

Histopathology of Colorectal Cancer after Neoadjuvant Chemoradiation Therapy

Maura O'Neil* and Ivan Damjanov

Department of Pathology and Laboratory Medicine, University of Kansas School of Medicine, Kansas City, KS, USA

Abstract: Neoadjuvant treatment has become the standard of care for locally advanced gastrointestinal carcinoma, hence, the pathologist increasingly encounters treated primary and metastatic colorectal tumors. The pathologic assessment of therapeutic response and evaluation of residual disease is important because the histologic response to therapy or tumor response grade (TRG) has been shown to correlate with survival. Therefore, it is important for the pathologist to be familiar with the changes seen in colorectal cancer and metastatic colorectal cancer after neoadjuvant therapy. This review discusses the treatment effects seen in colon, rectal and liver resections including lymph nodes and the evaluation of residual tumor tissue in these specimens.

Keywords: Colon, rectum, adenocarcinoma, radiation, chemotherapy.

INTRODUCTION

Neoadjuvant treatment has become the standard of care for locally advanced gastrointestinal tumors. Preoperative radiation (RT) and chemotherapy (CT) have been shown to improve outcome in patients with locally advanced rectal adenocarcinoma [1]. An increasing number of patients with stage IV colon cancer are also being managed with neoadjuvant chemotherapy, followed by resection when downstaging allows [2]. In addition, 15-25% of patients with colorectal cancer have liver metastases at the time of presentation and the 5-year survival can be improved if the liver metastases are reduced in size by chemotherapy, thereby allowing initially unresectable patients to become resectable [3].

It is for these reasons that the pathologist increasingly encounters treated colorectal primary and metastatic tumors, and the assessment of the therapeutic response and the evaluation of residual disease are important. The histologic response to therapy or tumor response grade (TRG) has been shown to correlate with survival [2, 4, 5].

HANDLING OF SURGICAL RESECTION SPECIMENS

Prior to the processing of surgically resected intestine containing the tumor treated by chemo- and/or radiation therapy, it is imperative for the pathologist to collect as much data about the patient and the course of the disease as possible. Information routinely required includes the pre-treatment biopsy diagnosis, specific site of tumor, and clinical and radiologic impressions of the treatment response. It has been reported that the clinical assessment of the response to treatment is often inaccurate resulting in an

underestimate of the objective and verifiable response. Accordingly, the pathologic assessment of the treatment response is considered to be essential for objective assignment of the TRG, planning of additional treatment, and prognosis [6].

Colorectal Resections

Currently, there are no standards for the handling of surgical colorectal specimens after neoadjuvant therapy. If tumor is grossly visible, standard processing protocols used for routine cases are appropriate. However, if viable tumor is not grossly visible, some authors suggest embedding the whole suspicious area with the application of step sectioning as appropriate. Using this technique, some authors have not found a single case of full pathologic remission, and vital tumor cells were demonstrable in all cases of treated colorectal cancer examined in that study [7]. Similarly, Rullier *et al.* always found residual tumor cells after exhaustive pathologic examination of mucin pools in rectal cancers treated prior to the resection [8]. Hiotis *et al.* and Shia *et al.* however, found complete pathologic response rates of 10% and 14%, respectively, indicating that a complete eradication of the malignant tumor is possible, even though only a minority of cases [5, 6]. Accordingly, if no residual tumor is seen on initial microscopic examination, it has been recommended that the entire tumoral region should be blocked. These blocks then should be serially sectioned and meticulously examined microscopically to identify any residual foci of adenocarcinoma. If there is any doubt about the presence of minute foci of adenocarcinoma, immunohistochemistry with antibodies to cytokeratins should be performed.

Lymph Node Dissection

The mesocolon should be extensively examined and a systematic search for lymph nodes should be undertaken. As many lymph nodes as possible should be submitted for microscopic examination. Meticulous microscopic examination of the lymph nodes identified during gross examination, as well all those tissues that appeared suspicious for

*Address correspondence to this author at the Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, MS 3045, 3901 Rainbow Blvd., Kansas City, KS 66160, USA; Tel: 913-588-7103; Fax: 913-588-8780; E-mail: moneil@kumc.edu

metastasis should be performed. It is important to note that even if there is no residual tumor in the intestinal wall, there may be residual malignancy in the lymph nodes.

Sampling of Liver Lesions

Segmental resections of the liver are performed for metastatic lesions. The suspicious areas, usually visible radiologically, are typically identified by the surgeon during exploration and resected with adequate perilesional margins. Care should be taken to block and evaluate all these hepatic parenchymal surgical margins. In addition, the tumor bed should be sampled from its center and the peripheral zones close to the normal liver surrounding them. Multiple sections must be taken because it may be difficult to identify viable tumor cells in the mucus comprising the bulk of some lesions. If the liver contains more than one dominant nodule, all those should be sampled in addition to the main mass. Small foci of abnormal tissue found during sectioning of the peritumoral liver parenchyma, especially those containing mucus, should also be submitted for microscopic examination.

MICROSCOPIC CHANGES

Rectal Adenocarcinoma

Preoperative neoadjuvant chemoradiation therapy is now standard treatment for T3, T4, and lymph node positive rectal adenocarcinomas. The use of preoperative chemoradiation modifies the histologic appearance of rectal cancer. Rectal cancers that respond to this treatment undergo significant regression, which may result in complete disappearance of carcinoma cells and replacement of the tumor by fibrous or fibroinflammatory tissue (Fig. 1). Hemosiderin-laden and foamy macrophages may also be present. The so called "colloid response" or "mucin lakes" are often seen in post-irradiated tumors.

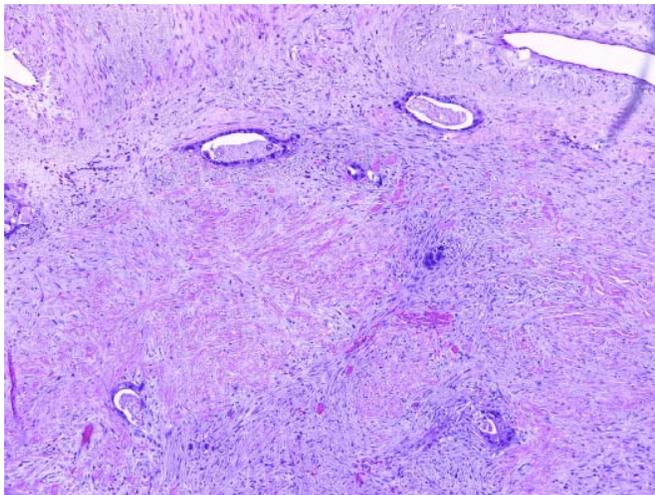


Fig. (1). Rectum, post-treatment. The morphologic features highlighted here include hyalinized fibrosis replacing neoplastic glands; lack of active tumor necrosis; and increased hyperchromasia/nuclear atypia in the remaining malignant cells. x100.

Predominant colloid changes after preoperative radiation were first described by Dworak *et al.* [7]. These large pools of mucin dissect the rectal wall (Fig. 2) and contain very few identifiable tumor cells (Fig. 3). This colloid change should

not be confused with colloid colorectal carcinoma. The irradiated tumors show a more prominent mucin component accounting for 80-90% of the lesion, which usually contain no or only rare isolated tumor cells. Furthermore such postirradiation mucin lakes are less basophilic than untreated colloid carcinomas.

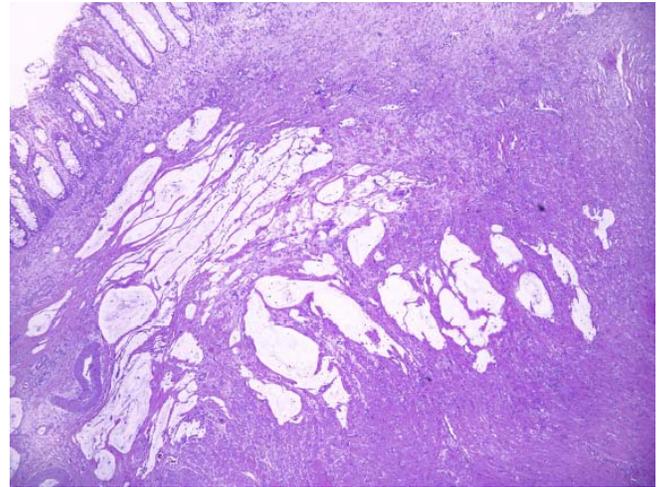


Fig. (2). Rectum, post-treatment. Colloid changes after preoperative radiotherapy are characterized by mucin substance or mucin pools dissecting through the rectal wall. x100.

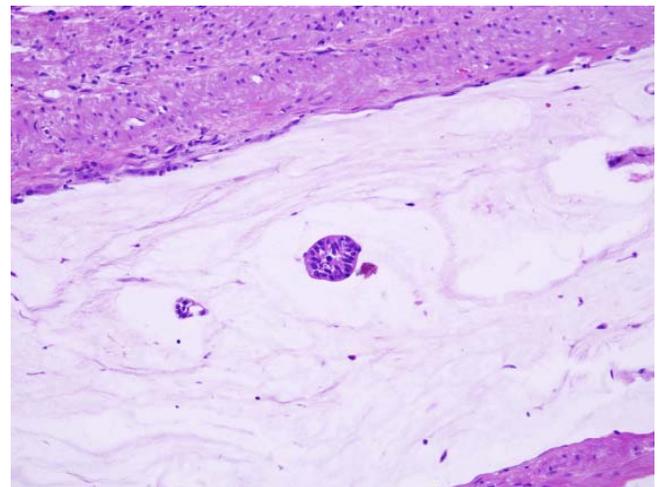


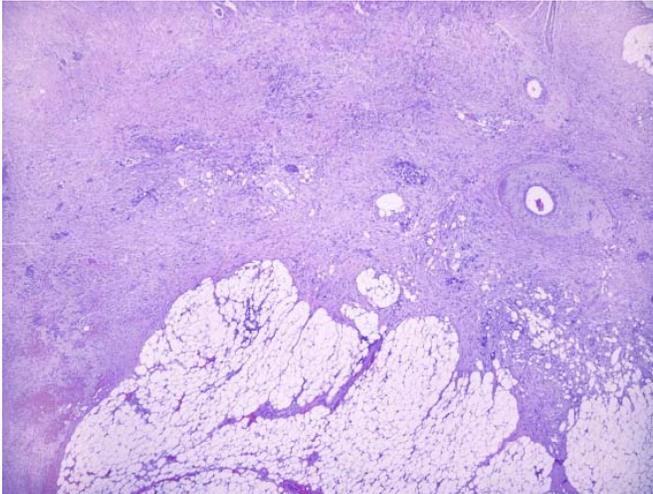
Fig. (3). Rectum, post-treatment. The remaining malignant cells present in colloid changes after preoperative radiotherapy are usually quite sparse, consisting of rare isolated cells or small clusters. x200.

Most authors do not consider acellular mucin lakes as residual tumor, however the presence of mucin lakes warrants a meticulous search for residual vital tumor cells. If mucin lakes are seen in the rectal resection margins on frozen section, extension of the surgery may be warranted if clinically possible. Such additional surgery is recommended even if no vital tumor cells are found in the mucus at the time of the intraoperative microscopic examination of frozen sections [7].

If residual viable tumor is present, it can be dispersed anywhere within the rectal wall or perirectal tissue (Fig. 4B). Even if the superficial tumor has completely regressed, viable malignant neoplastic glands can be found deep in the rectal wall (Fig. 4B). The residual malignant cells can

acquire an eosinophilic cytoplasm or undergo oncocytic differentiation (Fig. 5). Such tumor cells apparently contain densely packed mitochondria in their cytoplasm. Typically such cells show intensive immunohistochemical staining with anti-antibodies to mitochondria. Oncocytic cells differ in this respect from the cells of the same tumor seen in biopsies taken before the radiation therapy, i.e., cells from which they have most likely been derived [9].

(A)



(B)

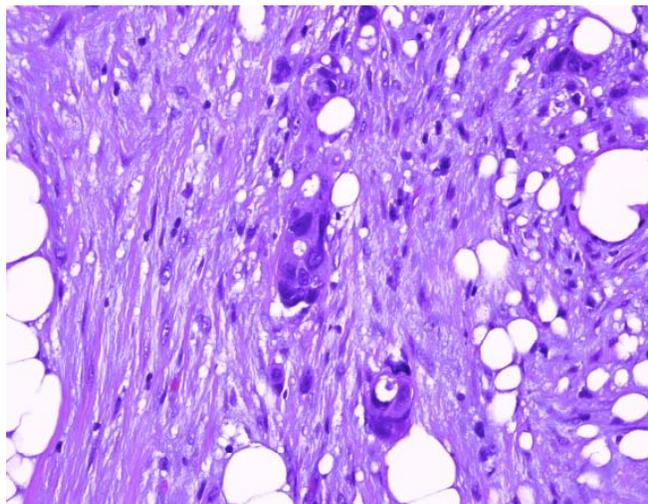


Fig. (4). Rectum, post-treatment. (A) Residual adenocarcinoma forms small nest and may be dispersed anywhere within the rectal wall or perirectal tissue. x40. (B) The mucosal portion of tumor may regress, leaving only distorted glands and single malignant cells deep within the rectal wall. x200.

Marked cytoplasmic eosinophilia, often in association with nuclear atypia is the most commonly seen type of cytologic alteration in tumors remaining in the intestine after radiation [5]. Some tumor cells may even acquire a more squamoid appearance (Fig. 6A, B). The individual tumor cells in treated rectal cancer may show marked nuclear atypia (Fig. 6C, D). The effects of treatment may be quite localized, with more typical areas of colorectal adenocarcinoma, characterized by elongated stratified basophilic cells, immediately adjacent to eosinophilic

markedly atypical cells (Fig. 6C). The nuclear hyperchromasia and atypia of treated colorectal adenocarcinoma cells may be quite marked, however mitoses are typically not seen or are found exceptionally rarely (Fig. 6D).

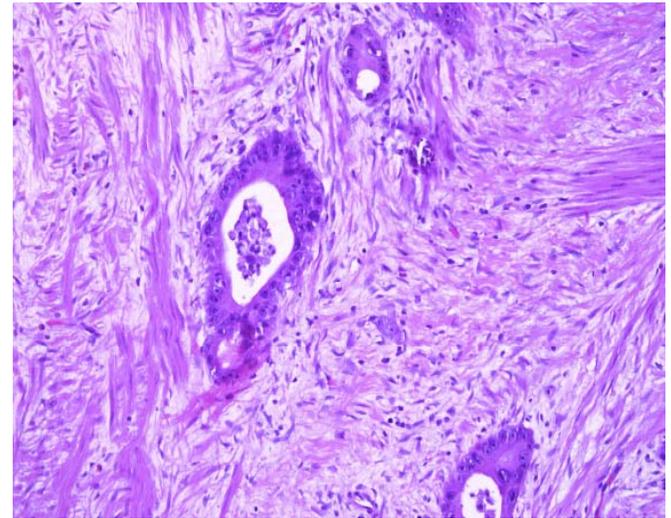


Fig. (5). Rectum, post-treatment. Oncocytic/eosinophilic changes are characterized by residual malignant cells with abundant pink cytoplasm and prominent nucleoli. x200.

Finally, it is worth mentioning that irradiated adenocarcinoma may show unexpected changes such as neuroendocrine differentiation. These changes can be readily recognized by routine microscopy. Neuroendocrine cells can also be demonstrated immunohistochemically with antibodies to chromogranin [10].

Dworak *et al.* [7] originally recommended standards for the pathological work up and regression grading of rectal carcinoma after radiation treatment. The grading of regression (GR) was established as follows:

- Grade 0: No regression;
- Grade 1: Dominant tumor mass with obvious fibrosis and or vasculopathy;
- Grade 2: Dominantly fibrotic changes with few tumor cells or groups (easy to find);
- Grade 3: Very few (difficult to find microscopically) tumor cells in fibrotic tissue with or without mucous substance;
- Grade 4: No tumor cells, only fibrotic mass (total regression or response).

Changes in Colon Adenocarcinoma

There are a growing number of patients with stage IV colon cancer who now receive chemotherapy prior to colectomy. Similar to rectal cancer, the predominant histologic changes seen after neoadjuvant chemotherapy include fibrosis and calcifications, (Fig. 7), acellular necrosis, and inflammation. The single most common histological response to chemotherapy in colorectal cancer is fibrosis overgrowth, and is observed in nearly 70% of primary tumors and is correlated to the response in the corresponding liver metastasis as well [1].

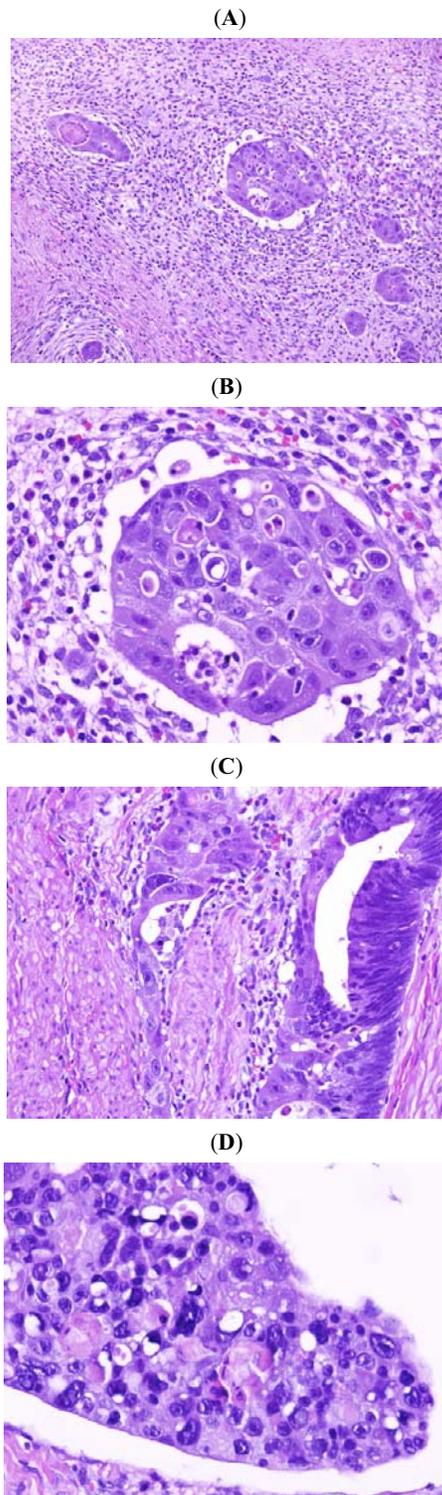


Fig. (6). Rectum, post-treatment. (A) These residual malignant cells show oncocytic/eosinophilic changes and marked nuclear atypia. x200. (B) Some may even acquire a more squamoid appearance. The squamoid nest of malignant cells shown are surrounded by a fibroinflammatory infiltrate with prominent eosinophils. x400. (C) The effects of treatment may be quite localized, with more typical areas of colorectal adenocarcinoma, characterized by elongated stratified basophilic cells, immediately adjacent to eosinophilic markedly atypical cells. x400. (D) The nuclear atypia of treated colorectal adenocarcinoma may be quite marked, however mitoses are unusual. x400.

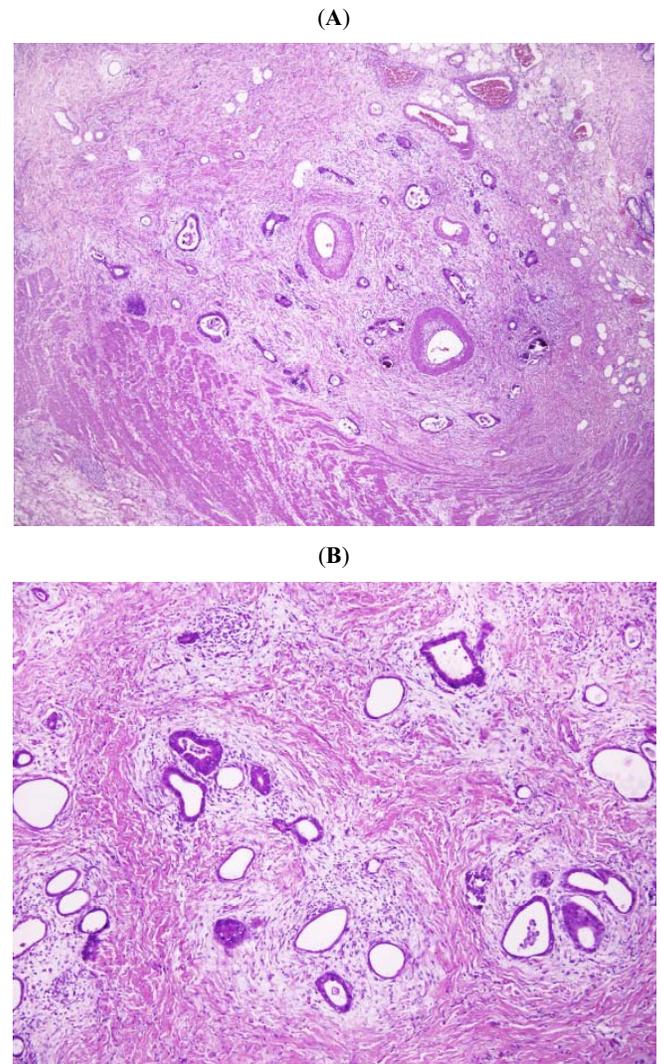


Fig. (7). Colon, post-treatment. The predominant histologic changes seen after neoadjuvant chemotherapy include fibrosis, acellular necrosis with or without calcifications, and inflammation. x100.

Residual malignant glands can undergo marked cystic dilatation and thus may be mistaken for benign glands when examined at low power magnification (Fig. 8A). On higher power magnification, however, the malignant nature of glandular cells is quite obvious and can be readily recognized (Fig. 8B). Changes at the tumor surface include nontumorous inflammatory ulceration and epithelial regenerative changes (Fig. 9A). Submucosal edema and inflammation may be prominent (Fig. 9B). Even if the colonic mucosal tumor has regressed completely and disappeared it is common to see residual malignant glands dispersed deep within the colonic wall (Fig. 9B).

In contrast to rectal carcinomas which are treated preoperatively with radiotherapy +/- chemotherapy [5, 8], most colon cancer patients receive only chemotherapy preoperatively. The presence of acellular mucin pools has not been significantly associated with tumor regression in colon adenocarcinoma, which suggests that this feature is more related to radiation therapy [11].

Tumor regression of colon cancer has been scored according to the scheme from Mandard *et al.* [12]. This

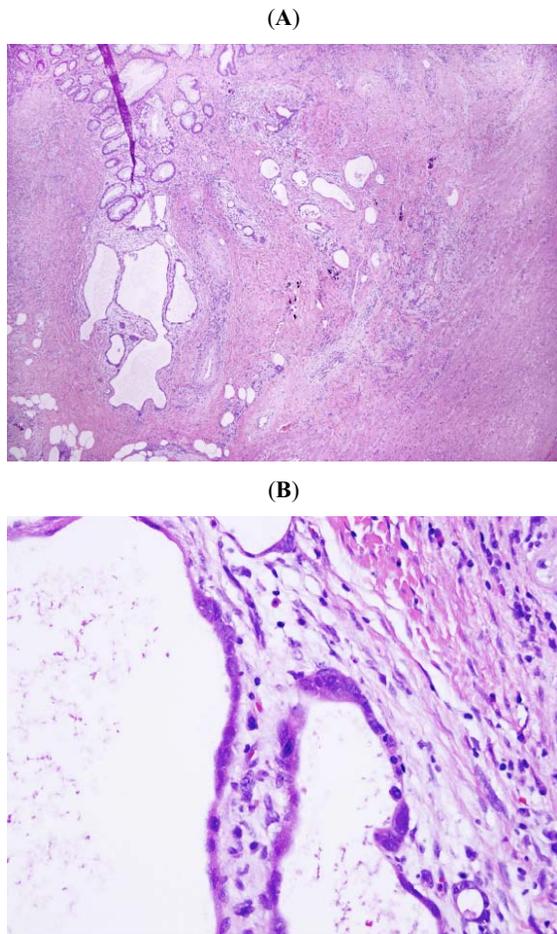


Fig. (8). Colon, post-treatment. (A) Treated malignant glands can undergo marked cystic dilatation, giving the false impression of benignity at low power. x40. (B) On higher power, however, the malignant nature of the glandular cells is appreciated. x400.

scoring system was originally developed for the assessment of pathologic regression in esophageal carcinomas, and was subsequently applied to the assessment of colon carcinomas. This score identifies five tumor regression grades on the basis of the presence of residual tumor and is as follows:

- TRG 1: No residual tumor
- TRG 2: Rare residual cancer cells
- TRG 3: Fibrosis outgrowing residual cancer
- TRG 4: Residual cancer outgrowing fibrosis
- TRG 5: Absence of regressive changes

It has been shown that the TRG in the primary tumor is significantly correlated with the TRG of the corresponding metastases [11].

Changes in Liver Metastases

In the last decade chemotherapy has been used preoperatively in patients with hepatic metastases of large intestinal cancer. Such treatment was found to reduce the size of hepatic colorectal metastases (HCRM), allowing previously unresectable patients to undergo resection. The

histologic response to chemotherapy in HCRM was characterized by Rubbia-Brandt *et al.* [4].

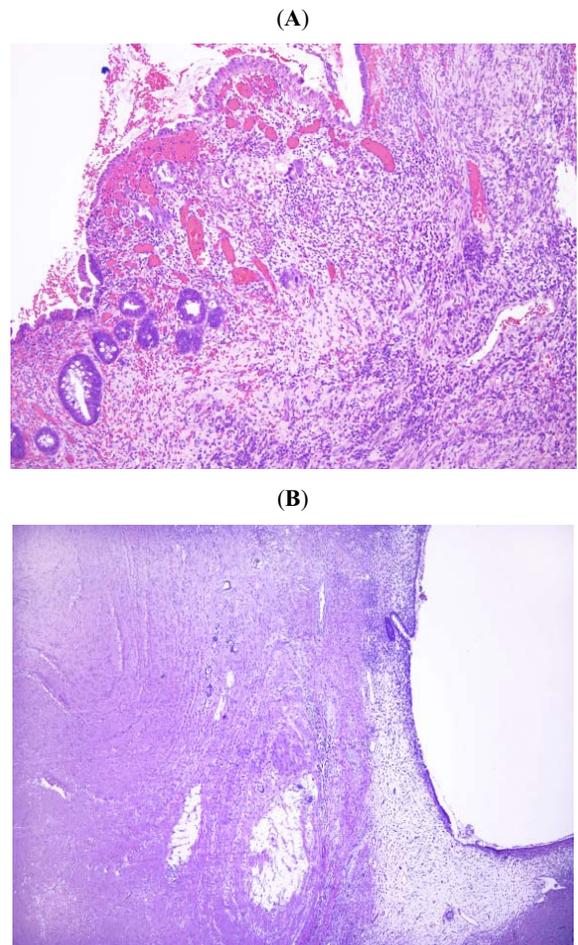


Fig. (9). Colon, post-treatment. (A) Changes at the tumor surface include inflammatory ulceration and epithelial regenerative changes, and submucosal edema and inflammation. x100. (B) As with rectal carcinoma, even if the mucosal tumor has regressed it is common to see residual malignant glands dispersed deep within the colonic wall. x100.

Untreated HCRM show large areas of viable tumor glands intermingled with zones of dirty necrosis (Fig. 10). Tumor regression of HCRM is characterized by fibrosis overgrowing the tumor cells, foamy macrophages, and decreased intra-tumoral "dirty" necrosis (Fig. 11A, B). When residual tumor is present it is located predominantly at the periphery of the treated HCRM (Fig. 12) and only rarely at the center. Rubbia-Brandt *et al.* [4] demonstrated that the TRG is related to the type of chemotherapy. These authors also established a correlation between the histological response and survival of the treated patients.

Systemic neoadjuvant chemotherapy in HCRM frequently causes morphologic lesions that involve the hepatic microvasculature of non-tumoral liver [13]. Rubbia-Brandt *et al.* [4] demonstrated sinusoidal obstruction, complicated by perisinusoidal fibrosis and veno-occlusive lesions of the non-tumoral liver in 30% of patients treated with chemotherapy. These changes were particularly prominent

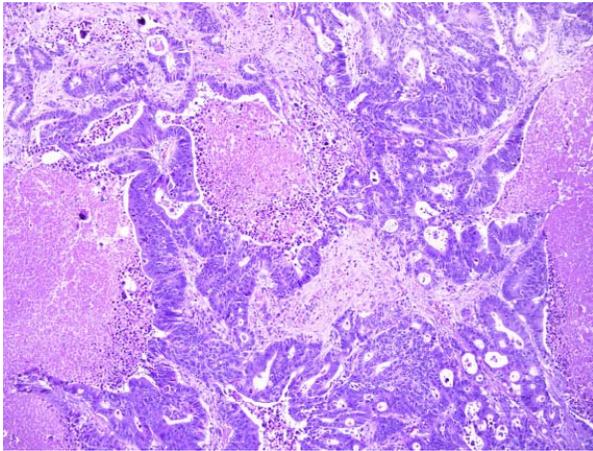
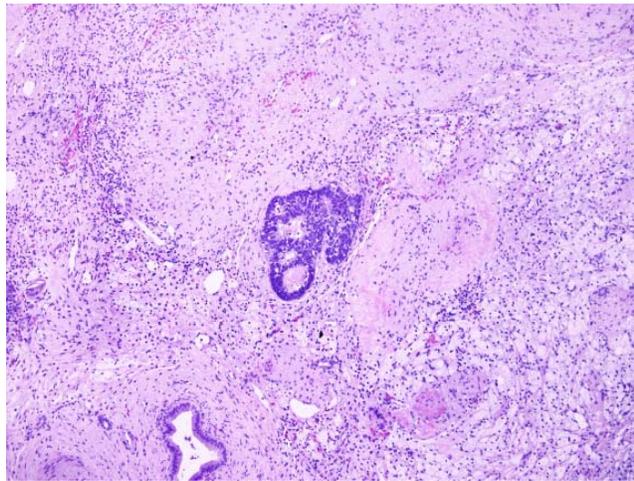


Fig. (10). Liver metastasis in a patient who did not receive neoadjuvant therapy. This untreated colorectal metastasis is characterized by large areas of viable malignant tumor glands intermingled with zones of “dirty” necrosis. x100.

(A)



(B)

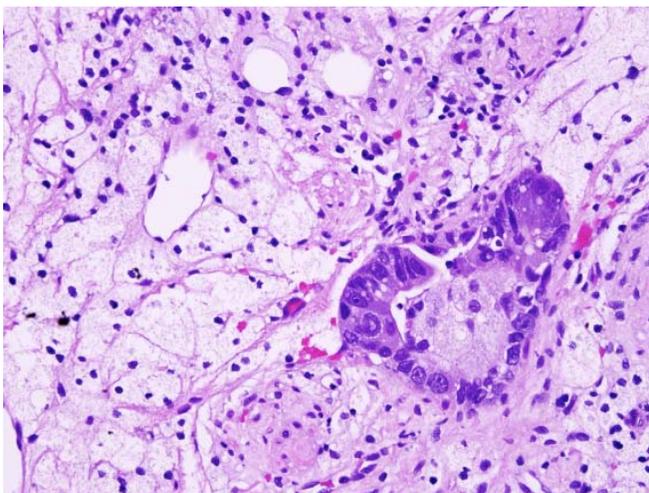


Fig. (11). Liver metastasis, post-treatment. (A) This residual group of malignant glands is surrounded by fibrosis and inflammation. A normal appearing bile duct is present in the lower part of the image. x100. (B) A higher power image shows foamy macrophages intermixed with the fibro-inflammatory tissue. x200.

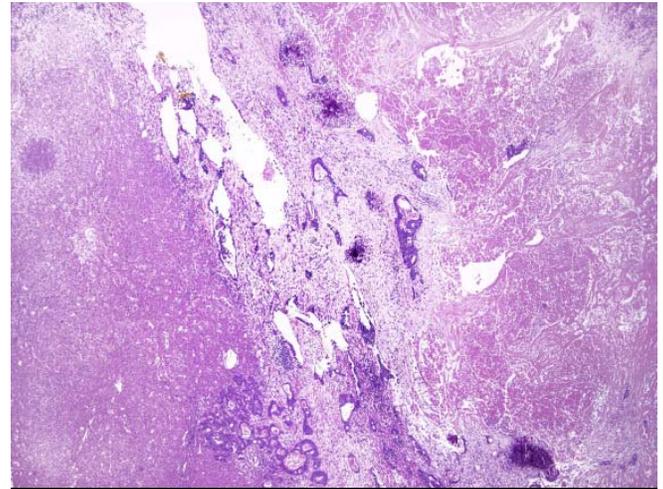


Fig. (12). Liver metastasis, post-treatment. When residual metastatic malignant glands remain, they are present predominantly at the periphery of the tumor bed. This image highlights a central area of acellular necrosis with calcification which is rimmed by residual malignant glands. These glands are present at the interface between the normal hepatic tissue and the necrotic tumor bed. x100.

in patients treated with oxaliplatin. Sinusoidal dilatation is readily identified in non-tumoral tissue (Fig. 13).

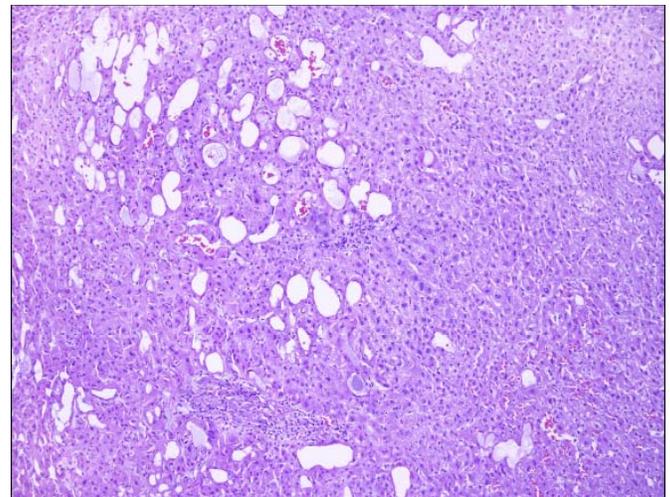


Fig. (13). Non-tumoral liver, post-treatment. Neoadjuvant systemic chemotherapy also has effects on the morphology of non-tumoral liver, and frequently causes lesions involving the hepatic microvasculature. This image highlights sinusoidal dilatation with erythrocyte congestion, which is the most common microvascular lesion seen in the liver after neoadjuvant chemotherapy. x100.

Changes in Lymph Nodes

The fibrotic changes seen in the primary treated colorectal cancer can also be seen in the lymph nodes. The lymph nodes can demonstrate fibrotic nodules with sparse lymphoid cells (Fig. 14A, B) or focal fibrotic changes with mucinous substance. Small foci of residual metastatic carcinoma can often be identified within the fibrosis (Fig. 14C, D). Radiation changes in the lymph node include a fibrotic capsule and irregular foci of fibrosis, especially in the sinus (Fig. 15).

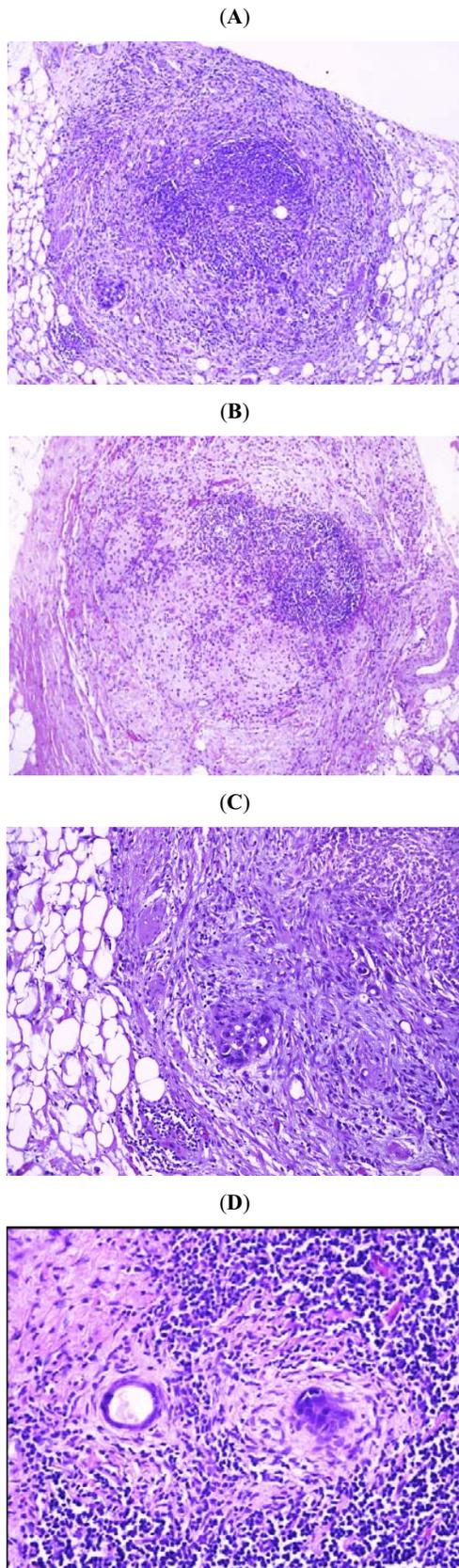


Fig. (14). Lymph node, post-treatment. (A) Changes in the lymph node related to treatment can range from mild fibrosis to (B) almost complete obliteration of the lymph node by fibrous tissue. (C and D) A careful search is warranted to identify any remaining metastatic tumor cells. A&B x100, C&D x200.

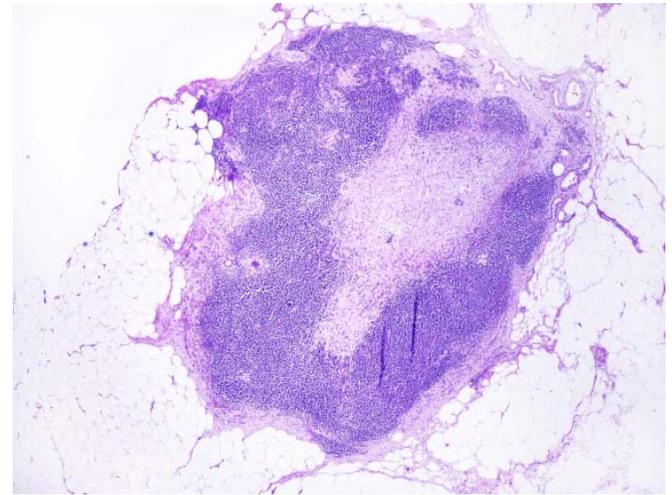


Fig. (15). Lymph node, post treatment. Radiation changes seen in lymph nodes include a fibrotic capsule and irregular foci of fibrosis, especially in the lymph node sinus. x40.

CONCLUSION

Chemotherapy and radiation therapy routinely used in the preoperative treatment of patients with colorectal cancer produce characteristic morphologic changes which can be readily recognized during microscopic pathologic examination of surgically resected intestine and adjacent lymph nodes. Proper histopathologic identification and interpretation of these changes are important for the exact assessment of the tumor response grade (TRG), planning of additional therapy, and formulation of the prognosis.

ABBREVIATIONS

CT	=	Chemotherapy
RT	=	Radiation therapy
TRG	=	Tumor regression grade

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Received: June 15, 2009

Revised: June 25, 2009

Accepted: July 5, 2009

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