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,2 Xiao-Tian Shi,3 and Guang-Wei Xu4
Departments of 1Thoracic Surgery, 2Genetics, 3Pathology, and 4General Surgery, Peking University School of Oncology, Beijing Cancer Hospital, Beijing, China; and 4Department 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 systematic 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 tissue from 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 HOX47, 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 paralogous clusters distributed on chromosomes: 7 (HOX1), 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
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 of CRB/C0.

#### Tissue Samples and Preparation.

Primary and antiprimer sequences were designed on the basis of the published sequences of human genes. The primers were synthesized on a DNA synthesizer (Model 3800A; Applied Biosystems, Foster City, CA) and purified with a standard protocol. Each primer (2.0 μmol/L) was stored at −20°C until use. RNA was extracted from the tissue samples with Trizol reagent (GIBCO, Grand Island, NY) according to the manufacturer’s instructions. The quality and quantity of RNA were determined by measuring the A260/A280 ratio with a spectrophotometer (GeneQuant; Amersham, Buckinghamshire, U.K.).

#### Primers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense</th>
<th>Antisense</th>
<th>Annealing temperature (°C)</th>
<th>Size (bp)</th>
<th>Intron</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOX1</td>
<td>ATGAACTCTCCTTCGGAGATA</td>
<td>CGTAATCTCCAACATTTCC</td>
<td>48</td>
<td>655</td>
<td>+</td>
</tr>
<tr>
<td>HOX2</td>
<td>AGCAGGAAAGGAAATGAAAACG</td>
<td>GGGCACCAGAGAGCTAA</td>
<td>54</td>
<td>102</td>
<td>−</td>
</tr>
<tr>
<td>HOX3</td>
<td>TGCAAAGAGGACACTACTAGA</td>
<td>CGTCGCGGCCAAGAG</td>
<td>51</td>
<td>126</td>
<td>−</td>
</tr>
<tr>
<td>HOX4</td>
<td>TGCATGCGGACAGCTGCT</td>
<td>TTGACCTGGCGCTACGAA</td>
<td>52</td>
<td>391</td>
<td>+</td>
</tr>
<tr>
<td>HOX5</td>
<td>TGCAGCAATGCAGCTGCTG</td>
<td>TTGAAATGCGAGCCTGCT</td>
<td>56</td>
<td>112</td>
<td>+</td>
</tr>
<tr>
<td>HOX6</td>
<td>ATGACGACGCCATGACTCTCGC</td>
<td>TGAGGCTCTGGAGATTGATGAC</td>
<td>58</td>
<td>250</td>
<td>+</td>
</tr>
<tr>
<td>HOX7</td>
<td>CAAAATGCGGAGGCCACTTG</td>
<td>TAGCGGAGCAGAAAAGG</td>
<td>58</td>
<td>146</td>
<td>−</td>
</tr>
<tr>
<td>HOX9</td>
<td>CAGCCAACTCGGCTCCTAGGC</td>
<td>CACTGCTTTTGCCTGCTGTC</td>
<td>53</td>
<td>229</td>
<td>−</td>
</tr>
<tr>
<td>HOX10</td>
<td>AGAGCAGCAAAGCGCTGCGGAGAAG</td>
<td>GGACGCTGACGTAATCTCTACG</td>
<td>62</td>
<td>198</td>
<td>−</td>
</tr>
<tr>
<td>HOX11</td>
<td>ACCGCGAAAGACGCTGCT</td>
<td>GAGCACTGAGGAAGCAGT</td>
<td>50</td>
<td>114</td>
<td>−</td>
</tr>
<tr>
<td>HOX12</td>
<td>GGCAGAAGAAGGCCGCGTG</td>
<td>CGTCGCGCGTAATCTCG</td>
<td>53</td>
<td>114</td>
<td>−</td>
</tr>
<tr>
<td>HOX13</td>
<td>CTTCTCTGAGTAGACTCCACTT</td>
<td>GCATCTTCAGCCTGCT</td>
<td>52</td>
<td>826</td>
<td>+</td>
</tr>
<tr>
<td>HOX14</td>
<td>TCTTCTCTTCTGAGCAACCTCCT</td>
<td>AGTAGAGATCTCTCTCCAGTCT</td>
<td>52</td>
<td>353</td>
<td>+</td>
</tr>
<tr>
<td>HOX15</td>
<td>AGTACAAAGAGAGCGAGCAGG</td>
<td>TGAGTGTAGCGGGTCATG</td>
<td>50</td>
<td>120</td>
<td>−</td>
</tr>
<tr>
<td>HOX16</td>
<td>GTGCCAGAACGCCGCCTGCAC</td>
<td>CGTGCAGGAGGTTGTTGATG</td>
<td>56</td>
<td>161</td>
<td>−</td>
</tr>
<tr>
<td>HOX17</td>
<td>GTCCTTTCACAGGCTT</td>
<td>TGTCCTTTCCTCCTACCT</td>
<td>52</td>
<td>122</td>
<td>−</td>
</tr>
<tr>
<td>HOX18</td>
<td>GGCGAGGCCGAGCAGCATC</td>
<td>ACTGCGCTGGTGTTCCTC</td>
<td>52</td>
<td>236</td>
<td>+</td>
</tr>
<tr>
<td>HOX19</td>
<td>AGAGTACACTGCTGGTACTA</td>
<td>TCTGCTCTCTCTCTCTCT</td>
<td>48</td>
<td>274</td>
<td>−</td>
</tr>
<tr>
<td>HOX20</td>
<td>AGCCTCTTGTGCAATGG</td>
<td>GTAACAAGTCGGACCACG</td>
<td>48</td>
<td>300</td>
<td>−</td>
</tr>
<tr>
<td>HOX21</td>
<td>AGCAGGGAAGGAGAAGGTA</td>
<td>CTTTCTCTTACACCTAG</td>
<td>48</td>
<td>250</td>
<td>−</td>
</tr>
</tbody>
</table>

#### acknowledge

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 manufacturer’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 a 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-phosphate 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-phosphate 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. 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.

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.
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, HOX9, 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. These five expressed HOX genes (HOXA7, HOXA9, and HOXC6) were used to perform the Fisher’s exact test and

<table>
<thead>
<tr>
<th>Gene</th>
<th>Positive cases in T (%)</th>
<th>Expression level (mean ± SD)*</th>
<th>Positive cases in N (%)</th>
<th>Expression level (mean ± SD)*</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOXA7</td>
<td>30 (83.3)</td>
<td>1.08 ± 0.38</td>
<td>12 (33.3)</td>
<td>0.84 ± 0.14</td>
<td>0.000</td>
</tr>
<tr>
<td>HOXA9</td>
<td>24 (66.7)</td>
<td>0.70 ± 0.22</td>
<td>9 (25.0)</td>
<td>0.66 ± 0.35</td>
<td>0.001</td>
</tr>
<tr>
<td>HOXC6</td>
<td>24 (66.7)</td>
<td>0.86 ± 0.31</td>
<td>9 (25.0)</td>
<td>0.62 ± 0.28</td>
<td>0.001</td>
</tr>
<tr>
<td>HOXA2</td>
<td>31 (86.1)</td>
<td>0.93 ± 0.29</td>
<td>36 (100.0)</td>
<td>1.12 ± 0.43</td>
<td>0.054</td>
</tr>
<tr>
<td>HOXC9</td>
<td>33 (91.7)</td>
<td>0.97 ± 0.25</td>
<td>36 (100.0)</td>
<td>0.48 ± 0.11</td>
<td>0.239</td>
</tr>
</tbody>
</table>

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

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.
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 differentiation 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 esophageus-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 (HOX42, HOX7, HOX9, 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); HOX7, HOX9, 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

<table>
<thead>
<tr>
<th>Item</th>
<th>RT-PCR (+)</th>
<th>Western blot (+)</th>
<th>RT-PCR (+) and Western blot (+)</th>
<th>RT-PCR (+) and Western blot (−)</th>
<th>RT-PCR (−) and Western blot (+)</th>
<th>RT-PCR (−) and Western blot (−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>24</td>
<td>13</td>
<td>13</td>
<td>11</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>N</td>
<td>9</td>
<td>12</td>
<td>9</td>
<td>0</td>
<td>3</td>
<td>24</td>
</tr>
</tbody>
</table>

NOTE. (+), positive cases; (−), negative cases. Total case number is 36.
Abbreviations: T, tumor tissue; N, noncancerous mucosa.
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 Sangeeta Cheema for linguistic advice and assistance of the revised article.

REFERENCES

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

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


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/11/3/1044

Cited articles
This article cites 21 articles, 5 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/11/3/1044.full.html#ref-list-1

Citing articles
This article has been cited by 12 HighWire-hosted articles. Access the articles at:
/content/11/3/1044.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.