Galectin-8 Is Upregulated in Keratinocytes by IL-17A and Promotes Proliferation by Regulating Mitosis in Psoriasis

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Psoriasis is a chronic inflammatory skin disease that develops under the influence of the IL-23/T helper 17 cell axis and is characterized by intense inflammation and prominent epidermal hyperplasia. In this study, we demonstrate that galectin-8, a β-galactoside–binding lectin, is upregulated in the epidermides of human psoriatic skin lesions as well as in a mouse model of psoriasis induced by intradermal IL-23 injections and in IL-17A–treated keratinocytes. We show that keratinocyte proliferation is less prominent in galectin-8–knockout mice after intradermal IL-23 treatment than in wild-type mice. In addition, we show that galectin-8 levels in keratinocytes are positively correlated with the ability of the cells to proliferate and that transitioning from mitosis into G1 phase is delayed in galectin-8–knockout HaCaT cells after cell-cycle synchronization and release. We demonstrate by immunofluorescence staining and immunoblotting the presence of galectin-8 within the mitotic apparatus. We reveal by coimmunoprecipitation and mass spectrometry analysis that α-tubulin interacts with galectin-8 during mitosis. Finally, we show that in the absence of galectin-8, pericentrin compactness is lessened and mitotic microtubule length is shortened, as demonstrated by immunofluorescence staining. We conclude that galectin-8 is upregulated in psoriasis and contributes to the hyperproliferation of keratinocytes by maintaining centrosome integrity during mitosis through interacting with α-tubulin.


INTRODUCTION

Psoriasis vulgaris is a chronic inflammatory skin disease characterized by a thickened epidermis and intense inflammatory cell infiltration in the involved skin. The pathogenesis of psoriasis is associated with hyperproliferative keratinocytes (Lynde et al., 2014), with fast keratinocyte turnover having been demonstrated in multiple studies and various keratinocyte mitogens having been identified (Krueger et al., 1990). In view of the dense infiltration of T cells within the psoriatic lesions and the phenotypic reversal observed with the T-cell inhibitor cyclosporine, psoriasis is considered a T-cell disease. Moreover, the role of IL-17A in the promotion of epidermal hyperplasia was proven in a mouse model of psoriasis (Rizzo et al., 2011). The mechanisms through which IL-17A regulates keratinocyte proliferation have been elucidated (Ha et al., 2014; Lai et al., 2012; Wu et al., 2015). However, the related mechanism in the setting of psoriasis is less well-studied.

Galectin-8 belongs to the galectin family of β-galactoside–binding proteins having conserved carbohydrate-recognition domains and broad tissue distribution (Zick et al., 2002). Like other galectins, galectin-8 is synthesized without a classical signal peptide for secretion and is present in the intracellular compartment, although it can be released into the extracellular space by unconventional pathways. Galectins have been shown to function both extracellularly and intracellularly to modulate a variety of cellular functions (Cumming et al., 2017; Liu and Rabinovitch, 2005). Currently, limited information is available on the role of galectin-8 in the skin keratinocytes. In a previous transcriptomic study (Choy et al., 2012) employing microarray, we found that the mRNA levels of galectin-8 were significantly higher in lesional skin than in nonlesional skin in patients with psoriasis (Supplementary Figure S1a). Because hyperplastic epidermis is a hallmark of IL-23–IL-17 pathway. Keratinocytes act as a target for IL-17 and provide positive feedback to the inflammatory cascade, therefore further promoting inflammatory cell infiltration (Kim and Krueger, 2017).

The concept that IL-17A is the main driver of psoriasis pathogenesis is consolidated by the finding that almost half of the patients with psoriasis can achieve PASI 90, 90% improvement, after treatment with IL-17A–based therapies (Langley et al., 2014; Lebwohl et al., 2015; Papp et al., 2018). Moreover, the role of IL-17A in the promotion of epidermal hyperplasia was proven in a mouse model of psoriasis (Rizzo et al., 2011). The mechanisms through which IL-17A regulates keratinocyte proliferation have been elucidated (Ha et al., 2014; Lai et al., 2012; Wu et al., 2015). However, the related mechanism in the setting of psoriasis is less well-studied.

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Abbreviations: Ctrl, control; Gal8L, long form of Gal-8; Gal8S, short form of Gal-8; KO, knockout; NHEK, normal human epidermal KC; PCM, pericentriolar material; PCTN, pericentrin; WT, wild type

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psoriasis, we hypothesized that galectin-8 may regulate keratinocyte proliferation in the setting of psoriasis.

In this study, we show that galectin-8 expression is induced in psoriatic keratinocytes, especially by IL-17A. During mitosis, intracellular galectin-8 is localized to the mitotic apparatus and associated with centrosomes. Moreover, galectin-8 contributes to the organization of the mitotic structure, which is known to be associated with cell proliferation. Our findings provide insight into how galectin-8 contributes to keratinocyte proliferation in psoriasis.

RESULTS
Galectin-8 is overexpressed in psoriatic epidermis
To define the expression pattern of galectin-8 protein in psoriatic skin, tissue sections from lesional and nonlesional skin were analyzed by immunofluorescence staining. The specificity of the antibody used was confirmed by immunohistochemical staining of wild-type (WT) and galectin-8–knockout (KO) HaCaT cells (Supplementary Figure S1b). We noted a significantly higher intensity of galectin-8 staining in the epidermis of psoriatic lesions than that of the nonlesional skin (Figure 1a). Although interpersonal variations were observed, a statistically significant difference in the intensity of galectin-8 was noted between the lesional and nonlesional skin from four patients with psoriasis (Figure 1b).

A mouse model of psoriasis was studied, which involved intradermal injections of recombinant mouse IL-23 (Figure 1c) (Chan et al., 2006). We found that galectin-8 mRNA was markedly induced in the epidermis of mice treated with recombinant mouse IL-23 in contrast to the epidermis of those treated with PBS (Figure 1d).

Galectin-8 is responsible for keratinocyte hyperproliferation in psoriasis
To define the role of galectin-8 in psoriasis, we compared the responses of Lgals8−/− mice with those of their WT littermates to intradermal recombinant mouse IL-23 injections. We noted a significantly lower epidermal thickness of the skin sections from Lgals8−/− mice than those from the WT mice after the injections (Figure 2a and b). Furthermore, we quantified the proliferating cells by the expression of minichromosome maintenance protein 2, which has been shown to be a sensitive marker for cell proliferation (Abdou et al., 2014; Shin et al., 2010) in the skin sections. Fewer minichromosome maintenance protein 2-positive keratinocytes were found in the IL-23–treated epidermis of Lgals8−/− mice than in that of the WT mice (Figure 2c and d). Moreover, we also noted that a larger proportion of minichromosome maintenance protein 2-positive keratinocytes was located suprabasally in the WT mice than in the Lgals8−/− mice (Figure 2e). This finding is consistent with the existing information that larger numbers of proliferating keratinocytes, especially suprabasal ones, are present in human psoriatic lesions (McKay and Leigh, 1995; Weinstein et al., 1985). These results suggest a positive role of galectin-8 in keratinocyte proliferation and indicate that induced galectin-8 in keratinocytes may contribute to the enhanced keratinocyte proliferation in psoriasis.

IL-23 and downstream cytokines have been demonstrated to be the main effectors responsible for most of the pathologic changes that occur in psoriasis (Levin and Gottlieb, 2014). Of these cytokines, IL-17 and IL-22 are potent stimulators of keratinocyte proliferation (Saba and Wolk, 2013; Zheng et al., 2007). To explore the factors that induce galectin-8 in keratinocytes in the context of psoriasis, we treated human-immortalized keratinocytes HaCaT cells and primary human keratinocytes, that is, normal human epidermal keratinocytes (NHEKs) with selected mitogenic cytokines, including EGF, IL-6, IL-17A, and IL-22. We noted that the total galectin-8 levels, including both long form of galectin-8 (Gal8L) and short form of galectin-8 (Gal8S),...
were increased, although only modestly, in IL-17–treated HaCaT cells compared with the untreated cells (Supplementary Figure S2a). We then confirmed that IL-17A dose-dependently induced galectin-8 expression in these cells (Figure 2f and g). Although there was a trend of a dose-dependent increase in the galectin-8 levels in the NHEKs (Supplementary Figure S2b), the difference was not statistically significant. IL-22 also induced galectin-8 in both HaCaT and NHEK but only very modestly and not obviously dose dependently (Supplementary Figure S2c). These results suggest that IL-17A, a downstream effector of IL-23, can induce galectin-8 in keratinocytes.

Galectin-8 levels in keratinocytes are positively correlated with proliferation ability
To further elucidate the role of galectin-8 in keratinocyte proliferation, we generated galectin-8–KO clones of HaCaT cells using the CRISPR/Cas9 system. The KO of galectin-8 was confirmed by immunoblotting (Figure 3a, left), and the specificity of the KO was demonstrated by the unaltered expression of other galectins (Supplementary Figure S3a and b). The growth curves of control (Ctrl) and galectin-8–KO clones were determined by sulforhodamine B assay, and the results revealed the impaired proliferation of galectin-8–KO clones compared with the Ctrl clone (Figure 3a, right).

In keratinocytes, Gal8S (35 kDa) and Gal8L (39 kilodaltons) are the two main isoforms differing in the length of the link peptide between the two carbohydrate-recognition domains (Zick et al., 2002). To validate the impact of galectin-8 in cell proliferation, FLAG-tagged galectin-8 was reconstituted into a galectin-8–KO HaCaT clone by lentiviral infection. Galectin-8 introduction was confirmed by immunoblotting with both anti-galectin-8 and anti-FLAG antibodies (Figure 3b, left). The impaired proliferation of galectin-8–KO HaCaT cells was rescued after reconstitution with both forms of galectin-8 but not with an empty vector (Figure 3b, right). Similarly, a dose-dependent effect of galectin-8 on proliferation was demonstrated in the NHEK overexpressing galectin-8 (Gal8S or Gal8L) generated by lentiviral infection compared with cells transfected with a Ctrl vector (Supplementary Figure S3c). These results confirm that galectin-8 plays a positive role in keratinocyte proliferation.
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Galectin-8 Promotes Mitosis in Keratinocytes

Cell-cycle progression in keratinocytes correlates positively with galectin-8 level

The above results suggest that galectin-8 may contribute to the regulation of cell-cycle progression. To analyze cell-cycle distribution, HaCaT cells were stained with a mitotic marker, phosphohistone H3 (Prigent and Dimitrov, 2003), and Hoechst to define the G1, S, G2, and M phases of the cell cycle (Figure 4a, left). A larger proportion of galectin-8–KO clones remained in the mitotic phase at 24 hours after passage than Ctrl HaCaT cells (Figure 4a, right). To further confirm the impact of galectin-8 loss on cell-cycle progression, HaCaT cells were synchronized at the G1/S phase by thymidine (Yoshizawa-Sugata and Masai, 2014), followed by their placement in complete fresh medium. In the first 8 hours after thymidine release, Ctrl and galectin-8–KO clones followed the same pace in their transition into the S and G2/M phases. After 10 hours of release, the cell number of Ctrl clone entering into the G1 phase increased, whereas over half of the KO cells remained in the G2/M phase. The transition into subsequent S and G2/M phases was further delayed in KO clones, as demonstrated at 14 hours (Figure 4b and c). Similar results were obtained with cells released from synchronization at the mitotic phase using nocodazole (Supplementary Figure S4). Thus, the impaired proliferation of galectin-8–KO clones can be explained by the delay in cell-cycle progression from mitosis to the G1 phase. We conclude that galectin-8 expression impacts cell-cycle progression in keratinocytes.

Galectin-8 is enriched in mitotic apparatus and associates with α-tubulin

By epifluorescence microscopy, we noted that the distribution of galectin-8 corresponded to the pattern of mitotic apparatus in mitotic cells (Supplementary Figure S5a). We isolated the mitotic apparatus from HaCaT cells sequentially synchronized by thymidine and nocodazole (Sauer et al., 2005) and confirmed that galectin-8 was present in the isolated material by immunoblotting (Figure 5a). To elucidate further how galectin-8 regulates mitosis, we pursued the identification of galectin-8–interacting proteins during the cell cycle. We reconstituted FLAG-tagged galectin-8 (Gal8S and Gal8L) into galectin-8–KO HaCaT cells by lentiviral infection, synchronized the resultant cells, and then treated them with a cell-permeable amine-reactive crosslinker. Galectin-8 with its interacting proteins was pulled down from the cell lysates by anti-FLAG agarose beads, and the isolated materials were analyzed by gel electrophoresis. We selected the protein bands around 55 kDa at G2/M group, where much higher protein densities were detected in both Gal8S and Gal8L over-expressing cells for mass spectrometric analysis (Supplementary Figure S6). α-Tubulin and β-tubulin were identified as two of the most abundant proteins in Gal-8–overexpressing clones and as being present in higher amounts in these clones than in the Ctrl clone (Supplementary Table S1). Because mitotic microtubules, composed of α-tubulin and β-tubulin heterodimers, are known to play a key role in the mitotic process, galectin-8 may regulate mitosis progression through interacting with these proteins. We confirmed that galectin-8 interacts with α-tubulin during mitosis by showing that FLAG-tagged galectin-8 was coimmunoprecipitated with α-tubulin from the lysates of synchronized HaCaT cells by an anti-α-tubulin antibody (Figure 5b). Because α-tubulin and β-tubulin exist as stable heterodimers, the above
results suggest that galectin-8 may also interact with β-tubulin at least indirectly through binding to α-tubulin.

We performed immunofluorescence staining, and significant colocalization of galectin-8 and α-tubulin was observed in mitotic cells, especially during prophase and metaphase (Figure 5c). The specificity of the antibody used was confirmed by staining of WT and galectin-8–KO HaCaT cells (Supplementary Figure S5b). However, this colocalization was not observed in the cells in interphase (Figure 5d). Significant galectin-8–α-tubulin colocalization in cells in metaphase was confirmed by Pearson correlation coefficient (Figure 5e). In NHEK cells, a similar pattern...
of galectin-8–α-tubulin colocalization was also observed in mitosis (Supplementary Figure S5c). In these assays, we noted that the distribution of galectin-8 expanded beyond the area of the centrosome, labeled by pericentrin (PCTN) (Supplementary Figure S5d). The above findings suggest that galectin-8 may regulate mitosis by interacting with α-tubulin in mitotic microtubules. Because α-tubulin is not a glycoprotein, the interaction is likely due to protein–protein interaction, similar to many other reported examples of interactions between galectins and their intracellular-binding partners (Cummings et al., 2017; Liu et al., 2002).

Galectin-8 maintains the integrity of the centrosome and mitotic spindles during mitosis

Immunofluorescence staining revealed that galectin-8 was mainly located on both sides of the mitotic apparatus (Figure 5c). Centrosomes are important structures for regulating mitosis and are composed of a pair of centrioles, surrounded by an organized protein complex, pericentriolar material (PCM). Of the PCM, PCNT plays a central role as a multifunctional scaffold in mitosis through recruiting other PCM-related proteins as well as regulating both cell-cycle checkpoint and microtubule organization and orientation (Delaval and Doxsey, 2010). Additional immunofluorescence assays revealed a significantly higher proportion of mitotic cells in galectin-8–KO clones with a dispersion of PCTN than in the Ctrl clone (Figure 6a). Moreover, the mitotic axes, as determined by the distance between PCTNs at the opposite poles, of galectin-8–KO cells were shorter than those of the Ctrl clone (Figure 6b). By contrast, other components of the centrosome, including centrin 2, which regulates centriole duplication, and CPAP, which regulates centriole elongation (Tang et al., 2009), remained compact in galectin-8–KO clones (Supplementary Figure S7a and b). Furthermore, there

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Figure 5. Galectin-8 interacts with α-tubulin on microtubules during mitosis. (a) Immunoblotting of whole-cell lysates from nonsynchronized cells and isolated mitotic apparatus released from nocodazole synchronization from parental and galectin-8 KO HaCaT cells. (b) Coimmunoprecipitation of FLAG-tagged galectin-8 with α-tubulin from HaCaT cells (representative results are from four independent experiments). (c) Immunofluorescence staining of HaCaT cells during mitosis (nucleus: DAPI, α-tubulin: FITC, Gal-8: rhodamine). Bar = 10 μm. (d) Immunofluorescence staining of HaCaT cells in interphase (nucleus: DAPI, α-tubulin: FITC, Gal-8: rhodamine). Bar = 20 μm. (e) PCCs for colocalization of α-tubulin and Gal-8 in metaphase and interphase, with 10 and 5 cells being analyzed, respectively. Error bars: mean ± SD, ****P < 0.001 by t-test. 8L, reconstituted with FLAG-tagged Gal8L; 8S, reconstituted with FLAG-tagged Gal8S; Gal-8, galectin-8; Gal8L, long form of Gal-8; Gal8S, short form of Gal-8; IP, immunoprecipitation; KC, keratinocyte; KO, galectin-8–knockout HaCaT cells; PCC, Pearson correlation coefficient; WT, parental HaCaT cells; V, galectin-8–KO HaCaT cells reconstituted with empty vector.
was no difference in the distribution of γ-tubulin, which is recruited robustly on mitotic entry, in galectin-8–KO clones (Supplementary Figure S7b). These findings imply that galectin-8 is instrumental for the compactness of PCTN within the centrosomes as well as the resulting microtubule formation during mitosis without interfering with the centriole formation.

**DISCUSSION**

In this study, we demonstrate that galectin-8 expression is increased in the epidermis of psoriatic lesions from human patients as well as a mouse model and in cultured human keratinocytes treated with IL-17A. We also report that galectin-8 positively regulates keratinocyte proliferation by maintaining mitotic structures and may therefore contribute to epidermal hyperplasia in psoriasis. We also found that galectin-8 interacts with mitotic microtubules and influences the centrosome compactness, and its expression is positively correlated with mitosis progression. We propose that galectin-8 enhances the integrity of centrosome protein complexes as well as the resulting microtubule formation during mitosis and facilitates the progression of the cell cycle from mitosis into G1 phase.

Most studies addressing the functions of galectin-8 employed recombinant galectin-8 protein added to cells in vitro. Relatively high concentrations of recombinant galectin-8 (up to 0.75 μM) were found to inhibit the adhesion of HaCaT cells (Hadari et al., 2000). Regarding the effect of galectin-8 on cellular proliferation, this protein was previously studied as a matrix protein by binding to cell-surface integrins and activating signaling cascades, including the activation of focal adhesion kinase (Levy et al., 2001). In another study, the addition of galectin-8 (up to 5 μM) to Chinese hamster ovary cells induced the expression of the cyclin-dependent kinase inhibitor p21, resulting in a cell-cycle arrest. In contrast, exogenous galectin-8 was shown to promote glioblastoma cell proliferation (Metz et al., 2016). However, for various reasons, results obtained by using galectins added to in vitro cultures composed of single-cell type may not reveal the intrinsic functions of endogenous galectins in vivo (Cummings et al., 2017).

In our study, the analysis of human skin through immunofluorescence (Figure 1a) revealed that endogenous galectin-8 was mainly distributed inside the cells but not in the extracellular areas of the epidermis. We then focused on the role of endogenous galectin-8 in keratinocytes, and our findings revealed that galectin-8 promotes keratinocyte proliferation without any detectable presence in the extracellular space of keratinocytes. Another study mentioned above addressed the function of galectin-8 by studying the effects of galectin-8–knockdown in a glioblastoma cell line, which resulted in impaired cell cycling (Metz et al., 2016). This is consistent with our findings in keratinocytes. Although those authors proposed that this was the result of cell-adhesion alteration induced by secreted galectin-8, it is also possible that the protein functions intracellularly.

Regarding the mechanism, we propose that galectin-8 positively regulates mitosis by functioning inside the centrosomes. The centrosome is composed of a pair of centrioles surrounded by the PCM, which undergoes maturation during mitosis through the robust expansion of PCM where PCTN plays a central role in recruiting proteins (Conduit et al., 2015; Lawo et al., 2012; Mennella et al., 2012). Our experiments demonstrated that the deletion of galectin-8 in keratinocytes resulted in the dispersion of PCTN in mitotic cells and a delay in cell-cycle progression from mitosis into G1 phase. However, the distribution of other centrosomal proteins such as centrin-2, CPAP, and γ-tubulin was not altered. Centrin-2 and CPAP are located at the central portion (zone I and II) of the centrosome, whereas PCTN and γ-tubulin are located more distally to centriole within the PCM (zone III and IV) (Fu et al., 2015). Because many protein components of the centrosome are recruited through the transportation of motor proteins along mitotic microtubules (Young et al., 2000), the dispersion of PCTN in galectin-8–KO clones and the interaction of galectin-8 with γ-tubulin in microtubules suggest that galectin-8 may affect the formation and maturation of centrosomes through the recruitment of the related proteins in microtubules. Accordingly, our findings support that galectin-8 promotes keratinocyte proliferation through maintaining the constituents of the centrosome, especially PCTN, during mitosis and cell-cycle progression. We propose that through this mechanism, galectin-8 contributes to epidermal hyperplasia, as observed in human and mouse psoriatic skin, in which the expression of galectin-8 is upregulated.

Recently, galectin-8 was demonstrated to maintain the polarity of the renal epithelial cell (Madin–Darby canine kidney) by targeting Gp135, also known as podocalyxin, to...
the apical surface of cells (Lim et al., 2017). In renal epithelial cells, the ring around the base of the primary cilium is a hot spot for Gp135 targeting. Moreover, delivery toward the periciliary ring and subsequent redistribution is microtubule dependent (Stoops et al., 2015). The structures of the centrosome and the basal body of primary cilium are similar (Bornens, 2012). Thus, analogous to its role in targeting Gp135 to the basal body of cilium, galectin-8 may mediate the targeting of proteins for centrosome maturation as well as the movement of these proteins toward or away from the centrosome along the microtubules. In addition, there is a reciprocal stabilization between mitotic microtubules and the kinetics of the centrosome structure, including PCM (Woodruff et al., 2014). Our data also revealed a shorter mitotic axis in galectin-8-KO cells compared with WT cells (Figure 6b). Further investigations are needed to elucidate the detailed mechanism of how galectin-8 regulates the structure of PCM and the functions of microtubules.

IL-23 as well as IL-17 and IL-22, which are induced by IL-23, are the main effectors responsible for the pathologic changes in psoriasis. Briefly, IL-23 secreted from dendritic cells can drive the polarization and expansion of IL-17-producing lymphocytes and the resultant robust production of IL-17A (Hawkes et al., 2018). We demonstrated a dose-dependent induction of galectin-8 in vitro after the treatment of both immortal human keratinocytes (HaCaT) and NHEK with IL-17A. In the mouse model of psoriasis induced by intradermal injections of IL-23, IL-17A was also shown to be the main factor promoting epidermal hyperplasia (Rizzo et al., 2011). We demonstrated that galectin-8 expression is increased in the epidermis of IL-23–treated mouse skin. With these findings together with our demonstration that the level of galectin-8 is related to the proliferative ability of keratinocytes, we propose that IL-17A promotes keratinocyte proliferation through the induction of galectin-8.

Asymmetric stem cell division contributes to the formation of transit-amplifying cells and the promotion of stratification in mammalian skin (Lechler and Fuchs, 2005; Williams et al., 2011). Recently, IL-17A was shown to induce asymmetric stem cell division in the keratinocytes in the context of psoriasis (Charruyer et al., 2017; Jia et al., 2016). In the IL-23 injection mouse model, we noted a less acanthotic epidermis and decreased suprabasal proliferative cells in Lgals8−/− mice, which implies that the proliferative ability and the formation of transit-amplifying cells from asymmetric cell division are impaired in the absence of galectin-8. This gives further evidence to the role of galectin-8 in IL-17A–promoted keratinocyte proliferation in the setting of psoriasis.

Whether the cells with increased galectin-8 expression in psoriasis reside in the epidermis or dermis remains to be discerned. In this study, we demonstrated the prominent upregulation of galectin-8 in the epidermis. Our unpublished findings however suggested that the protein was also overexpressed in infiltrating cells in the dermis of lesional skin. Accordingly, galectin-8 may contribute to the pathogenesis of psoriasis through both keratinocytes and infiltrating cells. It is possible that other cytokines expressed by inflammatory cells also contribute to keratinocyte proliferation. Additional experiments are warranted to delineate the role of galectin-8 within the subsets of inflammatory cells and the complex network of inflammatory cytokines.

In summary, our findings indicate that galectin-8 interacts with the mitotic microtubules and positively influences the integrity of the centrosome structure and promotes cell-cycle progression. The proliferating ability of cells is promoted, leading to epidermal hyperplasia in psoriasis when galectin-8 is upregulated. Moreover, our research provides a significant insight into how IL-17A induces keratinocyte proliferation.

**MATERIALS AND METHODS**

**Human skin samples and mouse experiment**

Human skin samples were collected under the regulation of the Ethics Committee of Taipei Tzu Chi Hospital (TCHIRB 03-X16-052) and with written informed consent from the patients. The analysis of human tissue samples was approved by the Institutional Review Board of Academia Sinica, Taiwan (AS-IRB01-16076). An animal model of psoriasis was developed under the approval of the animal protocol by the Animal Ethics Committee in the Institute of Biomedical Science, Academia Sinica, Taipei, Taiwan (10-11-090).

**Isolation of mitotic apparatus**

After synchronization at G2/M phase by serial treatments with thymidine and nocodazole, the mitotic apparatus were collected under taxol stabilization of microtubules (Sauer et al., 2005).

**Data availability statement**

The microarray data are available in the Gene Expression Omnibus repository (www.ncbi.nlm.nih.gov/projects/geo) under the accession number GSE153007.

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**CONFLICT OF INTEREST**

The authors state no conflicts of interest.

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**AUTHOR CONTRIBUTIONS**

Conceptualization: YHL, CSL, FTL; Data Curation: YHL, CSL, DFC; Formal Analysis: YHL; Funding Acquisition: FTL; Investigation: YHL, CYC, CCH, TJF; Methodology: YHL, HLC, CYC, CCH, THL; Project Administration: FTL; Resources: YHL, HLC, HYC; Supervision: FTL; Validation: CSL, JRA, HYC; Writing - Original Draft Preparation: YHL, CSL; Writing - Review and Editing: FTL

**SUPPLEMENTARY MATERIAL**

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.3106/j.jid.2020.07.021.

**REFERENCES**


SUPPLEMENTARY MATERIALS AND METHODS

Human skin samples
Gene expression of nonlesional and lesional skin from patients with psoriasis was assessed as previously described (Choy et al., 2012). The microarray data are available in the Gene Expression Omnibus repository (www.ncbi.nlm.nih.gov/projects/geo) under the accession number GSE153007. In brief, Agilent 2-color Whole Human Genome expression microarray analysis (Agilent, Santa Clara, CA) using a common reference design with a Universal Human Reference control color was conducted. Variance-stabilizing normalization was employed for background correction and between array normalization. Gene expression of each probe was normalized on the basis of test versus reference signals. For genes with multiple probes represented on the array, the featureFilter function from the genefilter package was employed to select the most variable probe. Human skin samples were collected under the regulation of the Ethics Committee of the hospital (TCHIRB 03-X16-052) and with written informed consent from the patients. Punch biopsies, 6 mm in size, were taken from patients with psoriasis at two sites: one from a fresh-onset active psoriatic plaque and one from normal skin at 3 cm distant from the lesion site. The samples were fixed in formalin and embedded in paraffin for further examination.

Mouse experiment and real-time quantitative PCR
Galectin-8–knockout (KO) sperms with a C57BL/6N background were acquired from the Knockout Mouse Project Repository, University of California, Davis, and used to derive galectin-8-KO mice at the National Laboratory Animal Center, Taiwan. The IL-23–induced mouse psoriasis-like model was established as previously described (Chan et al., 2006). Intradermal injections of 1 μg recombinant mouse IL-23 in 20 μl PBS or PBS only as a control were administered to the shaved back of anesthetized mice once a day for 5 days. Mice were euthanized on day 5. Parts of the harvested skin sheets were separated under microscopy (Chiang et al., 2013). Other parts of the skin were fixed in formalin and processed for H&E and immunohistochemical staining.

Total RNA was extracted from the detached epidermis using the RNeasy kit (Qiagen, Hilden, Germany). mRNA was then reverse transcribed into cDNA using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA), and the levels of mouse galecin-B and Gapdh were determined by quantitative real-time PCR using specific Universal Probe Library probes (Roche Holdings AG, Basel, Switzerland) and the primers targeting the gene products according to the manufacturer’s instructions. Relative levels of mRNAs were calculated, and fold changes were determined by the ΔΔCt method.

H&E and immunohistochemical staining
Sections of paraffin-embedded skin were prepared and stained with H&E at the Pathology Core Laboratory (Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan). Sections, 5 μm thickness, were deparaffinized and rehydrated for subsequent immunohistochemical staining. Heat-induced epitope retrieval was performed by incubating the sections in citrate buffer (Thermo Fisher Scientific, Waltham, MA) at 98 °C for 10 minutes, and endogenous peroxidase was quenched by incubating in 3% hydrogen peroxide in PBS for 5 minutes. Horse serum (Vector Laboratories, Burlingame, CA) was applied to the tissue sections for 1 hour to block nonspecific binding. Gal-8 in human skin was detected by incubation with rabbit anti–Gal-8 antibody and rhodamine-conjugated anti-rabbit IgG antibody. Fluorescent images were captured by a confocal LSM 700 (Carl Zeiss AG, Oberkochen, Germany) microscope, and the mean intensity of single chlorophores within the selected area was determined and analyzed by ZEN software (Carl Zeiss AG). MCM2 in mouse skin was detected by incubation with a rabbit anti-MCM2 antibody (catalog number CST3619, Cell Signaling Technology, Danvers, MA). After washing with PBS with Tween 20 (0.05%), the slides were incubated with a polymer horseradish peroxidase–conjugated horse anti-rabbit IgG antibody (Vector Laboratories) for 30 minutes. MCM2 staining was visualized using an ImmPACT DAB Peroxidase Substrate Kit (Vector Laboratories) as a red–brown precipitate. The nuclei were counterstained with Methyl green (Vector Laboratories). The images were captured on a DP80 microscope with CellSens software (Olympus, Tokyo, Japan). Epidermal thickness of each section was determined using the mean of 10 randomly selected epidermal segments of 100 μm in length with the measure module. MCM2-positive cells were quantified on the skin sections where epidermal hyperplasia was induced. The average numbers of MCM2-positive cells within the epidermis from three segments, 50 μm in length, of each section were determined. The MCM2-positive cells situated above the basal layer of keratinocytes were counted as suprabasal MCM2-positive cells.

Immunofluorescence staining
Immunofluorescence staining was performed on subconfluent nonsynchronized HaCaT and normal human epidermal keratinocytes (NHEKs). Primary antibodies against galectin-8, α-tubulin, pericentrin (catalog number ab4448, Abcam, Cambridge, United Kingdom), centrin 2, CPAP (Tang et al., 2009), and γ-tubulin were applied. Fluorescence-conjugated secondary antibodies were used, and the slides were mounted with ProLong Gold Antifade mountant with DAPI (Thermo Fisher Scientific). Fluorescent images were captured by LSM 700 (Carl Zeiss AG) confocal microscope. Pearson correlation coefficients for galectin-8 and α-tubulin within individual cells were obtained using ZEN software (Carl Zeiss AG).

Cell culture and treatment
The human-immortalized keratinocyte line HaCaT was maintained at 37 °C in DMEM (Gibco, CA) supplemented with 10% of fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin (Sigma), and 100 μg/ml streptomycin (Gibco). NHEKs were purchased from Gibco (catalog number C-001-5C). NHEKs were cultured at 37 °C in keratinocyte serum-free medium supplemented with 30 μg/ml bovine pituitary extract and 5 ng/ml recombinant human EGF (Gibco). HaCaT cells or NHEKs were incubated for 48 hours and then treated with indicated the concentrations of recombinant human IL-17A (catalog number 200-17, PeproTech, Rocky Hill, NJ) for 24 hours at 37 °C.
Generation of galectin-8–KO stable cells and Galectin-8–overexpressing stable cells (HaCaT and NHEK)

Galectin-8–KO HaCaT cells were generated by using the CRISPR/Cas9 system (GeneArt CRISPR Nuclease Vector Kit, ThermoFisher Scientific). The sequence of genomic target DNA of galectin-8 GATATCTGTAGGTGTATAGG, was designed in silico through the CRISPR Design Tool (http://tools.genome-engineering.org). Single-guide RNA sequence was cloned into an expression plasmid bearing a single-guide RNA scaffold backbone and Cas9. The resulting plasmid was then transfected into the HaCaT cells using Effectene (Qiagen). After sorting the fluorescence-expressing cells, single-cell colonies were obtained and assayed for targeted cleavage and successful missense mutations by gene sequencing. The absence of Gal-8 was further confirmed by immunoblotting, whereas the unaltered expression of other galectins was also confirmed.

Human galectin-8 mRNA, long and short forms, were amplified by pGal8L-GFP (our laboratory) and pGal8S-GFP (Kuo-I Lin’s laboratory, Academia Sinica, Taipei, Taiwan) and subcloned into a pIR3-3xFLAGGeG plasmid. The sequences of all plasmids were confirmed. FLAG-tagged galectin-8 cDNA was packaged into lentiviruses by the National RNAi Core Facility (Taipei, Taiwan). Lentiviruses expressing FLAG-tagged galectin-8 or vector only were transduced into the galectin-8–KO HaCaT clone KO1 or NHEK.

Western blotting and lactose pull-down assay

Cells were harvested and lysed in radio-immunoprecipitation assay lysis buffer containing 1% Triton X-100 and a protease inhibitor cocktail (Calbiochem, Darmstadt, Germany). Total protein concentrations were determined with a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). Equal amounts of sample lysates were subjected to SDS-PAGE, and the separated proteins were transferred to polyvinyl difluoride membranes (EMD Millipore, Burlington, MA). Primary antibodies against galectin-8 (catalog number PA5-50965, Thermo Fisher Scientific), α-tubulin (catalog number T9026, Sigma), γ-tubulin (catalog number T5192, Sigma), FLAG (catalog number 20543-1-AP, Proteintech), or GAPDH (catalog number PA5-50965, Thermo Fisher Scientific), or GAPDH (catalog number T9026, Sigma), FLAG (catalog number 20543-1-AP, Proteintech), or GAPDH (catalog number T9026, Sigma), FLAG (catalog number 20543-1-AP, Proteintech) were applied to detect the corresponding proteins. The membranes were subsequently incubated with horseradish peroxidase–conjugated secondary antibodies against mouse or rabbit IgG. Horseradish peroxidase substrate (EMD Millipore) was added, and proteins were visualized with a chemiluminescence image capture system (BioSpectrum UVP, Goteborg, Sweden) and then quantified by the area density module according to the manufacturer’s instructions. For enrichment of Gal-8, cell lysates were incubated with lactose beads at 4 °C overnight. After washing three times, the proteins bound on the beads were mixed with sample buffer and treated at 95 °C for 10 minutes in preparation for immunoblotting.

Cell proliferation assay

Individual CRISPR-generated control and KO (KO1, KO2, KO3) clones were seeded in 96-well plates, and cells were fixed with 10% (weight/volume) trichloroacetic acid at the indicated time. Fixed cells were stained with sulforhodamine B for 30 minutes, after which the excess dye was removed by washing repeatedly with 1% (volume/volume) acetic acid. The protein-bound dye was dissolved in 10 mM Tris base solution, and the optical density was determined at 515 nm using a microplate reader. A sulforhodamine B assay was performed to determine the growth curves of these clones.

Cell-cycle synchronization and analysis by flow cytometry

For synchronization experiments, cells were arrested in the early S phase by thymidine (2 mM) or at G2/M phase by nocodazole (50 ng/ml), as previously described (Tang et al., 2009). Then, the cells were placed in normal culture medium after being washed three times with PBS. Cells were collected at given time points and fixed in precooled 70% ethanol. Cells of the same batch were incubated in RNase A-containing propidium iodide solution and prepared for flow cytometric analysis by LSR II (BD, Franklin Lakes, NJ). Populations of cells in each stage of the cell cycle were determined by the cell-cycle model of software FlowJo, version 10 (LLC, Ashland, OR).

In-gel digestion and mass spectrometry analysis

FLAG-tagged galectin-8–overexpressing HaCaT cells were synchronized at the early S phase by thymidine or at G2/M phase by sequential thymidine and nocodazole treatment. After cross linking with dithiobis (succinimidyl propionate) (Sigma-Aldrich, St Louis, MO), lysates from FLAG-tagged galectin-8 stably expressing HaCaT cells were extracted and pulled down by anti-FLAG M2 beads (Sigma-Aldrich). FLAG-tagged galectin-8 was eluted from the beads with FLAG peptide. Eluted proteins were resolved by NuPAGE and Coomassie blue staining. Individual protein bands were excised from the gel and subjected to in-gel tryptic digestion. The digested peptides were extracted and then identified by Liquid Chromatography with tandem mass spectrometry (Proteomics Core Facility, Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan). Tandem mass spectrometry spectra were analyzed with the search engine Mascot (Matrix Science, London, UK). Only the proteins with a score exceeding 1,000 were considered further and annotated. Scaffold (version 4.10.0, Proteome Software Inc, Portland, OR) was used to validate tandem mass spectrometry–based peptide and protein identifications. The relative abundance of proteins was expressed as total spectrum counts, which is a label-free semiquantitative measure of protein abundance in proteomic studies (Lundegrd et al., 2010).

Coinmunoprecipitation

HaCaT cells expressing FLAG-tagged galectin-8 or vector were synchronized at the G2/M phase by sequential thymidine and nocodazole treatment. Then, the cells were placed in normal DMEM medium for 30 minutes, washed with PBS, and treated with 0.4 μg/ml dithiobis (succinimidyl propionate)–cell-permeable cross linker (Sigma-Aldrich) for 30 minutes at room temperature. To stop the cross-linking process, the cells were incubated with 0.1 M Tris hydrochloride (pH 7.5) for 15 minutes. Next, the cells were washed with lactose to remove Gal-8 that might be present on the cell surface through binding to glycoconjugates and then lysed in radio-immunoprecipitation assay buffer (50 mM Tris hydrochloride, pH 7.4, 15 mM sodium chloride, 0.25% deoxycholic
acid, 1% nonyl phenoxypolyethoxylethanol, 1 mM EDTA) with 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, 1 mM sodium fluoride, and protease inhibitor cocktail on ice for 30 minutes with consecutive pipetting cycles every 10 minutes. After brief sonication, lysates were centrifuged at 15,000 g for 10 minutes, and supernatant samples were collected. The precleaned lysates were incubated with anti-α-tubulin antibody or IgG Ctrl at 4 °C overnight and then mixed with protein A/G mix magnetic beads (Thermo Fisher Scientific). Proteins precipitated by A/G beads were washed with lysis buffer containing 25 mM lactose and resuspended in SDS sample buffer. The protein samples bound by beads were boiled for 10 minutes and subjected to immunoblotting analysis with anti-FLAG and anti-α-tubulin antibody.

Statistical analysis
All quantitative data are presented as mean ± SEM or SD. We applied a two-tailed t-test to compare the samples and a paired t-test to compare results within the same patient or from mouse littermates. A P-value < 0.05 denotes statistical significance. Quantitative results were analyzed using Prism 6 software (GraphPad Software, San Diego, CA).

SUPPLEMENTARY REFERENCES
Supplementary Figure S1. Galectin-8 mRNA is overexpressed in psoriatic skin. (a) Galectin-8 profiles in nonlesional and lesional psoriasis skin from microarray (n = 16). ***P < 0.001 by two-tailed paired t-test. (b) Immunohistochemical staining of galectin-8 in HaCaT cell pellets, WT and KO (Gal-8: Vector VIP, counterstain: methyl green). Bar = 50 μm. Gal-8, galectin-8; KO, knockout; WT, wild type.
Supplementary Figure S2. Galectin-8 protein is induced by IL-17A in keratinocytes. (a) Immunoblotting of HaCaT cells and NHEKs treated with various mitogens for keratinocytes. (b) Immunoblotting of NHEKs treated with rhIL-17A and densitometric analysis of the intensities of galectin-8 bands (Gal8L plus Gal8S) relative to α–tubulin from three independent experiments (n = 3). (c) Immunoblotting of HaCaT cells and NHEKs treated with rhIL-22 and densitometric analysis of the intensities of galectin-8 bands (Gal8L plus Gal8S) relative to α–tubulin from three independent experiments (n = 3). Error bar: mean ± SD, *P < 0.05, **P < 0.01, ***P < 0.005 by two-way ANOVA test. Gal-8, galectin-8; Gal8L, long form of Gal-8; Gal8S, short form of Gal-8; NHEK, primary human keratinocyte; rh, recombinant human.
Supplementary Figure S3. Validation of galectin-8–KO HaCaT cells generated by CRISPR/Cas9 technology. Galectin-8–overexpressed NHEKs are more proliferative. (a) Immunoblotting of whole-cell lysates of WT and galectin-8–KO HaCaT clones. (b) Affinity purification of lysates from WT and galectin-8–KO clones by lactosyl-sepharose followed by immunoblotting analysis. (c) Cell proliferation curve expressed by absorbance at 515 nm by SRB assay of NHEKs overexpressing FLAG-tagged galectin-8 (n = 12), confirmed by immunoblotting with anti-galectin-8 and anti-FLAG antibodies. Error bar: mean ± SEM, ***P < 0.001 by two-tailed t-test. Ctrl, control; Gal, galectin; Gal8L, long form of Gal-8; Gal8S, short form of Gal-8; Gal9L, long form of Gal-9; Gal9S, short form of Gal-9; KO, knockout; NHEK, primary human keratinocyte; SRB, sulforhodamine B; Vector, empty vector; WT, wild type.

Supplementary Figure S4. Transitioning from mitosis into G1 phase in galectin-8–KO keratinocytes is delayed. FACS analysis of the progression of cell cycles in synchronized cells after release from nocodazole synchronization at indicated time in Ctrl and galectin-8–KO HaCaT cells (representative results are from three independent experiments). Ctrl, CRISPR/Cas9 control; h, hour; k, thousand; KO, galectin-8–KO HaCaT cells; M, mock treatment.
Supplementary Figure S5. Galectin-8 colocalizes with α-tubulin on microtubules during mitosis in HaCaT cells and NHEKs. (a) Epifluorescence microscopy analysis of HaCaT cells (nucleus: DAPI, Gal-8: rhodamine). Bar = 20 μm. (b) Immunofluorescence staining of HaCaT cells, WT, and galectin-8–KO cells by confocal microscopy (nucleus: DAPI, α-tubulin: FITC, Gal-8: rhodamine). Bar = 50 μm. (c) Immunofluorescence staining of NHEKs during mitosis by confocal microscopy (nucleus: DAPI, α-tubulin: FITC, Gal-8: rhodamine). Bar = 10 μm. (d) Immunofluorescence staining of HaCaT cell at the spindle pole (nucleus: DAPI, PCTN: rhodamine, Gal-8: FITC). Bar = 10 μm. Gal-8, galectin-8; KO, knockout; NHEK, primary human keratinocyte; PCTN, pericentrin, WT, wild type.

Supplementary Figure S6. Coomassie blue stain of the FLAG-tagged galectin-8 and interacting proteins from synchronized galectin-8–overexpressed HaCaT cells on NuPAGE gel. The part of gel around 55 kDa of G2/M phase from vectors, Gal8S and Gal8L, was selected for mass spectrometric analysis. Gal8L, long form of galectin-8; Gal8S, short form of galectin-8.
Supplementary Figure S7. Centrosomal proteins during mitosis in HaCaT cells. Immunofluorescence staining of Ctrl and galectin-8–KO mitotic HaCaT cells. (a) Nucleus: DAPI, α-tubulin: FITC, CPAP: rhodamine. Inset: CPAP. Bar = 10 µm. (b) Nucleus: DAPI, γ-tubulin: FITC, centrin-2: rhodamine). Ctrl, CRISPR/Cas9 control clone; KO, galectin-8–KO HaCaT cells. Bar = 10 µm.
### Supplementary Table S1. List of 50 Proteins in the Sequence of Abundance According to Total Spectrum Count (an Indicator for Semiquantitative Measurement of Protein Abundance) of the Clone Transfected with Gal8S as Described in Supplementary Figure S6

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Abbreviations: Gal-8, galectin-8; Gal8L, long form of Gal-8; Gal8S, short form of Gal-8; ID, identification document.

1TUBB4B, cluster of tubulin beta-4B chain; TUBA1C, cluster of tubulin alpha-1C chain; TUBB6, tubulin beta-6 chain.