Aberrant expression of homeobox gene *HOXA7* is associated with müllerian-like differentiation of epithelial ovarian tumors and the generation of a specific autologous antibody response

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Autologous serum antibodies to molecules that are aberrantly expressed in tumors represent potential biomarkers for early diagnosis of cancer. In this study, we identified the homeobox gene *HOXA7* as encoding an antigen in epithelial tumors of the ovary. These tumors are thought to arise from the simple epithelium lining the ovarian surface, but they often resemble the specialized epithelia derived from the müllerian ducts. Expression of *HOXA7* was detected in ovarian tumors exhibiting müllerian-like features and correlated with the generation of anti-*HOXA7* antibodies by patients. In contrast, it was observed that healthy women lack anti-*HOXA7* antibodies ($P < 0.0001$) and that *HOXA7* expression is absent from normal ovarian surface epithelium. Interestingly, *HOXA7* expression was detected in the müllerian-like epithelium lining inclusion cysts in normal ovaries and in the müllerian duct-derived epithelium of normal fallopian tubes. Furthermore, ectopic expression of *HOXA7* enhanced the epithelial phenotype of immortalized ovarian surface epithelial cells, as indicated by the appearance of cobblestone morphology, induction of E-cadherin expression, and down-regulation of vimentin. These findings associate aberrant *HOXA7* expression with the müllerian-like differentiation of epithelial ovarian tumors and suggest diagnostic utility of serum antibodies to *HOXA7*.

Cancer patients can generate humoral and cell-mediated immune responses to molecules expressed in tumors but not in normal tissues, and also to self-antigens that are overexpressed in tumors (1). Although patients can also generate immune responses to cancer-independent autoantigens, the selective recognition of tumor antigens by the immune system provides a powerful means to screen for molecules associated with neoplasia. Given the difficulty of cloning T cell-recognized epitopes, antigens have been increasingly identified by using the antibody repertoire of cancer patients in a methodology termed SEREX (serologic identification of antigens by recombinant expression cloning). This approach involves screening tumor cDNA expression libraries with patient sera, and its application has uncovered antigens associated with various cancers such as melanoma, renal cell carcinoma, and colon cancer (2–4). SEREX-defined antigens with restricted tissue expression patterns represent candidate targets for immunotherapy (5). In addition, autologous antibody responses to SEREX antigens have been explored as serum biomarkers for cancer detection (6). Furthermore, SEREX antigens may contribute to pathogenesis—e.g., mutant p53 (3) and HOXB7 (7).

Metastatic ovarian carcinoma responds with limited success to current therapeutic modalities. Less than 12% of women with stage IV disease survive 5 years after diagnosis (8). The prognosis for women with organ-confined disease (stage I) is more optimistic, with a 5-year survival rate of 90%. However, ≈70% of patients with ovarian carcinoma present with disseminated disease at the time of initial diagnosis. Ovarian carcinomas are thought to arise from cells of the ovarian surface epithelium

(OSE) (9, 10). The OSE is a single layer of mesodermally derived cells surrounding the ovary and is morphologically similar to the simple mesothelial lining of peritoneal surfaces. In contrast, epithelial ovarian tumors often resemble the specialized, more architecturally complex epithelia of the female reproductive tract that derive from the müllerian ducts (10). A major impediment to early diagnosis of ovarian carcinoma has been the lack of defined precursor or premalignant lesions. No description of a stepwise sequence of molecular events exists for ovarian carcinogenesis analogous to that described for colorectal neoplasia (11). The use of serum tumor biomarkers for detection of ovarian cancer has been limited by their insufficient specificity and sensitivity, particularly for organ-confined early-stage disease. Elevated levels of CA125, the most widely used serum biomarker for ovarian cancer, occur in only 50% of stage I patients, and can also be detected in healthy women (12). Patients with ovarian carcinoma can generate tumor-reactive serum antibodies (13). However, very few antigens have been found to be widely expressed among ovarian carcinomas but absent from the OSE and other normal tissues. For example, HER-2/neu is known to be immunogenic, but amplification and overexpression of the *HER-2/neu* oncogene occurs in only 20% of ovarian cancers (14).

In this study, we applied SEREX methodology by using serum of a patient with serous ovarian carcinoma, the subtype responsible for the majority of ovarian cancer-related deaths, and we isolated the homeobox gene *HOXA7*. Serologic reactivity to *HOXA7* was surveyed, together with analyses of endogenous *HOXA7* expression patterns in tissue specimens and of ectopic *HOXA7* expression in OSE cells. The findings associate aberrant expression of *HOXA7* with müllerian-like differentiation of epithelial ovarian tumors and the generation of an autologous antibody response of potential diagnostic utility.

Materials and Methods

Human Tissues and Sera. Tissues excess to diagnosis were snap-frozen in liquid nitrogen. Sera were obtained from healthy female donors ($n = 30$), patients with benign ovarian cystadenomas ($n = 19$), and patients with primary ovarian carcinoma, where disease extended to the uterus and fallopian tubes (stage II, $n = 1$), to the abdomen and lymph nodes (stage III, $n = 38$), or involved distant metastasis (stage IV, $n = 12$). Tissue and sera were collected with informed consent of patients (protocol no. RPN98-03-02-01). Tumors were graded according to architectural and cytologic criteria described elsewhere (15).

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Abbreviations: OSE, ovarian surface epithelium; RT, reverse transcription.

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Recombinant Expression Cloning by Serologic Screening. A cDNA expression library was constructed in the bacteriophage λ ZAP-Express vector (Stratagene) from the OV-1063 cell line as previously described (7). This cell line was originally reported to be derived from a serous ovarian carcinoma (16). Although its origin has been disputed, the OV-1063 cell line has been recently found by serial analysis of gene expression to have a global profile of gene expression similar to those of primary ovarian carcinoma specimens (17). Serologic screening was performed as described (3). Briefly, *Escherichia coli* strain XLI-Blue MRF' was infected with recombinant phage, cDNA expression was induced by isopropyl β -D-thiogalactoside, and plaques were transferred to nitrocellulose membranes. Membranes were incubated with diluted patient serum (1:500), and reactivity of serum antibodies was detected by alkaline phosphatase-conjugated anti-human IgG antibody and 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium color development (Kirkegaard & Perry Laboratories). Positive phage were purified to monoclonality by repeated screening. pBK-CMV phagemids containing cDNAs were isolated and sequenced.

Reactivity of Multiple Patient Sera with Positive Phage. *E. coli* were infected with a 1:1 ratio of monoclonalized positive phage and nonreactive phage of the cDNA expression library as internal negative controls to achieve subconfluent lysis. Serum samples of patients were tested in parallel for reactivity by using the phage plaque immunoscreening assay described above.

ELISA of Serologic Reactivity to HOXA7. Full-length *HOXA7* coding sequences were amplified by PCR from pBK-CMV phagemids and cloned into the pPROEXHT vector (Life Technologies, Grand Island, NY). Preparation and purification of His-tagged recombinant protein and its use in ELISA are described elsewhere (7). Sera were tested in ELISAs at dilutions ranging from 1:100 to 1:50,000.

Analysis of HOXA7 Expression. Total RNA was isolated from tissue specimens by using TRIZOL (Life Technologies) and treated with DNase I. Reverse transcription, amplification of cDNAs for *HOXA7* and β -actin, and Southern blot analysis of reverse transcription (RT)-PCR products were performed as previously described (7, 18). Briefly, amplification was performed with a 2-min start at 94°C, denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min, for 40 cycles for *HOXA7* and 25 cycles for β -actin. Titrations were performed to ensure a linear range of amplification. Primers were as follows: *HOXA7*, 5'-GAGCTGGAGAAGGAGTTCCA-3' and 5'-CTTTCTTCCACTTCATACGA-3'; β -actin, 5'-ATGATATC-GCCGCGCTCG-3' and 5'-CGCTCGGTGAGGATCTTCA-3'. Southern blot analysis of RT-PCR products was conducted by using ³²P-labeled β -actin cDNA (CLONTECH) and the full-length *HOXA7* cDNA fragment described above, and hybridization signals were detected by PhosphorImager analysis (Molecular Dynamics). Immunohistochemical analysis was performed on sections of paraffin-embedded tissue specimens by using a rabbit polyclonal antiserum raised against human *HOXA7* peptide (Covance) (1:2,000) in a standard avidin-biotin-peroxidase method.

Transfection of IOSE-29 Cells with HOXA7. Full-length *HOXA7* cDNA was subcloned into the mammalian expression vector pcDNA4His (Invitrogen). The IOSE-29 cell line was cultured as described elsewhere (19). Subconfluent cultures of IOSE-29 cells were transfected with linearized DNA by using Lipofectamine PLUS reagent (Life Technologies) and selected with zeocin (400 μ g/ml). Experiments were performed using lines established from single colonies. Expression of E-cadherin and vimentin was analyzed in IOSE-29 cells and also OVCAR-3 cells

(obtained from American Type Culture Collection) by immunofluorescence as described elsewhere (20) using mouse anti-E-cadherin monoclonal antibody (clone HEC-1, Zymed) and mouse anti-vimentin monoclonal antibody (clone V9, Sigma) at 1:300 dilution.

Results

Isolation of HOXA7 cDNAs by Serologic Screening. We applied SEREX methodology using serum of a patient with stage III serous carcinoma of the ovary. Twenty-seven positive clones were isolated by immunoscreening >800,000 recombinant phage plaques, in addition to the four clones previously isolated in an initial screen of 200,000 plaques (7). Twenty-one of the 27 positive clones contained the 690-bp coding region of *HOXA7* (21) (GenBank accession no. NM006896) plus 167 bp and 120 bp, respectively, of 5'- and 3'-untranslated sequences. No mutations were observed among the *HOXA7* clones. Of the other six positive clones, four corresponded to *HOXB7*, which was also isolated in the initial screen (7), one encoded ADP-ribosylation factor-1, a small G-protein (GenBank accession no. AF052179), and sequences of another clone (44B.1) have yet to be verified.

Serologic Reactivity to HOXA7. Several SEREX-defined antigens are only immunogenic in an individual patient, whereas reactivity to others has been detected among healthy individuals (2-4). We initially examined reactivity to *HOXA7* in a small number of patient and healthy donor serum samples by the phage plaque assay. As shown in Fig. 1A, little or no reactivity with plaques of monoclonalized *HOXA7* positive phage was observed in sera of healthy women and of patients with poorly differentiated serous ovarian carcinomas. In contrast, strong reactivity was detected in sera of patients with histologically more differentiated serous carcinomas and with benign serous cystadenomas. Serologic reactivity to *HOXA7* was confirmed at a more quantitative level by ELISA using purified recombinant *HOXA7* protein. Because patients are unlikely to have been exposed to bovine papillomavirus capsid protein L2 (BPVL2), this recombinant antigen was used as a negative control protein (22). As shown in Fig. 1B, little serologic reactivity to BPVL2 was observed among healthy women and among patients. In contrast, a significant difference was observed between the reactivity to *HOXA7* of serum antibodies of healthy women and of patients with moderately differentiated serous carcinomas ($P < 0.0001$). Sixteen of 24 patients (67%) with these carcinomas were found to generate anti-*HOXA7* serum antibodies, where a positive reaction is defined as an optical density value that exceeds the mean optical density value of sera of healthy donors by three standard deviations (6). Thirteen of 19 patients (68%) with benign serous cystadenomas generated serum antibodies reactive with *HOXA7*. However, the serologic reactivity to *HOXA7* observed among patients with poorly differentiated serous carcinomas was found to be not significantly different from responses of healthy women. In fact, serum from only 1 of the 24 patients with poorly differentiated serous carcinoma gave a positive reaction as defined above. Our studies emphasize the serous histotype, as this is the most prevalent histologic differentiation pattern in epithelial ovarian tumors. However, analysis of a limited number of sera likewise revealed serologic reactivity to *HOXA7* in patients with endometrioid ovarian carcinomas that were histologically differentiated, but not those that were poorly differentiated (Fig. 1A).

Analysis of HOXA7 Expression in Benign and Malignant Ovarian Tumors. Significant *HOXA7* expression was detected by RT-PCR analysis in serous and endometrioid carcinomas that were moderately and well differentiated, but was almost undetectable in those that were poorly differentiated (Fig. 2A). *HOXA7* expression was also strongly detected in serous cystadenomas. The

A

B

Fig. 1. Serum antibody responses to HOXA7. (A) Antibodies to HOXA7 in sera (diluted 1:500) of patients with ovarian carcinomas (ca.) (blots 1–9) and with ovarian cystadenomas (blots 10, 11), and of healthy female volunteers (blots 12–14), were detected by their reactivity to plaques of monoclonalized HOXA7 positive phase. Patient code numbers are shown with the type and degree of histology of their tumors. Histology of carcinomas included those of the serous and endometrioid subtypes, which were poorly, moderately (mod.), and well differentiated (diff.). Background reactivity to plaques of monoclonalized HOXA7 positive phase of the secondary anti-human IgG antibody is shown in blot 15. (B) Reactivity of sera (1:500 dilution) with recombinant HOXA7 protein (dark bars) and to the negative control protein BPVL2 (light bars) was assessed by ELISA and measured in terms of optical density at 450 nm. Shown are values of statistical significance, as determined by the Mann–Whitney *u* test, for differences in reactivity to HOXA7 of sera of patients with moderately differentiated serous ovarian carcinomas ($n = 24$), with poorly differentiated serous ovarian carcinomas ($n = 24$), with serous ovarian cystadenomas ($n = 19$), and of healthy female volunteers ($n = 30$). Differences in reactivity to BPVL2 among the four groups of women were found to be not significant ($P > 0.05$). Horizontal bars indicate median values.

generation by patients of serum antibodies to HOXA7 correlated with HOXA7 expression patterns in their tumors (compare patient codes in Fig. 1A with those in Fig. 2A). Tissue expression patterns of HOXA7 were confirmed by immunohistochemical staining with HOXA7 antibody and found to be restricted to the epithelium, not the stroma. Intense HOXA7 staining was observed throughout the papillae of all six histologically differentiated serous carcinomas examined by RT-PCR analysis, as well as in seven additional cases (Fig. 2B). Glandular structures of differentiated endometrioid carcinomas were likewise HOXA7-positive. In contrast, little or no HOXA7 staining was observed in poorly differentiated serous and endometrioid carcinomas (Fig. 2B). Ovarian carcinomas vary in their degree of histologic differentiation, whereas cystadenomas tend to exhibit relatively homogenous histologic composition. Strong, uniform HOXA7 staining was observed throughout the epithelium of all 29 cases of serous cystadenoma analyzed (Fig. 2B).

HOXA7 Expression Patterns in Normal Tissue Specimens. HOXA7 expression was not detected by RT-PCR analysis in epithelial cells scraped from the surface of normal ovaries (Fig. 2A). Only very weak HOXA7 staining was observed in the single cell layer lining the ovarian surface in one normal specimen, whereas another 13 specimens were HOXA7-negative (Fig. 3A). In-

creased staining was observed in columnar cells lining invaginations of the ovarian surface (Fig. 3B). These invaginations are thought to create small cysts that eventually lose their connection to the surface (10, 23). Such benign inclusion cysts frequently are lined by müllerian-like epithelium that is rare on the ovarian surface, and they are believed to represent the site of origin of epithelial ovarian tumors (10). In contrast to the normal OSE, the epithelium lining inclusion cysts was found to be HOXA7-positive in all specimens of normal ovary examined (Fig. 3C and D). HOXA7 staining was observed throughout the müllerian duct-derived epithelium of normal fallopian tubes, which serous ovarian tumors histologically resemble (Fig. 3E and F). Interestingly, HOXA7 staining of the müllerian-like epithelia of normal and tumor tissues was primarily cytoplasmic. Cytoplasmic staining has been observed for other HOX proteins in different tissues, although its significance is unknown (24). Preliminary studies also revealed HOXA7 to be expressed in the normal epithelium of the endometrium, which is also of müllerian duct origin and shares histologic features with endometrioid ovarian carcinomas. These findings indicate that HOXA7 expression is associated with normal müllerian differentiation and with the aberrant müllerian-like phenotype of benign and malignant epithelial ovarian tumors.

Fig. 2. Expression patterns of *HOXA7* in benign and malignant epithelial ovarian tumors. (A) Shown are Southern blots of *HOXA7* and β -actin RT-PCR products amplified from RNA of ovarian carcinomas (lanes 1–15, 16, 21), in ovarian cystadenomas (lanes 17–20), in epithelial cells scraped off surfaces of normal ovaries (lanes 22–24), and in the simian virus 40-immortalized OSE cell line, IOSE-29 (lane 25). The specimen used for analysis shown in lane 4 (case SCM4) is the same as those in lanes 16 and 21. Specimens included those collected from the same patients whose sera were tested for reactivity to *HOXA7* by the phage plaque assay shown in Fig. 1A (refer to corresponding patient code numbers). (B) Shown are typical examples of intense staining using *HOXA7* antibody in a histologically differentiated serous carcinoma (case SCM4) and serous cystadenoma (case SCB2), and of weak staining in poorly differentiated serous (case SCP2) and endometrioid (case ECP1) carcinomas. ($\times 60$.)

Ectopic Expression of *HOXA7* in Immortalized OSE Cells. The OSE is of an “uncommitted” phenotype, with the potential to convert to epithelial or mesenchymal phenotypes in response to different stimuli (10, 25). Because *HOXA7* expression is normally absent in the OSE, we considered the possibility that expression of *HOXA7* in OSE cells could promote transition to a more epithelial phenotype. The IOSE-29 cell line was established by immortalizing normal human OSE cells with SV-40 large T antigen (19). IOSE-29 cells undergo mesenchymal conversion in culture and lack the epithelial cell adhesion molecule E-cadherin, as do most normal OSE cells (26). IOSE-29 cells were found to be *HOXA7*-negative (Fig. 2A). We therefore examined the effect of stably transfecting this cell line with *HOXA7*. IOSE-29 cells transfected with vector DNA alone grew in fibroblastic monolayers as observed for the parental cell line (19) (Fig. 4A and B). In contrast, *HOXA7*-transfected IOSE-29 cells grew closely packed in islands, exhibiting cobblestone morphology typical of epithelial cells such as the ovarian carcinoma cell line OVCAR-3 (Fig. 4C and D). E-cadherin was not detected in parental and vector-transfected IOSE-29 cells (Fig. 4E and F). However, *HOXA7*-transfected IOSE-29 cells expressed E-cadherin, which accumulated at cell–cell junctions as observed for OVCAR-3 cells (Fig. 4G and H). In contrast, expression of the mesenchymal marker vimentin was strongly detected in parental and vector-transfected IOSE-29 cells (Fig. 4I and J) and was markedly down-regulated in *HOXA7*-transfected IOSE-29 cells (Fig. 4K). It therefore seems that ectopic expression of *HOXA7* in IOSE-29 cells can promote their transition to the epithelial phenotype.

Discussion

An intriguing aspect of many epithelial ovarian tumors is their epithelial phenotype, which is more “committed” than that of the OSE, and their histologic resemblance to the müllerian duct-derived epithelia. Because the müllerian duct-derived epithelia and the OSE share a common embryonic precursor, the urogenital coelomic epithelium, the appearance of müllerian-like characteristics in ovarian tumors has been speculated to

reflect aberrant differentiation toward tissue types of related embryonic origin (26). It is thought that an early step in epithelial ovarian neoplasia involves aberrant epithelial differentiation driven by inappropriate expression of developmentally regulated genes (10, 27). In this study, we identified *HOXA7* as an immunogenic molecule that is expressed by müllerian-like epithelial ovarian tumors. Whereas surface epithelium of normal ovaries was found to express very little or no *HOXA7*, increased expression was observed in columnar cells lining invaginations of the ovarian surface, in müllerian-like epithelium of inclusion cysts, and also in normal epithelium of fallopian tubes. *HOXA7* expression therefore seems to be associated with increased commitment to müllerian-like epithelial differentiation, a notion supported by our findings that ectopic expression of *HOXA7* in immortalized OSE cells promoted their transition to a more epithelial phenotype. Our observations raise the possibility that *HOXA7* expression is associated with normal development of the müllerian duct-derived epithelia and that inappropriate expression of *HOXA7* in the OSE could give rise to aberrant epithelial differentiation. Our study suggests that the resemblance of ovarian tumors to the müllerian duct-derived epithelia reflects inappropriate reactivation of an embryonic differentiation program involving *HOXA7* expression that is recognized by the patient immune system.

Many molecules of etiologic relevance to carcinogenesis, such as oncogene products, are immunogenic in cancer patients (1, 28). The application of SEREX has uncovered numerous antigens in various cancers, and much recent attention has focused on their immunotherapeutic potential (5). However, the biologic relevance to neoplasia of most SEREX-defined antigens is unclear. Our study of *HOXA7* reveals a potential molecular link between mechanisms that regulate normal differentiation of the müllerian duct-derived epithelia and those that lead to aberrant epithelial differentiation associated with ovarian neoplasia. Further studies to elucidate this link are required, although suitable model systems for investigating “preneoplastic” events of ovarian neoplasia do not currently exist. *HOX* genes encode transcription factors and have been described as “master regulators”

Fig. 3. Analysis of HOXA7 expression in normal tissue. Shown are examples of minimal HOXA7 staining in cells lining the ovarian surface (A), increased staining in columnar cells lining invaginations of the ovarian surface (B), and intense staining in the epithelium lining inclusion cysts of histologically normal ovaries (C and D). Strong HOXA7 staining was observed throughout the epithelium of normal fallopian tubes (E), and was similar in intensity and uniformity to the staining observed in serous cystadenomas (F). (A–C, $\times 400$; D–F, $\times 160$.)

of normal cell growth and differentiation and key determinants of tissue identity (29, 30). *HOX* genes regulate differentiation of hematopoietic lineages, and their aberrant expression is linked to leukemogenesis (31). However, knowledge of the role of *HOX* genes in development of solid malignancies is limited. Loss of p53 expression in breast carcinomas has been attributed to silencing of *HOXA5* (32). We recently reported that overexpression of *HOXB7* in OSE cells enhances proliferation and up-regulates production of basic fibroblast growth factor, a potent stimulator of growth, angiogenesis, and cell migration (7). E-cadherin is expressed in normal müllerian duct-derived epithelia, inclusion cysts, and müllerian-like ovarian neoplasms but is generally absent from normal OSE (27). The parallel nature of HOXA7 and E-cadherin expression and the induction of E-cadherin in HOXA7-overexpressing OSE cells implicate the

Fig. 4. Ectopic HOXA7 expression in immortalized OSE cells. Cultures of parental IOSE-29 cells (A, E, I), IOSE-29 cells transfected with pcDNA4His vector (B, F, J), IOSE-29 cells transfected with pcDNA4His-HOXA7 construct (C, G, K), and OVCAR-3 cells (D, H, L) were photographed by phase-contrast microscopy (A–D), or fixed and stained by immunofluorescence for E-cadherin (E–H) and for vimentin (I–L). (A–D, $\times 140$; E–L, $\times 640$.)

E-cadherin gene as a target of HOXA7. However, recent studies indicate that the E-cadherin gene is primarily regulated by suppression of its promoter in nonepithelial cells rather than specific activation in epithelial cells (33), suggesting the link to HOXA7 is indirect. Numerous genes have been identified by SAGE and microarray analysis to be up- and down-regulated in ovarian carcinomas as compared with normal OSE (17, 34). It remains to be determined which of these genes are regulated by HOXA7. Several other normal tissues, in addition to the fallopian tube and endometrium, apparently express *HOXA7*, such as colon and kidney (35). Identifying downstream targets of *HOXA7* and also the signals that induce *HOXA7* expression will be of importance for understanding the roles of this *HOX* gene in normal differentiation and neoplasia in the reproductive tract and possibly other tissues.

The strong correlation found between HOXA7 expression in ovarian tumors and the generation of a specific anti-HOXA7 antibody response in cancer patients indicates that the mechanism underlying the immunogenicity of HOXA7 is likely to be similar to that of most self-antigens—i.e., overriding thresholds critical for the maintenance of tolerance (1). The possibility cannot be excluded, however, that tumor-associated posttranslational modification and/or alterations in antigen processing/presentation by tumor cells could also contribute to the immunogenicity of HOXA7 in ovarian cancer patients. The generation of anti-HOXA7 antibodies by patients with cystadenomas is somewhat surprising in its similarity to responses of patients with differentiated carcinomas, because the latter all had disseminated disease. It is possible that HOXA7 is quite immunogenic in women with cystadenomas who are otherwise healthy and that the immune system is exquisitely sensitive to respond to a level of HOXA7 that exceeds the normal systemic threshold. It has been reported that peptides derived from the homeodomain of Antennapedia, a *Drosophila* transcription factor closely related to HOXA7, can be efficiently internalized by cells in a receptor-independent manner (36). Proteins have been fused to Antennapedia peptides to promote their uptake by cells (37). A possible mechanism for HOXA7 immunogenicity therefore could involve its efficient uptake by antigen-presenting cells.

The generation of autologous serum antibodies to tumor antigens can be regarded as the systemic amplification by the host immune system of a signal indicating the presence of a tumor. As an amplified signal, autologous anti-tumor antibodies represent potential serum biomarkers, particularly for detecting small, early-stage lesions. The primary goal of ovarian cancer screening is to detect disease while still organ-confined. Although larger case/control studies are required to correlate antibody titers with stage of disease, we found that detectable levels of anti-HOXA7 serum antibodies are generated by 67% of women with histologically differentiated ovarian carcinomas of the most common serous subtype. This frequency of antibody response is markedly higher than that observed to SEREX antigens in other cancers, e.g., frequencies of antibody responses to SEREX-defined antigens in renal cell carcinoma range from only 5 to 25% (4). The clinical relevance of anti-p53 serum antibodies has been evaluated for various cancers but was found to be of limited value for ovarian carcinoma (38). An obvious deficiency in the utility of anti-HOXA7 serum antibodies as diagnostic biomarkers is that there is extremely poor sensitivity for detecting women with poorly differentiated ovarian carcinomas. We have found that the related homeobox gene *HOXB7* is

highly expressed in ovarian carcinomas that vary in their degree of histologic differentiation (7), and the diagnostic potential of anti-HOXB7 antibodies requires further study. Although the anti-HOXA7 antibody assay alone allows no distinction between benign and malignant ovarian tumors, it is able to discriminate patients with differentiated ovarian tumors from healthy women. This indicates that specific anti-tumor antibody responses can be detected when an epithelial ovarian tumor, albeit benign, is still organ-confined, and further application of SEREX to ovarian cancer to find additional tumor antigens is therefore warranted.

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