Hirschsprung's Disease Pathology

10

Kenichi Kohashi, Izumi Kinoshita, and Yoshinao Oda

10.1 Introduction

Although Harald Hirschsprung first described Hirschsprung's disease in 1888 [1], the pathological features were not understood until the 1940s, when Whitehouse and Kernohan [2, 3] demonstrated that aganglionosis within the distal colon or rectum was the cause of the functional obstruction.

In chronic constipation in childhood, diagnostic procedures such as radiographic contrast enema, anorectal manometry, and pathological approaches including acetylcholinesterase (AChE) histochemistry and rectal biopsy have been performed to differentiate idiopathic constipation from Hirschsprung's disease [4]. Although radiographic contrast enema is a useful tool for determining the distribution of lesions [5, 6] and a normal anorectal reflex in anorectal manometry is an adverse finding in Hirschsprung's disease, these methods have not been sufficiently developed to yield 100% diagnostic accuracy [6].

The pathological approach is a highly reliable and requisite method for diagnosis of Hirschsprung's disease [6]. However, it is sometimes difficult to distinguish Hirschsprung's disease from its allied disorders using only the pathological approach [7]. Therefore, to make a diagnosis of Hirschsprung's disease and its allied disorders, multilateral approaches based mainly on pathological diagnosis are needed.

The aim of this chapter is to discuss the histological features and frozen section diagnosis of Hirschsprung's disease.

K. Kohashi (✉) · I. Kinoshita · Y. Oda
Department of Anatomic Pathology, Pathological Sciences, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
e-mail: kohas@surgpath.med.kyushu-u.ac.jp

10.2 Pathological Definition

The key histological feature of Hirschsprung's disease is a congenital absence of ganglion cells in the continuous distal bowel beginning at the internal anal sphincter and extending proximally for varying distances [8–11].

10.3 Histological Features

Hirschsprung's disease is histologically characterized by the absence of ganglion cells (aganglionosis) in both the myenteric (Auerbach) plexus (between the smooth muscle layers of the gastrointestinal tract wall) and submucosal (Meissner) plexus (within the submucosa of the gastrointestinal tract wall) of a segment of the bowel [8–11]. The presence of hypertrophied, disorganized, and nonmyelinated nerve fibers of both adrenergic and non-adrenergic types is observed in the aganglionic segment; these abnormal fibers fail to properly innervate the intestinal smooth muscle [10–13]. In the aganglionic segment, fibro-collagenous fibers in the submucosal layer increase as time proceeds (Fig. 10.1).

In the transitional zone between normal ganglionosis and aganglionosis, the ganglion cells are immature and decreased in number, and hypertrophied and nonmyelinated nerve fibers are occasionally observed (Fig. 10.2). This transitional zone usually occurs over a short distance, but some patients have longer transitional zones than others [10].

Although mature ganglion cells are characterized by relatively large-sized rounded nuclei having coarsely granular chromatin, prominent nucleoli, and moderate-to-large amounts of amphophilic cytoplasm (Fig. 10.3), immature ganglion cells show small-sized nuclei, inconspicuous nucleoli, or small amounts of cytoplasm. In premature infants, it may be difficult to evaluate the immaturity of ganglion cells due to their relatively small size. Meanwhile, there are no definitive criteria in terms of numbers of ganglion cells to distinguish normoganglionosis from the transitional zone. In one previous morphometrical study, normal numbers of

T. Taguchi et al. (eds.), *Hirschsprung's Disease and the Allied Disorders*, https://doi.org/10.1007/978-981-13-3606-5_10

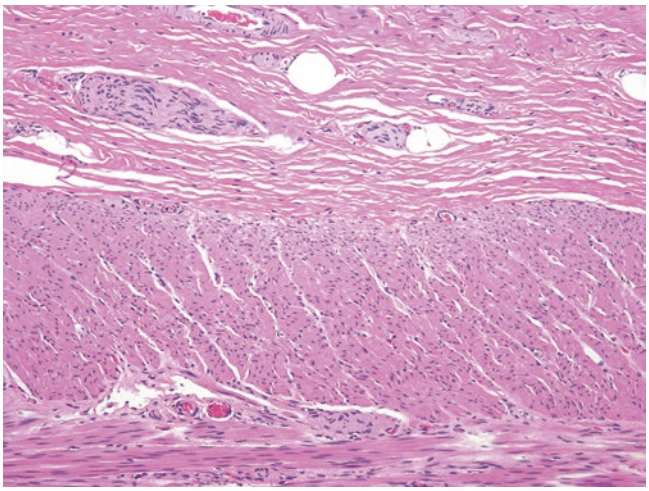

Fig. 10.1 Aganglionic segment. Some thickened nerve bundles in the submucosal and intramuscular layer are seen

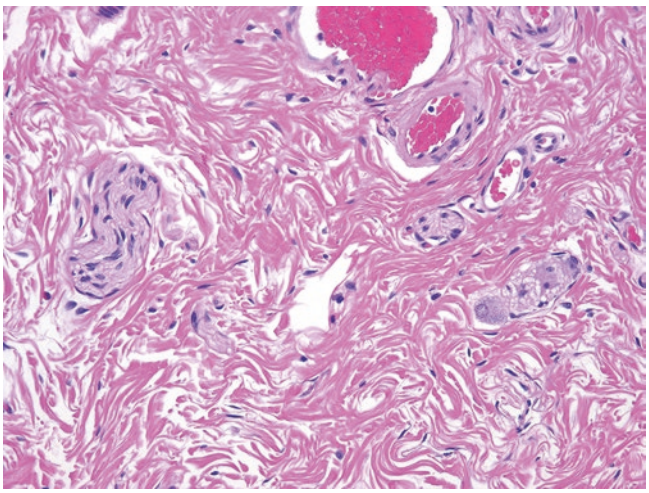

Fig. 10.2 Transitional zone. Although there are mature ganglion cells in Meissner plexus, thickened nerve bundles are scattered

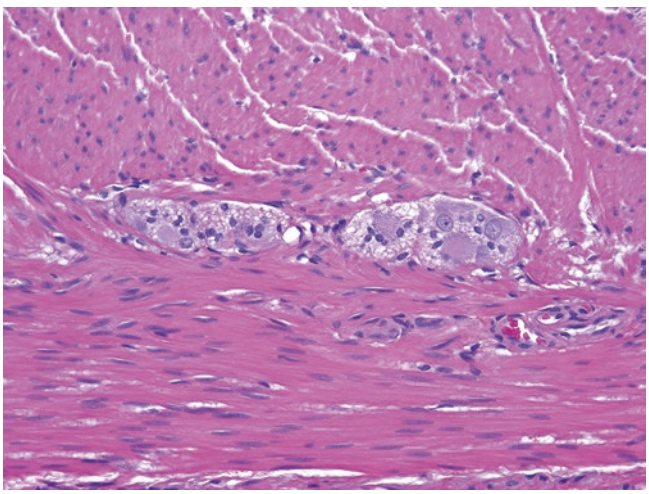

Fig. 10.3 Normoganglionic segment. More than one mature ganglion cells in one neural plexus is observed

ganglion cells of the intestine differed depending on age (<3 months, 117.9 ± 26.0/cm; 3 months–1 year, 63.3 ± 23.4/cm; 1–10 year, 64.8 ± 23.2/cm; >10 year, 38.6 ± 13.4/cm) and site (ileum, 84 ± 22.6/cm; transverse colon, 123.0 ± 24.3/cm; sigmoid colon, 137.3 ± 20.6/cm) [7], but the 50–100 ganglion cells per 1 cm of intestine reported by Taguchi et al. may provide an indication of the normal range in the neonatal bowel depending on the site or age.

10.4 Hirschsprung's Disease Form

Based on the extent and location of the aganglionic segment, several forms of the disease have been recognized [4, 10, 11, 14].

10.4.1 Short-Segment Form

Ultrashort form: The aganglionic segment is only a small area of the lower rectum, and this narrow segment is inconspicuous by radiographic contrast enema. The diagnosis of this variant can be missed if the biopsy is taken at too proximal point in the intestine.

Conventional short-segment form: The aganglionic segment involves several centimeters of the rectum and rectosigmoid.

10.4.2 Long-Segment Form

Conventional long-segment form: The aganglionic segment extends beyond the sigmoid or transverse colon.

Total colonic aganglionosis: The aganglionic segment is more extensive, involving most or all of the large bowel and up to 30 cm of the terminal ileum.

Extensive aganglionosis: The aganglionosis extends proximally more than 30 cm to the ileocecal valve.

10.5 Immunohistochemistry

Important diagnostic parameters of Hirschsprung's disease are numbers and distributions of the neural plexus, the existence or nonexistence of nerve fibers, and the numbers and maturity of ganglion cells in the neural plexus. However, it is difficult to evaluate these particulars using only HE staining. Immunoreactivities for some neural markers have the potential to become an ancillary parameter in the diagnosis of Hirschsprung's-related disease.

CD56, also known as the neural cell adhesion molecule (NCAM), is a homophilic binding glycoprotein located on the membranes of neurons and glia [15–18]. In the intestinal

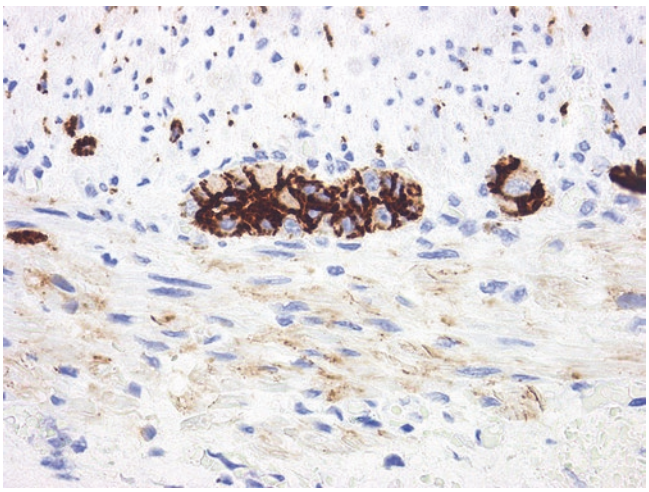

Fig. 10.4 All neural cells including neural fiber, enteric glia, and ganglion cells are positive for CD56

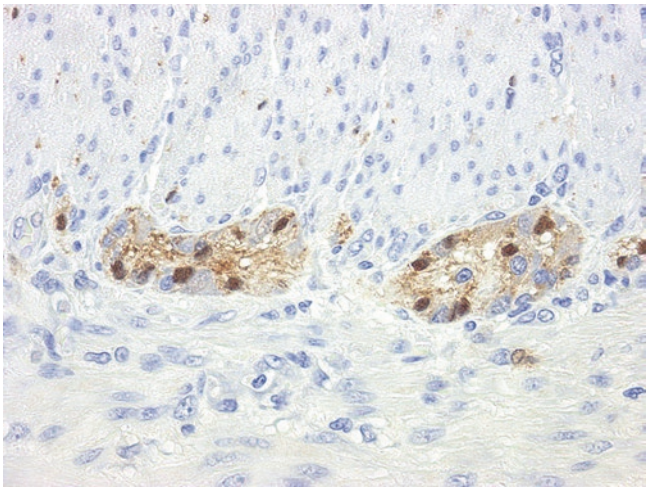

Fig. 10.5 Ganglion cells do not exhibit immunoexpression of S100 proteins

wall, all neural cells and networks are positive for CD56 (Fig. 10.4). Therefore, it is useful to understand the distributions of the neural plexus and nerve fibers.

The S100 proteins are a family of low-molecular-weight proteins and are characterized by two calcium-binding sites of the EF-hand type [19]. S100 proteins are normally present in cells derived from the neural crest (Schwann cells and melanocytes), chondrocytes, adipocytes, myoepithelial cells, macrophages, Langerhans cells, and dendritic cells. In the intestinal wall, neural fibers and enteric glia are positive for S100 proteins (Fig. 10.5). However, the ganglion cells do not exhibit immunoexpression of S100 proteins.

HuC/D, members of the Hu family, are the RNA-binding proteins that display neuron-specific expression and are involved in neuronal differentiation and the maintenance of the nervous system [20, 21]. In the intestinal wall, HuC/D

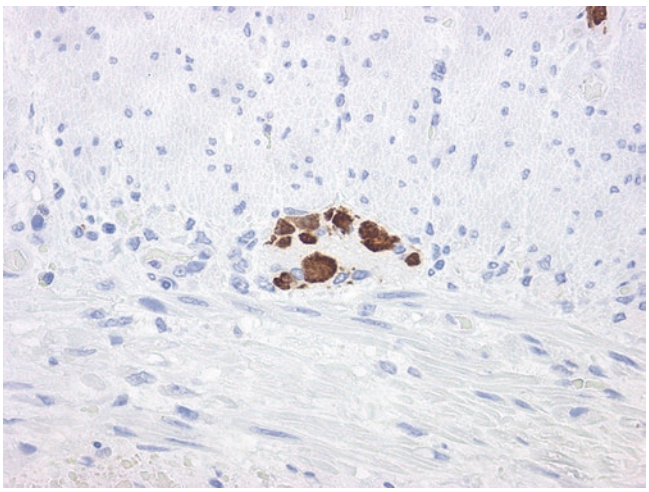

Fig. 10.6 Ganglion cells in the enteric neural plexus are positive for HuC/D

immunoreactivity reveals the ganglion cells, while the other neural cells and fibers do not show HuC/D immunoexpression (Fig. 10.6). It is thus useful to identify the numbers and sizes of the ganglion cells.

BCL-2 encodes an integral outer mitochondrial membrane protein that blocks the apoptotic death of some cells such as lymphocytes [22]. High levels of BCL-2 expression are maintained in sensory and sympathetic adult neurons [23]. In Hirschsprung's and its allied disorders, BCL-2 immunostaining reveals small or immature ganglion cells, but mature large neurons are negative or faintly stained for BCL-2 [24]. BCL-2 is the most valuable biomarker for discriminating immature small neurons [24].

Calretinin is a calcium-binding protein of 29 kDa that is a member of the family of so-called EF-hand proteins [25]. Calretinin is reported to be abundantly expressed in neurons. There is a lack of immunostaining for calretinin in aganglionic segments in patients with Hirschsprung's disease and in the nerve fibers in these areas, whereas both ganglion cells and nerve fibers show calretinin expression in ganglionic areas of Hirschsprung's disease and in the normal colon [26]. Although a clear distinction between transition zone tissue and normal tissue cannot be made using calretinin, it serves as a feasible fallback system in cases with inadequate biopsies that contain little or no submucosa [26].

10.6 Frozen Section Diagnosis

The transanal endorectal pull-through has been widely performed as a minimally invasive operative procedure to surgically correct the functional obstruction in Hirschsprung's disease. In such cases, intraoperative diagnosis of normoganglionosis in the frozen section is absolutely necessary.

Table 10.1 Pitfalls in the frozen section diagnosis

Surgical point
Full-thickness biopsy
Biopsy site: mesenteric side
Sample size: a length of at least 1 cm in the direction of the long axis
Pathological point
Maturity of ganglion cells
Absence of nerve bundles
Number of ganglion cells: 50–100 ganglion cells per 1 cm of intestinal wall

However, there are two pitfalls in the frozen section diagnosis: the first involves the site of intraoperative biopsy and the second involves the diagnosis of normoganglionosis (Table 10.1).

The transitional-aganglionic bowel junction on the mesenteric side tends to be more proximal than that on the antimesenteric side [27]. The circumferential distribution of ganglion cells in both the myenteric and the submucosal plexus in the transitional zone is uneven [28]. Even if a diagnosis of normoganglionosis is made based on intraoperative biopsy performed from the antimesenteric side, there remains the possibility of a transitional zone on the mesenteric side. Therefore, intraoperative biopsy should be implemented from the mesenteric side in frozen section diagnosis.

Checkpoints for diagnosis of normoganglionosis are the evaluations of maturity, the number of ganglion cells, and the presence or absence of nerve bundles. These assessment criteria have been previously noted. To this end, full-thickness biopsy is necessary to give an accurate diagnosis of normoganglionosis for several reasons. The transitional zone histologically shows heterogeneous maturation and numbers of ganglion cells. Seromuscular biopsy is not able to identify submucosal aganglionosis, hypoganglionosis, immaturity of ganglia, or nerve hyperplasia [6]. Moreover, a sample size with a length of at least 1 cm in the direction of the long axis is required for the evaluation of numbers of ganglion cells.

References

1. Hirschsprung H. Stuhltragheit neugeborener infolge von dilatation und hypertrophic des colons. Jb Kinderheilkd. 1888;27:1.
2. Whitehouse F, Kernohan J. Myenteric plexuses in congenital megacolon: study of 11 cases. Arch Intern Med. 1948;82:75.
3. Puri P, Montedonico S. Hirschsprung's disease: clinical features. In: Holschneider AM, Puri P, editors. Hirschsprung's disease and allied disorders. 3rd ed. Berlin: Springer; 2008. p. 107–13.
4. Suita S, Taguchi T, Ieiri S, Nakatsuji T. Hirschsprung's disease in Japan: analysis of 3852 patients based on a nationwide survey in 30 years. J Pediatr Surg. 2005;40:197–202.
5. Reid JR, Buonomo C, Moreira C, Kozakevich H, Nurko SJ. The barium enema in constipation: comparison with rectal manometry and biopsy to exclude Hirschsprung's disease after the neonatal period. Pediatr Radiol. 2000;30:681–4.
6. Martucciello G, Pini Prato A, Puri P, Holschneider AM, Meier-Ruge W, Jasonni V, Tovar JA, Grosfeld JL. Controversies concerning diagnostic guidelines for anomalies of the enteric nervous system: a report from the fourth International Symposium on Hirschsprung's disease and related neurocristopathies. J Pediatr Surg. 2005;40:1527–31.
7. Taguchi T, Masumoto K, Ieiri S, Nakatsuji T, Akiyoshi J. New classification of hypoganglionosis: congenital and acquired hypoganglionosis. J Pediatr Surg. 2006;41:2046–51.
8. Grosfeld JL. Hirschsprung's disease: a historical perspective 1691–2005. In: Holschneider AM, Puri P, editors. Hirschsprung's disease and allied disorders. 3rd ed. Berlin: Springer; 2008. p. 1–12.
9. Friedmacher F, Puri P. Current practice patterns of rectal suction biopsy in the diagnostic work-up of Hirschsprung's disease: results from an international survey. Pediatr Surg Int. 2016;32:717–22.
10. Fenoglio-Preiser CM, Noffsinger AE, Stemmermann GN, Lantz PE, Isaacson PG, editors. Hirschsprung disease, gastrointestinal pathology: an atlas and text. 3rd ed. Philadelphia: Lippincott Williams and Wilkins; 2008. p. 548–55.
11. Goldblum JR. Large bowel. In: Goldblum JR, Lamps LW, McKenney JK, Myers JL, editors. Rosai and Ackerman's surgical pathology. 11th ed. Philadelphia: Elsevier; 2018. p. 648–702.
12. Kamijo K, Hiatt RB, Koelle GB. Congenital megacolon. Gastroenterology. 1953;24:173–85.
13. Tam PK, Boyd GP. Origin, course, and endings of abnormal enteric nerve fibres in Hirschsprung's disease defined by whole-mount immunohistochemistry. J Pediatr Surg. 1990;25:457–61.
14. Taguchi T, Obata S, Ieiri S. Current status of Hirschsprung's disease: based on a nationwide current status of Hirschsprung's disease: based on a nationwide. Pediatr Surg Int. 2017;33:497–504.
15. Giles AP, Casaclang D, Keen CE. Diagnosis of Hirschsprung's disease. Pediatr Dev Pathol. 1998;1:571–2.
16. Oh JT, Han AR, Yang WI, Han SJ, Choi SH, Hwang EH. Morphometric evaluation of PGP9.5 and NCAM expressing nerve fibers in colonic muscle of patients with Hirschsprung's disease. Yonsei Med J. 2002;43:31–6.
17. Geramizadeh B, Akbarzadeh E, Izadi B, Foroutan HR, Heidari T. Immunohistochemical study of enteric nervous system in Hirschsprung's disease and intestinal neuronal dysplasia. Histol Histopathol. 2013;28:345–51.
18. Yoshimaru K, Taguchi T, Obata S, Takemoto J, Takahashi Y, Iwanaka T, Yanagi Y, Kuda M, Miyoshi K, Matsuura T, Kinoshita Y, Yoshioka T, Nakazawa A, Oda Y. Immunostaining for Hu C/D and CD56 is useful for a definitive histopathological diagnosis of congenital and acquired isolated hypoganglionosis. Virchows Arch. 2017;470:679–85.
19. Donato R. S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int J Biochem Cell Biol. 2001;33:637–68.
20. Phillips RJ, Hargrave SL, Rhodes BS, Zopf DA, Powley TL. Quantification of neurons in the myenteric plexus: an evaluation of putative pan-neuronal markers. J Neurosci Methods. 2004;133:99–107.
21. Swaminathan M, Kapur RP. Counting myenteric ganglion cells in histologic sections: an empirical approach. Hum Pathol. 2010;41:1097–108.
22. Haddad JJ. On the antioxidant mechanisms of Bcl-2: a retrospective of NF-kappaB signaling and oxidative stress. Biochem Biophys Res Commun. 2004;322:355–63.
23. Merry DE, Veis DJ, Hickey WF, Korsmeyer SJ. bcl-2 protein expression is widespread in the developing nervous system and retained in the adult PNS. Development. 1994;120:301–11.
24. Park SH, Min H, Chi JG, Park KW, Yang HR, Seo JK. Immunohistochemical studies of pediatric intestinal pseudoobstruction: bcl2, a valuable biomarker to detect immature enteric ganglion cells. Am J Surg Pathol. 2005;29:1017–24.

25. Camp AJ, Wijesinghe R. Calretinin: modulator of neuronal excitability. Int J Biochem Cell Biol. 2009;41:2118–21.
26. Guinard-Samuel V, Bonnard A, De Lagausie P, Philippe-Chomette P, Alberti C, El Ghoneimi A, Peuchmaur M, Berrebi-Binczak D. Calretinin immunohistochemistry: a simple and efficient tool to diagnose Hirschsprung disease. Mod Pathol. 2009;22:1379–84.
27. Gherardi GJ. Pathology of the ganglionic-aganglionic junction in congenital megacolon. Arch Pathol. 1960;69:520–3.
28. Das K, Kini U, Babu MK, Mohanty S, D'Cruz AJ. The distal level of normally innervated bowel in long segment colonic Hirschsprung's disease. Pediatr Surg Int. 2010;26:593–9.