TOPIC HIGHLIGHT

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Neuroendocrine differentiation: The mysterious fellow of colorectal cancer

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Abstract

Neuroendocrine differentiation in sporadic colorectal cancer has been recognized since decades, but its clinical impact is still controversially discussed. Detailed parameter analyses hint at the possibility that probably not neuroendocrine differentiation itself, but its association with poor grade of tumor differentiation, lymph node metastases, distant metastases and other unfavorable features contribute to worse clinical outcome. However, other studies deny a relationship between neuroendocrine differentiation and prognosis of colorectal cancer. This review elucidates, whether new insights into the origin of neuroendocrine differentiation in the intestinal epithelium, its regulation by mTOR pathway components and its possible link to the intestinal stem cell compartment could determine a role of neuroendocrine cells as prognostic marker and putative therapeutic target in sporadic colorectal cancer.

Key words: Neuroendocrine differentiation; Colorectal cancer; mTOR pathway; Neuroendocrine tumorigenesis; Targeted therapy

Core tip: Neuroendocrine differentiation in sporadic colorectal cancer has been recognized since decades. In contrast to the clinico-pathologically well-defined pure neuroendocrine tumors and mixed adenoneuroendocrine carcinomas of the colon and rectum, the clinical impact of focal neuroendocrine differentiation in colorectal carcinomas is still controversially discussed. Further insights into the regulation of neuroendocrine differentiation by mTOR pathway components and recent knowledge about a link of enteroendocrine cells to the intestinal stem cell compartment hint at a role of neuroendocrine cells as prognostic marker and putative therapeutic target in sporadic colorectal cancer.

INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the fourth leading cause of cancer-related death worldwide[1]. More than 50% of patients with CRC experience recurrence or metastases despite of curative operations[2]. Cytotoxic drugs applied as monotherapy or combined with monoclonal antibodies targeting proangiogenic vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) have been shown to prolong survival[3,4]. However, a proportion of patients gain little or even no benefit from these therapeutic regimens[5]. These facts underline the need to increase knowledge about special phenotypes, somatic genetic alterations and signaling pathways, which can be translated into prognostic markers or new molecularly defined targets for therapy of CRC.

The recent approval of new drugs for the treatment of advanced pancreatic neuroendocrine tumors brought neuroendocrine differentiation of tumor tissue, also beyond the pancreaticobiliary tract, into the focus of oncologists[6-8]. Whereas pure neuroendocrine tumors (NET) of the gastrointestinal tract have been established as well defined entities, the prognostic and therapeutic relevance of neuroendocrine differentiation in sporadic colorectal cancer has been less extensively evaluated. Insights into the histogenesis, epidemiology and pathogenetic links to known cancer pathways are necessary to elucidate the sufficiency of neuroendocrine cells as prognostic marker or new target for the therapy of CRC.

ORIGIN OF ENTEROENDOCRINE CELLS

Enteroendocrine cells comprise approximately 1% of epithelial cells in the gastrointestinal system and represent the largest population of hormone-producing cells in the body[9].

Detailed investigation revealed that enteroendocrine precursor cells differentiate immediately from self-renewing Lgr5+ intestinal stem cells[10]. This lineage differentiation depends on a regulatory cascade involving Notch signaling[10], the hairy enhancer of split (HES) transcription repressor and proendocrine basic helix-loop-helix (bHLH) transcription factors[11]. Expression of proendocrine bHLH factors, which is positively influenced by inactivation of Notch signaling[11], enables cells to differentiate toward divergent subsets of mature hormone-producing endocrine cells[12,13]. Several key transcription factors are involved in the regulation of enteroendocrine cell differentiation[14], which takes place within the crypts. Completely differentiated enteroendocrine cells are mainly determined to migrate upward along

the villus as mature hormone-producing cells[12,13]. However, a small population of enteroendocrine cells migrates downwards to the bottom of the crypt or stays localized at the crypt base[15], where they reside in a Wnt signaling active zone and express both stem and postmitotic mature endocrine cell markers[16]. In both, intestinal and enteroendorine cell populations, expression of stem cell markers and continuous exposure to Wnt signaling could be hallmarks of cells, which are susceptible to neoplastic transformation[16,17].

However, data from a mouse model indicate that probably not these terminally differentiated enteroendocrine cells, but their early precursors respond to abnormal Wnt signaling by developing serotonin-expressing adenomas of the small intestine[18]. The concept that exocrine and endocrine components of CRC have the same cellular origin is supported by studies on mixed adenoneuroendocrine carcinomas (MANEC) and neuroendocrine carcinomas with minor associated exocrine components, which could demonstrate that both components share somatic mutations in several genes as *APC, TP53, DCC, KRAS, BRAF, ATM, CTNNB1, ERBB4, JAK3, KDR, RB1, BCL9, FOXP1*[19-22] and display identical LOH pattern on different chromosome loci as 5q, 17p, 18q[23]. Evidence of an additional mutation (*SMARCA4*)[22] or LOH involving 6q, 11p, 18q, *APC* marker and chromosome 3[23] in the endocrine tumor cell population could indicate that this component corresponds to a higher grade transformation of the tumor[20].

According to these data from mouse and human models, higher grade tumor transformation *via* development of neuroendocrine compartments can occur in every stage of intestinal or colorectal carcinogenesis and, furthermore, it could be even therapy-related. Probably both, pre- and postoperative as well as cytotoxic and radiation therapy could induce trans-differentiation in carcinomas with completely developed phenotype as indicated by the finding of increased neuroendocrine cells in a subset of distant metastatic compared to primary colorectal carcinomas[24] and in neoadjuvant treated compared to untreated rectal carcinomas[25]. This trans-differentiation from non-neuroendocrine to neuroendocrine tumor cells has already been proven for prostate cancer in an androgen-deprived environment[26,27] and is essentially associated with the phosphatidylinositol 3-kinase-Akt-mammalian target of rapamycin (PI3K-Akt-mTOR) pathway[28]. Studies on both experimental and human sporadic neuroendocrine tumors (NETs) and on familial syndromes, in which NETs arise, point to the involvement of mTOR pathway components in neuroendocrine tumorigenesis in general[29]. Proven or putative activators of the mTOR pathway are mutations in upstream regulators of mTOR (*PTEN* and *TSC2*) and overexpression of a microRNA (miR-21) that targets PTEN and reduces
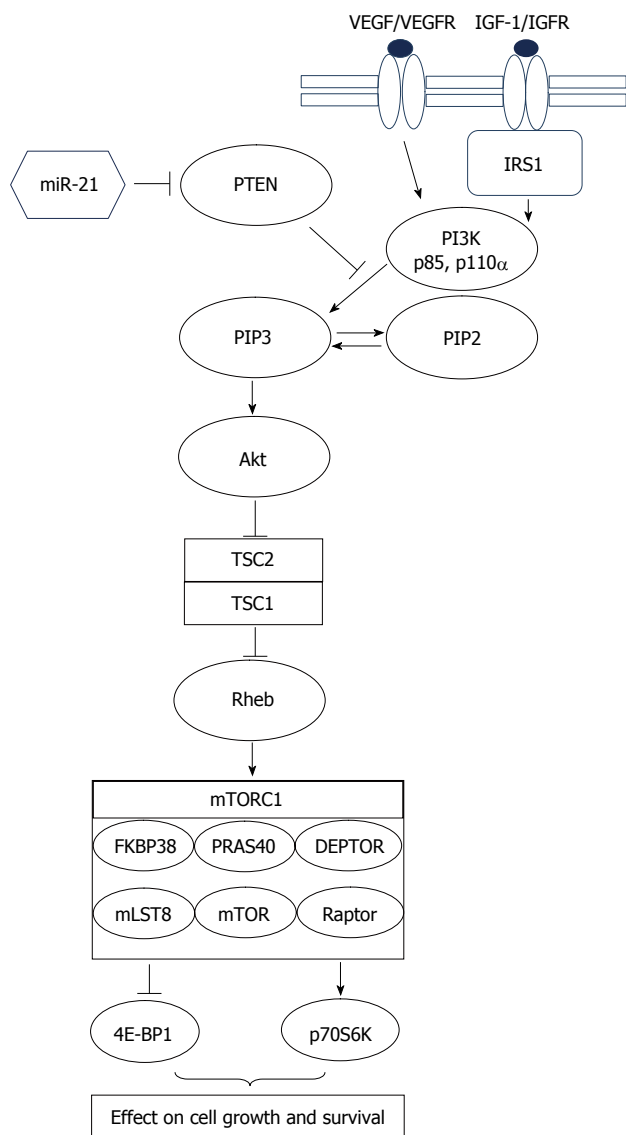
Figure 1 The PI3K/PTEN/Akt/mTOR-cascade (Modified according to Cingarlini *et al*[29] and McCubrey *et al*[32]). Phosphatidylinositol-3-kinase (PI3K) is a heterodimeric protein with an p85-kDA regulatory subunit and a p110α-kDa catalytic subunit (PIK3CA). PI3K phosphorylates membrane phospholipids, thereby forming the second messenger lipids phosphatidylinositol 3,4-biphosphate (PIP2) and phosphatidylinositol 3,4,5-triphosphate (PIP3). Pleckstrin-homology (PH) domain of kinase Akt binds to PIP3, thereby promoting activation of Akt *via* phosphorylation by phosphotidylinositide-dependent kinase 1 (PDK1, not displayed in the Figure). Akt inhibits tuberous sclerosis 2 (TSC2 or tuberin) function through direct phosphorylation. TSC2 phosphorylation by Akt represses activity of the TSC1/TSC2 complex, allowing the small GTPase Rheb to activate the protein kinase function of mTOR (mammalian target of rapamycin). mTOR forms the catalytic core of the mTORC1 complex, which comprises additionally Raptor (Regulatory associated protein of mTOR) adaptor protein, DEPTOR (DEP domain containing mTOR-interacting protein), mLST8 (member of the Lethal-with-Sec-Thirteen gene family), FKBP38 (FK506 binding protein 38), and PRAS40 (proline-rich Akt substrate 40 kDa protein). Activated mTOR phosphorylates p70S6K, which induces a negative feedback loop uncoupling IRS-1 (insulin receptor substrate-1) from PI3K, thus preventing further signal transduction through this pathway. Negative regulation of the PI3K pathway is primarily accomplished though the PTEN tumor suppressor protein, which dephosphorylates phosphoinositide substrates as PIP3. Expression of PTEN is regulated by microRNA miR-21.

its expression[29-31] as displayed in Figure 1 (modified according to Cingarlini *et al*[29] and McCubrey *et al*[32]). The association between neuroendocrine trans-differentiation and mTOR pathway could be important for new targeted therapy regimens as discussed later in this review.

NEUROENDOCRINE DIFFERENTIATION IN COLORECTAL TUMORS: DIAGNOSIS, CLASSIFICATION AND EPIDEMIOLOGY

Neuroendocrine cells in non-neoplastic and neoplastic tissue of the gastrointestinal tract and nerve elements express a panel of identical antigens, which are used as neuroendocrine markers[33]. The markers synaptophysin, chromogranin A, B and C, HISL-19, neuron-specific enolase (NSE), the proprotein convertases PC2 and PC3, the lymphoreticular epitope Leu-7, and the neural cell adhesion molecule (or CD56) are sufficient to reveal neuroendocrine differentiation[33,34] independent of hormone production[35]. An example for synaptophysin expression in a colorectal adenocarcinoma is displayed in Figure 2. In addition, syntaxin1, VAMP2, SNAP25, alpha/beta-SNAP[36] and L-dopa decarboxylase (DDC)[37] have been used as neuroendocrine markers. In the pre-immunohistochemistry era, the Churukian-Schenk argyrophil stain[38] and the Grimelius stain[39] were applied to demonstrate neuroendocrine cells, which are argyrophilic. The currently known 15 neuroendocrine cell types of the gastrointestinal tract and pancreas produce different hormones, but all of them express the general neuroendocrine marker synaptophysin[40].

In accordance with the consensus guidelines of the European Neuroendocrine Tumor Society (ENETS)[41,42], the current WHO classification for gastrointestinal neuroendocrine tumors[43] applies a grading system based on mitotic activity and the percentage of Ki-67 labeled proliferating cells: Grade 1, grade 2 and grade 3 (= neuroendocrine carcinoma) are defined by mitotic counts of < 2/10 high power fields (HPF), 2-20/10 HPF and > 20/10 HPF, respectively, and/or by Ki-67 indices of ≤ 2%, 3%-20% and > 20%, respectively. A fourth group, mixed adenoneuroendocrine carcinoma (MANEC) is morphologically recognizable as both gland-forming epithelial and neuroendocrine phenotype, with each component representing at least 30% of the lesion[43]. An additional category considering neuroendocrine differentiation less than 30%, but above the level reported for normal colorectal epithelium (> 1 cell/mm^2[44,45], > 2%[36]) similar to that proposed previously by Jansson *et al*[46] has not been defined by the current WHO classification[43]. However, a detailed study on colorectal tumors with mixed glandular-neuroendocrine differentiation[47] revealed that also neuroendocrine tumor components comprising less than the currently
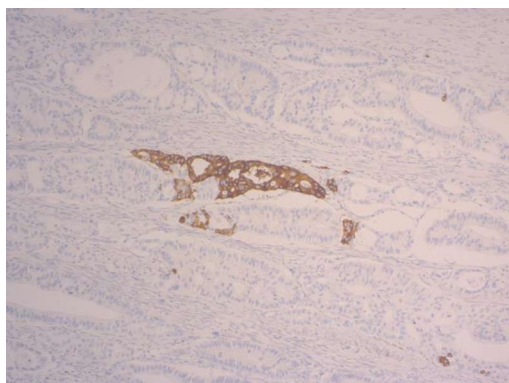
Figure 2 Primary colorectal cancer with focal synaptophysin expression (magnification × 200).

used 30% cut off could have negative impact on the clinical course and patient outcome, which sets the occasional finding of isolated neuroendocrine cells in colorectal cancer into a new focus.

In sporadic colorectal cancer, neuroendocrine cells have been identified in 8%-77.5% of cases[24,25,36,38,39,45,48-63], largely depending on the method used to assess the neuroendocrine cell population[24]. Neuroendocrine differentiation occurs also in hereditary non polyposis colorectal cancer (HNPCC, 51.4%)[64].

HISTOPATHOLOGICAL AND CLINICAL FEATURES OF SPORADIC COLORECTAL CANCER WITH NEUROENDOCRINE DIFFERENTIATION

Chromogranin A and synaptophysin are the most frequently used markers to study the link between neuroendocrine differentiation and clinicopathological characteristics. These markers are expressed in divergent patterns: Co-expression of chromogranin A and synaptophysin[63,65] occurs as well as predominance of one marker concomitant with absence of the other marker[65,66].

Studies focusing on the relationship between occasional neuroendocrine differentiation (*i.e.,* < 30%) and clinicopathological parameters in sporadic colorectal cancer are summarized in Table 1[24,25,39-71]. Detailed parameter analyses hint at the possibility that not neuroendocrine differentiation itself, but its association with poor grade of tumor differentiation, lymph node metastases, distant metastases and other unfavorable features contribute to the worse prognosis of sporadic CRC with neuroendocrine differentiation, which has been reported by several authors (Table 1). However, other authors deny a relationship between neuroendocrine differentiation and the prognosis of CRC (Table 1).

The assumption of a causal relationship between neuroendocrine differentiation and tumor differentiation

is growing stronger after introduction of a new histologic grading system based on the number of poorly differentiated cell clusters (PDC) in CRC[72,73]. In a study of 20 consecutive CRCs with high grade PDCs (≥ 10 clusters, grade Ⅲ CRCs), the PDCs, but not the glandular part expressed synaptophysin[74]. This could be the morphologic correlate for the previously discussed "trans-differentiation", which initiates the development of a more aggressive tumor[20].

The presence of neuroendocrine cells in the proliferative compartments of gastrointestinal adenocarcinomas is well-documented[38,63]. Moreover, according to recent knowledge obtained from an adult Drosophila midgut model, enteroendocrine cells could function as local regulators of intestinal stem cell proliferation through modulation of the mesenchymal stem cell niche[75]. These findings could hint at the importance of neuroendocrine cells for both, maintenance and progression of tumors, thus contributing to the development of CRC with high survival potential and aggressiveness.

Further evidence for an indirect impact of neuroendocrine cells on the clinical outcome of CRC is given by the previously published link between chromogranin A/antioxidant enzyme co-expressing CRC cells and unfavorable prognosis, probably due to activated antioxidant defense and higher metabolic activity of the tumors[76]. In addition, high expression of MTOR or its downstream targets p-RPS6KB1, p-RPS6 and p-EIF4EBP1 is associated with adverse clinical outcomes in neuroendocrine tumors[77], but this link has not been investigated for neuroendocrine foci within sporadic CRC.

NEUROENDOCRINE DIFFERENTIATION AND THERAPY OF SPORADIC COLORECTAL CANCER

Neuroendocrine differentiation has been recognized as result of therapy and is getting into the focus of oncologist as target for new treatment approaches.

Shia *et al*[25] demonstrated an increased frequency and density of cells with an endocrine phenotype in rectal adenocarcinomas treated with neoadjuvant radiochemotherapy and found that the extent of endocrine cells appeared proportional to the degree of treatment response. This therapy-related endocrine differentiation of tumor cells could be induced by cytotoxic insult[25].

The approval of new drugs for the treatment of advanced pancreatic neuroendocrine tumors[6-8] harbored the possibility to extend the therapeutic spectrum also for neuroendocrine differentiated tumors beyond the pancreaticobiliary tract. Everolimus plus octreotide long-acting repeatable (LAR) showed significant benefits and improved outcomes for patients with advanced colorectal neuroendocrine tumors[78].

Table 1 Relationship between neuroendocrine differentiation and clinico-pathological parameters

Clinical parameter	Link to neuroendocrine differentiation	Ref.
Age	No	[39, 55, 63]
Gender	No	[39, 55, 63]
Preoperative conditions	Association with lower CEA levels	[63]
Tumor markers		
DNA ploidy	No	[50]
TP53 expression	No	[50, 67]
	Similar abnormal expression as in conventional adenocarcinomas	[25]
BCL-2 expression	Yes	[68]
Ki-67 labeling index	Very low (< 5%)	[25]
	No	[24]
Tumor localization	No	[24, 25, 39, 45, 48-50, 53, 55, 63, 66]
	Yes	[38, 59]
Tumor morphology		
Polypoid *vs* ulceration	No	[55, 63]
Tumor differentiation	No	[24, 39, 45, 48-50, 52, 53, 63, 66]
	Yes	[38, 56, 57, 60, 69]
Tumor size	No	[55, 63]
Tumor stage	No	[24, 39, 45, 48-50, 53, 55, 66]
	Yes	[56]
Lymphatic and venous invasion	No	[55, 63]
Perineural invasion	No	[63]
Lymph node metastases	No	[55]
	Yes	[57, 58]
Distant metastases	Yes	[47, 51]
Clinical stage	No	[53, 55]
	Yes	[36]
Therapy response	Associated with better response to radiochemotherapy	[25]
Prognosis	No	[38, 44, 50, 53, 55, 62, 63]
	Better prognosis	[60]
	Shorter survival from time of metastasis	[24]
	More aggressive behavior	[48]
	Poor prognosis	[36, 39, 45, 46, 54, 56, 57, 59, 61 (stage Ⅱ), 66 (stage Ⅲ and Ⅳ), 70, 71]

Phase Ⅰ trials including CRC patients demonstrated a positive effect on stable disease for one of these drugs, everolimus, when it was combined with cetuximab[79] or with 5-fluorouracil/leucovorin (5-FU/LV) or with mFOLFOX6 (5-FU/LV + oxaliplatin)[80]. Everolimus inhibits the PI3K/PTEN/Akt pathway by connecting to the FK-506 binding protein 12 to block mTOR (mammalian target of rapamycin) activation[79]. Considering a possible importance of the PI3K-Akt-mTOR pathway for neuroendocrine differentiation[28], neuroendocrine cells within sporadic CRC could be the putative target for mTOR-inhibitor therapy (for example everolimus). The published pharmacodynamics trials[79,80] did not consider special CRC phenotypes. However, according to new insights into genotype-phenotype correlations, pretreatment histomorphological characterization of CRC could possibly help to increase efficacy of mTOR-inhibitor therapy.

CONCLUSION

A possible role of neuroendocrine differentiation as prognostic marker and therapeutic target in sporadic colorectal cancer should be further elucidated by large cohort studies.

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