Heterotopic Ossification in Rectal Cancer: Rare Finding With a Novel Proposed Mechanism

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The rare finding of heterotopic ossification in a case of primary rectal adenocarcinoma is described along with a review of the literature. Immunohistochemistry for a bone morphogenic protein (BMP-2) and fibroblast growth factor (FGF-2), both of which induce and stimulate bone formation, was performed and revealed overexpression of BMP-2 by the tumor cells, elucidating a possible mechanism which up to now had been based merely on speculation.


KEY WORDS: bone morphogenic protein; rectal adenocarcinoma; ossification

Heterotopic bone formation has been reported in malignancies involving the kidneys, liver, breast, and skin [1–4]. Ossification in the gastrointestinal tract is extremely rare; nevertheless, it has been reported in association with benign colonic polyps, with carcinomas and carcinoid of the stomach, and with mucocele of the appendix [5–8]. Approximately 34,700 new cases of rectal adenocarcinoma were diagnosed in the United States in 1999 [9]. Despite the high incidence of these tumors, heterotopic ossification within primary rectal adenocarcinoma is an exceedingly rare event, even though the most common location for ossification in a gastrointestinal tumor is in the lower gastrointestinal tract [10].

In 1923, Hasegawa [11] was the first to describe two cases of rectal carcinoma with bone formation in the stroma. In 1939, Dukes [12] was the first investigator in the English literature to describe ossification of primary rectal carcinoma in two cases. We report a case, which is only the eighth report of heterotopic bone formation in primary rectal adenocarcinoma (Table I), and one of the first with preoperative evidence of ossification as documented by computed tomography (CT) scan. More importantly, prior reports have only speculated on the possible mechanisms of this unusual pathologic finding, and only recently has there been a report of bone morphogenic protein (BMP) expression in a case of colon cancer with heterotopic ossification [13]. In the current report, we demonstrate, for the first time, expression of a bone morphogenic protein (BMP-2) within the tumor cells of a rectal adenocarcinoma, revealing a potential signaling mechanism for the transformation of mesenchymal cells into bone.

CASE REPORT

A 38-year-old otherwise healthy, white woman presented with a 3-month history of crampy abdominal pain in the left lower quadrant, and bright red blood per rectum. There was no family history of colon cancer. Hemoglobin was 12.7 g/dl, and stools were guaiac positive. The carcinoembryonic antigen (CEA) level was 218 ng/ml. A flexible sigmoidoscopy was performed, which revealed a partially obstructing immobile lesion in the rectum. Biopsy showed infiltrating moderately differentiated adenocarcinoma. CT scan demonstrated a polypoid mass with extensive calcification extending into the lumen of the rectum (Fig. 1), with no evidence of metastatic disease. Staging by endoscopic ultrasound showed a T3N1MX lesion.

The patient received preoperative radiation therapy consisting of 4,500 cGy as well as 5-fluorouracil (5-FU)/eniluracil 1.5 mg/15 mg bid for 4 weeks. Four months after initial presentation, she underwent exploratory surgery. Intraoperative evaluation of the abdomen indicated...
no metastatic disease. An abdominoperineal resection was performed. Postoperatively, the patient did well, and she was discharged home on the sixth day. Adjuvant chemotherapy consisted of 5-FU/Leucovorin. Thirty-three months later, she is doing well with no evidence of recurrent disease.

The resected specimen contained a 4.5 × 4.0 × 4.0-cm rectal tumor. Histology was consistent with invasive, moderately differentiated adenocarcinoma extending through the muscularis propria, but not involving the serosal surface. There was extensive heterotopic ossification and calcification, but no necrosis was seen within the tumor (Fig. 2). The bone had a benign appearance. The margins were free of tumor, and 18 lymph nodes were negative. The adenocarcinoma showed a positive reaction for cytokeratin and negative reaction for vimentin. The stroma showed a positive reaction for vimentin and a negative reaction for cytokeratin.

Specimens of tumor with and without heterotopic ossification were quick frozen in liquid nitrogen, embedded in OCT compound (Sakura Finetek, Torrance, CA), and sectioned for staining. After blocking for 1 h with normal donkey serum in phosphate-buffered saline (PBS), primary antibody, either BMP-2 or FGF-2 (Santa Cruz Biotechnology, Santa Cruz, CA) was applied to the slides in a humidified chamber and was incubated for

### TABLE I. Cases of Rectal Adenocarcinoma Containing Heterotopic Ossification

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Author</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Haque et al. [14]</td>
<td>78</td>
<td>M</td>
<td>Adenocarcinoma</td>
<td>APR</td>
</tr>
<tr>
<td>2</td>
<td>Dukes [12]</td>
<td>69</td>
<td>M</td>
<td>Adenocarcinoma</td>
<td>Perineal resection</td>
</tr>
<tr>
<td>3</td>
<td>Dukes [12]</td>
<td>32</td>
<td>F</td>
<td>Adenocarcinoma</td>
<td>APR</td>
</tr>
<tr>
<td>4</td>
<td>Ansari et al. [10]</td>
<td>54</td>
<td>F</td>
<td>Adenocarcinoma</td>
<td>LAR</td>
</tr>
<tr>
<td>5</td>
<td>Urbanke [18]</td>
<td>55</td>
<td>F</td>
<td>Adenocarcinoma</td>
<td>APR</td>
</tr>
<tr>
<td>6</td>
<td>Christie [32]</td>
<td>44</td>
<td>F</td>
<td>Adenocarcinoma</td>
<td>APR</td>
</tr>
<tr>
<td>7</td>
<td>Byard et al. [33]</td>
<td>72</td>
<td>M</td>
<td>Adenocarcinoma</td>
<td>Unknown</td>
</tr>
<tr>
<td>8</td>
<td>Kypson et al. (present study)</td>
<td>38</td>
<td>F</td>
<td>Adenocarcinoma</td>
<td>APR</td>
</tr>
</tbody>
</table>

APR, abdominoperineal resection; LAR, low anterior resection.

Fig. 1. Computed tomograph showing thickening of the lateral aspects of the rectum. Areas of increased attenuation demonstrate heterotopic ossification.
30 min at room temperature. Slides were washed three times in PBS, and a secondary antibody, biotinylated donkey anti-goat IgG, was applied and incubated for 30 min. After PBS wash, streptavidin-horseradish peroxidase (HRP) conjugate was applied for 30 min. Slides were then developed with diaminobenzidine tetrahydrochloride (DAB), washed in dH2O, counterstained with hematoxylin and eosin (H&E), dehydrated through graded alcohols, and dried in xylene. Slides were then developed with diaminobenzidine tetrahydrochloride (DAB), washed in dH2O, counterstained with hematoxylin and eosin (H&E), dehydrated through graded alcohols, and dried in xylene. Slides were sealed with Cytoseal XYL (Stephens Scientific, Riverdale, NJ) and coverslipped. Immunohistochemical staining for fibroblast growth factor (FGF-2) was negative, however, BMP-2 staining was positive when compared to two negative controls of rectal adenocarcinoma without heterotopic ossification (Fig. 3).

**DISCUSSION**

Adenocarcinoma of the rectum is a common lesion and is increasing in frequency. Heterotopic ossification involving primary adenocarcinoma of the rectum is extremely rare, with only a handful of cases in the literature (Table I), as opposed to the 60 cases of heterotopic ossification involving the entire gastrointestinal tract [10,13–15].

Nevertheless, the overall incidence of heterotopic ossification as suggested by Dukes [12] is a mere 0.4%. A detailed review of these specific cases involving the rectum (Table I) shows a male-to-female ratio of almost 1:2 and a mean age of 55 years. The course and prognosis of adenocarcinoma of the lower gastrointestinal tract...
with ossification does not differ from carcinoma without ossification, although it appears to be more common in slow-growing lesions and in younger patients [12].

Pathologic calcification implies the abnormal deposition of calcium salts. It is a process that does occur with some frequency in the presence of an alkaline environment or in a variety of pathologic conditions secondary to excess calcium. Ossification refers to the formation of heterotopic bone and is a process that is more complex, probably requiring the presence of osteoblasts.

The mechanism of heterotopic bone formation within gastrointestinal adenocarcinoma is not completely understood and is therefore highly debated. Do tumor cells undergo osseous metaplasia, or do they secrete substances that induce the condition? A review of the literature shows that osseous metaplasia has been observed in histologic settings that include (1) association with tumor necrosis [12], (2) within pools of mucin [3], and (3) within the stroma of the tumor. In his 1939 report, Dukes [12] noted that all four cases of heterotopic bone formation occurred in the setting of low-grade malignancy and contained areas of necrosis. However, other investigators have noted that although necrosis commonly occurs in intestinal adenocarcinomas, heterotropic ossification is a rare event. Furthermore, when heterotropic ossification is observed it is usually associated with mucinous adenocarcinomas [16]. Nevertheless, ossification in our specimen exhibited neither mucin nor areas of necrosis, indicating some other mechanism for the formation of heterotopic bone formation in intestinal adenocarcinoma.

Leriche and Policard [17] stated that heterotopic bone develops by metaplasia from connective tissue. This conclusion is supported by Urbanke [18], who demonstrated all the transitional forms of cells between inactive fibrocytes, fibroblasts, and typical plump and polygonal osteoblasts in his specimens of heterotopic bone formation in rectal cancer. Urbanke concluded that osteoblasts probably developed in situ from the fibroblasts in the connective tissue stroma.

It has been speculated that tumor cells themselves may secrete a substance that stimulates bone formation [10]. However, no chemical factor able to induce osseous metaplasia has yet been isolated from tumor cells. Randall et al. [19] found alkaline phosphatase activity in osteoblasts, in proliferating mesenchymal cells surrounding osseous foci, as well as on the apical membranes of colonic adenocarcinoma cells. These investigators concluded that colonic carcinomas “can promote heterotopic ossification, and that alkaline phosphatase is intimately associated with bone formation” in these situations. It could be speculated that the pluripotent mesenchymal cells, under the influence of unknown stimuli generated from the malignant epithelial cells, are transformed into osteoblasts, as reported by Rhone and Horowitz [20]. The osteoblasts, in turn, produce metamorphic bone. Interestingly, osseous metaplasia is always composed of benign bone [21]. Heterotopic bone formation in colorectal cancer should not be confused with carcinomas, which carries a much worse prognosis [22].

Recently, numerous regulators have been identified that exert modulatory effects on cells with an osteoblastic phenotype. Bone morphogenic proteins are a group of related proteins originally identified by their presence in bone-inductive extracts of demineralized bone and are members of the tumor growth factor-β (TGF-β) superfamily [23]. BMPs are characterized as low-molecular-weight glycoproteins that act as cytokines and that generally target immature, multipotent cells [24]. Once they bind to extracellular receptors and activate them via phosphorylation, BMPs initiate a chain of intracellular events that culminate in the increased expression of genes coding for various growth factors and bone matrix proteins [25,26]. Binding to the appropriate receptors causes stimulation of mesenchymal cell differentiation into osteoblasts, which then manufacture bone. Early difficulty in BMP research resulted from limited natural quantities; however, through the use of molecular cloning technology, recombinant forms of BMP of human origin have been developed and are now synthesized commercially.

Thirteen BMPs have been identified, and all but BMP-1 are believed to have osteogenic properties [24]. The BMP proteins can be divided into subgroups based on the primary amino acid sequence in the mature regions of the molecule. BMP-2 and BMP-4 are closely related molecules, being 92% identical in the cystine portion of the mature region, while BMP-5, -6, and -7 form another subgroup with about 90% amino acid identity [27]. It has been reported that BMP-2, -4, and -7 have the ability to induce ectopic bone formation in vivo [28]. Furthermore, BMP-6 has a greater effect on osteoblast formation compared with BMP-2 or BMP-4, as measured in a fetal rat cell culture model [29]. However, there are other data to support that BMP-2 and BMP-4 act more potently than BMP-6 in osteoblast differentiation [30]. In particular, BMP-2 is an active inducer of osteoblastic differentiation of both immature osteoblasts and less committed cells. Furthermore, studies have shown that BMP-2 increases expression of osteoblastic indicators from pluripotent stem cell cultures, which suggests that BMP-2 regulates the entrance of uncommitted cells into specific differentiation pathways [31].

Basic fibroblast growth factor (bFGF), a member of a family of growth factors that stimulate proliferation of cells of mesenchymal, epithelial, and neuroectodermal origin, has been suggested to be a strong mitogen for bone-derived cells. Immunohistochemical staining for
FGF-2 was negative. Staining for BMP-2 showed extensive expression of this protein within tumor cells as compared with the controls (rectal adenocarcinoma without heterotopic ossification). This finding is in contrast to the report by Imai et al. [13], who found strong expression of BMP-5 and -6 in tumor cells with weak expression of BMP-2. In the current study, we did not stain for other BMPs, but we demonstrated, for the first time, overexpression of BMP-2 in rectal adenocarcinoma cells. This implies that an osteogenic signal exists and is found within the tumor cells. This may be the mechanism responsible for initiating the induction of osteoblasts ultimately leading to the presence of heterotopic bone. Why some tumors secrete BMP-2 is unknown, but it is most likely a random activation of BMP expression, with probably very little growth advantage for the neoplasm. One would expect that if there were some sort of survival benefit to the expression of BMP, one would expect to see heterotopic ossification more commonly. Nevertheless, in these rare cases, some reason must account for the presence of bone formation.

In summary, we present a 38-year-old woman with heterotopic bone formation in the setting of primary rectal adenocarcinoma—only the eighth such case report in the English literature. Her preoperative CT scan showed a large area of calcification within the tumor. Of note, the tumor had no areas of necrosis, did not contain mucin, and had heterotopic bone formation that was histologically benign. The precise mechanism of heterotopic ossification is still largely unknown but, for the first time, we demonstrate, with immunohistochemical staining, the presence of a bone morphogenetic protein known to induce bone formation. Awareness of heterotopic bone formation in colorectal adenocarcinoma is important if one wants to avoid a misdiagnosis of carcinosarcoma. The fact that gastrointestinal cancers can calcify and ossify has a clinical relevance, because a diagnosis of carcinosarcoma should always be taken into account when calcification of the gastrointestinal tract has been detected radiographically.

REFERENCES