DNA methylation profile of psoriatic skins from different body locations

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Aim: To understand whether the anatomical location of origin plays a role in shaping the DNA methylation (DNAm) landscape of psoriatic skins. **Patients & methods:** A number of 108 psoriatic and 57 control skin samples were grouped based on their anatomical locations. Two group t-tests were used to identify those differentially methylated sites and regions. Target region methylation loci were validated by bisulfate conversion sequencing. The correlations of DNAm with pathological features, DNAm and gene expression were also interrogated. **Results:** Our analysis revealed 315 location-specific differentially methylated sites for back, 291 for the extremities and 801 for abdomen. Moreover, we observed that the extremity-specific loci cg21942490 located on *HOXA9* is associated with hyperkeratosis. We further observed that *HOXA5* and *KIAA1949* are differential methylation regions. **Conclusion:** Our study shown evidence of anatomical location-dependent DNAm pattern in psoriasis skins, and thus provided new insights into the pathogenesis of this disease.

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Psoriasis is a chronic, immune-mediated skin disorder affecting 2–3% of the world's population. It has a higher prevalence in American and Canadian populations (4.6 and 4.7%, respectively), compared with 0.4–0.7% in African, African-American and Asian populations [1,2]. The pathogenesis of psoriasis remains incompletely understood, but there is increasing evidence indicated that aberrant DNA methylation (DNAm) patterns are one of the most likely causes, encompassing differential methylation sites (DMSs) and differential methylation regions (DMRs) [3–5].

Pathological, therapeutic and molecular variations have been noticed among psoriasis originated from different anatomical body locations, despite the relatively consistent clinical manifestation of the disease. First, the severity varies across different body locations. Specifically, in case of extremities, torsos are prone to have more severe symptoms [6,7]. Second, the location of the lesions on the body may affect the response to drugs in patients with immune diseases. There is some evidence that DMSs in psoriatic skin can revert, after phototherapy or after 1 month of anti-TNF- α therapy, to that observed in normal tissue. This observation might indirectly confirm the association of DNAm with the clinical outcome of interindividual locations [4,8]. Third, it has been previously reported that many DMRs, particular those in hypomethylated regions, have tissue-specific functions that is tightly linked to the anatomical locations that raised the disease [9–13]. Fourth, gene expression profile of psoriatic skins might be influenced by their biopsy locations [14]. These lines of findings suggest that body location may play an important role in psoriasis.

Despite previous progresses in terms of aberrant DNAm landscape in psoriasis, the potential influence of different body locations has not been examined. Furthermore, a recent study [15] on the role of DNAm in the clinical cardinal histopathological regulation of psoriasis inspired us to explore the relationship among DNAm, histopathologies and body location. We have previously obtained genome-wide DNAm data by using HumanMethylation450 BeadChip. Here, we conducted concrete association analysis among them.

Patients & methods

Sample preparation

We previously performed a genome-wide DNAm study which included 114 psoriatic skin and 62 normal skin samples [3]. Here we grouped these samples based on their anatomical locations and disease state. Some samples were excluded because they were not the entry objects of our research. All samples were collected from the Department of Dermatology, the First Affiliated Hospital, Anhui Medical University, China. The patients' clinical and histopathological information, including sex, age and Psoriasis Area and Severity Index (PASI) scores, was showed in Supplementary Tables 1 and 2.

Identifying DMSs

There are two types of probes with different hybridization chemistries for Illumina 450k methylation BeadChip (Illumina Inc, CA, USA). Such design of including both type-I and -II probes may pose certain bias if not well adjusted. To account for this, we first performed probe normalization by using BMIQ (β Mixture Quantile dilation), which is a model-based intra-array normalization strategy [16]. We then used t-test to compare the difference between groups. DMSs were considered those fulfilling the criteria of $\delta\beta > 0.1$ and Benjamini–Hochberg (BH) corrected $p < 0.001$. All statistical analyses were performed using the R package ChAMP [17].

Algorithm for detecting DMRs

The Bumhunter algorithm was used to detect DMRs. This algorithm is not based on previous DMP output. Initially, all methylation probes were separated into clusters. Each cluster contained at least seven probes, and the maximum distance between two nearby probes was set to 300 base pairs (bp). Probes from different chromosomes were separated. We then fitted a statistical model to calculate the value of bumps for each probe. Consecutive probes meeting a cut-off value of 0.05 were classified into the same region.

$$Y_{ij} = \beta_0(l_j) + \beta_1(l_j)X_j + \epsilon_{ij}$$

where i represents the subject, l_j represents the j th probe, X_j is the disease status. ϵ_{ij} is the measurement error, $\beta_0(l_j)$ is a baseline and $\beta_1(l_j)X_j$ is the parameter of interest, which is a function of probes. We assume that $\beta_1(l)$ will be equal to zero over most of the genomes, and we want to identify stretched where $\beta_1(l) \neq 0$, which we call bumps. Permutations were performed to create null distributions for the candidate regions. For each observed candidate region, we determined how many null regions were 'more extreme' (longer and higher average value). The p-value was calculated as the percent of candidate regions obtained from the permutations that are as extreme as the observed region. Family-wise error rate (FWER) was the proportion of permutations that had at least one region as extreme as the observed region. We also computed the p-value and FWER for the area of the regions. The result of Bumhunter analysis contained all the DMRs detected, with their length, clusters and number of CpGs annotated.

Gene Ontology & the Kyoto Encyclopedia of Genes & Genomes

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analyses were carried out for the differentially methylated genes between groups using R package cluster Profiler [18]. Gene sets that showed differential methylation in three different body locations were searched against a default Homo sapiens population background, and these result were matched with GO biological processes and KEGG pathways. Enrichment test was based on the hypergeometric distribution. The level of statistic significance was set to a Benjamini–Hochberg (BH) corrected $p < 0.05$.

Figure 1. Comparison of the number of differential methylation sites identified in psoriatic skin versus normal skin samples among back, extremities and abdomen.

Bisulfite sequencing

The DNA methylation level was analyzed using MethylTarget™ (Genesky Biotechnologies, Inc., Shanghai, China), a next-generation sequencing (NGS) based multiple-targeted CpG methylation analysis method. Specifically, the genomic regions of interest were analyzed and transformed to bisulfite-converted sequences by the geneCpG software. PCR primer sets were designed from bisulfate-converted DNA using the Methylation Primer software.

Genomic DNA (400 ng) was subjected to sodium bisulfite treatment using EZ DNA Methylation™-GOLD Kits (Zymo Research Corporation, CA, USA) according to the manufacturer's protocols. Libraries from different samples were quantified and pooled together, followed by sequencing on the Illumina NextSeq platform (Illumina Inc, CA, USA) according to the manufacturer's protocols. Sequencing was performed with a 2×150 bp paired-end mode. Statistical tests were performed by t-test and Analysis of Variance (ANOVA).

Results

Location-specific DMSs for psoriasis

During our genome-wide methylation analysis, we considered three pairwise comparisons: PP_{back} versus NN_{back}; PP_{extremities} versus NN_{extremities} and PP_{abdomen} versus NN_{abdomen}. At a cutoff of $p_{BH-adjusted} < 0.001$ and $\delta - \beta > 0.1$, we identified 873 DMSs for the back, 861 for the extremities and 1618 for the abdomen. Among these DMSs 247 are shared across the three comparisons (Supplementary Tables 3, 4 & 5 & Figure 1).

For illustration, we selected two most significantly hypomethylated and two hypermethylated DMSs for each body location (Figure 2). We noticed that some of these genes are involved in immune response, one of the most fundamental processes that contributes to psoriasis development. One example is the extremity-specific site cg23394673 in the body of *TNFRSF4* ($p = 1.88 \times 10^{-7}$, $\delta = 0.106$). *TNFRSF4* was regarded as a major signal source of NF- κ B involved proliferation and differentiation of keratinocytes [19,20]. Abdomen-specific hypomethylated site cg07964527 was located inside the gene body of *PDE8A*, implicating CD4⁺ T cell immunological activation processes [21,22]. However, another interesting loci cg22272840 was located in the gene body of *SOX7* ($\delta = 0.115$, $p = 1.82 \times 10^{-4}$), which is required for immune vascular development [23].

To further validate our findings, we selected four DMSs for each body location (12 in total) and carried out target region bisulfate conversion sequencing using 43 PP and 47 NN samples (back: 12 PP vs 16 NN; extremity: 14 PP vs 13 NN; abdomen: 17 PP vs 18 NN). Due to technical limitations, two DMSs failed to design target capture probes. After all, we found ten DMSs showing significant changes for the remaining loci, suggesting considerable reliability for our array results (Table 1 & Supplementary Table 6).

We next performed the functional annotation for DMS-related genes. GO analysis of variably methylated genes indicated some specific features for the back, extremities and abdomen. The biological processes 'microtubule binding and cell adhesion molecule binding' were significantly enriched in samples from the back, whereas 'actin binding and transcription co-activator activity' were enriched in the extremities. Abdomen-specific GO terms included 'TGF- β binding and phosphatidylinositol binding'. Besides location-specific GO classes, we also found the common functional terms 'growth factor binding' among the three locations, suggesting its fundamental roles for disease manifestations (Figure 3). Detailed GO results can be found in Supplementary Table 8.

Figure 2. DNA methylation level of the most significant CpG site in psoriatic skin versus normal skin samples corresponding locations. (A–C) PP is highlighted in green, NN are depicted in blue. β -value: DNA methylation levels.

Table 1. Ten location-specific differential methylation sites were validated by bisulfate conversion sequencing.								
Body location	CpG loci annotation	Target	Chr	Genome position	Distance to TSS	Average PP	Average NN	p-value
Back	cg22272840	<i>SOX7</i>	8	10,586,882	1202	0.22	0.34	3.75E-06
	cg11910652	<i>TJAP1</i>	6	43,457,275	-46	0.80	0.71	1.21E-03
	cg04883026	<i>SPTBN2</i>	11	66,487,938	932	0.35	0.46	4.77E-05
Extremities	cg23394673	<i>TNFRSF4</i>	1	1,149,212	336	0.27	0.35	4.97E-06
	cg20090283	<i>SRCIN1</i>	17	36,718,550	43,633	0.15	0.19	2.00E-02
	cg07852840	<i>SPSB4</i>	3	140,813,919	43,676	0.49	0.30	3.65E-06
Abdomen	cg02479022	<i>SLC39A14</i>	8	22,250,005	-337	0.50	0.32	4.55E-06
	cg07964527	<i>PDE8A</i>	15	85,534,864	9660	0.69	0.83	5.95E-05
	cg22729681	<i>PBX1</i>	1	164,717,625	189,029	0.49	0.35	2.06E-05
	cg01866518	<i>PXN</i>	12	120,673,236	14,728	0.64	0.50	1.42E-06

Chr: Chromosome number; NN: Normal skin; PP: Psoriatic skin; TSS: Transcription start site.

A total of 247 common-shared sites were identified among the three locations, 58.2% (144) of which overlapped with DMSs identified in previous publications [3]. The top 25 sites are listed in Table 2. The 247 DMSs uniquely mapped to 178 genes, 146 of which were located in the gene body (Figure 1 & Supplementary Table 7). In addition, KEGG pathway analysis showed that the 178 genes are significantly enriched in ‘regulation of actin cytoskeleton’ and ‘proteoglycans in cancer’ (Supplementary Table 8).

Figure 3. Gene Ontology comparative analysis with differentially methylated genes across the three locations. Each circle is colored in accordance with p-value. Size of a circle as a proportional adjustment to geneRatio size. The abscissa of each circle corresponds to the differential methylation gene, and the ordinate corresponds to the relevant gene functional region.

Histopathological features related to location-specific DMSs

We performed pathological examination of all the 108 PP samples, and collected data pertaining to physiological features, including hyperkeratosis, Munro's microabscess, parakeratosis, elongation of rete pegs, focal hypergranulosis, thinning of granulos, thickening of acanthosis and vascular dilatation and congestion. We compared PP samples with and without histopathological features and found that the extremities are more prone to hyperkeratosis than the back and abdomen (Table 3). No significant DMSs were identified between hyperkeratosis-present and -absent groups. We further investigated whether the histopathological features affected location-specific DMSs among the three body locations, and observed hyperkeratosis was closely related to some extremity-specific DMSs, suggesting a contribution to body location differences in psoriasis. For example, the methylation level of cg21942490 in extremities is significantly lower than in the back or abdomen. This locus is located in 1500 bp upstream of the *HOXA9* transcription start site, possibly indicating variable roles of *HOXA9* across different anatomical body locations. We also found some loci of the *HOX* family genes displaying methylation changes in the PP_{extremity} versus PP_{back} and PP_{extremity} versus PP_{abdomen} comparisons, further suggesting the contribution of the *HOX* family to hyperkeratosis. By integrating our previous RNA-seq dataset of 20 samples: 4 extremities, 11 back and 5 abdomen, we found that *HOXB9* and *HOXD4* were more downregulated in the extremity than in the abdomen or back, even in such a small sample (Table 4). However, we did not find significant differences from the paired analysis of PASI scores among

Table 2. Top 25 unique mapped genes identified among back, extremities and abdomen.

DMS	Chr	Gene [†]	Context [‡]	Back		Extremities		Abdomen	
				p-value [§]	β [¶]	p-value [§]	β [¶]	p-value [§]	β [¶]
cg22724943	1	MAN1C1	Body	8.31E-06	-0.137	2.85E-08	-0.170	1.28E-04	-0.153
cg04930596	12	NCOR2	Body	4.46E-04	-0.102	3.86E-08	-0.155	5.22E-04	-0.137
cg01678309	15	CORO2B	Body	2.26E-05	-0.114	3.89E-08	-0.130	2.50E-04	-0.119
cg13855435	17	YPEL2	Body	1.97E-04	-0.119	3.89E-08	-0.150	6.83E-04	-0.131
cg09490124	7	PUS7	Body	2.00E-04	-0.110	6.42E-08	-0.143	2.07E-04	-0.145
cg12636499	10	SH3PXD2A	Body	4.60E-05	-0.118	7.73E-08	-0.152	1.16E-04	-0.160
cg14541011	9	RALGDS	Body	1.06E-04	-0.143	7.73E-08	-0.185	6.25E-04	-0.155
cg10926487	17	TIMP2	Body	4.65E-04	-0.102	8.09E-08	-0.148	3.19E-04	-0.134
cg13086983	1	ECE1	Body	1.18E-05	-0.142	8.61E-08	-0.156	1.43E-04	-0.156
cg18735015	10	SH3PXD2A	Body	3.08E-04	-0.112	8.61E-08	-0.172	3.75E-04	-0.146
cg22330763	6	SLC29A1	5'UTR	2.94E-04	-0.111	8.61E-08	-0.162	2.77E-04	-0.144
cg01912455	22	SMARCB1	TSS1500	3.95E-05	-0.126	9.54E-08	-0.146	1.77E-04	-0.159
cg02442900	2	DGKD	Body	9.48E-06	-0.114	9.54E-08	-0.125	1.50E-04	-0.127
cg03052794	19	C19orf22	Body	1.51E-05	0.110	9.54E-08	0.120	1.87E-04	0.109
cg17825194	12	NCOR2	Body	1.15E-04	-0.101	9.54E-08	-0.139	3.99E-04	-0.130
cg23601468	22	SYN3	Body	1.75E-05	-0.133	9.54E-08	-0.140	1.49E-04	-0.146
cg24216770	20	DLGAP4	Body	5.60E-05	-0.133	9.54E-08	-0.146	2.78E-04	-0.143
cg10863207	17	RFFL	5'UTR	2.38E-05	0.161	1.00E-07	0.199	3.08E-04	0.161
cg02550308	16	SNTB2	Body	1.36E-04	-0.117	1.09E-07	-0.135	5.66E-04	-0.119
cg04099543	11	P2RY6	5'UTR	9.10E-05	0.122	1.19E-07	0.155	3.33E-04	0.136
cg11829253	15	PSTPIP1	Body	1.57E-04	-0.110	1.26E-07	-0.123	4.14E-04	-0.110
cg12196406	19	FXVD1	TSS200	4.53E-04	-0.101	1.26E-07	-0.127	1.10E-04	-0.136
cg11826961	17	THRA	5'UTR	1.52E-04	-0.116	1.27E-07	-0.145	3.16E-04	-0.147
cg05049448	7	SNX8	Body	3.67E-04	-0.115	1.32E-07	-0.153	4.83E-04	-0.135
cg00288598	8	EIF2C2	Body	4.63E-05	0.182	1.34E-07	0.213	2.69E-04	0.173

[†]Gene: gene name, according to the HUGO Gene Nomenclature Committee.

[‡]Context: location of the gene-associated CpG-site(s) with respect to the gene context.

[§]Adjusted p-value: t-test p-values adjusted for Benjamini-Hochberg corrections.

[¶]β: methylation differences between the cases and controls.

Chr: Chromosome number; DMS: Differentially methylated site.

Table 3. Comparative analysis of pathological features among the three locations.

Physiology features	Back		Extremities		Abdomen		p-values: statistic significance		
	Present	Absent	Present	Absent	Present	Absent	Back vs extremities	Back vs abdomen	Extremities vs abdomen
Hyperkeratosis	25	7	36	2	29	9	0.038	0.85	0.022
Parakeratosis	23	9	35	3	31	7	0.025	0.33	0.17
Munro microabscess	12	20	9	29	8	30	0.21	0.13	0.78
Elongation of rete pegs	14	18	11	27	16	22	0.19	0.89	0.23
Focal hypergranulosis	0	32	1	37	1	37	N/A	N/A	N/A
Granulosa thinning	5	27	10	28	10	28	0.28	0.28	1
Acanthosis	23	9	31	7	25	13	0.33	0.58	0.11
Vascular dilatation and congestion	25	7	34	4	33	5	0.19	0.33	0.72

p-values: χ^2 test were analyzed by R package.

different body locations.

Table 4. *Hox* family genes and extremity-specific differentially methylated site associated with hyperkeratosis.

Gene name	CpG locus	Features	Methylation				Expression			
			Extremity (X ± S)	Back (X ± S)	Abdomen (X ± S)	P ^{Extremity vs back}	Extremity (X ± S)	Back (X ± S)	Abdomen (X ± S)	P ^{Extremity vs back}
<i>HOXA11</i>	cg05977669	Body	0.324 ± 0.056	0.396 ± 0.061	0.401 ± 0.046	1.56E-05	0.51 ± 0.25	0.25 ± 0.15	0.21 ± 0.11	0.118
<i>HOXA10</i>	cg14188840	TSS1500	0.797 ± 0.036	0.851 ± 0.032	0.846 ± 0.041	1.81E-07	19.75 ± 3.0	15.91 ± 5.82	15.83 ± 1.97	0.094
<i>HOXB13</i>	cg08594218	3'UTR	0.186 ± 0.082	0.116 ± 0.036	0.121 ± 0.065	2.08E-04	0.09 ± 0.08	0.03 ± 0.03	0 ± 0.01	0.205
<i>HOXB9</i>	cg00846995	Body	0.190 ± 0.052	0.239 ± 0.044	0.242 ± 0.060	5.78E-05	0.16 ± 0.06	0.12 ± 0.07	0.24 ± 0.07	0.284
<i>HOXA2</i>	cg00188704	Body	0.832 ± 0.059	0.782 ± 0.063	0.774 ± 0.076	2.84E-03	0.15 ± 0.18	0.26 ± 0.31	0.21 ± 0.28	0.421
<i>HOXD4</i>	cg01152019	TSS1500	0.516 ± 0.074	0.608 ± 0.058	0.585 ± 0.091	2.79E-06	0.71 ± 0.51	2.32 ± 1.94	1.76 ± 0.74	0.032
<i>HOXA9</i>	cg21942490	TSS1500	0.196 ± 0.052	0.290 ± 0.112	0.292 ± 0.102	5.05E-05	19.75 ± 3.0	15.91 ± 5.82	15.83 ± 1.97	0.094

p-values: t-tests were analyzed by R package.
X ± S: Mean ± standard deviation.

Figure 4. DNAm level distribution of the CpG sites for two common genes identified in the two most significant shared differentially methylated regions (DMR1 and DMR2) across the three locations. The y-axis denotes the DNA methylation level and the x-axis is the CpG sites in *HOXA5*, and the green dot represents PP, NN are depicted in red. Blue and pink solid wavy lines represent PP mean and UC mean, respectively. PP loess and UC loess are depicted as brownish green and yellow, respectively. These CpG loci correspond to the gene region below the abscissa, whereas the color of each gene region is consistent with the corresponding annotation in the upper right corner. (Light blue: 1st Exon; light green: 5'UTR; Red: TSS1500; dark blue: TSS200; pink: island). The description of *KIAA1949* gene is almost identical to the characterization of the above *HOXA5* gene. DMR: Differentially methylated region; PP: Psoriatic skin.

Differences in DMRs between extremities, back & abdomen in psoriasis

Finally, to explore whether the region of methylation affected psoriasis, we used the Bumhunter algorithm to detect DMRs in different locations. A total of 457 DMRs were identified, of which 108 (23.6%) mapped to the back areas, whereas the remaining 198 (43.2%) and 151 (33.0%) mapped to the abdomen and extremities, respectively. There were 34 common DMRs present at all three locations (Supplementary Table 9).

Here we showed the two most representative DMRs ($p < 1.86 \times 10^{-5}$) that overlap *HOXA5* and *KIAA1949*. Specifically, the *HOXA5* DMRs are hypermethylated, whereas the *KIAA1949* DMRs are hypomethylated, in PP compared with NN (Figure 4). The starting and ending point of *HOXA5* DMRs ranged from 27,183,196 to 2,718,4821 on chromosome 7, which contained 35 CG sites; while the starting and ending point of *KIAA1949* DMRs ranged from 30,652,202 to 30,652,829 on chromosome 6, which included 11 CG sites. Both these DMRs are related to gene function and development. After annotating the shared *HOXA5* DMRs harboring DMSs, we found that about 70% of CpGs were located on CpG islands and TSS1500, whereas the CpGs of *KIAA1949* DMRs were located in non-CGI regions and gene body (Figure 4). Furthermore, our result indicate that the expression of *HOXA5* ($p = 6.55 \times 10^{-2}$) and *KIAA1949* was not significant.

Discussion

Our previous genome-wide methylation study identified strong associations between nine CpG loci and psoriasis [3]. Here for the first time, we separately analyzed the genome-wide DNAm profiles of skin samples from the three anatomical body locations, that is, back, extremities and abdomen. We focused on the disparate locations of the psoriatic plaques, because psoriatic lesions in disparate locations of psoriasis show different severity and differ in response to treatment, a phenomenon that may be associated with abnormal DNA methylation [8,14]. Because DNAm variation affects the expression of genes or nearby genes with positive or negative correlations, DNAm has become a research hotspot in the pathogenesis of psoriasis [3,24].

We observed extensive differences in DNAm in PP skin compared with NN across three somatic locations and also found that 178 shared unique mapped sites were strongly associated with psoriasis. However, these sites mainly occurred within the gene body and not in the promoters, indicating that these loci might be involved in the regulation of gene expression and related to tissue or cell development discrepancies previously identified [11,12,25,26]. The differences in DNAm are highly specific among distinct locations, further molding specific function pattern of these differentiated tissues. About 42% of the common-shared sites we identified differ from the original data because of our small sample sizes and methodological discrepancies as well as the incorporation of data from heterogeneous locations.

We performed GO analysis on differentially methylated genes in each location and found the same hypermethylated gene functional enrichment region annotated with ‘growth factor binding’ as well as specific regions. Growth factors closely related to excessive hyperproliferation and differentiation of psoriatic keratinocytes and blood vessels, such as VEGF [27], IGF I [28], FGF [29,30], NGF-regulating neuropeptides and angiogenesis [31], were encoded by hypermethylated genes and almost exclusively secreted by inflammatory T-cell-dominated psoriatic pathogenesis [32]. The results were similar to those previously reported by Park *et al.* [33], indicating that the hypermethylation pattern of CD4⁺ T cells in psoriatic lesions caused epidermal and vascular proliferation of psoriatic lesions. It appears that these enriched hypermethylated genes may be involved in the regulation of growth factor expression in psoriasis. Meanwhile, we also identified specific processes among them. Differences in GO analysis results at each location may be related to differences in gene expression, further triggering a variation in inflammation. Furthermore, we found specific hypomethylated gene enrichment in the back, but not in the limbs and abdomen, suggesting that these specifically enriched genes can be considered as location-specific markers. Consistent with previous findings, hypomethylated genes are more likely to be linked to tissue-specific functions [12]. From the results of our study, it appears that shared biological pathways connected with development were enriched in different locations of psoriasis.

The location-specific methylation genes-*SOX7* in the back, *TNFRSF4* in the extremities and *PDE8A* in the abdomen – could be used as specific markers for functional annotations, such as enhancers, DNase I hypersensitivity sites and transcription factors, and used in comparison studies to further understand the pathogenesis of psoriasis in different body locations. Previous study has shown that the *SOX7* is expressed specifically in endothelial cells of vessel, providing positive feedback to VEGF signaling via the mTOR pathway [23]. *TNFRSF4*, known as *OX40* in CD4⁺ T cells, is motivated by the NF- κ B signaling pathway to increase the expression of antiapoptotic molecules, whereas NF- κ B signaling pathway is closely associated with epidermal proliferation and differentiation [20,34]. The abdomen-specific *PDE8A* acted as a potential drug target gene regulating CD4⁺ T cell function via the *PDE8A-Raf-1* kinase signaling pathway [35]. These results show that efforts to develop and test inhibitors of these location-specific gene pathways could provide a novel and efficient way to treat psoriasis.

The DNAm profile varies between different anatomical locations, possibly because the infiltration of inflammatory cells in psoriatic lesions and different pathological features change tissue components and DNAm [12,36]. Previous study has shown that DNAm and pivotal pathogenic genes might be involved in regulating the cardinal histopathological features detected in psoriasis [15]. Although significant DMSs were not observed between PP and NN at the same locations with or without pathological characteristics, we found extremity-specific DMSs associated with hyperkeratosis among the three locations. Both are caused due to comparative analysis by choosing PP versus NN or other confounding factors, such as cellular heterogeneity, interindividual discrepancy and the limited presence of conspicuous pathological characteristics. Furthermore, the detection of some *HOX* family gene loci related to hyperkeratosis as well as the extremity-specific differential methylation site cg21942490 located on *HOXA9* further indicate that *HOX* family gene DMSs mediated unique psoriatic pathological characteristics at different locations. Our gene expression analyses showed that *HOXB9* and *HOXD4* were downregulated in the

extremities. These data indicate that the expression of the *HOX9* paralogues *HOXA9* and *HOXB9* is related to FGFs in a limb-specific manner [37–39]. We observed no significant differences among PASI scores in these locations, which could be related to a decreased severity of psoriatic lesions. However, a recent study found that T lymphocyte infiltration can affect psoriatic epidermal differentiation and proliferation, and change the composition of each layer [36]. Some defects in the PASI score and histopathology of our original samples made it infeasible to further explore the correlation between DNAm and lymphocyte infiltration level from different locations. In future, we intend to pursue further research using appropriate samples.

Furthermore, we identified the most common *HOXA5* DMRs and *KIAA1949* DMRs. *HOXA5* is a member of the *HOX* gene family, which encodes a transcription factor controlling development and differentiation, which specifies regional differences along the anterior–posterior axis [40,41]. We found that *HOXA5* is a hypermethylated gene in PP versus NN, as found in previous study [42]. However, the *HOXA5* expression that we observed was inconsistent with that reported by Li B team [43], probably demonstrating the limitations imposed by our small sample size. Moreover, *HOXA5* is also underexpressed in some human tumors, including gastric cancer [44], colorectal cancer [45], breast cancer [46], liposarcomas [47] and, most notably, chronic myeloid leukemia [48], in which *HOXA5* hypermethylation was the target of gene inactivation. In addition, the presence of *HOXA5*-induced apoptosis indicates that the *HOXA5* gene may have the ability to activate antagonist genes against the release of growth factors, which mainly implicates inhibition of epidermal formation and keratinocyte differentiation [49].

As a novel candidate hypomethylated gene implicated in psoriasis, the *KIAA1949* encoded phosphatase 1 F-actin cytoskeleton targeting subunit protein. Previous studies have shown that *KIAA1949*, which is located between the *HLA-C* and *HLA-E* gene clusters on human chromosome 6, associates with protein phosphatase 1 capable of modulating actin dynamics [50,51]. The role of *KIAA1949* in the pathogenesis of psoriasis has not yet been reported. Therefore, the *KIAA1949* gene expression in our study is not significant, but its expression is low in breast cancer [52]. Our results indicate that the differences in DMRs at different body locations are involved in the pathogenesis of psoriasis.

Conclusion

We have shown that location-specific and location-shared DMSs and DMRs contribute to our understanding of psoriatic pathogenesis. Although the DNAm and pathological data that we collected had some limitations, our results, validated using external datasets, suggest the presence of a number of location-specific DMSs that are associated with psoriatic histopathological features. In particular, the *HOX* family genes are closely correlated with hyperkeratosis, with *HOXA9* being specific to the extremities. DMRs analysis among the three locations identified the *HOXA5* and *KIAA1949* regions as potentially vital to the modulation of keratinocyte development in psoriasis. We were also able to show that growth factors contribute to psoriatic pathological changes. Identification of the common and location-special genes involved in regulation of psoriatic pathogenesis may be valuable in developing drugs for the better treatment of psoriasis. Our results need to be further developed using a larger sample size and additional pathological *HOX* family characteristics.

Future perspective

The data analysis and functional studies of DMSs and DMRs are particularly important in the investigation of psoriasis in different locations. By analyzing the histopathology of DMSs and DMRs in psoriasis in different locations, we identified the similarities and differences among the different locations. These similarities and differences will help in deepening our understanding of the pathogenesis of psoriasis and could provide a basis for the development of new drugs. Further research should focus on the mechanism by which the *HOX* family gene plays a regulatory role in psoriatic pathogenesis in different locations. This can be achieved by increasing the sample size and using more appropriate pathological tissues, for example, in PP versus PN. It should then be possible to select multiple overlapped *HOX* genes with differential expression among the three locations and perform pathway and functional analysis. These location-specific and location-shared differential methylation gene function and pathways could potentially contribute to the development of tailored responses to highly targeted agents.

Author contributions

F Zhou, X Zhang and M Wu conceived and designed the study. M Wu, F Zhou, X Li, C Zhang, C Zhang, D Qian, J Ma, M Cai, L Tang, H Cheng, C Shen and G Chen accomplished the acquisition of data and verification experiment. F Zhou, M Wu and X Zheng

processed the data and performed the statistical analyses. M Wu and F Zhou drafted the manuscript. All authors reviewed the results and contributed to the final manuscript.

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Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

Written informed consent was obtained from all of the subjects, under an Anhui Medical University approved protocol, and the study was performed according to the Declaration of Helsinki principles.

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Availability of data & materials

The epigenome-wide data generated and analyzed during the current study are available from the corresponding author on reasonable request. The raw data had been submitted to Gene Expression Omnibus under accession GSE73894.

Summary points

- We revealed 315, 291 and 801 location-specific differentially methylated sites (DMS) for back, extremities and abdomen, respectively.
- We confirmed the reliability of these location-specific DMSs by bisulfite sequencing, such as *SOX7* in the back, *TNFRSF4* in the extremities and *PDE8A* in the abdomen.
- These location-specific methylated genes were significantly enriched in 'cell adhesion molecule binding', 'transcription coactivator activity' and 'phosphatidylinositol binding'.
- We also found 178 location-common differentially methylated genes and referred genes were enriched in 'growth factor binding' as implicated by Gene Ontology analysis.
- Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis showed that 178 unique-mapped genes were significantly enriched in 'regulation of actin cytoskeleton' and 'proteoglycans in cancer'.
- We observed that hyperkeratosis is tightly related to some extremity-specific DMSs, especially for the extremity-specific differential methylation site cg21942490 located on *HOXA9*, possibly indicating variable roles of *HOXA9* across different anatomical body regions.
- We further observed 457 differential methylation regions, in which 23.6, 43.2 and 33.0% were mapped to back, abdomen and extremities, respectively. *HOXA5* and *KIAA1949* are the top overlapped differential methylation regions.
- *HOXB9* and *HOXD4* were more downregulated in the extremity than in the abdomen or back, but the expression of *HOXA5* and *KIAA1949* gene was not significant.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Perera GK, Di Meglio P, Nestle FO. Psoriasis. *Annu. Rev. Pathol.* 7, 385–422 (2012).
 2. Parisi R, Symmons DP, Griffiths CE, Ashcroft DM. Global epidemiology of psoriasis: a systematic review of incidence and prevalence. *J. Invest. Dermatol.* 133(2), 377–385 (2013).
 3. Zhou F, Wang W, Shen C *et al.* Epigenome-wide association analysis identified nine skin DNA methylation loci for psoriasis. *J. Invest. Dermatol.* 136(4), 779–787 (2016).
- **Allows to understand the differences in DNA methylation (DNAm) levels and their expression associations in patients with psoriasis from the perspective of genome-wide level.**

4. Roberson ED, Liu Y, Ryan C *et al.* A subset of methylated CpG sites differentiate psoriatic from normal skin. *J. Invest. Dermatol.* 132(3 Pt 1), 583–592 (2012).
5. Zhang P, Zhao M, Liang G *et al.* Whole-genome DNA methylation in skin lesions from patients with psoriasis vulgaris. *J. Autoimmun.* 41, 17–24 (2013).
- **Allows to understand differentially methylated regions and their function in patients with psoriasis from the perspective of genome-wide DNAm patterns.**
6. Chen K, Wang G, Jin H *et al.* Clinic characteristics of psoriasis in china: a nationwide survey in over 12000 patients. *Oncotarget* 8(28), 46381–46389 (2017).
7. Hagg D, Sundstrom A, Eriksson M, Schmitt-Egenolf M. Severity of psoriasis differs between men and women: a study of the clinical outcome measure psoriasis area and severity index (PASI) in 5438 Swedish register patients. *Am. J. Clin. Dermatol.* 18(4), 583–590 (2017).
8. Gu X, Nylander E, Coates PJ, Fahraeus R, Nylander K. Correlation between reversal of DNA methylation and clinical symptoms in psoriatic epidermis following narrow-band UVB phototherapy. *J. Invest. Dermatol.* 135(8), 2077–2083 (2015).
- **Allows to learn that DNAm pattern was reversed at the end of narrow-band UVB phototherapy in psoriatic epidermis showing excellent clinical improvement.**
9. Ai R, Hammaker D, Boyle DL *et al.* Joint-specific DNA methylation and transcriptome signatures in rheumatoid arthritis identify distinct pathogenic processes. *Nat. Commun.* 7, 11849 (2016).
- **The report shows differences in biological pathways and DNA signatures between rheumatoid arthritis knee and hip fibroblast-like synoviocytes.**
10. Schultz MD, He Y, Whitaker JW *et al.* Human body epigenome maps reveal noncanonical DNA methylation variation. *Nature* 523(7559), 212–216 (2015).
11. Deaton AM, Webb S, Kerr ARW *et al.* Cell type-specific DNA methylation at intragenic CpG islands in the immune system. *Genome Res.* 21(7), 1074–1086 (2011).
12. Lokk K, Modhukur V, Rajashekar B *et al.* DNA methylome profiling of human tissues identifies global and tissue-specific methylation patterns. *Genome Biol.* 15(4), r54 (2014).
13. Zhang B, Zhou Y, Lin N *et al.* Functional DNA methylation differences between tissues, cell types, and across individuals discovered using the M&M algorithm. *Genome Res.* 23(9), 1522–1540 (2013).
- **Allows to learn that there were no differences in gene expression profile among the locations of psoriatic plaques. While the gene expression profiles of involved psoriatic skin correlated with psoriasis area and severity index score.**
14. Quekenborn-Trinquet V, Fogel P, Aldana-Jamayrac O *et al.* Gene expression profiles in psoriasis: analysis of impact of body site location and clinical severity. *Br. J. Dermatol.* 152(3), 489–504 (2005).
- **Makes realize that there were differences in the DNAm distribution and function of different tissues or cells in human body.**
15. Chandra A, Senapati S, Roy S, Chatterjee G, Chatterjee R. Epigenome-wide DNA methylation regulates cardinal pathological features of psoriasis. *Clin. Epigenetics* 10(1), 108 (2018).
16. Teschendorff AE, Marabita F, Lechner M *et al.* A β -mixture quantile normalization method for correcting probe design bias in Illumina Infinium 450 k DNA methylation data. *Bioinformatics* 29(2), 189–196 (2013).
17. Morris TJ, Butcher LM, Feber A *et al.* Champ: 450 k chip analysis methylation pipeline. *Bioinformatics* 30(3), 428–430 (2014).
18. Gentleman RC, Carey VJ, Bates DM *et al.* Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol.* 5(10), R80 (2004).
19. Croft M. The role of tnfr superfamily members in T-cell function and diseases. *Nat. Rev. Immunol.* 9(4), 271–285 (2009).
20. Kaufman CK, Fuchs E. It's got you covered. NF- κ B in the epidermis. *J. Cell Biol.* 149(5), 999–1004 (2000).
21. Dong H, Osmanova V, Epstein PM, Brocke S. Phosphodiesterase 8 (PDE8) regulates chemotaxis of activated lymphocytes. *Biochem. Biophys. Res. Commun.* 345(2), 713–719 (2006).
22. Vang AG, Ben-Sasson SZ, Dong H *et al.* PDE8 regulates rapid TEFF cell adhesion and proliferation independent of ICER. *PLoS ONE* 5(8), e12011 (2010).
23. Kim K, Kim IK, Yang JM *et al.* Sox1 transcription factors are positive feedback regulators of VEGF signaling. *Circ. Res.* 119(7), 839–852 (2016).
24. Izzi B, Pistoni M, Cludts K *et al.* Allele-specific DNA methylation reinforces PEAR1 enhancer activity. *Blood* 128(7), 1003–1012 (2016).
25. Maunakea AK, Nagarajan RP, Bilenky M *et al.* Conserved role of intragenic DNA methylation in regulating alternative promoters. *Nature* 466(7303), 253–257 (2010).
26. Baribault C, Ehrlich KC, Ponnaluri VKC *et al.* Developmentally linked human DNA hypermethylation is associated with down-modulation, repression, and upregulation of transcription. *Epigenetics* 13(3), 275–289 (2018).
27. Vegfors J, Ekman AK, Stoll SW, Bivik Eding C, Enerback C. Psoriasis (S100A7) promotes stress-induced angiogenesis. *Br. J. Dermatol.* 175(6), 1263–1273 (2016).

28. Wraight CJ, White PJ, Mckean SC *et al.* Reversal of epidermal hyperproliferation in psoriasis by insulin-like growth factor I receptor antisense oligonucleotides. *Nat. Biotechnol.* 18(5), 521–526 (2000).
29. Kovacs D, Falchi M, Cardinali G *et al.* Immunohistochemical analysis of keratinocyte growth factor and fibroblast growth factor 10 expression in psoriasis. *Exp. Dermatol.* 14(2), 130–137 (2005).
30. Cheng H, Xu M, Liu X *et al.* TWEAK/Fn14 activation induces keratinocyte proliferation under psoriatic inflammation. *Exp. Dermatol.* 25(1), 32–37 (2016).
31. Raychaudhuri SK, Raychaudhuri SP. NGF and its receptor system: a new dimension in the pathogenesis of psoriasis and psoriatic arthritis. *Ann. N Y Acad. Sci.* 1173, 470–477 (2009).
32. Deng Y, Chang C, Lu Q. The inflammatory response in psoriasis: a comprehensive review. *Clin. Rev. Allergy Immunol.* 50(3), 377–389 (2016).
33. Park GT, Han J, Park SG, Kim S, Kim TY. DNA methylation analysis of CD4⁺ T cells in patients with psoriasis. *Arch. Dermatol. Res.* 306(3), 259–268 (2014).
34. Rogers PR, Song J, Gramaglia I, Killeen N, Croft M. Ox40 promotes BCL-XL and BCL-2 expression and is essential for long-term survival of CD4 T cells. *Immunity* 15(3), 445–455 (2001).
35. Basole CP, Nguyen RK, Lamothe K *et al.* PDE8 controls CD4(+) T cell motility through the PDE8A-RAF-1 kinase signaling complex. *Cell Signal.* 40, 62–72 (2017).
36. Lorthois I, Simard M, Morin S, Pouliot R. Infiltration of T cells into a three-dimensional psoriatic skin model mimics pathological key features. *Int. J. Mol. Sci.* (2019). <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6479293/>
37. Cohn MJ, Patel K, Krumlauf R *et al.* HOX9 genes and vertebrate limb specification. *Nature* 387(6628), 97–101 (1997).
38. Xu B, Wellik DM. Axial HOX9 activity establishes the posterior field in the developing forelimb. *Proc. Natl Acad. Sci. USA* 108(12), 4888–4891 (2011).
39. Raines AM, Magella B, Adam M, Potter SS. Key pathways regulated by HOXA9,10,11/HOXD9,10,11 during limb development. *BMC. Dev. Biol.* (2015). <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4506574/>
40. Sinha P, Singh K, Sachan M. High resolution methylation analysis of the HOXA5 regulatory region in different somatic tissues of laboratory mouse during development. *Gene. Expr. Patterns* 23–24 (2017). 59–69
41. Morgan BA. HOX genes and embryonic development. *Poult. Sci.* 76(1), 96–104 (1997).
42. Nobeyama Y, Umezawa Y, Nakagawa H. Silencing of homeobox A5 gene in the stratum corneum of psoriasis. *Exp. Dermatol.* 26(11), 1068–1074 (2017).
- **Allows to learn that there were differences in methylation and expression of *HOXA5* gene in the psoriatic stage of epidermal maturation.**
43. Li B, Tsoi LC, Swindell WR *et al.* Transcriptome analysis of psoriasis in a large case-control sample: RNA-seq provides insights into disease mechanisms. *J. Invest. Dermatol.* 134(7), 1828–1838 (2014).
44. Peng X, Zha L, Chen A, Wang Z. HOXA5 is a tumor suppressor gene that is decreased in gastric cancer. *Oncol. Rep.* 40(3), 1317–1329 (2018).
45. Mo JS, Park YR, Chae SC. MicroRNA 196b regulates HOXA5, HOXB6 AND GLTP expression levels in colorectal cancer cells. *Pathol. Oncol. Res.* 25(3), 953–959 (2019).
46. Teo WW, Merino VF, Cho S *et al.* HOXA5 determines cell fate transition and impedes tumor initiation and progression in breast cancer through regulation of e-cadherin and CD24. *Oncogene* 35(42), 5539–5551 (2016).
47. Lee DH, Forscher C, Di Vizio D, Koeffler HP. Induction of p53-independent apoptosis by ectopic expression of HOXA5 in human liposarcomas. *Sci. Rep.* 5, 12580 (2015).
48. Strathdee G, Holyoake TL, Sim A *et al.* Inactivation of HOXA genes by hypermethylation in myeloid and lymphoid malignancy is frequent and associated with poor prognosis. *Clin. Cancer Res.* 13(17), 5048–5055 (2007).
49. Liang Y, Xia L, Du Z *et al.* HOXA5 inhibits keratinocytes growth and epidermal formation in organotypic cultures *in vitro* and *in vivo*. *J. Dermatol. Sci.* 66(3), 197–206 (2012).
50. Lai NS, Wang TF, Wang SL *et al.* Phostensin caps to the pointed end of actin filaments and modulates actin dynamics. *Biochem. Biophys. Res. Commun.* 387(4), 676–681 (2009).
51. Kao SC, Chen CY, Wang SL *et al.* Identification of phostensin, a PP1 F-actin cytoskeleton targeting subunit. *Biochem. Biophys. Res. Commun.* 356(3), 594–598 (2007).
52. Su YA, Yang J, Tao L, Nguyen H, He P. Undetectable and decreased expression of KIAA1949 (phostensin) encoded on chromosome 6p21.33 in human breast cancers revealed by transcriptome analysis. *J. Cancer* 1, 38–50 (2010).