

# Serological identification of embryonic neural proteins as highly immunogenic tumor antigens in small cell lung cancer

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**Serological analysis of expression cDNA libraries (SEREX) derived from two small cell lung cancer (SCLC) cell lines using pooled sera of SCLC patients led to the isolation of 14 genes, including 4 SOX group B genes (SOX1, SOX2, SOX3, and SOX21) and ZIC2. SOX group B genes and ZIC2 encode DNA-binding proteins; SOX group B proteins regulate transcription of target genes in the presence of cofactors, whereas ZIC2 is also suspected to be a transcriptional regulator. These genes are expressed at early developmental stages in the embryonic nervous system, but are down-regulated in the adult. Although SOX2 mRNA can be detected in some adult tissues, ZIC2 is expressed only in brain and testis, and SOX1, SOX3, and SOX21 transcripts are not detectable in normal adult tissues. Of SCLC cell lines tested, 80% expressed ZIC2 mRNA, and SOX1, SOX2, and SOX3 expression was detected in 40%, 50%, and 10%, respectively. SOX group B and ZIC2 antigens elicited serological responses in 30–40% of SCLC patients in this series, at titers up to 1:10<sup>6</sup>. In sera from 23 normal adults, no antibody was detected against SOX group B or ZIC2 proteins except for one individual with low-titer anti-SOX2 antibody. Seroreactivity against SOX1 and 2 was consistently higher titered than SOX3 and 21 reactivity, suggesting SOX1 and/or SOX2 as the main antigens eliciting anti-SOX responses. Although paraneoplastic neurological syndromes have been associated with several SCLC antigens, neurological symptoms have not been observed in patients with anti-SOX or anti-ZIC2 antibodies.**

**S**mall cell lung cancer (SCLC) is a highly aggressive form of lung cancer that accounts for approximately 20% of all cases of lung cancer (1). Although initially responsive to chemotherapy, SCLC is almost invariably fatal. SCLC is a carcinoma of neuroendocrine origin, in contrast to non-SCLC (NSCLC), which is of bronchial epithelial origin. Dense-core neurosecretory granules and other neuroendocrine markers are characteristic features of SCLC (2, 3). Some of these markers, including synaptophysin, chromogranin A, and neuron-specific enolase, are expressed normally by neuroendocrine cells throughout development (4). In contrast, other SCLC gene products have been detected only in the embryonic nervous tissue and have not been found in normal adult tissues. The restricted expression of human achaete-scute homologue (hASH) is one such example. hASH1 has been shown to be essential for the development of neuroendocrine cells in the lung and for the maintenance of the neuroendocrine features of SCLC (5, 6).

The association of SCLC with paraneoplastic neurological syndromes is well known (7, 8). These syndromes, defined as neurological disorders that are related to cancer but cannot be accounted for by metastasis or treatment complications, are rare manifestations of a small subset of malignancies, including SCLC (8, 9). Current knowledge points to immune-mediated neuronal injury as the cause of these paraneoplastic syndromes, resulting from the amplified or aberrant expression of immunogenic neural antigens by the cancer. The elicited humoral and/or cell-mediated immune responses react with normal neuronal

cells, leading to structural damages and clinical manifestations. The presence of high-titered antibodies in patients with paraneoplastic disorders has facilitated the identification of antigenic targets associated with these syndromes (9). A number of these antigens have been isolated by using antibodies from SCLC patients, and all of them are expressed normally by selected neuronal cell populations. The current list of SCLC-related antigens associated with paraneoplastic neurological syndromes includes HuD, a neuronal antigen homologous to *Drosophila* Elav and SxI genes (10); recoverin, a photoreceptor protein (11, 12); amphiphysin, a 128-kDa synaptic vesicular protein (13); Ri (Nova), an antigen with homology to RNA-binding proteins, expressed in the developing motor neurons (14); VGCC, located at the presynaptic region of the neuromuscular junction (15); and CRMP-5, a neuron-specific collapsin-response mediator protein (16). Clearly, SCLC is a highly immunogenic tumor likely the result of the expression of a wide array of normal neuroectodermal antigens to which the host is not tolerant.

To extend the search for SCLC antigens with immunogenicity in humans, we have analyzed the humoral immune response of SCLC patients by using serological analysis of expression cDNA libraries (SEREX), an approach that is being applied to a broad range of different human tumor types (17–19). SEREX analysis, which involves serological screening of cDNA expression libraries with sera from cancer patients, has shown that cancer patients mount a vigorous humoral immune response to a wide variety of cellular antigens, including differentiation antigens, mutational antigens, cancer-testis (CT) antigens, and amplified antigens (20). What has been clear from SEREX is that high-titered IgG response to tumor antigens is not a rare event and such responses are not limited to patients with paraneoplastic syndromes. In the present study, SEREX of SCLC cell lines has identified several genes with expression patterns predominantly restricted to the embryonic nervous system.

## Materials and Methods

**Cell Lines, Tissues, and Patient Sera.** Cell lines were obtained from the repository maintained at the Ludwig Institute for Cancer Research (LICR), New York Branch at the Memorial Sloan-Kettering Cancer Center, or from American Tissue Culture Collection. Eleven SCLC cell lines were used, including nine classical (SK-LC-13, NCI-H69, 128, 146, 187, 209, 378, 889, and 740) and two variant (NCI-H82 and 526) forms. The variant SCLC lines differ from the classical lines in lacking or having

Abbreviations: SEREX, serological analysis of recombinant cDNA expression libraries; SCLC, small cell lung cancer; HMG, high-mobility group; RT-PCR, reverse transcription-PCR.

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diminished neuroendocrine features, as well as in other biochemical, morphological, and growth properties (21, 22). Normal and tumor tissues were obtained from the departments of Pathology, New York Presbyterian Hospital (NYPH), and Memorial Sloan-Kettering Cancer Center (MSKCC). Patient sera were obtained from the Department of Medicine, NYPH, and from the LICR Melbourne Branch, Australia.

**Immunoscreening of SCLC Cell Line Libraries and Characterization of Immunoreactive Clones.** Construction of cDNA expression libraries from NCI-H740 and SK-LC-13 SCLC cell lines in the  $\lambda$ -ZAP vector (Stratagene) and immunoscreening of the libraries were done as described previously (20, 23), with the following modifications. Sera from five SCLC patients (Lu94, Lu100, Lu101, Lu104, and Lu113) were pooled and absorbed (19). The pooled serum was diluted 1:200 (final dilution, 1:1,000 for each serum) in Tris-buffered saline (TBS) containing 1% BSA and .02%  $\text{NaN}_3$  and used to screen the NCI-H740 library [ $5.6 \times 10^5$  plaque-forming units (pfu)] and the SK-LC-13 library ( $2.2 \times 10^5$  pfu). Immunoreactive clones were isolated and sequence was analyzed as described previously (23). Selected immunoreactive clones were tested for reactivity against 10-fold serially diluted sera (1:10<sup>3</sup> to 1:10<sup>6</sup>) from individual lung cancer patients and normal individuals by using the same plaque assay. A  $\lambda$ -ZAP clone without an insert was coplated and included in each assay as a negative control.

**Reverse Transcription-PCR (RT-PCR) Analysis.** RT was performed with total RNA isolated from tissue or cell lines by the guanidium thiocyanate/CsCl method (24). Primers used to amplify *ZIC2* were designed based on the published sequence (AF104902): *ZIC2A1*, 5'-CATGAATATGAACATGGGTATGAACATGG; and *ZIC2B1*, 5'-TCGCAGCCCTCAAACCTCACTG. PCR was performed with initial denaturation and AmpliTaq Gold (Perkin-Elmer) activation at 94°C for 10 min, followed by 35 cycles of amplification (denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and primer extension for 1 min at 72°C), followed by 6-min extension at 72°C. Amplification products were analyzed by agarose gel electrophoresis and visualized by ethidium bromide staining.

**Northern Blot Analysis.** Adult normal tissue mRNA blots were obtained from CLONTECH and contained 2  $\mu\text{g}$  of poly(A)<sup>+</sup> RNA per lane. SCLC cell line total RNA was isolated as described above, and poly(A)<sup>+</sup> mRNA was prepared by using the Microfast Track kit (Invitrogen). Two micrograms of mRNA was transferred to nylon membranes (Schleicher & Schuell) after denaturing gel electrophoresis. Hybridization was carried out in the ExpressHyb buffer (CLONTECH); hybridization and washing conditions were as described by the manufacturer. The probes used for Northern blot analysis were 450-bp *SOX2* fragment (nucleotides 630-1,080, GenBank accession no. Z31560), 751-bp *SOX1* fragment (nucleotides 1,520-2,271, Y13436), 330-bp *SOX3* fragment (nucleotides 442-772, X71135), 680-bp *SOX21* fragment (nucleotides 2,720-3,400, AF104902), and full-length *ID4* cDNA (1,322 bp, U28368).

## Results

**Isolation of Immunoreactive Clones from SCLC Cell Lines by SEREX.** SEREX of the SCLC cell line NCI-H740 with a pool of five sera from SCLC patients resulted in the isolation of 37 clones coding for 8 known gene products (Table 1). These eight genes were given SEREX gene designations of *NY-SCLC-1* to *NY-SCLC-8*. The most frequently isolated genes were *SOX2* and *ZIC2*, comprising 51% and 24% of all clones. A single clone corresponding to *SOX1* was also isolated from this library. *SOX*- and *ZIC2*-encoding clones showed very strong immunoreactivity with the SCLC patient sera. Other genes isolated included *ID4*,

**Table 1. Genes isolated by SEREX of the SCLC cell line NCI-H740**

Gene designation	Gene/sequence identity (GenBank accession no.)	No. of clones (% of total)
<i>NY-SCLC-1</i>	<i>SOX2</i> (Z31560)	19 (51)
<i>NY-SCLC-2</i>	<i>SOX1</i> (Y13436)	1 (3)
<i>NY-SCLC-3</i>	<i>ZIC2</i> (AF104902)	9 (24)
<i>NY-SCLC-4</i>	<i>ID4</i> (U28368)	2 (5)
<i>NY-SCLC-5</i>	<i>MAZ</i> (M94046)	1 (3)
<i>NY-SCLC-6</i>	<i>MPP11</i> (X98260)	3 (8)
<i>NY-SCLC-7</i>	<i>eIF2B</i> (U23028)	1 (3)
<i>NY-SCLC-8</i>	<i>RBP-1</i> (L07872)	1 (3)
		Total: 37

*MPP11*, *MAZ*, *eIF2B*, and *RBP-1*. *ID4* protein is a member of the dominant negative helix-loop-helix (HLH) protein family (25). This protein can interact with other HLH proteins (such as *Achaete-scute*), and, by virtue of not containing a DNA-binding domain, it acts as a repressor (25). *ID4* mRNA expression in normal tissues was found to be universal by Northern blot analysis (data not shown). Seroreactivity against *ID4* was of moderate intensity. MPP11 (MPM2-reactive phosphoprotein 11), another HLH protein-binding factor, originally was identified in HeLa cells by M-phase protein-recognizing antibodies (26). Seroreactivity against MPP11 was strong. MPP11 has been identified previously by SEREX of gastric and breast cancer (SEREX database: <http://www.licr.org/SEREX.html>; clones NGO-St-58 and LONY-BR-7), and MPP11 mRNA is universally expressed. The other genes isolated from the NCI-H740 library—myc-associated Zinc-finger protein *MAZ*, the eukaryotic translation initiation factor *eIF2B*, and the J- $\kappa$  recombination signal-binding protein (*RBP-1*)—also have been identified by SEREX. *MAZ*, *eIF2B*, and *RBP-1* mRNA are broadly expressed in normal adult tissues.

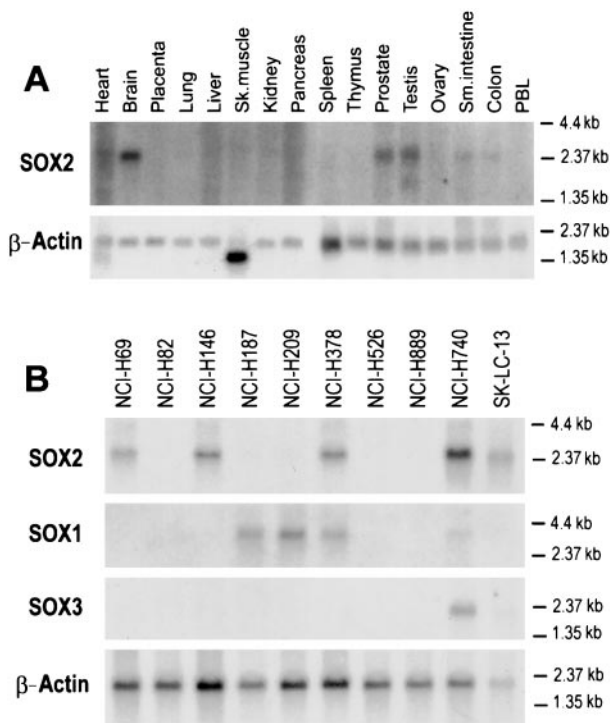
SEREX of the second SCLC line SK-LC-13 with the same pooled sera from SCLC patients resulted in the identification of 14 clones corresponding to 10 genes (Table 2). Four genes were identical to those isolated from NCI-H740, and six were distinct (*NY-SCLC-9* to *NY-SCLC-14*). *SOX2* was isolated twice, and *SOX3* and *SOX21* each were represented by a single clone. *ZIC2* was isolated twice. Other genes isolated in both SK-LC-13 and NCI-H740 libraries were *ID4*, isolated once, and *MPP11*, which was represented by three immunoreactive clones. *NY-SCLC-11* (*KIAA0963*) is an unknown gene with identical expressed sequence tag sequences derived from many tissues. Two novel genes (*NY-SCLC-13* and *NY-SCLC-14*) showed no sequence identity to current GenBank entries. These two genes are intriguing because their DNA sequences contain homopolymers

**Table 2. Genes isolated by SEREX of the SCLC cell line SK-LC-13**

Gene designation	Gene/sequence identity (GenBank accession no.)	No. of clones (% of total)
<i>NY-SCLC-1</i>	<i>SOX2</i> (Z31560)	2 (14)
<i>NY-SCLC-9</i>	<i>SOX3</i> (X71135)	1 (7)
<i>NY-SCLC-10</i>	<i>SOX21</i> (AF107044)	1 (7)
<i>NY-SCLC-3</i>	<i>ZIC2</i> (AF104902)	2 (14)
<i>NY-SCLC-4</i>	<i>ID4</i> (U28368)	1 (7)
<i>NY-SCLC-6</i>	<i>MPP11</i> (X98260)	3 (21)
<i>NY-SCLC-11</i>	<i>KIAA0963</i> (AB023180.1)	1 (7)
<i>NY-SCLC-12</i>	<i>LAG-3</i> (X51985)	1 (7)
<i>NY-SCLC-13</i>	<i>Novel-1</i>	1 (7)
<i>NY-SCLC-14</i>	<i>Novel-2</i>	1 (7)
		Total: 14







**Fig. 3.** Northern blot analysis of *SOX* group B gene expression. *SOX2* expression in normal tissues (A) and *SOX1*, 2, and 3 expression in SCLC cell lines (B). A  $\beta$ -actin probe was used to assess RNA quality and quantity. SCLC blots were exposed for 24 hr (*SOX2*), 72 hr (*SOX1*), or 1 week (*SOX3*). Normal tissue blot for *SOX2* was exposed for 1 week.

**Expression of *SOX* Group B Genes.** Because *SOX* group B genes are intronless (29–32), their gene expression was evaluated by Northern blot analysis. Among normal tissues, *SOX2* mRNA could be detected in brain, testis, and prostate and at lower levels in small intestine and colon, but not in heart, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, ovary, and peripheral blood leukocytes (Fig. 3A). *SOX1*, *SOX3*, and *SOX21* mRNA was not detected in normal adult tissues, which is

consistent with the current literature (33, 34). In tumor cell lines, *SOX2* was expressed in 5 of 10 SCLC cell lines, including NCI-H740 and SK-LC-13 (Fig. 3B). *SOX2* message was not detected in three NSCLC lines (SK-LC-7, 8, and 17) or in eight melanoma cell lines (SK-MEL-10, 12, 14, 24, 26, 28, and 37 and Mz19) (data not shown). *SOX1* mRNA was detected in 4 of 10 SCLC cell lines, *SOX3* mRNA was detected in 2 of 10 lines, and *SOX21* was not detected even after prolonged exposure (1 week) (Fig. 3B). *SOX1* and *SOX3* required longer exposure times than *SOX2*, indicating lower expression levels. The two variant SCLC cell lines, NCI-H82 and NCI-H526, had no detectable *SOX* group B gene expression.

**High-Titer Antibodies to *SOX* and *ZIC2* Proteins in SCLC Patients.** Sera from 17 SCLC patients and 23 normal adults were titrated against phage clones coding for *SOX1*, 2, 3, or 21 or *ZIC2*. Only 1 of the 23 normal sera showed weak reactivity against *SOX2* (titer, 1:1,000). In contrast, 7 of 17 SCLC patients (41%) had antibodies reactive with *SOX1*- and *SOX2*-containing phagemids, whereas 29% (5 of 17) and 35% (6 of 17) had antibodies to *SOX3* and *SOX21*, respectively. Five of 17 (29%) patients had detectable anti-*ZIC2* antibodies. Antibody titers measured up to 1:10<sup>6</sup> (Table 4 and Fig. 4). The five patients with antibodies against *ZIC2* also had reactivity against *SOX* proteins; one (Lu113) showed high-titered reactivity (1:10<sup>6</sup>), whereas another (Lu139) was reactive at a 1:10<sup>3</sup> dilution. Two patients (Lu100 and A6) with strong *SOX1* and *SOX2* reactivity showed no reactivity with *ZIC2* (Table 4 and Fig. 4). All patients with antibodies against *SOX3* or *SOX21* had higher reactivity against *SOX1* and *SOX2*. The presence of consistently higher-titer antibodies against *SOX1* and 2 suggests *SOX1* and/or 2 as the main immunogenic tumor antigen in these patients; seroreactivity to *SOX3* and *SOX21* might be secondary to the shared antigenic epitopes located within the highly conserved HMG box of *SOX* proteins.

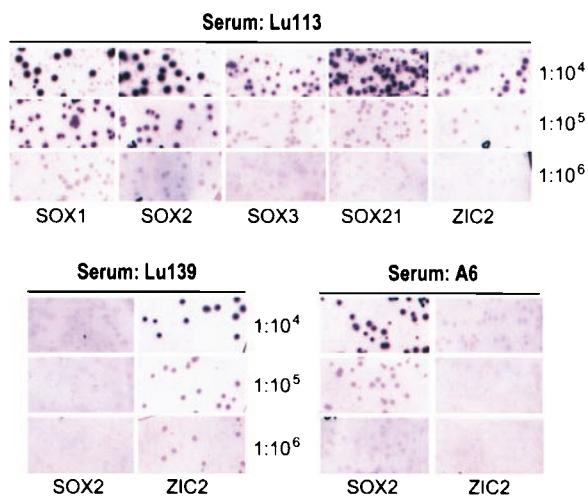
## Discussion

*SOX* family proteins (Sry-like HMG box), currently with more than 30 members in vertebrates, belong to the HMG box superfamily of DNA-binding proteins (33, 34). They originally were identified based on their homology within the HMG box to the prototype protein *SRY*, encoded by the testis-determining gene on chromosome Y (35). Within the DNA-binding 79-aa

**Table 4.** *SOX* and *ZIC2* reactivity of sera from SCLC patients

Patient	Serum/ protein	<i>SOX1</i>	<i>SOX2</i>	<i>SOX3</i>	<i>SOX21</i>	<i>ZIC2</i>
1	Lu 94*	1:10 <sup>5</sup>	1:10 <sup>5</sup>	1:10 <sup>4</sup>	1:10 <sup>4</sup>	1:10 <sup>5</sup>
2	Lu 100*	1:10 <sup>5</sup>	1:10 <sup>5</sup>	1:10 <sup>4</sup>	1:10 <sup>4</sup>	—
3	Lu 101*	—	—	—	—	—
4	Lu 104*	—	—	—	—	—
5	Lu 113*	1:10 <sup>6</sup>	1:10 <sup>6</sup>	1:10 <sup>5</sup>	1:10 <sup>5</sup>	1:10 <sup>5</sup>
6	Lu 139	1:10 <sup>3</sup>	1:10 <sup>3</sup>	—	—	1:10 <sup>6</sup>
7	Lu 159	—	—	—	—	—
8	A1	1:10 <sup>5</sup>	1:10 <sup>6</sup>	1:10 <sup>5</sup>	1:10 <sup>5</sup>	1:10 <sup>4</sup>
9	A2	—	—	—	—	—
10	A3	—	—	—	—	—
11	A4	—	—	—	—	—
12	A5	—	—	—	—	—
13	A6	1:10 <sup>5</sup>	1:10 <sup>5</sup>	1:10 <sup>5</sup>	1:10 <sup>4</sup>	—
14	A7	—	—	—	—	—
15	A8	—	—	—	—	—
16	A9	—	—	—	—	—
17	A10	1:10 <sup>4</sup>	1:10 <sup>4</sup>	—	1:10 <sup>5</sup>	1:10 <sup>4</sup>
		7/17 (41%)	7/17 (41%)	5/17 (29%)	6/17 (35%)	5/17 (29%)

\*Sera contributing to serum pool used in SEREX of the SCLC cell lines.



**Fig. 4.** Seroreactivity of SCLC patients (Lu113, Lu139, and A6) for SOX1, 2, 3, and 21 and ZIC2. Serum dilutions and cloned gene products are indicated.  $\lambda$ ZAP phages without insert are mixed with test clones and serve as internal negative controls; these are visible as a background to the positive clones (see 1:10<sup>4</sup> dilution of Lu113 serum). Assays are scored positive only if test clones are clearly distinguishable from control phages. Lu113 seroreactivity can be observed up to 1:10<sup>6</sup> for SOX1 and SOX2 and 1:10<sup>5</sup> for SOX3, SOX21, and ZIC2. Lu139 serum showed strong reactivity with ZIC2 but not SOX2, and A6 serum is reactive with SOX2 but not ZIC2.

HMG box domain, SOX proteins share more than 50% identity. They are classified into groups A–G, with SRY being the sole member of group A (33, 34). Within each group, the HMG sequences are more than 90% identical and the homology extends into N- and C-terminal regions. Group B SOX proteins (SOX1, 2, 3, 14, and 21) bind similar DNA sequences (32, 36). SOX1, 2, and 3 have been shown to be transcriptional activators (37), and SOX3 recently has been shown to regulate Wnt signaling in *Xenopus* by binding to  $\beta$ -catenin (38). Whereas the mouse *Sox2* knockout is embryonic lethal (39), *Sox1* knockout mice are viable at birth but develop seizures and are defective in eye and lens development (40).

The expression of human SOX 1, 2, 3, and 21 gene counterparts in other vertebrates has been studied in detail (33, 34). They are generally expressed within the developing nervous system of the vertebrate embryo, and their expression either ceases or is reduced at later stages. The genes have spatial and temporal patterns of expression that are distinct but mostly overlapping. Chick *Sox3* (*cSox3*) is detectable throughout the epiblast (the layer from which the embryonic tissues will develop) during Hamburger–Hamilton (HH) stage 1 before gastrulation (41), and *cSox2* is first expressed in the presumptive neural ectoderm at HH stage 3–4 (41, 42). In both chicken and mouse, *Sox1*, 2, 3, and 21 are expressed predominantly in the developing central nervous system and are down-regulated as neural differentiation progresses (36, 37, 39, 42–46). Outside the nervous system, low-level mouse *Sox* group B gene expression has been reported in gut epithelium, developing limb buds, and genital ridges of the embryo (36, 47). Although this embryonic expression pattern generally holds true for all species examined, some interspecies variations in temporal expression of *Sox* group B genes exist. For example, in contrast to chick *Sox2*, mouse *Sox2* is expressed before gastrulation. Also, whereas *Sox3* is expressed in the mouse gonads, chick and *Xenopus Sox3* are not (36, 43–45, 47).

The *ZIC* genes (Zinc-finger gene of the cerebellum), currently with four members, also encode DNA-binding proteins (48–50). *ZIC* proteins are structurally similar to each other, particularly

within a 150-aa domain that encodes five Zinc-finger motifs (51). Mouse *Zic* genes, although unrelated to *Sox* group B genes, have similar temporal expression patterns, and *Sox2* and *Zic1* are subject to common developmentally regulatory elements (52). All *Zic* genes are expressed from the gastrula stage on and show different temporal expression patterns (51, 53). *Xenopus Zic2* expression commences earlier than *XZic1* and 3 (51). In the adult mouse, *Zic2* and 3 expression is restricted to the granule cells of the cerebellum, whereas *Zic1* expression is detectable in the olfactory bulb and cerebrum in addition to the cerebellum (48, 50). Mutations in human *ZIC2* and *ZIC3* are related to holoprosencephaly and situs abnormalities, respectively, whereas *mZic1* knockout mice develop severe ataxia because of a hypoplastic cerebellum (54–56).

Two possibilities could account for the expression of *SOX* and *ZIC2* in SCLC. One is that the normal progenitor cell of SCLC, generally presumed to be the neuroendocrine Kulchitsky cell (57), expresses *ZIC2* and *SOX* group B genes; thus, the expression of these antigens in SCLC represents the persistence of these differentiation characteristics during neoplastic clonal expansion. Although we have not detected *ZIC2* and *SOX* group B expression in normal lung, this cannot be taken as evidence for absence of expression in normal Kulchitsky cells, because this is a rare cell population poorly represented in normal adult lung (in contrast to a greater abundance of Kulchitsky cells in fetal and neonatal lung). The other possibility is that *SOX* group B and *ZIC2* genes are not expressed in normal adult Kulchitsky cells or bronchial epithelium and that the expression of these genes in SCLC represents a reactivation of lineage-specific embryonic markers, reflecting the developmental stage at which *ZIC2* and *SOX2* are coexpressed. Neither of the two explanations, however, can account for the finding that the *ZIC2* gene is expressed in many cancer types unrelated to neuroendocrine lineage. This suggests that various gene activation or derepression mechanisms are involved in the tumor expression of *ZIC2* and maybe *SOX* genes.

From an immunological standpoint, the high frequency and high titers of anti-*SOX* and anti-*ZIC2* antibodies in these SCLC patients are striking. At present, we can only speculate as to why these antigens elicit such a strong humoral immune response. A likely reason is that SCLC expresses a range of neuroectodermal antigens, including *SOX* group B and *ZIC2*, primarily restricted to the nervous system and that immune tolerance has not been established against these antigens. Growing outside the brain–blood barrier, SCLC would provide a potent immunogenic stimulus to the host. In our series, anti-*ZIC2* antibody was observed in 29% and anti-*SOX* antibody was observed in 41% of the SCLC sera. These sera were collected from a heterogeneous group of SCLC patients who were at different stages of their diseases, receiving various treatments, and having variable responses; indeed, one of the antibody-positive patients (Lu113) had no clinical evidence of residual disease when serum was collected, but had a subsequent recurrence of tumor. A large-scale survey of anti-*SOX* and anti-*ZIC2* antibodies in SCLC patients, correlating the seroreactivity with tumor stage, treatment, and survival, is needed to explore the clinical significance and possible utility of these antibodies as tumor markers.

In addition to the immunodiagnostic potential of *SOX* group B and *ZIC2* gene products, the possibility that these antigens could serve as cancer vaccine targets should be considered. The expression of these genes in brain is a major concern, particularly given the paraneoplastic syndromes associated with SCLC (8, 9). However, despite the presence of high-titer anti-*SOX* and anti-*ZIC2* antibodies, none of the seven antibody-positive patients in this study had neurological manifestations related to the disease. In fact, the only patient in this series with a paraneoplastic syndrome involving the cerebellum (patient A9) had no detectable anti-*SOX* group B or *ZIC2* antibodies. Thus, immune

responses against SOX group B and ZIC2 may not lead to autoimmune neurological disorders, but this must be explored in a much larger series of patients. Because *SOX* and *ZIC2* genes are conserved in mice, *SOX* and/or *ZIC2* vaccination can be assessed in an experimental model. Indeed, HuD antigen, one of the neuronal antigens associated with paraneoplastic syndromes (8, 9), recently has been used as a vaccine target in a murine model of SCLC, and antitumor activity was observed without neurological disease (58, 59). In considering immunological approaches to the therapy of SCLC, a major obstacle is obtaining tumor tissue for antigen analysis. SCLC usually is diagnosed by endoscopic biopsies rather than surgical resection, and an ade-

quate specimen for RNA extraction and RT-PCR typing cannot be obtained in most cases. A further complication is the fact that *SOX Group B* genes are intronless and RT-PCR typing is often unreliable. Immunohistochemical typing with specific antibody reagents should circumvent these problems, and the generation of mAbs against individual *SOX* and *ZIC2* gene products is underway.

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