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Immunohistochemical detection of bone morphogenetic protein-2 and transforming growth factor beta-1 in tracheopathia osteochondroplastica

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Abstract Tracheopathia osteochondroplastica (TO) is an unusual condition characterized by cartilaginous or bony submucosal nodules in the tracheobronchial tree. Bone morphogenetic protein-2 (BMP-2) and transforming growth factor beta-1 (TGF-β1) are potent inducers for new bone formation. We studied the precise localization of BMP-2 and TGF-β1 in two autopsied cases of TO, using immunohistochemical methods. Positive BMP-2 immunoreactivity was detected in numerous mesenchymal cells and chondroblasts lining the nodules in the tracheal submucosa. BMP-2 was not found in mature lamellar bony nodules. TGF-β1 was not seen in mesenchymal cells, though it did appear in chondrocytes and osteocytes in the nodules. These results suggest that BMP-2 plays an important role in nodule formation and acts synergistically with TGF-β1 to promote the nodules inductive cascade in the tracheal submucosa.

Key words Tracheopathia osteochodroplastica · Bone morphogenetic protein-2 · Transforming growth factor beta-1 · Immunohistochemistry

Introduction

Tracheopathia osteochondroplastica (TO) is an unusual condition of the tracheobronchial tree, characterized by cartilaginous and bony submucosal nodules covered by intact mucosa. While this disease may often go unrecognized because of its asymptomtic nature, it occasionally produces narrowing of the airways with a variety of symptoms, such as dry cough, dyspnoea, hoarseness, and recurrent respiratory tract infections [11, 13].

The aetiology and pathogenesis of TO remain unknown. The basic causes of TO have been postulated to be chronic infections, a congenital basis, chemical or mechanical irritation, metabolic disturbances, degenerative processes, ecchondrosis and exostosis, or metaplasia of elastic tissue [10, 12, 20].

Bone morphogenetic protein-2 (BMP-2) is a member of the transforming growth factor beta (TGF-β) gene superfamily that has been implicated in catilage and bone formation [22]. The BMP proteins induce ectopic bone formation in vivo [4–6, 21]. The expression of BMP-2 used with the monoclonal antibody in normal, osteosarcoma [19], and pleomorphic adenomas of the salivary gland [7] have been reported. We therefore investigated the precise localization of BMP-2 and TGF-β1 in TO by immunohistochemistry.

Materials and methods

Autopsy specimens were fixed in buffered formalin and embedded in paraffin. Sections were routinely stained with haematoxylin and eosin. Immunohistochemical studies were also performed on paraffin sections using the avidin–biotin peroxidase complex technique (Dakopatts, Copenhagen, Denmark). The antibody panel used included monoclonal anti-BMP-2 (Austral Biological), monoclonal anti-TGF-β1 (Anogen), monoclonal antibodies to CD45RO (Biomeda), CD43 (Organon Teknika), CD74 (Nichirei), CDW75 (Nichirei), HLA-DR (Nichirei), CD68 (Dakopatts), CD15 (Becton Dickinson), and polyclonal antibody to S-100 protein (Dakopatts). Appropriate positive controls were included.

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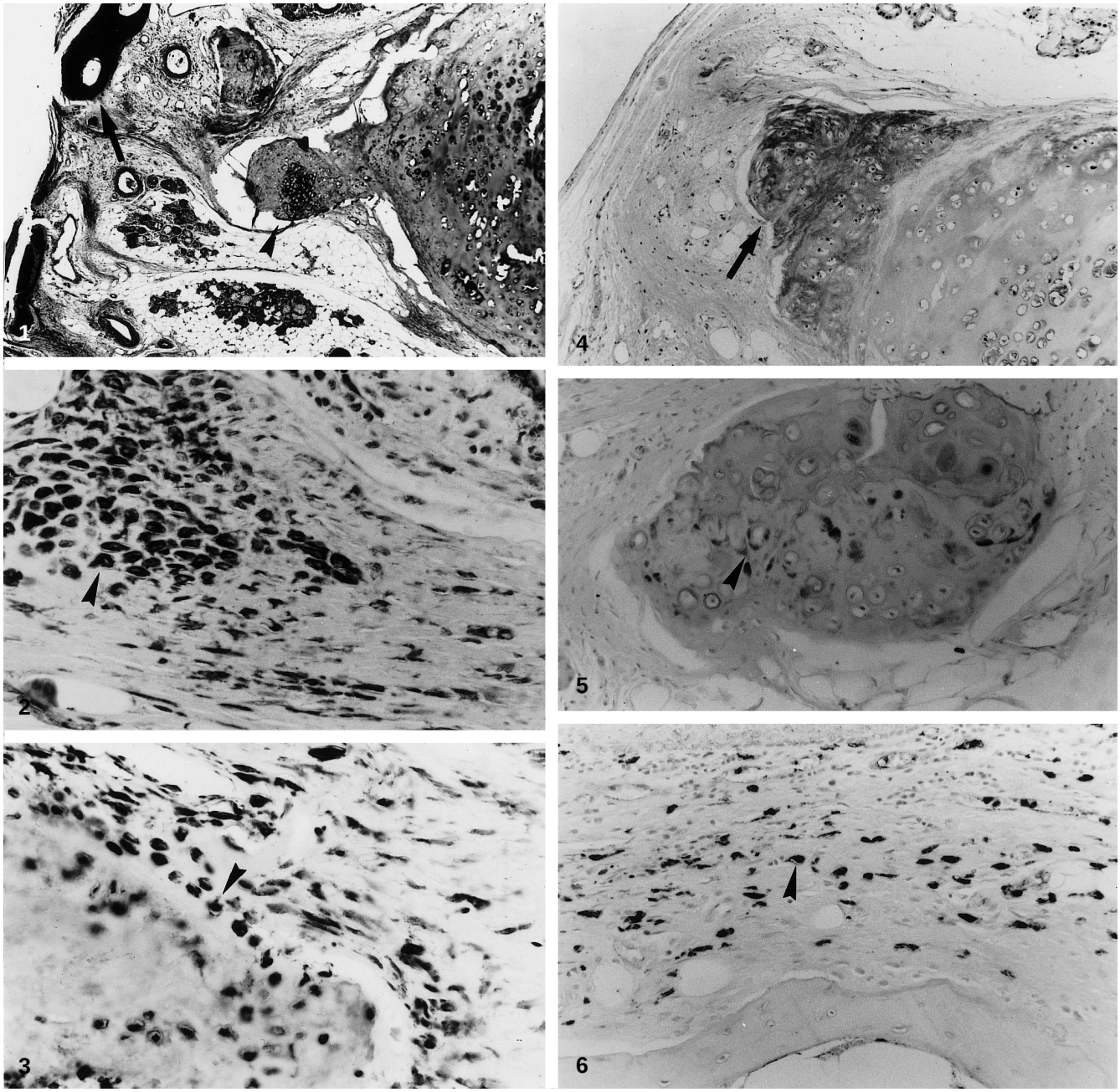

Fig. 1 Nodules with lamellar bone (*arrow*) in the submucosa and osseocartilaginous tissues of nodules (*arrow head*) subjacent to the tracheal cartilage. Moderate inflammatory cells in the submucosa. Elastica-van Gieson, ×25

Fig. 2 Immunostaining for BMP-2 shows strong cytoplasmic staining of mesenchymal cells (*arrowhead*) around the nodules. ABC method, ×200

Fig. 3 BMP-2 immunoreactivity shows an immature matrix of cartilage and chondroblasts (*arrowhead*) lining the new cartilage tissue. ABC method, ×200

Fig. 4 BMP-2 immunoreactivity is seen in the nodule (*arrow*) subjacent to tracheal cartilage. ABC method, ×50

Fig. 5 TGF-β1 immunoreactivity is seen in chondrocytes and osteocytes (*arrowhead*). ABC method, ×200

Fig. 6 Granulocytes-monocytes lineage cells (*arrowhead*) in collagenous tissues around a nodule. ABC method, ×200

Clinical findings

Case 1

A 57-year-old woman presented with dyspnoea, cough, hoarseness, and dysphasia of 3 days' duration. She had a long history of recurrent hoarseness and chronic sinusitis. She was admitted to the Division of Internal Medicine, Kahoku Hospital, in October 1993. Physical examination revealed a temperature of 38 °C, dehydration, cyanosis, and a respiratory rate of 35 breaths/min. Auscultation exhibited coarse, low-pitched wheezes with a prolonged expiratory phase. Radiography of the chest disclosed severe lobar pneumonia of both lungs and mild cardiomegaly. Indirect laryngoscopy revealed slight inflammatory changes in the vocal cords and trachea, with sputum retention in the hypopharynx. The laboratory data at admission included: haemoglobin 12.3 g/dl, white blood cell count 25,830/mm^3, asparate aminotransferase 167 IU/l, alanine aminotransferase 118 IU/l, and lactate dehydrogenase 522

IU/l. Arterial blood gas analysis with the patient breathing room air revealed P_aO_2 of 45 mmHg, P_aCO_2 of 48 mmHg, and pH of 7.42. She became increasingly dyspnoeic despite optimal therapy with antibiotics and prednisone. The patient died of pneumonia 2 days after admission. No other relevant changes were seen.

Case 2

A 75-year-old man with a perianal abscess was admitted to the hospital in October 1992. His past history included a myelodysplastic syndrome (MDS: FAB, RAEB) of 2 years' duration. The patient had no history of pulmonary disease. Physical examination revealed normal findings in the chest. The laboratory data demonstrated the following: haemoglobin 8.8 g/dl, white blood cell count 3,150/mm^3 (myeloblasts: 3%), thrombocytopenia (28,000/mm^3). The patient underwent drainage of his perianal abscess. On the following day, he developed haematemesis, melaena, and severe pain in the right hypochodrium. The patient had recurrent melaena and died 2 weeks after the drainage. Autopsy findings, in addition to TO, were advanced gastric cancer at the lower corpus on the lesser curvature with no distant metastasis, latent prostatic cancer, hypoplastic marrow, acute cholecystitis with localized bile peritonitis, chronic bronchitis, and pulmonary emphysema.

Pathological findings in the trachea

Histological features

At autopsy the trachea had identical appearances in both cases. Multiple sessile nodules projected into the lumen. The nodules were distributed from the level of the first tracheal ring to the proximal main bronchi. They were covered by normal mucosa. The nodular areas of the trachea were examined histologically in their entirety and serially sectioned. The nodules demonstrated newly formed bone or cartilage both beneath the mucosal surface and deep in the lamina propria mucosae (Fig. 1). They consisted of hyaline cartilage with areas of lamellar bone or bony nodules with haematopoietic bone marrow. Two different developmental processes were found in the submucosa: one had connections of bone, cartilage, or collagenous tissue continuous with the perichodrium of the tracheal cartilage; the second did not directly connect to the perichondrium. Bony nodules of a lamellar type displayed fatty and haematopoietic bone marrow. The surface epithelium overlying the osseocartilaginous nodules revealed predominantly squamous metaplasia. The submucosa was attenuated over the nodules. Moderate mononuclear cell infiltrate was present in the submucosa.

Immunohistochemical findings

Extensive BMP-2 was distributed in numerous mesenchymal cells around the oseocartilagenous nodules in the submucosa (Fig. 2). BMP-2 was located in new cartilage, the immature matrix of cartilage, and chondroblasts lining the new cartilage tissue (Fig. 3). Little BMP-2 was found in hypertrophic chondrocytes, or osteocytic cells of the mature lamellar bone. Significant BMP-2 immunoreactivity was also recognized in the nodules subjacent to the tracheal cartilage (Fig. 4). No BMP-2 was detected in the mature calcified matrix of nodules. Little BMP-2 was distributed in normal adjacent cartilage.

TGF-β1 was detected in chondrocytes and osteocytes in the nodules and normal adjacent cartilage (Fig. 5). Little TGF-β1 was found in the calcified matrix and osseous cels of the lamellar bone. TGF-β1 could not be seen in mesenchymal cells. TGF-β1 was also expressed in bronchial epithelial cells, especially in the basal layer.

The pattern of immunohistochemical localization of S-100 protein resembled the distribution of TGF-β1. S-100 protein was found in the chondrocyte nuclei and cytoplasm.

Numerous CD15-positive (granulocytes-monocytes) and CD68-positive (monocytes) cells were detected in collagenous tissues around newly formed nodules, haematopoietic bone marrow, and intra- or perivascular tissues in the submucosa (Fig. 6). There was a paucity of CD45RO-positive cells (T cells) in the submucosa.

Discussion

We have used immunohistochemical methods to detect the precise localization of BMP-2 and TGF-β1 in the trachea with TO. The data show that BMP-2 is localized in mesenchymal cells, osteoblastic cells, chondroblastic cells around the new nodules in the submucosa, and the nodules subjacent to the tracheal cartilage. TGF-β1 was not detected in mesenchymal cells, but did appear in mature chondrocytes and osteocytes in the nodules. These results suggest that both BMP-2 and TGF-β1 play an important part in the histopathogenesis of TO.

Two main pathogenetic theories of TO have been proposed. Virchow regarded the lesion as a kind of ecchondrosis or exostosis arising from the tracheal cartilage. Supporters of this theory maintain that the osseocartilaginous nodules have a connection to the perichodrium of the tracheal cartilage from which they arise [10, 12]. The other theory was proposed by Dalgaard [2], who stated that the nodules arose as a result of a metaplastic process in the submucosa and lamina propria; mesenchymal cells in the submucosa lost their immature character and developed into chodrocytes through metaplasia, forming new nodules. In our two cases, serial sections of nodules revealed both ecchondrial processes from the tracheal cartilage and metaplastic processes in the submucosa.

BMP-2 is known to be a member of the TGF-β1 gene superfamily as well as a factor in ectopic bone and cartilage formation by the induction of differentiation of mesenchymal cells to osteoprogenitor cells [5, 6, 15, 21]. In TO, extensive BMP-2 was found in mesenchymal cells around newly formed nodules in the submucosa, whereas little BMP-2 was found in the mature lamellar bones. This suggests that during the metaplastic process in the submucosa, mesenchymal cells may produce and deliver BMP-2, which can then induce differentiation into osteoblasts and chondroblasts, and finally into newly formed nodules. BMP-2 was also localized in the nodules of the

immature chondromatrix and lining cells subjacent to the tracheal cartilage. This implies that BMP-2 may participate in the process of ecchondrosis from the tracheal cartilage.

The observation that numerous CD15- and CD68-positive granulocytes-monocytes infiltrate at the periphery of nodules in the submucosa is consistent with a previous report on a demineralized bone matrix implant system devised to study bone formation at a heterotopic site [3]. The report shows that monocyte lineage cells migrate at the periphery of the matrix in the early staes, disappearing at later stages to be replaced by chondrocytes. BMP proteins stimulate the chemotaxis of monocytes and mesenchymal cells, and at high concentrations increase the expression of TGF-β1 transcripts [1]. TGF-β1 also has a chemotactic function in the case of monocytes and mesenchymal cells [17]. Moreover, TGF-β1 stimulates the production of extracellular matrix proteins by chondrocytes [14]. It is noteworthy, however, that TGF-β1 alone cannot induce cartilage and bone formation in the in vivo bioassay. In our cases, TGF-β1 was detected in chondrocytes in newly formed nodules in the submucosa. Thus, BMP-2 may act synergistically with TGF-β1 to promote the nodules' inductive cascade in TO. We speculate that BMP-2 may stimulate the chemotaxis of monocytes in the submucosa, though the precise mechanism of the initial expression of BMP-2 is not known. The monocytes may release various cytokines and growth factors that recruit mesenchymal cells and stimulate the production of extracellular matrix proteins. In addition, previous studies demonstrated that an acidic environment culd enhance new bone formation by BMP proteins [18]. The acidic environment could be induced with migration of inflammatory cells, including that of monocyte-lineage cells. It is possible that the monocyte-lineage cells in the submucosa may enhance the nodules formation associated with BMP-2.

BMP-2 has been identified in a variety of embryonic epithelial, mesenchymal tissues, and bone-forming cells [8, 9]. In contrast, no BMP-2 immunoreactivity has been detected by immunohistochemical analysis [6, 19] of adult tissues, including the bone matrix and lungs. Previous work was based on the assumption that the initial BMP proteins diffuse from the resorbed bone matrix and that after the stimulation of BMP proteins, mesenchymal cells start to synthesize and secrete BMP proteins, which could induce the other mesenchymal cells to differentiate [15]. In the process of ecchondrosis in TO, BMP-2 may spread from the tracheal cartilage. However, chondrogenesis and osteogenesis are complex, multistep processes that invole the interactions of multiple cell types, local pH, growth factors, and the extracellular matrix. In TO, the precise mechanism of the expression of BMP-2 remains unclear.

S-100 protein is a calcium-binding protein. Chondrocytes in BMP-induced osteogenesis are characterized by marked S-100 protein staining [16]. In our two cases, marked S-100 protein immunoreactivity was detected in the chondrocytes that were in the process of ecchondrosis. S-100 protein in the chondrocytes may participate in a calcium signalling mechanism.

In these two cases of TO, we investigated the precise localization of BMP-2 and TGF-β1. Both BMP-2 and TGF-β1 may have important and distinct roles in the histopathogenesis of TO.

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