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Neuregulin 1 (NRG1) and its receptors in the enteric nervous system and other parts of the gastrointestinal wall

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Summary. Neuregulin 1 (NRG1) belonging to the transmembrane growth factors family is widespread in living organisms. It acts through ErbB family receptors and first of all takes part in embryogenesis, as well as in developmental, regenerative and adaptive processes occurring in various internal organs and systems. It is known that NRG1 and its receptors are present in various parts of the gastrointestinal (GI) tract. First of all NRG1 and ErbB receptors have been detected in the enteric nervous system (ENS) localized in the wall of the esophagus, stomach and intestine and regulating the majority of the GI tract functions, but also in the mucosal and muscular layers of the GI tract. The NRG1/ErbB pathway is involved in the development and differentiation of the ENS and regulation of the intestinal epithelium functions. Moreover, dysregulation of this pathway results in a wide range of gastrointestinal diseases. However, till now there are no summarizations of previous studies concerning distribution and functions of NRG1 and its receptors in the GI tract. The present review fills this gap.

Key words: Neuregulin 1, ErbB receptors, Intestinal innervation, Intestine, NRG1/ErbB pathway

Introduction

The functions of the gastrointestinal (GI) tract are regulated by many neuronal and endocrine factors. The neuronal control of activity of the esophagus, stomach and intestine is exercised by the enteric nervous system (ENS), which is located in the wall of the digestive tract and characterized by significant independence from the central nervous system (Mawe et al., 2023; Sharkey and Mawe, 2023). For this reason, as well as due to its complex structure, the ENS is often called the second or intestinal brain (Schneider et al., 2019). It is known that neurons belonging to the ENS are distinguished by exceptional diversity in terms of morphology, functions and neurochemical characterization (Furness, 2000). Previous studies have confirmed that in addition to acetylcholine, which is the main neurotransmitter within the ENS, enteric neurons have the ability to synthesize dozens of other active substances acting as neurotransmitters, neuromodulators, enzymes and/or transporters (Furness, 2000; Makowska et al., 2022). The best known of them are vasoactive intestinal polypeptide (VIP), galanin, nitric oxide, somatostatin, calcitonin gene related peptide and substance P (Furness, 2000; Spencer and Hu, 2020). However, it is known that the enteric neurons show the presence of a wide range of many other active substances, whose functions in the GI tract are relatively unknown. One of them is neuregulin

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Abbreviations. AR, amphiregulin; ARIA, acetylcholine receptor-inducing activity; BACE, β-site of amyloid precursor protein cleaving enzyme; BTC, betacellulin; C-terminal, carboxy-terminal; EGF-epithelial growth factor; ENS, enteric nervous system; EPR, epiregulin; ErbB, epidermal growth factor receptor family; ERK, extracellular signal-regulated kinase; GDNF, glial cell line-derived neurotrophic factor; GGF, glial growth factor; GI tract, gastrointestinal tract; HB-EGF, heparin binding-EGF; HRG, heregulin; Ig, immunoglobulin; ISP, inner submucous plexus; JAK, Janus kinase; kDa, kilodalton; MAPK, mitogen-activated protein kinase; MP-myenteric plexus; N-terminal, amino-terminal; NDF, neu differentiation factor; NRG1-4,neuregulin 1-4; OSP, outer submucous plexus; phox2b, paired-like homeobox 2b; PI3 K, phosphatidylinositol-3 kinase; PKC, protein kinase C; PLCγ, phospholipase Cγ; RAF, rapidly accelerated fibrosarcoma; RAS, rat sarcoma virus; RET, rearranged during transfection; SHH, sonic hedgehog signaling molecule; SMDF, sensory and motor neuron-derived factor; Sox10, Sry-related HMg-Box gene 10; SP, submucous plexus; SSE, stratified squamous epithelial; STAT, signal transducer and activator of transcription; TACE, tumour necrosis factor-α converting enzyme; TGF-α, transforming growth factor- alpha; VIP, vasoactive intestinal polypeptide

1 (NRG1).

NRG1 is a member of the transmembrane growth factors family. It is widespread in the organism and first of all takes part in embryogenesis, as well as in developmental and regenerative processes in various internal organs and systems, including among others the nervous system, heart, mammary glands and reproductive organs (Shi and Bergson, 2020). NRG1 acts through the ErbB family of receptor tyrosine kinases (Wu et al., 2015). The presence of NRG1 and its receptors has also been reported in the GI tract, both in the enteric neurons and other elements of the wall of the esophagus, stomach and intestines (Barrenschee et al., 2015; Szymańska et al., 2020).

This review aims to collect and summarize the current knowledge on the distribution and function of NRG1 and its receptors in the ENS and other parts of the wall of the GI tract, both in physiological conditions and during pathological states.

NRG1 structure

NRG1 is a 44-kD glycoprotein and it is the most studied and the best known member of a group of structurally related substances called neuregulins, that also includes NRG2, NRG3 and NRG4 (Gambarotta et al., 2013). Neuregulins, encoded by four different genes, are transmembrane growth factors belonging the large family of epithelial growth factor (EGF) proteins (Gambarotta et al., 2013). The history of research on NRG1 dates back to the early nineties, when it was discovered independently by several different groups of scientists and described under different names, such as neu differentiation factor (NDF), heregulin (HRG), glial growth factor (GGF), acetylcholine receptor-inducing activity (ARIA), and sensory and motor neuron-derived factor (SMDF) (Falls, 2003; Esper et al., 2006). At present it is known that NRG1 has above 30 isoforms, which are grouped into six types, including type I (NDF/HRG/ARIA), type II (GGF), type III (SMDF) and less known type IV, V and VI. Among these types, the most studied are types I, II and III, the presence of which has been confirmed in various groups of vertebrates. Types IV, V and VI are known to a lesser extent, but it is known that type IV is present only in mammals, and types V and VI have been described only in the primates (Chou and Ozaki 2010; Gambarotta et al., 2013).

NRG1 types are synthetized as precursors, which are anchored in the cell membrane. Molecules of pro-NRG1 consist of extra- and intracellular parts. For most types of NRG1, the amino (N) - terminal region is located outside the cell and the carboxy (C-) - terminal region in the cytoplasm (Mei and Xiong, 2008). The only exception is NRG1 type III, where there is a cysteine rich domain in the N-terminal region, which is a transmembrane domain, and therefore both N- and C-terminal regions of NRG1 type III are positioned inside the cell (Gambarotta et al., 2013; Zhang et al., 2017). The basis for the classification into the six classes of NRG1 mentioned above are differences in the structure of N-terminal domains. N-terminal domains are connected with EGF-like domain through an immunoglobulin (Ig)-like domain with or without spacer domain (in types I, II, IV and V) or directly (in type III and VI) (Gumà et al., 2010; Gambarotta et al., 2013). All NRG1 isoforms contain an EGF-like domain, which is the most important part of the molecule, because it is responsible for the receptor activation and initiation of intracellular signaling pathways (Falls, 2003; Gambarotta et al., 2013). Differences between particular NRG1 types may also result from different structures of exons between EGF-like domain and transmembrane region, as well as within the cytoplasmic carboxy-terminal region of the molecule (Falls, 2003, Gumà et al., 2010; Gambarotta et al., 2013; Zhang et al., 2017). Activation of pro-NRG1 to active form is done through proteolytic cleavage at the juxta-membrane region of the molecule. This reaction may be catalyzed by three types of enzymes, including tumour necrosis factor-α converting enzyme (TACE), β-site of amyloid precursor protein cleaving enzyme (BACE) and meltrin beta (Zhang et al., 2017; Shi and Bergson, 2020). The scheme of the NRG1 molecule is presented in Figure 1.

NRG1 receptors

Neuregulins act on transmembrane tyrosine kinase receptors of the ErbB family. The name of family ErbB comes from the name of erythroblastic leukemia viral oncogene, which is a viral oncogene homologous to these receptors. Till now four members of the ErbB family receptors have been described: ErbB1, ErbB2, ErbB3 and ErbB4 (Britsch, 2007). All these receptors have a similar structure, in which an extracellular ligand-

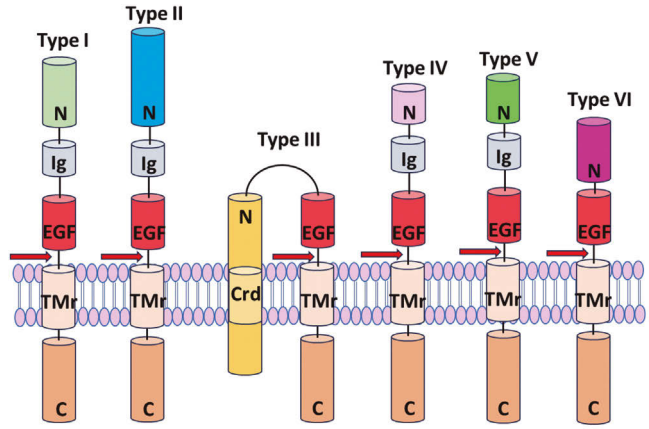

Fig. 1. Types of neuregulin 1 (NRG1): structure, location on the cellular membrane and cleavage sites (indicated with red arrows): N, amino-terminal region - different in each type of NRG1; Ig, immunoglobulin-like domain; EGF, EGF-like domain, TMr, transmembrane region; C, carboxy-terminal region; Crd, cysteine-rich domain in the amino-terminal region of type III.

binding N-terminal, transmembrane, cytoplasmic tyrosine kinase and cytoplasmic C-terminal domains can be distinguished (Seroogy et al., 2013). Contrary to other types of ErbB family receptors, ErbB3 receptor does not show tyrosine kinase activity. Moreover, it is known that there are many isoforms of ErbB receptors, which differ from each other by different sequences of amino acids in the extracellular juxta-membrane and/or C-terminal regions of the molecule (Britsch, 2007; Seroogy et al., 2013).

After binding of neuregulin molecule to ErbB receptor, receptors form homo- or heterodimers, which in turn activates autophosphorylation of specific tyrosine residue in the C-terminal regions (Seroogy et al., 2013). This process may initiate various intracellular pathways. The main pathways activated by ErbB receptors are the Ras/ Raf/ERK mitogen-activated protein kinase (MAPK) pathway, the phosphatidylinositol-3 kinase (PI3 K) pathway, the PLCγ/PKC pathway and the JAK/STAT pathway. As mentioned above ErbB3 receptor has no tyrosine kinase activity and therefore it may stimulate the above mentioned pathways only by forming heterodimers with other types of ErbB receptors (Britsch, 2007; Seroogy et al., 2013; Sabbah et al., 2020).

Previous studies have shown that neuregulins can directly bind only to ErbB3 and ErbB4 receptors (Seroogy et al., 2013). Moreover, it is know that all neuregulins (NRG1, NRG2, NRG3 and NRG4) can directly activate ErbB4 receptor, whereas only NRG1 and NRG 2 can directly bind to both ErbB3 and ErbB4 receptors (Seroogy et al., 2013; Sabbah et al., 2020). In turn, ErbB2 receptors can be activated by neuregulins only indirectly, by heterodimerization of these types with ErbB3 or ErbB4 connected with neuregulins (Seroogy et al., 2013). ErbB1 receptor can be activated by a wide range of substances, including EGF, transforming growth factor- alpha (TGF-α), amphiregulin (AR), betacellulin (BTC); heparin binding-EGF (HB-EGF) and epirgulin (EPR) (Normanno et al., 2005; Seroogy et al., 2013). Moreover, it is known that (in addition to NRG1-4) BTC, HB-EGF, EPR and tomoregulin can directly bind to ErbB4 receptor (Normanno et al., 2005; Sabbah et al., 2020). In turn, ErbB2 receptor does not directly bind any known ligand (Normanno et al., 2005). The structure of ErbB receptors and formation of ErbB homo- and heterodimers under the impact of NRG1are presented in Figure 2.

Organization of the ENS

The ENS is characterized by a large number of neurons, which in humans is estimated at about 200-600 million (Furness, 2000). Neurons within the ENS are grouped in ganglia located in the wall of the GI tract from esophagus to the rectum. Intramural ganglia are connected to each other with a dense network of nerve fibers and form ganglionated plexuses (Furness, 2000; Sharkey and Mawe, 2023). The exact organization and localization of these plexuses depend both on the mammal species and segment of the GI tract.

In small mammals (for example in rodents) the ENS in all segments of the GI tract is built with two kinds of ganglia. The first of them are myenteric ganglia, which are located in the muscular layer, between longitudinal and circular muscles, and the second, submucous ganglia located in the submucosal layer in close proximity to the muscularis mucosae of the mucosal layer (Makowska et al., 2022, 2023). Myenteric ganglia are interconnected with dense networks of nerve fibers and form the

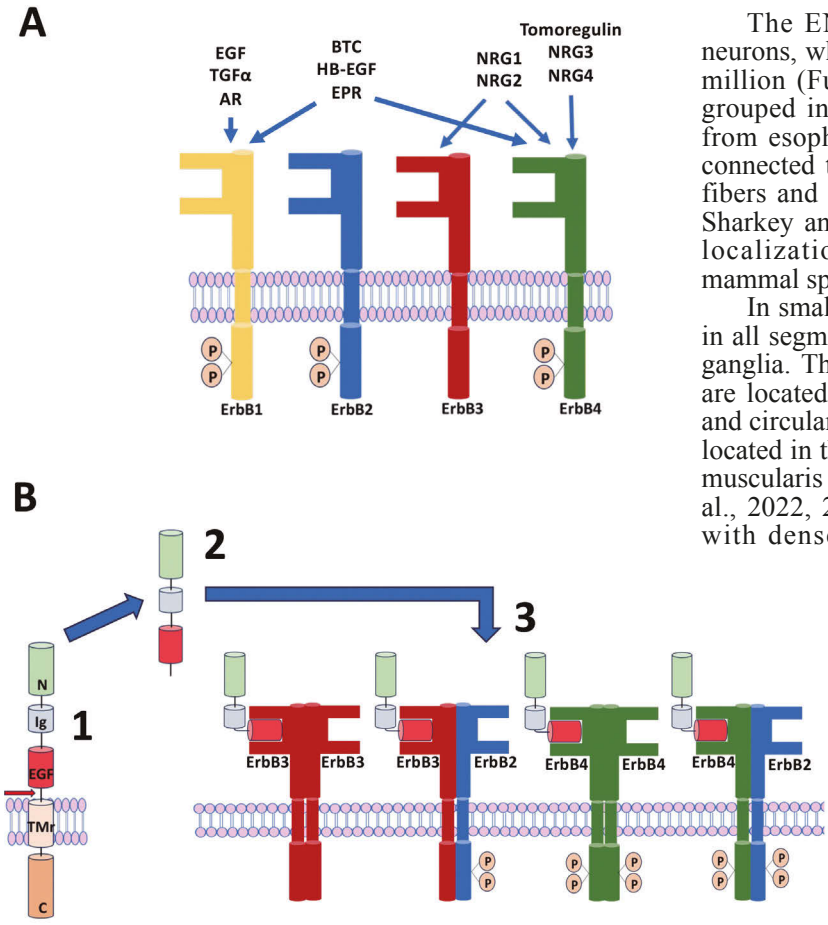

Fig. 2. ErbB receptors: **A.** types of ErbB receptors and their ligands: AR, amphiregulin, BTC, betacellulin, EGF, epithelial growth factor, EPR, epirgulin, HB-EGF, heparin binding-EGF, NRG1-4, neuregulin 1-4, p, tyrosine residue; TGF-α, transforming growth factor-alpha. **B.** dimerization of ErbB receptors under the impact of neuregulin 1: 1) preneuregulin 1: N, amino-terminal region; Ig, immunoglobulin-like domain; EGF, EGF - like domain; TMr, transmembrane region; C, carboxy-terminal region. cleavage sites indicated with red arrow; 2) active form of NRG1; 3) Homo- and hetero-dimers of ErbB receptors forming under the influence of NRG1: p, tyrosine residue.

myenteric plexus (MP) along the entire length of the GI tract. Submucous ganglia form plexus (submucous plexus - SP) only in small and large intestines (Fig. 3A), while in the esophagus and stomach neuronal networks between submucous ganglia are definitely less dense (Makowska et al., 2022, 2023).

In big mammals (for example in the domestic pig) the organization of the ENS in the esophagus and stomach is the same as in rodents (Wojtkiewicz et al., 2017; Palus et al., 2019). In turn, in the small and large intestines submucous plexus is divided into two types: outer submucous plexus (OSP) located in close proximity to the inner side of the circular muscle layer and the inner submucous plexus (ISP) is located like the submucous plexus in rodents, closer to the intestinal lumen (Fig. 3B) (Szymańska and Gonkowski, 2019; Szymańska et al., 2020).

In humans the structure of the ENS in the esophagus and stomach is similar to other mammals. In turn the organization of the ENS in the human intestines, especially regarding submucous plexus, is still discussed. Some authors describe three submucous plexuses and apart from OSP and ISP, they distinguish an intermediate submucous plexus, which does not contain clusters of nerve cells located between them (Ibba-Manneschi et al., 1995). Other authors distinguish two submucous plexuses in the human intestine (like in big mammals). However, contrary to other species, ISP in the human intestine is multi-layered, and particular ganglia included in it are at a different depth of the submucous layer. (Jabari et al., 2014; Zetzmann et al., 2018). Moreover, previous studies draw attention to dissemination of submucous ganglia throughout the entire submucosal layer in the human intestine and significant inter-individual differences in organization of this part of the ENS (Graham et al., 2020).

Distribution of NRG1 and its receptors in the ENS

Knowledge about distribution of NRG1 and its receptors in the ENS is relatively limited. First of all this substance has been observed in the nervous structures located in the human GI tract. The most detailed description of the distribution of NRG1, ErbB2 and ErbB3 in the human ENS was provided by Barrenschee et al. (2015, 2019). The authors have found NRG1 in nervous structures located within the distal colonic segments collected from patients after partial colectomy for colorectal carcinoma from a place located at a safe distance from the tumor (without any pathological changes) (Barrenschee et al., 2015). NRG1 has been observed both in the submucous and myenteric plexuses in the neuronal somata and (with higher density) in nuclei of neurons, as well as in neuropil located in the enteric ganglia. The authors have also shown that NRG1 in the enteric plexuses colocalizes with pan-glial marker S100b, which strongly suggests that this substance is present also in glial cells located in the ENS. Weaker signals have been noted also in nerve fibers in the muscular layer of the colonic wall (Barrenschee et al., 2015). The same authors have also confirmed the high expression of mRNA for NRG1 within the MP (Barrenschee et al., 2015).

ErbB2 and ErbB3 receptors, similarly to NRG1,

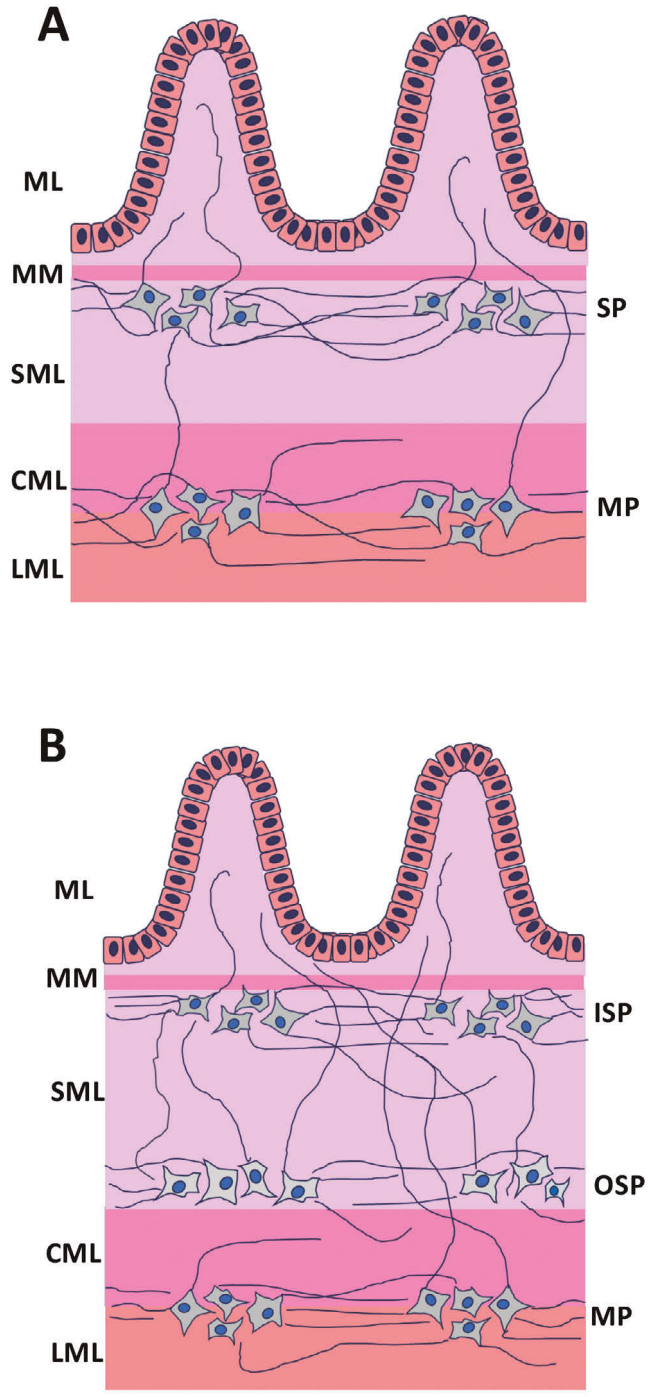

Fig. 3. Organization of the enteric nervous system in the intestine of rodents **(A)** and domestic pig **(B)**. ML, mucosal layer; MM, muscularis mucosae; SL, submucosal layer; CML, circular muscular layer; LML, longitudinal; muscular layer; SP, submucous plexus; MP, myenteric plexus; ISP, inner submucous plexus; OSP, outer submucous plexus.

have also been found in the submucous plexuses and MP of human distal colon in neuronal somata and to a lesser extent neutropil, as well as in glial cells. Interestingly, ErbB2 receptors have been found in some glial cell nuclei, while ErbB3 receptors were generally absent in this part of the cell (Barrenschee et al., 2015). ErbB2 and ErbB3 receptors have also been found in small amounts of intramuscular nerves in the human distal colon (Barrenschee et al., 2015). The same authors have described low expression of mRNA for ErbB2 and ErbB3 receptors in the MP.

The next study of Barrenschee et al. (2019) has confirmed the presence of NRG1, ErbB2 and ErbB3 in the MP of the human sigmoid colon. Strong NRG1 immunoreactive signals were noted in neurons and glial cells. In turn ErbB2 was observed mainly in neuronal somata and ErbB3 both in neuronal somata and ganglionic neutropil. Moreover, expression of mRNA for NRG1, ErbB2 and ErbB3 has been found in the MP of human sigmoid colon (Barrenschee et al., 2019). The presence of NRG1 and ErbB2 receptor in the MP of the human colon has also been confirmed by Garcia-Barcelo et al. (2009).

Distribution of NRG1 in the ENS has also been described in the domestic pig. It should be underlined that organization of the enteric innervation in terms of neurochemical characterization of neuronal cells in human and domestic pig is similar. The domestic pig has often been used as an animal model for research on human ENS and therefore knowledge of the active substances in the porcine ENS is relatively good. NRG1 has been observed in neuronal cells located in all types of the enteric plexuses in various segments of the porcine large intestine, such as caecum, ascending and descending colon (Szymanska et al., 2020). The percentage of neurons containing NRG1 in relation to all cells immunoreactive to pan-neuronal marker protein gene product 9.5 fluctuated from about 20% to about 27% and depended both on the type of the enteric plexus and segment of the large intestine (Szymanska et al., 2020). Contrary to humans (Barrenschee et al., 2015), NRG1 has not been found in the neuropil within the enteric ganglia and intramural nerve fibers in the porcine large intestine (Szymanska et al., 2020).

It is also known that NRG1 in the ENS of the porcine large intestine may co-localize in a wide range of other neuronal active factors. This fact confirms the presence of NRG1 in various classes of the enteric neurons. Makowska et al. (2021) have shown that enteric neurons in the porcine caecum, ascending and descending colon containing NRG1 may also contain substance P, VIP and galanin. The presence of NRG1 has also been observed in nitrergic enteric neurons (Makowska et al., 2021). The degree of colocalization of NRG1 with other factors in the enteric neurons clearly depends on the type of the enteric plexus and segment of the large intestine, but generally NRG1 was relatively most frequently observed in nitrergic and VIP-ergic neurons (Makowska et al., 2021). Knowledge about distribution of NRG1 and its receptors in the ENS of other species is extremely scanty. Namely, expression of NRG1 has been observed in the muscular layer of the stomach and intestine of mouse embryos, and the localization of this expression was in accordance with the distribution of myenteric ganglia (Orr-Urtreger et al., 1993). Apart from NRG1, the presence of ErbB3 receptor has been described in the glial and neuronal cells located in the myenteric and submucous ganglia of mouse intestine (Chalazonitis et al., 2011). Moreover, expression of ErbB2 and ErbB3 have been detected in the small intestine and colon of mouse, mainly in the glial cells, enteric progenitors and Schwann cell precursors, while lower expression was noted in the enteric neurons (Le et al., 2021). It should be pointed out that the expression of ErbB2 and ErbB3 receptors has also been observed in the neural crest cells, which colonize the gastrointestinal tract during development (Britsh et al., 1998).

Distribution of NRG1 and its receptors in other parts of the GI wall

Studies on the presence of NRG1 and its receptors in the GI tract started in the late eighties and early nineties in the twentieth century. In the 80-s the presence of ErbB2 receptor was described in the GI tract of human and rat fetuses (Coussens et al., 1985; Kokai et al., 1987; Quirke et al., 1989). In rats (both in fetuses and adult animals) this receptor was found in epithelial cells of the intestinal villi (Kokai et al., 1987), and in human fetuses in the epithelium of esophagus, stomach, small intestine and colon, as well as (in smaller quantities) in the intestinal muscular layer (Quirke et al., 1989). In turn, the expression of mRNA for NRG1 was described in the human intestine, but not in the stomach (Holmes et al., 1992).

Later studies confirmed these observations. In humans, during analysis of biopsy specimens, the expression of mRNA for NRG1 and ErbB3 and ErbB4 receptors was found in the mucosal layer of the esophagus, stomach and duodenum (Kataoka et al., 1998). The highest expression of mRNA for NRG1 and its receptors has been observed in the duodenum (Kataoka et al., 1998). Moreover, ErbB4 receptors were found using immunohistochemistry method in normal gastric mucosa in surface epithelial cells, but not in gastric fundic and pyloric glandular cells (Kataoka et al., 1998). NRG1 and its receptors in the human gastric mucosa have also been described by Noguchi et al. (1999), who found NRG1 in scattered lamina propria mesenchymal cells corresponding to fibroblasts, but not in the surface epithelial cells or glandular cells. In turn, ErbB2 receptor has been noted mainly in glandular epithelial cells. The same authors have also described mRNA for NRG1 in gastric fibroblasts (Noguchi et al., 1999).

Expression of mRNA for NRG1, as well as NRG1 protein have been detected in whole wall of the human

colon (Tang et al., 2012a,b; Gunadi et al., 2022). More thorough investigations have been conducted by Barrenschee et al. (2015, 2019). These authors described the expression of mRNA for NRG1, as well as ErbB2 and ErbB3 receptors in circular and longitudinal muscles in the human distal (Barrenschee et al., 2015) and sigmoid colon (Barrenschee et al., 2019). Levels of mRNA for ErbB2 and ErbB3 in the colonic muscular layer were significantly higher than those noted in the MP localized in this intestinal segment. Especially it was visible in the case of ErbB3, in which mRNA levels were up to sixfold higher in longitudinal muscular layer and about 17-fold higher in circular muscular layer in comparison to values noted in the MP (Barrenschee et al., 2015). Histochemistry staining also confirmed the presence of NRG1, ErbB2 and ErbB3 receptors in the muscular cells located in the circular muscle layer of the human colon, and in the case of NRG1, strong signals were visible not only in muscular cell bodies, but also in the nuclei (Barrenschee et al., 2015).

There is also a comprehensive study on the distribution of NRG1 in the various segments of the rhesus monkey gastrointestinal tract (Zhao, 2013). This study has reported clear differences in the number and distribution of cells containing NRG1, ErbB2 and ErbB4 depending on the segment of the GI tract with the highest expression of NRG1, ErbB4 and/or ErbB2 in the stomach and the small intestine (Zhao, 2013). In the esophagus NRG1 and ErbB4 receptors were found in the stratified squamous epithelial (SSE) cells bordering the lamina propria, but the degree of colocalization of NRG1 and ErbB4 in the same cells was rather small. In other parts of the esophageal wall the number of cells showing the presence of NRG1 and ErbB4 receptors was extremely scanty, and the presence of ErbB2 receptors in the esophagus was not confirmed (Zhao, 2013). In the rhesus monkey stomach NRG1 was noted mainly in the parietal cells of the mid and upper regions of the glands, where this substance co-localized with ErbB2 and ErbB4 receptors. NRG1 was also noted in the chief cells of the gastric glands but without co-localization with receptors (Zhao, 2013). In the rhesus monkey small intestine NRG1 was present in the cells of the lamina propria. The number of mucosal cells containing ErbB2 receptors was extremely scanty. In turn, ErbB4 receptors were noted in intestinal cells similar to enteroendocrine cells. In the colon NRG1 was present in a small number of membranes of the absorptive columnar cells, in which ErbB2 and ErbB4 were almost undetectable (Zhao, 2013).

Knowledge about distribution of NRG1 and its receptors in other species is relatively scanty. In addition to the previously mentioned description of ErbB2 in epithelial cells of the rat intestinal villi (Kokai et al., 1987), it is known that also mRNA for ErbB3 are present in the intestinal wall of rat fetuses (Chalazonitis et al., 2011). ErbB receptors have been described in rat intestine goblet cells (Gu et al., 2008). In turn, mRNA for NRG1 has been detected in goblet cells, as well as stratified squamous cells, fibroblasts, immune cells, nerves, and conjunctival stromal cells in rat intestine (Gu et al., 2008). Moreover, the presence of ErbB3 was noted in the mouse fetal intestine, in which immunoreactivity to this receptor was observed in cells concentrated in a band of the fetal bowel neighboring to the developing neurons (Chalazonitis et al., 2011). As regards the studies on NRG1 in the mouse intestine, mRNA for this peptide has been found in mesenchymal cells of the intestine and stomach in early organogenesis and in the lamina propria and inside the intestinal villi in later stages of development (Meyer and Birchmeier, 1994). Moreover, in the mouse intestine expression of NRG1 has been noted in the stromal cells, macrophages and epithelial cells, and ErbB3 was rarely observed in mesenchymal cells but was detected in basolateral membranes of epithelial cells (Jardé et al., 2020). Other studies described the presence of NRG1 in gastrointestinal fibroblasts located in the mouse small intestine and colon (Lemmetyinen et al., 2023). NRG1 has also been found in the gastrointestinal of Zebrafish, in which this substance has been described both in the intestinal epithelium, enterocytes and muscular external layer in adult individuals, as well as in the gut tube of the zebrafish larvae (Pu et al., 2017).

Summarization of previous studies concerning distribution of NRG1 and its receptors in the GI tract is presented in Table 1.

Functions of NRG1 and its receptors in the GI tract

NRG1 is commonly known as a factor which plays an essential role in the regulation of cell proliferation, survival, and differentiation in many internal systems and organs, including among others, heart, brain, neuromuscular synapses, lungs and reproductive system (Sandrock et al., 1997; Mei and Xiong, 2008; Kawashima et al., 2014; Mishra et al., 2019; Grego-Bessa et al., 2023). Similar functions of NRG1 have been described in the GI tract.

Studies conducted on zebrafish have shown that NRG1 is not only involved in the ENS development, but also takes part in the maintenance of normal morphology of neuronal structures in the intestinal wall (Pu et al., 2017). Such conclusions are supported by the facts that a decrease in NRG1 expression results in the reduction of the number of the enteric neurons with simultaneous decrease of expression of genes - markers of the ENS development, including crestin, ret, gdnf, shh, sox10 and phox2b. Moreover, a loss of NRG1 in adult individuals causes changes in nerves located in the intestinal wall. Nerves become shorter, thicker and disorderly arranged (Pu et al., 2017). Important functions of NRG1/ErbB signaling in the development and postnatal maintenance of the ENS have also been described in mice. In this species the roles of the NRG1/ErbB pathway in regulation of the migration of vagus nerve-associated neural crest cells to the gut have been found (Espinoza-Medina et al., 2017). Moreover, it has been shown that

the loss of ErbB2 results in a dramatic reduction in the number of enteric neurons and glial cells in the intestine (Crone et al., 2003), and mice lacking the ErbB3 gene show a total loss of enteric glia and reduced ganglionic number in the duodenum (Erickson et al., 1997; Riethmacher et al., 1997).

NRG1 is known as a factor which stimulates enteric neurons *in vitro*, which is manifested in the development of neuronal networks and an increase in full nerve fiber length, as well as a number of branching points in the course of nerve fibers and ganglion-like aggregates (Barrenschee et al., 2015). Other studies described NRG1 as an neurotrophic factor, which promotes the growth and differentiation of the enteric neurons in the postnatal period through regulation of expression of the nicotinic acetylcholine receptor (Barrenschee et al., 2019). Moreover, it is known that the influence of NRG1 on neurogenesis/gliogenesis, and therefore ENS development and differentiation, takes place through the balance between expression of NRG1 and glial cell derived neurotrophic factor (GDNF). NRG1 inhibited GDNF-induced neuronal differentiation and in turn GDNF negatively affected NRG1/ErbB signaling (Gui et al., 2013).

Due to important roles of NRG1/ErbB signaling in the development and differentiation of the ENS, any disorders in the expression of NRG1 and/or its receptors promote pathological processes within the intestine. A relatively large number of previous studies have described a connection between aberrant NRG1 gene expression and development of Hirschsprung disease - the most frequent developmental anomaly of the ENS, characterized by the lack of intramural enteric ganglia in the intestine and consequent disorders in the intestinal motility (Garcia-Barcelo et al., 2009; Tang et al., 2016, 2018; Li et al., 2017; Zhang et al., 2018; Gunadi et al., 2018, 2022; Le et al., 2021). Interestingly, the occurrence of correlations between aberrant NRG1 gene expression and Hirschsprung disease depends on the human population studied. Namely, such correlations have been reported in Chinese, Caucasian, and Thais (Tang et al., 2012b, Luzon-Toro et al., 2012, Phusantisampan et al., 2012, Kapoor et al., 2015), whereas in Spanish patients association of NRG1 variants with Hirschsprung disease phenotype has not been observed (Luzón-Toro et al., 2012). In patients suffering from Hirschsprung disease changes in the degree of NRG1 methylation have also been visible. Namely, during this disease the percentage of partially methylated NRG1 is higher both in the ganglionic (81%) and aganglionic (75%) fragments of the colon in comparison to the colon in physiological conditions where this value amounted to 59% (Gunadi et al., 2022).

Dysregulation of the NRG1/ErbB pathway may also result in other disorders within the GI tract, including chronic intestinal pseudo-obstruction (Le et al., 2021) and diverticular disease (Barrenschee et al., 2019). During diverticular disease down regulation of NRG1 and ErbB3 has been observed in the myenteric plexus. The lack of NRG1 influenced the composition of enteric neurotransmitter receptors and decreased the expression of cholinergic receptors, which in turn resulted in the intestinal motility disorders observed during diverticular disease (Barrenschee et al., 2015).

Other studies described the participation of the NRG1/ErbB pathway in processes connected with

Table 1. Distribution of NRG1 and its receptors in the gastrointestinal tract according to previous studies.

Species	Distribution	Reference
Human	ErbB2 receptor in the epithelium of esophagus, stomach, small intestine and colon, as well as in the intestinal muscular layer	Quirke et al., 1989
	NRG1 in the intestine	Holmes et al., 1992
	NRG1, ErbB3 and ErbB4 receptors in mucosal layer of esophagus, stomach and duodenum	Kataoka et al., 1998
	NRG1 and ErbB2 in gastric mucosa	Noguchi et al., 1999
	NRG1 and ErbB2 receptor in the colonic myenteric plexus	Garcia-Barcelo et al., 2009
	NRG1 in the colonic wall	Tang et al., 2012a,b; Gunadi et al., 2022
	NRG1, ErbB2 and ErbB3 receptors in the colonic enteric ganglia, intramuscular nerve fibers and muscular layer	Barrenschee et al., 2015
	NRG1, ErbB2 and ErbB3 receptors in the myenteric plexus and muscular layer of the sigmoid colon	Barrenschee et al. 2019
Rhesus monkey	NRG1, ErbB2 and ErbB4 receptors in mucosal layer of various segments of the gastrointestinal tract from esophagus to colon	Zhao, 2013
Domestic pig	NRG1 in all types of the enteric ganglia of the caecum, ascending and descending colon	Szymanska et al., 2020; Makowska et al., 2021
Rat	ErbB2 receptor in epithelial cells of the small intestine	Kokai et al., 1987
	ErbB3 receptor in the intestinal wall	Chalazonitis et al., 2011
	NRG1 in the intestine; ErbB2, ErbB3 and ErbB4 in the intestinal goblet cells	Gu et al., 2008
Mouse	NRG1 in stromal cells, macrophages and epithelial cells of the intestine, ErbB3 in epithelial cells	Jardé et al., 2020
	NRG1in fibroblasts in small intestine and colon	Lemmetyinen et al., 2023

neoplasms. It has been shown that NRG1 gene deletion occurred during rectal mucosal melanoma (Li et al., 2021) and NRG1/ErbB signaling may modulate colorectal carcinogenesis (Tvorogov et al., 2009; Westendorp et al., 2021), which takes place through the influence of NRG1 on vascular endothelial growth factor secretion through autocrine and paracrine mechanisms (Yonezawa et al., 2009). It is also known that the NRG1/ErbB pathway may contribute to intestinal adenoma formation, and overexpression of NRG1 may result in the outgrowth of ileal organoids (Nguyen et al., 2020). In turn, ErbB4 deletion from colorectal cancer cells promotes apoptosis and inhibits proliferation (Lee et al., 2009). A relatively large number of investigations report the overexpression of ErbB receptors during gastric cancer, which suggests the possibility that these receptors may contribute to the growth of the neoplastic processes in the stomach (Lemoine et al., 1991; Kimura et al., 2004, 2005; Tvorogov et al., 2009; Moghbeli et al., 2019). It is also known that ErbB1 and ErbB3 co-overexpression during gastric cancer is considered as a sign of poor prognosis for the patient (Moghbeli et al., 2019).

Some previous studies described multidirectional roles of NRG1/ErbB signaling in the intestinal epithelium. It is known that both NRG1 and ErbB receptors expression increases in the intestinal fibroblasts during experimental inflammation (Lemmetyinen et al., 2023). In human and mouse colitis the overexpression of ErbB4 receptors has been noted, which was associated with the inhibition of colon epithelial apoptosis. This, in turn, suggests that overexpression of ErbB4 during chronic inflammation may contribute to colitis-associated tumorigenesis in the GI tract (Frey et al., 2010). Moreover, increased expression of ErbB4, induced by tumor necrosis factors - an important proinflammatory cytokine, promotes survival of the colon epithelial cells (Frey et al., 2009). This, in turn, suggests that ErbB4 is an important factor in regulatory processes connected with survival of the intestinal epithelial cells during inflammatory processes. Other studies suggest that ErbB4 may be an important regulatory factor in colitis severity (Schumacher et al., 2021).

Moreover, it has been reported that after irradiation - induced damage of the intestine NRG1 takes part in *de novo* crypt formation and contributes to intestinal epithelium integrity. NRG1 also induces regenerative processes and remodels the epithelial actin cytoskeleton (Lemmetyinen et al., 2023). Participation of NRG1 in regenerative processes in the intestinal epithelium has also been described by Jardé et al. (2020), who have shown that exogenous NRG1 regenerates intestinal crypts after experimental injury, which is done through supporting the function of intestinal stem cells (Jardé et al. 2020; Abud et al., 2021). Previous investigations have also described participation of the NRG1/ErbB pathway in regulation of intestinal epithelium secretory activity. Namely, it is known that NRG1 inhibits the activity of the epithelium (Keely and Barrett, 1999) and disorders in ErbB signaling dysregulate intestinal epithelial transport and secretion (O'Mahony et al., 2008). Other studies described stimulatory effects of NRG1 on activity and proliferation of the intestinal goblet cells (Gu et al., 2008). There are also assumptions that NRG1 is involved in the protection of gastric glandular cells against self-damage by hydrochloric acid and processes connected with parietal cell differentiation and renewal (Zhao, 2013), as well as in adaptive and/or neuroprotective processes under the impact of toxins in food (Szymańska et al., 2020; Makowska et al., 2021).

Summarization

Previous studies described the presence both of NRG1 and its receptors in various parts of the wall of the GI tract, including enteric neurons and glial cells, nerve fibers, mucosa and muscular layers. Distribution of this these factors clearly depends on the segment of the digestive tract and species studied. In the light of previous studies the NRG1/ErbB pathway plays important multidirectional functions in regulation of the gastrointestinal functions. First of all, NRG1 and its receptors are involved in development, growth, differentiation and survival of the nervous structures and glial cells within the ENS, and therefore they are important factors affecting intestinal motility and secretion. Moreover, it is known that dysregulation in the NRG1/ErbB pathway may result in a wide range of intestinal disorders and diseases, including Hirschsprung disease, diverticular disease, chronic intestinal pseudo-obstruction, and gastric and colorectal cancers. It is also known that NRG1 ensures proper functions of the gastrointestinal epithelium. Moreover, NRG1 is an important factor taking part in intestinal inflammatory processes, as well as in adaptive reactions under the impact of toxic substances in the food. However, despite such important functions of the NRG1/ErbB pathway in the GI tract, many aspects connected with its activity in the stomach and intestine are still not clear and require further studies.

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