Overexpression of Vascular Endothelial Growth Factor A in Invasive Micropapillary Colorectal Carcinoma

Marilyn Rosa, MD, Maisoun Abdelbaqi, MD, Katherine M. Bui, Aejaz Nasir, MD, MPhil, Marilyn M. Bui, MD, PhD, David Shibata, MD, and Domenico Coppola, MD

Background: Invasive micropapillary carcinoma (IMPC) is a rare variant of colorectal cancer with an adverse prognosis. “Retraction artifact” around tumor cells is a feature of IMPC. The aim of this study was to assess the nature of the retractions around the tumor cells and to describe the histopathological features of a group of 18 cases of IMPC.

Methods: A pathology review of 128 consecutive colorectal cancers identified 18 cases of histologically proven IMPC using 5% of the total tumor volume comprised of a micropapillary component as the diagnostic criterion. Immunostains for D2-40, CD31, CD34, vascular endothelial growth factor A (VEGF-A), and mucin 1 (MUC-1) were performed using the avidin-biotin complex method.

Results: Cases of IMPC were characterized by pseudomicropapillae surrounded by lacunar-like clear spaces. These structures exhibited the inside-out growth pattern as highlighted by MUC-1 staining. The lining of the lacunar spaces was immunoreactive to CD31 but not CD34 or D2-40, indicating that they are neovascular structures. Furthermore, the tumor cells strongly and diffusely expressed VEGF-A.

Conclusions: The strong coexpression of VEGF-A and CD31 suggests a prominent role of neoangiogenesis in these tumors.

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the third leading cause of cancer death in both men and women in the United States. Invasive micropapillary carcinoma (IMPC) has recently been described as a rare variant of CRC with a poor prognosis independently of stage. Although a focal micropapillary pattern can be seen in conventional cases of CRC, the diagnosis of the micropapillary variant of CRC requires that at least 5% of the total tumor volume has a micropapillary component. The proportion of the micropapillary component has also been suggested to have an important predictive factor for nodal metastasis, but this finding has not been consistent.

IMPC has been recognized and studied at other anatomical locations such as the ovary, breast, urinary
bladder, salivary glands, and lung.\textsuperscript{6-10} In its breast counterpart, IMPC shows a tendency to invade lymphatic vessels and to spread to regional lymph nodes. Whether the lacunar-like spaces surrounding the micropapillary structures are true vascular channels or “retraction artifacts,” as is commonly considered, remains a subject of debate.\textsuperscript{11,12} Some published studies suggest that retraction clefts are likely the result of an altered tumor–stromal interaction.\textsuperscript{11,13} In addition, these retraction clefts may be potential spaces that represent prelymphatic spaces involved in the facilitation of the initial lymphatic invasion and may undergo endothelialization under the influence of growth factors secreted by tumor cells.\textsuperscript{11}

The histopathological, immunohistochemical, and molecular features of colorectal IMPC have been previously defined.\textsuperscript{1,5} In this study, we review a single institution series of colorectal IMPC cases and characterize their vascular immunohistochemical phenotype to understand whether the presence of retraction clefts correlates with lymphangiogenesis and, thus, is a factor facilitating metastatic tumor spread.

**Methods**

**Participants**

This study was carried out in accordance with a research protocol approved by the Institutional Review Board at the H. Lee Moffitt Cancer Center & Research Institute and the University of South Florida in Tampa, Florida. A total of 128 consecutive colon resections performed at the Moffitt Cancer Center during a 5-year period from 2005 to 2010 were retrospectively selected from the anatomical pathology files. Two pathologists (DC and MA) reviewed the pathology reports and the hematoxylin and eosin–stained microscopic slides of each tumor to confirm the diagnosis and to select a representative block for immunohistochemical studies. As in previously published studies, tumors were classified as IMPC when at least 5% of the tumor was comprised of a micropapillary component.\textsuperscript{2,5} In case of a discrepancy, the slides were reviewed again by both pathologists at a multiheaded microscope to reach a diagnostic consensus. Collected clinicopathological variables included tumor size, grade, stage, histological type, and the presence of nodal metastases, distant metastases, or both.

**Immunohistochemistry**

Immunohistochemical analysis was performed on 5-µm unstained sections from formalin-fixed, paraffin-embedded representative tumor blocks with an autostainer using monoclonal antibodies against CD31, CD34, D2-40, vascular endothelial growth factor A (VEGF-A), and mucin 1 (MUC-1). All stains were run with appropriate controls and stained in accordance with the manufacturer’s recommended protocol. Results were considered positive when cytoplasmic staining, membranous staining, or both were present in any percentage of the cells of interest and considered negative when there was a complete lack of staining.

**Results**

Our review identified a total of 18 (14% of consecutive colorectal carcinomas) bona fide cases of IMPC. The age of the patients ranged between 34 and 93 years (mean, 52.3 years). Twelve patients were men and 6 patients were women. Eight tumors were located in the right colon (2 in the cecum), 4 in the left colon, 4 in the rectum, 1 at the splenic flexure, and 1 in the terminal ileum. The tumor size ranged from 2.0 to 6.1 cm (mean, 4.0 cm). Twelve tumors were diagnosed as moderately differentiated and 6 were poorly differentiated. The pathological stages of the tumors were as follows: 4 carcinomas were stage 1, 3 were stage 2A, 2 were stage 3B, 8 were stage 3C, and 1 was stage 4. Three patients received preoperative chemotherapy (Table).

<table>
<thead>
<tr>
<th>Location</th>
<th>Size (cm)</th>
<th>Grade</th>
<th>% of Micropapillary Component</th>
<th>No. of LNs</th>
<th>Morphology of LN Metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascending colon</td>
<td>4.5</td>
<td>3</td>
<td>90%</td>
<td>0/15</td>
<td>NA</td>
</tr>
<tr>
<td>Ileocecal valve</td>
<td>4.3</td>
<td>2</td>
<td>10%</td>
<td>1/11</td>
<td>Micropapillary</td>
</tr>
<tr>
<td>Right colon</td>
<td>3.5</td>
<td>3</td>
<td>10%</td>
<td>3/26</td>
<td>1/3 micropapillary</td>
</tr>
<tr>
<td>Sigmoid colon</td>
<td>5.0</td>
<td>2</td>
<td>80%</td>
<td>14/20</td>
<td>9/14 micropapillary</td>
</tr>
<tr>
<td>Rectum</td>
<td>3.3</td>
<td>2</td>
<td>10%</td>
<td>2/31</td>
<td>Micropapillary</td>
</tr>
<tr>
<td>Rectum</td>
<td>6.0</td>
<td>2</td>
<td>20%</td>
<td>7/16</td>
<td>Micropapillary</td>
</tr>
<tr>
<td>Terminal ileum</td>
<td>3.5</td>
<td>3</td>
<td>30%</td>
<td>13/34</td>
<td>Slides not available</td>
</tr>
<tr>
<td>Rectum</td>
<td>3.2</td>
<td>3</td>
<td>10%</td>
<td>0/23</td>
<td>NA</td>
</tr>
<tr>
<td>Right colon</td>
<td>4.0</td>
<td>3</td>
<td>10%</td>
<td>18/18</td>
<td>3/18 micropapillary</td>
</tr>
<tr>
<td>Rectum</td>
<td>2.6</td>
<td>2</td>
<td>10%</td>
<td>0/24</td>
<td>NA</td>
</tr>
<tr>
<td>Cecum</td>
<td>3.5</td>
<td>2</td>
<td>10%</td>
<td>4/5</td>
<td>Micropapillary</td>
</tr>
<tr>
<td>Cecum</td>
<td>4.5</td>
<td>2</td>
<td>10%</td>
<td>7/46</td>
<td>5/12 micropapillary</td>
</tr>
<tr>
<td>Ileocecal valve</td>
<td>6.5</td>
<td>3</td>
<td>50%</td>
<td>15/49</td>
<td>13/15 micropapillary</td>
</tr>
<tr>
<td>Sigmoid colon</td>
<td>4.0</td>
<td>2</td>
<td>30%</td>
<td>0/18</td>
<td>NA</td>
</tr>
<tr>
<td>Sigmoid colon</td>
<td>2.5</td>
<td>2</td>
<td>60%</td>
<td>3/13</td>
<td>No micropapillary</td>
</tr>
<tr>
<td>Splenic flexure</td>
<td>0.6</td>
<td>3</td>
<td>90%</td>
<td>0/15</td>
<td>NA</td>
</tr>
<tr>
<td>Right colon</td>
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<td>2</td>
<td>40%</td>
<td>7/13</td>
<td>Micropapillary</td>
</tr>
<tr>
<td>Left colon</td>
<td>2.0</td>
<td>2</td>
<td>20%</td>
<td>1/15</td>
<td>Micropapillary</td>
</tr>
</tbody>
</table>

LN = lymph node, NA = not applicable.
Histologically, all micropapillary areas exhibited the characteristically features of IMPC as previously described.\textsuperscript{2-5} Tumors were composed of clusters of cells growing in a micropapillary (or pseudo-micropapillary) pattern surrounded by lacunar-like clear spaces lined by elongated endothelial-like cells (Fig 1A). Fibrovascular cores were not identified (Fig 1B). Tumor cells were columnar to polygonal and had a moderate amount of eosinophilic cytoplasm and conspicuous nucleoli. Characteristically, the micropapillae exhibited a rotation of cell polarization known as an “inside-out” growth pattern, or “reverse cell polarity,” which was also highlighted by MUC-1 immunostaining (Fig 1C). Collections of neutrophils were seen in some cases to infiltrate the micropapillae and occasionally to spill into the lacunar-like spaces (Fig 1D). In addition, the micropapillary features represented at least 10% of the tumors in all of our cases. The transition between conventional adenocarcinoma and IMPC was abrupt, and, in general, the IMPC component was identified at the advancing, infiltrating edge of the tumors.

Immunohistochemically, we observed a strong and diffuse expression of VEGF-A in the tumor cells within the lacunar-like spaces (Fig 2A). With regard to the vascular markers, CD31 — but not CD34 — positivity highlighted the cells lining the clear spaces surrounding the micropapillae, thus suggesting that they are immature vessels (Fig 2B and C). D2-40 also failed to stain this lining, suggesting that the peritumoral spaces are not lymphatic (Fig 2D).

**Discussion**

IMPC is an unusual and aggressive variant of colorectal adenocarcinoma commonly associated with lymphovascular invasion, lymph node metastasis, and poor clinical outcome.\textsuperscript{2-5} In our retrospective review of 128 cases of CRC, we identified 18 cases of IMPC (approximately 14%), similar to what was reported by Haupt et al\textsuperscript{4} (19% of 178 cases of CRC with IMPC-like features). Histologically, our cases also exhibited similar features to those previously described — in particular, the presence of micropapillae without a fibrovascular core surrounded by a clear lacunar space.\textsuperscript{2-5}

The “micropapillary” appearance, which was first described in tumors of the breast,\textsuperscript{10,11} has been attributed to a peculiar rotation of cell polarization seen in these tumors and is described as an inside-out growth pattern. This characteristic feature has been supported by electron microscopy studies, which have shown that the microvilli of the cellular surface are at the stromal–epithelial interface and not toward the lumen of the neoplastic gland.\textsuperscript{2} This inside-out pattern is better observed with MUC-1 immunostain\textsuperscript{2} and has been linked to stromal and vascular invasion.\textsuperscript{2,5}

Metastasis to local lymph nodes via the lymphat-
ly identified in this tumoral area may explain this early metastatic and local progression. In our series, of the removed 392 lymph nodes, 95 were positive for metastatic disease; the metastasis was of micropapillary morphology in 53 lymph nodes. Although the percentage of the micropapillary component did not appear to correlate with the presence or number of positive lymph nodes, a slight increase of papillary morphology was seen in the metastatic tumors (56% of lymph node metastases were of micropapillary morphology; see Table).

To explore the role of peritumoral spaces in angiogenesis, tumor progression, and metastatic spread of IMPC of a colorectal origin, we studied a panel of vascular markers (CD31, CD34, D2-40) and VEGF-A.

CD31, also known as platelet-endothelial cell adhesion molecule 1 (PECAM-1), is a 130k-Da transmembrane glycoprotein expressed at high levels on early and mature endothelial cells, platelets, and most leukocyte subpopulations. In addition to its role in the adhesion and transmigration of inflammatory cells, CD31 has an important role in angiogenesis. DeLisser et al. showed that PECAM-1 is involved in angiogenesis and suggested that endothelial cell–cell adhesion molecules are important in the formation of new vessels. PECAM-1 also plays a complex role in tumor-induced angiogenesis in which neovascularization arises from the interactions between multiple stimulatory factors (e.g., VEGF, basic fibroblast growth factor, interleukin 8, angiogenin). CD31 is involved in the regulation of hematopoietic progenitor cell compartmentalization between the peripheral blood and bone marrow as well as in maintaining the levels of the matrix-degrading enzyme matrix metalloproteinase 9 in the bone marrow vascular niche. In all of our IMPC cases, the cells lining the lacunar-like spaces surrounding the micropapillae strongly stained for CD31 but not for CD34, suggesting that they are newly formed immature blood vessels. The same cells were negative for D2-40, which is a sensitive and relatively specific marker for lymphatic endothelium in all parenchymatous organs.

VEGF-A is a vascular-related protein widely regarded as a classic angiogenic cytokine because of its role in stimulating various endothelial cell responses necessary for angiogenesis. It belongs to the VEGF–platelet-derived growth factor gene family, which also includes VEGF-B, VEGF-C, VEGF-D, and VEGF-E. While VEGF-C and VEGF-D are involved in lymphangiogenesis, the expression of VEGF-A has been identified as an early change in the formation of new blood vessels. In our study, we used a monoclonal antibody that recognized the 189, 165, and 121 isoforms of VEGF. While the 2 smaller isoforms VEGF-165 and VEGF-121 are secreted proteins that act as diffusible agents, the larger isoform VEGF-189 remains cell associated. Presumably, VEGF and CD31 are related biomarkers in neoangiogenesis and may partially contribute to the biological aggressive behavior of IMPC. The coexpression of VEGF and CD31 in IMPC is a novel finding that warrants further investigation, particularly with respect to the possibility that targeted antiangiogenic therapy may be more efficacious in this subset of CRC.

In certain cases of IMPC, we observed collections of neutrophils infiltrating the micropapillae and occasionally spilling into the lacunar-like spaces, thus mimicking microabscesses. Neutrophils are a critical source of cytokines, such as interleukin 8, VEGF, and matrix metalloproteinases 2 and 9. Therefore, it is possible that they may also have a role in the neoangiogenesis associated with IMPC; however, further studies comparing IMPC with nonmicropapillary colorectal carcinomas are necessary to draw specific conclusions.

Conclusions

The results of this study demonstrate that the lacunar spaces surrounding tumor cells in colonic micropapillary carcinoma represent vascular channels and vascular endothelial growth factor A is produced by tumor cells. Our findings of strong CD31 and vascular endothelial growth factor A positivity in the lining tumor cells of the retraction clefts of invasive micropapillary colorectal carcinoma may represent neoangiogenesis around tumor clusters. This also suggests a prominent role of neoangiogenesis in the characteristics of early tumor progression, local invasion, and metastatic spread seen in invasive micropapillary colorectal carcinoma.

References

11. Acs G, Paragh G, Rakosy Z, et al. The extent of retraction clefts correlates with lymphatic vessel density and VEGF-C expression and predicts...


