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Rapid communication

Overexpression of bone morphogenic protein (BMP)-4 mRNA in gastric cancer cell lines of poorly differentiated type

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Abstract: Gastric cancer is classified as the poorly differentiated and the well differentiated type, depending on its histological, biological, and genetic characteristics. Bone metastasis is more frequently detected in the poorly differentiated type than in the well differentiated type. The prognosis of patients with diffuse osteoplastic bone metastasis of gastric cancer is extremely poor. In this study, we examined the mRNA expression of bone morphogenic proteins (BMPs) in seven gastric cancer cell lines: OKAJIMA, TMK1, MKN45, and KATO-III derived from the poorly differentiated type; and MKN7, MKN28, and MKN74, derived from the well differentiated type. BMP-2 was expressed only in TMK1. BMP-4 mRNA was overexpressed in OKAJIMA, MKN45 and KATO-III, weakly expressed in MKN7, MKN28, and MKN74, but was not expressed in TMK1. Although the biological roles of BMP-2 and BMP-4 expression in gastric cancer remain to be elucidated, these results indicate that BMP-4 mRNA is preferentially overexpressed in the poorly differentiated type of gastric cancer.

Key words: gastric cancer, bone morphogenic protein-2, bone morphogenic protein-4

Introduction

Gastric cancer is one of the most common maligrancies worldwide.[1] Better control of the primary tumor and improved patient prognosis have led to the increased importarce of distant metastasis for patient management. Bone metastasis of gastric cancer is detected in 0.99%–2.1% of patients who have been surgically operated, and in 7.9%–20.0% of autopsied cases.[2–4] Bone metastasis is more frequently detected in the poorly differentiated type of gastric cancer than in the well differentiated type.[3] Some patients with gastric cancer of the poorly differentiated type show an extremely poor clinical course because of diffuse osteoplastic bone metastasis associated with diffuse intravascular coagulation.[4]

The bone morphogenic proteins (BMPs) BMP-2/BMP-2A, BMP-3, BMP-4/BMP-2B,[5] BMP-5, BMP-6, and BMP-7,[6] which belong to the transforming growth factor β (TGF-β) superfamily, are implicated not only in the induction of bone but also in the morphogenesis of several organs other than bone, including brain, kidney, heart, and skin.[7,8] BMPs are classified into three subgroups: BMP-2 and BMP-4 (subgroup 1); BMP-3 (subgroup 2); and BMP-5, BMP-6, and BMP-7 (subgroup 3). BMP-2 and BMP-4 show the most potent bone-inducing activity.[6]

BMPs are expressed in several tumor cell lines, including the osteosarcoma cell line, U-2 OS, and the salivary gland adenocarcinoma cell line, HSG-S8.[5,9] In this study, we examined the mRNA expression of BMP-2 and BMP-4 in seven gastric cancer cell lines, and found that BMP-4 is preferentially overexpressed in the poorly differentiated type of gastric cancer.

Materials and methods

Cell lines

The human gastric cancer cell lines, OKAJIMA (poorly differentiated adenocarcinoma; por), TMK1 (por), MKN7 (tubular adenocarcinoma; tub), MKN28 (tub), MKN45 (por), MKN74 (tub), and KTAO-III (signet-ring cell carcinoma) were cultured in RPMI-1640 supplemented with 10% fetal calf serum, as described previously.[10]

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Poly(A)$^+$ RNA extraction and cDNA-polymerase chain reaction (cDNA-PCR)

Poly(A)$^+$ RNA was extracted from the gastric cancer cell lines by the Fast Track Kit (Invitrogen, San Diego, Calif.). The first strand of cDNA was synthesized from 200 ng of poly(A)$^+$ RNA by reverse transcriptase (Amersham Amersham, UK) with oligo-dT primer, and was used as the template for cDNA-PCR.[11] Primers PA1 and PA2 corresponded to the published nucleotide sequence of BMP-2, and primers PB1 and PB2 corresponded to BMP-4.[5]

The nucleotide sequences of the primers were: primer PA1 (sense), 5′-ACACTGAGACGCTGTTCC CA-3′; primer PA2 (antisense), 5′-CGATACAGGTC TAGCATGTA-3′; primer PB1 (sense), 5′-CAGGG ACCTATGGAGCCATT-3′; and primer PB2 (antisense), 5′-CGGTAAAGATCCCGCATGTA-3′.

Northern blot analysis

Two μg of poly(A)$^+$ RNAs were separted on 1.0% agarose gels containing 17.9% formaldehyde, transferred to nitrocellulose filters, and then hybridized with ^{32}P-labeled probes under highly stringent conditions.[12]

Results and discussion

cDNA-PCR with primers PA1 and PA2 amplified 448-bp bands from the poly(A)$^+$ RNAs of TMK1 cells, but not from those of MKN45 cells. The purified PCR product corresponded to the 5′-noncoding region and a part of the open reading frame of BMP-2 (nucleotides 127–574 of BMP-2), and was renamed BMP-2 specific probe, BMP-2N. Southern blot hybridization with the BMP-2N probe did not reveal gene amplification of BMP-2 in the gastric cancer cell lines examined (data not shown). Northern blot hybridization with the BMP-2N probe revealed that 3.8-kb BMP-2 mRNA was expressed only in TMK1 cells (Fig. 1A). The quality and quantity of RNAs on the filters were confirmed by hybridization with a β-actin probe (Fig. 1C).

The BMP-4 specific probe, BMP-4N, corresponding to the 5′-noncoding region and part of the open reading frame of BMP-4 (nucleotides 250–657 of BMP-4), was amplified by cDNA-PCR with primers PB1 and PB2 from the poly (A)$^+$ RNA of MKN45 cells. BMP-4 gene amplification was not detected in the gastric cancer cell lines examined (data not shown). Northern blot analysis showed that 2.2-kb BMP-4 mRNA was overexpressed in OKAJIMA, MKN45, and KATO-III cells, and weakly expressed in MKN7, MKN28, and MKN74; but was not expressed in TMK1 (Fig. 1B).

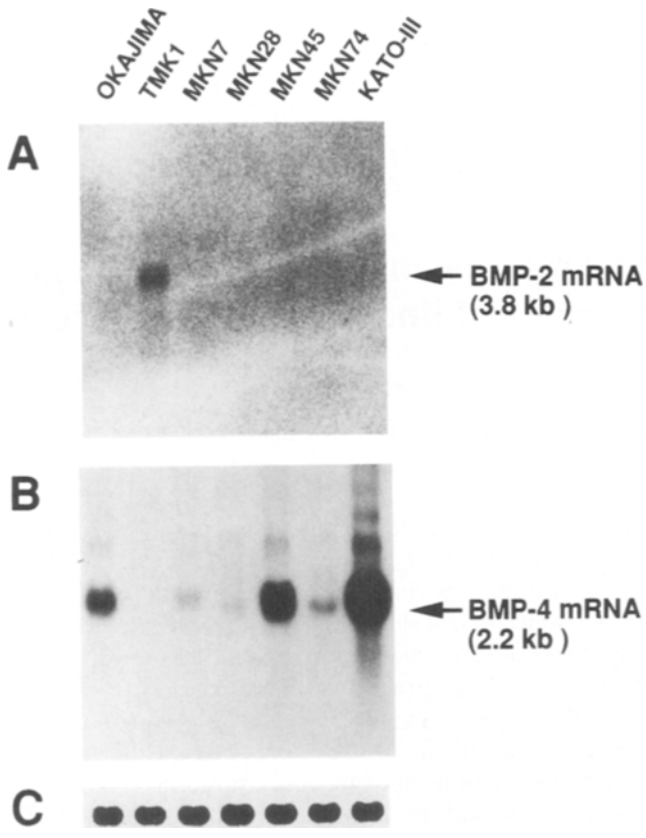

Fig. 1A–C. Northern blot analysis of bone morphogenic protein (BMP)-2 and BMP-4 mRNA expression in gastric cancer. Poly(A)$^+$ RNA from the seven gastric cancer cell lines (2 μg per lane) was fractionated in agarose gel, transferred onto nitrocellulose filters, and then probed with **A** ^{32}P-labeled BMP-2N, **B** BMP-4N, or **C** β-actin

As stated above, gastric cancer is classified, as the poorly differentiated type (or diffuse type) and the well differentiated type (or intestinal type), depending on its histological, biological, and genetic characteristics. Bone metastasis is more frequently detected in the poorly differentiated type of gastric cancer, while liver metastasis is more frequently detected in the well differentiated type. The K-*sam* gene and the c-*erb*B2 gene, respectively, are amplified in the poorly differentiated type and in the well differentiated type.[13] OKAJIMA, TMK1, MKN45, and KATO-III are derived from the poorly differentiated type, while MKN7, MKN28, and MKN74 are derived from the well differentiated type. Our results indicate the preferential overexpression of BMP-4 mRNA in the poorly differentiated type of gastric cancer.

The process of bone metastasis consists of a series of steps, including invasion of the surrounding normal stroma, detachment from the primary lesion, migration to the bone, attachment to the bone, and growth in the bone. Although the outcome of bone metastasis

depends mainly on the blood flow, as well as on the lymphatic flow, there may be other factors involved in the formation of bone metastasis, such as the interaction of gastric cancer cells with the microenvironment in the bone.[14]

The molecular basis of the intrinsic capacity of the poorly differentiated type of gastric cancer cells to colonize the bone is now under intense investigation. Our results suggest an association between BMP overexpression and the increased bone metastatic potential of the poorly differentiated type of gastric cancer. Gastric cancer cells overexpressing BMPs could migrate to the bone and stimulate osteogenesis, which, in turn, could provide a more desirable environment for gastric cancer cells to proliferate in the bone. Further analysis of the expression of BMPs in the primary lesion, as well as in the bone-metastatic lesions of gastric cancer, may elucidate the relationship between BMP overexpression and increased bone-metastatic potential.

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References

1. Parkin DM, Laara E, Muir CS. Estimate of the worldwide frequency of 16 major cancers in 1980. Int J Cancer 1980;41: 184–197.
2. Abrams HL, Sprio R, Goldstein N. Metastasis in carcinoma. Analysis of 1000 autopsied cases. Cancer 1950;3:74–85.
3. Yoshikawa K, Kitaoka H. Bone metastasis of gastric cancer. Jpn J Surg 1983;13:173–176.
4. Katoh M, Sugano K, Natomi H, et al. Two cases of an advanced gastric cancer with a diffuse carcinomatosis of the bone marrow showing a widespread osteoplastic bone metastasis (in Japanese with English abstract). Jpn J Cancer Clin 1990;36:551–556.
5. Wozney JM, Rosen V, Celeste AJ, et al. Novel regulators of bone formation: Molecular cloning and activities. Science 1988; 242:1528–1534.
6. Celeste AJ, Iannazzi JA, Taylor RC, et al. Identification of transforming growth factor β family members present in bone-inductive protein purified from bovine bone. Proc Natl Acad Sci USA 1990;87:9843–9847.
7. Lyons KM, Pelton RW, Hogan BL. Pattern of expression of murine Vgr-1 and BMP-2A RNA suggests that transforming growth factor-β-like genes coordinately regulate aspects of embryonic development. Genes Dev 1989;3:1657–1668.
8. Lyons KM, Pelton RW, Hogan BLM. Organogenic and pattern formation in the mouse: RNA distribution patterns suggest a role for bone morphogenic protein-2A (BMP-2A). Development 1990;109:833–844.
9. Hatakeyama S, Ohara-Nemoto Y, Kyakumoto S, et al. Expression of bone morphogenic protein in human adenocarcinoma cell line. Biochem Biophys Res Commun 1993;190:695–701.
10. Katoh M, Yazaki Y, Sugimura T, Terada M. c-*erb*B3 gene encodes secreted as well as transmembrane receptor typrosine kinase. Biochem Biophys Res Commun 1993;192:1189–1197.
11. Katoh M, Hirai M, Sugimura T, Terada M. Cloning and characterization of MST, a novel putative serine/threonine kinase with SH3 domain. Oncogene 1995;10:1447–1451.
12. Katoh M, Hattori Y, Sasaki H, et al. K-*sam* gene encodes secreted as well as transmembrane receptor tyrosine kinase. Proc Natl Acad Sci USA 1992;89:2960–2964.
13. Katoh M, Terada M. Oncogenes and tumor suppressor genes. In: Nishi M, Ichikawa H, Nakajima T, et al. (eds) Gastric cancer. Tokyo Berlin Heidelberg New York: Springer 1993; 196–208.
14. Katoh M, Unakami M, Hara M, Fukuchi S. Bone metastasis from colorectal cancer in autopsy cases. J Gastroenterol 1995; 30:615–618.