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Cross-sectional study of proteomic differences between moderate and severe psoriasis

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Although an ongoing understanding of psoriasis vulgaris (PV) pathogenesis, little is known about the proteomic differences between moderate and severe psoriasis. In this cross-sectional study, we evaluated the proteomic differences between moderate and severe psoriasis using data-independent acquisition mass spectrometry (DIA-MS). 173 differentially expressed proteins (DEPs) were significantly differentially expressed between the two groups. Among them, 85 proteins were upregulated, while 88 were downregulated (FC ≥ ± 1.5, $P < 0.05$). Eighteen DEPs were mainly enriched in the IL – 17 signalling pathway, Neutrophil extracellular trap formation, Neutrophil degranulation and NF – kappa B signalling pathway, which were associated with psoriasis pathogenesis. Ingenuity pathway Analysis (IPA) identified TNF and TDP53 as the top upstream up-regulators, while Lipopolysaccharide and YAP1 were the top potential down-regulators. The main active pathways were antimicrobial peptides and PTEN signalling, while the inhibitory pathways were the neutrophil extracellular trap pathway, neutrophil degranulation, and IL-8 signalling. 4D-parallel reaction monitoring (4D-PRM) suggested that KRT6A were downregulated in severe psoriasis. Our data identify Eighteen DEPs as biomarkers of disease severity, and are associated with IL – 17 signalling pathway, Neutrophil extracellular trap formation, NF – kappa B signalling pathway, and defence response to the bacterium. Targeting these molecules and measures to manage infection may improve psoriasis's severity and therapeutic efficacy.

Keywords Proteomics, Psoriasis vulgaris, 4D-parallel reaction monitoring, Data-independent acquisition mass spectrometry, Ingenuity pathway analysis, Biomarker

Psoriasis vulgaris (PV) is a common, chronic and immune-related skin disease which can also cause multisystem damage like depression (especially in young people)[1,2]and psoriatic arthritis[3]. Recent research has focused on the crucial roles of immune molecules in PV, such as IL-17 A/F, IL-23, and TNF-α[4]. Biological agents have significantly improved psoriasis efficacy, but a loss of effectiveness[5] often occurs in clinical practice. Therefore, it is necessary to explore this disease further to discover more critical immune molecules involved in pathogenesis and develop new drugs.

In the last decade, proteomic approaches have been used to identify and characterize proteins that participated in the pathogenesis of psoriasis or other skin diseases. For instance, recent studies have shown the circulating proteomic landscape of psoriasis or hidradenitis suppurativa (HS) using a smaller Olink proteomic platform[6–11]. In a large-scale proteomic study using a novel extended Olink platform assessing 1536 biomarkers, Navrazhina K et al.. found that HS presents a more extraordinary serum inflammatory burden[12]. The above results suggest that detecting more biomarkers could help us discover critical immune molecules and deepen our understanding of disease pathogenesis.

Several studies have identified proteins associated with the severity of psoriasis, such as some Antimicrobial peptides (AMPs) (S100A7, S100A8, S100A9 and PI3)[8,13–15]. Still, they mainly evaluate blood samples or the stratum corneum (SC) cells, or the platforms used in these studies have limited ability to detect more proteins.

The most common approach to large-scale proteomics is data-dependent acquisition (DDA) mass spectrometry, such as label-free[16] and TMT/iTRAQ[17,18]. However, DDA has been reported to decline when sample complexity[19] which leading to limited reproducibility, bias toward high abundance peptides and undersampling[20]. One possible approach to overcome these limitations is data-independent acquisition mass spectrometry (DIA-MS)[21]. DIA-MS is a proteomic technology that combines deep proteome coverage

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capabilities with quantitative consistency and accuracy[22], which has been successfully used for the investigation of skin disease[23–26], as described in our previous study[27]. Until now, no studies have directly compared proteomic differences between severe and moderate psoriasis in psoriatic lesions. Here, we used DIA-MS to characterize the proteomic differences between severe PV (sPV) and moderate PV (mPV) in psoriatic lesions and explore biomarkers associated with disease severity. Finally, we validated selected differentially expressed proteins (DEPs) with 4D-parallel reaction monitoring (4D-PRM) technology[28].

Results

Quantitative analysis of proteins in psoriatic lesions using DIA

For DIA detection, fifteen samples were collected from patients with sPV($n=7$) and mPV($n=8$). Then, 4D-PRM was performed for validation in twelve newly collected cases. The characteristics of included patients are shown in Table 1.

A total of 6418 proteins were quantified in the proteomic experiment, with a false discovery rate (FDR) of less than 1% at both the peptide and protein levels. Protein identified in technical replicates and control samples showed a relatively low coefficient variance (CV) (see Supplementary Fig. S2 online).

Bioinformatics analysis and functional description of DEPs in moderate and severe psoriasis

173 DEPs were significantly changed in the sPV group compared to the mPV group (FC ≥ ± 1.5, $P<0.05$). Among them, 85 proteins were upregulated, while 88 were down-regulated (see Supplementary Table S1 online). Top nine upregulated proteins were OSBPL6, CYFIP2, MYOCD, AGO3, DCD, PCBP4, LRIF1, DLG5 and KRT15, while top nine down-regulated proteins were MTMR10, CCNDBP1, STK3, LAMP1, UBE2D1, APBB3, BPI, SULT1B1 and IGHM (Fig. 1).

To further investigate the molecular functions of these DEPs, we performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis[29–31] in the sPV and mPV groups. The top 10 significant difference clusters of GO terms were displayed in Fig. 2a. DEPs in our data highlighted involvement in defence response to the bacterium or stimulus, cell proliferation, and expression related processes.

The top 20 RichFactor of enriched pathways were illustrated in Fig. 2b. Data shown that few DEPs are involved in well-known pathways associated with psoriasis, such as IL-17 signalling pathway, Neutrophil extracellular trap formation, and NF-kappa B signalling pathway.

	DIA test		PRM validation	
Variables	sPV groups($n=7$)	mPV groups($n=8$)	sPV groups($n=6$)	mPV groups($n=6$)
Sex- no. (%)				
Male	6(85.7%)	4(50.0%)	5(83.3%)	2(33.3%)
Female	1(14.3%)	4(50.0%)	1(16.7%)	4(66.7%)
Age, year				
Mean ± SD.	51.6 ± 16.8	42.4 ± 15.5	48.8 ± 14.3	33.5 ± 7.9
Median (IQR)	47.0(34.0–66.0)	41.0(29.3–59.0)	45.5(36.8–59.8)	33.0(28.8–38.5)
Range	34.0–76.0	22.0–64.0	36.0–74.0	22.0–46.0
Course, year				
Mean ± SD.	24.3 ± 16.0	13.8 ± 14.9	11.3 ± 8.0	4.6 ± 3.9
Median (IQR)	21.5(11.0-40.8)	10.0(2.3–20.3)	8.5(6.0-15.8)	4.0(0.9-8.0)
Range	6.0–50.0	0.5–46.0	6.0–27.0	0.5–11.0
Previous treatment - no. (%)				
Topical therapy	6(85.7%)	8(100.0%)	5(83.3%)	5(83.3%)
Systemic therapy	7(100.0%)	6(75.0%)	4(66.7%)	4(66.7%)
Phototherapy	4(57.1%)	3(37.5%)	1(16.7%)	2(33.3%)
Biologic therapy	2(28.6%)	1(12.5%)	0(0.0%)	0(0.0%)
Family history - no. (%)				
Yes	3(42.9%)	1(12.5%)	3(50.0%)	1(16.7%)
No	4(57.1%)	7(87.5%)	3(50.0%)	5(83.3%)
Comorbidity- no. (%)				
Yes	4(57.1%)	0(0.0%)	1(16.7%)	1(16.7%)
No	3(42.9%)	8(100.0%)	5(83.3%)	5(83.3%)
PASI (0 weeks), score				
Mean ± SD.	24.6 ± 5.8	11.2 ± 2.4	30.6 ± 10.6	9.0 ± 2.2
Median (IQR)	21.0(20.1–31.5)	11.6(10.0-12.2)	25.8(22.8–43.6)	8.8(7.0-11.2)
Range	19.1–33.3	6.8–15.0	20.2–44.6	6.8–11.8
no. (%), number; SD, standard deviation; IQR, interquartile range.				

Table 1. Baseline characteristics of Psoriasis patients.

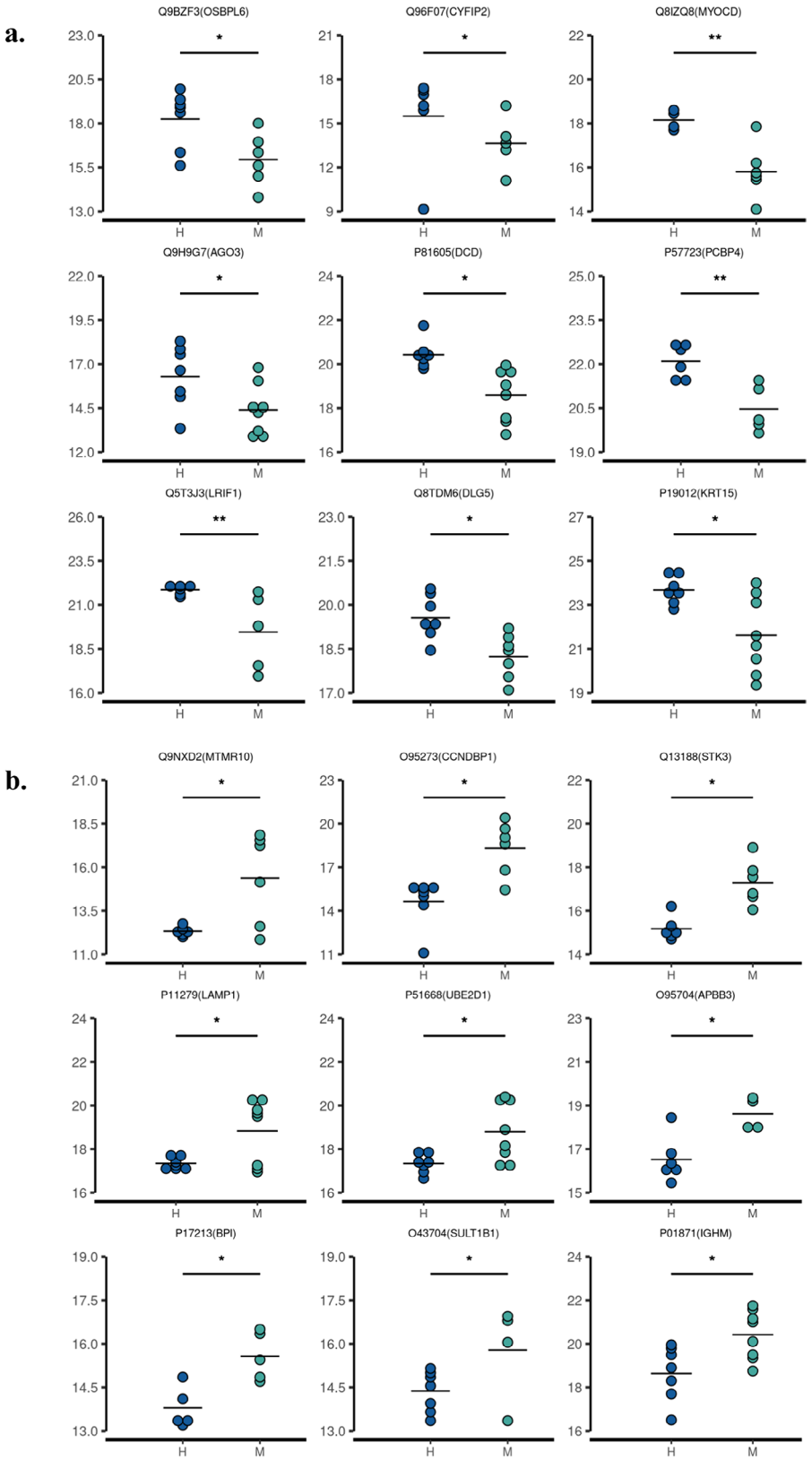


Fig. 1. Box plot analysis of nine proteins with the highest ± FC in the upregulated and down-regulated DEPs, respectively. (**a**)Nine proteins (OSBPL6, CYFIP2, MYOCD, AGO3, DCD, PCBP4, LRIF1, DLG5 and KRT15) with the highest + FC in the up-regulated DEPs. (**b**)Inversely, the nine proteins with the highest -FC were MTMR10, CCNDBP1, STK3, LAMP1, UBE2D1, APBB3, BPI, SULT1B1 and IGHM in the down-regulated DEPs. The statistical analysis was performed using Student's t-test with a p-value ≤ 0.05. *, ** and *** represent the p-values less than 0.05, 0.005 and 0.0005, respectively.

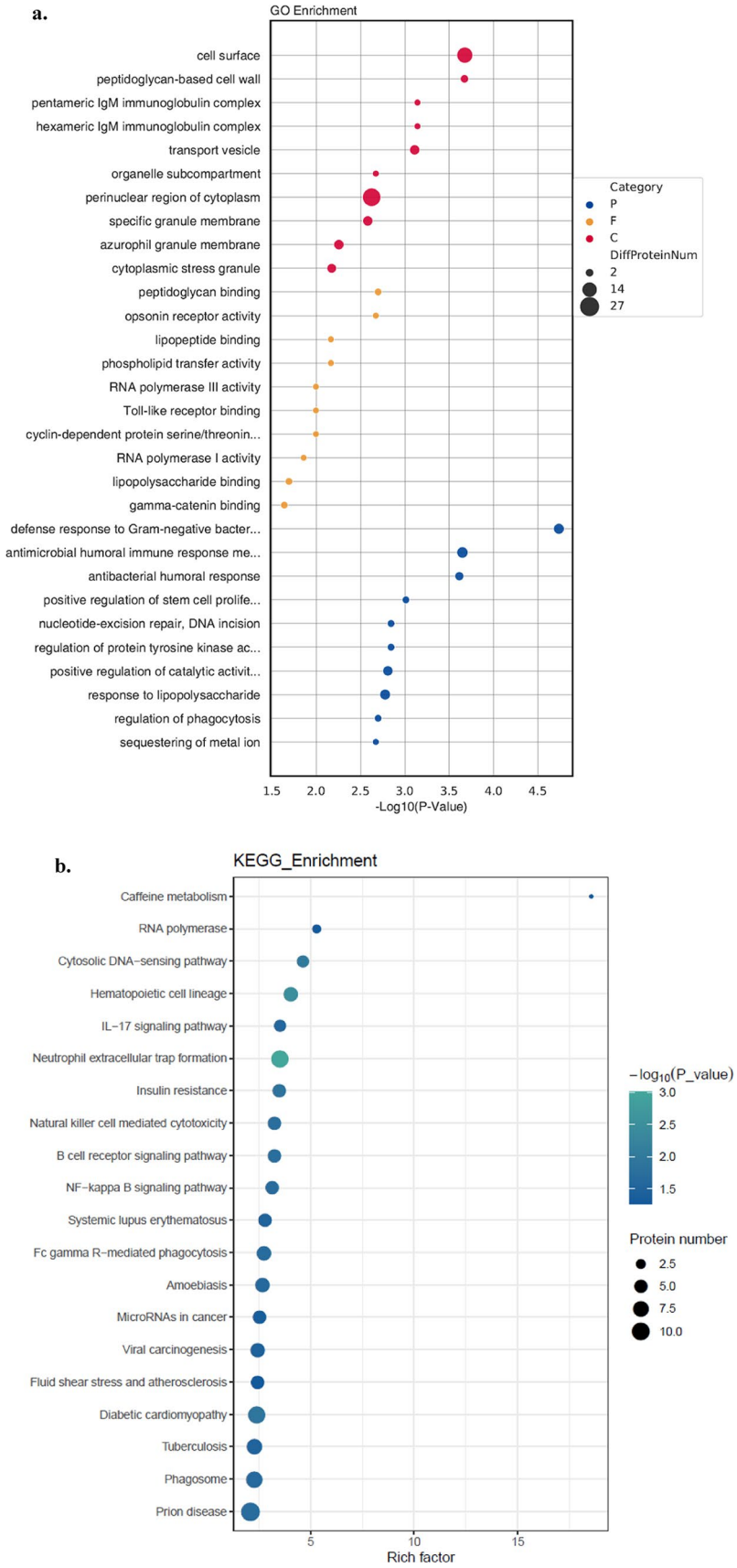


Fig. 2. GO and KEGG enrichment analyses were performed in 173 DEPs (FC ≥ ± 1.5, *P*-value < 0.05). Dotplot displayed the top 10 significant difference GO terms (**a**) and the top 20 enriched pathways in Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis (**b**). A *p*-value < 0.05 was used for biological process selection.

Identification of proteins and pathways associated with psoriasis pathogenesis and disease severity

As shown in Supplementary Table S2, DEPs in the sPV group, compared to the mPV group, participated in the IL−17 signalling pathway, Neutrophil extracellular trap formation and NF−kappa B signalling pathway. Neutrophil extracellular trap formation was predicted to be down-regulated according to the Z score using ingenuity pathway analysis (IPA) (see Supplementary Fig. S3 online). In detail, 4 DEPs are involved in the IL−17 signaling pathway. Among them, HSP90AA1, S100A7, S100A7L2 were upregulated, while CASP3 was down-regulated. Five proteins participate in the NF-kappa B signalling pathway. Among them, MALT1 was upregulated, whereas IGHM, CD14, SYK, and IGHV3-49 were down-regulated in sPV.

We then performed Pearson correlation analyses to explore the associations between DEPs ($FC \geq \pm 2.0$, $P < 0.05$) and Psoriasis Area and Severity Index (PASI). The positive and negative correlation data are plotted in Fig. 3. Specifically, in the sPV group, CFAP47 and OSBPL6 have the highest positive Pearson correlation coefficient with PASI. Other proteins, such as CD36 and CD14, showed no statistical difference despite appearing to be associated with PASI. For the mPV group, ANO7 and S100A7L2 have the highest p-PCC, while CCNDBP1, GFPT1, YTHDF2, TELO2 and BPI have the highest negative Pearson correlation coefficient (see Supplementary Table S3 online).

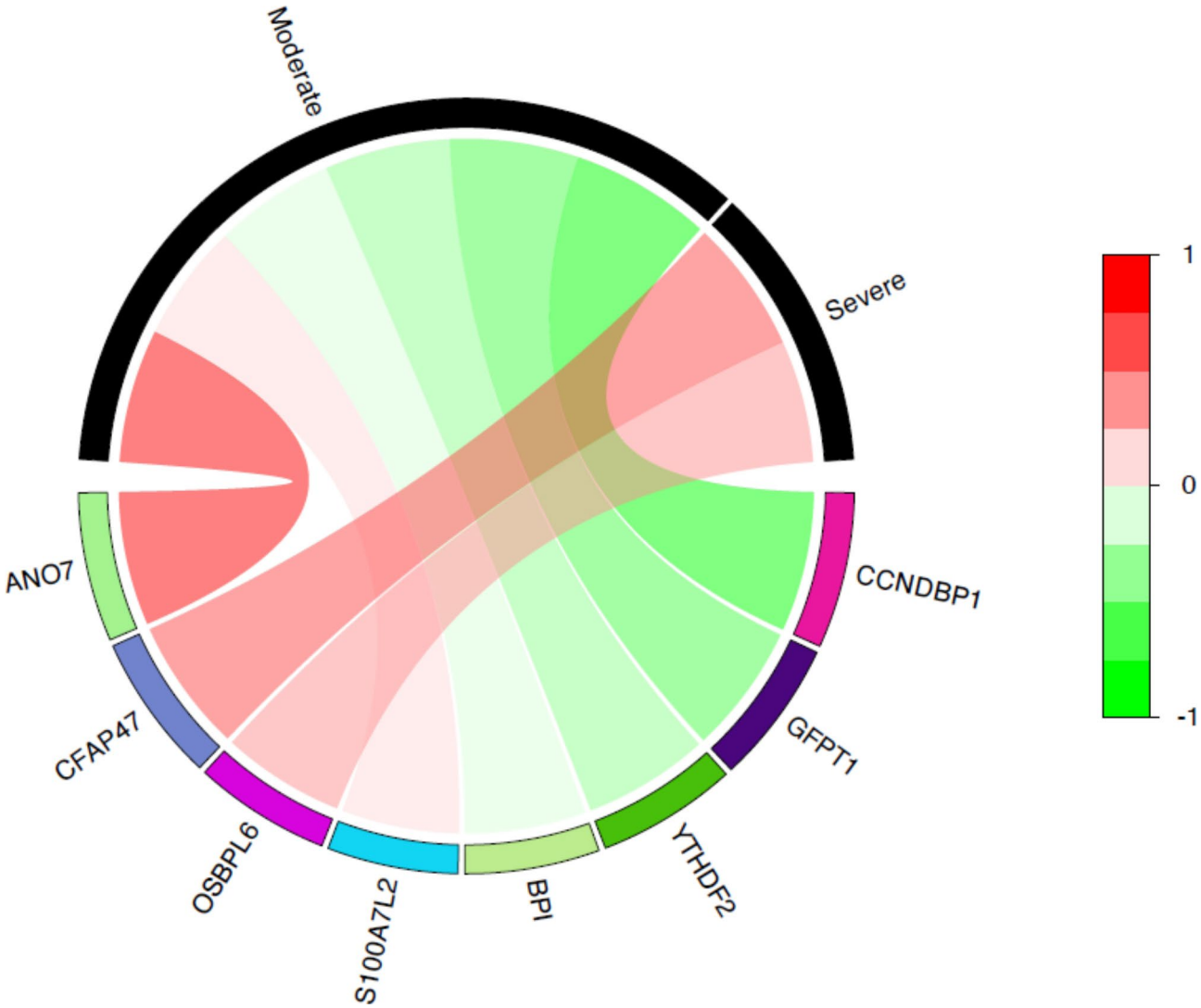


Fig. 3. Correlation between DEPs ($FC \geq \pm 2.0$, P-value < 0.05) and clinical PASI score. Circos plot presenting the Pearson correlation between DEPs and the PASI score ($|cor| > 0.6$, p-value < 0.05). Red ribbons indicate positive Pearson correlation coefficients. Green ribbons represent negative Pearson correlation coefficients. The width of the ribbons indicates the correlation value.

Protein interaction network showed two sets of molecules interacting with chaperone proteins HSP90AA1

To clearly show the interactions among these DEPs, we constructed an interaction network among molecules using the STRING database (see Supplementary Table S4 online). We found two molecular clusters interacting with chaperone proteins (mainly HSP90AA1). One cluster included CD36 and CD14, which are associated with HSP90AA1 through their effects on LGALS3 or ITGB3. The other contained STK3 and PANX1, which linked with HSP90AA1 through their effects on CASP3 (Fig. 4).

We further analyzed these proteins using IPA. It identified the top potential upstream up-regulators were TNF (p-value = 6.84e-10, z-score 0.266) (Fig. 5a) and TDP53 (p-value = 6.26e-8, z-score = 0.286) (Fig. 5b), while the top potential down-regulators were Lipopolysaccharide (p-value = 1.12e-6, z-score=-1.494) (Fig. 5c) and YAP1 (p-value = 2.52e-6, z-score=-0.814) (Fig. 5d).

Enriched interaction analysis contains 13 networks; the top was selected based on the Z-score. The highest score network (score 46) was centred on 22 proteins (Fig. 5e). Pathway analysis showed that the main active pathways were Antimicrobial peptides and PTEN signalling. In contrast, the main inhibitory pathways were the Neutrophil extracellular trap pathway, Neutrophil degranulation, and IL-8 signalling (see Supplementary Fig. S3 online).

The expressions of KRT6A were downregulated in sPV by 4D-PRM

To validate the results obtained from the DIA, we collected nine new samples and three original samples for validation. In a separate cohort study, we screened thirty-two proteins for further validation using 4D-PRM.

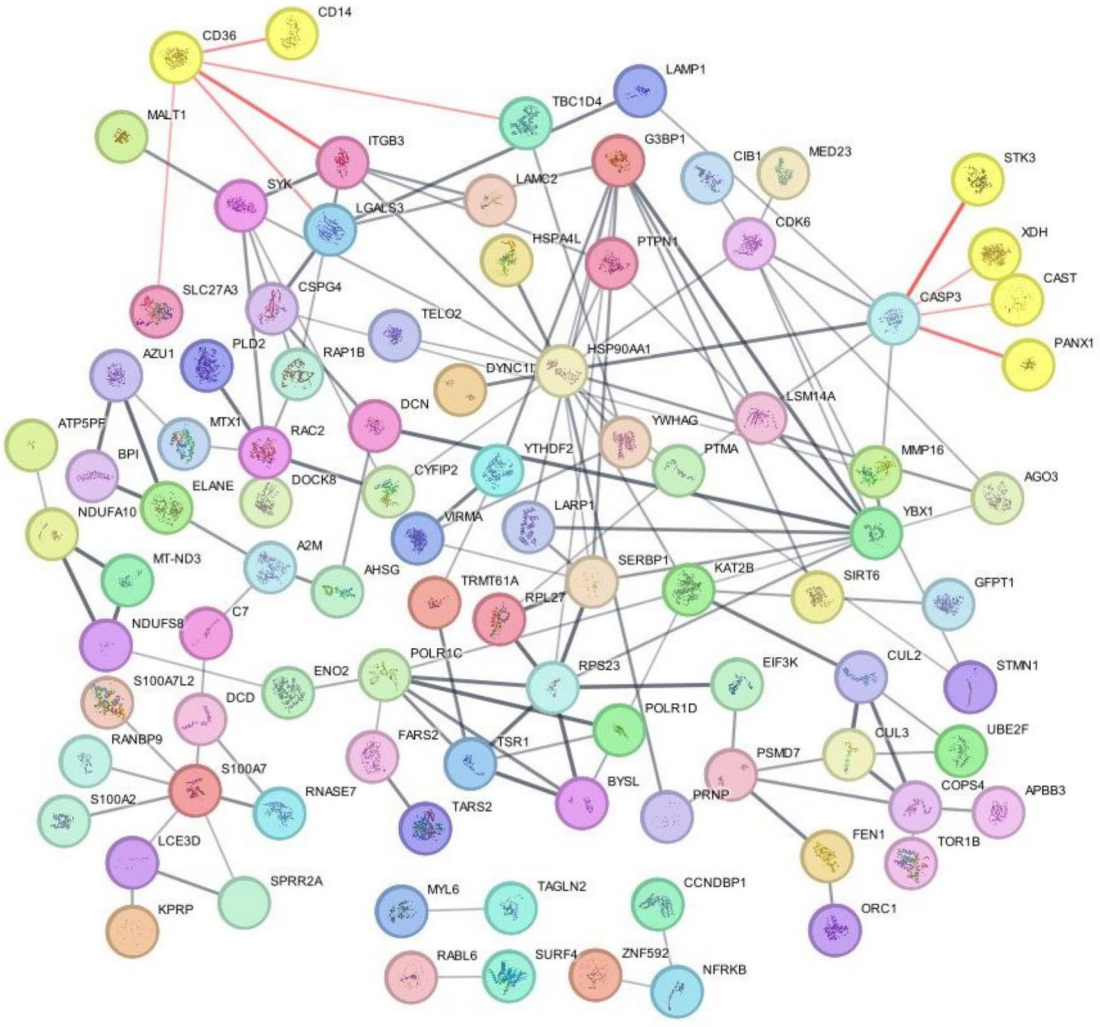


Fig. 4. Protein-protein interaction networks of DEPs (FC ≥ ± 1.5, P-value < 0.05) were obtained from STRING (Confidence score > 0.5). Cytoscape (Version 3.10.2) was used to visualize the networks. Nodes represent proteins, and edges represent the interaction between them. Edge width corresponds to the combined score of specific protein pairs. A larger edge width indicated a higher score. PPI: protein-protein interaction.

Seventeen proteins were quantitated, and the results demonstrated that KRT6A were downregulated in sPV (see Supplementary Table S5 online). The PRM peptide quantitative results was shown in Supplementary Table S6 online. At the same time, we added the peptide standard curve of the peptide APFDLFENR_.2 as an example (see Supplementary Fig. S4 online).

Discussion

Here, we use DIA-MS to evaluate proteomic differences in psoriatic lesions between sPV and mPV and simultaneously determine some biomarkers associated with disease severity. We quantified 6418 proteins in the DIA-MS test, which was significantly higher than that in a previous proteomic study using LC-MS/MS[16,32] or the Tandem Mass Tags (TMT) approach[17]. These results show that DIA-MS has significantly advanced global protein quantification across multiple samples. Our research identified 173 DEPs in the sPV group compared to the mPV group, which likely plays a crucial role in psoriasis and were associated with disease severity. The main active pathways were antimicrobial peptides and PTEN signalling, while the inhibitory pathways were the neutrophil extracellular trap pathway, neutrophil degranulation, and IL-8 signalling.

We quantified a higher amount of protein than most studies. The reasons for the differences in research results were firstly due to the different detection methods. For the same skin sample, the identification results of different techniques are quite different among Olink high-throughput proteomics[8,11], LC–MS/MS[16,32], Tandem Mass Tags (TMT)-MS/MS[33,34]. Secondly, the type of sample also affects the identification result. For example, also using DIA mass spectrometry, the amount of protein in the skin identified in our study was significantly higher than that in the serum[23,25,26], which may be caused by the wide range of protein concentrations and the detection capability in blood. More rigorous self-controlled studies, such as Lesional (PP) compared with uninvolved (PN) skin samples, can help to confirm that the proteomic changes they have observed were indeed related to psoriasis. However, the implementation of such studies was difficult and may cause harm to participants, such as scarring at the site of the sampled, and triggering new psoriatic lesions.

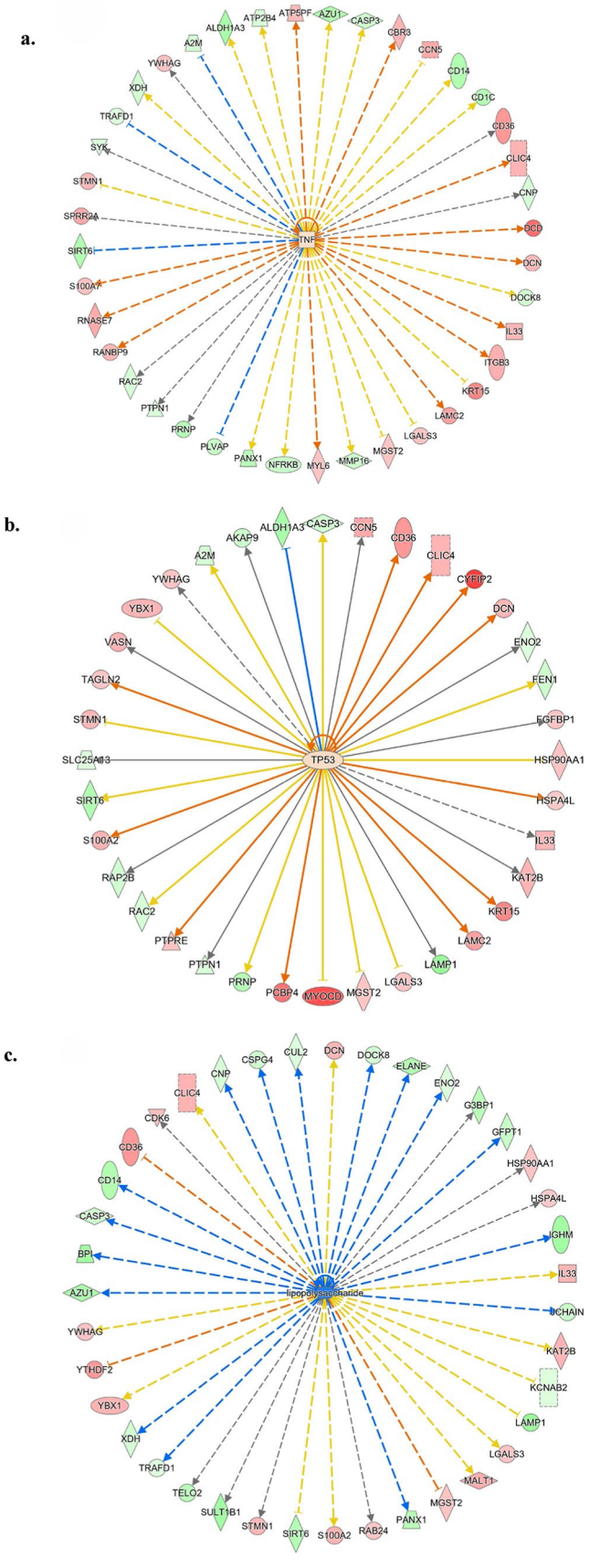
To verify the effectiveness of our proteomic approach we compared major known psoriatic biomarkers published in previous proteomic studies with our dataset. The results varied widely among studies. Known psoriatic biomarkers also found in our study includes S100A7[15,35], CD14[25]. We found that CFAP47 and OSBPL6 were associated with disease severity and have the highest positive Pearson correlation coefficient with PASI. KRT6A is one of the stress keratins that regulate keratinocyte differentiation and participate in EGFR and retinoic acid signalling[36,37]. Our results demonstrated that it was upregulated in DIA but downregulated in the PRM test, which the conclusions of the previous study can explain that individual stress keratin genes are associated with partially distinct gene networks[38].

We then performed literature search for known functions of proteins found to be altered in our study to explore which they may potentially participate in the pathogenesis of psoriasis. Most studies compared protein differences between lesional and uninvolved skin[16,32], lesional and healthy controls[8,11,33,34], and we did not search for studies that directly compared protein differences in severe and moderate psoriasis lesions. Compared to these studies, DEPs in our data highlighted involvement in some overlap biological process, such as cell proliferation[39], response to stimulus[40], expression related processes[41].

It is worth noting that some detected DEPs participate in the defence response to the bacterium, such as defence response to Gram-negative bacterium, antimicrobial humoral immune response mediated by AMP, antibacterial humoral response and response to Lipopolysaccharide. These proteins include AMPs, serine protease, chaperone protein, co-receptor and other molecules. Our work confirms the initial role of bacterial origin elements in psoriasis and the excessive innate immune responses induced by them[4]. Given the initial role of trauma and infection in the onset of psoriasis, it is essential to strengthen skin care, such as enhanced emollients, and to prevent local infections, especially in the progression of psoriasis. Patients should be educated to avoid washing skin lesions with salt or hot water (Especially among the Chinese) to avoid aggravating them.

Consistent with previous studies, KEGG enrichment and IPA analysis found that few DEPs predominantly participated in the IL – 17 signalling pathway (HSP90AA1, S100A7, S100A7L2, and CASP3)[15] and NF – kappa B signalling pathway, which plays a vital role in the pathogenesis of psoriasis[42]. S100A7 (Psoriasin) is one of the AMPs belonging to the S100 family, produced by keratinocytes and leukocytes stimulated with IL-17, IL-22 and TNF; therefore, it plays an essential role in innate immunity and angiogenesis[13,43]. In our study, it was upregulated in sPV compared to mPV, which agrees with previous work showing higher serum levels of psoriasin in patients with severe psoriasis[13,15] and a reduction after treatment with biological agents[27]. HSP90AA1 is a molecular chaperone which plays an essential role in cell survival, cytokine signalling, and immune response. It can bind bacterial Lipopolysaccharide (LPS) and mediates LPS-induced inflammatory response, including monocyte TNF secretion[44]. Furthermore, HSP90AA1 released by stressed keratinocytes activate DCs to secrete proinflammatory cytokines and AMPs such as S100A7[45]. Despite previous research finding a significantly decreased expression of HSP90AA1 in both keratinocytes and lymphocytes from psoriatic skin[46], this study shows that it is upregulated in sPV. Our data is consistent with another study showing that it is significantly upregulated in epidermal keratinocytes and mast cells of psoriatic lesions and down-regulated after ustekinumab treatment[45] .

CD14 is one of the proteins involved in NF – kappa B signalling pathway which activates the transcription of hundreds of genes involved in immune response, growth control, or protection against apoptosis. It acts as a co-receptor for toll-like receptors (TLRs) to activate multiple signal pathways of innate immunity responses to pathogens or tissue injury in diverse cells. This function can be achieved by LBP-dependent combination of the CD14-LPS complex or independently of TLRs[47]. Studies have shown that CD14 + DC3s increased in psoriatic lesions and co-produced IL1B and IL23A[48]; in contradiction to this, IL-17 A blockade induced higher expression of CD1C and CD14, which are markers for CD1c + CD14 + dendritic cell (DC) that suppress antigen-specific T-cell responses, in post-treatment regulatory semimature DCs[49]. Accordingly, CD14 are down-


a.
ATP5PF
AZU1
ATP2B4
ALDH1A3
CASP3
A2M
CBR3
CCN5
YWHAG
CD14
XDH
CD1C
TRAFD1
CD36
SYK
CLIC4
STMN1
CNP
SPRR2A
DCD
TNF
SIRT6
DCN
S100A7
DOCK8
RNASE7
IL33
RANBP9
ITGB3
RAC2
KRT15
PTPN1
LAMC2
PRNP
LGALS3
PLVAP
MGST2
PANX1
NFRKB
MYL6
MMP16
b.
ALDH1A3
CASP3
AKAP9
CCN5
CD36
A2M
CLIC4
YWHAG
CYFIP2
YBX1
DCN
VASN
ENO2
TAGLN2
FEN1
STMN1
EGFBP1
SLC25A13
TP53
HSP90AA1
SIRT6
HSPA4L
S100A2
IL33
RAP2B
KAT2B
RAC2
KRT15
PTPRE
LAMC2
PTPN1
LAMP1
PRNP
PCBP4
MYOCD
MGST2
LGALS3
c.
CSPG4
CUL2
DCN
DOCK8
ELANE
CNP
ENO2
CLIC4
G3BP1
CDK6
GFPT1
CD36
HSP90AA1
CD14
HSPA4L
CASP3
IGHM
BPI
IL33
AZU1
lipopolysaccharide
IGCHAIN
YWHAG
KAT2B
YTHDF2
KCNAB2
YBX1
LAMP1
XDH
LGALS3
TRAFD1
MALT1
TELO2
MGST2
SULT1B1
PANX1
STMN1
SIRT6
S100A2
RAB24

◀**Fig. 5**. Upstream regulator analysis (URA) of 173 DEPs (FC ≥ ± 1.5, *P*-value < 0.05) according to Z-score using ingenuity pathway analysis (IPA), with $P < 0.05$, Z score > 0 or < 0). It determines likely upstream regulators that are connected to data set genes through a set of direct or indirect relationships. The top potential upstream up-regulators were TNF (**a**) and TDP53 (**b**), while the top potential down-regulators were Lipopolysaccharide (**c**) and YAP1 (**d**). Causal network analysis of DEPs using IPA. The highest-score network is displayed. The relationship among molecules is represented by lines (solid lines for direct association and dotted lines for indirect association) (**e**).

regulated, along with CD1c, in sPV according to our proteomic dataset. These findings indicate that, although the CD14-mediated immune response to pathogenic microorganisms is weakened, the reduction of CD14 on specific dendritic cell subpopulations attenuates the inhibitory effect on T cells, which leads to the persistence and aggravation of inflammation in psoriasis. This study found CD36, another co-receptor associated with the TLR2/TLR6, was upregulated in sPV. Meanwhile, CD36 has a more vigorous activity for modulating TLR4-TLR6 signal transduction than CD14. So, we speculate that the reduction of CD14 may be related to the competition of the CD36 receptor pathway and the regulation of other proteins.

Other proteins involve in the NF-kappa-B signalling pathway are MALT1 and IL-33. MALT1 is a protease that is involved in the activation of NF-kB and MAP signalling[50], Inhibition of MALT1 in keratinocytes can reduce proinflammatory cytokines (TNF, IL-1b, and IL-17 C), chemokines, and antimicrobial peptides[51]. So, it may play a role in the pathogenesis of psoriasis and provide a basis that MALT1 inhibitors can be used as a treatment for psoriasis[52]. IL-33 is a cytokine that signals through the IL1RL1/ST2 receptor, which can activate NF-kappa-B and MAPK pathways[53], and is a proinflammatory molecule and modulator in psoriasis[54–56]. However, other studies found that IL-33 exert anti-inflammatory and protective activities in psoriatic skin[57,58], which only manifests when the amount of IL-33 is excessive. Considering that IL33 was significantly elevated in sPV, our results agree it is a risk factor for psoriasis. Meanwhile, consistent with previous research, our data show that IL-33 can function both as a cytokine and as a nuclear transcriptional regulator[58] since it can interact with chaperone proteins to regulate the transcription machinery.

Neutrophil elastase (NE) is a primary proteinase in neutrophils that participates in microbicidal activity[59]. Previous research has found that the levels and activity of NE reflect disease state and severity[60] and augmented staining in the low-density granulocytes (LDGs) of psoriasis[61]. However, our results show that NE is down-

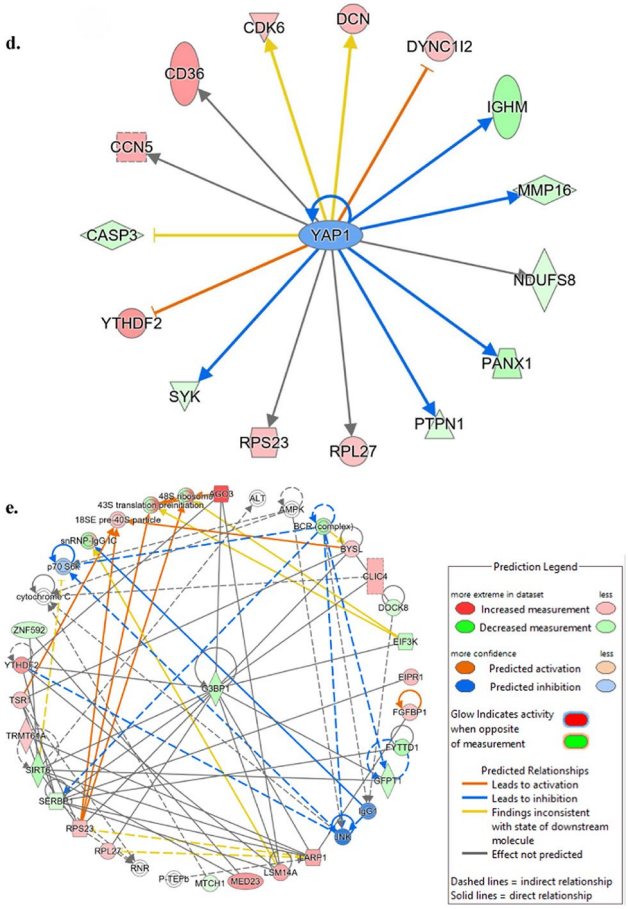


Figure 5. (continued)

regulated in sPV. Moreover, SLPI is a reversible NE inhibitor that dynamically controls NE activity. Although SLPI expression decreased in sPV, there was no statistical difference between the two groups. These results indicate that NE has a pleiotropic feature; that is, it has not only antibacterial effects and promotes proinflammatory but also impairs innate immunity[60].

In our research, there are sex differences in patients between the two groups. Several studies have indicated that women have less severe psoriasis than men[62,63] which may be caused by differences in protein molecules, such as estradiol hormones[64], but in most regions, women and men are affected equally[65]. Therefore, whether gender affects disease severity and proteome difference in patients with psoriasis needs further study.

Limitations of the study: (1) The sample size was relatively small which may limit the generalizability of the results. (2) The detection limits of the DIA method, for example, DIA did not detect some low-abundance proteins, as we discussed in our previous study. (3) There are sex differences in patients between the two groups, which may affect the proteomic profiles (Table 1). (4) Potential biases in patient selection. (5) The selection criteria for DEPs is loose, which could potentially invalidate the results of pathway and PPI analysis. (6) The validation analysis by 4D PRM is not an orthogonal method (for example, western blot) to DIA assay. These confounding factors might affect the results.

Materials and methods

Patients and samples

This study enrolled patients with moderate ($5 < PASI \leq 15$) to severe psoriasis ($PASI > 15$) from June 2020 to February 2024 in the Dermatology Hospital of Zhejiang Province. Patients were not receiving treatment at the time of enrollment or receiving treatment other than systematic immunosuppressants or combined with phototherapy. Participants were assessed and sampled at baseline. The 12 samples for PRM validation in our study came from another independent cohort. Other inclusion and exclusion criteria of the patients, and strategies for evaluating disease severity have been formulated in our previous studies[27]. The study was approved by ethical committees at the Dermatology Hospital of Zhejiang Province (LL-2020-15) and performed according to the Declaration of Helsinki. Patients provided written informed consent before sampling. We used a biopsy punch with a diameter of 4 mm for sampling lesional (LS) plaques. The skin specimens were placed in 5-mL Eppendorf tubes, immediately frozen in liquid nitrogen for 5–10 min, and stored at −80 °C.

Proteomic data acquisition

DIA analysis

After sample preparation and liquid nitrogen grinding + SDT lysis, 1ug of peptides was collected from each sample, iRT peptides mixed lagging samples, separated by nano-LC and analysed by online electrospray tandem mass spectrometry. The whole liquid mass series system was as follows: (1) liquid phase system: Easy nLC system (Thermo Fisher Scientific); and (2) mass spectrometry system: Orbitrap Exploris 480 (Thermo Fisher Scientific).

DIA data analysis

Data from DIA were processed and analysed by Spectronaut 14.6 (Biognosys AG, Switzerland) with default settings, and the retention time prediction type was set to dynamic iRT. Data extraction was determined by Spectronaut X based on extensive mass calibration. Spectronaut 14.6 will dynamically determine the ideal extraction window depending on iRT calibration and gradient stability. The Q value (FDR) cut-off at the precursor and protein levels was 1%. Decoy generation was set to mutate, similar to scrambled but applying only a random number of AA position swamps (min = 2, max = length/2). All selected precursors passing the filters were used for quantification. MS2 interference removes all interfering fragment ions except the three least interfering ones. The average top 3 filtered peptides that passed the 1% Q value cut-off were used to calculate the significant group quantities.

Quality control of proteome data

DIA analysis of 15 samples was performed using the database constructed by deep DIA[27]. To ensure the accuracy of the quantification for the results obtained, we first normalized the DIA results, which excluded systematic errors to some extent (see Supplementary Fig. S1 online). The CV value interval statistics and sample consistency assessments were then performed. The quality of proteomic data was ensured at multiple levels.

4D-PRM

0.2ug peptide sample were analyzed on nanoElute (Bruker, Bremen, Germany) coupled to a timsTOF Pro (Bruker, Bremen, Germany) equipped with a CaptiveSpray source. Peptides were separated on a 25 cm × 75 μm analytical column, 1.6 μm C18 beads with a packed emitter tip (IonOpticks, Australia). The timsTOF Pro (Bruker, Bremen, Germany) was operated in PRM-PASEF mode. The detection ion mode is positive, the scanning range of the parent ion is 100–1700 m/z, the range of ion mobility 1/K0 is 0.6–1.6 V·s/cm2, the Accu time and Ramp time is 50 ms, the Lock Duty Cycle is 100%, the Capillary Voltage is 1500 V, the Dry Gas speed is 3 L/min, the Dry Temp is 180 °C. The charge range is 0–5, and the CID collision energy is 10 eV.

Bioinformatic analysis

GO analysis of the DEPs was analyzed in the GO database and displayed by Cytoscape. Pearson correlation was used to show the correlation between DEPs and clinical severity (PASI). Circus plot was generated using the circle package in R language (version 4.0.1)[66]. The KEGG database was used for pathway analysis. The STRING database performed the interaction network among proteins. Upstream regulator analysis (URA) and interaction analysis were implemented by IPA (Version 24.0.1) (with $P < 0.05$, Z score > 0 or < 0)[67].

Statistical analysis

Student's t test was performed for pair of groups to be compared, and p-value < 0.05 was defined as statistically significant, according to published studies[25,68]. The selection criteria of the DEPs for bioinformatic analysis were cut off with $P < 0.05$ and FC ≥ 1.5[12]. Statistical analysis was performed in R software (version 4.0.1).

Conclusions

In conclusion, this study presents a proteomic difference between sPV and mPV in psoriatic lesions. These proteins comprise AMPs, serine protease, chaperone protein, co-receptor and other molecules. At the same time, they were associated with the IL – 17 signalling pathway, Neutrophil extracellular trap formation, Neutrophil degranulation, NF – kappa B signalling pathway, and defence response to a bacterium, which is closely associated with psoriasis pathogenesis. Our data identified eighteen DEPs as biomarkers of disease severity, and it suggested that targeting these molecules and preventing infection may improve the severity and therapeutic efficacy of psoriasis.

Data availability

The data are available from the corresponding author on reasonable request. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (https://proteomecentral.proteomexchange.org) via the iProX partner repository with the dataset identifier PXD052886.

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Author contributions
L.L.W. and C.C. contributed equally as co-first authors to the study's investigation and the writing of the original draft. B.B.X. and N.N.S. conducted the investigation. L.H.H and J.H. were responsible for resource acquisition. Q.D. was pivotal in conceptualization, funding acquisition, methodology development, and project administration. Q.D. and C.C. were instrumental in reviewing and editing the manuscript.All authors reviewed the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

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