Gene expression and transcriptomic profiles of invasive behavior in extra-mammary Paget's disease

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To the Editor: Extra-mammary Paget's disease (EMPD) is a rare cutaneous intra-epidermal adenocarcinoma with a controversial origin, and frequently occurs in the penoscrotal region in males. Primary EMPD, which originates within the epidermis or apocrine glands, is usually an *in situ* disease confined to the epidermis with a good prognosis, while secondary EMPD is considered to be associated with underlying internal malignancy. EMPD *in situ* has the potential to become invasive EMPD which has a higher metastasis rate and worse prognosis.[1] Thus, it is important to identify transcriptomic profiles related to the biological behavior of invasion to improve the treatment of invasive EMPD in the future.

The research participants comprised 61 male patients diagnosed with primary penoscrotal EMPD without secondary malignancy or history of cancer. Corresponding tumor samples from each patient were collected during surgery and conducted bulk RNA-sequencing (RNA-seq), which was performed on a Hiseq 4000 platform (Illumina) following the manufacturer's recommendations at Novogene Bioinformatics Institute, Beijing, China. The study was conducted in accordance with the *Declaration of Helsinki* principles and was approved by the Ethics Committee of Peking University First Hospital (No. 2020-127). Written informed consents were provided by patients who participated in this study.

Patients were then divided into non-invasion ($n = 36$) and invasion ($n = 25$) groups based on postoperative hematoxylin and eosin-stained tissue sections [Figure 1A]. Paget cells (PCs) of non-invasive EMPD are *in situ* or could be found within the epithelial sheaths of hair follicles and sweat glands. Conversely, PCs of invasive EMPD break through the basement membrane zone and invade the dermis or subcutaneous tissue. As a sensitive marker of PCs, cytokeratin 7 immunohistochemical staining was used to reassess the location of PCs to verify the accuracy of grouping.

The comparison analysis revealed a total of 37 differentially expressed (7 upregulated and 30 downregulated) genes in invasion group compared with non-invasion group, which were defined as genes with differential expression levels >2-fold change and false discovery rates (FDR) <0.05 [Figure 1B, C]. The upregulated genes were *STMN1*, *CDC20*, *KIF2C*, *UBE2C*, *ASF1B*, *MYBL2*, and *ARPC1B* according to the FDR value from low to high. These seven genes are closely related to cell proliferation, cancer progression, and poor prognosis. Gene ontology analysis categorized seven upregulated genes into the following biological processes: mitotic spindle organization (FDR = 6.24×10^{-3}), mitotic nuclear division (FDR = 2.3×10^{-3}), and cell division (FDR = 1.52×10^{-2}). Conversely, biological processes of skin development (FDR = 3.02×10^{-12}) and establishment of the skin barrier (FDR = 1.99×10^{-6}) were significantly downregulated in invasion group compared with non-invasion group [Figure 1D]. These findings indicated that cell cycle-related genes contribute to the invasive behavior in EMPD.

To further explore the tumor microenvironment (TME) of invasive EMPD, the CIBERSORT algorithm was performed to analyze the composition of tumor-infiltrating immune cells (TICs) in 22 immune cell subsets. The main constituent cells in the TICs of invasive EMPD were resting memory $CD4^+$ T cells (15%), $CD8^+$ T cells (13%), plasma cells (12%), M2 macrophages (12%), and resting mast cells (9%) [Figure 1E]. Compared with the non-invasion group, the infiltration of naive B cells and plasma cells increased in the invasion group, while resting mast cells and resting dendritic cells decreased ($a < 0.05$;

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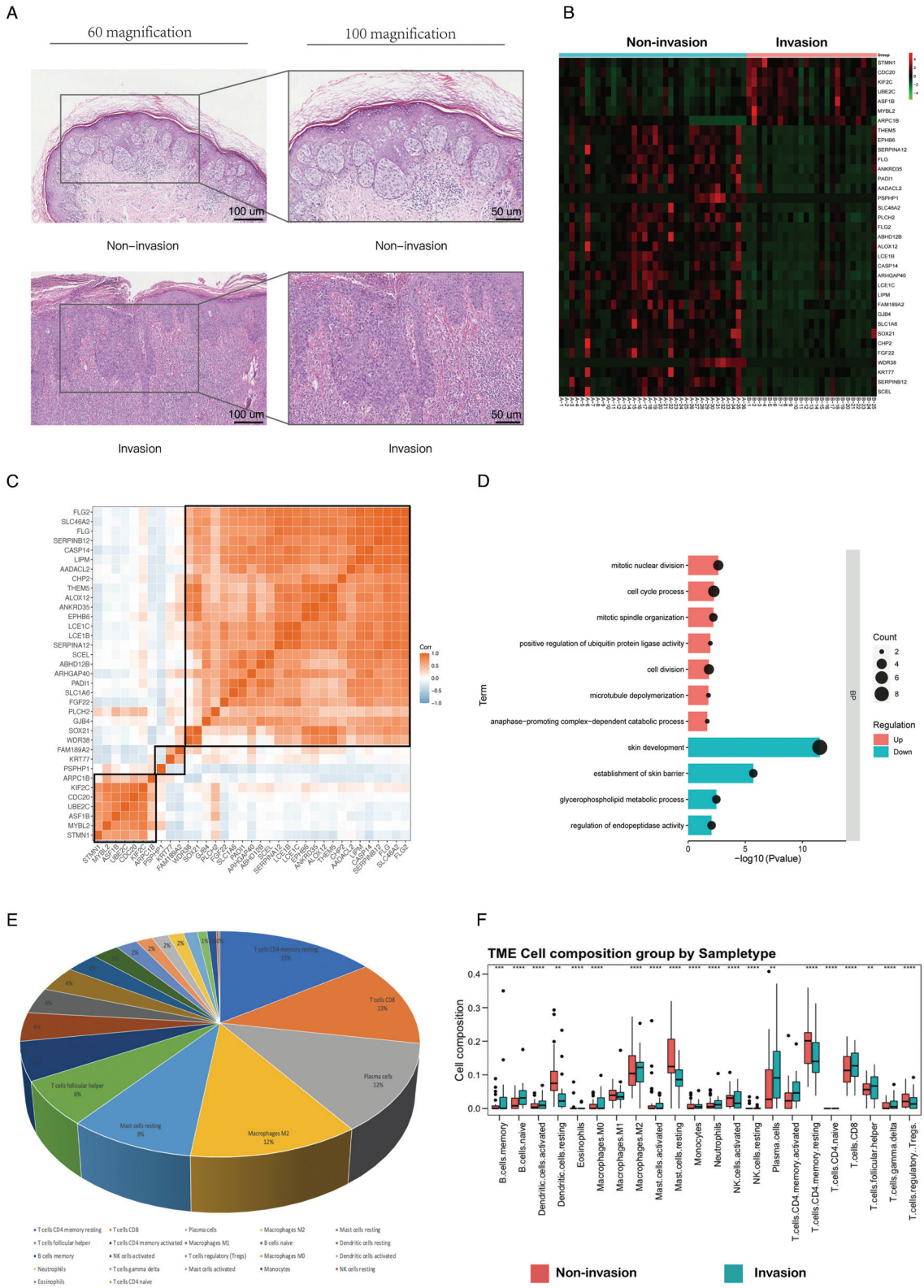

Figure 1: Gene expression and transcriptomic profiles of invasive EMPD. (A) H&E-stained histological manifestations of non-invasive and invasive EMPD. (B) Heatmap of DEGs in invasive and non-invasive EMPD, in which 7 upregulated and 30 downregulated genes were identified. (C) Correlation clustering of upregulated and downregulated DEGs in invasive EMPD. (D) GO term enrichment analysis of upregulated and downregulated DEGs in invasive EMPD. (E) A pie chart shows the composition of TICs present in invasive EMPD. (F) Differences in the composition of TICs between invasive and non-invasive EMPD. DEGs: Differentially expressed genes; EMPD: Extra-mammary Paget's disease; GO: Gene ontology; H&E: hematoxylin and eosin; TICs: Tumor-infiltrating immune cells; TME: Tumor microenvironment.

Figure 1F). These results elaborated the cellular differences in TME between invasive EMPD and non-invasive EMPD.

EMPD is a slow-growing cutaneous adenocarcinoma. Most of them are carcinoma *in situ*, in which PCs are confined to the epidermis for a prolonged duration and spread horizontally. However, once being invasive, PCs can spread vertically and have a stronger ability for proliferation and migration. The seven genes identified in this study exhibit oncogenic functions related to promoting cell division and proliferation, especially *CDC20* and *UBE2C*, which are both biomarkers of rapid cellular proliferation. They are implicated in the growth and invasion of various tumors and have been considered as potential therapeutic targets. However, the association of these genes with invasive EMPD has not been reported. *STMN1* is an oncogene involved in various cellular processes, including invasion, cell cycle progression, and mitotic division. Increased *STMN1* expression is closely associated with malignant behavior and poor prognosis in multiple adenocarcinomas such as prostate cancer, colon cancer, breast cancer, pancreatic cancer, and lung adenocarcinoma.[2] High expression of *CDC20* can exhibit an oncogenic effect that is associated with malignant progression, tumor metastasis, and poor prognosis in various cancers, including prostate cancer.[3] *ASF1B* is highly expressed in 22 types of cancer, in which it can promote cell proliferation and influence cell cycle progression.[4] In addition, multiple studies have found that *KIF2C*, *UBE2C*, *MYBL2,* and *ARPC1B* can indicate poor prognosis in multiple cancers including hepatocellular carcinoma, endometrial carcinoma, breast cancer, and colon cancer.[5,6] Altogether, these upregulated genes exhibit features of enhanced tumor cell proliferation and migration capacity in invasive EMPD. When high expression of these genes is observed in slow-growing EMPD, it is necessary to be vigilant against the occurrence of invasion.

Invasive EMPD is characterized by extensive immune infiltration, including lymphocytes, macrophages, neutrophils, and eosinophils. By interacting with tumor cells, these immune cells play an important role in tumor progression and metastasis. Besides, changes in the cellular proportion of TICs may also reflect differences in immunological status in invasive EMPD. B cells are composed of distinct functional phenotypes which play important roles in antigen presentation and antibody production in tumors. Chemokines secreted by tumor cells may attract more naive B cells into TME and promote their differentiation, while plasma cells may exert whether anti-tumor or pro-tumor effects through producing antibodies locally.[7] The changes of TICs in invasive EMPD and their functions need further study, which may provide new ideas for immunotherapy in advanced EMPD.

In conclusion, our study identified seven genes closely related to the invasive behavior of EMPD, which have the potential to serve as biomarkers and therapeutic targets. Changes in the cellular proportion of TICs in invasive EMPD have been elaborated. These findings may contribute to the treatment of invasive EMPD in the future and therefore warrant further study.

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Conflicts of interest

None.

Data availability

Original sequencing data for bulk RNA-seq in this study can be found in the National Genomics Data Center and are accessible through the GSA-Human series accession number HRA001914. The datasets used and analyzed during this study are available from the corresponding author on reasonable request.

References

1. Ishizuki S, Nakamura Y. Extramammary Paget's disease: diagnosis, pathogenesis, and treatment with focus on recent developments. Curr Oncol 2021;28:2969–2986. doi: 10.3390/curroncol28040260.
2. Xun G, Hu W, Li B. PTEN loss promotes oncogenic function of STMN1 via PI3K/AKT pathway in lung cancer. Sci Rep 2021;11:14318. doi: 10.1038/s41598-021-93815-3.
3. Cheng S, Castillo V, Sliva D. CDC20 associated with cancer metastasis and novel mushroom-derived CDC20 inhibitors with antimetastatic activity. Int J Oncol 2019;54:2250–2256. doi: 10.3892/ijo.2019.4791.
4. Hu X, Zhu H, Zhang X, He X, Xu X. Comprehensive analysis of pan-cancer reveals potential of ASF1B as a prognostic and immunological biomarker. Cancer Med 2021;10:6897–6916. doi: 10.1002/cam4.4203.
5. Huang R, Liu J, Li H, Zheng L, Jin H, Zhang Y, *et al*. Identification of hub genes and their correlation with immune infiltration cells in hepatocellular carcinoma based on GEO and TCGA databases. Front Genet 2021;12:647353. doi: 10.3389/fgene.2021.647353.
6. Zhang W, Gao L, Wang C, Wang S, Sun D, Li X, *et al*. Combining bioinformatics and experiments to identify and verify key genes with prognostic values in endometrial carcinoma. J Cancer 2020;11:716–732. doi: 10.7150/jca.35854.
7. Sharonov GV, Serebrovskaya EO, Yuzhakova DV, Britanova OV, Chudakov DM. B cells, plasma cells and antibody repertoires in the tumour microenvironment. Nat Rev Immunol 2020;20:294–307. doi: 10.1038/s41577-019-0257-x.

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