Heterotopic Ossification in Metastatic Colorectal Carcinoma: Case Report with Morphoproteomic Insights into the Histogenesis

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Abstract. A case of metastatic colorectal carcinoma with heterotopic ossification is described. Objective. Morphoproteomic analysis was performed to help define the histogenesis of the heterotopic ossification in this context. Design. Immunohistochemical stains for Gli2, α-SMA, SPARC (osteonectin), and nestin were performed and the expression level (chromogenic signal intensity) and subcellular compartmentalization of these protein analytes were assessed in the tumor cells vis-à-vis the companionate stromal cells and osteoblasts in a morphoproteomic application. Results. This analysis revealed that the heterotopic ossification is more likely the result of pluripotent stromal cells that undergo differentiation to form osteoblasts rather than the tumor cells undergoing osseous metaplasia. Conclusion. Morphoproteomics provides evidence that the histogenesis of heterotopic ossification in this case of metastatic colon cancer is from the stromal cells in the tumoral microenvironment.

Key Words: Heterotopic Ossification, and Morphoproteomics.

Introduction

Heterotopic ossification occurs when bone tissue forms outside the skeleton. This phenomenon has been observed in the liver, breast, skin, and kidneys [1-4]. Though rarely, it has also been observed in the gastrointestinal (GI) tract. Since the first cases of osseous metaplasia in the GI tract were described [5], reports of heterotopic ossification in association with a wide range of GI diseases have been published [6-8]. However, we only found a few cases of metastatic colorectal adenocarcinoma with heterotopic ossification in the literature [9]. Here, we report a case of a 72-year-old male with such a presentation.

To further investigate the histogenesis of this rare entity, we performed morphoproteomic studies on the core biopsy of the metastatic colorectal adenocarcinoma from the patient. Morphoproteomics includes the immunohistochemical assessment of various protein analytes in tumor cells and their microenvironment in an attempt to better define the biology of the tumor and possible interactions with companionate cells in the microenvironment [10].

Case Report

A 72-year-old Hispanic male with a history of metastatic colon cancer and early stage prostate cancer presented to an outpatient clinic with increasing discomfort and severe pain in the sacral region and a 15-pound weight loss over a few weeks. The patient is status post-radiotherapy for his prostate cancer and chemotherapy for his metastatic colon cancer (FOLFOX, FOLFIRI, and more recently oxaliplatin/cetuximab).

A restaging computed tomography positron emission tomography (CT PET) scan was ordered revealing a 3.0 cm lesion in the periumbilical area, as well as lymphadenopathy in the pre-renal and pre-sacral areas. Blood chemistries, complete blood count, and prostate specific antigen (PSA) were within normal limits; however, carcinoembryonic antigen (CEA) was elevated at 10.4 ng/mL (expected range: 0 – 3.0 ng/mL).

Fine needle aspiration and core biopsy of the periumbilical mass were performed using a 20-gauge needle with ultrasound guidance by an interventional radiologist.
The biopsy was not in close proximity to any bone. A cell block was prepared from the formalin-fixed core biopsy material.

The cell block showed numerous malignant glands. The malignant glands displayed morphologic features consistent with a colorectal primary to include glandular architecture with central necrosis. Additionally, there was heterotopic ossification in association with the malignant cells (Figure 1). Immunohistochemical stains were positive for CDX2 (Pre-diluted; Ventana; Tuscan, Arizona, USA), variable for cytokeratin (CK)20 (Pre-diluted; Ventana; Tuscan, Arizona, USA), and negative for CK7 (Pre-diluted; Ventana; Tuscan, Arizona, USA) and prostate specific antigen (PSA) (Pre-diluted; Ventana; Tuscan, Arizona, USA). The immunohistochemical and histologic features are consistent with a colorectal primary.

To further investigate the histogenesis of the heterotopic ossification with morphoproteomics, immunohistochemical stains for Gli2 (glioma-associated oncogene protein 2) (Dilution 1:400; Abcam; Cambridge, Massachusetts, USA), alpha-smooth muscle actin (α-SMA) (Dilution 1:500; Dako; Glostrup, Denmark), secreted protein, acidic and rich in cysteine (SPARC, otherwise known as osteonectin) (Dilution 1:100; Leica; Newcastle, UK), and nestin (Dilution 1:400; Abcam; Cambridge, Massachusetts, USA) were performed on the cell block with accompanying positive and negative controls (Table 1). Gli2 was expressed in stromal and tumor cell nuclei (Figure 2A and B). Nestin was moderately positive in the osteoblast/osteocytes and stroma and negative in the tumor cells (Figure 2C and D). Osteonectin was strongly positive in the osteoblasts/osteocytes and stroma and was weakly expressed in the tumor cells (Figure 2E). Alphah(α)-SMA was positive in stromal myofibroblasts (Figure 2F).

Discussion

Heterotopic ossification of the GI tract, especially of metastatic colon carcinoma, is a rare entity. Furthermore, the histogenesis of this rare entity remains elusive. We investigated its histogenesis through the use of morphoproteomics. Here, morphoproteomics gave us additional supporting evidence for existing hypotheses and identified additional pathways in the histogenesis of tumor-related heterotopic ossification.

Previous authors have tried to study the histogenesis of heterotopic ossification through the association of histologic features. However, no consistent correlation can be made between these associations [11, 12]. More recently, some authors hypothesized that osteoblast metaplasia of tumor cells resulted in heterotopic ossification [13], while others hypothesized that the metaplasia of a stromal pluripotent cell into an osteoblast by factors secreted by the cancer cells might be the cause of heterotopic ossification [14].

Currently, the latter hypothesis is more widely accepted, and our morphoproteomic analysis supports this hypothesis. First, osteonectin was strongly positive in the osteoblasts/osteocytes and stroma and weakly positive in the tumor cells. Osteonectin (SPARC) serves as an indicator of bone formation. It is one of the main non-collagenous components of bone that induces calcium deposition [15] and promotes osteoblast differentiation and survival [16-18]. The strong staining in the stroma, as opposed to the weak staining in the tumor cells with this bone formation indicator, provides evidence that the heterotopic bone comes from pluripotent cells in the tumoral microenvironment. Secondly, we see positive staining of nestin in the stroma, while the tumor cells are negative. Nestin, an intermediate filament protein, is abundant in embryonic stem-derived progenitor cells that can differentiate into various lineages, including mesodermal [19]. Here, it can serve as an indicator of the bone precursors, which are mesodermal in origin. Similar to SPARC, the positive staining of nestin in the stroma lends more support to the hypothesis that the osteoblastic differentiation occurs in the stroma and not the tumor cells.
Both the literature and our morphoproteomic analysis support the hypothesis that it is the metaplasia of stromal pluripotent cells into osteoblasts by factors secreted by tumor cells that causes heterotopic ossification. However, the factors secreted by the tumor cells causing the ossification are not well defined. Some authors had proposed alkaline phosphatase as a possible factor in the past [20]. More recently, two reports have implicated bone morphogenetic protein (BMP) in heterotopic ossification [7, 21]. Specifically, Imai et al [21] have shown through immunohistochemistry that BMP-2, BMP-4, BMP-5, and BMP-6 were present in tumor cells; and in another study, Kypson et al [7] have also shown through immunohistochemistry that BMP-2 was present in the tumor cells. Almost all of the BMPs have been associated with osteogenic activity [22], meaning that they are substances that can stimulate bone formation. Hence, the presence of BMP by immunohistochemistry in the tumor cells suggest that the tumor cells are secreting these factors into the microenvironment, and these factors are possibly stimulating the pluripotent stromal cells and causing heterotopic metaplasia. Through the use of morphoproteomics, we have additional evidence that BMP is one of the factors involved in osseous metaplasia. Here, Gli2 was positive in the stromal cells and tumor cells. Gli2 is a factor that can stimulate the production of BMP [23-25]. So, the presence of Gli2 in the microenvironment leads us to believe that BMP was also present in the microenvironment of the tumor in our patient.

We sought to investigate additional mechanisms of osseous metaplasia through morphoproteomics. One such mechanism we investigated involves the activation of transforming growth factor–beta (TGF-β), which has been shown to increase osteoblast differentiation marker [26]. First, TGF-β activates Smad (through the Smad pathway) and p38MAPK (through the non-Smad-dependent pathway), and these two factors converge at Runx2 to induce bone formation [27]. Secondly, TGF-β activity strongly enhances BMP-2 to increase osseous formation [28]. Here, we have immunohistochemical evidence of TGF-β activation, which stimulates bone formation through the two mechanisms mentioned above, namely the expression pattern of Gli2 and α-SMA. Gli2 stained positively in the stromal and tumor cells, signifying its presence in the microenvironment. The TGF-β/Smad pathway has been shown to induce Gli-2 [29-31], our first evidence of TGF-β activity in the microenvironment. Also, α-SMA was positive in the occasional stromal myofibroblast. Similarly, TGF-β is known to induce α-SMA expression in fibroblast [32-34], which further implicates the presence of TGF-β activity in the microenvironment. Morphoproteomics has provided evidence of TGF-β activity in the microenvironment of the heterotopic ossification seen in our patient, providing us with an additional mechanism of this phenomenon.
Figure 2. Gli2 shows nuclear positivity in stromal and tumor cell (A, x400; B, x600). Nestin is moderately expressed in the cytoplasmic compartment of the osteoblast/osteocytes and stroma and negative in the tumor cells (C, x400; D, x400). Osteonectin (SPARC) is strongly positive in the cytoplasm of the osteoblasts/osteocytes and stromal cells and is weakly positive (upper left) in the tumor cells (E, x600). Lastly, α-SMA is positive in occasional stromal myofibroblasts (F, x200).
In conclusion, here we report a rare case of heterotopic ossification in a metastatic colon carcinoma and have investigated its histogenesis. Through morphoproteomics, we have additional evidence that it is the differentiation of stromal pluripotent cells rather than tumor cells into osteoblasts. Secondly, Gli2 serves as evidence for the role of BMP in osseous metaplasia. Lastly, Gli2 and α-SMA in the microenvironment is additional evidence for the role of TGF-β pathway in heterotopic ossification.

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References