Should immunohistochemical expression of mismatch repair (MMR) proteins and microsatellite instability (MSI) analysis be routinely performed for poorly differentiated colorectal neuroendocrine carcinomas?

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Summary

Colorectal poorly differentiated neuroendocrine carcinomas (NECs) are typically associated with poor outcomes. The mechanisms of their aggressiveness are still being investigated. Microsatellite instability (MSI) has recently been found in colorectal NECs showing aberrant methylation of the *MLH1* gene and is associated with improved prognosis. We present a 76-year-old lady with an ascending colon tumour showing features of a pT3 N0 R0, large cell NEC (LCNEC) following right hemicolectomy. The adjacent mucosa showed a sessile serrated lesion (SSL) with low-grade dysplasia. Immunohistochemistry showed loss of expression for MLH1 and PMS2 in both the LCNEC and dysplastic SSL. Molecular analysis indicated the sporadic nature of the MLH1 mismatch repair (MMR) protein-deficient status. Our patient did not receive adjuvant therapy and she is alive and disease-free after 34 months follow-up. This finding, similar to early-stage MMR-deficient colorectal adenocarcinoma, is likely practice-changing and will be critical in guiding the appropriate treatment pathway for these patients. We propose that testing of MMR status become routine for early-stage colorectal NECs.

Learning points:

- Colorectal poorly differentiated neuroendocrine carcinomas (NECs) are known to be aggressive and typically associated with poor outcomes.
- A subset of colorectal NECs can display microsatellite instability (MSI) with mismatch repair (MMR) protein-deficient status.
- MMR-deficient colorectal NECs have been found to have a better prognosis compared with MMR-proficient NECs.
- MMR status can be detected using immunohistochemistry.
- Immunohistochemistry for MMR status is routinely performed for colorectal adenocarcinomas.
- Immunohistochemical expression of MMR protein and MSI analysis should be performed routinely for early-stage colorectal NECs in order to identify a subgroup of MMR-deficient NECs which are associated with a significantly more favourable prognosis.

Background

Colorectal poorly differentiated neuroendocrine carcinomas (NECs) are rare and known to be aggressive. The mechanisms of their carcinogenesis and aggressiveness are still being investigated. Recently, Takizawa *et al.* (1) showed that the molecular features of colorectal NECs are similar to those of adenocarcinoma as opposed to those of neuroendocrine tumours (NETs). Both the loss of Rb expression and high expression of p16 and Bcl-2 were more evident in small cell NECs (SCNECs) compared with large cell NECs (LCNECs). This is supported by case reports of the coexistence of colorectal NECs and conventional adenoma/adenocarcinoma (2). Stelow *et al.* (3) showed that DNA mismatch repair proteins were intact in 14/15 colorectal SCNECs. A case of LCNEC arising from a sessile serrated lesion (SSL) has been reported before, suggesting a rare but potentially novel endpoint for the microsatellite instability (MSI) pathway (4). Sahnane *et al.* have recently demonstrated that a subset of colorectal NECs exhibiting MSI and extensive gene hypermethylation showed improved survival compared with NECs without these features (5). Six out of their 11 MSI-NEC cases were LCNECs.

Herein we report a case of a colorectal LCNEC associated with an SSL, showing loss of mismatch repair protein expression and 34 months disease-free follow-up.

Case presentation

A 76-year-old lady initially presented to her general practitioner with change in bowel habit. Her past medical history included hypertension and hypercholesterolaemia. There was a significant family history of cancer in that her sister was diagnosed with breast cancer and her mother and her brother were diagnosed with lung cancer. She was found to have an ascending colon tumour on colonoscopy and biopsy revealed a neuroendocrine

carcinoma with a Ki-67 of 70%. There were no metastases on CT chest, abdomen and pelvis. The referring team arranged a ^{68}Ga Gallium DOTATATE PET/CT, which showed low-grade uptake in the ascending colon tumour indicating poor somatostatin receptor expression. She underwent a right hemicolectomy. The macroscopic examination revealed a 24 mm tumour in the ascending colon, displaying histological features of a pure LCNEC (Fig. 1B). No glandular differentiation was seen, excluding a mixed neuroendocrine–non-neuroendocrine neoplasm (MiNEN). Tumour necrosis was present (<30%). The mitotic activity was high (approximately 35 mitoses/10 high power fields) and included both typical and atypical mitotic figures. Immunohistochemistry showed positivity of the LCNEC for pancytokeratins, chromogranin (Fig. 2A), synaptophysin, CD56 and CDX2. Overexpression of p53 was seen. The proliferation index with Ki-67 was high, approximately 70% (Fig. 2B). There was no host lymphoid response and tumour infiltrating lymphocytes were not seen. The tumour invaded the full thickness of the colonic wall and extended for <1 mm beyond the muscularis propria into the subserosa with no serosal breach (pT3, ENETS 2007 TNM stage). Nine lymph nodes were retrieved and were all uninvolved by tumour (pN0). Intramural venous invasion was seen, but no extramural venous invasion was present. There was no perineural invasion. All surgical margins were negative (R0 resection). The mucosa overlying the LCNEC showed an SSL with a focus of conventional adenomatous-type low-grade dysplasia (LGD) (Fig. 1A). Immunostains for mismatch repair (MMR) proteins were performed and showed loss of expression for MLH1 (Fig. 3A) and PMS2 in both the LCNEC and dysplastic SSL; normal MMR expression was seen within the non-dysplastic SSL. There was normal expression for MSH2 (Fig. 3B) and MSH6. Molecular analysis revealed WT RAS, a BRAF p.Val600Glu, c.1799T>A mutation and a TP53 p.Arg248Gln, c.743G>A mutation.

Figure 1

(A) Large cell neuroendocrine carcinoma (LCNEC) is seen invading the submucosa. A sessile serrated lesion (SSL) is seen in the overlying mucosa, at the edge of which is a focus of low-grade adenomatous-type dysplasia (LGD) (H&E $\times 4$). (B) Higher magnification of the LCNEC shows intermediate/large-sized tumour cells with abundant eosinophilic cytoplasm and pleomorphic nuclei with prominent nucleoli and arranged in solid growth pattern. High mitotic activity is noted (H&E $\times 30$).

Figure 2

(A) Immunohistochemistry shows positivity of the neoplastic cells for chromogranin (×20). (B) Immunostain for Ki-67 shows a high proliferation index, 70% (×20).

Investigation

In view of the MLH1 MMR-deficient status of the LCNEC and significant family history of lung and breast cancer, it was recommended that the patient be referred for discussion with a geneticist. The genetics clinic review concluded that the patient's cancer had occurred as an age-related event rather than due to hereditary factors, as the *BRAF* mutation indicates the sporadic nature of the MLH1 MMR-deficient status and lung and breast cancers are not part of the spectrum of cancers seen in Lynch syndrome.

The patient was referred to the Royal Free NET Unit, a certified European NET Centre of Excellence, after the surgery for a management opinion.

Review of the patient's post-operative CT and ¹⁸F-fluorodeoxyglucose (FDG) PET showed no evidence of residual or recurrent disease.

Treatment

Given that no lymph nodes were involved and the R0 resection status, adjuvant chemotherapy was not recommended, particularly in light of MLH1 and PMS2 deficiency which may confer reduced benefits of chemotherapy in this setting.

Outcome and follow-up

The patient continues on 6-monthly CT surveillance and 12-monthly endoscopy surveillance with no evidence of recurrent disease after 34 months follow-up.

Discussion

Colonic poorly differentiated NECs are by definition high-grade NECs according to the latest WHO 2019 GI-NET classification (6) and can be small cell type or large cell type. They are morphologically similar to SCNEC and LCNEC of the lung. From a pathologic point of view, a number of observations from the study by Shia *et al.* (7) indicate that small cell and non-small cell variants have different characteristics that may imply different biology. Differences were found particularly in regard to tumour location; NECs arising from the squamous mucosa of the gastrointestinal tract (oesophagus and anal canal) were most frequently SCNECs but in contrast, NECs arising from the glandular mucosa (especially the large bowel) were more often LCNECs. In addition, the frequency and type of a non-neuroendocrine component, consistent with MiNEN, also differed. A non-neuroendocrine carcinoma component occurred in 61% of LCNECs and 35% of SCNECs; this was predominantly adenocarcinoma

Figure 3

(A) Immunostain for MLH-1 shows normal expression (dark brown positive nuclei) within the sessile serrated lesion (SSL) and loss of expression within the large cell neuroendocrine carcinoma (LCNEC) and the focus of low-grade dysplasia (LGD). Internal positive control can be seen within the normal colonic crypts and inflammatory cells (ICs). (B) Immunostain for MSH-2 shows diffuse normal expression within SSL, LCNEC and the focus of LGD (×4).

however the few cases of associated squamous cell carcinoma were only found in SCNECs.

Shia *et al.* (7) and Takizawa *et al.* (1) showed that patients with NEC of the GI tract have a poor prognosis. The median overall survival in the Shia *et al.* (7) series was only 15.7 months (95% CI: 11.3–19.7). No significant prognostic difference was seen between SCNECs and LCNECs, although according to Takizawa *et al.* (1) the morphological features of SCNECs and LCNECs differ significantly by definition. Furthermore, both Shia *et al.* (7) and Takizawa *et al.* (1) demonstrated that there are some differences in molecular features between SCNECs and LCNECs, proving that tumour morphology is often a reflection of its underlying molecular abnormalities. In this context, SCNECs and LCNECs should remain separate pathologic categories.

We describe a case of LCNEC associated with an overlying SSL with LGD. NECs have been rarely reported to coexist with adenoma and/or adenocarcinoma (2, 7, 8, 9) and only one case of LCNEC in association with an SSL (4) has previously been described. The simultaneous occurrence of both tumours suggests a common histogenesis. In support of this, the immunohistochemistry and molecular analysis demonstrate that both the LCNEC and the dysplastic SSL share the MLH1 MMR-deficient status.

Serrated colorectal polyps are a heterogeneous group of lesions characterised morphologically by a serrated architecture of the epithelial compartment. Cytological dysplasia is not present in uncomplicated SSL, but develops with progression toward carcinoma. SSLs are prone to methylation of the promoter regions of a number of genes, the most important of which is *MLH-1*, a DNA MMR gene. When methylation occurs, SSLs develop MSI. Carcinomas arising from premalignant SSLs follow the proposed MSI pathway.

The association of our present case of LCNEC with a dysplastic SSL supports the La Rosa group's (5) findings that the MMR defect seems to occur early in the tumourigenic pathway of MSI-NECs. The La Rosa group investigated the incidence of the MSI phenotype in 89 patients with GEP-NEC/MANECs and MSI was observed in 11 (12.4%): seven intestinal and four gastric. MSI-NEC/MANECs were observed in gastric and colorectal sites with very similar frequency to that reported for MSI gastrointestinal adenocarcinomas. The pathogenetic mechanisms, as well as the clinicopathologic and the molecular profiles of MSI-NEC/MANECs, closely resemble those described for sporadic gastric and colorectal MSI-adenocarcinomas. All but two MSI-cases showed *MLH1* methylation and loss of

MLH1 protein. The remaining two MSI-cancers showed lack of MSH2 or PMS2 immunohistochemical expression. Six out of 11 cases were of the large cell subtype and, interestingly, no carcinomas showed distant metastases at the time of diagnosis, being classified as stage II or III depending on the nodal status. GEP-NEC/MANECs showing aberrant methylation of the *MLH1* gene were associated with a better outcome, with a median overall survival of 61.5 months compared with 6 months for MLH1-unmethylated cases ($P=0.01$). Regarding the prognostic value of the Ki67 index, the La Rosa group (5) observed an unexpected finding. The 55% threshold index of the Nordic NEC series (10) failed to prognosticate NEC/MANECs when applied to their whole series, including both MSI- and microsatellite-stable (MSS) cases. However, it successfully identified two different prognostic groups when only MSS carcinomas were considered, after excluding MSI carcinomas from the analysis ($P=0.049$), suggesting that the Ki-67 index has a different prognostic value in MSI-positive and in MSI-negative neoplasms.

As for conventional colorectal adenocarcinoma, early-stage MMR-deficient NECs have been found to have a better prognosis compared with MMR-proficient NECs (5). Multiple retrospective and population-based studies have shown that patients with early-stage MMR-deficient colorectal adenocarcinomas have a more favourable prognosis than those with MMR-proficient tumours (11). Furthermore, there is evidence of lack of benefit of 5-fluorouracil-based adjuvant chemotherapy in stage II MMR-deficient colorectal adenocarcinoma and adjuvant chemotherapy is not recommended in this setting (12, 13). Recently, several clinical trials have demonstrated long-term immunotherapy-related responses and improved prognosis in patients with MMR-deficient metastatic colorectal adenocarcinoma treated with immune checkpoint inhibitors (14) and an adjuvant trial with an anti-programmed cell death ligand-1 (anti-PD-L1) monoclonal antibody is currently in progress (NCT02912559).

In contrast to colorectal adenocarcinoma, there is scarce evidence to guide treatment decisions in the colorectal NEC population confined to retrospective series and small non-randomised clinical trials. Guidance (15, 16) is, therefore, based on the much more common small cell carcinoma of the lung and platinum-based chemotherapy regimens, commonly cis/carboplatin and etoposide, are typically used. Based on the fact that NECs of colorectal origin have a molecular profile similar to adenocarcinoma (1), oxaliplatin-based (XELOX, FOLFOX) and irinotecan-based (FOLFIRI, IP) regimens are also used,

often in the salvage setting following progression after first-line platinum/etoposide (15). Given the high relapse rates following resection of early-stage disease in the wider colorectal NEC population, most oncologists would advocate for platinum-based adjuvant chemotherapy (15). However, in the setting of early-stage MMR-deficient colorectal NEC, the prognosis is likely more favourable and, similar to early-stage MMR-deficient colorectal adenocarcinoma, the benefit of adjuvant chemotherapy may be far less. Similarly, there may also be a role for immune checkpoint inhibitors in MMR-deficient colorectal NEC and, should benefit be proven in the future, it may become important to determine MMR status in both early and advanced-stage colorectal NEC. Prospective and preferably randomised studies would be required to validate these extrapolations.

Our patient with resected pT3 N0 M0 R0 ascending colon LCNEC with deficient MMR status shows no evidence of disease recurrence after 34 months follow-up and in the absence of adjuvant therapy.

Colorectal adenocarcinoma patients with MMR-deficient tumours have distinct clinical and pathological features compared with their MMR-proficient counterparts, including proximal colon predominance, poor differentiation and/or mucinous histology and increased numbers of tumour infiltrating lymphocytes. The current case presented as a poorly differentiated NEC and was located in the right colon. However, no host lymphoid response or brisk tumour infiltrating lymphocytes were seen. The MSI group of the La Rosa *et al.* study included 3/21 (14.3%) right colonic and 1/12 (8.3%) left colonic neoplasms. Approximately 54% of MSI-carcinomas showed prominent intraperitumoural lymphoid infiltration. Interestingly, using CD3-immunostained sections, intratumoural lymphoid infiltration was statistically higher ($P=0.01$) in MSI than in MSS cases. Similarly, peritumoural lymphoid infiltration was also statistically higher ($P=0.0002$) in MSI cases than in MSS ones (5).

In conclusion, there is emerging evidence of right colon LCNECs reported in association with SSLs and loss of MMR protein, suggesting that some colorectal LCNECs may develop through the microsatellite instability pathway rather than the conventional pathway of colorectal adenocarcinomas. Routine testing of colorectal adenocarcinoma at the time of diagnosis for deficient MMR status (by MSI testing or MMR immunohistochemistry) is now recommended by the Royal College of Pathologists in the United Kingdom and, therefore, MMR status, assessed by either method, is considered a core data item of the

histopathology report. Similarly, immunohistochemical expression of MMR protein and MSI analysis should be performed routinely for early-stage colorectal NECs in order to identify a subgroup of MMR-deficient NECs which are associated with a significantly more favourable prognosis. This finding, similar to early-stage MMR-deficient colorectal adenocarcinoma, is likely practice-changing and will be critical in guiding the appropriate treatment pathway for these patients.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Patient consent

Written informed consent has been obtained from the patient.

Author contribution statement

T V L made the histological diagnosis. T V L and Z N wrote the first draft of the manuscript. A H provided clinical data and follow-up information. All authors commented on previous versions of the manuscript and approved the final version.

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Received in final form 29 May 2020

Accepted 13 July 2020