Nerves in gastrointestinal cancer

Nerves in gastrointestinal cancer: from mechanism to modulations

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Abstract | Maintenance of gastrointestinal health is challenging as it requires balancing multifaceted processes within the highly complex and dynamic ecosystem of the gastrointestinal tract. Disturbances within this vibrant environment can have detrimental consequences, including the onset of gastrointestinal cancers. Globally, gastrointestinal cancers account for ~19% of all cancer cases and ~22.5% of all cancer-related deaths. Developing new ways to more readily detect and more efficiently target these malignancies are urgently needed. Whereas members of the tumour microenvironment, such as immune cells and fibroblasts, have already been in the spotlight as key players of cancer initiation and progression, the importance of the nervous system in gastrointestinal cancers has only been highlighted in the past few years. Although extrinsic innervations modulate gastrointestinal cancers, cells and signals from the gut's intrinsic innervation also have the ability to do so. Here, we shed light on this thriving field and discuss neural influences during gastrointestinal carcinogenesis. We focus on the interactions between neurons and components of the gastrointestinal tract and tumour microenvironment, on the neural signalling pathways involved, and how these factors affect the cancer hallmarks, and discuss the neural signatures in gastrointestinal cancers. Finally, we highlight neural-related therapies that have potential for the management of gastrointestinal cancers.

With more than 3.6 million diagnoses and 2.2 million related deaths in 2020 worldwide, gastrointestinal cancers (oesophagus, stomach and intestine) represent a major health burden, accounting for 22.5% of all cancer-related deaths worldwide[1]. Proper management of gastrointestinal cancers is challenging because the gastrointestinal tract is a vibrant and dynamic ecosystem wherein each component — epithelial and various other cell types, luminal contents, the microbiota and extracellular matrix (ECM) — can be an accomplice to the pathogenesis of these cancers, as they serve various biological roles. Collectively, these components constitute the so-called tumour microenvironment (TME)[2,3]. In the early days of cancer research, scientists believed that a tumour was a homogeneous mass of cancer cells. However, a plethora of long-term and in-depth investigations uncovered that tumour development closely resembles normal organogenesis and should be regarded as a heterogeneous entity resulting from the dynamic, reciprocal interactions between cancer cells and their surrounding microenvironment[4,5]. Moreover, the coordinated cellular and molecular processes that enable the body to manage its homeostatic balance will be governed by the tumour itself[1,6]. A well-understood phenomenon driving this feature is the ability of tumours to foster the formation of blood and lymph vessels by which necessary nutrients are received and by which spreading to distant sites is conferred[4,6]. To this end, the 'established' TME strongly influences the behaviour of a primary tumour, determines whether it will disseminate to other organs and affects responses to therapy[6,7]. Whereas immune, endothelial and cancer (epithelial) cells have been in the spotlight for many years, justifying the development of targeted therapies, the role of the nervous system only entered the limelight in the past couple of years, which is remarkable given the extensive innervation of the gastrointestinal tract.

The gastrointestinal tract is innervated by the three main divisions of the autonomic nervous system, which provide both extrinsic and intrinsic neural control of gut function[8,9]. The parasympathetic and sympathetic nervous systems supply the gastrointestinal tract with extrinsic innervation, that is, parasympathetic and sympathetic neurons have their cell bodies outside the gut wall, whereas cells of the enteric nervous system (ENS) are located within the bowel itself, providing intrinsic control of gut function (FIG. 1 and BOX 1). In mammals, the ENS forms an intricate neural network embedded along the gastrointestinal wall that consists of different types of enteric neurons and glia, which are

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Key points

- The gut is a highly innervated, dynamic ecosystem wherein nerves are key for intestinal functioning and homeostasis by communicating with a variety of cell types and the gut microbiome.
- Neural contributions to gastrointestinal cancers represent a flourishing area of investigation as both intrinsic and extrinsic nerves influence gastrointestinal tumorigenesis via their interplay with cancer cells.
- Neural-related signals and pathways can influence the cancer hallmarks, interfering with several cancer cell characteristics (metabolism and (epi)genomic stability) and/or supporting a cancer-promoting microenvironment (immune infiltration, extracellular matrix).
- While neoneurogenesis and axonogenesis are emerging within the gastrointestinal cancer field, both topics require in-depth investigation to identify their exact origin and driving mechanisms.
- Cancer cells are able to hijack (embryonic) neural pathways to promote their own fitness.
- Targeting neural cell-derived messengers and their respective receptors holds great promise in the treatment of gastrointestinal cancers.

predominantly clustered within interconnected ganglia of the submucosal and myenteric plexus[10]. Although the inner workings of the gut are modulated by the ENS, extensive extrinsic inputs fine-tune gut function[11]. By collaborating with a variety of cell types, such as smooth muscle cells and interstitial cells of Cajal as well as epithelial, endothelial and immune cells, the enteric neuron–glial network controls motility patterns, supports mucosal barrier function, aids in immunological defence and controls mucosal secretions[12]. The ENS has been implicated in the pathogenesis of various gastrointestinal disorders, with an obvious role in the aetiology of enteric neuropathies[13]. Moreover, increased understanding of its contribution to several other diseases, including those associated with intestinal inflammation, is currently arising[14]. Despite the recognized importance of neurons in cancer[4], the role of the nervous system in gastrointestinal carcinogenesis is only now emerging[15,16].

In this Review, we focus on the blossoming area of neural contributions to the pathogenesis of gastrointestinal cancers. We address how neurons interact with the gut microbiota and various TME components and how these interactions affect the hallmarks of cancer. Neural and neural-related intracellular signalling pathways and the neural signature in gastrointestinal cancers are also highlighted. Supported by the resulting insights, we discuss current and potential future therapeutic strategies.

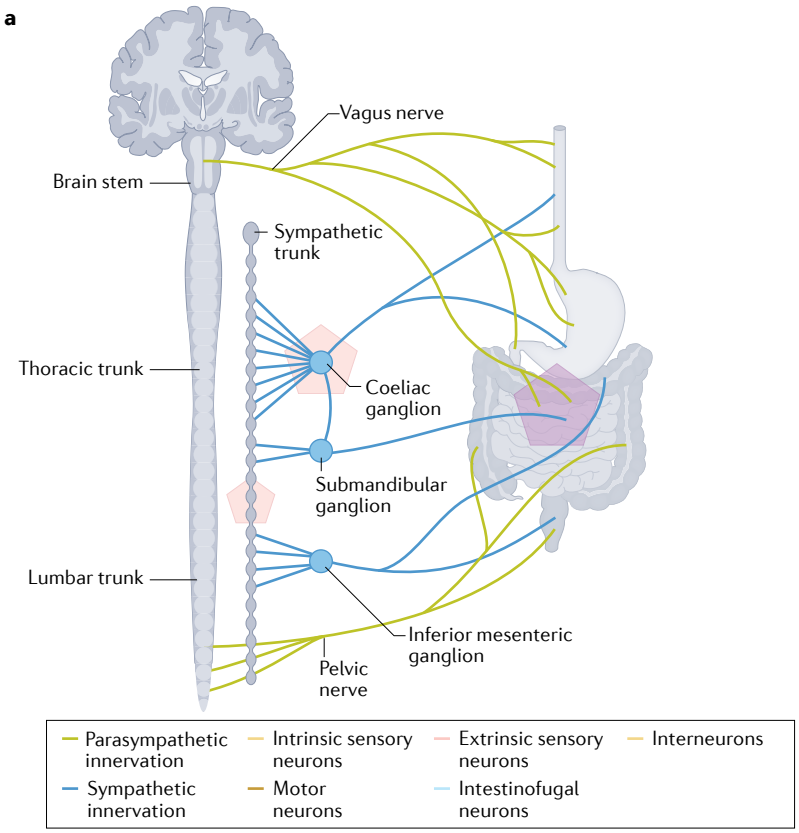
Crosstalk between cancer and neural cells

In the past decade, several landmark papers have shown that tumour innervation promotes the malignant phenotype of cancer cells. For oesophageal, stomach and colorectal cancers, it has been established that perineural invasion (BOX 2) is an independent prognostic factor associated with worse prognosis and poor clinical outcome[17–22]. Similarly, axonogenesis (that is, the formation of new axons or increased nerve density[23,24]) and (neo)neurogenesis (that is, de novo formation of nerve cells[25,26]) confer tumours a growth advantage. From the early 2000s onwards, pioneering in vivo studies started to use a variety of tools to interfere with tumour innervation and explore their mechanistic and functional importance for the aetiology of gastrointestinal cancers.

Denervation

Both surgical cutting of nerves and injection of neurotoxic drugs have been used to study the effect of denervation in cancer. In humans, extrinsic vagal denervation, termed vagotomy, either unilateral or bilateral, was first used in the management of gastric and duodenal ulcers[27,28]. This treatment seemed to increase the risk of developing gastric cancer due to delayed gastric emptying and concomitant hypochlorhydria, which could increase the levels of *N*-nitroso compounds[29–31]. Similarly, unilateral vagotomy increased the numbers of gastric tumours that were formed upon treatment of Wistar rats with the nitrosoguanidine derivative *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG)[32]. However, later papers have shown that, for gastric and small intestinal cancers, vagal denervation has antitumorigenic effects. Using a spontaneous (Ins-Gas), carcinogen-induced (MNU) and bacteria-infected (*Helicobacter pylori*–H^+/K^+–ATPase-Il1β) mouse model of gastric cancer, Zhao et al. demonstrated that surgical denervation (bilateral or unilateral truncal vagotomy) or a botulin toxin injection (which blocks acetylcholine release from axon endings) suppressed tumorigenesis[23]. In agreement, gastric cancer is more prevalent in the lesser as opposed to the greater curvature in mice and humans[33,34], which parallels the higher nerve density in the lesser curvature[23]. The discrepant outcomes between earlier and later studies are most likely explained by the fact that later studies applied vagotomy with an accompanying drainage procedure (pyloroplasty) to improve gastric emptying[31]. In a genetic intestinal cancer mouse model ($APC^{Min/+}$), tumour development in the small intestine was suppressed by subdiaphragmatic vagotomy[35]. Interestingly, for colorectal cancer (CRC), truncal vagotomy with accompanying drainage procedure had no clear effect on carcinogenesis in rats[36,37] whereas, in humans, this treatment for gastric or duodenal ulcers increased the risk of developing CRC across a range of studies of varying cohort sizes[38–40]. Further research is needed to specify if this increased risk results from delayed emptying and increased levels of gastrin, because these findings were observed in patient cohorts treated with different gastric surgeries with or without accompanying drainage.

Conflicting data have also been reported following surgical and pharmacological sympathetic denervation. The most widely used compound to achieve chemical sympathectomy is 6-hydroxydopamine, a neurotoxin that destroys catecholaminergic neurons at the injection site. Using Wistar rats, Tatsuta et al. found that prolonged administration of 6-hydroxydopamine markedly reduced azoxymethane-induced colonic tumour incidence as well as gastric tumorigenesis (MNNG-induced)[41,42]. Even though Sadighparvar et al. observed substantially fewer aberrant crypt foci following sympathetic denervation (coeliac–mesenteric ganglionectomy and guanethidine sulfate administration) in Wistar rats during 1,2-dimethylhydrazine (DMH)-induced carcinogenesis, no effect on colon tumour incidence or size was observed[43]. Surgical sympathectomy



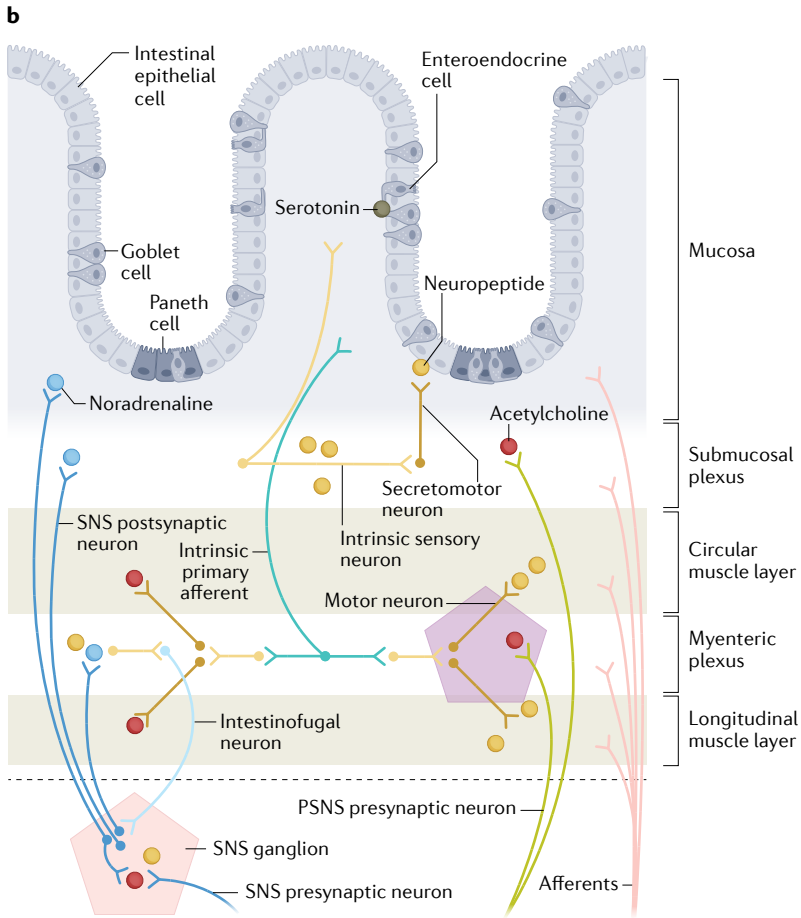


Fig. 1 | **Nervous signalling paths and modulators of the gastrointestinal tract in mammals.** Each part of the gastrointestinal tract (that is, oesophagus, stomach, small and large intestine) is differentially innervated, both extrinsically and intrinsically. **a** | Extrinsic innervation of the gastrointestinal tract. Parasympathetic (cholinergic) innervation (green trajectories) via the vagus nerve is very dense at the beginning of the gastrointestinal tract, yet becomes sparse whilst moving distally, with most parasympathetic innervation of the distal colon originating from preganglionic neurons within S1–S4 lumbosacral spinal cord regions. Parasympathetic preganglionic neurons synapse with myenteric postganglionic neurons in the gastrointestinal tract (purple pentagon). Sympathetic (adrenergic) innervation (blue trajectories) varies per organ of the gastrointestinal tract, with the cervical and thoracic trunk from spinal segments T1–T10 innervating the oesophagus, T6–T9 and T9–10 thoracic neurons supplying the stomach and small intestine, respectively, and L2–L5 lumbar regions innervating the colon. Sympathetic preganglionic neurons synapse with postganglionic neurons in the prevertebral ganglia or, yet less likely, in the paravertebral ganglia (rose pentagons). Visceral afferent fibres travel along the vagus and spinal nerves as well as the sympathetic nerves and transduce sensory signals from the enteric nervous system (ENS) (not shown). **b** | Intrinsic innervation and major synapses in the gastrointestinal tract. Schematic overview of a variety of neurotransmitters and synapses in the ENS with a specific focus on those that have been shown to be involved in gastrointestinal carcinogenesis. The ENS is embedded along the entire gastrointestinal wall with a variable design depending on the intestinal segment. The myenteric plexus continues from the upper oesophagus to the internal anal sphincter, whereas the submucosal plexuses are absent in the oesophagus, contain few ganglia in the stomach, and are only fully established in the small and large intestine. ENS neurons are depicted in various colours to distinguish subpopulations (see key). Enteric glial cells are not shown in this figure. PSNS, parasympathetic nervous system; SNS, sympathetic nervous system.

of the small intestine did not affect *Apc*-driven intestinal carcinogenesis in mice[35]. Compared with normal tissue, human gastric[44–46] and colon cancer[47] tissues were characterized by markedly reduced sympathetic nerve fibres or noradrenaline levels, which were gradually restored with increasing distance from the tumour site. However, these conclusions were drawn from studies with small patient cohorts (82 and 5, respectively) and do not provide conclusive data regarding potential positive effects of sympathetic innervation on gastrointestinal carcinogenesis.

For intrinsic denervation in animal models, benzalkonium chloride is the chemical that has mostly been used to locally damage the myenteric plexus[48]. Whereas application of benzalkonium chloride to the serosal surface of the gastrointestinal tract initially induces hyperplasia of gastric G cells, oesophageal cells and descending colonic epithelial cells[49–51], it hampers gastric and colon tumour development and growth in an MNNG and DMH rat model of gastric[52] and colon cancer[53], respectively. This finding could be mediated by an imbalance in the levels of several neural factors as well as by the interplay between benzalkonium chloride, the acidic environment and various neuropeptides. Upon

Box 1 | **Innervation of the gastrointestinal tract**

The nervous system in vertebrates is built from two main parts: the central nervous system and the peripheral nervous system, with the first comprising the brain and spinal cord, and the latter consisting of neural cells located outside of the brain and spinal cord. The peripheral nervous system can be separated into the somatic and autonomic nervous systems, with the autonomic nervous system further subdivided into the parasympathetic (FIG. 1a, green trajectories), sympathetic (FIG. 1a, blue trajectories) and enteric nervous systems[12].

Acetylcholine is the primary neurotransmitter to relay signals in parasympathetic preganglionic and postganglionic synapses as well as from preganglionic sympathetic fibres. Noradrenaline, on the other hand, is the main neurotransmitter released from postganglionic sympathetics (FIG. 1b). The native receptors via which acetylcholine and noradrenaline exert their actions are muscarinic or nicotinic cholinergic receptors, and the α-adrenoceptor and β-adrenoceptor, respectively. Acetylcholine released within ganglionic synapses primarily activates nicotinic receptors on postganglionic neurons to transmit autonomic signals from preganglionic to postganglionic neurons. Postganglionic neurons extend further to visceral organs, including the gastrointestinal tract, where parasympathetic acetylcholine binds muscarinic receptors (M_1–M_5) and sympathetic noradrenaline binds α-adrenergic receptors (α_1 and α_2) and β-adrenergic receptors (especially β_1 and β_3) on target gastrointestinal cells, including epithelial, immune and other stromal cells. Next to neuronal sources of these neurotransmitters, systemic adrenaline produced by adrenal glands or members of the tumour microenvironment can reach target cells in the gastrointestinal tract[286].

Sensory information is conveyed via visceral afferents that make up most of the extrinsic gastrointestinal innervation and largely follow autonomic fibres to reach the central nervous system following two distinct routes. Splanchnic and pelvic afferents, which have their cell bodies in thoracolumbar and lumbosacral dorsal root ganglia, mainly follow the sympathetic and parasympathetic chain, respectively, to enter the brain via the spinal cord. The nodose or jugular ganglia harbour cell bodies of vagal afferents and project to the nucleus tractus solitarius within the brainstem[287–290] (FIG. 1a). The enteric nervous system harbours region-specific circuits that integrate information from the gut lumen and gut wall via intrinsic primary afferent neurons, interneurons and motor neurons to regulate gut motility and secretomotor and vasomotor responses[287,291,292] (FIG. 1b). In addition, intestinofugal neurons project outside the gut wall and convey afferent information to sympathetic prevertebral ganglia[292]. Enteric neuron subtypes communicate with one another and with target cells, using neurotransmitters, such as acetylcholine nitric oxide and serotonin, as well as neuropeptides such as neuropeptide Y, substance P and vasoactive intestinal peptide. Collectively, these messengers assist in controlling gastrointestinal homeostasis and motility[293,294] (FIG. 1b).

benzalkonium chloride treatment, levels of substance P and vasoactive intestinal polypeptide (the two main neuropeptides driving MNNG-induced carcinogenesis) decrease, leading to an inflamed and acidic gastric environment, which reduces the effectiveness of MNNG[52,54]. Correspondingly, benzalkonium chloride-induced denervation of the rat oesophagus[51] and distal colon[55] is associated with megaoesophagus and megacolon, with the latter condition also hindering colorectal tumorigenesis in humans[56]. By contrast, a small increased risk of developing oesophageal cancer has been observed in several cohorts of patients with megaoesophagus[57]. Given that Munari et al. ascribed this risk to prolonged contact with food, microbial overgrowth and increased nitrate levels[57], it is questionable whether the absence of nerves is really accountable for the discrepant gastrointestinal cancer risks rather than the influence of the distended gut wall or obstructed luminal content on the carcinogenic process. To accommodate for this aspect, Zhao et al. used a mouse gastric model wherein they performed denervation after completion of the cancer induction protocol. The finding that vagotomy also inhibited gastric tumorigenesis in this model suggests that this is a nerve-driven inhibitory effect[23].

Before these denervation studies can be translated to the patient, it is important to fully understand the neuroanatomy and biology during carcinogenesis, which is currently far from being fully established. The manner (surgical versus chemical), location, period (short term versus long term) and timing (before or during cancer onset or progression) of the employed interference represent fundamental denominators for the experimental outcomes and could account for the discrepancies that have been described. For instance, although it seems that, especially in the human situation, denervation before the onset of carcinogenesis (for example, treatment of ulcers) inhibits tumour formation, Zhao et al. demonstrated equally positive effects of denervation before or after the establishment of gastric cancer on tumour incidence and progression in mice[23]. Moreover, the various techniques described have limitations due to their incomplete denervation, subsequent nerve regeneration or unintended secondary effects on other members of the TME. In effect, the observation that parasympathetic but not sympathetic denervation reduces intestinal carcinogenesis in the *Apc*$^{Min/+}$ mouse model might be explained by the difference in complete parasympathetic versus partial sympathetic denervation of the small intestine[35,58,59].

Modulation of neural factors

Several neurotransmitters, neurotrophic factors and their respective receptors have been shown to regulate intestinal epithelial growth and proliferation and are commonly overexpressed in gastrointestinal cancer tissue[15]. As a consequence, multiple in vitro and in vivo studies have applied a variety of techniques, such as knockdown, overexpression, blocking or (ant)agonizing, to assess the influence of neurotrophin and neurotransmitter signalling on gastrointestinal carcinogenesis. Neurotrophic factors are defined as endogenous molecules that regulate survival, growth and morphological plasticity of neurons. Considering their name, neurotrophic molecules were initially thought to be uniquely related to the nervous system. However, since the mid-1900s, it has been shown that other cells, including cancer cells, also possess the machinery to produce, secrete and respond to neural factors[60]. Interestingly, different receptor subtypes have been identified on a variety of cell types within the gastrointestinal tract. However, the implication in gastrointestinal carcinogenesis is restricted to the muscarinic M_3R receptor and, to a lesser extent, the M_1R receptor as well as the α_2-adrenoceptor, β_2-adrenoceptor and β_3-adrenoceptor for neurotransmitters and the Trk and p75^{NTR} receptors for neurotrophins[16,61]. A comprehensive overview of the main neurotrophic factors and neurotransmitters involved in gastrointestinal carcinogenesis is provided elsewhere[15,62]; however, within these studies, the origin of these messengers was often not defined. In the following section, we elaborate on the neuronal cell-derived messengers and their respective receptors on cancer cells.

Cholinergic signalling in gastrointestinal cancers. During gastric tumorigenesis, cholinergic nerve density increases with tumour progression and both mouse

and human oesophageal, gastric and colon cancer cells upregulate the expression of M_3R[23,63–67] (FIG. 2). Binding of acetylcholine to M_3R activates intracellular EGFR–ERK–AKT signalling in gastric and colon cancer cells in vitro[68,69] and in vivo (azoxymethane-induced CRC mouse model)[70]. M_3R receptor activation also induces ligand-independent Wnt signalling through YAP in vivo (MNU gastric mouse model), with a concomitant upregulation of, amongst other Wnt targets, CD44 and Lgr5 as well as nuclear translocation of β-catenin. Both pathways trigger proliferation and expansion of the gastrointestinal epithelium[63,68–72]. In addition, activating the M_3R–AMPK–MACC1 and matrix metalloproteinase (MMP) pathway by acetylcholine promotes gastric and colon cancer migration and invasion in vitro[73] and in vivo[74,75]. Progression towards a cancerous gastrointestinal epithelium reinforces bidirectional crosstalk as human gastric and colon (cancer) cells can synthesize and release acetylcholine, which subsequently stimulates neighbouring nerve fibres and axonogenesis[63–65,69,76,77].

Adrenergic signalling in gastrointestinal cancers. Given that adrenergic stimulation, for example, via α_2-adrenergic-induced EGFR–MEK–ERK signalling, in the gastrointestinal epithelium supports cell migration and wound healing[73,78], it is not surprising that dysregulation of these signalling cues is involved in the pathogenesis of gastrointestinal cancers (FIG. 3). Moreover, adrenergic signals and receptors are commonly overexpressed within cancer tissues, further suggesting that adrenergic signals take part in gastric[79] and colorectal[80] tumorigenesis. According to several in vitro and in vivo studies[79–86], the carcinogenic influences are mostly mediated by β_2-adrenoceptor and α_2-adrenoceptor, attenuating critical cell functions such as apoptosis and immune responses, inducing epithelial–mesenchymal transition, and promoting metastatic and invading capacities. Both in vitro[79,84] and in vivo[79] studies have shown that this process is mediated via activation of the VEGF–MMP and STAT3–ERK–MAPK pathways. For instance, noradrenaline promotes cell survival through AMPK-dependent autophagy in gastric cancer cell lines[85]. Similarly, β_2-signalling enhances the survival of gastric cancer xenografts, whereas pharmacological blocking of the β_2-adrenoceptor promotes apoptosis of these xenografts (propranolol induced)[79] and of CRC cells in vitro[86]. In agreement, elevated levels of the β_2-adrenoceptor have been identified as a clinically significant prognostic marker for CRC in humans[80]. Notably, the studies described mainly focus on extrinsic innervation and no knowledge is available on the ENS.

Targeting neurotrophin signalling. As mentioned earlier, acetylcholine can immediately fuel a reciprocal nerve–cancer communication by activating M_3R on epithelial cells. This process stimulates nerve growth factor (NGF) production, which subsequently targets its respective TrkA receptor on nerves and triggers cholinergic neurite growth[63]. Functional blocking or inhibition of either M_3R or TrkA receptors reduces epithelial cell proliferation and tumour innervation in mouse gastric cancer models, thereby emphasizing the contribution of neurotrophic signals to the pathogenesis of gastric cancer[63]. Likewise, oesophageal carcinogenesis can be enhanced by neurotrophic factors and their receptors. Human oesophageal cancer tissues overexpressing NGF are characterized by the presence of nerve bundles and neuropeptide-immunoreactive nerve fibres expressing the TrkA receptor[17,87]. Moreover, oesophageal cancer cells undergo apoptosis upon silencing of the non-specific neurotrophin receptor $p75^{NTR}$ (REF.[88]). However, in CRC, this receptor is often silenced in human tissues to counteract its tumour suppressive effects, that is, inhibiting proliferation and promoting apoptosis in vitro[89,90].

Box 2 | **Perineural invasion in gastrointestinal cancers**

One of the key factors affecting the aggressiveness of a cancer cell is its ability to disseminate and migrate towards remote tissue sites. Although lymph and blood vessels are considered the primary routes for cancer cells to migrate, metastatic spread via nerve fibres was identified in the mid-1800s and termed perineural invasion (PNI). To date, PNI has been recognized as an important hallmark and a prognostic feature for different cancer types. Even though it is well established that this process comprises neoplastic spreading via nerves, a universal definition for PNI is still lacking.

Originally, PNI was thought to be a passive process, being defined as "silent extensions of malignant cells along the nerve sheath" by Mohs and Lathrop in 1952 (REF.[295]). Later on, Batsakis broadly defined PNI as "tumour cell touching and invasion in, around and through nerves"[296]. This definition covers all of the histopathological characteristics that have been observed for PNI, yet might vary according to subjective interpretations. The following suggestion by Veness to cite PNI "only when tumour cells are able to invade the perineurium" seems too stringent given that the nerve sheath consists of three connective tissue layers: epineurium, perineurium and endoneurium[297]. Consequently, to further limit variable interpretations of this definition, different papers (Fagan et al.[298], Bockman et al.[299] and Nagakawa et al.[300]) have described that 'at least 33% of the nerve circumference should be surrounded by tumour cells' to classify it as PNI. Liebig and colleagues then advocated to define PNI as tumour cell touching and invasion in, around and through nerves, with tumour cells in any of the three nerve sheath layers, or involving at least 33% of the nerve circumference[301]. Importantly, it has become apparent that PNI is not a simple, passive process, but rather an active process involving reciprocal communication between nerves and tumour cells via paracrine signalling. Such a mechanism was first described by Ayala et al. using mouse dorsal root ganglia and prostate cancer cells co-cultured in a Matrigel matrix[302].

For gastrointestinal cancers specifically, the prevalence of PNI varies from 4% to 76%, depending on the specific gastrointestinal site and the definition that has been used[18,19,22,303,304]. Nevertheless, it has been identified as a prognostic factor associated with poor prognostic outcomes in a variety of gastrointestinal cancer types. For oesophageal cancer, PNI was found to be an independent predictor for shorter disease-free and disease-specific survival[17,18]. Similarly, high levels of PNI have been observed in gastric cancer, corresponding with disease progression and also predicting gastric cancer recurrence in patients who underwent curative resection[19,20]. For colorectal cancer, PNI has been characterized as a prognostic marker associated with a worse clinical outcome because of increased local recurrences and shorter 5-year disease-free survival. Its prognostic value reportedly compares with that of other well-established markers such as differentiation grade and depth of invasion[21,22]. Moreover, work by Duchalais et al. provided a mechanistic understanding of how cancer cells adhere to nerves: with tumour epithelial cells preferentially and directly adhering to enteric neurons via N-cadherin and L1CAM, predominantly at the invasive front, and faithfully following the neural trajectory[305].

These findings underscore that PNI should be respected as a high-risk feature and supports its implementation in the standardized reporting criteria of (gastrointestinal) cancers. However, determination of the PNI pattern can be challenging in clinical practice due to potential subjective pathological interpretation among certified pathologists and the requirement of deeper sections for immunohistochemistry. Thus, the identification of proper neural-related biomarkers for diagnostic purposes would be desirable[306–308].

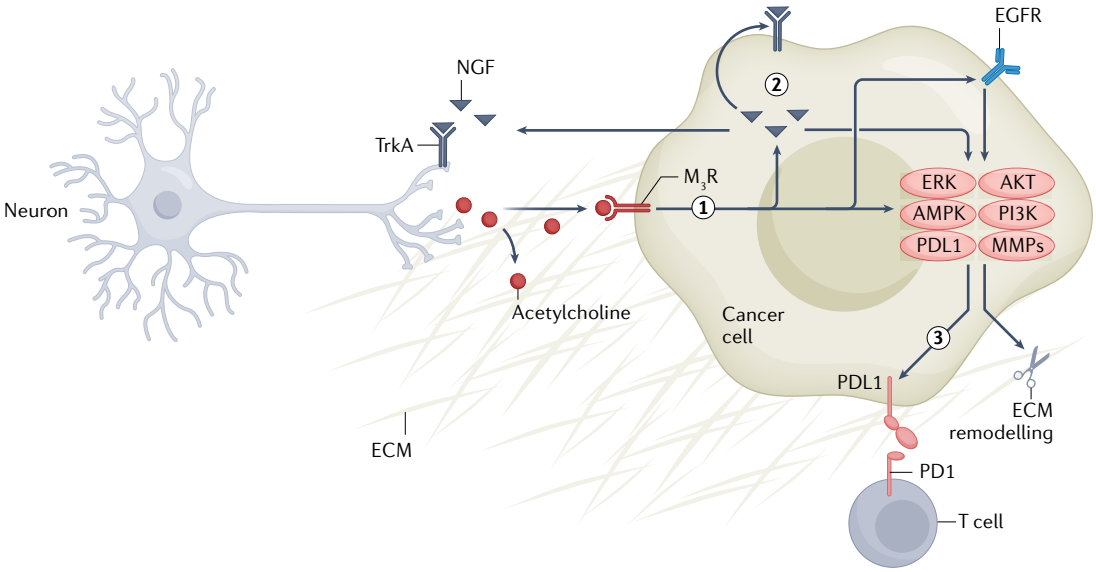


Fig. 2 | **Cholinergic signalling in gastrointestinal cancers.** Gastrointestinal cancer cells can upregulate the expression of muscarinic M_3R receptors. (1) By binding to these receptors, acetylcholine activates intracellular EGFR–ERK–AKT signalling as well as matrix metalloproteinases (MMPs), which promotes cancer cell invasion and migration. (2) M_3R activation can also stimulate nerve growth factor (NGF) production, which subsequently targets its respective TrkA receptor on nerves and triggers cholinergic neurite growth. (3) In addition, cholinergic signalling induces the expression of PDL1 ligands in colon cancer cells. ECM, extracellular matrix.

Similar to acetylcholine, noradrenaline also directly nourishes bidirectional crosstalk as its binding to the β_3-adrenoceptor increases the levels of brain-derived neurotrophic factor (BDNF), which consequently stimulates axonogenesis and tumour growth and/or progression via its native NTRK2 (also known as TrkB) receptor on nerve cells[79,91–93]. BDNF–TrkB signalling can also instantaneously modify cancer cell characteristics, favouring malignant progression in vitro[94,95]. This aspect is achieved by transactivation of the EGF pathway on cancer cells, thereby promoting the proliferation of small and large intestinal cancer cell lines[94,95], a mechanism that confers resistance to EGFR inhibitors in CRC[96,97]. Moreover, this pathway activates ERK and AKT signalling[92,98], which leads to the upregulation of anti-apoptotic proteins, such as BCL-2, in vitro. Such survival effect is abolished by BDNF knockdown in CRC cell lines[99]. These data might justify studying the benefits of therapeutic co-administration of TrkB and EGFR inhibitors in patients with CRC.

Taken together, these studies emphasize that a variety of neural signalling mechanisms fuel the interaction between the nervous system and intestinal epithelium. However, even though some mechanistic insights have established how cancer cells regulate nerve recruitment, we need to better understand which cells secrete which neural factors and which receptor is targeted. Interestingly, this nerve dependence in cancer follows a long-reported nerve dependence during tissue regeneration and tissue remodelling, and common cellular and molecular mechanisms have been described. Here, outgrowth and infiltration of nerves have also been observed, and the release of transferrin[100], neuregulin[101] and the neurotransmitter substance P[102] by nerve endings can rescue the regeneration capacity in several experimental degeneration models; factors for which also an oncogenic potential has been described. Although the concept of neoneurogenesis during cancer development and/or progression is discussed in several papers, this aspect remains mainly a 'black box' in gastrointestinal cancers. It has been described in vivo that neural progenitor cells from the brain escape the blood–brain barrier to colonize prostate cancer[28] and that cancer stem cells have the potential to differentiate into neural-like cell populations both in vitro and in vivo[25,26]. However, these findings need more in-depth investigation, particularly in the context of gastrointestinal cancers. In fact, as the cancerous gastrointestinal tract contains cells with neurogenic potential[103], future studies need to define whether they contribute to neoneurogenesis in gastrointestinal cancers.

Neural cells, the TME and hallmarks of cancer

In 2000, Hanahan and Weinberg introduced the hallmarks of cancer, a rationalizing set of six acquired biological properties that govern neoplastic transformation[104]. A decade later, they revised this list, resulting in ten hallmarks: "sustaining proliferative signalling", "evading growth suppressors", "resisting cell death", "enabling replicative immortality", "inducing angiogenesis", "activating invasion and metastasis", "tumor-promoting inflammation", "genome instability and mutation", "deregulating cellular energetics" and "avoiding immune destruction"[6]. As reasoned earlier, the interaction between nerves and their environment clearly influences the survival, growth and dissemination of tumour cells. Based on these findings, Senga and Grose recommended appending "neuronal signalling" to the hallmarks, in addition to "dedifferentiation and transdifferentiation", "epigenetic dysregulation" and "altered microbiome"

(discussed later)[105]. Although limited data are available on how the nervous system influences cancer hallmarks with respect to gastrointestinal cancers, in the following sections, we discuss studies that provide evidence on the role of neural signalling in this context (FIGS. 2–4).

Angiogenesis and the neuroendothelial unit

In 1971, Folkman reported angiogenesis as an important trait of tumours as it enables the supply of necessary nutrients to ensure tumour cell survival[106]. This tumour-associated neovasculature was initially thought to be vital only for aggressive and rapidly growing tumours. Yet, nowadays, angiogenesis has a full-standing status within the hallmarks of cancer as it also contributes to the initial cancer stages[6]. Neuroangiogenesis refers to the process wherein nerves bundle along blood vessels, which is key for organ development and wound healing but also seems to contribute to the carcinogenic process[107–110]. Within this partnership, endothelial cells are partially guided by neural signals and vice versa; that is, angiogenic factors promote neural growth[79,111,112]. During prostate cancer, it is well established that adrenergic cues (such as noradrenaline), induce an 'angiogenic switch' in endothelial cells, thereby tuning the initiation and patterning of angiogenesis in vivo[113]. Such mechanism is not well defined for gastrointestinal cancers yet is supported by the fact that activation of either β-adrenergic receptors or exogenous administration of (nor)adrenaline upregulate levels of angiogenic factors such as VEGF, MMPs and IL-8 in CRC and gastric cancer both in vivo and in vitro — an effect that is abolished by blocking the β_2-adrenoceptor[79,114]. Similarly, the inhibition of β_1-adrenergic receptors suppresses endothelial cell proliferation via inhibition of the glycolytic flux, which limits tumour formation of CRC cells orthotopically injected in the caecum of immunocompromised mice[111]. Again, this current knowledge is restricted to extrinsic innervation; however, exogenously added serotonin could also exert pro-angiogenic effects by activating endothelial cells directly[115] or by inhibiting expression of the angiogenic inhibitor MMP12 in tumour-infiltrating macrophages within colon cancer allografts[116]. By contrast, dopamine has an antiangiogenic effect by binding D2 receptors on endothelial cells, leading to the suppression of VEGFR2 phosphorylation and downstream MAPK and FAK signalling in colon and gastric cancer-bearing rodents, thereby inhibiting endothelial cell proliferation and migration whilst promoting tumour cell apoptosis[117–120]. Thus, specific studies of the ENS in this regard are needed.

Activating invasion and metastasis

Structural and compositional changes of the ECM scaffold within the TME have a leading role in tumour growth and metastasis[4]. Initially, the ECM provides a physical barrier that limits tumour development and prevents immune cell infiltration[4,121]. Several neurotrophic factors, neurotransmitters and neuropeptides

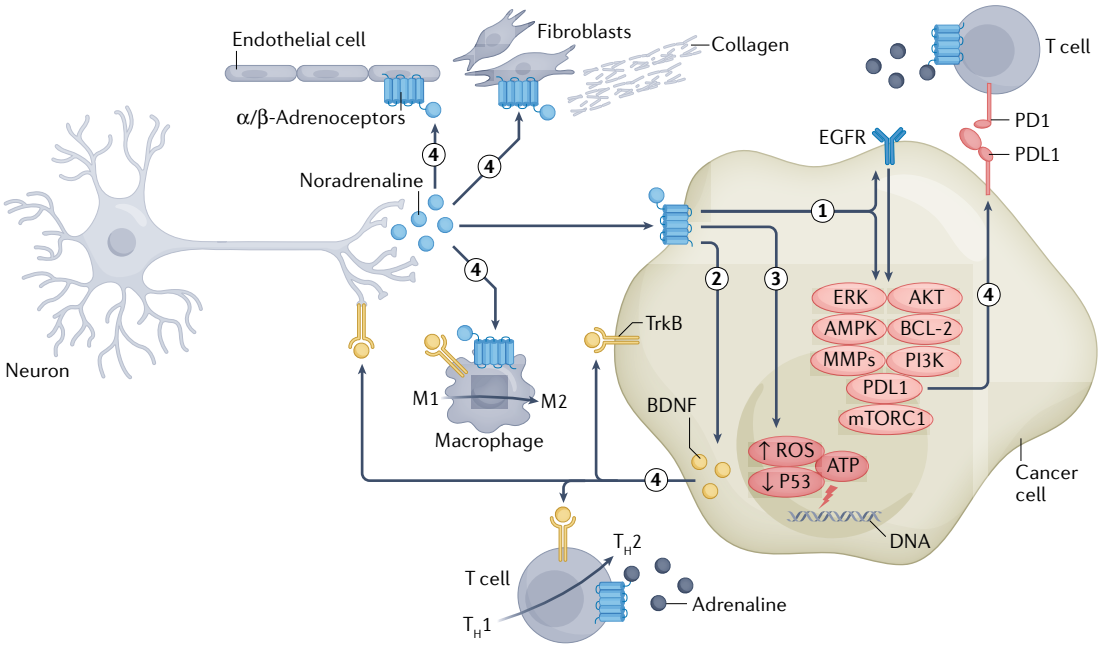


Fig. 3 | **Adrenergic signalling in gastrointestinal cancers.** (1) Signalling via α-adrenoceptors and β-adrenoceptors activates the VEGF–matrix metalloproteinase (MMP) and STAT3–ERK–MAPK pathways, attenuating apoptosis and immune responses, inducing epithelial–mesenchymal transition and promoting metastatic capacities of the tumour. (2) β_3-Adrenoceptor activation increases the levels of brain-derived neurotrophic factor (BDNF), which stimulates axonogenesis. BDNF–TrkB signalling transactivates the EGF pathway on cancer cells. (3) Adrenergic signalling stimulates the degradation of p53, thereby leading to the accumulation of DNA damage in cancer cells. Upregulation of BCL-2 inhibits their apoptosis. (4) Moreover, β-adrenergic signalling increases type I collagen fibres via activation of cancer-associated fibroblasts, induces an angiogenic switch in endothelial cells, mediates the transformation of M1 (pro-inflammatory) to M2 (anti-inflammatory) macrophages, shifts cytokine production from T helper 1 (T_H1) to T_H2 pattern, controls lymphocyte trafficking, and blocks effector $CD8^+$ T cell activation through PDL1 upregulation. ROS, reactive oxygen species.

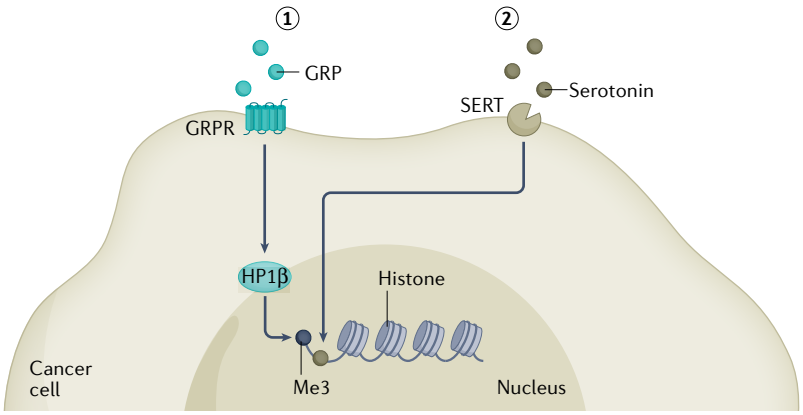


Fig. 4 | **Neural-related factors alter the epigenome in gastrointestinal cancer cells.** (1) Gastric-releasing peptide (GRP) upregulates the heterochromatin protein 1β (HP1β) in colon cancer cells. HP1β mediates chromatin condensation and gene silencing stabilization. (2) Serotonylation of the glutamine residue on histone 3 H3Q5 stabilizes H3K4 methylation. GRPR, gastric-releasing peptide receptor.

upregulated during carcinogenesis can introduce changes within this scaffold in favour of tumour progression and spreading, in particular by directly activating MMPs or indirectly triggering TME cells that actively produce and remodel the ECM (FIGS. 2 and 3).

In accordance with its overexpression in human gastric cancer and CRC cells and tissues[23,63,122], expression levels of M_3R have been shown to correlate with cancer stage as well as lymph node metastasis in gastric cancer[64]. Activation of M_3R on cancer cells in vitro results in the upregulation of several MMPs, including MMP1, MMP7 and MMP10, which facilitate CRC cell migration and invasion; this effect can be reversed by blocking MMP1 activation[74,75,122–124]. Additionally, MMP2, MMP7 and MMP9 levels are increased in human gastric cancer and oesophageal cancer tissues[125–127] but their potential regulation by neural factors is currently not established. Furthermore, (nor)adrenaline can also stimulate migratory capacities of gastric cancer xenografts[79] and colon cancer cells in vitro, respectively, which can be inhibited by the β-adrenergic blocker propranolol[128]. Interestingly, dependent on which receptor NGF activates, contradictory effects on ECM remodelling have been reported. TrkA activation facilitates CRC cell migration and invasion in vitro via MAP–ERK signalling and enhances MMP2 and MMP9 activity[129]. By contrast, NGF signalling via $p75^{NTR}$ suppresses gastric cancer cell metastasis by attenuating urokinase-type plasminogen activator (a cell motility factor) and MMP9 levels, whilst increasing levels of the tissue inhibitor of matrix metalloproteinase 1 (TIMP1)[130]. This finding is reflected by upregulated $p75^{NTR}$ levels in non-metastatic compared with metastatic human gastric cancer tissues[130]. During gastric and colon cancer, the BDNF–TrkB pathway promotes invasion and suppresses anoikis in vitro and in vivo (intravenously injected), presumably by boosting the epithelial–mesenchymal transition[94,95,131]. Finally, in gastric xenografts, inhibition of TrkB receptors proved sufficient to abrogate this effect[131].

Besides MMPs, noradrenaline can cause an increase in type I collagen fibres via activation of cancer-associated fibroblasts (CAFs)[2,132]. According to evidence in experimental models, this step favours tumour progression, in part by supporting migration of blood vessels and nerve fibres and paving the way for tumour cells to invade[4,133]. CAFs are one of the most dominant members of the TME, representing a heterogeneous group of activated fibroblasts that are involved in several hallmarks of cancer. Their extensive functions in gastrointestinal cancers are comprehensively reviewed elsewhere[134–136]. Importantly, in addition to CAFs, enteric neurons themselves are also capable of synthetizing and secreting ECM molecules as first evidenced by the Goldstein group[137,138]. In 2021, Vaes et al. also uncovered that mature enteric neurons can secrete ECM molecules, such as Nidogen 1 and Fibulin 2, thereby promoting colorectal carcinogenesis[139]. Furthermore, myenteric denervation by benzalkonium chloride administration has been shown to be associated with an increased frequency of reticular and elastic fibres within the non-cancerous gastric mucosa, whereas it shifts the fibrillary component towards more elastic fibres in gastroadenocarcinomas (benzalkonium chloride plus MNNG rat model)[140]. Such observation correlates with increased expression of elastin in human colorectal tumour tissues and cancer cells[141]. This finding suggests that, in this situation, elastic fibres have protective effects and that degradation of these fibres, as observed in non-denervated gastric adenocarcinomas in MNNG-treated rats, supports aggressive tumour growth[140,142].

Genome instability and epigenetics

Tumour formation represents a multistep process driven by (epi)genomic alterations in non-neoplastic cells[6]. It has been described that various neurotransmitters can influence the cellular genome mainly via pathways involved in DNA damage and repair. For instance, via its β_1-adrenoceptor, noradrenaline protects DNA in CRC cell lines from oxidative stress[111,143,144]. Similarly, serotonin protects the colonic epithelium against carcinogen-induced DNA damage in tumorigenic mouse models[145]. On the other hand, adrenaline has been shown to stimulate degradation of p53 and the production of reactive oxygen species via β_2-adrenergic receptors in vitro, thereby leading to DNA damage in stem cells and cancer cells[146–149]. Next to the genome, the cellular epigenome can also be regulated by several neuropeptides and neurotransmitters such as gastric-releasing peptide (GRP) and serotonin (FIG. 4). In cultured human colon cancer cells, GRP-induced signalling enhances the expression of the heterochromatin protein 1β (HP1β)[150,151]. Even though HP1β is a known epigenomic reader and modulator with critical effects on chromatin structure[152], the exact consequences on heterochromatin status in gastrointestinal cancers are currently unknown and warrant further investigation[150]. Nevertheless, antagonizing GRP-induced signals in CRC cells in vitro reduces HP1β levels and the concomitant invasive characteristics as well as cell growth via EGF signalling[153–156]. Importantly, when combined with 5-fluorouracil, an anti-metabolic drug widely used in cancer chemotherapy, GRP antagonists synergistically inhibit CRC cell growth in vitro[157]. Serotonin has

been shown to bind the glutamine residue on histone 3 (H3Q5) next to the critical lysine residue (H3K4). The H3K4 residue represents a major methylation site that is known for its global effects on gene expression in normal and cancer cells[158]. This so-called 'serotonylation' of Q5 stabilizes H3K4 methylation[158]. Moreover, serotonylation can influence the functioning of proteins such as the mTORC1 oncoprotein. Inhibiting this serotonylation leads to diminished cancer cell proliferation in vitro and reduced tumour size in an ectopic CRC mouse model[159]. Again, with most data being derived from in vitro experiments, it cannot be deduced where the neuronal messengers are derived from.

Reprogramming energy metabolism

To fuel their own growth and survival, cancer cells adapt their metabolism to comply with the high energetic demand of carcinogenic processes. Oncogenic mutations and subsequent mitochondrial dysfunction render tumour cells, including gastrointestinal cancer cells, dependent on glutamine metabolism[160–164]. In 2021, Rabben et al. uncovered that vagal innervation of gastric tumours maintains glutaminolysis in mice, whereas vagotomy re-established energy production by oxidative phosphorylation[160]. This innervation-induced metabolic reprogramming effect is mediated by modifying functions of metabolic regulators such as HIF1A and SIRT1 (REF.[165]). Besides, adrenergic signalling affects cancer cell metabolism as it has been implicated in mitochondrial respiration of (colon) cancer cell lines that express the β_1-adrenoceptor. More specifically, nebivolol, a common β_1-receptor blocker, inhibited mitochondrial respiration and subsequent ATP synthesis in several cancer cell types (including colon cancer) in vitro by upregulating ATPase inhibitory factor 1 (IF1) levels and impairing the phosphorylation of components of respiratory complexes I and V. Additionally, in vivo, when colon cancer cells were injected subcutaneously in nude mice, nebivolol impaired energy production and proliferation of colon cancer cells, while enhancing their apoptotic rate[111], thereby pinpointing towards the glycolytic-inducing capacities of noradrenaline.

Inflammation and immune evasion

Even though the neuroimmune crosstalk is increasingly recognized as a crucial regulator of digestive function and gastrointestinal homeostasis[166], mechanistic understanding of neuroimmune interactions in the context of gastrointestinal cancers is limited. Although, so far, the focus has been on innate macrophages as well as on adaptive T cells, a role for neural signalling to other types of immune cells is also likely as these cells express a variety of neurotransmitter receptors that interfere with their ability to attack tumour cells[167–169]. However, here, we only focus on established neuroimmune interactions during gastrointestinal carcinogenesis.

Neural signals and innate immune cells. For breast, prostate and pancreatic cancer, extensive evidence depicts the opposing effects of sympathetic (enhancive) and parasympathetic (suppressive) innervation on the recruitment of tumour-associated macrophages (TAMs)[170]. However, this process is less well established for gastrointestinal cancers. Chronic restraint stress[79,85] and unresolved inflammation represent an important risk factor for the development of gastric cancer, causing immune cell infiltration and subsequent promotion of epithelial proliferation. As a result, the TME of patients with gastric cancer is usually characterized by high levels of inflammation and recruited TAMs[171,172]. Importantly, stress and infection-induced inflammation activate β-adrenergic signalling, to which TAMs and muscularis macrophages can respond. Thus, sympathetic signalling modulates macrophage infiltration turning the TME into a tumour-promoting environment, an aspect observed in different cancer types[173]. β-Adrenoceptor signalling also results in suppressed immune activity, potentially via transformation of M1 (pro-inflammatory) to M2 (anti-inflammatory) macrophages, and the subsequent production of polyamines[166,174–177]. This process represents a plausible mechanism given the observation that the presence of polarized M2 macrophages (anti-inflammatory) correlates with tumour progression in patients with gastric cancer ($n = 113$)[178] and CRC ($n = 30$ (REFS.[179,180]), $n = 205$ (REF.[181])). Still, the role of TAMs in CRC is rather conflicting, partly dictated by their position within the TME. In fact, TAMs are considered oncogenic because they can promote cancer cell invasion and metastasis while suppressing antitumour immune responses. At the same time, their absence can induce similar effects, probably through a compensatory mechanism. Given that the presence of TAMs within tumour stroma negatively correlates with survival of patients with CRC and their position at the tumour front has the opposite effect, it could well be that their location within the TME determines their eventual antitumorigenic or protumorigenic capacity[182].

Neural signs and adaptive immune cells. Infiltration of $CD8^+$ effector T cells (or cytotoxic T lymphocytes) in the TME is associated with improved survival for patients in many types of cancer, including gastrointestinal cancers[183–185]. In human CRC tissues ($n = 39$), perineural invasion was associated with decreased effector memory T cells[186]. This finding is supported by the observation that β_2-adrenergic-mediated signalling controls lymphocyte trafficking in mice and blocks effector $CD8^+$ T cell activation both in vivo[187,188] and in vitro[181]. Such an effect can be reversed by blocking β_2-adrenergic signalling, resulting in T cell activation and tumour shrinkage in mice[168,189,190]. Interestingly, Tavazoie et al. observed that liver X receptor (LXR) agonists reduce the abundance of innate, myeloid-derived suppressor cells, which triggers $CD8^+$ T cell responses and tumour regression, in both mouse and humans with colon cancer[191]. In human gastric cancer, increased $CD8^+$ T cell density combined with elevated PDL1 expression correlates with metastatic and more advanced disease stages, which, according to research from other cancer types, can be induced by tumour innervation[192–194]. Adrenergic signalling, for instance, is associated with an increase in PDL1 expression in pancreatic cancer cells in vitro[195] and nerve fibres themselves can produce PDL1 in prostate cancer[196]. Interestingly, work published as a preprint by Kuol et al.

suggests that expression of PDL1 ligands in CRC cell lines can be induced by cholinergic signalling[197]. Based on these data, a combined treatment effect of neuromodulators and immune-checkpoint inhibitors warrants future investigation. Nerves have similar tumour-supporting effects via T helper cells. Hou et al. revealed a shift from T helper 1 (T_H1) to T_H2 cytokine production in a mouse stress model of CRC[198], whereas suppression of β-adrenergic signalling redirected the balance towards the T_H1 side[199]. Similarly, Mitsui et al. showed that truncal vagotomy in mice limited T_H1 and T_H2 cytokine levels within the small intestine[200].

Although it is apparent from the studies highlighted already that neural signalling can influence cancer hallmarks, there is still a largely undiscovered field of investigation with huge potential to better understand gastrointestinal cancer pathogenesis and to identify novel targets for proper gastrointestinal cancer management. As it is already known that various subtypes of immune cells have different effects on cancer hallmarks[201], future studies should also investigate the influence of different neuronal subtypes on cancer hallmarks and further distinguish local and systemic influences. In experimental animal models of melanoma and pancreatic cancer, it is described that sensory neurons are also involved in tumour initiation and progression, whereas, to our knowledge, this is not studied in other gastrointestinal cancers[187,202]. Interesting, but not discussed within this Review, is the role of stress on gastrointestinal cancers, especially as we know that stress hormones can also affect cancer hallmarks as discussed earlier. For more information on this matter, we would like to refer to other reviews covering this topic[146,188].

Finally, we would like to address that, despite an increasing body of literature showing that glial cells are vital for nervous system function and homeostasis[203], glial cells are not specifically considered in the neuronal signalling hallmark. Enteric glia are the non-neuronal cells within the ENS that not only provide structural support to neurons but also regulate several tasks important for gut function such as intestinal motility and epithelial barrier integrity[103,204,205]. Enteric glia are active partners in ENS activity and possess the required machinery to integrate and transmit information along enteric neural circuits[206,207]. Although the exact role of enteric glia is not clear, the ENS is involved in several mucosal functions, including the maintenance of epithelial integrity and the gastrointestinal stem cell niche in the so-called neural–glial–epithelial unit[208,209]. During gastrointestinal carcinogenesis, these neuroglial networks exhibit structural abnormalities, with a denser and more branched network towards cancer cells as well as a changed cellular subtype distribution with increased levels of neuroprotective messengers like PGE2, TGFβ and galanin, as observed in vitro and in mouse and human tissues[210–213]. Additionally, when activated by colon cancer cells, enteric glial cells, at least in vitro, stimulate stem cell expansion and tumour formation via the EGFR–ERK pathway, highlighting the reciprocal communication within the TME[210]. Also, depletion of $GFAP^+$ enteric glia prior to the induction of CRC in mice (azoxymethane–dextran sodium sulfate and $Apc^{Min/+}$) markedly reduces tumour burden (about 80–90% and 30%, respectively), whereas $GFAP^+$ glial cell depletion after tumour formation does not affect tumour growth or number[214]. This finding supports the notion that enteric glial cells mainly contribute to the initial phases of colorectal carcinogenesis — a finding that is further evidenced by Baghdadi et al., who revealed that $GFAP^+$ enteric glia regulate the regeneration of the intestinal stem cell niche via WNT signalling[215,216]. Together, even though there is much to uncover, also in the context of their possible contribution to neoneurogenesis[217], these data pinpoint to the addition of glial cells to the cancer hallmarks.

Neural relationship with the microbiota

Together with the enteric nervous, immune and endocrine systems, the multifaceted intestinal microbial community (BOX 3) orchestrates intestinal responses to pathophysiological challenges[3,218,219]. Even though thorough experimental evidence linking the nervous system, microbiota and gastrointestinal cancers is currently lacking, the findings discussed next suggest functional associations between these three components, as the ENS and microbial community are both altered during gastrointestinal cancers and reciprocally communicate and modulate each other's composition and functioning during health and disease.

Using a *sox10* mutant zebrafish line, a Hirschsprung model characterized by absence of the ENS, Rolig et al. described profound alterations in the microbiota with an excess of pro-inflammatory microorganisms and a lack of anti-inflammatory lineages[218]. Vice versa, the microbiota influences the structural organization of enteric neuron–glial networks as well as ENS and gut functioning (for example, intestinal permeability and ion transport) in a region-specific fashion[220–224]. However, whether these are direct or indirect effects remains to be investigated. Notwithstanding possible links with gut innervation, the importance of a diverse yet balanced microbial

Box 3 | **The gut microbiota**

The complex ecological system of microorganisms colonizing our digestive system — the gut microbiota — has a long-standing reputation of preserving our intestinal health[232,309]. As early as 1901, enriched levels of bacteria within the gastrointestinal tract were discovered[310]; thereafter, the presence of viruses, fungi and archaea was confirmed, with increased density whilst moving along the gastrointestinal tract[311]. Together, these species, both commensal and pathogenic, account for more than 100 trillion microorganisms, forming the largest reservoir of microorganisms communal to humans. This reservoir has co-evolved with its host to establish a sophisticated and mutually favourable relationship.

The gut microbiota is mostly established at birth but its composition changes swiftly over the first couple of years of life, reaching a diverse, adult-like microbial composition at ~2.5 years of age[312]. In humans, this system comprises three primary phylae, Bacillota (synonym Firmicutes), Bacteroidota (synonym Bacteroidetes) and Actinomycetota (synonym Actinobacteria)[313], and offers benefits to the host by strengthening intestinal epithelial barrier integrity[314], regulating host immunity[315] and protecting against pathogens[316]. Despite its relatively constant composition, gut microbiota can be influenced and/or disrupted by environmental factors, including diet, lifestyle and antibiotic use. This disruption can lead to an imbalance between intestinal microorganisms, or 'dysbiosis', which impairs intestinal homeostasis and could set the stage for the development of several intestinal diseases such as inflammatory bowel disease and cancer[232].

community and the fact that imbalances herein might have deleterious consequences are evidenced by various experimental models[225]. Disruptions of the commensal microbial community in mice have been shown to be associated with the development of (gastrointestinal) diseases, including gastrointestinal cancer[226–228]. In this regard, the gut microbiota has been proposed as a novel hallmark of cancer[105].

Microbiota influence intestinal health by producing a diverse metabolite repertoire from dietary products within the gut, which, amongst other functions, strengthen the intestinal epithelial barrier and immune defence mechanisms[229]. However, upon intestinal dysfunction and/or carcinogenic transformation, the intestinal epithelial barrier gets compromised, providing microorganisms with the opportunity to infiltrate the internal environment, thereby setting the stage for disease progression[230–233]. One of the best-characterized commensal microorganisms residing in the gastric and duodenal lumen is *H. pylori*[226,234]. However, this strain, alone or in combination with other aetiological factors, also represents one of the main risk factors for the development of gastric cancer and potentially increases the risk of CRC[235]. Infection with *H. pylori* can affect the release of different neurotransmitters, such as vagal acetylcholine, which influences ENS morphologically (for example, neuronal and axonal degeneration) and functionally (for example, changes in neuropeptide levels such as vasoactive intestinal peptide and c-fos) as well as the composition of the gut microbiota[236,237]. Likewise, during *Citrobacter rodentium* infection in DMH-induced and $Apc^{Min/+}$ mouse models of CRC, adrenergic signalling via the β_2-adrenoceptor leads to recruitment of $ChaT^+$ T cells, thereby promoting colonic tumour development[238]. Additionally, gut microbiota have the ability to influence CRC susceptibility and progression by producing protumorigenic, neuroactive metabolites and modifying cancer hallmarks (for example, inflammation and genome instability) as evidenced by human-derived microorganism screenings[239,240]. This phenomenon is further reflected by the observation that the gut microbiome of patients with CRC displays prominent differences compared with healthy individuals, including reduced butyrate-producing bacterial lineages and enriched levels of pathogenic bacteria such as *Escherichia coli*, *Salmonella* and *Shigella*. Such bacteria have been shown to strongly adhere to colonic epithelial cells and induce DNA damage in vitro (rat cell lines) and in vivo (CRC mouse models)[3,241–243]. Patients with CRC also display an altered virome (such as presence of cytomegalovirus) and mycobiome (for example, abundance of fungal *Malassezia*), when compared with healthy individuals, but no link with neural messengers has been established yet[228].

Neural stemness and signatures in cancers

Cancer biology represents an intriguing process that seems to combine the fundamentals of embryonic development and organogenesis. Embryonic stem cells have the ability to differentiate into all three germ layers. However, their native identity is neural as they differentiate into a neural lineage (ectoderm) in vitro in the absence of external cues, that is, under minimal culturing conditions[244,245]. It is arguable that the neural-default state of embryonic stem cells goes along with their tumorigenic ability because it is well established that embryonic stem cells are capable of forming 2D colonies and 3D teratomas, that is, tumours, when propagated in vitro and in vivo, respectively[246]. Analogue observations have been described for neural precursor cells. With increased potential for tumorigenesis — in terms of the required number of injected cells and tumour size, when compared with embryonic stem cells — neural precursor cells could be regarded as 'potent' tumorigenic cells[247,248]. Some derivatives of the neural precursor lineage, like neural crest cells, also display potent disseminating capacities as they can undergo an epithelial–mesenchymal transition and migrate away through xeno-environments to their destinations[249]. Interestingly, both embryonic stem cells and neural precursor cells lose their tumorigenicity when forced to differentiate by retinoic acid in vitro[248]. In most common gastrointestinal cancer types, cancer cells are primarily restricted to an epithelial lineage identity yet they still express potency markers such as MYC, OCT4 and SOX2 (REF.[250]). Key effectors crucial for neural stemness (for example, nervous system polycomb 1 and Musashi 1) have also been shown to be important for colon and gastric cancer cells[251–254]. Advances in multi-omics have highlighted that neural-related genes are more prone to DNA hypermethylation and, subsequently, are more often downregulated in human gastrointestinal cancers[255–258]. Although this finding seems counterintuitive, these downregulated pathways are related to neuron formation and functions[255–258]. In this regard, it is tempting to suggest that cancer cells hijack neural reprogramming pathways to promote their tumorigenicity and potency. As described earlier, gastrointestinal cancer stem cells are able to generate a neuron-like phenotype, mimicking neuronal cells both in vitro and in vivo, as indicated by the protein expression of neural markers such as Tuj1 (REF.[26]). However, functional neuronal properties, such as action potential firings, have not been evaluated and are needed to further identify the functional role of these cells during gastrointestinal carcinogenesis. Similar to embryonic stem cells and neural precursor cells, gastrointestinal cancer cell lines treated with retinoic acid to stimulate neuronal differentiation have less tumorigenic capacities[259,260]. Altogether, these findings propose a connection between neural identity and pluripotency as well as tumorigenicity.

Therapeutic potential

In view of the importance of the nervous system and neural signalling pathways in gastrointestinal cancer development, progression and dissemination, the modulation of neural–cancer crosstalk as well as of neural and neural stemness pathways in cancer cells is gaining more ground in the approach against gastrointestinal cancers. These strategies mainly comprise antagonizing parasympathetic and sympathetic activity and modulation of other neurotrophic signalling mechanisms, though they are not yet aimed at targeting the ENS.

Targeting sympathetic signals

Blocking sympathetic β-adrenergic receptors has shown promise in lowering the recurrence and long-term mortality of several (gastrointestinal) cancer types[261,262], but failed to reduce short-term mortality in patients with oesophageal cancer[263,264]. Moreover, a meta-analysis of the observational studies in CRC have shown no improvement in overall survival (HR 0.90, 95% CI 0.93–1.10)[265]. Based on the involvement of β-adrenergic signalling in gastrointestinal carcinogenesis and the ability of β-blockers to enhance radiation efficacy in colon tumours in vivo[266,267], intervention-designed clinical trials in patients with gastric cancer and CRC are currently being conducted. Such trials assess the potential benefits of these beta-blockers either preoperatively (gastrointestinal cancer[268]), in combination with chemotherapy and/or radiotherapy (gastric cancer[269], oesophageal adenocarcinoma[270]), or perioperatively with a prostaglandin inhibitor, aiming to ameliorate stress-inflammatory responses (CRC[271]).

Targeting parasympathetic signalling

Inhibition of the acetylcholine pathway (botulinum toxin injection) weakens colonic tumour growth and invasiveness and sensitizes gastric cancer cells to chemotherapy. This process results in prolonged survival of mice with gastric cancer[64,69,272,273]. As it has been shown that surgical denervation and botulinum toxin suppresses gastric cancer and recurrence in mice and humans[23,31], botulinum toxin was tested in a pilot phase II trial consisting of six patients with gastric cancer[274]. Despite promising safety, its application to treat gastric cancer still needs broader investigations[160].

Targeting neurotrophic receptors

Owing to the well-described role of neurotrophins and their Trk receptors in tumorigenesis, they represent possible targets in the treatment of gastrointestinal cancers. Blocking NGF–TrkA or BDNF–TrkB signalling reduces innervation and the size of gastric tumours in genetic mice models[63] and suppresses gastric cancer growth in vitro and in vivo[131]. In this respect, larotrectinib and entrectinib, two potent small-molecule inhibitors of Trk, have been approved by the FDA to treat Trk fusion-positive tumours[275]. Clinical trials and development of second-generation Trk inhibitors are also ongoing to overcome resistance issues (phase I[276–278] and phase II[279,280]).

Neural stem cells as drug transporters

Neural stem cells are known for their tropism towards cancer cells in vitro and in vivo[281]. Consequently, they have been used as vehicles to specifically express anticancer drugs and prodrug-activating enzymes at the tumour site, thereby reducing potential adverse effects[282,283]. This approach is under phase I clinical trials for brain cancer[284,285] but has not yet been explored in the context of gastrointestinal cancers.

Conclusions

The multifaceted characteristics of carcinogenesis mirror the essential processes that drive the development, growth and survival of multicellular organisms. Within these processes, cells are in close and permanent contact with their neighbourhood, with every cell, even cancer cells, expressing similar messengers and receptors to communicate. During carcinogenesis, cancer cells utilize nearby and remote resources to serve their increasing energy demand. It has been established that neurons reside within the TME and communicate with cancer cells as well as with other TME members. Nerves not only provide a 'railway' for dissemination but are also deployed by cancer cells to constantly send out a variety of signals that favour their growth and survival. As a consequence, manipulating the nervous system shows promise in the treatment of gastrointestinal cancers. However, within the reciprocal neural–cancer crosstalk, many enigmas still have to be unravelled before proper therapies can be designed. This situation is complicated by the fact that several messenger molecules have dual roles, being either protumorigenic or antitumorigenic, depending on the cell type, receptor and intracellular targets they influence as well as the tissue wherein they exert their actions. In addition, the complex gastrointestinal environment in which several other tissue components, such as the immune system and intestinal microbiota, are implicated, complicates the development of neural-oriented treatment strategies for gastrointestinal cancers. Finally, the findings in this field are mainly established using in vitro and in vivo assays, whereas translation to the human situation is often missing. Nerves are identified in the tumour stroma of patients with gastrointestinal cancer and affect patient outcomes but in-depth molecular profiling of tumour-associated neurons in humans is lacking. This research might be hindered as neurons represent a rare intestinal cell population that is difficult to capture, and long-lived culture potential is restricted. Altogether, these aspects emphasize the need to adopt technologies and tools from the neuroscience field and to establish strong collaborations between neuroscientists and cancer biologists. Further in-depth basic and translational research is warranted to understand the role of neurons in gastrointestinal cancers and to identify the best therapeutic targets.

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