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Multiplex immunohistochemistry reveals histological features of three different intestinal polyp subtypes in pediatric patients

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Abstract

Background Histologically, our understanding of intestinal polyps remains limited in scope, particularly regarding the diverse subtypes observed in pediatric patients. To enhance our comprehension, three different polyp subtypes including solitary juvenile polyps (SJPs), juvenile polyposis syndrome (JPS)-related polyps, and Peutz–Jeghers syndrome (PJS)-related polyps were investigated.

Methods This study used advanced multiplex immunohistochemistry (mIHC) technology to analyze polyps comprising 4 SJP, 4 JPS and 4 PJS polyps from 12 individual patients who underwent colonoscopies or radical surgical procedures. subtypes.

Results These mIHC analyses revealed some differences among these polyp subtypes. PJS-related polyps, specifically, displayed epithelial dysplasia with dendritic gland hyperplasia and distinct villous structures adorned with finger-like projections on their surfaces. In contrast, SJP and JPS polyps exhibited cystic glandular dilation, with their surfaces lined with continuous but eroded epithelia. Furthermore, PJS polyps had an abundance of microvessels and thick smooth muscle fibers, whereas SJP and JPS polyps were characterized by lymphoid follicle-like structures.

Conclusions These findings not only deepen our structural understanding of various intestinal polyp subtypes but also offer valuable insights that may inform the diagnosis of patients with these conditions.

Keywords Pediatric intestinal polyps, Multiplex immunohistochemistry, Epithelial dysplasia, Cystic glandular dilation

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Background

Intestinal polyps occur in 1% of preschool-age children, and juvenile polyps account for 80% of all childhood polyps [1, 2]. Solitary juvenile polyps (SJPs), juvenile polyposis syndrome (JPS) and Peutz–Jeghers syndrome polyps (PJSs) are the most common intestinal polyps in children [3, 4]. SJPs result from restructuring of the mucosal architecture secondary to chronic inflammatory processes. In contrast, nearly half of JPS-associated polyps occur in individuals harboring heterozygous mutations in the *SMAD4* or *BMPR1A* genes. Furthermore, PJS-associated polyps are the defining feature of Peutz–Jeghers syndrome, an autosomal dominant, hereditary polyposis disorder caused by germline mutations in the serine/threonine kinase 11 (*STK11/LKB1*) genes [1, 5, 6]. In clinical practice, hematoxylin and eosin (HE) staining is routinely employed to differentiate various types of polyps, providing crucial evidence for accurate clinical diagnosis [7]. Compared with HE staining, multiplex immunohistochemistry (mIHC) technology, which has emerged as a powerful technique in the field of biomedical research and clinical diagnostics, represents a significant advancement in the traditional immunohistochemistry approach, enabling the simultaneous detection of multiple antigens within a single tissue sample and providing unprecedented insights into biological processes and disease mechanisms [8, 9].

Recently, we revealed the heterogeneous cellular microenvironment of colonic polyps from pediatric patients via single-cell RNA sequencing (scRNA-seq) as well as multiplex immunohistochemistry (mIHC) technology [10]. In this study, mIHC analysis was focused primarily on validating the frequencies of distinct cell types, as revealed by the scRNA-seq data, thus confirming the differences observed at the transcriptomic level. However, our analysis did not delve deeply into the spatial characteristics of the polyps, which could have provided additional layers of information and insights. In this paper, we present a detailed description and comparative analysis of the spatial characteristics of three distinct polyp subtypes found in pediatric patients. Our findings will contribute to a more comprehensive appreciation of the pathological features of pediatric polyps and facilitate more targeted and effective diagnostic and therapeutic approaches.

Methods

Pediatric polyp sample collection

Polyp samples were procured from residual tissues that had been previously utilized for pathological examinations.

mIHC experiment

For mIHC analysis, formalin-fixed and paraffin-embedded (FFPE) polyp samples from pediatric patients were sectioned at a precise thickness of 4 μm. A panel of primary antibodies targeting CD3 (MXB Biotechnologies, 1:5, #MAB-0740), CD19 (Cell Signaling Technology, 1:2000, #90176), EpCAM (Abcam, 1:2000, #ab223582), PECAM1 (MXB Biotechnologies, 1:5, #MAB-0720), CD11C (Cell Signaling Technology, 1:2000, #45581), α-SMA (Boster Biological Technology, 1:2000, #BM0002) and DAPI (2 μg/ml, Invitrogen, #D1306) were used. mIHC staining was achieved via an Opal Polaris 7 Color IHC Detection Kit from Akoya Bioscience (#NEL871001KT). The resulting multispectral images were then scanned using a PhenoImager HT system from Akoya Bioscience for comprehensive analysis.

Results

mIHC analysis of three different types of intestinal polyps in pediatric patients

To characterize the diverse types of intestinal polyps observed in pediatric patients, we conducted mIHC analysis on a cohort of 12 intestinal polyps comprising 4 SJP, 4 JPS and 4 PJS polyps from 12 individual patients who underwent colonoscopies or radical surgical procedures. The demographic characteristics, clinical manifestations and pathological diagnoses of the pediatric patients are summarized in Table 1. As shown in Fig. 1a and b, we employed a panel of antibodies, excluding DAPI, that specifically target markers of epithelial cells (EpCAM), endothelial cells (PECAM1), smooth muscle cells (α-SMA), T-cells (CD3), B-cells (CD19), and myeloid cells (CD11C), thereby enabling the comprehensive mapping of these cell types within the polyp microenvironment. On the basis of these mIHC experiments, we clearly observed the delicate histological structures of these three different types of intestinal polyps in children.

SJP and JPS polyps exhibit cystic glandular dilation, whereas PJS polyps exhibit epithelial dysplasia with dendritic gland hyperplasia

Pathological examination indicated that the characteristics of SJP and JPS polyps were cystically dilated glands filled with mucus, whereas PJS polyps were characterized by unique crisscrossed smooth muscle bundles. As shown in Fig. 2a, JPS and SJP polyps were characterized by regular and normal glands, with occasional secondary branches of excessively dilated glandular lumens. In some cases, the outlets of the glands were obstructed, leading to mucus retention and cystic dilation of the glands. The surrounding glands undergo reactive hyperplasia in response to compression, presenting as relatively small glandular lumens. The glandular manifestations in SJP polyps are generally similar to those in JPS polyps,

Table 1 Patient characteristics and clinical presentation

Patient	Gender	Age (years)	Clinical presentation					Diagnosis
			Hematochezia	Abdominal pain	Intussusception	Prolapse	Other	
1	Male	7	NO	NO	NO	Yes	NO	SJP
2	Female	6	Yes	NO	NO	NO	NO	SJP
3	Female	6	Yes	NO	NO	Yes	NO	SJP
4	Female	6	Yes	Yes	NO	NO	NO	SJP
5	Male	13	Yes	Yes	NO	NO	NO	JPS
6	Male	10	Yes	Yes	NO	Yes	NO	JPS
7	Male	6	Yes	Yes	NO	Yes	NO	JPS
8	Male	8	Yes	NO	Yes	Yes	Diarrhoea	JPS
9	Male	7	NO	Yes	Yes	Yes	Mucocutaneous Pigmentations	PJS
10	Female	15	Yes	NO	NO	Yes	Mucocutaneous Pigmentations	PJS
11	Male	15	Yes	Yes	Yes	NO	Mucocutaneous Pigmentations	PJS
12	Female	4	NO	Yes	Yes	NO	Mucocutaneous Pigmentations	PJS

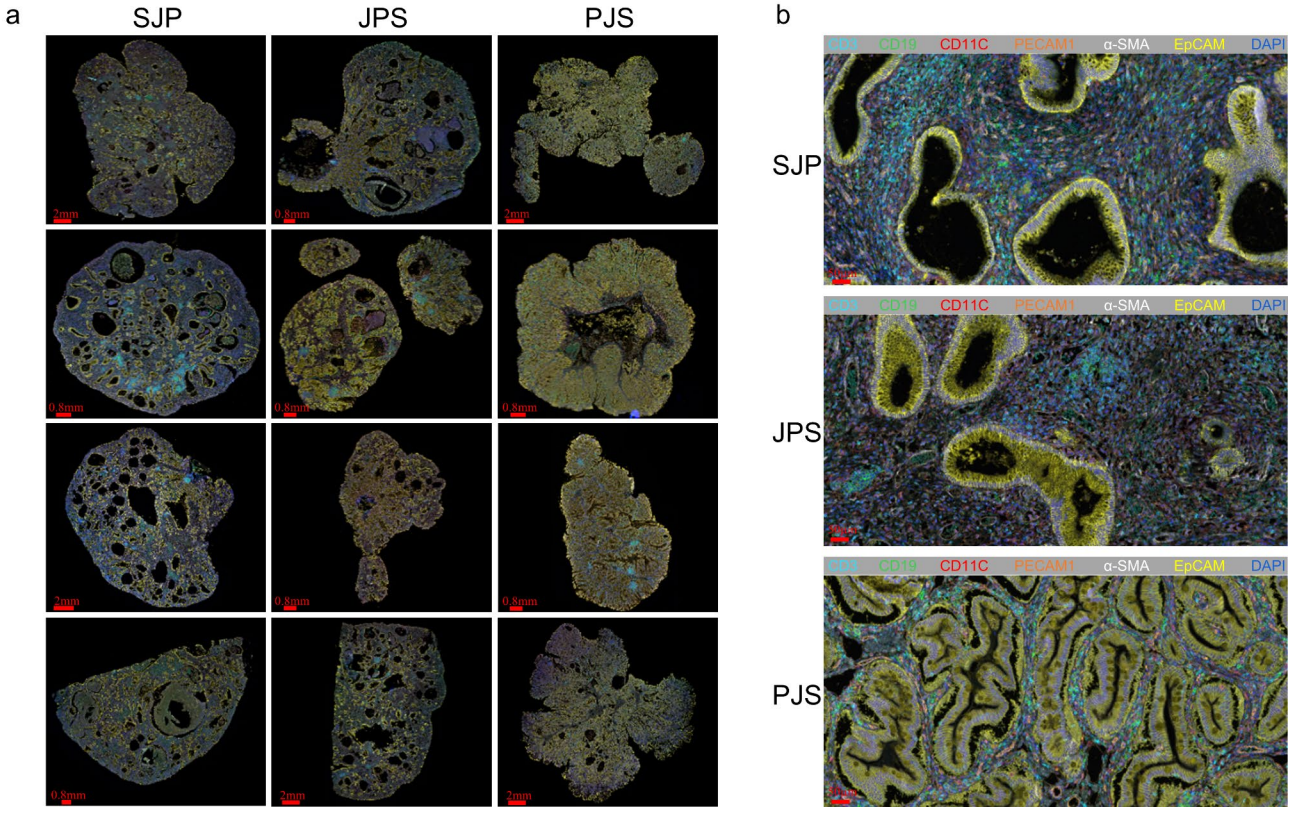

Fig. 1 mIHC analysis of three different types of intestinal polyps in pediatric patients. **(a)** mIHC images of 12 polyp samples showing staining for CD3 (T-cells), CD19 (B-cells), CD11C (myeloid cells), PECAM1 (endothelial cells), α-SMA (smooth muscle cells), EpCAM (epithelial cells) and DAPI (cell nuclei) in three different polyp subtypes. The scale bars are as indicated in each image. **(b)** Representative enlarged mIHC images of three different polyp subtypes. Scale bar, 50 μm

with the main difference being that the glandular densities in JPS polyps are slightly greater than those in SJP polyps. In addition, we found that, compared with SJP and JPS polyps, PJS polyps presented greater densities of glandular distribution. Moreover, most of the glands in PJS polyps displayed structural disarray and abnormal differentiation of glandular epithelial cells, manifested as multiple branches within the glandular lumen. In addition, we have previously reported that, compared with normal tissue, PJS polyps presented significantly increased frequency of goblet cells on the basis of the analysis of scRNA-seq data [10]. Thus, we further

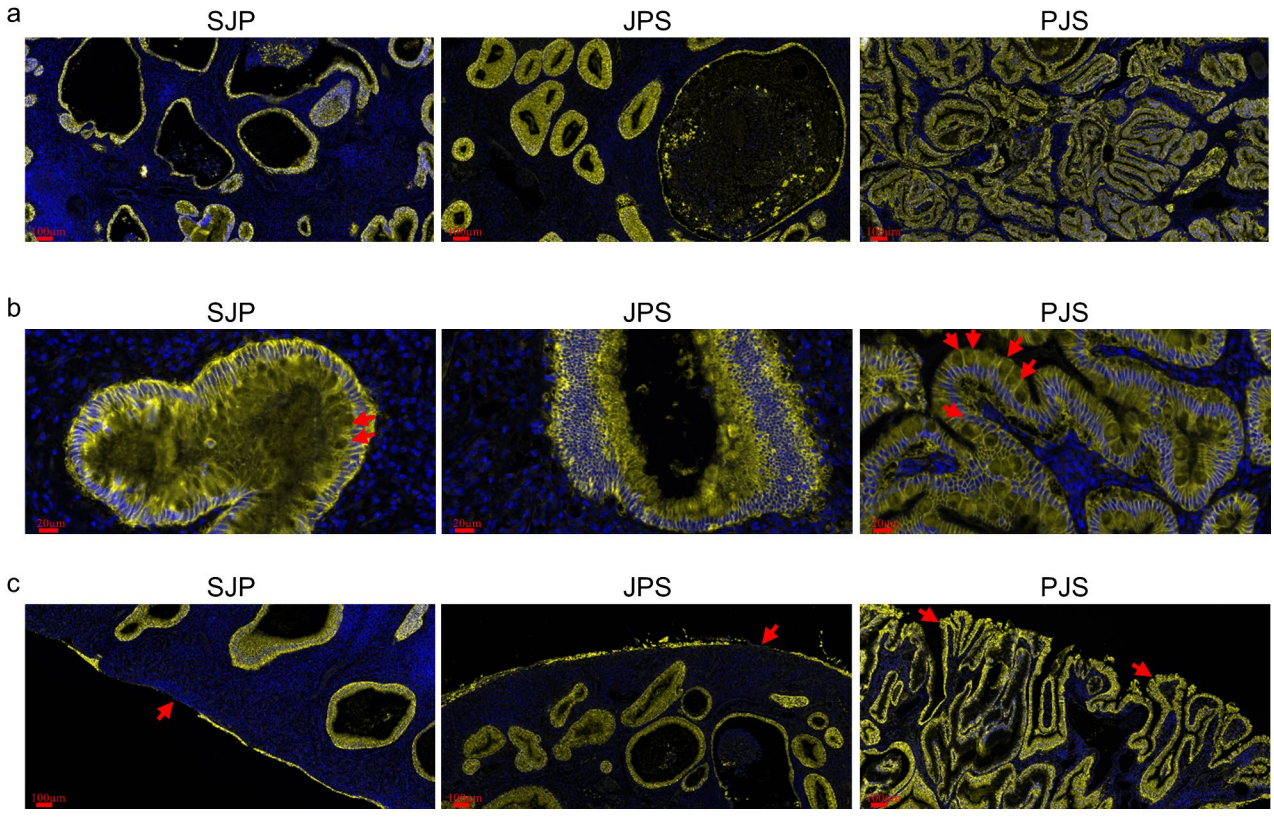

Fig. 2 mIHC showing the glands, goblet cells and surfaces of three different polyp subtypes. The epithelial cells were stained with EpCAM antibody (yellow), and the cell nuclei were stained with DAPI (blue). **(a)** Representative mIHC images of glands across three different polyp subtypes. Scale bar, 100 μm. **(b)** Representative enlarged mIHC images of goblet cells (red arrow) across three different polyp subtypes. Scale bar, 100 μm. **(c)** Representative mIHC images of the surfaces of three different polyp subtypes. The red arrow indicates surface erosion. Scale bar, 100 μm

amplified these mIHC images and observed that PJS polyps had more goblet cells than SJP and JPS polyps in each gland did (Fig. 2b).

The surfaces of SJP and JPS polyps are lined with continuous but eroded epithelia, whereas PJS polyps exhibit a villous structure with finger-like projections

As shown in Fig. 2C, the surfaces of SJP and JPS polyps were lined with successive layers of epithelial cells, while surface erosion was also observed in both JPS and SJP polyps. Unlike SJP and JPS polyps, PJS polyps had an irregular surface with finger-like projections of villous structures, and crypts were also be observed because this phenomenon usually occurs at the surface of the intestinal mucosa. These results suggest that there are differences in the underlying pathological mechanisms between PJS-associated polyps and the other two polyp subtypes.

PJS-associated polyps are characterized by abundant microvessels and thick smooth muscle fibers

By conducting a thorough analysis of the expression colocalization of PECAM1 and α-SMA, which are markers of endothelial cells and smooth muscle cells, respectively, we were able to distinguish between vascular smooth muscle tissue and muscle fiber tissue, which allowed us to precisely identify and characterize these anatomical structures. As shown in Fig. 3, these images revealed that the densities of both PECAM1 and α-SMA expression were significantly greater in PJS polyps than in either JPS or SJP polyps. Additionally, compared with JPS and SJP polyps, PJS polyps presented a more abundant distribution of microvessels, whereas JPS and SJP polyps presented a greater proportion of larger, lumen-containing blood vessels. Moreover, we observed that PJS polyps had typical thick smooth muscle fibers interspersed within the glandular spaces, forming a cord-like structure, whereas neither SJP nor JPS polyps had this structure.

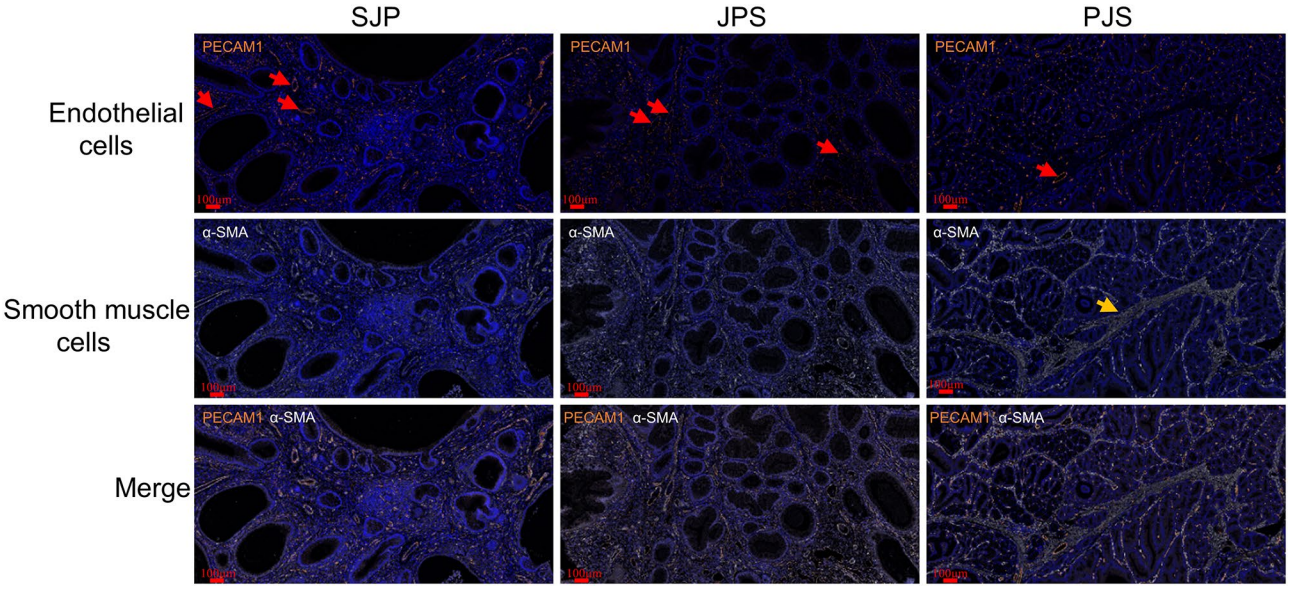

Fig. 3 mIHC image showing the distributions of endothelial cells and smooth muscle cells in three different polyp subtypes. The endothelial cells were stained with a PECAM1 antibody (orange), the smooth muscle cells were stained with an α-SMA antibody (white), and the cell nuclei were stained with DAPI (blue). The red arrow indicates lumen-containing blood vessels, and the yellow arrow indicates smooth muscle fibers. Scale bar, 100 μm

Immune cell infiltration occurs in all three polyp subtypes

Using CD3, CD19 and CD11C antibodies, we identified T-cells, B-cells and myeloid cells, respectively, in these polyps. We found that, compared with those in PJS polyps, T-cells and B-cells tended to aggregate, forming structures resembling lymphoid follicles in SJP and JPS polyps, while myeloid cells were widely distributed among these glands across all three polyp subtypes (Fig. 4).

Discussion

As one of the most common causes of rectal bleeding in pediatric patients, the vast majority of intestinal polyps are histologically classified as juvenile polyps with negligible risk for malignant transformation. At present, pathological examination, which is mainly based on HE staining, can distinguish between different types of polyps, but it still cannot make a more detailed differentiation of polyps. In this study, we provide a detailed atlas of the spatial structures of three different polyp subtypes from pediatric patients via mIHC analysis.

Compared with SJP polyps, which are typically solitary and rarely recurrent, both JPS and PJS polyps, which have a more definitive pathogenesis, are often found in multiples and are often recurrent [11, 12]. However, histologically, JPS polyps are distinct from PJS polyps but share some features with SJP polyps. For example, the macroscopic appearances of most SJP and JPS polyps typically exhibit smooth, rounded surfaces without fissures or lobulations, whereas PJS polyps are usually multilobulated. Moreover, the surfaces of SJP and JPS polyps are smooth and covered with a successive layer of epithelial cells, whereas PJS polyps exhibit a villous structure with finger-like projections, and crypts can be observed on their surfaces. This difference might reflect their different pathological mechanisms. It is currently speculated that the formation of SJP and JPS polyps arises from obstruction at the outlet of a particular gland within the intestinal mucosa, resulting in mucus retention. The retained mucus subsequently compresses and obstructs the surrounding glandular lumens, leading to a progressive blockage of an increasing number of glands and, ultimately, the growth and enlargement of polyps. However, PJS polyps result from autonomous hyperplasia of the glands, accompanied by asynchronous and uneven hyperplasia of the surface epithelium. Our recent study revealed that colonocytes in all three polyp types presented downregulated expression of pathway markers associated with cell cycle checkpoints, whereas markers of the p53 pathway and apoptosis pathway showed uniquely downregulated expression in colonocytes of PJS polyps [10]. These results suggest that *STK11/LKB1* mutation in PJS patients promotes epithelial dysplasia and polyp progression.

Another distinctive characteristic of PJS polyps is their abundance of microvessels, whereas SJP and JPS polyps have more lumen-containing blood vessels. These abundant microvessels might provide essential nutrients for the growth and function of the numerous glands present in the polyps, which may explain why PJS polyps grow rapidly, with their large size contributing to the formation of giant polyps, which in turn can lead to intussusception and intestinal obstruction. Conversely, hematochezia and

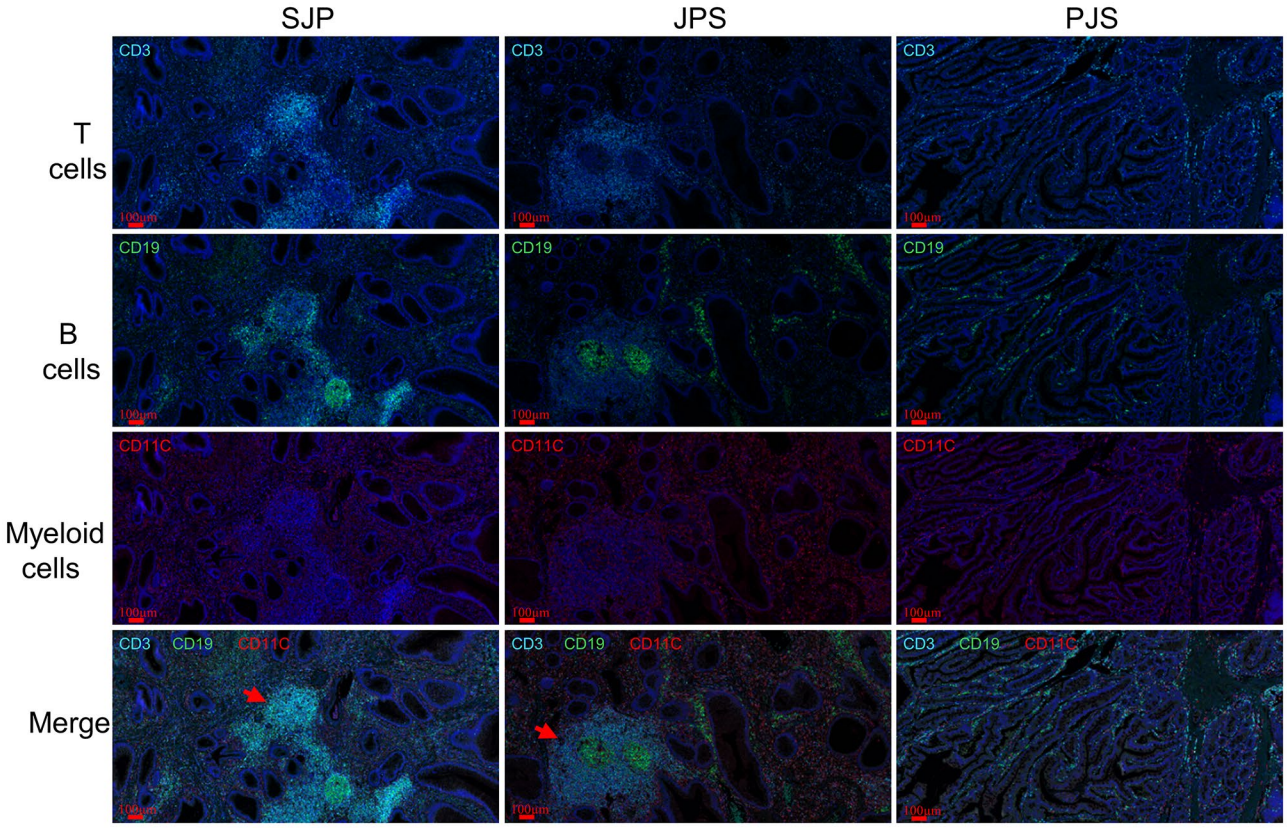

Fig. 4 mIHC results showing the distributions of T-cells, B-cells and myeloid cells in three different polyp subtypes. The T-cells were stained with a CD3 antibody (sky blue), the B-cells were stained with a CD19 antibody (green), the myeloid cells were stained with a CD11C antibody (red), and the cell nuclei were stained with DAPI (blue). The red arrow indicates lumen-containing blood vessels, and the yellow arrow indicates smooth muscle fibers. Scale bar, 100 μm

anemia are common clinical manifestations observed in patients with SJP and JPS [13–15].

In addition, the formation of lymphoid follicle-like structures is a characteristic feature of SJP and JPS polyps but not PJS polyps. Generally, the formation of lymphoid follicles is an immune response to inflammation and adverse stimuli [16]. These phenomena suggest that SJP and JPS polyps have a strong inflammatory response and that these inflammatory mediators repeatedly stimulate the glands to secrete mucus, leading to continuous expansion of the glands, whereas PJS polyps exhibit a relatively weak inflammatory response.

Overall, our mIHC analysis meticulously delineates the intricate structures of various types of polyps, offering valuable insights and evidence that support the pathological diagnosis of polyps.

Conclusion

Pathological examination plays a crucial role in the diagnosis of clinical diseases. Our study utilizing mIHC technology has significantly broadened our histological understanding of intestinal polyps, particularly in pediatric patients, by elucidating the distinct structural and morphological features of these three different polyp subtypes. The identified differences, such as the unique epithelial dysplasia with dendritic gland hyperplasia in PJS polyps and the prevalence of microvessels and thick smooth muscle fibers, alongside the contrasting features of SJP and JPS polyps, underscore the importance of this approach in refining diagnostic accuracy. These insights represent a valuable contribution to the field, enhancing our ability to characterize and potentially better manage patients with these intestinal polyp subtypes.

Abbreviations

mIHC	Multiplex immunohistochemistry
SJPs	Solitary juvenile polyps
JPS	Juvenile polyposis syndrome
PJS	Peutz–Jeghers syndrome
STK11/LKB1	Serine/threonine kinase 11
HE	Hematoxylin and eosin
scRNA-seq	Single-cell RNA sequencing
FFPE	Formalin-fixed and paraffin-embedded

Author contributions

LL Huang and XJ Liu completed the main analyses of the article and wrote the manuscript. SY Li, YJ Liu, WJ Chen and YN Li collated the data and the results of the analyses. HY Peng and XY Wang checked and revised the manuscript. PW Xiong, QL Yang and ST Wu collected data on the research participants. L Che, HM Zhao and YF Deng proposed research ideas and provided guidance and

assistance during the research process. All authors read, revised, and approved the final manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Hunan Children's Hospital. The ethics committee approval code was HCHLL-2020-61. Before the implementation of this study, the use of intestinal polyp tissue from patients for this research was consented to by the participants' parents or legal guardians.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Soyer T. Polypoid disease of colon in children. Pediatr Surg Int. 2020;36(4):447–55.
2. Adolph VR, Bernabe K. Polyps in children. Clin Colon Rectal Surg. 2008;21(4):280–5.
3. Cauchin E, Touchefeu Y, Matysiak-Budnik T. Hamartomatous Tumors in the gastrointestinal tract. Gastrointest Tumors. 2015;2(2):65–74.
4. Ma H, Brosens LAA, Offerhaus GJA, Giardiello FM, de Leng WWJ, Montgomery EA. Pathology and genetics of hereditary colorectal cancer. Pathology. 2018;50(1):49–59.
5. Poffenberger MC, Metcalfe-Roach A, Aguilar E, Chen J, Hsu BE, Wong AH, Johnson RM, Flynn B, Samborska B, Ma EH, et al. LKB1 deficiency in T cells promotes the development of gastrointestinal polyposis. Science. 2018;361(6400):406–11.
6. Sachatello CR, Griffen WO Jr. Hereditary polypoid diseases of the gastrointestinal tract: a working classification. Am J Surg. 1975;129(2):198–203.
7. Hamada Y, Katsurahara M, Umeda Y, Ikenoyama Y, Shigefuku A, Fujiwara Y, Beppu T, Tsuboi J, Yamada R, Nakamura M, et al. Endoscopic resection for a solitary Peutz-Jeghers type polyp in the duodenum: a case report with literature review. DEN Open. 2023;3(1):e226.
8. Wu Y, Yang S, Ma J, Chen Z, Song G, Rao D, Cheng Y, Huang S, Liu Y, Jiang S, et al. Spatiotemporal Immune Landscape of Colorectal Cancer Liver Metastasis at single-cell level. Cancer Discov. 2022;12(1):134–53.
9. Zhang W, Song ZJ, Zhang BY, Wang JL, Guo Q, Sun ZW, Tang H. Multiplex immunohistochemistry indicates biomarkers in colorectal cancer. Neoplasma. 2021;68(6):1272–82.
10. Deng Y, Li C, Huang L, Xiong P, Li Y, Liu Y, Li S, Chen W, Yin Q, Li Y, et al. Single-cell landscape of the cellular microenvironment in three different colonic polyp subtypes in children. Clin Translational Med. 2024;14(1):e1535.
11. Dal Buono A, Gaiani F, Poliani L, Laghi L. Juvenile polyposis syndrome: an overview. Best Pract Res Clin Gastroenterol 2022, 58–9:101799.
12. Kay M, Eng K, Wyllie R. Colonic polyps and polyposis syndromes in pediatric patients. Curr Opin Pediatr. 2015;27(5):634–41.
13. Kılıç S, Atıcı A, Soyköse-Açıkalın Ö. Peutz-Jeghers syndrome: an unusual cause of recurrent intussusception in a 7-year-old boy. Turk J Pediatr. 2016;58(5):535–7.
14. Schreibman IR, Baker M, Amos C, McGarrity TJ. The hamartomatous polyposis syndromes: a clinical and molecular review. Am J Gastroenterol. 2005;100(2):476–90.
15. Chow E, Macrae F. A review of juvenile polyposis syndrome. J Gastroenterol Hepatol. 2005;20(11):1634–40.
16. Yadava K, Bollyky P, Lawson MA. The formation and function of tertiary lymphoid follicles in chronic pulmonary inflammation. Immunology. 2016;149(3):262–9.

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