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RNA Sequencing Reveals the Long Non-Coding RNA Signature in Psoriasis Keratinocytes and Identifies CYDAER as a Long Non-Coding RNA Regulating Epidermal Differentiation

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Keywords: CYDAER | keratinocytes | Long non-coding RNAs | psoriasis | RNA sequencing | RP11-295G20.2

ABSTRACT

Psoriasis is a common chronic inflammatory skin disease determined by genetic and environmental factors, resulting in the activation of IL-23/IL-17-mediated immune response, epidermal hyperproliferation, and keratinocyte activation. Long non-coding RNAs (lncRNAs) are non-protein-coding transcripts > 500 nucleotides with diverse regulatory functions; their role in epidermal dysfunction in psoriasis is poorly understood. To identify epidermal transcripts with potential roles in psoriasis, including lncRNAs, we performed RNA sequencing on keratinocytes from psoriasis and healthy skin. We identified 889 differentially expressed lncRNAs, many of which with yet unknown functions. RP11-295G20.2 was identified as a lncRNA significantly induced in psoriasis keratinocytes, and this was verified by qRT-PCR and by single-molecule in situ hybridisation. Analysis of subcellular fractions of epidermis revealed a cytoplasmic localisation in line with results of single molecule in situ hybridisation. We report that RP11-295G20.2 has a skin-enriched expression, and within skin it is mainly expressed in suprabasal epidermal layers. Moreover, RP11-295G20.2 is induced by the key psoriasis cytokine IL-17A and shows a dynamic regulation during keratinocyte differentiation with upregulation during early differentiation and downregulation in the late stage. Knockdown of RP11-295G20.2 in keratinocytes promotes terminal differentiation. Based on our findings, we named RP11-295G20.2 Cytoplasmic Differentiation-Associated Epidermal RNA, CYDAER. In summary, our study provides a comprehensive characterisation of the non-coding RNA landscape of psoriasis keratinocytes and identifies CYDAER as a skin-enriched lncRNA regulating keratinocyte differentiation. Our data suggest that overexpression of CYDAER may contribute to altered differentiation in psoriatic epidermis.

Jan Cedric Freisenhausen and Longlong Luo contributed equally to this work.

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1 | Introduction

At least 60 million people worldwide are living with psoriasis, a chronic inflammatory disease [1]. The most common subtype of psoriasis is plaque psoriasis, presenting as inflammatory skin lesions with silvery scales [2], which are characterised by immune cell infiltration, increased keratinocyte proliferation and abnormal epidermal differentiation [3, 4]. Psoriasis is a multifactorial disease to which both genetic and environmental risk factors contribute. To date, 109 distinct psoriasis susceptibility loci have been reported, but even these can only explain an estimated 60% of heritability [5].

Under normal conditions, keratinocytes undergo a well-coordinated differentiation process, culminating in the formation of a functional, protective skin barrier. In psoriasis, the epidermis contains an increased number of proliferating keratinocytes, and while differentiated keratinocytes are present, they undergo an aberrant, wound-healing-like differentiation programme with impaired terminal differentiation and thickened epidermis and the retention of nucleated cells in the stratum corneum as result [4, 6].

The IL-23/IL-17 signalling axis is central to the pathogenesis of psoriasis, which is evidenced by the efficacy of treatments targeting these cytokines or their receptors [7]. IL-23 drives innate and adaptive immune cells to produce IL-17, that, together with other cytokines, causes activation, hyperproliferation and abnormal differentiation of keratinocytes, leading in turn to the release of antimicrobial peptides, chemokines and alarmins recruiting immune cells to the skin and a vicious circle, thus establishing chronic inflammation [8].

Long non-coding RNAs (lncRNAs) are long (> 500 nt) transcripts with no protein-coding ability [9], and while previously largely neglected, they are increasingly recognised as important regulatory factors in the cells [10]. Their functions depend on their localisation as well as their protein or nucleic acid interacting partners. Nuclear lncRNAs can alter chromatin accessibility through interaction with chromatin-modifying enzymes, regulate transcription through recruitment of transcription-enhancing or -repressing protein complexes [11] and also influence splicing [12]. Cytoplasmic lncRNAs can interact with messenger RNAs (mRNAs), ribosomal RNAs and regulatory RNAs and control RNA stability or the translation process [13], or the assembly of riboprotein complexes. Roles for lncRNAs have been identified in tissue homeostasis, inflammation, proliferation and differentiation. However, the function of the majority of lncRNAs is yet unknown. In skin, the lncRNA ANCR has been shown to regulate epidermal proliferation/differentiation [14, 15], and the lncRNA PRINS is induced in nonlesional psoriasis epidermis and regulates stress response [16], highlighting lncRNA-mediated gene regulation in epidermal homeostasis. Moreover, a set of lncRNAs are dysregulated in psoriasis skin [17]. However, little is known about the contribution of lncRNAs to epidermal dysfunction in psoriasis.

Here we aimed to explore the long non-coding RNA landscape in psoriasis keratinocytes, to uncover lncRNAs that contribute to epidermal dysfunction in the disease. To this end, we performed RNA sequencing in psoriasis keratinocytes, allowing us to identify a set of lncRNAs with yet uncharacterised functions, which may contribute to the disease. RP11-295G20.2 was identified as an IL-17A-induced, skin-enriched lncRNA overexpressed in psoriasis keratinocytes and regulating keratinocyte differentiation. On the basis of our observations and with the permission of the Human Genome Organisation Gene Nomenclature Committee, we have named this lncRNA cytoplasmic differentiation-associated epidermal RNA (CYDAER).

2 | Results

2.1 | Comprehensive Characterisation of the Coding and Non-Coding Transcriptome of Psoriasis Keratinocytes

To identify lncRNAs with potential roles in epidermal dysfunction in psoriasis, we performed a comprehensive characterisation of the long non-coding transcriptome of sorted keratinocytes in psoriasis. CD45-negative epidermal cells (predominantly keratinocytes) were sorted from skin biopsies from psoriasis lesions (PP, $n = 7$), and from skin of healthy controls (H, $n = 8$), followed by ribosomal RNA-depletion and RNA sequencing thereby capturing both polyadenylated- and non-polyadenylated transcripts (Figure 1). RNA sequencing generated a total of 640 million reads, on average, 40 million base pair (bp) paired-end reads per sample. We performed separate analysis for protein-coding and non-coding transcripts.

Analysis of mRNAs showed clear separation of transcriptomic profiles in H and PP keratinocytes, as shown by principal component analysis (PCA) (Figure 1). Among 14 302 mRNAs detected to be reliably expressed in our samples, 3413 were significantly upregulated while 2950 were significantly downregulated in PP as compared to H keratinocytes (|fold change| > 1.5, |False Discovery Rate| (FDR) < 0.05) (Figure 1d), highlighting substantial transcriptomic changes in psoriasis keratinocytes. Among the top upregulated mRNAs were alarmins—immune-activating, chemotactic and antimicrobial peptides—such as defensins (DEFB4A), members of the S100A family (S100A7A, -A12, -A8), and small proline-rich proteins (SPRR2F, -2A -2B), while the top downregulated mRNAs included late cornified envelope proteins (LCE1D, -1E, -5A), cell adhesion-related proteins (CADM3, COL19A1) and solute carrier family proteins (SLC18A, -8A). The top 30 differentially expressed mRNAs are listed in Table S1.

The significantly differentially expressed mRNAs with at least 2-fold change were analysed for enrichment in Gene Ontology biological processes using Enrichr [18]. Among the upregulated mRNAs, processes associated with metabolism (e.g., glycolysis and mitochondrial oxidative phosphorylation), proliferation, inflammatory signalling pathways (e.g., TNF, NF-kB) and cytokine production were enriched, in agreement with the hyperproliferative and activated state of psoriasis keratinocytes (Figure 1f). The downregulated mRNAs were enriched in terms related to epidermal development, cellular calcium-transport and -response, signalling pathways (e.g., bone morphogenic protein, semaphorin, Notch). Additional processes enriched among downregulated mRNAs were related to cell adhesion, migration, and extracellular matrix, and negative regulation of epithelial proliferation. Analysis of the differentially expressed protein-coding genes using the Reactome pathway knowledgebase [19], resulted in similar categories (Figure S1).

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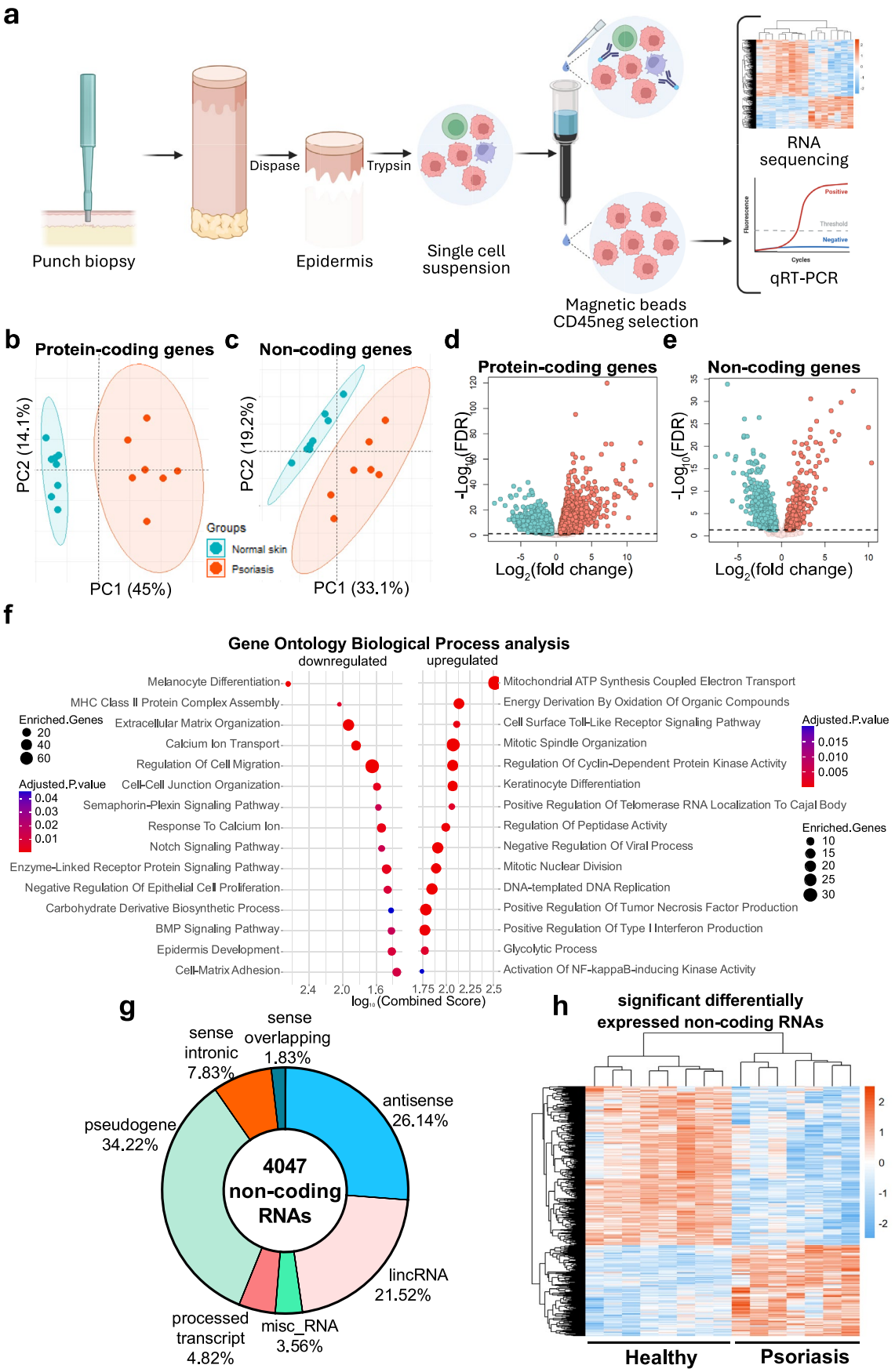

FIGURE 1 | Legend on next page.

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FIGURE 1 | Characterisation of the coding and non-coding transcriptome in healthy and psoriasis epidermal keratinocytes. Graphical representation of the workflow (created with Biorender) (a). Principal component analysis of protein showing clustering of samples based on protein-coding (b) and non-coding (c) transcripts. Volcano plot showing significantly differentially upregulated (in red) or downregulated (in blue) protein-coding (d) and non-coding transcripts (e) detected by RNAseq in PP vs. H. (f) Top 15 enriched Gene Ontology (GO) biological process categories among significantly down- and upregulated protein-coding genes in PP vs. H. (g) Classification of the biotype of lncRNAs differentially expressed between psoriasis and healthy CD45^{neg} epidermal cells. (h) Heatmap showing the expression of 889 significantly differentially expressed (FC > 1.5; FDR < 0.05) lncRNAs between PP vs. H keratinocytes. H, healthy; PP, psoriasis lesional.

To explore lncRNAs altered in psoriasis keratinocytes, separate analyses were performed for non-coding transcripts. There were 4592 annotated non-coding transcripts identified to be reliably expressed. PP and H keratinocytes were clearly separated based on their expression of non-coding transcripts (Figure 1c). The majority of these were pseudogenes (34%), antisense (26%) and long intergenic ncRNAs (22%) (Figure 1h). Differential expression analysis identified 314 significantly upregulated and 575 significantly downregulated lncRNAs in PP as compared to H keratinocytes (|fold change| > 1.5, FDR < 0.05, CPM > 1) (Figure 1e and Table S2). Among the top upregulated lncRNAs were the pseudogenes SPRR2C, CYP4Z2P and VNN3 and long intergenic RNA LINC01215, while the top downregulated lncRNAs included PHF2P2, the pseudogenes FRG2DP and several poorly characterised lncRNA genes such as RP11-1008C21.1 and RP11-307C19.2 (Table S2).

2.2 | Long Non-Coding RNAs CYDAER (RP11-295G20.2), LINC01123, and LINC01215 Are Significantly Overexpressed in Keratinocytes in Psoriasis Lesions

Our RNAseq analyses identified lncRNAs with differential expression in PP versus H keratinocytes (Figure 2a). To validate these results for selected lncRNAs, three lncRNAs were analysed by qRT-PCR in an extended cohort including psoriasis lesional (PP) and non-lesional skin samples (PN) and skin from healthy controls (H). CYDAER (RP11-295G20.2) was increased 7.4-fold between PP and H in RNAseq (Figure 2b). In line with this, qRT-PCR analyses demonstrated a 6.9- and 4.6-fold increase in PP vs. H and PP vs. PN, respectively ($p < 0.0001$; Figure 2c). Two other candidate lncRNAs, LINC01123 and LINC01215, were increased 2.1-, and 116.6-fold, in the RNAseq data, respectively (Figure 2d,f). Confirming RNAseq results, qRT-PCR analysis demonstrated that LINC01123 was increased 2.6-fold between PP and H ($p < 0.0001$) and 1.8-fold between PN and PP ($p < 0.001$; Figure 2e,g). Interestingly, LINC01215 was not detectable in the majority of H and PN keratinocytes by qRT-PCR, while it was expressed in keratinocytes from all PP samples (Figure 2g).

2.3 | CYDAER Is a Skin-Enriched Cytoplasmatic Long Non-Coding RNA

Next, we focused our investigations on CYDAER, because we identified it as a relatively abundant lncRNA highly overexpressed in PP keratinocytes, and its regulation and functions in skin have been unknown. CYDAER is encoded by a gene located on chromosome 1q42.2, and the exon structure of the 465 base pair long predominant isoform ENST00000416221.5 is shown in the coverage plot (Figure 3a). Analysis of coding potential using CPC2 [20] indicated a very low protein-coding probability (Figure 3b), comparable to that of the known non-coding RNAs MALAT1 and UCA1 [21] (Figure S2), suggesting that CYDAER functions as a non-coding RNA. To get insight into its expression across various tissues, publicly available RNAseq data in the Genotype-Tissue expression (GTex) database [22] were interrogated. Notably, CYDAER showed a highly skin-enriched expression pattern, suggesting specific functions for this lncRNA in the skin (Figure 3c).

To further explore the expression pattern of CYDAER in healthy and psoriasis skin we performed single molecule fluorescence in situ hybridisation (RNAscope). Our results showed an increased signal of CYDAER in psoriasis as compared to healthy epidermis, confirming the overexpression of CYDAER in psoriasis epidermis (Figure 3d).

Since the subcellular localisation of lncRNAs can provide clues to their function, we next investigated CYDAER expression in nuclear and cytoplasmic subcellular fractions from healthy human epidermis. qRT-PCR results showed that CYDAER is mainly localised to the cytoplasm, similar to transcripts with known cytoplasmic localisation such as the HPRT mRNA and 18S rRNA (Figure 3e). In line with this, single molecule in situ hybridisation for CYDAER revealed predominantly cytoplasmic localisation in both healthy and psoriasis epidermis (Figure 3d).

2.4 | CYDAER Is Induced by IL-17A in Keratinocytes

To explore whether the inflammatory cytokine milieu in psoriasis can be responsible for the upregulation of CYDAER in keratinocytes in psoriasis plaques, we analysed the expression of CYDAER in cultured human primary keratinocytes treated with the key psoriasis cytokine IL-17A. qRT-PCR results demonstrated that IL-17A significantly induced CYDAER in cultured keratinocytes starting from 24 h onwards (Figure 4a). In line with this, CYDAER was significantly induced by IL-17A in 3D epidermal models as measured by qRT-PCR (Figure 4b) and shown also by single molecule in situ hybridisation (Figure 4c).

2.5 | CYDAER Regulates Keratinocyte Differentiation

Analysis of the distribution of the single molecule in situ hybridisation signal revealed an interesting pattern: CYDAER was

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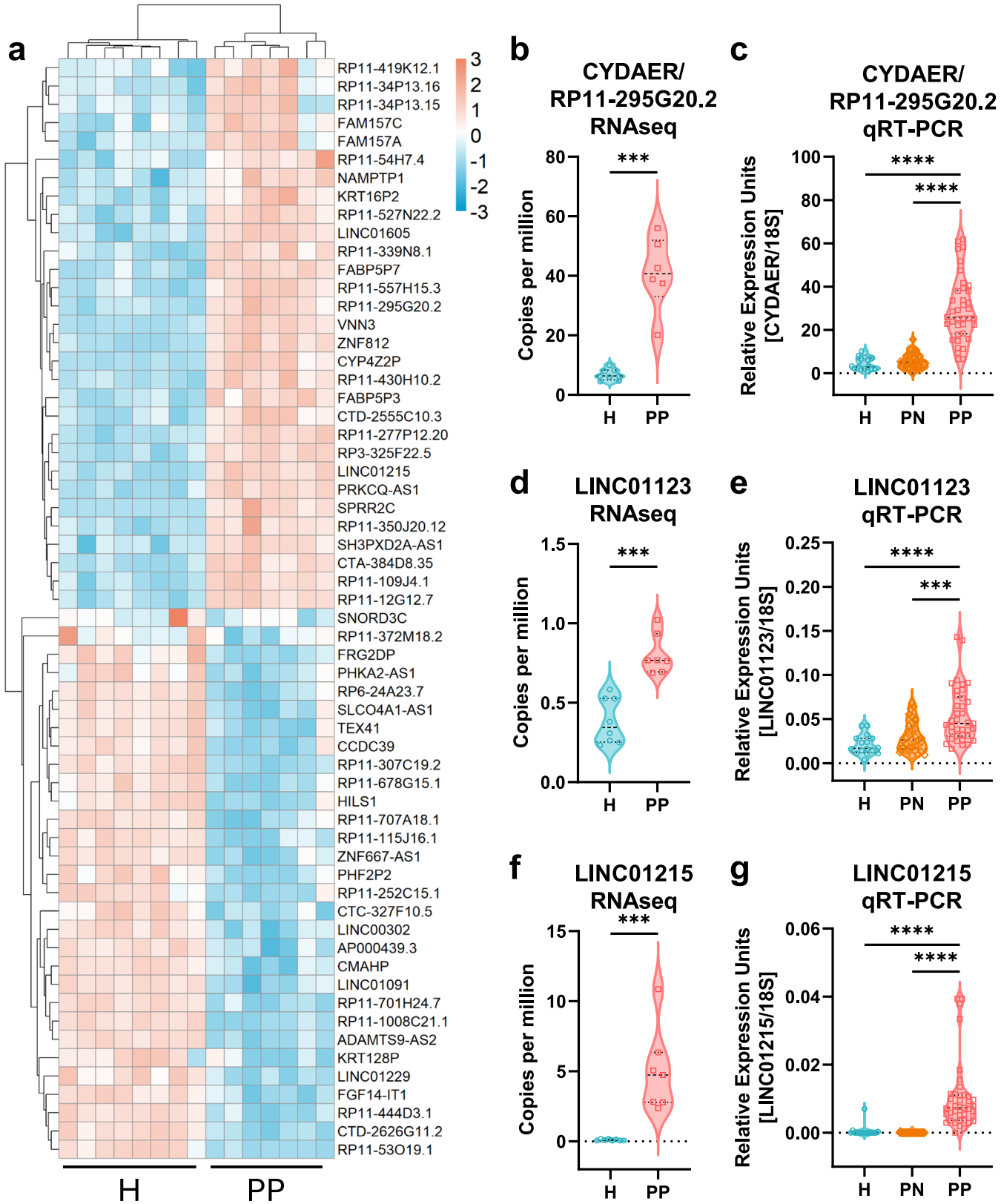

FIGURE 2 | Differentially expressed lncRNAs in psoriasis keratinocytes. (a) Heatmap illustrating the 30 top up- and downregulated significantly differentially expressed lncRNAs between PP and H. (b, d, f) Violin plots showing the RNAseq normalised readcounts for CYDAER (b), LINC01123 (d) and LINC01215 (f), respectively. (c, e, g) qRT-PCR analysis of CYDAER (c), LINC01123 (e) and LINC01215 (g), on an extended cohort of $CD45^{neg}$ epidermal cells from lesional and non-lesional skin from psoriasis patients ($n = 37$), and healthy skin ($n = 17$). ***$p < 0.001$, ****$p < 0.0001$, Mann–Whitney test (b, d, f), Kruskal-Wallis test with Dunn's multiple comparison (c, e, g).

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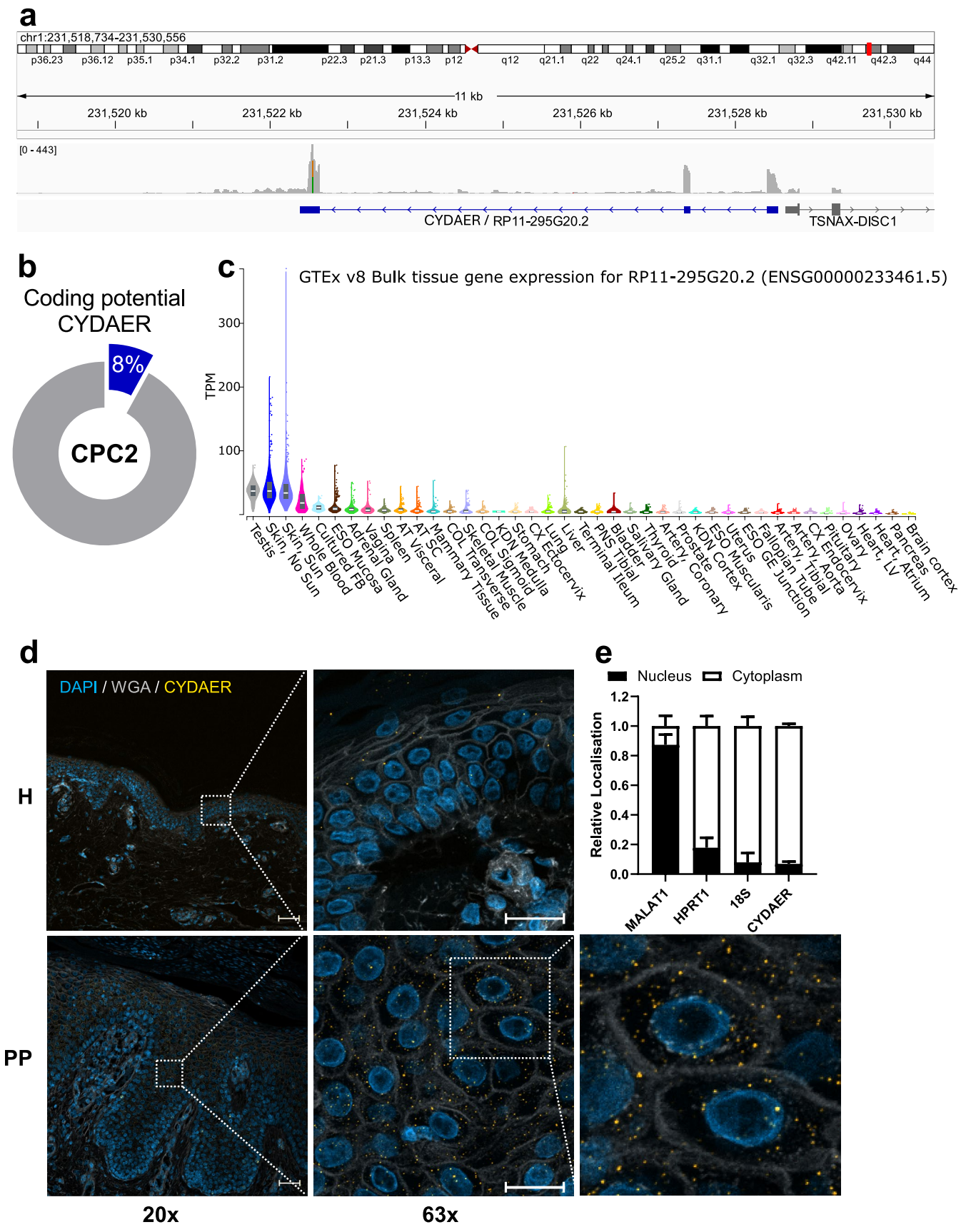

FIGURE 3 | Legend on next page.

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FIGURE 3 | CYDAER is a skin-enriched cytoplasmic lncRNA. (a) Illustration of the genomic context and exon structure of CYDAER; IGV Genome Browser view of sequencing read coverage for CYDAER together with the best matching isoform of a representative PP RNAseq sample. (b) Coding probability of the RP11-295G20.2 transcript according to the coding potential calculator 2 (CPC2). (c) Expression of CYDAER in healthy human tissues, RNAseq data from Gtex v8. (d) Single molecule fluorescence in situ hybridisation (RNAScope) of CYDAER in healthy (H, upper panels) and psoriasis lesional (PP, lower panels). Scale bar 50 μm (20×, left panels), 20 μm (63×, middle panels). (e) qRT-PCR analysis of MALAT1 (known nuclear lncRNA), HPRT1 and 18S (mainly cytoplasmic RNAs), and CYDAER in nuclear and cytoplasmic fractions of healthy human epidermis ($n = 4$). Data are expressed as mean ± SD. AT = adipose tissue, COL = colon, CX = cervix, ESO = oesophagus, FB = fibroblasts, GE = gastroesophageal, KDN = kidney, LV = left ventricle, PNS Tibial = tibial nerve, SC = subcutaneous.

rarely detected in the basal cell layers, instead, it was predominantly localised in the suprabasal layers of both healthy and psoriasis epidermis (Figure 3d). Combination of single molecule fluorescence in situ hybridisation with immunofluorescence staining for keratin 10, a marker for early terminal differentiation of keratinocytes, showed co-localisation of CYDAER with keratin 10 both in psoriasis epidermis (Figure 4d), as well as the 3D epidermal model (Figure 4c). This expression pattern suggested that CYDAER may be regulated during the differentiation process of keratinocytes. To test this, we analysed the expression pattern of CYDAER in in vitro models for epidermal differentiation. First, we analysed its expression in time-course experiments in a calcium-induced differentiation model in primary human keratinocytes. We found that CYDAER was significantly induced after 2 and 5 days, while decreasing at a later stage of differentiation, on day 12 (Figure 4e). Notably, keratin 10 showed a similar expression pattern, with the highest expression between 2 and 5 days and decreased expression afterwards (Figure 4e). Second, the expression pattern of CYDAER was analysed in 3D epidermal models harvested at different timepoints during its development, representing different stages of epidermal differentiation and stratification. Here we observed a similar pattern: CYDAER was induced during early stages of differentiation (one day before airlifting), and shortly after airlifting (day 2 and 5 post-airlifting) but decreased on day 11. Keratin 10 increased continuously up to day 11 in this model in parallel with CYDAER until day 5 (Figure 4f).

The dynamic regulation of CYDAER during differentiation suggested that it may be involved in the regulation of keratinocyte differentiation. To test this hypothesis, we knocked down CYDAER expression using an siRNA pool in primary human keratinocytes, followed by calcium-induced differentiation (Figure 4g) (Figure S3). qRT-PCR analysis revealed that the late differentiation markers filaggrin and loricrin were significantly increased at day 12 by CYDAER knockdown relative to controls, with loricrin already showing a prominent increase by day 5 of calcium-induced differentiation. The early differentiation marker keratin 10 was only slightly increased by CYDAER knockdown (Figure 4g).

In summary, our results show that CYDAER is induced by the central psoriasis cytokine IL-17A, is localised to suprabasal epidermal layers, and its expression is dynamically regulated during keratinocyte differentiation. Moreover, siRNA-mediated knockdown of CYDAER promotes keratinocyte differentiation, suggesting that CYDAER acts as a negative regulator of epidermal differentiation.

3 | Discussion

Keratinocytes are essential players in psoriasis: their hyperproliferation, aberrant differentiation and activation are hallmarks of the disease, sustaining and reinforcing a vicious circle of inflammation. Identification of novel regulators of these processes can lead to a better understanding of the disease and uncovering of potentially actionable pathways as well as a better understanding of normal epidermal development. Gene regulation by long non-coding RNAs is a fundamental regulatory mechanism in biology; however, its contribution to epidermal homeostasis and dysfunctions has been largely unexplored. While lncRNAs dysregulated in psoriasis plaques have been identified already in 2005 [16] followed by more recent studies by several groups worldwide [17, 21, 23–28], their cellular and subcellular expression patterns and functional contribution are still unknown apart from a few examples [25, 27, 28].

In the present study we performed a comprehensive analysis of coding and long non-coding transcripts in keratinocytes sorted from psoriasis plaques using RNA sequencing and identified lncRNAs with potential functional relevance. Our analyses showed substantial transcriptomic changes in the psoriasis keratinocytes, in agreement with previous studies [29, 30], with enrichment of biological processes related to among others metabolic, cell proliferation and inflammatory pathways among upregulated genes. Interestingly, glycolysis, citric acid cycle and aerobic respiration were among the enriched processes, presumably reflecting the high metabolic activity of highly proliferative cells. These findings are in line with a recent study showing increased expression of glucose and lactate transporters in psoriatic keratinocytes [31].

Our analyses demonstrate that—similar to mRNAs—a large proportion of the detected lncRNAs are dysregulated in psoriasis keratinocytes. RP11-295G20.2 (also known as AL445524.2, LOC122526782), now given the name CYDAER, was one of the significantly upregulated lncRNAs, which was abundantly expressed. The function of CYDAER in the skin was previously unstudied, except for being among upregulated lncRNAs in public data sets of psoriasis lesions compared to non-lesional and healthy skin [26], making this study warranted. Interestingly, CYDAER displays a skin-enriched expression when compared to over 50 other healthy tissues, suggesting an important function for this lncRNA in skin biology. We found that knockdown of CYDAER promotes calcium-induced terminal differentiation, judged by the increased expression of the late differentiation markers filaggrin and loricrin. This result together with

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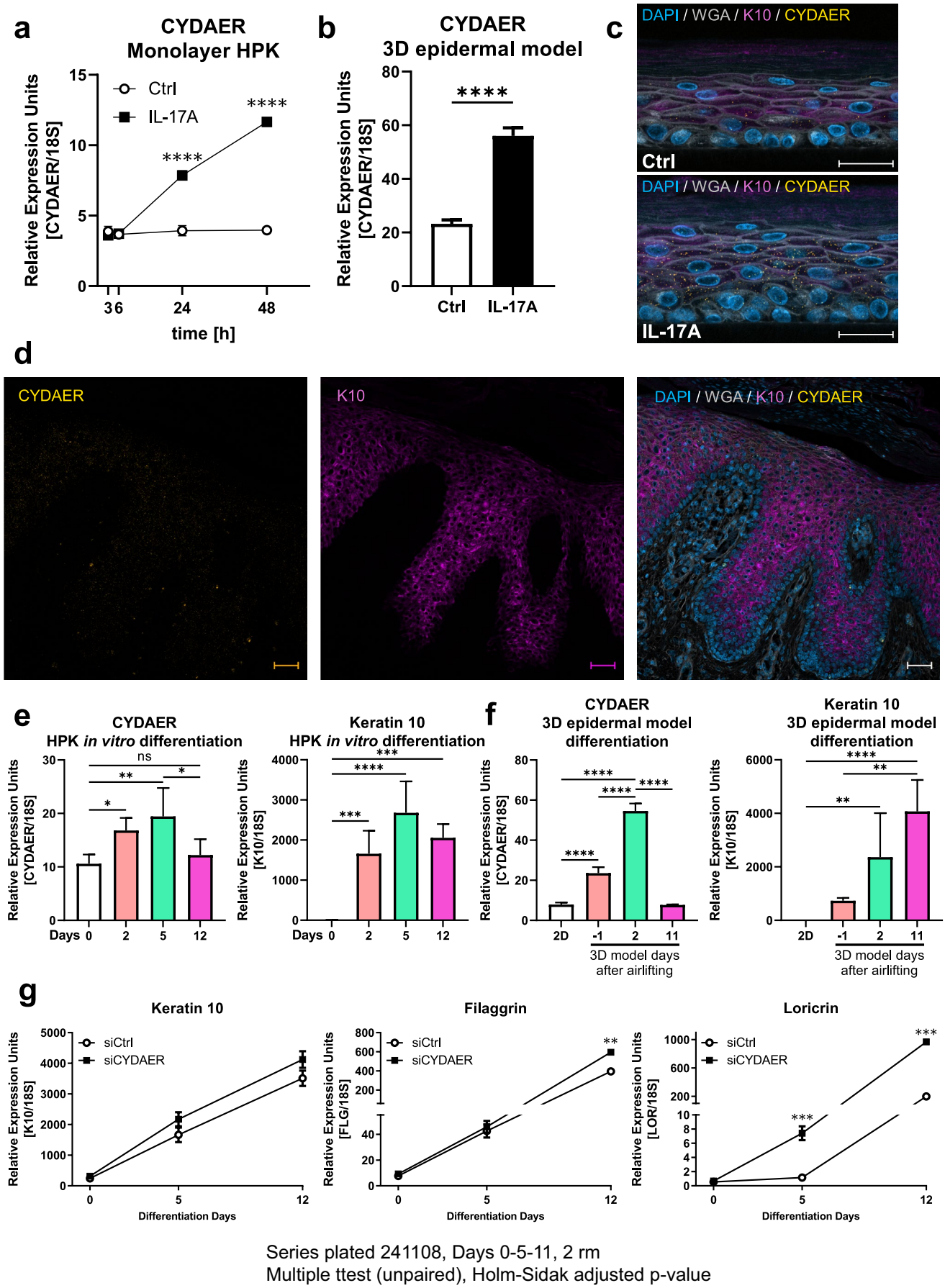

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FIGURE 4 | CYDAER is regulated by IL-17A and during differentiation in keratinocytes and its inhibition promotes keratinocyte differentiation. (a) qRT-PCR analysis of CYDAER in cultured primary human epidermal keratinocytes treated with IL-17A for the indicated timepoints. (b, c) 3D epidermal models were treated with IL-17A and CYDAER was analysed by qRT-PCR (b) and single molecule in situ hybridisation combined with immunofluorescent staining for Keratin 10 and WGA (wheat germ agglutinin, for plasma membrane labelling) (c). (d) Single molecule in situ hybridisation for CYDAER combined with immunofluorescent staining for keratin 10 in psoriasis epidermis. (e) qRT-PCR analysis of the CYDAER and K10 in primary human keratinocytes treated with 1.8 mM $CaCl_2$ for the indicated times. (f) qRT-PCR analysis of the CYDAER and K10 in 3D epidermal equivalents harvested at the indicated timepoints relative to air-lifting. (g) qRT-PCR analysis of K10, FGL and LOR in primary human keratinocytes reverse transfected with an siRNA pool for CYDAER (siCYDAER) or control siRNA (siCtrl) and treated with 1.8 mM $CaCl_2$ for the indicated times. Data in (a–b, e–g) are expressed as mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, Unpaired *t*-tests followed by Holm-Šídák correction for multiple comparisons (a, g), unpaired *t*-test (b), One-way ANOVA, Tukey's multiple comparison test (e, f). Scale bars = 20 μm (d), 50 μm (e)

TABLE 1 | TaqMan qRT-PCR primers.

Gene		Sequence 5′–3′
18S	Forward	CACGGACAGGATTGACAGATT
	Probe	AGTTGGTGGAGCGATTTGTCTGGT
	Reverse	GCCAGAGTCTCGTTCGTTATC
LINC01215	Forward	GCCATGCTGAACTGTCTCT
	Probe	ACCCAAGTGTTACCAGTGAAGGGT
	Reverse	TCTTTGTTCAAAGCGCCAAG
LINC01123	Forward	TCTTGCAACAGTGGCCG
	Probe	ACCACTGTAGTCAGAGAGGCCAGT
	Reverse	GTCAGCAGCAGCAGTGAG
CYDAER	Forward	GCTCTGGCACGAAGACAA
	Probe	CGATCTTGGCTCACTGCAACCTCT
	Reverse	CTCAGGTGGGTTGTCTGTAATC
KRT10	IDT Predesigned Assay ID	Hs.PT.58.38635764
FLG		Hs.PT.58.24292320.g
LOR		Hs.PT.58.27761511
MALAT1		Hs.PT.58.26451167.g
HPRT1		Hs.PT.58v.45621572

the predominant expression of CYDAER in the lower suprabasal layers of the epidermis and its dynamic regulation during differentiation (with upregulation during early differentiation and downregulation in later stages) suggests that this lncRNA acts as a negative regulator of late-stage epidermal differentiation in normal skin. In psoriasis, upregulation of CYDAER may contribute to the altered path of keratinocyte differentiation with expansion of the lower suprabasal layers. We showed that IL-17A, a central cytokine in the pathogenesis of psoriasis [32], induces CYDAER in keratinocytes and 3D epidermal models, suggesting that IL-17A, perhaps in combination with other factors, can contribute to the overexpression of CYDAER in psoriasis epidermis.

In corneal epithelium, CYDAER was described as one of six lncRNAs undergoing alternative splicing when the splicing-related protein pinin was depleted from human corneal epithelial cells [33]. The exon expression profile of pinin-depleted human corneal epithelial cells changed to match more closely the profile observed in this study. Interestingly, pinin was described to be crucial for the development and maintenance of epithelial tissues [34].

In hepatocellular carcinoma, as in many cancers, CYDAER was increased and promoted cell growth through regulation of autophagy through direct interaction with PTEN [35]. CYDAER has further been suggested to be a favourable prognostic biomarker in oesophageal squamous cell carcinoma [36]. In contrast, in lung adenocarcinoma CYDAER has been reported to be overexpressed and linked to poor prognosis; in lung cancer cell lines, knockdown of CYDAER reduced proliferation, endoplasmic reticulum stress, migration and invasions [37]. Interestingly, we found that unfolded protein response was enriched among genes significantly upregulated in psoriasis epidermis (Figure S1). Moreover, ER stress has been implicated in psoriasis [38, 39], but whether regulation of ER stress may be involved in the role of CYDAER in psoriasis remains to be determined.

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Both single molecule in situ hybridisation and subcellular fractionation experiments showed a predominantly cytoplasmic localisation of CYDAER in keratinocytes in line with results from subcellular fractions of HCC cell lines [35, 40] but in contrast to in situ hybridisation experiments on human cornea [33]. This suggests that it can function by interacting with RNA or proteins in the cytoplasm and may interact with cytoplasmic RNAs or riboprotein complexes to regulate translation or protein modifications [41, 42]. Future studies will determine the interacting partners and function of CYDAER in epidermal homeostasis and dysfunction.

In addition to CYDAER, LINC01123 and LINC01215 were found to be overexpressed in psoriasis keratinocytes. Consistent with our previous findings, LINC01215 was also overexpressed in full-depth biopsies of psoriasis skin in another patient cohort and considered a potential biomarker in psoriasis [26, 43]. In epithelial ovarian cancer, LINC01215 was shown to regulate the RUNX3 promotor methylation leading to reduced RUNX3 expression and increased proliferation, migration and invasion, but reduced apoptosis [44]. LINC01123 is still very poorly characterised, and its expression and function in skin or psoriasis is as yet unknown. However, two independent studies have presented its role as a competing endogenous RNA for VEGFA [45, 46]. The function of these lncRNAs and their potential contribution to psoriasis remains to be determined in future studies.

Our study identified a set of lncRNAs with yunidentified functions that may not only contribute to epidermal dysfunction in psoriasis but may also be important regulators in epidermal biology. A limitation of our study is that by using sorted $CD45^{neg}$ epidermal cells, keratinocytes in the late differentiation stages may have been lost through the trypsinisation and purification column processes, since these may fail to effectively dissociate highly crosslinked epidermal keratinocytes. However, at the same time, our approach enabled the identification of a keratinocyte lncRNA-signature, excluding lncRNAs from dermal cell population or epidermal immune cells. Moreover, the use of RNA sequencing with ribosomal depletion rather than the commonly used polyA+ selection allowed for the identification of non-polyA lncRNAs in addition to polyA transcripts [9].

In conclusion, this study expands our knowledge on the regulatory mechanisms in psoriasis epidermis and opens up for future functional studies aiming to understand the role of lncRNAs in skin biology and psoriasis.

4 | Materials and Methods

4.1 | Skin Biopsies and Patients

After written informed consent, 4 mm skin punch biopsies were collected from lesional ($n = 39$) and non-lesional ($n = 37$) skin of chronic plaque psoriasis patients and skin of seventeen healthy controls from the lower back or buttocks region. Patients had not received systemic immunosuppressive treatment or phototherapy for at least 4 weeks and topical therapy for at least 2 weeks before collecting the skin biopsy. The study was approved by the Stockholm Regional Ethics Committee at Etikprövningsmyndigheten/Swedish Ethical Review Authority, and all procedures were in accordance with the Declaration of Helsinki principles.

4.2 | Isolation of CD45-Negative Epidermal Cells and RNA Isolation

$CD45^{neg}$ cells were isolated from human epidermis as described previously [30]. Briefly, biopsies were incubated with PBS + 5 U/mL dispase (Thermo Fisher Scientific, Waltham, MA, USA) overnight at 4°C, and epidermal sheets were separated from the dermis using forceps and an epidermal single cell suspension was prepared by incubating the epidermis with trypsin/EDTA (Thermo Fisher Scientific, Waltham, MA, USA) for 15 min followed by resuspension. The cell suspension was subjected to CD45-negative selection using CD45 microbeads and MACS magnetic cell separation (Miltenyi Biotec, Bergisch Gladbach, NRW, Germany). Keratinocytes were collected with the flowthrough and stored in Qiazol (Qiagen, Hilden, Germany) at −80°C until RNA isolation. RNA was isolated using the Qiagen miRNeasy kit (Qiagen, Hilden, Germany).

4.3 | RNA Sequencing and Data Analysis

cDNA libraries were prepared of 300 ng of RNA from seven samples from psoriasis lesions and eight healthy epidermis samples, obtained as described above, using the Illumina Ribo-Zero Plus ribosomal depletion (Illumina, San Diego, CA, USA). The cDNA was sequenced on an Illumnia NovaSeq 6000 machine using 50 bp paired-end sequencing on a single SP flow cell at the SNP&SEQ Technology Platform in Uppsala. On average, 40 million paired reads were obtained per sample. For each sample, quality control was performed (FastQC 0.11.8) and adapters were trimmed using Trim Galore! 0.6.4. We used STAR-2.6.1 [47] to align the reads to the human reference genome (build 38).

Read counts were filtered by their expression level (minimum 10 reads in at least seven samples, minimum 40 total reads and expression in > 70% of samples per condition), normalised using the TMM weighted trimmed mean of *M*-values method and differential expression analysis was conducted using edgeR 3.42.4 [48]. The edgeR gene-wise negative binominal generalised model was applied to fit the data, and differential expression was subsequently analysed using likelihood ratio testing. biomaRt 2.56.1 was used to retrieve gene information from the Ensembl database [49], and coding and non-coding genes were subset using the Ensembl gene biotypes. Principal component analysis (PCA) was performed using base R. Volcano plots were made using the R plot function. To generate heatmaps, pheatmap 1.0.12 was used. Gene Ontology analysis was performed using enrichR [18] on significant differentially expressed coding genes ($|FC| > 2$, CPM > 1, FDR < 0.05).

4.4 | Cell Culture and Treatments

Primary human epidermal keratinocytes (Thermo Fisher Scientific, Waltham, MA, USA) were cultured in Epilife Growth medium supplemented with 1% Penicillin/Streptomycin antibiotics and 1% Human Keratinocyte Growth Supplement (both Gibco, Waltham, MA, USA). Primary keratinocytes were treated with human recombinant IL-17A (100 ng/mL) (R&D Systems, Minneapolis, MN, USA). For the calcium-induced differentiation, cells were grown to confluency and their medium

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was changed to supplement-free medium with 1.8 mM calcium chloride. For siRNA transfection, pools of siRNAs targeting LOC122526782 and negative control were purchased (siTOOLs, Planegg, Germany). One hundred thousand primary human epidermal keratinocytes were reverse transfected using RNAiMAX (Thermo Fisher Scientific, Waltham, MA, USA) into 24 well plates using the siTOOLs protocol. Twenty-four hours after transfection, the medium was changed to growth medium until confluency, then to supplement-free medium with 1.8 mM calcium chloride.

4.5 | 3D Human Epidermal Models

Human epidermal models were generated using 24-well Nunc cell culture polycarbonate insert carrier plate system (Thermo Fisher Scientific, Waltham, MA, USA) seeded with 175 000 human primary keratinocytes. The cells were cultured submerged in medium for 2 days and then cultured exposed to the air-liquid interface for up to 11 days to form fully differentiated epidermal models. Day 1 sample was harvested 1 day before air-lifting, day 2 and day 11 were harvested 2 and 11 days after air-lifting, respectively. Medium was changed every other day, and during the last 4 days, the models were stimulated with 50 ng/mL IL-17A (R&D Systems, Minneapolis, MN, USA).

4.6 | qRT-PCR

Up to 300 ng of RNA were reverse transcribed using the RevertAid First strand synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA). qRT-PCR TaqMan primers and probes were designed with the IDT Primer Quest tool or ordered predesigned (Table 1). Amplification of cDNA was performed using the QuantStudio 6 Real-time PCR system (Thermo Fisher Scientific, Waltham, USA). Expression levels were calculated using the delta-Ct-method through normalisation to the housekeeping gene 18S.

Statistical analyses and plots were generated with GraphPad Prism 9.

4.7 | Single Molecule In Situ Hybridisation and Immunofluorescence

A custom RNAscope Multiplex probe matching the ENST00000416221.5 transcript of CYDAER was ordered from Advanced Cell Diagnostics (ACD, Newark, CA, USA). Formalin-fixed (4%) paraffin-embedded (FFPE) skin sections from patients with psoriasis or healthy donors were sectioned onto microscope slides at 7 μm thickness. The RNAscope Multiplex Immunofluorescence integrated co-detection reagents were used as stated in the manufacturer's instructions. Mouse Anti-cytokeratin 10 [SPM261] (Novusbio, Abington Oxon, UK) 1:100 was used together with anti-mouse AF-647, and counterstaining was done with Fluorescein Wheat Germ Agglutinin 1:100 and DAPI (4′,6-diamidino-2-phenylindole, dihydrochloride) 1:1000 (both Thermo Fisher Scientific, Waltham, MA, USA). For imaging, a Zeiss LSM 900 Airy2 confocal microscope was used.

Author Contributions

E.S. and A.P. were involved in the conceptualisation of the study, study funding, supervision and manuscript editing. J.C.F. was involved in the experimental design, carried out the majority of experiments and data analysis and wrote the manuscript in consultation with E.S. L.L. conducted sample sorting and preparation for RNA sequencing, participated in experimental design, carried out experiments and performed data analysis. E.K., J.E. and V.S. carried out experiments. All authors critically reviewed the manuscript.

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Ethics Statement

The study was approved by the Stockholm Regional Ethics Committee at Etikprövningsmyndigheten (Swedish Ethical Review Authority, Dnr. 2021-01568 and 2022-01804-02) and all procedures were in accordance with the Declaration of Helsinki principles. All patients and healthy donors gave their written informed consent prior to inclusion.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in Gene Expression Omnibus at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE274560, reference number GSE274560.

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