Expression of 11 *HOX* Genes Is Deregulated in Esophageal Squamous Cell Carcinoma

Ke-Neng Chen,[1] Zhen-Dong Gu,[1] Yang Ke,[2] Ji-You Li,[3] Xiao-Tian Shi,[5] and Guang-Wei Xu[4]

Departments of [1]Thoracic Surgery, [2]Genetics, [3]Pathology, and [4]General Surgery, Peking University School of Oncology, Beijing Cancer Hospital, Beijing, China; and [5]Department of Thoracic Surgery, Anyang Tumor Hospital, Anyang City, China

ABSTRACT

Purpose: ***HOX*** **genes are vital for all aspects of mammalian growth and differentiation, and recent data have shown that their deregulated expression is related to carcinogenesis. To date, there has been no systemic study on expression of *HOX* genes in esophageal carcinoma. We investigated the expression pattern of 39 known *HOX* genes in cancerous and noncancerous tissue from 36 patients with esophageal squamous cell carcinoma to determine whether their expression is altered in esophageal cancer.**

Experimental Design: **Thirty-six patients with resectable esophageal squamous cell carcinoma (ESCC) were enrolled in this study. Specific primers were designed for each of 39 *HOX* genes, and reverse transcription-PCR was done in cancerous and noncancerous samples of these 36 patients. Furthermore, the expression of HOXA9 protein was subjected to Western blot analysis in all 36 paired tissue samples.**

Results: **Eight of 39 *HOX* genes were expressed in cancerous but not in noncancerous tissue. Five of 39 *HOX* genes were expressed both in cancerous and noncancerous tissue. Of the latter, expression of *HOXA7*, *HOXA9*, and *HOXC6* was significantly higher in cancerous tissue ($P < 0.05$). The remaining 26 *HOX* genes were not detected in either types of tissue. HOXA9 protein was expressed in both kinds of tissue (cancer tissue versus noncancerous mucosa: 0.34 ± 0.32 versus 0.24 ± 0.27, $P = 0.121$).**

Conclusions: **This is the first comprehensive survey of 39 *HOX* gene expression in ESCC and noncancerous mucosa. Five of the 39 *HOX* genes were expressed in both types of tissue indicating their possible role in maintaining normal structure and function of adult esophageal mucosa. Eleven of the 39 *HOX* genes were deregulated in cancer tissue. These genes possibly participate in the carcinogenesis of ESCC.**

INTRODUCTION

Esophageal cancer is the sixth most common cancer in the world, of which esophageal squamous cell carcinoma (ESCC) accounts for >90% of the cases (1, 2). The highest incidence of ESCC in the world occurs in north-central China (3). Although tremendous advances in diagnosis and treatment have been achieved recently, ESCC is still one of the most lethal malignancies mainly because of advanced of stage at discovery and the lack of an efficacious system both in understanding its carcinogenic mechanism and in clinical evaluation. This understanding could lead to a better clinical evaluation and treatment for individual patients. Many of the molecular pathways that underlie carcinogenesis are aberrations of the normal processes (4, 5). The fetal cellular features of tumor cells suggest that neoplasia arises through a process of defective ontogeny. *HOX* genes code transcription factors that orchestrate organogenesis patterning and maintain tissue homeostasis. Thus, if defective ontogeny is a mechanism in cancer development, it can be hypothesized that tumor cells should express the *HOX* genes, which are normally expressed by the embryonic cells of that tissue. Because of their global importance in development and differentiation and their frequent deregulation in cancer, *HOX* genes are ideal subjects for exploration the intimate relationship between carcinogenesis and embryogenesis.

HOX genes constitute a family of transcription factors key to developmental processes (6). They all contain the homeobox, a highly conserved 183-nucleotide sequence encoding a 61-amino-acid domain (the homeodomain) responsible for the recognition and binding of sequence-specific DNA motifs. In humans, the 39 *HOX* genes identified thus far are clustered into 13 paralogue groups distributed on chromosomes: 7 (*HOXA*), 17 (*HOXB*), 12 (*HOXC*), and 2 (*HOXD*). During embryonic morphogenesis, these *HOX* genes determine positional identity along anterior-posterior axes according to the colinearity rule (7). They are also expressed in some normal adult organs with characteristic patterns, suggesting a role in the maintenance of tissue-specific architecture (8). A *HOX* gene is normally required for differentiation of a particular tissue, and it can play a causal role in carcinogenesis of that same tissue if expressed at the wrong time, at the wrong level, or in the wrong context. *HOX* genes were first believed to be transcriptional activators that enhance oncogenesis through their up-regulation in carcinoma cells. The actual situation seems to be much more complicated, as both losses and gains in *HOX* gene

Received 8/30/04; revised 11/7/04; accepted 11/12/04.
Grant support: Beijing New Star of Science and Technology Plan from Beijing Municipal Science and Technology Commission grant 954813600, Institutional research fund of Peking University School of Oncology, Beijing Cancer Hospital, Research fund of Beijing Municipal Science and Technology Commission grant H020920030390.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Note: Dr. K. Chen is currently a postdoctoral fellow at the Department of Plastic Surgery of University of Texas M.D. Anderson (Houston, Texas).
Requests for reprints: Ke-Neng Chen, Department of Thoracic Surgery, Peking University School of Oncology, Beijing Cancer Hospital, Beijing, China, 100036. Phone: 86-10-88121122; Fax: 86-10-88122437; E-mail: chenkeneng@yahoo.com.cn.

expression are related to carcinogenesis (4). Therefore, the current state of our knowledge is insufficient to establish a precise, causal relationship between individual *HOX* genes and the specific cancer phenotypes to which they contribute, or to understand how *HOX* genes contribute to the tissue-specific features of cancer. Comprehensive analysis of the expression patterns of *HOX* genes in different cancers is needed to fill these gaps in knowledge. Researchers have shown that the deregulation of *HOX* genes is associated both with leukemia and solid malignancies (9–21). To date, no persuasive reports have yet been published on *HOX* gene expression patterns in ESCC (22). However, knowing the expression pattern of this gene family in ESCC may contribute to our understanding of what role *HOX* genes play in this specific cancer phenotype.

This report is the first systemic study on 39 *HOX* gene expression portraits both in tissues of ESCC and matched noncancerous mucosa. We found unique *HOX* gene expression pattern in both kinds of tissue. These observations indicate that 11 *HOX* genes possibly participate in the carcinogenesis of ESCC.

MATERIALS AND METHODS

Samples Collection and Preparation. Tissue samples were removed from 36 patients with ESCC who underwent surgical resection at the Thoracic Surgery Department of Peking University School of Oncology from February 2003 to June 2003. All of the patients underwent radical esophagectomy without any preoperative chemotherapy or radiotherapy. They include 29 men and seven women ages 40 to 69 years (mean 60 years). The pathologic diagnosis of ESCC was confirmed by two independent senior pathologists from the Pathologic Department of Peking University School of Oncology. These tumors were classified into stage IIa (n = 10), IIb (n = 9), and III (n = 17) according to the criteria of the Unio Internationale Contra Cancrum tumor-node-metastasis system of malignant tumors (1987). During operation, each of 500 mg tissue samples was taken both from non-necrotic ESCC tissue and noncancerous mucosa and immediately snap frozen in liquid nitrogen and stored at −80°C until RNA or protein was extracted. Noncancerous mucosa referred the tissue taken from at least 5 cm away from cancerous margin. This study got the approval

Table 1 Specific primers for *HOX* genes

Gene	Primers		Annealing temperature (°C)	Size (bp)	Intron
	Sense	Antisense			
HOXA1	ATGAACTCCTTCCTGGAATA	CGTACTCTCCAACTTTCC	48	655	+
HOXA2	ACAGCGAAGGGAAATGTAAAAGC	GGGCCCCAGAGACGCTAA	54	102	–
HOXA3	TGCAAAAAGCGACCTACTACGA	CGTCGGCGCCCAAAG	51	126	–
HOXA4	TGCATGCGAGCCACGTCCT	TTGACCTGGCGCTCAGACAA	52	391	+
HOXA5	TGCGCAAGCTGCACATAAGTCATG	TTGAAGTGGAACTCCTTCTCCAGC	56	112	+
HOXA6	GATGCAGCGCATGAACTCCTGCG	TGGGCTGCGTGGAATTGATGAGC	58	250	+
HOXA7	CAAAATGCCGAGCCGACTT	TAGCCGGACGCAAAGGG	58	146	–
HOXA9	CAGCCAACTGGCTTCATGCG	CACTCGTCTTTTGCTCGGTC	53	229	–
HOXA10	AGAGCAGCAAAGCCTCGCCGGAGAAG	GGACGCTGCGGCTAATCTCTAGGCG	62	198	+
HOXA11	ACCCGCAAAAAGCGCTGC	GAGCATGCAGGACAGTTG	50	114	–
HOXA13	GGGAGAAAGAAGCGCGTG	CGTCGTGGCTGATATCCG	53	114	–
HOXB1	CCTTCTTAGAGTACCCACTCTG	GCATCTCCAGCTGCCTCCTT	52	826	+
HOXB2	TCCTCCTTTCGAGCAAACCTTCC	AGTGGAATTCCTTCTCCAGTTCC	52	353	+
HOXB3	AGTACAAGAAGGACCAGAAGGC	TGGAGTGTAAGGCGTTCATG	50	120	–
HOXB4	GTGCAAAGAGCCCGTCGTCTACC	CGTGTCAGGTAGCGGTTGTAGTG	56	161	+
HOXB5	GTTCCACTTCAACCGCTACC	TGTCCTTCTTCCACTTCATGC	52	122	–
HOXB6	GGCGAGGCCGCCAGACATAC	ACTCGGCCTGTTTTTCTTCC	52	236	–
HOXB7	AGAGTAACTTCCGGATCTA	TCTGCTTCAGCCCTGTCTT	48	274	+
HOXB8	AGCCTCCTTGTGCAATTG	GTAACAATTGCCCACAGC	48	300	–
HOXB9	GAGCAGGGCAAAGAGTAA	CTTTCTCCTGACACCTAG	48	250	–
HOXB13	CTGGAACAGCCAGATGTGTT	TTGGCGAGAACCTTCTTCTC	50	300	+
HOXC4	GAAGGAGAAGGATCGAGATCG	GACCTGACTTTGGTGTTGGG	53	124	–
HOXC5	TGGATGACCAAACTGCACATGAGC	CAAGTTGTTGGCGATCTCTATGCG	54	149	+
HOXC6	CACCTTAGGACATAACACACAGACC	CACTTCATCCGGCGGTTCTGGAACC	58	317	+
HOXC8	GCAACTCCCTTGAAGTTTCGT	GGGGGAAGTCCAAAGGTAATA	51	172	–
HOXC9	ACGAGGAAGAAGCGCTGCCCC	GAGAACCCGGGCCACCTCATA	60	133	–
HOXC10	CTACCGCCTGGAACAACCTGTTGG	ATGGTCTTGCTAATCTCCAGGCG	54	662	+
HOXC11	GTGTATATCAACAAAGAGAAGCGG	GATAGAGGGTCCAGGAGGTTCCGG	54	322	–
HOXC12	TAATCTCCTGAATCCCGGGTTT	TGGGTAGGACAGCGAAGGC	52	124	–
HOXC13	TCCCTAGCTCGCTGCCTCT	GGTTCACGTTGTGCGACAGG	53	478	–
HOXD1	CGACCCCCATCCCTATCTAGAC	TGGAACTCGGAAGCCAACTAAA	52	115	–
HOXD3	CATCAGCAAGCAGATCTTCC	AGCGGTTGAAGTGGAATTCC	50	187	+
HOXD4	TGGATGAAGAAGGTGCACG	ACTTCATCCTCCGGTTCTGG	50	220	+
HOXD8	GGATACGATAACTTACAGAGAC	TAGAGTTTGGAAGCGACTGT	48	219	+
HOXD9	GATGAGCAAGGAGAAATGCC	AGTCGCTGGAGAGTTTCTGG	52	140	–
HOXD10	TACTCCAACCAGCAATTGGC	CTCGGATTCGATTCTCTCGG	52	233	–
HOXD11	CTTCGACCAGTTCTACGAG	CAGACGGTCTCTGTTCAGT	50	457	+
HOXD12	AGCAGGCTAAGTTCTATGCG	CAATCTGCTGCTTCGTGTAG	50	380	+
HOXD13	AGAATGGAGAACGAGTATGCC	CGGTTCTGAAACCAAATGG	48	114	–

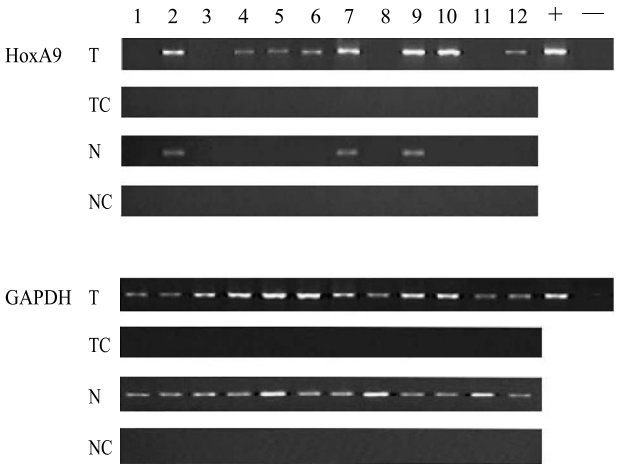

Fig. 1 Expression of the *HOXA9* gene in ESCC tissue and noncancerous mucosa, as detected by RT-PCR, in the first 12 of 36 patients. T, tumor tissue; TC, genomic DNA control for tumor tissue; N, noncancerous mucosa; NC, genomic DNA control for noncancerous mucosa; +, positive control; −, negative control. That both of the genomic DNA controls for tumor tissue and noncancerous mucosa are negative indicates there was no genomic DNA contamination in extracted RNA. GAPDH acts as a loading control.

from the ethics committee of Peking University School of Oncology, and informed consent was obtained from all individuals.

RNA Extraction. Total RNA was extracted from homogenized tissue samples with TRIzol (Invitrogen, Carlsbad, CA) according to the manufacture's instructions. To exclude the possibility of contamination with genomic DNA, we incubated total RNA with RNase-free DNase (DNA-free kit, Ambion, Austin, TX) at 37°C for 1 hour. Then RNA was quantified by absorbance reading at 260 nm.

Reverse Transcription-PCR. To determine whether *HOX* genes are expressed in ESCC tissue and noncancerous mucosa, we did reverse transcription-PCR (RT-PCR). RT was done in 20-μL reaction mixture containing 2 μg of total RNA, 500 ng of oligo(dT), and 50 units of the Superscript II reverse transcriptase (Superscript First-Strand Synthesis System, 11904-018, Invitrogen) for 1 hour at 42°C; 1 μL of RT reaction was used for PCR amplification. Each PCR reaction began with a 5 minutes hot start at 95°C followed by a denaturing step at 94°C for 45 seconds, an annealing step at the appropriate temperature for 45 seconds, and an extension step at 72°C for 1 minute for 35 cycles. A final extension run was conducted at 72°C for 7 minutes. We designed specific primers for each *HOX* gene (Table 1). For each PCR reaction, two negative controls were done, one is omission of the reverse transcriptase, which we called genomic DNA control, and the other is omission of the target cDNA which we called negative control. Glyceraldehyde-3-phospate dehydrogenase was used as a loading control, which was run for each sample in a second reaction using the same conditions with *HOX* genes except that the number of cycles was 24. PCR products were separated on a 2% agarose gel, stained with ethidium bromide, and visualized by the AlphaEase FC Imaging System (Alpha Innotech, San Leandro, CA). The expression level of RT-PCR products was calculated by the intensity of each band of the individual *HOX* gene compared with that of glyceraldehyde-3-phospate dehydrogenase, which was electrophoresed in parallel. At least two independent RT-PCR experiments were done for each *HOX* gene. A positive control using genomic DNA as the target gene assured the accuracy of the primers when it was negative for all samples.

Sequence Analysis. For each *HOX* gene, the representative PCR product was ligated to the plasmid (pGEM-T Easy Vector System I, Promega Co., Madison, WI) and transformed to competent cells (JM109, Promega). The successfully transformed cells were selected by PCR amplification, and then nucleotide sequence analysis was done to ensure that each PCR product was a specific fragment of the target gene.

Western Blot Analysis. RT-PCR is a sensitive detector of mRNA expression but cannot detect expression of protein, which is the real effector in many biological processes. We did Western blot to detect the expression of HOXA9 protein. One hundred milligrams of each frozen tissue sample were minced with a homogenizer and then bathed in ice for 1 hour with 1 mL of protein extraction buffer solution containing protease inhibitor (one tablet in 10 mL of extraction solution, Complete Mini, 1 836 153, Roche, Basel, Switzerland). We separated 100 μg of total protein on SDS-polyacrylamide gel consisting of 6% stacking gel and 12% separating gel at 100 V for 90 minutes. The proteins were then transferred onto a polyvinylidene difluoride membrane (Hybond Enhanced Chemiluminescence, Amersham Biosciences, Buckinghamshire, United Kingdom) at

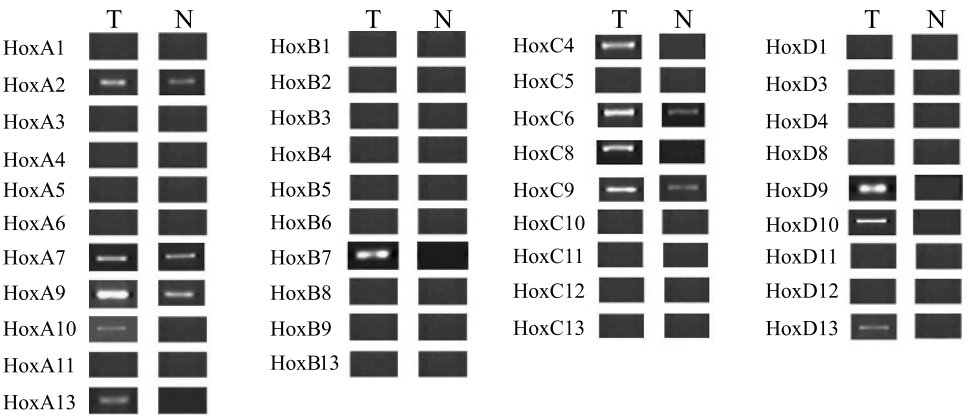

Fig. 2 Expression of 39 *HOX* genes in ESCC tissue and noncancerous mucosa, as detected by RT-PCR. T, tumor tissue; N, noncancerous mucosa. Representative result for each *HOX* gene to show the expression profile of 39 *HOX* genes.

Table 2 *HOX* genes expressed only in ESCC

Gene	Positive cases in T (%)	Expression level (mean ± SD)*	Positive cases in N
HOXA10	3 (8.3)	0.67 ± 0.14	0
HOXA13	27 (75.0)	1.08 ± 0.41	0
HOXB7	21 (58.3)	0.96 ± 0.21	0
HOXC4	12 (33.3)	0.52 ± 0.13	0
HOXC8	15 (41.7)	0.55 ± 0.12	0
HOXD9	6 (16.7)	0.40 ± 0.03	0
HOXD10	15 (41.7)	0.62 ± 0.25	0
HOXD13	15 (41.7)	0.52 ± 0.09	0

NOTE. Total case number is 36.
Abbreviations: T, tumor tissue; N, noncancerous mucosa.
*When the mean value of expression level was calculated, the samples that did not express *HOX* genes were not included.

100 V for 90 minutes at 4°C, using a Tris-glycine buffer with 20% methanol. The membrane was stained with Ponceau S to locate molecular weight standards. After blockage of the membrane with 5% nonfat dried milk in TBS [10 mmol/L Tris (pH 7.4) and 50 mmol/L NaCl] plus 0.1% Tween 20 for 1 hour at room temperature, we identified antigens by using goat polyclonal antibody against HOXA9 (sc-17155, Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:100 in blocking solution for 2 hours at room temperature. Afterwards, the membrane was incubated with peroxidase-conjugated rabbit anti-goat immunoglobulin G (305-035-003, Jackson Immuno-Research Laboratories, West Grove, PA) diluted 1:1,000 in blocking solution for 30 minutes at room temperature and the bands were visualized with the enhanced chemiluminescence system (Amersham Biosciences). We used actin as the loading control and quantified the results by scanning densitometry using the ratio of the HOXA9 signal to the actin signal for comparison within experimental groups.

Statistical Analysis. The software program SPSS 10.0 was used to perform the Fisher's exact test and *t* test to analyze the difference in *HOX* gene expression in ESCC and noncancerous mucosa. The data to show the expression level were presented as mean ± SDs. $P < 0.05$ was considered significant.

RESULTS

Thirty-Nine *HOX* Genes Expression in Esophageal Squamous Cell Carcinoma Tissue and Noncancerous Mucosa. The *HOX* gene expression in ESCC tissue, noncancerous mucosa, and genomic DNA control for carcinoma tissue and noncancerous mucosa from each patient were determined (Fig. 1 shows the representative results). The whole pattern of *HOX* gene expression detected by RT-PCR is that 8 of 39 *HOX* genes (*HOXA10*, *HOXA13*, *HOXB7*, *HOXC4*, *HOXC4*, *HOXC8*, *HOXD9*, *HOXD10* and *HOXD13*) were expressed in some of the ESCC tissue samples but not in any of the noncancerous mucosa samples; another five genes (*HOXA2*, *HOXA7*, *HOXA9*, *HOXC6*, and *HOXC9*) were expressed in some of the ESCC tissue samples and noncancerous mucosa samples (Fig. 2; Tables 2-3), among these five genes, *HOXA7*, *HOXA9*, and *HOXC6* were significantly higher in ESCC tissue than in noncancerous mucosa ($P < 0.05$). Thus, 11 *HOX* genes were deregulated in ESCC. However, the other two genes, *HOXA2* and *HOXC9*, tended to have lower expression in ESCC than in noncancerous mucosa but did not show significant differences (Fig. 2; Table 3). The remaining 26 *HOX* genes were silent in both cancerous and noncancerous tissues (Fig. 2).

Expression of HOXA9 Protein in Esophageal Squamous Cell Carcinoma and Noncancerous Mucosa. We subjected ESCC tissue and noncancerous mucosa to Western blot to assess the level of HOXA9 protein expression. HOXA9 was expressed in both ESCC tissue and noncancerous mucosa (Fig. 3). Expression tended to be higher in ESCC tissue, although statistically the difference was not significant (ESCC versus noncancerous mucosa: 0.34 ± 0.32 versus 0.24 ± 0.27, $P = 0.121$). The results of Western blot were consistent with that of RT-PCR (Table 4).

DISCUSSION

This is the first systemic report on the expression of 39 *HOX* genes in both noncancerous esophageal mucosa and in ESCC. In noncancerous mucosa, we found that 5 of 39 *HOX* genes (*HOXA2*, *HOXA7*, *HOXA9*, *HOXC6*, and *HOXC9*) were expressed, and others were not detectable in our study. Three of these five expressed *HOX* genes (*HOXA7*, *HOXA9*, and *HOXC6*)

Table 3 *HOX* genes expressed in noncancerous mucosa and ESCC

Gene	Positive cases in T (%)	Expression level (mean ± SD)*	Positive cases in N (%)	Expression level (mean ± SD)*	$P^{\dagger}$
HOXA7	30 (83.3)	1.08 ± 0.38	12 (33.3)	0.84 ± 0.14	0.000
HOXA9	24 (66.7)	0.70 ± 0.22	9 (25.0)	0.66 ± 0.35	0.001
HOXC6	24 (66.7)	0.86 ± 0.31	9 (25.0)	0.62 ± 0.28	0.001
HOXA2	31 (86.1)	0.93 ± 0.29	36 (100.0)	1.12 ± 0.43	0.054
HOXC9	33 (91.7)	0.97 ± 0.25	36 (100.0)	0.48 ± 0.11	0.239

NOTE. Total case number is 36.
Abbreviations: T, tumor tissue; N, noncancerous mucosa.
*When the mean value of expression level was calculated, the samples that did not express *HOX* genes were not included.
†*P* from Fisher exact test.

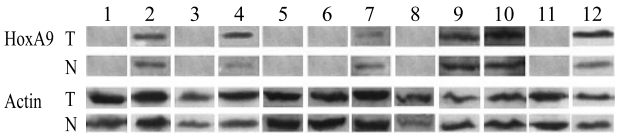

Fig. 3 Expression of HOXA9 protein in ESCC tissue and noncancerous mucosa, as detected by Western blot, in the first 12 of 36 patients. T, tumor tissue; N, noncancerous mucosa. Actin was used as a loading control.

were also expressed in ESCC, and the expression level is significantly lower in noncancerous mucosa than in cancer tissue. It is widely accepted that *HOX* genes play a crucial role as molecular address labels in early embryogenesis by conferring cell fate and establishing regional identity in tissues. However, *HOX* gene expression is not restricted to early development but also observed in differentiated cells of adult tissues. To better understand the functionality of *HOX* gene expression in adult tissues in physiologic and pathologic phenomena, and the potential role in pathogenesis, it is important to determine the expression profiles of *HOX* genes in specific tissues. However, there is no systemic study on 39 *HOX* gene expression in normal adult esophageal mucosa, which would be essential to understand the possible roles of *HOX* gene expression in carcinogenesis of ESCC. When concerns are concentrated on the difference of cancerous tissues and normal tissues, researchers always deem noncancerous mucosa as normal tissue. Thus, we infer that the five genes (*HOXA2*, *HOXA7*, *HOXA9*, *HOXC6*, and *HOXC9*), which we found to be expressed in noncancerous mucosa, play a role in the maintenance of normal adult esophagus-specific architecture as well as function. Indeed, there are reports of such systemic research in other tissues. Yamamoto et al. (23) systematically examined *HOX* gene expression in 26 different adult tissues. The results showed tissue-specific differential expression. They also revealed that posterior tissues generally express more *HOX* genes than anterior tissues and that the genes located centrally in the *HOX* gene complexes are expressed in more tissues than the genes located at the 5′ or 3′ end of the complexes. Other normal adult organs, such as the other part of the digestive tract, express different *HOX* gene patterns with more genes expressed (14, 23, 24). Takahashi et al. (21) and Yahagi et al. (24) also reported this observation. The unique expression patterns of *HOX* genes in individual organs indicate the genes' role in establishing the regional identity of organs and in maintaining organ specificity. In our study, of course, the noncancerous mucosa might not exactly represent the real normal tissue. We, therefore, will continue to evaluate these findings in different subgroups of population, including normal healthy adults.

In the present study, we showed that 13 of 39 *HOX* genes (*HOXA2*, *HOXA7*, *HOXA9*, *HOXA10*, *HOXA13*, *HOXB7*, *HOXC4*, *HOXC6*, *HOXC8*, *HOXC9*, *HOXD9*, *HOXD10*, and *HOXD13*) were expressed in ESCC tissue. Of these 13 expressed genes, eight *HOX* genes (*HOXA10*, *HOXA13*, *HOXB7*, *HOXC4*, *HOXC8*, *HOXD9*, *HOXD10*, and *HOXD13*) were only expressed in ESCC tissue but not in noncancerous mucosa. The other five *HOX* genes (*HOXA2*, *HOXA7*, *HOXA9*, *HOXC6*, and *HOXC9*) can be detected in both kinds of tissue. Among these five genes, the expression of *HOXA7*, *HOXA9*, and *HOXC6* is significantly higher in ESCC ($P < 0.05$). The remaining 26 of 39 *HOX* genes were not detectable in all materials examined. We, therefore, believe that the expression of 11 *HOX* genes is deregulated in ESCC. The concept that *HOX* genes might be involved in cancer is recent. A putative role of *HOX* genes in malignant processes has been well documented in leukemia (9–11). Several *HOX* genes are also implicated in solid tumors. Preliminary studies indicated that HOX proteins were augmented in various human malignant tumors, such as breast, colonic, rectal, gastric, lung, renal, and testicular carcinomas. Moreover, alterations in expression of *HOX* genes have been reported in renal, colorectal, lung, and cervical carcinomas (4, 5, 12–20). It seems that whereas some *HOX* genes have the same expression in normal and malignant tissues, others exhibit altered expression in cancer lesions, suggesting an association with cancer progression. These 11 deregulated *HOX* genes described in our study have been reported to be associated with other neoplasms. As examples, *HOXA7* and *HOXA9* expression is associated with leukemia (10); *HOXA7*, *HOXA9*, and *HOXA10* are highly expressed in lung cancer (15); *HOXB7* overexpression is correlated with enhanced tumor-associated angiogenic response (16); *HOXC4* and *HOXC8* are overexpressed in malignant prostate cell lines and lymph node metastasis (17); *HOXC8* is highly expressed in cervical carcinoma cell lines but not in normal cervical tissues (18); *HOXD9* is expressed in normal thyroid gland but not in thyroid cancer cell lines (21); and *HOXD10* and *HOXD13* are highly expressed in breast cancer (19). In our study, the finding that 11 *HOX* genes were deregulated in ESCC tissue is a specific expression pattern of *HOX* genes in ESCC because it differs from that of noncancerous mucosa that we observed and from those of other carcinoma tissues observed by other researchers (12–21). Our data, therefore suggests that these 11 *HOX* genes may be involved in the process of the carcinogenesis of ESCC.

In addition to the mRNA quantification of specific *HOX* gene expression, we also determined the protein level of HOXA9 by Western blot analysis. HOXA9 was chosen because our RT-PCR data showed a remarkable difference of HOXA9 mRNA level in the cancerous and noncancerous control. Although there is a lack of specific antibodies for all 39 individual *HOX* gene products, the antibody to HOXA9 is currently available. Our Western blot results show that the HOXA9 protein is detected in

Table 4 Correlation of HOXA9 expression detected by RT-PCR and Western blot

Item	RT-PCR (+)	Western blot (+)	RT-PCR (+) and Western blot (+)	RT-PCR (+) and Western blot (−)	RT-PCR (−) and Western blot (+)	RT-PCR (−) and Western blot (−)
T	24	13	13	11	0	12
N	9	12	9	0	3	24

NOTE. (+), positive cases; (−), negative cases. Total case number is 36.
Abbreviations: T, tumor tissue; N, noncancerous mucosa.

13 of 36 cancerous samples and 12 in noncancerous tissues. Although the overall HOXA9 protein levels (normalized by the actin level) in cancerous tissue and noncancerous control were not statistically different, there is a trend that the HOXA9 protein level (0.34 ± 0.32) is higher than that of noncancerous control (0.24 ± 0.27, $P = 0.121$). With improved availability of specific antibodies against individual *HOX* gene's products and advancement of proteomics technique, the expression level of individual HOX proteins and their roles in carcinogenesis of ESCC will be determined.

In summary, this is the first comprehensive survey of 39 *HOX* gene expression in ESCC tissue and noncancerous mucosa. Eleven of 39 *HOX* genes were deregulated in ESCC, suggesting the possibility that these genes are involved in the carcinogenesis of ESCC. Five of 39 *HOX* genes were expressed both in ESCC and noncancerous mucosa indicating their possible role in maintaining normal structure and function of adult esophageal mucosa. Further studies are needed to elucidate the role of *HOX* genes in esophageal carcinogenesis. However, our present study did not include high-risk-but-normal people, patients with different stages of ESCC nor subjects of with precancerous lesion or carcinoma *in situ* that are only seen in certain high-risk areas through large-scale screening for ESCC. Anyang City (Linxian or Linzhou locates within this area) is one of the highest incidence areas of ESCC in the world. With unique similar geographic, humanistic, lifestyle, and environmental factors and genetic background combining the high incidence of ESCC, the people of this area provide as an ideal natural research model of carcinogenesis of ESCC. We have successfully built a large-scale screening system in this area and have done large-scale screening for ESCC (25, 26). In future studies, we will test the unique expression profile of *HOX* genes confirmed in the present study in tissues from the individuals who accept the screening examination, including high-risk-but-normal population, atypical hyperplasia, carcinoma *in situ*, and patients with different Unio Internationale Contra Cancrum tumor-node-metastasis stages to ascertain the role of *HOX* genes in the carcinogenesis and the development of ESCC, which we believe will provide considerable evidence to our understanding of the relationship of deregulated embryogenesis and carcinogenesis and the functions of *HOX* genes in general and specific phenotypes of carcinogenesis.

ACKNOWLEDGMENTS

We thank the editor and reviewers of Clinical Cancer Research for detailed reviews of the article and Drs. Dah H. Ho, Xuanzheng Shi, Cynthia A. Frey, Andrea Gobin, and Sangeata Cheema for linguistic advice and assistance of the revised article.

REFERENCES

1. Pisani P, Parkin DM, Ferlay J. Estimates of the worldwide mortality from eighteen major cancers in 1985. Implications for prevention and projections of future burden. Int J Cancer 1993;55:891–903.

2. Macfarlane GJ, Boyle P. The epidemiology of oesophageal cancer in the UK and other European countries. J R Soc Med 1994;87:334–7.

3. Yang CS. Research on esophageal cancer in China: a review. Cancer Res 1980;40:2633–44.

4. Abate-Shen C. Deregulated homeobox gene expression in cancer: cause or consequence? Nat Rev Cancer 2002;2:777–85.

5. Cillo C, Cantile M, Faiella A, Boncinelli E. Homeobox genes in normal and malignant cells. J Cell Physiol 2001;188:161–9.

6. Gehring WJ, Hiromi Y. Homeotic genes and the homeobox. Annu Rev Genet 1986;20:147–73.

7. Krumlauf R. HOX genes in vertebrate development. Cell 1994;78:191–201.

8. Nakamura T, Largaespada DA, Lee MP, et al. Fusion of the nucleoporin gene NUP98 to HOXA9 by the chromosome translocation t(7;11)(p15;p15) in human myeloid leukaemia. Nat Genet 1996;12:154–8.

9. van Oostveen J, Bijl J, Raaphorst F, Walboomers J, Meijer C. The role of homeobox genes in normal hematopoiesis and hematological malignancies. Leukemia 1999;13:1675–90.

10. Ayton PM, Cleary ML. Transformation of myeloid progenitors by MLL oncoproteins is dependent on Hoxa7 and Hoxa9. Genes Dev 2003;17:2298–307.

11. Quentmeier H, Dirks WG, Macleod RA, Reinhardt J, Zaborski M, Drexler HG. Expression of HOX genes in acute leukemia cell lines with and without MLL translocations. Leuk Lymphoma 2004;45:567–74.

12. Cillo C, Barba P, Freschi G, Bucciarelli G, Magli MC, Boncinelli E. HOX gene expression in normal and neoplastic human kidney. Int J Cancer 1992;51:892–7.

13. De Vita G, Barba P, Odartchenko N, et al. Expression of homeobox-containing genes in primary and metastatic colorectal cancer. Eur J Cancer 1993;29A:887–93.

14. Vider BZ, Zimber A, Hirsch D, et al. Human colorectal carcinogenesis is associated with deregulation of homeobox gene expression. Biochem Biophys Res Commun 1997;232:742–8.

15. Calvo R, West J, Franklin W, et al. Altered HOX and WNT7A expression in human lung cancer. Proc Natl Acad Sci U S A 2000;97:12776–81.

16. Care A, Felicetti F, Meccia E, et al. HOXB7: a key factor for tumor-associated angiogenic switch. Cancer Res 2001;61:6532–9.

17. Miller GJ, Miller HL, van Bokhoven A, et al. Aberrant HOXC expression accompanies the malignant phenotype in human prostate. Cancer Res 2003;63:5879–88.

18. Alami Y, Castronovo V, Belotti D, Flagiello D, Clausse N. HOXC5 and HOXC8 expression are selectively turned on in human cervical cancer cells compared to normal keratinocytes. Biochem Biophys Res Commun 1999;257:738–45.

19. Cantile M, Pettinato G, Procino A, Feliciello I, Cindolo L, Cillo C. *In vivo* expression of the whole HOX gene network in human breast cancer. Eur J Cancer 2003;39:257–64.

20. Svingen T, Tonissen KF. Altered HOX gene expression in human skin and breast cancer cells. Cancer Biol Ther 2003;2:518–23.

21. Takahashi Y, Hamada J, Murakawa K, et al. Expression profiles of 39 HOX genes in normal human adult organs and anaplastic thyroid cancer cell lines by quantitative real-time RT-PCR system. Exp Cell Res 2004;293:144–53.

22. Maroulakou IG, Spyropoulos DD. The study of HOX gene function in hematopoietic, breast and lung carcinogenesis. Anticancer Res 2003;23:2101–10.

23. Yamamoto M, Takai D, Yamamoto F, Yamamoto F. Comprehensive expression profiling of highly homologous 39 HOX genes in 26 different human adult tissues by the modified systemicmultiplex RT-pCR method reveals tissue-specific expression pattern that suggests an important role of chromosomal structure in the regulation of HOX gene expression in adult tissues. Gene Expr 2003;11:199–210.

24. Yahagi N, Kosaki R, Ito T, et al. Position-specific expression of HOX genes along the gastrointestinal tract. Congenit Anom (Kyoto) 2004;44:18–26.

25. Li T, Lu ZM, Guo M, et al. p53 codon 72 polymorphism (C/G) and the risk of human papillomavirus-associated carcinomas in China. Cancer 2002;95:2571–6.

26. Li T, Lu ZM, Chen KN, et al. Human papillomavirus type 16 is an important infectious factor in the high incidence of esophageal cancer in Anyang area of China. Carcinogenesis 2001;22:929–34.

Clinical Cancer Research

Expression of 11 *HOX* Genes Is Deregulated in Esophageal Squamous Cell Carcinoma

Ke-Neng Chen, Zhen-Dong Gu, Yang Ke, et al.

Clin Cancer Res 2005;11:1044-1049.