38 MAP kinase activation) or UVB radiation (cell damage stress) with or without actinomycin D (transcriptional inhibition). IL-20 mRNA levels and IL-20 gene transcription were evaluated using quantitiative PCR primers that discriminate IL-20 mRNA and IL-20 precursor mRNA (pre-mRNA). IL-20 exhibited strong mRNA induction in response to anisomycin and UVB exposure (Figures 1a and b, respectively). Under identical conditions, IL-20 pre-mRNA did not increase, except in response to 20 mJ cm^{-2} UVB and 8 hours after anisomycin treatment (Figures 1d and c, respectively), at which dose and time the increase was limited and not significant. This modest variation in the transcriptional rate could suggest that IL-20 mRNA stability is a limiting step for IL-20 mRNA accumulation. The kinetics of mRNA stability following the inhibition of transcription with actinomycin D indicated that IL-19, IL-24, and, to a greater degree, IL-20 were unstable in non-stressed cells but were efficiently stabilized following UVB irradiation (Figure 1e). Collectively, these results indicated that (i) IL-20 mRNA was extremely unstable in non-stressed cells, and (ii) its induction in response to cellular stresses was predominantly mediated by its stabilization. Conversely, IL-19 and IL-24 mRNA induction was associated with both transcriptional and mRNA stabilization mechanisms. Consistent with these results, post-transcriptional regulatory elements present within the 3′ untranslated region (3′UTR) of the IL-20 cytokine subfamily were more numerous and relevant in IL-20 (and IL-22) than in IL-19 and IL-24 (and IL-26; Figure 1f).

IL-20 mRNA is stabilized in psoriatic skin

We used immunohistochemistry (IHC) to confirm that KCs are the primary source of IL-20 in psoriatic lesions (Figure 2a). DEFB4A, a marker of the severity of psoriasis (Jansen *et al.*, 2009), was increased ($\geqslant$2-fold) at the mRNA level in LS compared with non-lesional skin (NLS) in the entire patient cohort ($n = 17$; Figure 2b). IL-20 mRNA was overexpressed in LS in all tested patients, and IL-22 and IL-24 mRNA were increased in 16 of 17 patients, IL-26 in 15 of 17 patients, and IL-19 in 12 of 17 patients (Figure 2c). These results are consistent with previous studies (Sabat and Wolk, 2011). The IL-20 pre-mRNA level was not significantly increased in LS versus NLS and does not account for the upregulation of IL-20 mRNA in psoriasis. This result suggested that the upregulation in LS occurred at the mRNA level via a post-transcriptional mechanism(s) (Figure 2d). Similar results were obtained with IL-22 pre-mRNAs. Because of patient variations, IL-24 pre-mRNA was not significantly upregulated. In contrast, the levels of IL-19 and IL-26 pre-mRNAs were significantly increased in LS compared with NLS, suggesting that the increased gene transcription was a major contributor to the induction of mRNA in LS. We next established the lack of a correlation between the mRNA and the pre-mRNA levels in NLS for each of the five cytokines (Figure 2e). In contrast, the mRNA levels in LS appeared to significantly correlate with the pre-mRNA levels for IL-20, IL-19, IL-22, and IL-24. Indeed, the mechanisms uncoupling the mRNA levels from the pre-mRNA levels in NLS were not present in LS. Because IL-20 mRNA was regulated by the mRNA decay machinery and no increase in IL-20 pre-mRNA (i.e., transcriptional upregulation) was found in LS, IL-20 mRNA may be (i) destabilized in NLS and (ii) stabilized in LS. Comparatively, IL-22 mRNA was also regulated via an increase in stability in LS, whereas IL-19, IL-24, and IL-26 mRNAs were (i) destabilized in NLS and (ii) regulated via an increase in mRNA transcription and stability in LS.

HuR regulates IL-20 mRNA stability and relocalizes to the cytoplasm of psoriatic KCs

Figure 2 indicates that the pathways involved in the stabilization of messengers were activated in psoriasis; therefore, we focused on this aspect. p38 MAP kinases, one of the best-characterized mRNA stabilization pathways through 5′-AUUUA-3′ motifs in the mRNA 3′UTR (Winzen *et al.*, 1999), have been described to be involved in chronic inflammatory diseases, including psoriasis (Johansen *et al.*, 2006). IL-20 contains numerous AUUUA motifs in its 3′UTR, and the inhibition of p38 MAP kinases nearly abrogated the IL-20 response to cellular stress (Supplementary Figures S1A and S1B online). However, it was shown that epidermal p38α, but not myeloid p38α, is not essential in skin inflammation in mice (Kim *et al.*, 2008). Our results supported the relative importance of p38 MAP kinases and/or the target MK2 in the inflammatory response of KCs, as p38 and MK2 activation, estimated by IHC, was limited to (prometa-)metaphasic KCs (Supplementary Figures S1C–S1F online) and is thus the consequence of a high mitotic rate in the psoriatic epidermis.

Another mechanism that may account for the mRNA stabilization is mediated by the RNA-binding protein HuR (Kim and Gorospe, 2008). Indeed, the IL-20 3′UTR contains the highest affinity HuR heptamer-binding sites (5′-UUUUUUU-3′; Lebedeva *et al.*, 2011), and we demonstrated that HuR could bind to IL-20 mRNA (Figure 3a). As shown in Figure 3b, the increase in IL-20 mRNA induced by anisomycin was severely impaired by small interfering RNA-mediated HuR knockdown, stressing the importance of HuR in IL-20 mRNA overexpression. HuR mRNA and protein levels were found to be similar in LS compared with NLS (Figures 3c and d). However, IHC analysis indicated that the intracellular

distribution of HuR in KCs was predominantly nuclear in NLS and predominantly cytoplasmic in LS epidermis (Figure 3e). Because HuR stabilizes mature mRNAs in the cytoplasm (Srikantan and Gorospe, 2012), these results suggested that HuR could stabilize IL-20 mRNA and numerous other mRNAs by interacting with compatible 3′UTRs in LS epidermis.

HuR binds numerous transcripts involved in the pathogenesis of psoriasis

To identify RNA targets of HuR in psoriatic skin, we performed RIP-Seq experiments (Srikantan and Gorospe, 2011) (Supplementary Data S1 online). Pre-mature (i.e., non-spliced) RNAs and mature (i.e., spliced) RNAs targeted by HuR and identified in our RIP-Seq in NLS and LS represented 34.2 and 17.4% of the entire transcriptome, respectively, which is consistent with previous studies investigating HuR targets using photoactivatable-ribonucleoside-enhanced crosslinking and immunoprecipitation (Lebedeva et al., 2011; Mukherjee et al., 2011). Moreover, 42.9% of the pre-mature RNA and 45.8% of the mature RNA targets identified in LS have been described as HuR targets by at least one photoactivatable-ribonucleoside-enhanced crosslinking and immunoprecipitation study (Figure 4a). Mature RNAs with increased HuR binding in psoriatic skin (log2 ≥ 0.5) belong to the epithelial lineage, whereas mature RNAs with the lowest fold binding (log2 ≤ − 0.5) belong to other lineages (Figure 4b). HuR activity was displaced from the nucleus to the cytoplasm in LS based on a 55.5% decrease in total pre-mature RNA reads and a 48.4% increase in total mature RNA reads compared with NLS (Figure 4c).

The intersection between our RIP-Seq and expression array studies (Suárez-Fariñas et al., 2010) indicated an over-representation of upregulated (47.6%) compared with downregulated (18.4%) transcripts (Figure 5a). This over-representation was more pronounced when we considered only transcripts with an increased fold binding for HuR in LS (log2 ≥ 0.5): 47.6% compared with the transcripts that were downregulated (2.6%; Figure 5b).

To investigate the relevance of our HuR RIP-Seq, we used quantitative PCR to evaluate certain genes expressed in KCs (Figure 5c). All three evaluated transcripts that were upregulated in psoriasis and present in the RIP-Seq (CXCL-10, DEFB4A, and SLC6A14) were downregulated by at least 50% in HaCaT KCs (unstimulated or anisomycin-stimulated) following small interfering RNA-mediated HuR knockdown and appeared to be relevant HuR targets. The upregulated transcripts that failed to bind HuR in the RIP-Seq exhibited only a mild reduction (<25% downregulation); however,

CYP7B1, which was excluded from the RIP-Seq for minor criteria (9 instead of 10 RIP-Seq reads, see Supplementary Materials and Methods online), exhibited greater than 50% inhibition. In contrast, two evaluated genes (CCL27 and FA2H) known to be downregulated in psoriasis and not present in HuR RIP-Seq were not affected by HuR knockdown. Interestingly, 12 of 34 transcripts of genes that have been implicated in psoriasis by genome-wide association studies (Yu et al., 2008) appeared to be targeted by HuR (Figure 5d). Numerous targets important for psoriatic patho-physiology bound to HuR in LS more abundantly than in NLS (e.g., CXCL-10, IL17RA, IVL, HERC6, HLA-DPA1, HLA-E, IL36G, KRT17, LIPG, NAT9, PIM1, PSMA6, PSORS1C2, RPS6KB2, REL, RHEB, SERPINB13, or SPINK5); however, the RIP-Seq analysis was unable to detect IL-20 subfamily cytokine mRNA or the mRNA of other cytokines, such as IL-8, which is targeted by HuR (Nabors et al., 2001). This lack of detection was likely associated with the difficulty encountered in immunoprecipitating low-level transcripts from skin samples and indicated that our analysis may have underestimated the number of HuR-targeted transcripts. Next, we explored pathways that were modulated by HuR in LS by cross-matching HuR mature RNA targets (exhibiting a log2 increase ≥ 0.5-fold) using the Kyoto Encyclopedia of Genes and Genomes pathway database (Figure 5e), and we identified several pathways that have been implicated in the pathogenesis of psoriasis (see Discussion section).
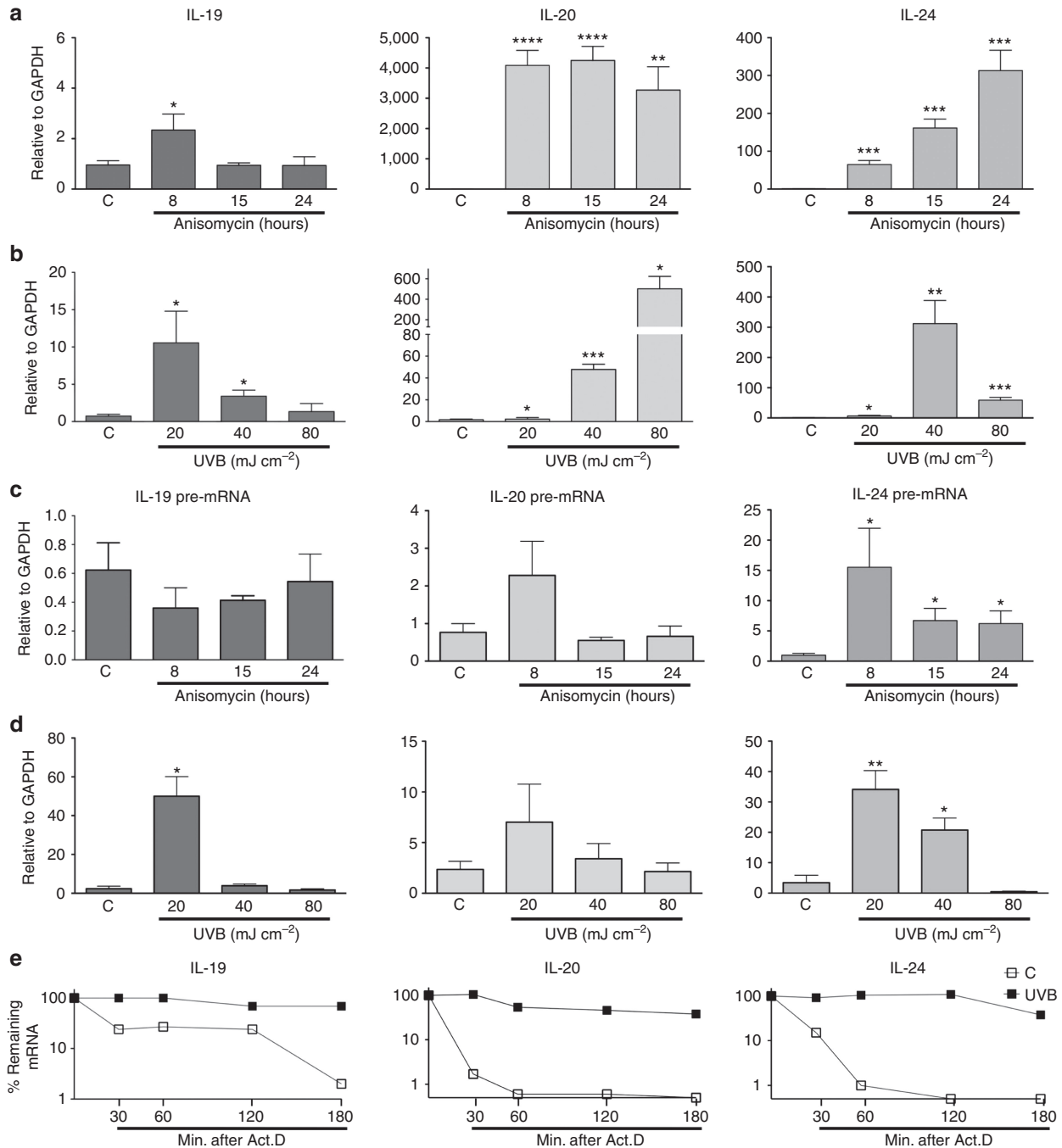
AMPK inactivation leads to HuR cytoplasmic localization and IL-20 mRNA stabilization and initiates epidermal changes in mouse skin

Using IHC, we explored whether known HuR shuttling regulators (Kim and Gorospe, 2008) were affected in psoriatic skin. Phosphorylated Cdk1 and protein kinase Cα labeling were similar in LS and NLS (data not shown). Phosphorylated AMPK labeling was predominantly detected in the basal layer of NLS epidermis (Figure 6a). Conversely, phosphorylated AMPK labeling was essentially absent in LS. To investigate the potential role of a decrease in AMPK activity, we examined the influence of compound C (CompC), a specific AMPK inhibitor (Zhou et al., 2001), on HuR subcellular localization and IL-20 mRNA stabilization. As shown in Figure 6b, HuR was primarily nuclear in untreated HaCaT cells, whereas exposure to CompC triggered an increase in cytoplasmic HuR. IL-20 mRNA decay following actinomycin D addition was primarily counteracted by CompC, as 30% of the mRNA remained after 120 minutes (Figure 6c). These observations suggested that AMPK inactivation in psoriasis may be responsible for the relocalization of

Figure 1. IL-20 production is regulated at the post-transcriptional level in HaCaT cells. (**a**) IL-19, IL-20, and IL-24 mRNA expression in response to anisomycin (400 ng ml^{-1}). (**b**) UVB dose–dependent mRNA expression of IL-19, IL-20, and IL-24 (± SEM; $n=3$; c, control). (**c**) IL-19, IL-20, and IL-24 pre-spliced mRNA expression in response to anisomycin (400 ng ml^{-1}). (**d**) IL-19, IL-20, and IL-24 pre-spliced mRNA expression in response to increasing UVB doses. **a–d** ± SEM; $n=3$; *$P<0.05$, **$P<0.005$, ***$P<0.001$, and ****$P<0.0001$. (**e**) Actinomycin D (ActD; 5 µg ml^{-1})-mediated termination of transcription in growing (open squares) versus UVB (filled squares)-irradiated (30 mJ cm^{-2} 4 hours prior to ActD) cells for IL-19, IL-20, and IL-24 mRNAs (one representative is shown; $n=3$). (**f**) Post-transcriptional regulatory elements in IL-20 family 3′UTRs, such as AU-rich elements (5′-AUUUA-3′, 5′-UAUUUAU-3′, and 5′-WWWWAUUUAW WWW-3′), HuR motifs (5′-UUUUUUU-3′, 5′-UUUAUUU-3′, and 5′-UUUGUUU-3′), and microRNAs with conserved matches.

HuR to the cytoplasm of KCs to promote the stabilization of IL-20 and other mRNAs. To address this question, we used a mouse skin model. Normal mouse epidermis shares molecular features of human epidermis: cytoplasmic phosphorylated AMPK labeling, predominant nuclear HuR localization, and minimal IL-20 levels (Figure 6d). The daily application of

|	AREScore	AUUUA	UAUUUAU	WWWWAUUUAWWWW	UUU (U/A/G) UUU	microRNAs
IL-19	1.3	1	–	–	– / – / –	–
IL-20	12.5	8	6	1	1 / 3 / –	miR-876-3p; miR-634, miR-548p; miR-3121-3p; miR-3674
IL-22	12.1	8	6	1	1 / 4 / 1	miR-3663-5p; miR-3688-3p; miR-3163; miR-944
IL-24	3.9	3	2	–	2 / 1 / –	miR-203
IL-26	2.6	2	–	–	– / – / –	–
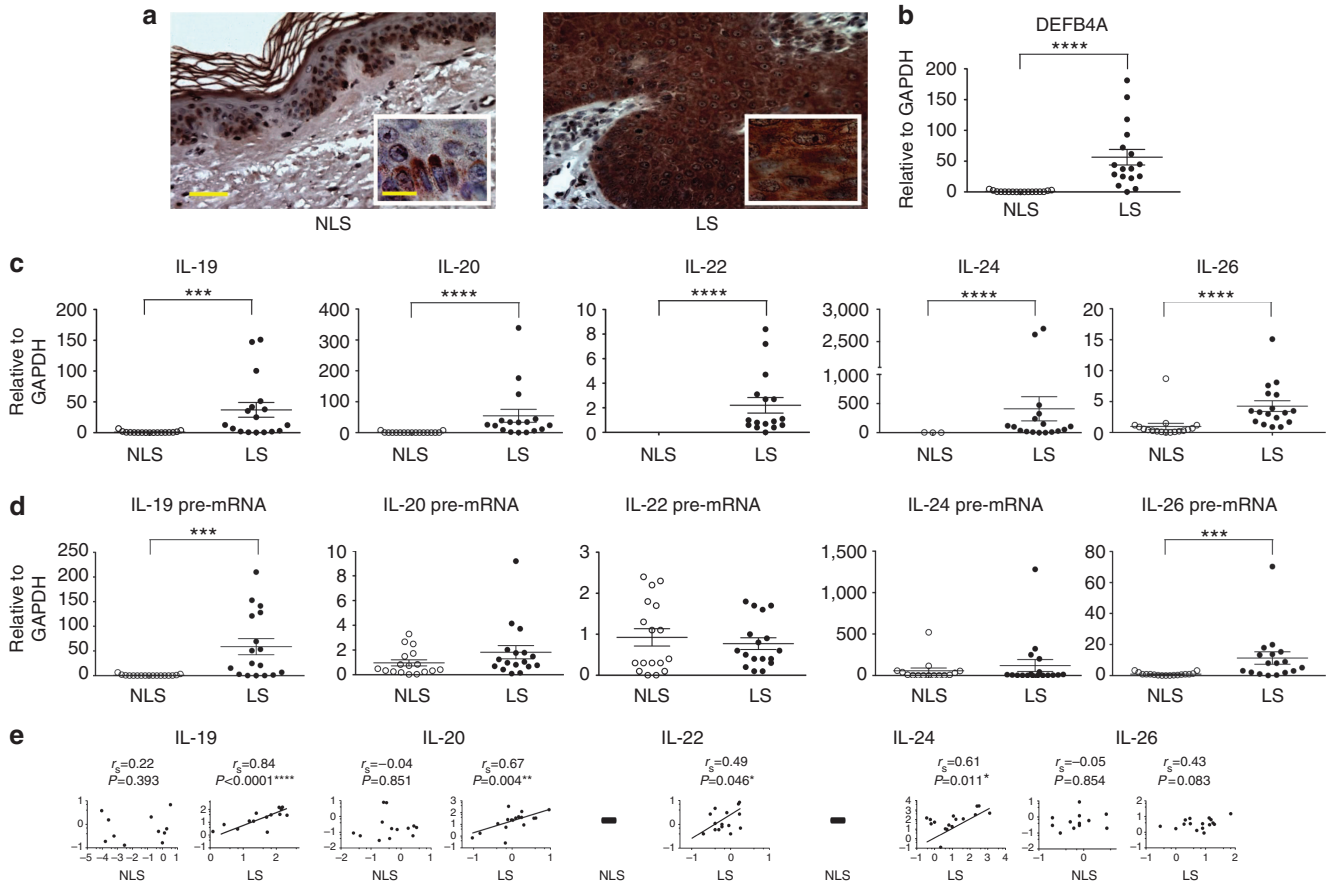
Figure 2. IL-20 overexpression in psoriasis is a consequence of mRNA stabilization. (**a**) Immunohistochemical (IHC) staining of IL-20 in non-lesional skin (NLS) and lesional skin (LS; one patient, representative of five). Scale bars = 200 μm for original magnification of 20× and 50 μm for original magnification of 100×. (**b**) DEFB4A mRNA expression in punch biopsies (± SEM; $n = 17$; ****$P < 0.0001$) for NLS (open circles) and LS (filled circles). (**c**) Identical results for IL-19, IL-20, IL-22, IL-24, and IL-26 mRNA expression (***$P < 0.001$, ****$P < 0.0001$). (**d**) IL-19, IL-20, IL-22, IL-24, and IL-26 pre-mRNA expression (***$P < 0.001$ for NLS (open circles) or LS (filled circles)). (**e**) Spearman's rank correlation analysis and log10 plots between pre-mRNA (abscissa) and mRNA expression (ordinate) levels of IL-19, IL-20, IL-22, IL-24, and IL-26 in NLS and LS (*$P < 0.05$, **$P < 0.005$, and ****$P < 0.0001$).

CompC on shaved mouse skin ($n = 6$; Figure 6d) induced histologically focal epidermal acanthosis and hyperkeratosis that were less widespread and pronounced than those observed following SDS application (Kim *et al.*, 2008). Moreover, CompC application led to a decrease in phosphorylated AMPK labeling, HuR cytoplasmic relocalization, and an increase in IL-20 labeling in mouse epidermis. SDS-treated mice also exhibited epidermal relocalization of HuR and an increase in IL-20 labeling but to a lesser extent. Collectively, these results suggest that AMPK inactivation in KCs promotes the cytoplasmic relocalization of HuR, stabilization of IL-20 mRNA, and overproduction of IL-20, which in turn induces epidermal changes.

DISCUSSION
Changes in mRNA abundance have a dominant role in determining the protein levels (Jovanovic *et al.*, 2015), whereas RNA processing degradation affects few genes, albeit with important functions (Rabani *et al.*, 2014). Indeed, the role of transcriptional regulation in psoriasis has been well documented, e.g., the inhibition of NF-κB in IL-24 induction

(Kumari *et al.*, 2013), and it is supported in the present study by the behavior of IL-19, IL-24, and IL-26. Here, we focused on the post-transcriptome because it is implicated in the expression of all IL-20 family members and because overexpression of IL-20 appears to be primarily a consequence of post-transcriptional regulation. We provide compelling evidence implicating HuR in the stabilization of mRNAs in psoriatic skin. IL-20 mRNA is targeted and regulated by HuR in human KCs, and IHC and RIP-Seq analyses support the translocation of HuR from the nucleus to the cytoplasm in lesional epidermis. Nuclear HuR can interact with pre-mature RNAs and affect their processing (Lebedeva *et al.*, 2011; Mukherjee *et al.*, 2011), and cytoplasmic HuR translocation is critical for HuR-mediated stabilization of mRNAs (Wang *et al.*, 2002). HuR mRNA targets are considered to comprise a large portion of the transcriptome (approximately 15%) and to be important for cell growth, proliferation, death, and immune responses (Srikantan and Gorospe, 2012). HuR translocation has been suspected in chronic inflammatory diseases, such as asthma (Fan *et al.*, 2011), and has been reported in certain cancers (Srikantan and Gorospe, 2012). However, to the best
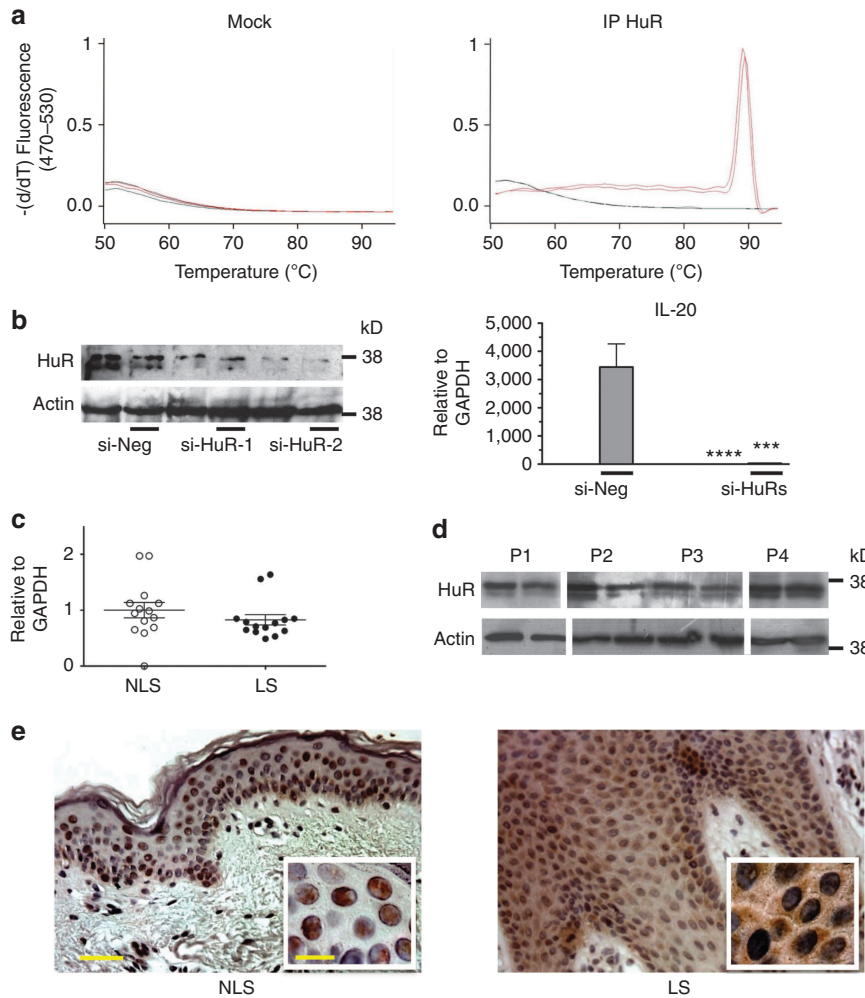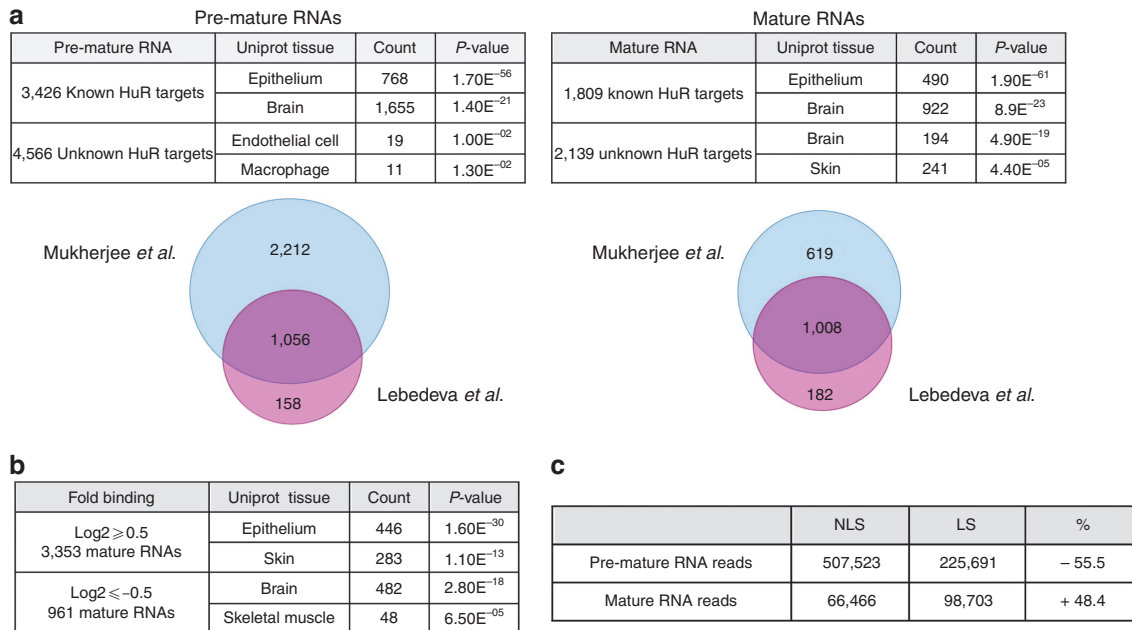
Figure 3. HuR regulates IL-20 mRNA and relocalizes in the cytoplasm of keratinocytes in psoriasis. (**a**) DNA melting curve analysis from IL-20 quantitative PCR following mock or HuR immunoprecipitations (IPs). HaCaT cells were previously irradiated (red line) or not (black line) for 4 hours with UVB (30 mJ cm^{-2}). (**b**) HaCaT cells were transfected with a control small interfering RNA (siRNA) or independently with two HuR-specific siRNAs and stimulated with anisomycin (400 ng ml^{-1} for 7 hours, short dark line) or not stimulated. Left: HuR protein expression. Right: IL-20 mRNA level, average of both HuR-specific siRNAs ($\pm$ SEM; $n = 3$ for each siRNA; ***$P < 0.0005$ and ****$P < 0.0001$). (**c**) HuR mRNA expression in non-lesional skin (NLS; open circles) and lesional skin (LS; filled circles; $\pm$ SEM; $n = 14$). (**d**) Western blot analysis of samples from patients P1–P4. Left: NLS. Right: LS. (**e**) Immunohistochemistry for HuR in NLS and LS (one patient, representative of five). Scale bars = 200 μm for original magnification of 20 × and 50 μm for original magnification of 100 ×.

of our knowledge, HuR targets have not been determined in any human disease, including psoriasis.

We found that pathways that could be affected by HuR cytoplasmic localization in psoriasis include adherens junction and focal adhesion pathways that participate in morphological and transduction changes in psoriatic KCs. Interestingly, innate and adaptive immune responses, which are an important feature in psoriasis pathophysiology, are also targeted by HuR. Indeed, KCs are an important component of the immune system, expressing FcgRIII, which is present in RIP-Seq, and molecules that are implicated in internalization (Gutowska-Owsiak and Ogg, 2012). Finally, the phosphatidylinositol 3-kinase/AKT/mammalian target of the rapamycin (PI3K/AKT/mTOR) pathway is also targeted by HuR cytoplasmic relocalization, as indicated by the Kyoto Encyclopedia of Genes and Genomes occurrences of inositol phosphate metabolism, mTOR, phosphatidylinositol, and insulin

signaling pathways. Positive feedback of HuR in the AKT pathway, which is involved in the pathogenesis of psoriasis, (Mitra *et al.*, 2012; Roller *et al.*, 2012; Buerger *et al.*, 2013), has been reported (Singh *et al.*, 2013).

Finally, AMPK activity, which is a sensor of cellular energy and an antagonist of the PI3K/AKT/mTOR pathway (Inoki *et al.*, 2012), appears to decrease in psoriatic lesional epidermis. Moreover, CompC, an AMPK inhibitor, induces HuR cytoplasmic localization, stabilizes IL-20 mRNA in human KCs, and initiates certain epidermal psoriatic histological features. Alterations in carbohydrate metabolism have been described in psoriasis for decades and are consistent with our results. AMPK activation leads to the stimulation of energy-producing pathways such as glycolysis. When AMPK is inactivated, ATP-consuming pathways such as glycogenesis are promoted. Indeed, a high level of glucose utilization without an increase in glycolysis, leading to the use of glucose-

a

Pre-mature RNAs

Pre-mature RNA	Uniprot tissue	Count	*P*-value
3,426 Known HuR targets	Epithelium	768	$1.70E^{-56}$
	Brain	1,655	$1.40E^{-21}$
4,566 Unknown HuR targets	Endothelial cell	19	$1.00E^{-02}$
	Macrophage	11	$1.30E^{-02}$

Mature RNAs

Mature RNA	Uniprot tissue	Count	*P*-value
1,809 known HuR targets	Epithelium	490	$1.90E^{-61}$
	Brain	922	$8.9E^{-23}$
2,139 unknown HuR targets	Brain	194	$4.90E^{-19}$
	Skin	241	$4.40E^{-05}$

Mukherjee *et al.*   2,212   1,056   158   Lebedeva *et al.*

Mukherjee *et al.*   619   1,008   182   Lebedeva *et al.*

b

Fold binding	Uniprot tissue	Count	*P*-value
Log2 ⩾ 0.5 3,353 mature RNAs	Epithelium	446	$1.60E^{-30}$
	Skin	283	$1.10E^{-13}$
Log2 ⩽ -0.5 961 mature RNAs	Brain	482	$2.80E^{-18}$
	Skeletal muscle	48	$6.50E^{-05}$

c

	NLS	LS	%
Pre-mature RNA reads	507,523	225,691	− 55.5
Mature RNA reads	66,466	98,703	+ 48.4

Figure 4. HuR binding to mature RNAs is increased in psoriasis. (**a**) Venn diagrams comparing our biopsies for HuR RIP-Seq with published HuR PAR-CLIPs for cell lines (Lebedeva *et al.*, 2011; Mukherjee *et al.*, 2011). Annotation of pre-mature RNAs belonging to the UCSC intronic regions and mature RNAs belonging to the University of California Santa Cruz (UCSC) transcript. (**b**) UniProt tissue analysis of mature RNAs with increased (log2 ⩾0.5) or decreased (log2 ⩽ -0.5) HuR binding in involved versus uninvolved skin. (**c**) Total pre-mature RNA and mature RNA reads in the RIP-Seq for NLS and LS. LS, lesional skin; NLS, non-lesional skin; PAR-CLIP, photoactivatable-ribonucleoside-enhanced crosslinking and immunoprecipitation; RIP-Seq, ribonucleoprotein immunoprecipitation analyzed by high-throughput sequencing.

6 phosphate via the pentose phosphate pathway (Herdenstam 1962; Halprin and Ohkawara, 1966) and to glycogen accumulation (Harmon and Phizackerley, 1984), has been described in the psoriatic epidermis. Moreover, numerous common drugs used to treat psoriasis are considered to enhance AMPK activity, such as vitamin D (Hadad *et al.*, 2008) or retinoic acid (Lee *et al.*, 2008). Furthermore, methotrexate induces the accumulation of aminoimidazole carboximide ribonucleotide (Beckers *et al.*, 2006), which is a potent AMPK activator. Collectively, these findings related to metabolism, molecular interactions, and drug therapy reinforce the importance of AMPK inhibition in psoriasis. Notably, UVB is known to decrease AMPK activity and relocalize HuR to the cytosol (Zhang and Bowden, 2008), and we demonstrated that UVB promotes IL-20 induction in KCs, apparently in contradiction with the efficacy of UVB treatment for psoriasis. However, UVB therapy involves many other mechanisms and cell lineages as for example the UVB-induced immune suppression mediated by Treg cells following amphiregulin stimulation by basophils (Meulenbroeks *et al.*, 2015).

In summary, we provide compelling evidence for the existence of a pathogenic cascade linking AMPK inactivation, HuR cytoplasmic relocalization, and increased IL-20 stabilization in the psoriatic epidermis. Our findings further support the important post-transcriptional regulatory role of HuR in the pathogenesis of psoriasis and implicate HuR in the psoriatic KC response to metabolic stress. Understanding the contribution of this potential pathway to psoriasis will be enhanced by future studies that will include both therapeutic

targeting of AMPK and HuR and relevant genetically modified animal models.

MATERIALS AND METHODS
Human tissue samples
We used tumor bank facilities offered by the University Hospital Carémeau, Nîmes (France). This was an open study that was approved by the ethics committee of the University Hospital of Carémeau, Nîmes (France), in accordance with French laws and regulations, and thus all patients provided written consent prior to study participation. The included patients with chronic plaque-type psoriasis had not received topical anti-psoriatic therapy during the previous 2 weeks or systemic therapy during the previous 4 weeks. Keratome biopsies, which were used for IHC, quantitative real-time reverse transcriptase–PCR, and RIP-Seq, were harvested from LS and NLS (at a distance of at least 5 cm from a lesional plaque) in patients with plaque-type psoriasis.

Histology
For human samples, formalin-fixed paraffin-embedded tissue sections (7 μm) were used for immunoperoxidase staining. The sections were counterstained with Harris hematoxylin (Sigma-Aldrich, St. Louis, MO). Serial sections (4 μm) of formalin-fixed paraffin-embedded mouse back skin were stained with hematoxylin-eosin-safran or used for immunoperoxidase staining and counterstained with Harris hematoxylin. For human and mouse immunoperoxidase staining, negative controls were processed with a matched control antibody. All sections were observed with an Olympus AX70 microscope (Olympus, Tokyo, Japan).
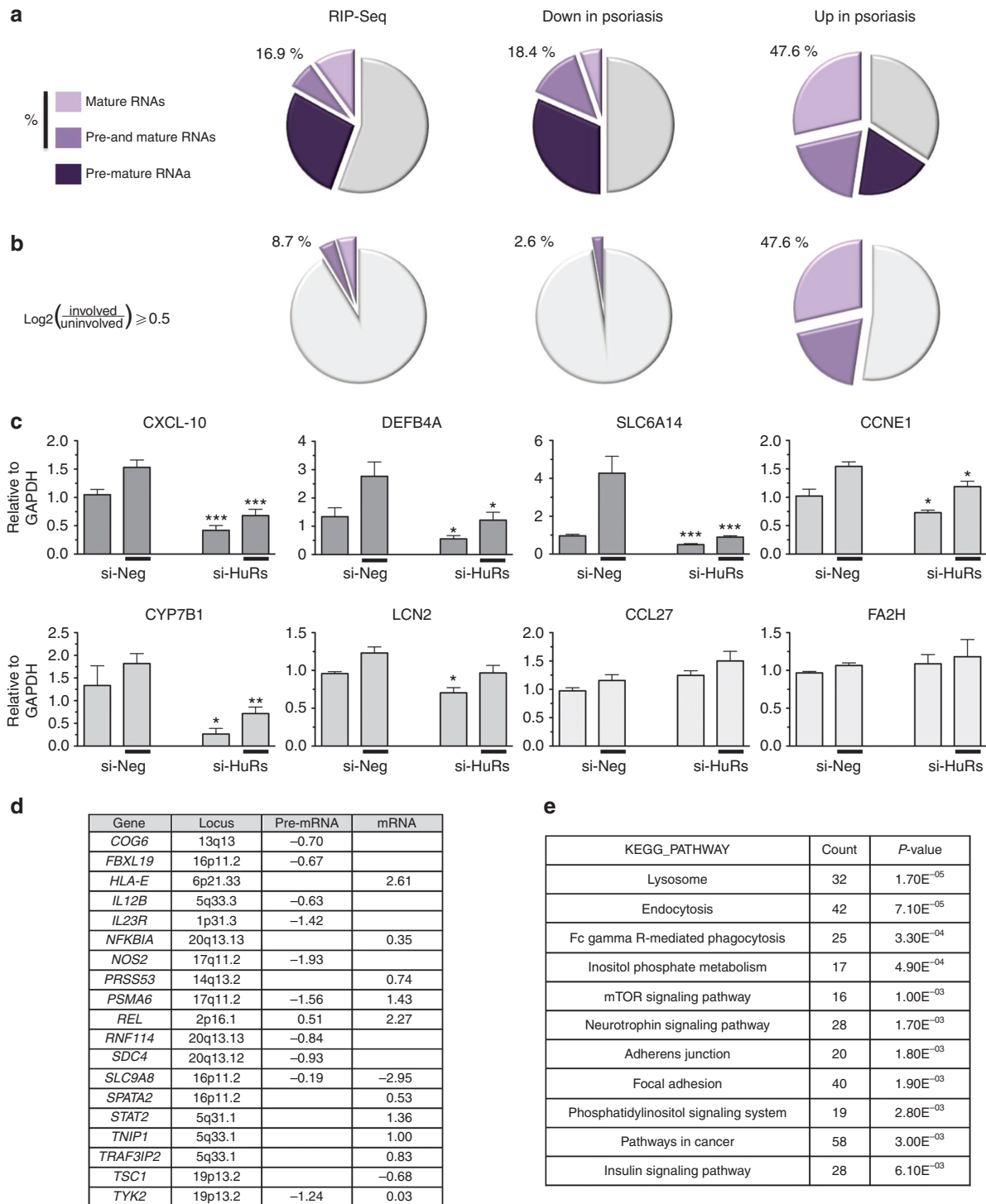
Figure 5. HuR binds numerous transcripts involved in psoriasis pathophysiology. (**a**) Comparison of ribonucleoprotein immunoprecipitation analyzed by high-throughput sequencing (RIP-Seq) HuR mRNA targets (Supplementary Data S1 online, exonic sheet) with up- and downregulated genes found in published psoriasis transcriptomes (Suárez-Fariñas *et al.*, 2010). (**b**) The same analysis with RIP-Seq HuR mRNA targets exhibiting an increased fold binding (log2 ≥ 0.5) to HuR in lesional skin (LS) versus non-lesional skin (NLS). (**c**) Small interfering RNA (siRNA) knockdown of HuR. HaCaT cells were transfected and stimulated as described in Figure 3b (± SEM; $n = 3$ for each siRNA; $*P < 0.05$, $**P < 0.005$, and $***P < 0.0005$), and the expression of certain transcripts was determined by qPCR. (**d**) Table of genes suggested to be implicated in psoriasis by genome-wide association studies (Yu *et al.*, 2008) that appeared to be HuR targets in our RIP-Seq analysis (log2-fold binding in LS). (**e**) Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of HuR mature RNA targets exhibiting a log2 ≥ 0.5-fold increased binding in LS using DAVID bioinformatics resources. Pathways with $P < 0.001$ are included in the table. Counts represent the number of matching genes for each KEGG pathway.
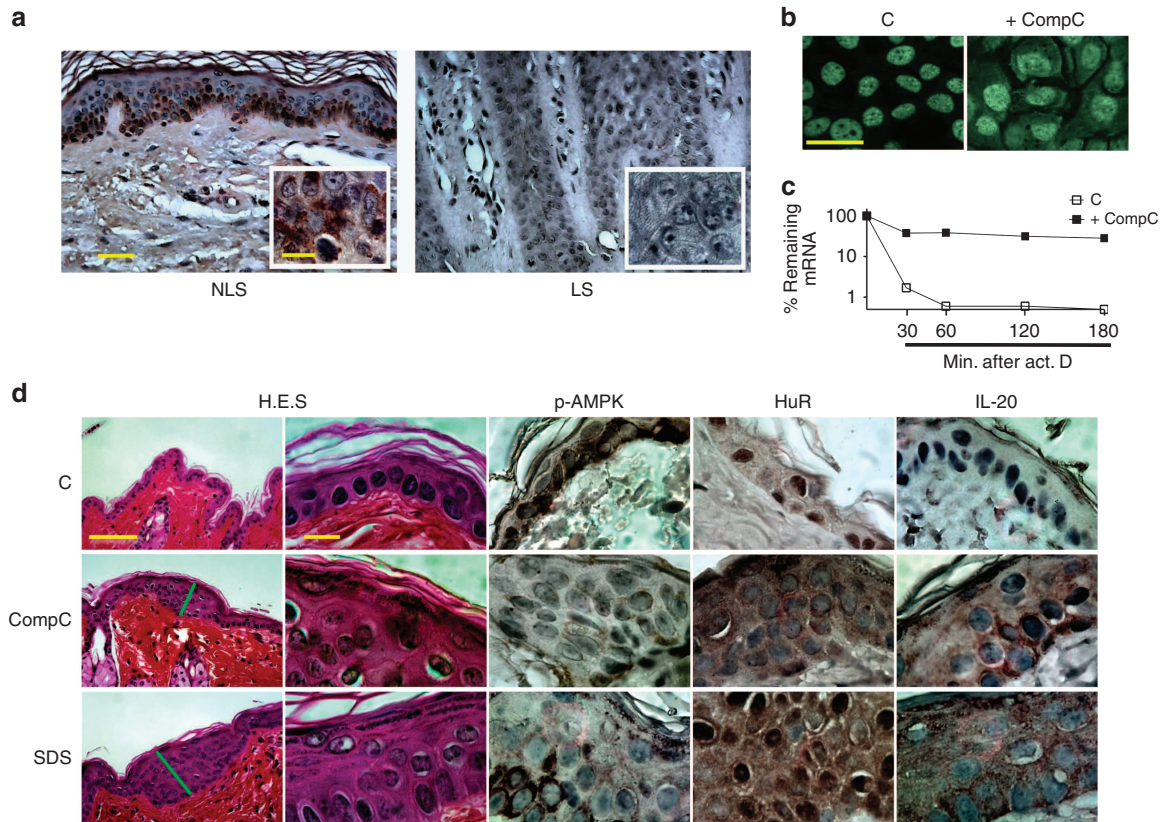
Figure 6. AMP-activated protein kinase (AMPK) inactivation leads to HuR cytoplasmic localization and IL-20 mRNA stabilization and initiates psoriatic features. (**a**) Immunohistochemistry (IHC) for phosphorylated AMPK (p-AMPK) in non-lesional skin (NLS) and lesional skin (LS; one patient, representative of five). Scale bars = 200 μm for original magnification of 20× and 50 μm for original magnification of 100×. (**b**) Confocal analysis of HuR localization in HaCaT cells that were treated or not (C) with Compound C (CompC; 20 μM for 15 hours). Scale bar = 100 μm. (**c**) IL-20 mRNA stability in HaCaT cells that were treated (filled squares) or not (open squares) with CompC (20 μM) for 4 hours (one representative; $n = 3$) (**d**) Dorsal mouse skin that was treated daily with control C (50% ethanol), SDS (5% PBS), or CompC (100 μM, 50% ethanol) for 2 weeks. Hematoxylin-eosin-safran (H.E.S.) staining (green line, acanthosis); $n = 6$. IHC staining for p-AMPK, HuR, and IL-20; $n = 3$. Scale bars = 400 μm for original magnification of 20× and 50 μm for original magnification of 100×.

RNA purification and quantitative real-time PCR

Total RNA was prepared using TRIzol (Invitrogen, Carlsbad, CA) for cellular samples or the MagNA Pure Compact RNA Isolation Kit (Roche Applied Science, Mannheim, Germany) for frozen tissue samples (−80 °C). RNAs were checked for DNA contamination with the appropriate primers. Reverse transcription was conducted using reverse-transcribed murine Moloney leukemia virus reverse transcriptase (Invitrogen) with oligo(dT) primers (Invitrogen) for mRNA quantification and random primers (Promega, Madison, WI) for pre-mRNA quantification.

Ribonucleoprotein immunoprecipitation analyzed by high-throughput sequencing

To limit individual genetic variation, we pooled five LS and five NLS samples for each HuR or mock IP. Frozen samples (−80 °C) were disrupted using the MagNA Pure Instrument (Roche Applied Science). IPs of endogenous RNA-protein complexes from LS and NLS were evaluated as previously described (Peritz et al., 2006) using Protein A-Sepharose (Sigma-Aldrich, St Louis, MO) that had been pre-coated with 60 μg of anti-HuR or isotype control (mock) antibodies. RNA-Seq is described in the Supplementary Materials and Methods online.

Skin inflammation models

C57BL/6 mice (Charles River Laboratories, Chatillon-sur-Chalaronne, France) were treated daily on shaved back skin with either 5% SDS in phosphate-buffered saline, CompC-HCl (Merck, Darmstadt, Germany) in 50% ethanol, or the control (50% ethanol). Two weeks later, the mice were killed, and the skin was excised. Two independent experiments consisting of at least three mice in each group were performed. The Ethics Committee on Animal Research of Languedoc-Roussillon, France, has approved this study (CEEA-LR-12069).

Statistical analyses

The values are expressed as the means ± SEM. The data were analyzed using an unpaired two-tailed Student's t-test, or a paired Wilcoxon test, or Spearman's rank correlation. Changes were identified as significant if the P-value was < 0.05. P-values associated with RIP-Seq functional annotations were derived using DAVID bioinformatics resources (Huang et al., 2009).

CONFLICT OF INTEREST
The authors state no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Armstrong AW, Harskamp CT, Armstrong EJ (2013) Psoriasis and metabolic syndrome: a systematic review and meta-analysis of observational studies. *J Am Acad Dermatol* 68:654–62

Beckers A, Organe S, Timmermans L *et al.* (2006) Methotrexate enhances the antianabolic and antiproliferative effects of 5-aminoimidazole-4-carboxamide riboside. *Mol Cancer Ther* 5:2211–7

Blumberg H, Conklin D, Xu WF *et al.* (2001) Interleukin 20: discovery, receptor identification, and role in epidermal function. *Cell* 104:9–19

Buerger C, Malisiewicz B, Eiser A *et al.* (2013) mTOR and its downstream signalling components are activated in psoriatic skin. *Br J Dermatol.* 169:156–9

Fan J, Ishmael FT, Fang X *et al.* (2011) Chemokine transcripts as targets of the RNA-binding protein HuR in human airway epithelium. *J Immunol* 186:2482–94

Gutowska-Owsiak D, Ogg GS (2012) The epidermis as an adjuvant. *J Invest Dermatol* 132:940–8

Hadad SM, Fleming S, Thompson AM (2008) Targeting AMPK: a new therapeutic opportunity in breast cancer. *Crit Rev Oncol Hematol* 67:1–7

Halprin KM, Ohkawara A (1966) Carbohydrate metabolism in psoriasis: an enzymatic study. *J Invest Dermatol* 46:51–69

Harmon CS, Phizackerley PJ (1984) Glycogen metabolism in psoriatic epidermis and in regenerating epidermis. *Clin Sci* 67:291–8

He M, Liang P (2010) IL-24 transgenic mice: *in vivo* evidence of overlapping functions for IL-20, IL-22, and IL-24 in the epidermis. *J Immunol* 184:1793–8

Herdenstam CG (1962) On the *in vitro* metabolism of labeled glucose in normal and psoriatic skin slices. *Acta Derm Venereol Suppl (Stockh)* 42(Suppl 47):1–62

Huang DW, Sherman BT, Lempicki RA (2009) Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res* 37:1–13

Inoki K, Kim J, Guan K-L (2012) AMPK and mTOR in cellular energy homeostasis and drug targets. *Annu Rev Pharmacol Toxicol* 52:381–400

Jansen PAM, Rodijk-Olthuis D, Hollox EJ *et al.* (2009) β-defensin-2 protein is a serum biomarker for disease activity in psoriasis and reaches biologically relevant concentrations in lesional skin. *PLoS One* 4:e4725

Johansen C, Funding AT, Otkjær K *et al.* (2006) Protein expression of TNF-alpha in psoriatic skin is regulated at a posttranscriptional level by MAPK-activated protein kinase 2. *J Immunol* 176:1431–8

Jovanovic M, Rooney MS, Mertins P *et al.* (2015) Immunogenetics. Dynamic profiling of the protein life cycle in response to pathogens. *Science* 347:1259038

Kim C, Sano Y, Todorova K *et al.* (2008) The kinase p38α serves cell type–specific inflammatory functions in skin injury and coordinates pro- and anti-inflammatory gene expression. *Nat Immunol* 9:1019–27

Kim HH, Gorospe M (2008) Phosphorylated HuR shuttles in cycles. *Cell Cycle* 7:3124–6

Kumari S, Bonnet MC, Ulvmar MH *et al.* (2013) Tumor necrosis factor receptor signaling in keratinocytes triggers interleukin-24-dependent psoriasis-like skin inflammation in mice. *Immunity* 39:899–911

Lebedeva S, Jens M, Theil K *et al.* (2011) Transcriptome-wide analysis of regulatory interactions of the RNA-binding protein HuR. *Mol Cell* 43:340–52

Lee YM, Lee JO, Jung J-H *et al.* (2008) Retinoic acid leads to cytoskeletal rearrangement through AMPK-Rac1 and stimulates glucose uptake through AMPK-p38 MAPK in skeletal muscle cells. *J Biol Chem* 283:33969–74

Meulenbroeks C, van Weelden H, Schwartz C *et al.* (2015) Basophil-derived amphiregulin is essential for UVB irradiation-induced immune suppression. *J Invest Dermatol* 135:222–8

Mitra A, Raychaudhuri SK, Raychaudhuri SP (2012) IL-22 induced cell proliferation is regulated by PI3K/Akt/mTOR signaling cascade. *Cytokine* 60:38–42

Mukherjee N, Corcoran DL, Nusbaum JD *et al.* (2011) Integrative regulatory mapping indicates that the RNA-binding protein HuR couples pre-mRNA processing and mRNA stability. *Mol Cell* 43:327–39

Nabors LB, Gillespie GY, Harkins L *et al.* (2001) HuR, a RNA stability factor, is expressed in malignant brain tumors and binds to adenine- and uridine-rich elements within the 3′ untranslated regions of cytokine and angiogenic factor mRNAs. *Cancer Res* 61:2154–61

O'Neill LAJ, Hardie DG (2013) Metabolism of inflammation limited by AMPK and pseudo-starvation. *Nature* 493:346–55

Peritz T, Zeng F, Kannanayakal TJ *et al.* (2006) Immunoprecipitation of mRNA-protein complexes. *Nat Protoc* 1:577–80

Rabani M, Raychowdhury R, Jovanovic M *et al.* (2014) High-resolution sequencing and modeling identifies distinct dynamic RNA regulatory strategies. *Cell* 159:1698–710

Roller A, Perino A, Dapavo P *et al.* (2012) Blockade of phosphatidylinositol 3-kinase PI3Kδ or PI3Kγ reduces IL-17 and ameliorates imiquimod-induced psoriasis-like dermatitis. *J Immunol* 189:4612–20

Rutz S, Wang X, Ouyang W (2014) The IL-20 subfamily of cytokines—from host defence to tissue homeostasis. *Nat Rev Immunol* 14:783–95

Sa SM, Valdez PA, Wu J *et al.* (2007) The effects of IL-20 subfamily cytokines on reconstituted human epidermis suggest potential roles in cutaneous innate defense and pathogenic adaptive immunity in psoriasis. *J Immunol* 178:2229–40

Sabat R (2010) IL-10 family of cytokines. *Cytokine Growth Factor Rev* 21:315–24

Sabat R, Wolk K (2011) Research in practice: IL-22 and IL-20: significance for epithelial homeostasis and psoriasis pathogenesis. *J Dtsch Dermatol Ges* 9:518–23

Singh M, Martinez AR, Govindaraju S *et al.* (2013) HuR inhibits apoptosis by amplifying Akt signaling through a positive feedback loop. *J Cell Physiol* 228:182–9

Srikantan S, Gorospe M (2011) UneCLIPsing HuR nuclear function. *Mol Cell* 43:319–21

Srikantan S, Gorospe M (2012) HuR function in disease. *Front Biosci* 17:189–205

Suárez-Fariñas M, Lowes MA, Zaba LC *et al.* (2010) Evaluation of the psoriasis transcriptome across different studies by gene set enrichment analysis (GSEA). *PLoS ONE* 5:e10247

Wang W, Fan J, Yang X *et al.* (2002) AMP-activated kinase regulates cytoplasmic HuR. *Mol Cell Biol* 22:3425–36

Winzen R, Kracht M, Ritter B *et al.* (1999) The p38 MAP kinase pathway signals for cytokine-induced mRNA stabilization via MAP kinase-activated protein kinase 2 and an AU-rich region-targeted mechanism. *EMBO J* 18:4969–80

Yu W, Gwinn M, Clyne M *et al.* (2008) A navigator for human genome epidemiology. *Nat Genet* 40:124–5

Zhang J, Bowden GT (2008) UVB irradiation regulates Cox-2 mRNA stability through AMPK and HuR in human keratinocytes. *Mol Carcinog* 47:974–83

Zhou G, Myers R, Li Y *et al.* (2001) Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 108:1167–74