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Original Article

Expression pattern of the class I homeobox genes in ovarian carcinoma

Jin Hwa Hong[1], Jae Kwan Lee[1], Joong Jean Park[2], Nak Woo Lee[1], Kyu Wan Lee[1], Jung Yeol Na[1]

Departments of [1]Obstetrics and Gynecology, [2]Physiology, Korea University College of Medicine, Seoul, Korea

Objective: Although some sporadic reports reveal the link between the homeobox (HOX) genes and ovarian carcinoma, there is no comprehensive analysis of the expression pattern of the class I homeobox genes in ovarian carcinoma that determines the candidate genes involved in ovarian carcinogenesis.
Methods: The different patterns of expression of 36 HOX genes were analyzed, including 4 ovarian cancer cell lines and 4 normal ovarian tissues. Using a reverse transcription-polymerase chain reaction (RT-PCR) and quantification analysis, the specific gene that showed a significantly higher expression in ovarian cancer cell lines than in normal ovaries was selected, and western blot analysis was performed adding 7 ovarian cancer tissue specimens. Finally, immunohistochemical and immunocytochemical analyses were performed to compare the pattern of expression of the specific HOX gene between ovarian cancer tissue and normal ovaries.
Results: Among 36 genes, 11 genes had a different level of mRNA expression between the cancer cell lines and the normal ovarian tissues. Of the 11 genes, only HOXB4 had a significantly higher level of expression in ovarian cancer cell lines than in normal ovaries ($p=0.029$). Based on western blot, immunohistochemical, and immunocytochemical analyses, HOXB4 was expressed exclusively in the ovarian cancer cell lines or cancer tissue specimens, but not in the normal ovaries.
Conclusion: We suggest HOXB4 may be a novel candidate gene involved in ovarian carcinogenesis.

Key Words: Homeobox gene, Ovarian neoplasms, Carcinogenesis

INTRODUCTION

Despite numerous clinical studies involving multi-modality therapies, ovarian cancer is the fourth most common cause of cancer death in women.[1] Most cases present at an advanced stage and have a poor outcome, so efforts to detect ovarian cancer at an early stage would have a significant impact on the prognosis of ovarian cancers.

Ovarian carcinogenesis has been evaluated at the molecular level and some candidate genes, such as p53,[2] PTEN,[3] and BRCA,[4] have been proposed to have roles in oncogenic transformation. Recently, various embryonic genes have been shown to be involved in the postnatal regulation of differentiation and cellular growth. Mutations in some of these embryonic genes have been reported to lead to the generation of many forms of human cancer.[5] One of the classes of such oncodevelopmental genes is the homeobox gene family.

Homeobox (HOX) genes constitute a family of transcription factors which function during embryonic development to control pattern formation, differentiation, and proliferation.[6] They were initially highlighted as master genes controlling the segment identity of *Drosophila* and were evaluated to determine their involvement in embryonic development.[6] They all contain a 61 amino-acids region called the homeodomain, which binds DNA and the sequence of this region determines their classification into different subsets.[7] Until now, 39 class I homeobox genes have been identified and they are organized into 4 different clusters (A, B, C, and D) located in 4 separate chromosomes.[2,7] These clustered HOX genes are important regulators of development and are expressed in overlapping domains along the anterior-posterior axis of the vertebrate embryo in multiple tissues.[8]

In addition to their dominant role during embryogenesis, the expression of homeobox genes has also been detected in adults.[9,10] It has been shown that HOX genes may play an important role in hematopoietic differentiation.[11] Moreover, HOX genes are also expressed in endothelial cells and are involved in the acquisition of the angiogenic phenotype.[12]

Importantly, an association between the deregulation of HOX gene expression and oncogenic transformation has re-

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Correspondence to **Jung Yeol Na**
Department of Obstetrics and Gynecology, Korea University Anam Hospital, 5 Seongbuk-gu, Anam-dong, Seoul 136-705, Korea
Tel: 82-2-920-5332, Fax: 82-2-921-5357
E-mail: jhblue5@naver.com

cently been reported in human tumors. In leukemia, in which the deregulation of HOX gene expression was well described, chromosomal translocations or DNA rearrangement result in over-expression of the homeodomain containing protein, such as HOX11, pbx-1 and HOXB8.[13,14] Similar data regarding solid tumors have been generated more recently and expression surveys have revealed differences between normal and tumor samples involving renal, colorectal, and lung cancers.[9,10,15]

HOX genes are evolutionarily highly conserved regulators of embryonic differentiation and are also expressed in normal adult reproductive tissue where they are involved in regulating differentiation. Currently, in gynecological field, there have been several reports suggesting that the abnormal expression of particular HOX genes is associated with cervical, ovarian, and endometrial cancers.[16-19] As for the cervical cancers, several studies have shown a different pattern of expression of specific HOX genes between normal cervix and cervical cancer, including a report which analyzed 39 HOX gene expression profiles.[16] Unfortunately, there have been no studies performing such a comprehensive analysis of the different expression of HOX genes between normal ovary and ovarian cancer. In the current study, we created expression profile of 36 HOX genes using ovarian-derived samples and determined which genes were over-expressed in ovarian cancer cell lines or tissues as compared to normal ovarian tissue.

MATERIALS AND METHODS

1. Cell lines and tissue samples

For the current study, we used ovarian cancer cell lines, normal ovarian tissues, and ovarian cancer tissues. The cells in the first passage, designated TOV-21G, OV-90, SK-OV3 and SW 626, were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Tissue samples were obtained from the Korea Lung Tissue Bank assigned and supported by the Korea Science and Engineering Foundation in the Ministry of Science and Technology under approval by the Institutional Review Board for Research on Human Genes at the Korea University Guro Hospital and with informed consent of the patients.

2. Cell culture

The TOV-21G and OV-90 cells were maintained in MCDB 105 medium (Sigma, St. Louis, MO, USA) supplemented with 1.5 g/L sodium bicarbonate and 15% fetal bovine serum (CAMBREX Inc., Charles, IA, USA) and Medium 199 (Gibco-BRL, Life Technologies, Gaithersburg, MD, USA) with 15% fetal bovine serum (CAMBREX Inc.), respectively. The SK-OV3 cells were maintained in McCoy's 5a medium (Gibco-BRL) with 10% fetal bovine serum (CAMBREX Inc.) and the SW 626 cells were maintained in Leibovitz's L-15 Medium (Gibco-BRL) with 10% fetal bovine serum (CAMBREX Inc.). Each medium was supplemented with 500 units/mL of penicillin and 500 μg/mL of streptomycin. The cells were seeded into a T75 flask. Cultures were maintained at 37°C in a humidified atmosphere with 5% CO2. Three-to-five days after initiating incubation, the small digested residues were removed and the culture was continued. The medium was replaced one-to-two times every week.

3. Assessment of cell viability and cell number in culture

The cultured cells were detached from culture dishes with 0.05% trypsin-EDTA (Gibco-BRL) after 72 hours of culture. The cells were stained with trypan blue (Gibco-BRL), and the viable cells without staining were counted on a hemocytometer.

4. Total RNA isolation and reverse-transcriptase reaction

RNA extraction and purification were done using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA) as described in the manufacturer's protocol. The concentration of RNA was measured using a spectrophotometer (DU®530, Beckman, Fullerton, CA, USA) and the RNA quality was confirmed on agarose gels. A total RNA sample (1 μg/sample) using the Maxime RT PreMix Kit (Intron Biotechnology Inc., Seongnam, Korea) was used in a 20 μL scale to generate cDNA. The reaction was run at 45°C for 60 minutes and 95°C for 5 minutes.

5. HOX gene single PCR

A total of 36 primers for PCR amplification were obtained from Seegene (Seegene Institute for Life Science, Seoul, Korea). PCR amplification was performed using a Maxime PCR PreMix Kit (Intron Biotechnology Inc.), 1 μL of a HOX gene primer (10 pmole/μL), 18 μL of ddH_2O, and 1 μL of synthesized first-strand cDNA. After a pre-heating step at 94°C for 15 minutes, 40 amplification cycles were carried out in the thermal cycler under the following conditions: denaturation at 94°C for 30 secconds, annealing at 63°C for 90 seconds and extension at 72°C for 90 seconds. Amplification was completed with a final extension step at 72°C for 10 minutes. The amplified PCR products were separated on 2% agarose gel containing ethidium bromide.

6. Western blot

Protein lysates were obtained with a buffer containing 50 mM HEPES (pH 7.5), 150 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 10% glycerol, 1% Triton X-100, a mixture of protease inhibitors (aprotinin, PMSF, and sodium orthovanadate). Total tissues and cell lysates were prepared by homogenization and sonication, respectively. The extracted protein concentration was measured according to the method of Bradford. Equal amounts of total protein were resolved on a 12% SDS-polyacrylamide gel. Proteins were transferred to a nitrocellulose membrane (Hybond^{TM}-P, Amersham Biosciences, Piscataway, NJ, USA). After blocking (TBS, 0.1% Tween-20) at 4°C overnight, the membranes were incubated with primary antibody for anti-goat HOXB4(dilution 1 : 1000, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) for 24 hours, followed by incubation with secondary antibody linked to HRP, anti-mouse GAPDH (dilution 1 : 2,000, Bio-Rad, Hercules, CA, USA). Immunoreactive proteins were visualized by chemiluminescence using SuperSignal West Dura Extended Duration Substrate (Pierce Chemical Co., Rockford, IL, USA) and signals were detected on X-ray film.

7. Immunohistochemistry

Immunohistochemical staining was used to localize and to compare the distribution of HOXB4. Tissue sections (4 μm) were deparaffinized and then rehydrated and blocked with 3% H_2O_2 in methanol for 30 minutes followed by universal blocking (normal serum, 1.5%; Vector Laboratories, Burlingame, CA, USA). Antibodies reactive to anti-goat HOXB4 (Santa Cruz Biotechnology Inc.) was used in dilutions of 1: 100 for 1 hour. After the primary antibody was applied, the slides were incubated overnight at room temperature. Detection was made by using secondary antibody (Vector Laboratories). All samples were counterstained with Mayer's hematoxylin before mounting with Immunomount (Lab vision, Fremont, CA, USA).

8. Immunocytochemistry

TOV-21G, OV-90, SK-OV3 and SW 626 at a density of 2×10^3 cells per well were placed on the eight-well chamber slides (Nalge Nunc International, Rochester, NY, USA) and maintained in each medium at 37°C in 5% CO_2. Each cell was fixed in 4% paraformaldehyde in phosphate buffered saline. Immunostaining was carried out using standard protocols, and the anti-goat HOXB4 (Santa Cruz Biotechnology Inc.) antibody was used. After the primary antibody was applied, the slides were incubated overnight at room temperature. Detection was made by using secondary antibody (Vector Laboratories). All samples were counterstained with Mayer's

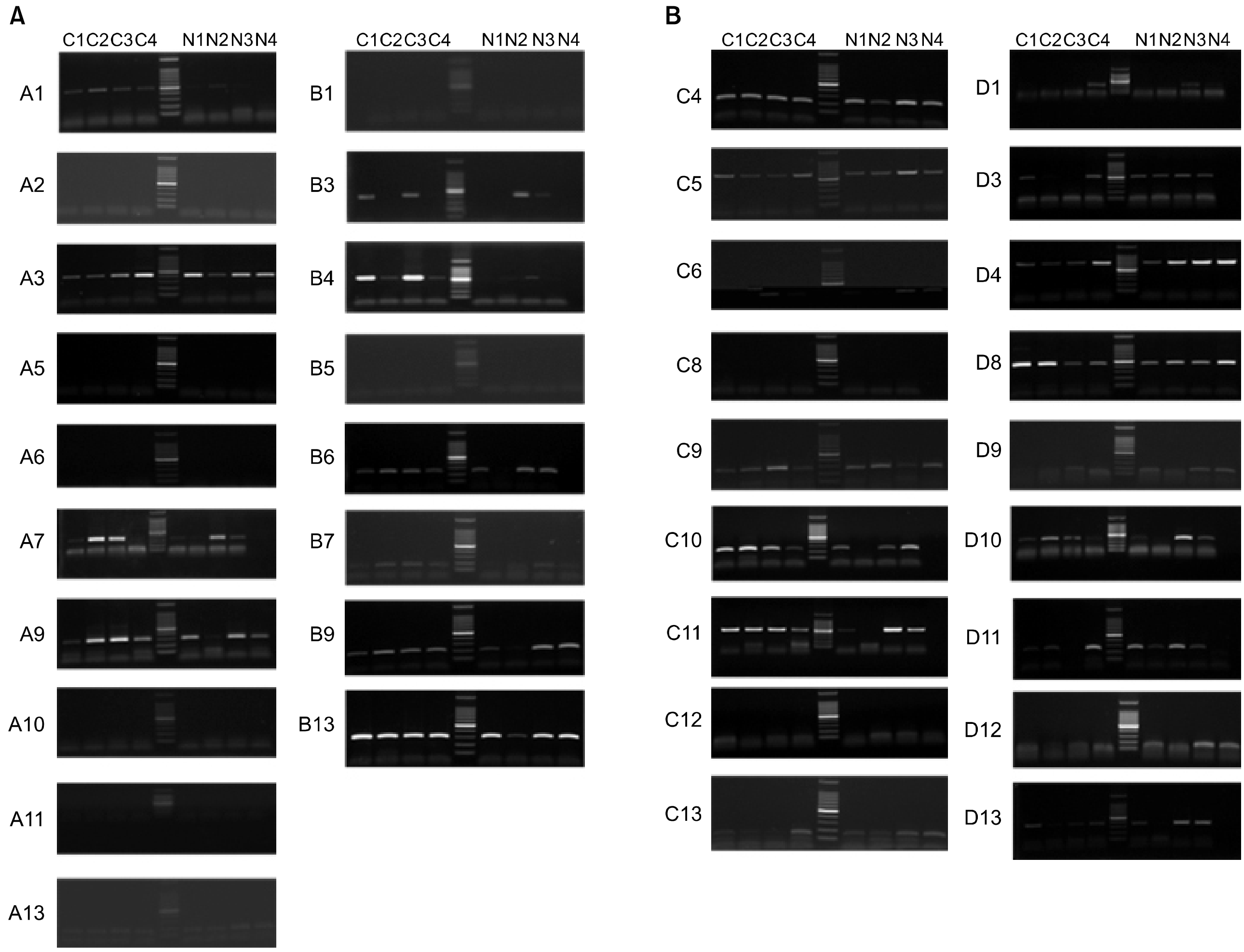

Fig. 1. RT-PCR results of 36 homeobox (HOX) genes in 4 ovarian cancer cell lines and 4 normal ovarian tissues. (A) Reverse transcription-polymerase chain reaction (RT-PCR) results of 10 HOXA and 8 HOXB genes. (B) RT-PCR results of 9 HOXC and 9 HOXD genes. C: ovarian cancer cell line, C1: SK-OV3, C2: TOV-21G, C3: SW626, C4: OV-90, N 1-4: normal ovarian tissues.

hematoxylin before mounting with Immunomount (Lab vision).

9. Statistics

The quantification analysis was performed on the band of genes that showed different expression pattern between ovarian cancer cell lines and normal ovarian tissues presented by RT-PCR. The values of each HOX gene were normalized to values obtained with GAPDH. Then, mean values of HOX to GAPDH ratio were created. These values were calculated through at least three independent experiments. They were analyzed using the Mann-Whitney U-test. SPSS ver. 12.0 (SPSS Inc., Chicago, IL, USA) was used and $p<0.05$ was considered statistically significant.

Table 1. 36 homeobox (HOX) genes expression profile in 4 ovarian cancer cell lines and 4 normal ovarian tissues (N: normal ovarian tissues)

	SK-OV3	TOV-21G	SW 626	OV-90	N1	N2	N3	N4
HOX A1	■	■	■	■	■	■	■	■
HOX A2	□	□	□	□	□	□	□	□
HOX A3	■	■	■	■	■	■	■	■
HOX A5	□	□	□	□	□	□	□	□
HOX A6	□	□	□	□	□	□	□	□
HOX A7	■	■	■	□	■	■	■	■
HOX A9	■	■	■	■	■	■	■	■
HOX A10	□	□	□	□	□	□	□	□
HOX A11	□	□	□	□	□	□	□	□
HOX A13	■	■	■	■	■	■	■	■
HOX B1	□	□	□	□	□	□	□	□
HOX B3	■	□	■	□	□	□	■	■
HOX B4	■	■	■	■	■	■	■	□
HOX B5	□	□	□	□	□	□	□	□
HOX B6	■	■	■	■	■	□	■	■
HOX B7	■	■	■	■	■	■	■	■
HOX B9	■	■	■	■	■	■	■	■
HOX B13	■	■	■	■	■	■	■	■
HOX C4	■	■	■	■	■	■	■	■
HOX C5	■	■	■	■	■	■	■	■
HOX C6	■	■	■	■	■	■	■	■
HOX C8	□	□	□	□	□	□	□	□
HOX C9	■	■	■	■	■	■	■	■
HOX C10	■	■	■	■	■	□	■	■
HOX C11	■	■	■	■	■	□	■	■
HOX C12	□	□	□	□	□	□	□	□
HOX C13	■	■	■	■	■	■	■	■
HOX D1	■	□	□	■	□	□	■	■
HOX D3	■	■	□	■	■	■	■	■
HOX D4	■	■	■	■	■	■	■	■
HOX D8	■	■	■	■	■	■	■	■
HOX D9	□	□	□	□	□	□	□	□
HOX D10	■	■	□	■	■	■	■	■
HOX D11	■	■	■	■	■	□	■	■
HOX D12	■	■	■	■	■	■	■	■
HOX D13	■	■	■	■	■	□	■	■

■ : expressed HOX gene, □ : unexpressed HOX gene.

RESULTS

1. RT-PCR analysis

We performed RT-PCR analysis on cDNA derived from 4 ovarian carcinoma cell lines and 4 normal ovarian tissues to reveal the patterns of expression of 36 human HOX genes in ovarian carcinoma. RNAs from both origins were reverse-transcribed into cDNA and PCR-amplified with specific primers for each HOX gene. The results of RT-PCR analysis on the expression of the 36 HOX genes are presented in Fig. 1 and the expression profile was schematically represented in Table 1. HOXA1, A3, A9, A13, B7, B9, B13, C4-6, C9, C13, D4, D8 and D12 genes were all expressed in both the ovarian cancer cell lines and the normal ovarian tissues. In contrast, HOXA2, A5, A6, A10, A11, B1, B5, C8, C12 and D9 were neither expressed in cancer cell lines nor expressed in normal ovarian tissues.

There were 11 genes which showed a different pattern of expression between ovarian cancer cell lines and normal ovarian tissues. HOXA7, D3, and D10 were expressed in 75% (3/4) of the cancer cell lines and 100% (4/4) of the normal ovarian tissues, whereas HOXB4, B6, C10, C11, D11 and D13 were expressed in 100% (4/4) of the cancer cell lines and 75% (3/4) of the normal ovarian tissues. Finally, HOXB3 and D1 were expressed in 50% (2/4) of both cell lines and normal tissues.

In order to determine which genes showed a significantly higher mRNA expression in ovarian cancer cell lines than in normal ovarian tissues, the quantification analysis was performed on the band of 11 genes presented by RT-PCR using Kodak EDAS 290 Imaging System (Eastman Kodak Co., New Haven, CT, USA). The values of each HOX gene were normalized to values obtained with GAPDH. Mean values of HOX/GAPDH expression were calculated for both the cancer cell lines and the normal ovarian tissues. As a result, we found that HOXB4 was the only gene that had significantly higher expression in ovarian cancer cell lines than in the normal ovarian tissues ($p=0.029$) (Fig. 2). The remainder failed to show a statistically significant difference in the pattern of expression between ovarian cancer cell lines and normal ovarian tissues.

2. Western blot analysis

Based on the RT-PCR results, we then performed western blot analysis to further confirm the specific expression of HOXB4 in ovarian cancer (Fig. 3). In western blot analysis, we included 7 ovarian cancer tissue specimens in addition to cell lines and normal tissues to strengthen our finding. The cancer tissue specimens were all serous papillary types in histology. The expression of HOXB4 was observed in the ovarian cancer cell lines and the seven ovarian cancer tissues, but none of the normal ovarian tissues. Based on this finding, we could confirm the specific expression of HOXB4 in ovarian cancer, not in the normal ovarian tissues, in protein level.

3. Immunohistochemical & immunocytochemical analysis

Next, as the final steps of our study, we performed im-

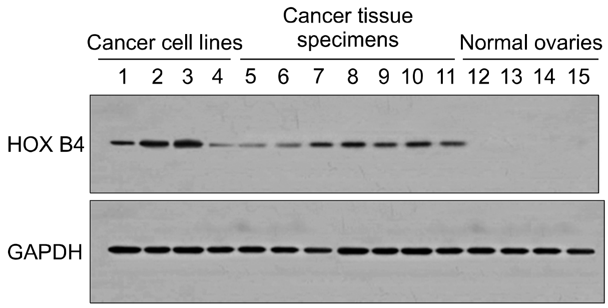

Fig. 3. Detection of homeobox B4 (HOXB4) by western blot in the 4 cancer cell lines, 7 cancer tissue specimens and 4 normal ovaries. 1: SK-OV3, 2: TOV-21G, 3: SW 626, 4: OV-90, 5-11: ovarian cancer tissues, 12-15: normal ovarian tissues.

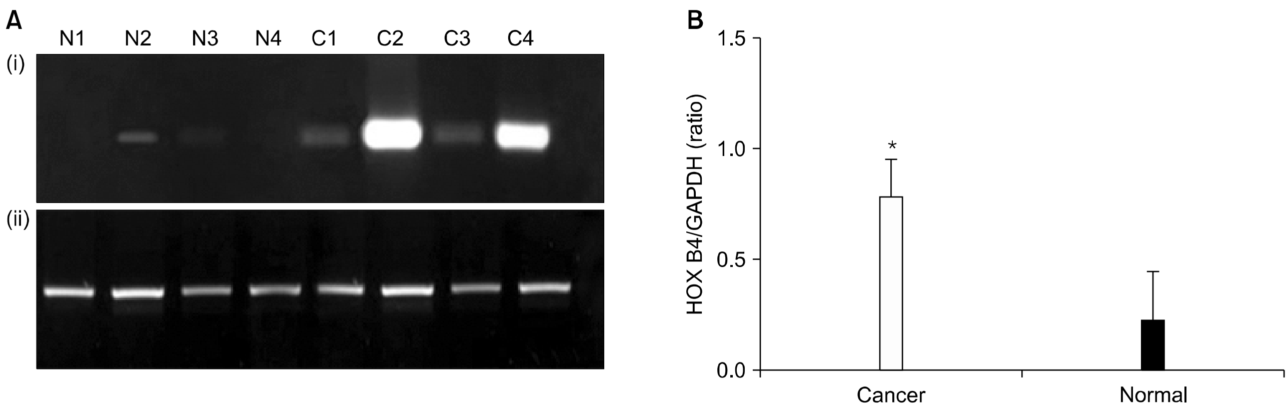

Fig. 2. A Expression pattern of homeobox B4 (HOXB4) by reverse transcription-polymerase chain reaction (RT-PCR) in normal ovaries and ovarian cancer cell lines. (A) HOX B4. (B) GAPDH B quantification analysis of HOX B4. HOX B4 expression was significantly higher in ovarian cancer cell lines than in normal ovaries (*p=0.029). N 1-4: normal ovarian tissues, C: ovarian cancer cell line, C1: OV-90, C2: SW-626, C3: TOV-21G, C4: SK-OV3.

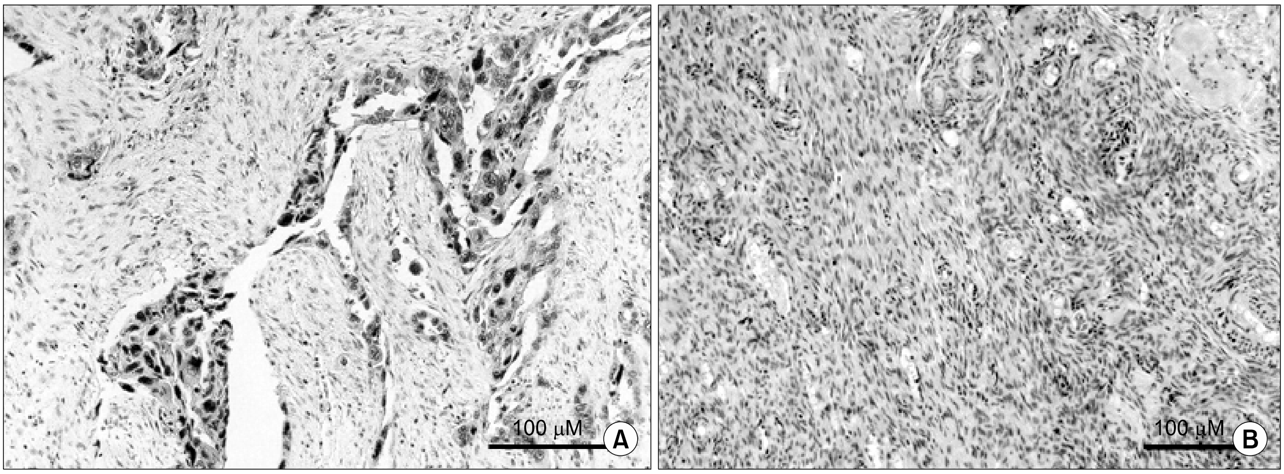

Fig. 4. Immunohistochemical staining of homeobox B4 (HOXB4) in ovarian cancer tissue (A) and normal ovarian tissue (B). (A) Mainly cytoplasmic with some perinuclear staining was seen exclusively in the epithelial cell, not in the stroma. All of the ovarian cancer tissue was serous in histology (×400). (B) No anti-HOXB4 antibody activity in the normal ovarian epithelial cell (×100).

munohistochemical and immunocytochemical analyses of ovarian cancer tissues and the four ovarian cancer cell lines to confirm the aberrant expression of HOXB4 in ovarian cancer. The immunohistochemical and immunocytochemical results were consistent with those observed by western blot analysis (Figs. 4 and 5). In immunohistochemistry, HOXB4 staining was observed exclusively in the epithelial cells, not the stroma. Moreover, the staining was primarily cytoplasmic with some perinuclear involvement in the ovarian cancer tissues, whereas no staining was observed in normal ovarian tissues. Also, in immunocytochemistry, strong cytoplasmic HOXB4 staining was observed in 4 ovarian cancer cell lines, but there was no anti-HOXB4 antibody activity in the normal ovarian epithelium. These findings indicate that HOXB4 expression may be associated with ovarian cancer development.

DISCUSSION

Recent studies have reported that aberrant HOX expression is involved in a wide variety of diseases, ranging from developmental defects[20,21] to cancers.[22-24] For example, mutation of the HOXD13 gene results in human synpolydactyly[20] and human hand-foot-genital syndrome is an autosomal dominant syndrome due to a HOXA13 mutation.[21] HOXB6, HOXB8, HOXC8 and HOXC9 are overexpressed in both premalignant polyps and colorectal cancer,[22] and HOX genes can be upregulated (HOXC11) or downregulated (HOXB5, HOXB9) in primary renal carcinomas.[23] In leukemia, the experimental overexpression of HOXA10 and HOXB8 has been reported.[24]

The association of the HOX gene with these various types of diseases may be derived from its diverse role in the cell-cycle regulation, cell proliferation, angiogenesis, and invasion. HOXB4 is involved in stem cell regulation in hematologic cells,[25] while HOXB7 is associated with angiogenesis.[26] HOXA10 has a role in invasion of breast cancer cells[27] and HOXB13 has been shown to be associated with differentiation of epidermal tissues.[28] These data show that the HOX genes have multiple roles in human developmental processes and the association of HOX genes with several types of cancers may be one of the functions of HOX genes.

Since the link between leukemia and HOX genes has been revealed, reports suggesting the association of various HOX genes with other solid tumors have followed. Among these, in the gynecological field, small numbers of studies have been reported. Alami et al.[29] reported that the vast majority of HOX genes were expressed in normal cervical keratinocytes, but only HOXA2, A7, C5, C8 and D12 were silent. They observed that this pattern was conserved in the SiHa cervical carcinoma cell line, except for the appearance of HOXC5 and C8 mRNA, and suggested that HOXC5 and/or HOXC8 could be involved in the process leading to the oncogenic transformation of cervical keratinocytes. Another study performed by Hung et al.[16] showed the results of RT-PCR analysis of 39 HOX genes in 11 cervical carcinoma cell lines. According to their study, HOXA1, B2, B4, C5, C10 and D13 genes were expressed in the majority of cervical carcinoma cell lines, but not in any of the normal cervical tissue samples. The discrepancy between the two studies with respect to the expression of HOXC8 in cervical carcinoma cell lines was attributed to the different culture conditions. In addition, Lopez et al. evaluated the different expression of HOXB between the cervical cancer tissues and the normal cervical tissue specimens by RT-PCR and *in situ* hybridization.[30] In their study, HOXB2, B4, and B13 gene expression was found only in tumor tissues. As in the case of our study, HOXB4 may be a potential candidate gene for oncogenic transformation in cervical carcinoma as well as in ovarian carcinoma.

There are few studies about the homeobox gene expression

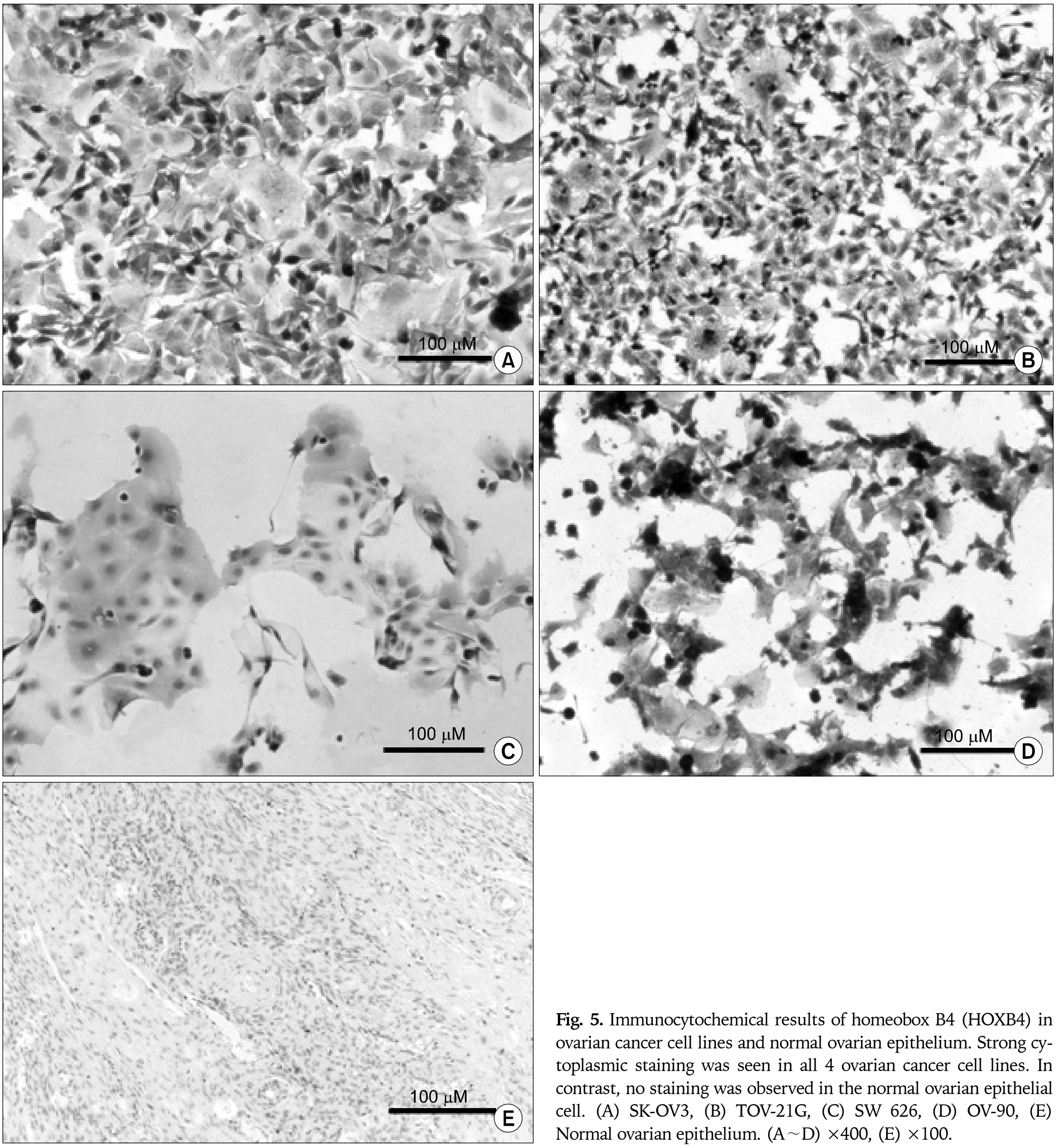

Fig. 5. Immunocytochemical results of homeobox B4 (HOXB4) in ovarian cancer cell lines and normal ovarian epithelium. Strong cytoplasmic staining was seen in all 4 ovarian cancer cell lines. In contrast, no staining was observed in the normal ovarian epithelial cell. (A) SK-OV3, (B) TOV-21G, (C) SW 626, (D) OV-90, (E) Normal ovarian epithelium. (A~D) ×400, (E) ×100.

in endometrial cancer. Lane et al.[31] compared the pattern of HOXA10 gene expression in the normal endometrium, endometrial hyperplasia, and endometrial cancer tissues. Based on Northern blot analysis and immunohistochemistry, they observed that the expression of HOXA10 was increased by 25% in high nuclear grade endometrial cancer compared with normal and hyperplastic endometrium, and low nuclear grade endometrial adenocarcinoma. According to their results, they concluded that the aberrant regulation of HOX gene expression was associated with abnormal differentiation of endometrial tissue. Another study reported by Zhao et al.[32] revealed that HOXB13 was overexpressed in endometrial cancer cells and tissues. Moreover, after transfecting antisense HOXB13/pcDNA3.1 plasmid vector into endometrial cancer cells, the invasive ability of antisense transfectants showed a 90% reduction compared with controls.

With respect to ovarian cancer, there have been sporadic reports suggesting the association of homeobox genes with ovarian carcinogenesis.[17,28] However, to our knowledge, there have been no reports providing a comprehensive analysis on the expression profiles of the majority of HOX genes in ovarian cancer, as in the current study. Specifically, HOXA7, B3, B4, B6, C10, C11, D1, D3, D10, D11, and D13 genes showed a different pattern of expression between ovarian cancer cell lines and normal ovarian tissues by RT-PCR. After performing quantification, only HOXB4 was shown to be expressed significantly higher in ovarian cancer cell lines than normal ovarian tissues. This finding was verified by western blot analysis using ovarian cancer tissue specimens. It is worthwhile to confirm the differential expression of HOXB4 gene between ovarian cancer and normal ovary in protein level, because there has been no report to verify the expression of HOX gene by western blot analysis. In the current study, HOXB4 was expressed exclusively in the 4 ovarian cancer cell lines and 7 ovarian cancer tissues, but not in any of the normal ovarian tissues. Furthermore, there was strong cytoplasmic staining in ovarian cancer cell lines and tissue specimens in the immunohistochemical/immunocytochemical analyses. Cytoplasmic staining has been observed for other HOX proteins in different tissues, although its significance is unknown.[33] Based on our results, we strongly suggest that HOXB4 has a potential role in ovarian carcinogenesis.

In addition to our study, HOXB4 has been suggested as a cancer-related gene in the various types of cancers, such as leukemia, breast cancer, osteosarcoma and lung cancer.[34-37] In leukemia, leukemic cells had dysregulated expression of oncogenes, a block in myeloid differentiation, and overexpression of HOXB4, whereas HOXB4 knockdown restored differentiation in leukemic cells.[34] Bodey et al.[35-37] confirmed the expression of HOXB3, B4, and C6 genes in breast cancer, osteosarcoma, and lung cancer by immunocytochemical analysis and suggested their association with the development of these cancers.

In contrast to our results, Naora et al.[17] reported that ovarian carcinoma was found to express HOXB7 at markedly higher levels than normal ovarian surface epithelium. However, in the current study, HOXB7 were expressed in both the cancer cell lines and the normal ovarian tissues. The basis for this discrepancy may be attributed to the different study design, which they initially searched for tumor antigens by immunoscreening a cDNA expression library with ovarian cancer patients' sera and then they found that 7 clones corresponding to the homeobox gene HOXB7. By RT-PCR and Western blot analysis using ovarian cancer cell lines, normal ovarian cell lines and ovarian cancer tissue specimens, they revealed HOXB7 overexpression in ovarian cancer. Other possible reasons are the differences in types of cell lines and culture conditions. Another study by Naora et al.[18] showed significant HOXA7 expression in ovarian cancer and benign ovarian tumors exhibiting müllerian-like features, whereas there was little or no expression on the surface of normal ovaries. This study raises the possibility that HOXA7 expression is associated with normal development of the mullerian duct-derived epithelium and that inappropriate expression of HOXA7 in the ovarian surface epithelium could give rise to aberrant epithelial differentiation. In contrast, HOXA7 were expressed in all normal ovarian tissues and in 3 of 4 ovarian cancer cell lines in the current study. In addition, some studies suggesting the possible role of non-HOX genes, such as BARX2, CDX2 and PAX8, have been reported in ovarian cancer development.[38-40]

The discrepancy of the pattern of HOX gene expression in gynecologic cancer from study to study may derive from the lack of knowledge of exact function of various HOX genes or diverse role of HOX gene in differentiation and developmental process. Furthermore, contribution of HOX genes may not be a singular factor in ovarian carcinogenesis like the phenomenon seen in embryogenesis. However, a growing number of studies about the HOX genes related to ovarian carcinogenesis would contribute to an understanding of the molecular mechanism of the pathophysiology involved and also to the development of targeted gene therapy in the future. Our study is limited by the small sample number, but this study has value in that provides preliminary data about the expression profile of HOX genes in ovarian cancer.

In conclusion, we suggest HOXB4 can be a novel candidate gene involved in ovarian carcinogenesis and further study with larger samples is needed.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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