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Review

The role of enteric neurons in the development and progression of colorectal cancer

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ABSTRACT

The enteric nervous system (ENS) is the intrinsic neural network of the gastrointestinal tract, which is essential for regulating gut functions and intestinal homeostasis. The importance of the ENS is underscored by the existence of severe gastrointestinal diseases, such as Hirschsprung's disease and intestinal pseudo-obstruction, which arise when the ENS fails to develop normally or becomes dysregulated. Moreover, it is known that enteric neurons are involved in intestinal inflammation. However, the role of the ENS in colorectal cancer (CRC) carcinogenesis remains poorly understood, even though processes like perineural invasion and neoneurogenesis are important factors in CRC. Here we summarize how enteric neurons are affected during CRC and discuss the influence of enteric neurons, either direct or indirect, on the development and/or progression of CRC. Finally, we illustrate how the ENS could be targeted as a potential anti-cancer therapy, establishing the ENS as an integral part of the tumor microenvironment.

1. Introduction

Over the last twenty years, the enteric nervous system (ENS), the complex neural network embedded in the wall of the gastrointestinal (GI) tract, has become an increasingly important topic in the study of developmental gastrointestinal diseases, functional GI disorders and even diseases of the central nervous system, such as Parkinson's disease [1,2]. The ENS, also referred to as the 'second brain' or 'minibrain' [3,4], consists out of an extensive network of enteric neurons and enteric glial cells organized in ganglia interconnected by nerve fiber bundles [5,6]. Enteric nerve fibers innervate the entire thickness of the gut wall, and are in close contact with the intestinal epithelium. Enteric ganglia are arranged in two major plexuses: the submucosal plexus and the myenteric plexus [7]. The submucosal plexus in humans is composed of an inner plexus located at the border of the muscularis mucosae and the submucosa, and an outer plexus that lies adjacent to the circular muscle. The submucosal plexus is in all species exclusively located in the small and large intestines, but not found in the stomach [8]. The myenteric plexus, on the other hand, is found along the length of the entire GI tract and is located between the circular and longitudinal muscle layers [8,9]. The ENS controls every function of the GI tract *via* signaling involving both enteric neurons as well as enteric glial cells [1,5,10–13]. When GI disease severely impacts the ENS, gut functions are seriously compromised, which can even be life-threatening [14]. Although the ENS has been extensively studied in the context of developmental, inflammatory and functional diseases, its role in the development and progression of colorectal cancer (CRC) is understudied and poorly understood.

Abbreviations: 5-HT, serotonin; Ach, acetylcholine; AOM, azoxymethane; ATP, adenosine triphosphate; CAC, colitis-associated cancer; cAMP, cyclic adenosine monophosphate; CGRP, calcitonin-gene related peptide; CNS, central nervous system; CRC, colorectal cancer; DCC, deleted in colorectal cancer; DSS, dextran sodium sulfate; ENS, enteric nervous system; ERK, extracellular signal-regulated kinases; GDNF, glial-derived neurotrophic factor; GI, gastrointestinal; GLP-2, glucagon-like peptide 2; IBD, inflammatory bowel disease; IEB, intestinal epithelial barrier; IL, interleukin; NDRG4, N-myc downregulated gene 4; NK-1, neurokinin-1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NGF, nerve growth factor; NO, nitric oxide; NOS, nitric oxide synthase; NPY, neuropeptide Y; NSE, neuron specific enolase; NT, neurotensin; OT, oxytocin; P2X7R, P2X purinoceptor 7; PACAP, pituitary adenylate cyclase-activating polypeptide; PDAC, pancreatic ductal adenocarcinoma; PGD_2, prostaglandin D_2; PNI, perineural invasion; RNS, reactive nitrogen species; ROS, reactive oxygen species; SNAP25, synaptosomal-associated protein 25; STAT3, signal transducer and activator of transcription 3; TNBS, trinitrobenzene sulfonic acid; TNF-α, tumor-necrosis factor-α; VAMP-3, vesicle associated membrane protein 3; VIP, vasoactive intestinal peptide; YAP, yes-associated protein

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CRC is the third most common cancer worldwide with estimated 1.4 million new cases per year [15]. Additionally, with a mortality rate of 8.5%, corresponding to 700,000 people, CRC is also the third most common cause of cancer death in the world [15]. The highest incidence and mortality of CRC is found in developed countries in North America and western Europe, with an increasing incidence found in countries which are becoming more westernized [16,17]. Initially, the development and progression of CRC was considered to be caused by the accumulation of genetic and epigenetic events [18,19]. Nowadays it has been recognized that the tumor microenvironment plays a key role in carcinogenesis [20]. Within the tumor microenvironment a large variety of cell types has been identified, including endothelial cells, pericytes, fibroblasts, myofibroblasts, immune cells and nerve cells [20,21]. The cells in the microenvironment are able to promote cancer cell growth, proliferation, survival, invasion/metastasis, and angiogenesis *via* the release of many different growth factors and cytokines [21–24]. While some cell types of the microenvironment, like endothelial or immune cells, have been extensively studied and show promising results as therapeutic targets in CRC, the putative role of the ENS in the development and progression of CRC is largely unknown. Recently, different landmark papers described the importance of neurons as critical microenvironmental determinants of cancer progression in prostate [25], pancreatic [26], skin [27] and gastric [28,29] cancers. In addition, it has been shown that enteric neurons have an important role in gut homeostasis and regeneration [12,29] which address a central role for enteric neurons in colorectal carcinogenesis. Most mechanisms described so far, by which enteric neurons interact with epithelial and tumor cells to promote gut homeostasis and/or malignant growth and invasion, involve the release of neuromodulators. In this review, we give a brief account of the physiology of the ENS and explore the current knowledge of enteric neurons on epithelial homeostasis/inflammation, and their involvement in the development and progression of CRC.

2. The major functions of the enteric nervous system

Enteric neurons number between 400 and 600 million in humans and 1.2 million in mice; the sheer size of the ENS suggests it is important [1,30], and this is borne out by developmental studies showing that its absence has lethal consequences [14]. Many subtypes of enteric neurons have been identified based on differences in morphology, electrical properties, projections and function [1,6,31]. It is now widely accepted that these subtypes of enteric neurons are functionally relevant and are able to control motility, and regulate intestinal permeability, secretion, blood flow and other activities of the gut [1,12,13].

The ENS plays an important role in the regulation of gastrointestinal motility *via* a peristaltic reflex circuit. The reflex is initiated by the release of enteroendocrine hormones, notably serotonin (5-HT) or direct mechanical stimulation of intestinal primary afferent neurons. Intestinal primary afferent neurons project to local interneurons which innervate motor neurons upstream and downstream of the origin of the stimulus. The ascending interneurons activate excitatory motor neurons in order to initiate contractions of the smooth muscle *via* the release of acetylcholine (ACh) and substance P, while downstream interneurons activate inhibitory motor neurons which release nitric oxide (NO) and vasoactive intestinal peptide (VIP), to relax the descending portion of the gut. Together this pattern of upstream contraction and downstream relaxation establishes a pressure gradient that leads to propagation of luminal content and forms the basis of propagated gastrointestinal motility [32–34]. Segmental motility of the intestine, where local movements occur to aid digestion, has also been well defined [35,36].

Intestinal epithelial barrier function is tightly regulated and controls the permeability of the intestines which is essential to prevent microbial products, microbes, antigens and other harmful substances from leaving the lumen and entering the bloodstream. ACh and substance P released by enteric neurons causes an increase in paracellular and transcellular permeability [12,37], while VIP reduces intestinal permeability [38]. Therefore VIP is able to balance the effects caused by permeability-increasing factors like ACh, substance P, but also inflammatory mediators and pathogens [38,39]. Intestinal barrier function is regulated by enteric neurons in the short term through post-translational modification of the myosin light chain phosphorylation, but also over more extended periods of time through changes in the expression of tight junction proteins [38–40].

The ENS also regulates secretion of H_2O, electrolytes and mucus, and plays a role in fluid exchange between the intestinal lumen and the gut mucosa. Active secretion and fluid exchange is initiated by intrinsic enteric reflexes [13]. Stimulation of intestinal primary afferent neurons activates secretomotor neurons which initiate secretion by the release of ACh, VIP, adenosine triphosphate (ATP), and substance P, while inhibition of the secretomotor neurons is mainly regulated *via* the sympathetic nervous system and by interneuronal-released somatostatin [41,42].

Overall, less is known about the role of enteric neurons in the regulation of proliferation, repair, wound healing and blood flow. Even though it is well known that the enteroendocrine peptide glucagon-like peptide 2 (GLP-2) exerts trophic effects on the mucosal epithelium, it remains to be determined which other neuromodulators are involved in this process. Several studies have suggested that ACh, substance P and VIP are important stimulators for epithelial cell proliferation although contradictory evidence indicates that VIP also has antiproliferative effects [43–46]. Other neuromodulators that take part in the regulation of the epithelium/epithelial homeostasis are 5-HT, which is involved in epithelial growth and [47] endocannabinoids have a role in mucosal healing [48]. Finally, enteric neurons can regulate blood flow within the gut in order to maintain a proper balance between the absorption of nutrients and fluid exchange [12,42]. Acetylcholine released by vasodilator/secrotomotor neurons interacts with the blood vessels to release NO from the endothelium hereby inducing vasodilation [42]. Guan et al. showed that also GLP-2 is able to stimulate blood flow. As GLP-2 receptors are localized on neurons expressing NO and VIP, it is suggested that these neurotransmitters act as additional regulators for blood flow [49]. Calcitonin gene-related peptide (CGRP) and substance P are also neurotransmitters with vasodilatory effects, but the signaling of these transmitters in the context of vasodilatory modulation originates from splanchnic primary afferent neurons which have their cell bodies located in the dorsal root ganglia [50]. However, signaling from the sympathetic nervous system (SNS) for blood flow is also essential for intestinal vasoconstriction. The SNS stimulates vasoconstriction *via* α1-adrenergic pathways in particular induced by norepinephrine/noradrenaline (NE) [51]. Additionally, dopamine, the precursor of NE, and neuropeptide Y, which is regarded as a co-transmitter with NE in the SNS, exert vasoconstrictor effects, although several lines of evidence indicate that these transmitters can also attenuate vasoconstriction depending on the binding to specific subtypes of receptors [52–54].

This brief overview of the functions of the ENS outlines many of the major transmitters and provides important background about the importance of the ENS. Currently most of our knowledge of the ENS in intestinal pathophysiology is based on studies on genetic ENS diseases, like Hirschsprung's disease, and on intestinal inflammatory conditions, like IBD. This needs to be considered when examining the role of enteric neurons and enteric neurotransmitters in intestinal inflammation and CRC.

3. Linking gut inflammation, a predecessor for CRC, and the ENS

The ENS plays a pivotal role in orchestrating the inflammatory processes in the gastrointestinal tract. Margolis et al. showed how an altered enteric neuronal density can contribute to the severity of gut inflammation. They treated two different mouse models, each characterized with an altered number of neurons with 2,4,6-trinitrobenzenesulfonic acid (TNBS) and dextran sodium sulfate (DSS) to