Neuroendocrine differentiation in sporadic CRC and hereditary nonpolyosis colorectal cancer[1]

M.H. Sun[*]
State Key Laboratory of Genetic Engineering; Department of Pathology, Cancer Hospital/Cancer Institute, Fudan University, 200032 Shanghai, P.R. China

Abstract. Extent neuroendocrine differentiation can be encountered in many human neoplasm derived from different organs and systems using immunohistochemistry and ultrastructural techniques. The tumor cells' behaviors resemble those of neurons and neuroendocrine cells. The presence of neuroendocrine differentiation reputedly appears to be associated with a poorer prognosis than the adenocarcinoma counterparts in sporadic human neoplasm. In this review the neuroendocrine carcinoma and the adenocarcinoma with neuroendocrine differentiation of colon and rectum both in sporadic colorectal carcinoma and the hereditary nonpolyposis colorectal cancer, the relationship of neuroendocrine differentiation and some possible molecular pathways in tumorogenesis of colorectal cancer will be discussed. Possible treatment strategy will also be addressed.

Keywords: Colorectal neoplasm, neuroendocrine differentiation, hereditary nonpolyposis colorectal cancer

1. Introduction

Extent neuroendocrine differentiation can be encountered in many human neoplasm derived from different organs and systems using immunohistochemistry and ultrastructural techniques [1–5]. The tumor cells' behaviors resemble those of neurons and neuroendocrine cells, having the ability to uptake/set free the neuroendotransmitters and different neuroendocrine substances [6], but the secretion is out of control. The colorectal carcinoma with neuroendocrine differentiation associates with a poorer prognosis than the adenocarcinoma counterparts in sporadic human

neoplasm [7–12], while such more aggressiveness of the tumor was not found in some other studies [13–17].

In this article we will discuss the neuroendocrine carcinoma and the adenocarcinoma with neuroendocrine differentiation of colon and rectum both in sporadic colorectal carcinoma and the hereditary nonpolyposis colorectal cancer, the relationship of neuroendocrine differentiation and some possible molecular pathways in tumorogenesis of colorectal cancer. Possible treatment strategy will also be addressed.

2. Neuroendocrine carcinoma and adenocarcinoma with neuroendocrine differentiation (NE) in colon and rectum

The typical neuroendocrine colorectal cancer is the rare small cell undifferentiated cancer [10]. According to WHO classification for tumors (2000) of the colon and rectum such tumors are classified to poorly

differentiated or undifferentiated carcinoma. The tumors possess predominant neuroendocrine differentiation (NE) microscopically and ultrastructurally [11]. Colonic small cell undifferentiated carcinoma had an extremely poor prognosis [7–12]. There have been a dozen of articles focusing on the tumors with NE [18–23]. According to Staren's group [20], all poor differentiated colorectal cancer selected from "normal carcinoma" can be divided into four groups depending on the status of NE differentiation: 1) pure exocrine carcinomas, 2) pure NE carcinomas, 3) mixed exocrine and NE carcinomas, and 4) exocrine carcinomas with occasional NE cells. Thus, phenotypically mixed exocrine and NE carcinomas comprise the largest group while the second largest group exhibits exclusive features of exocrine phenotype. Staren studied 683 colorectal cancers with microscope, immunohistochemistry and electromicroscope and defined 13 cases of solid clusters or ribbons of round to fusiform, small to intermediate-sized cells with variably abundant mitoses [20]. In "normal" or "routine" colorectal cancer as well as some cell lines derived from colorectal cancer NE is also a demonstrated common event [13,21–24]. Poorly differentiated colorectal cancer appeared to have more frequent neuroendocrine differentiation [10].

Most commonly used NE markers are NSE, chromogranin A and synaptophysin showing a suitable sensitivity and specificity. Neuroendocrine differentiation of immunohistochemistry displays three different staining patterns: 1) diffuse staining, mostly in poorly differentiated and undifferentiated colorectal cancer with all tumor cells stained positively; Most of the immunohistochemical markers stain the cytoplasm and display the form of the cells with round, oval or irregular shape without polarization [17]. The diffuse pattern is also described as the extent or pure pattern of NE. 2) more than 25% of all tumor cells are positive for neuroendocrine differentiation markers; The tumors were moderately or poorly differentiated but glandular structure is prominent; The positive cancer glandular structure located between the similar structured but negative stained tumor tissue. 3) more than 2% tumor cells are positive with NE; The positive cells scatter in the glandular structure, resembling the neuroendocrine cells in the normal mucous; The standard of > 2% cut is according to the 1% NE cell in normal mucous [22,25]. It is suggested that the colorectal cancer with extended expression of neuroendocrine markers should be defined as the neuroendocrine caner and the cancer containing more than 2% and 25% NE component could be defined as the cancer with neuroendocrine differentiation. But

usually both nomenclatures are used to define the tumor with expression of neuroendocrine differentiation markers.

3. Prognosis of neuroendocrine colorectal cancer and the colorectal cancer with neuroendocrine differentiation

The patients with typical neuroendocrine carcinoma had an extremely poorly prognosis [10]. Staren's group [20] has identified 43 undifferentiated colorectal cancer distinguished from all 683 colorectal cancers divided them into four groups as mentioned above: I) pure exocrine ($n = 8$), II) pure neuroendocrine ($n = 4$), III) mixed exocrine and neuroendocrine carcinomas ($n = 23$), and IV) predominant exocrine carcinomas with occasional neuroendocrine cells ($n = 9$). Group I had the best prognosis. Survival among groups II and III appeared to be less than group I. The cases in the group III with predominantly neuroendocrine mixed carcinomas also showed affirmed poor prognosis. That means that most of the poorly differentiated colorectal carcinoma has variant neuroendocrine differentiation. The extension of the neuroendocrine differentiation also plays a role in the prognosis. The median survival of the neuroendocrine differentiation ranged from 5 months to 22 months. The median survival of the patients with unique neuroendocrine and predominant neuroendocrine cells was five months; the patients with equal endo- and exocrine differentiation had a median survival of 22 months. In their later study, CRC with NE selected with NE markers showed similar results [18]. Some studies revealed that the poor prognosis of CRC with NE is related with marked tumor invasion of lymphatic and veins resulting in liver metastases, more aggressiveness, lymph node and distant metastasis [22,24,26,27]. Colorectal neuroendocrine neoplasm usually showed a poor differentiation. And carcinomas with neuroendocrine differentiation showed usually worse prognosis compared with the "normal" colorectal carcinomas in the same stage [28]. Gaffey found that the carcinoma with neuroendocrine differentiation had similar worse follow-up no matter whether the cancer is poor or moderately morphologically differentiated. Therefore the neuroendocrine markers for immunostainning had been suggested as a follow-up parameter [11]. Elevated serum level of the same human hormone or peptide expressed in colorectal tumor tissues had been demonstrated [29,30]. An in vivo xenograft model of NE tumor in nude mice

has been established and a higher serum level of hormone detected was seen [31]. Neuroendocrine protein or neuroendocrine transmitter in serum of patients with colorectal cancer could also serve as a prognostic indicator.

Several recent studies focused on the variant neuroendocrine differentiation in "routine" or "convenient" colorectal cancer. The results were however controversial [21,28,32,33]. Atasoy studied 50 cases of colorectal cancer with the antibodies against chromogranin A, neuron-specific enolase and synaptophysin. Positive staining of Chromogranin A was correlated with the grade and stage of the tumors and was associated with a decreased effect on survival [21,33]. But another study could not find any correlation between the NE and the prognosis [17,34]. A convincing relationship could only be settled when a large number of patient collective were put into the retrospective study. Further study of the area should be addressed to give a clear clue to evaluate the significance of NE in colorectal cancer.

4. Neuroendocrine differentiation in hereditary nonpolyposis colorectal cancer

There have been few articles about neuroendocrine differentiation in HNPCC. Kim studied the sporadic RER+ colorectal cancer using epithelial and neuroendocrine differentiation markers cytokeratin, NSE, chromogranin and synaptophysin. Most RER+ cancers were positive stained for cytokeratin but only 2/23, 3/23 and 0/23 were positive for NSE, chromogranin and synaptophysin. All ERE+ tumors showed poor differentiation and prominent peritumoral reaction [35]. This suggests that there is no correlation of RER+ and neuroendocrine differentiation in sporadic colorectal cancer. However, RER+, poor differentiation and prominent lymphocyte infiltration are all the features of HNPCC. According to Kim's study there was less neuroendocrine differentiation in RER+ cancers. In HNPCC poor differentiated colorectal cancer is more frequent than the sporadic ones [36,37]. To our knowledge there have been no published studies on the neuroendocrine differentiation in HNPCC. Our group has analyzed 34 HNPCC and 23 sporadic colorectal cancers using the neuroendocrine markers NSE, chromogranin A and synaptophysin, which were considered both specific and sensitive. The total positive frequencies of the staining were 43.48% (30.43% for NSE, 4% for chromogranin A, and 34.78% for synaptophysin) in

sporadic colorectal cancer and were 51.42% (47.1% for NSE, 17.6% for chromogranin and 35.3% for synaptophysin) in HNPCC. Statistical analysis revealed that there was no significant difference in the markers of NSE and synaptophysin, whereas the staining of chromogranin A showed significant difference between sporadic (4%) and hereditary nonpolyposis colorectal cancer (17.6%), but the positive cases were very low in both groups. The sporadic CRCs with neuroendocrine differentiation showed no tight correlation with the grade of differentiation (2/10 poor differentiated). Also the MSI status and neuroendocrine differentiation had no obvious link. A poor prognosis of the tumor with neuroendocrine differentiation in sporadic colorectal cancer was seen. It was however not demonstrated in the group of HNPCC. In our study there was only one death in HNPCC group, a patient in Dukes D [25] (unpublished data). In one case fulfilling Amsterdam Criteria two huge synchronous colonic cancers with extended neuroendocrine differentiation [38] of HNPCC was defined. Two tumors were located in ascending colon and transversal colon. While the tumor in transversal colon showed only poor and undifferentiated component, the tumor in ascending colon showed additional tendency of gland forming. Common positive staining of NES, pp, Gastrin, Somatostanin, Glusagon, common negative staining of chromogranin, ACTH in both tumors and alternative staining of Calcitonin, Serotonin, Synaptophysin in either tumor were detected. The tumors displayed microsatellite instability (4/5, 5/5) using the ICG-HNPCC recommended panel. The patient possesses a germline 24 bp deletion in exon 11 of hMLH1 gene. A 50 months disease free follow up was recorded by the submission of this study. NE in HNPCC is frequent but not a poor prognostic indicator. Vortmeyer et al. studied 9 colorectal neuroendocrine carcinomas using microsatellite analysis after microdissection. High LOH at APC, DCC and p53 genes were observed in 6 out of 8 informative tumors [39]. This is in agreement with the allelic deletion of tumor suppressor genes in the majority of sporadic CRC. These results indicate that CRC from HNPCC and sporadic CRC with neuroendocrine differentiation possess different genetic features of tumor development. HNPCC should undergo different carcinogenesis as sporadic CRC with NE. Of note, 3/10 sporadic CRCs with NE possess also loss expression of mismatch repair gene. In summary, the clue in distinguishing sporadic and hereditary nonpolyposis colorectal cancer with neuroendocrine differentiation remains to be found.

5. The mechanism and the significance of the neuroendocrine differentiation in sporadic colorectal cancer and HNPCC

The study of neuroendocrine differentiation in colorectal cancer has begun since 1978 [40]. Typical neuroendocrine cancer of colon and rectum showed an extremely poor prognosis. After that the neuroendocrine differentiation in "routine" colorectal cancer was also studied, most studies demonstrated that neuroendocrine differentiation was also a poor prognosis in "normal" glandular carcinoma. How the neuroendocrine differentiation affects the prognosis remains unclear. But there are some indicative studies revealing the relationship between neuroendocrine differentiation and some tumor associated genes or factors. Double immunostaining for the Bcl-2 as well as human vasoactive intestinal polypeptide, pancreatic polypeptide and somatostatin showed simultaneous expression in a study of 52 advanced colorectal cancers and their surrounding mucosa. Co-existing expression of Bcl-s and neuroendocrine markers suggests Bcl-2 protein does not only inhibit apoptosis but also influence gut neurohormonal polypeptide/amine production, which closely correlates to the occurrence of tumor [41]. Other study showed that the IL-1 expression coexisted in some colorectal carcinoma cell lines expressing CGA and IL-1RI. Furthermore, exogenously added IL-1 could cause a decrease in CGA, but an increase in CEA secretion, suggesting an inverse relationship between IL-1 and NE differentiation, as well as a direct relationship between IL-1 and CEA expression in colon carcinoma [23]. It also suggests that IL-1, a factor implicated in the growth and metastasis of several malignancies is involved in the tumorogenesis [42–44]. Also, IL-1 is known to induce the expression of matrix metalloproteinases in several cell types. And high CEA levels have been associated with metastasis in colon cancer [45]. That IL-1 can influence both exocrine and endocrine pathway is a very interesting clue for developing new treatment strategy of colorectal cancer with neuroendocrine differentiation. It is a complex relationship between cytokine and expression of exocrine or endocrine secretion of the colorectal cancer that needs thorough study. The pathway of the co-existing Bcl-2 and chromogranin and the inverse effect of IL-1 to the differentiation of chromogranin could be a new clue to block the differentiation towards neuroendocine direction of colorectal cancer. Interestingly, all 7 cell lines (Colo320DM, Lovo, SW403, SW1116, SW1417, LS123 and LS174t) displayed CGA expression [23]. Among the 7 cell lines LoVo and LS174t were demonstrated to possess microsatellite instability [46]. It seems that microsatellite instability correlates with IL-1 alpha expression but not with IL-1 beta. The tumorogenesis do not correlate to the microsatellite status of the cell lines [23]. It is another thread that two main molecular pathways of tumorogenesis of colorectal cancer i.e. the chromosome instability and the microsatellite instability switch on some common procedures in tumor behavior.

The studies of the clonality of the dominant exocrine adenocarcinoma with neuroendocrine differentiation showed that both tumor components possess similar genetic alteration. Loss of heterozygosity analysis revealed most of the cases with different multidirectional differentiated tumor components displayed identical genetic alterations in the loci studied [47,48]. Such changes resemble the ones observed in most sporadic colorectal cancers. This suggests likely that either a pluripotent epithelial stem cell or an adenocarcinoma precursor cell could be the origin of the neuroendocrine differentiated content. However, identical genetic alteration could not be found in different morphological parts of the same tumors [47,49]. It lacks the data about the difference of the tow main pathways as in the majority of sporadic colorectal cancer and hereditary nonpolyposis colorectal cancer. Study on this field is of important and necessary.

6. Treatment of colorectal cancer with neuroendocrine differentiation

There has been no standard therapy of the colorectal cancer with neuroendocrine differentiation [21]. The main strategy of treatment of neuroendocrine differentiated tumor is to make use of the analogues of the hormones uncontrolled secreted by tumor cells with neuroendocrine differentiation. Biotherapy with interferon-alpha and somatostatin analogues and chemotherapy are used in the neuroendocrine differentiated tumors. Somastostatin analogues are widely used in patients with symptoms and with carcinoids of low differentiation grade [50]. Somastostatin is also the most common hormone detected in neuroendocrine cancer or cancer with neuroendocrine differentiation. Somatostatin analogues is considered to be effective [51]. Chemotherapy is active in patients with poorly differentiated neuroendocrine tumors but it lacks long term regimen. The chemotherapeutic regimen commonly used is the combination of cisplatinum and etoposide. Radioisotope treatment according to the

transporter systems might be suitable to treat the tumor with different hormone secretion.

Recently, Hoepfner has used radioactive-labeled meta-iodobenzylguanidine (MIBG), a monoamine morepinepherine analogue in the culture of neuroendocrine gastrointestinal tumor cell line STC-1 to see the effect of MIBG in antiproliferation, cytotoxity, cell cycle and apoptosis. MIBG could induce apoptosis by changing mitochondrial membrane potential, activating caspase-3 and DNA fragmentation. Some genes involved in proliferation, apoptosis and stress responses could be altered by MIBG. MIBG showed no effect on the colorectal cancer cell line without NE differentiation. It may be a promising approach to use monoamine transporters in the plasma membrane of neuroendocrine gastrointestinal tumor cells for innovative and specific treatment strategies of these tumors [52]. It might be valuable to demonstrate if the hormone inhibition can go into effect in the advanced HNPCC associated tumors.

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