ECSA/DPPA2 is an Embryo-Cancer Antigen that Is Coexpressed with Cancer-Testis Antigens in Non–Small Cell Lung Cancer

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Abstract

Purpose: Cancer cells recapitulate many behaviors of pluripotent embryonic cells such as unlimited proliferation, and the capacity to self-renew and to migrate. Embryo-cancer sequence A (ECSA), later named developmental pluripotency associated-2 (DPPA2), is an embryonic gene initially isolated from pluripotent human preimplantation embryos. We hypothesized that ECSA/DPPA2 would be quiescent in most normal tissues but expressed in cancers and may therefore be a useful target for immunotherapy.

Experimental Design: ECSA/DPPA2 expression was examined in a panel of normal and tumor tissue by reverse transcription PCR, quantitative real-time PCR, and immunohistochemistry. A panel of 110 non–small cell lung cancers (NSCLC) were further investigated for the presence of ECSA/DPPA2 transcripts and several cancer testis antigens (CTA). Sera from 104 patients were analyzed for spontaneous ECSA/DPPA2 antibody production by ELISA and Western blot.

Results: ECSA/DPPA2 transcripts were limited to normal testis, placenta, bone marrow, thymus, and kidney but expressed in a variety of tumors most notably in 30% of NSCLC. Enrichment for CTAs in ECSA/DPPA2-positive NSCLC was observed. Immunohistochemistry confirmed nuclear and cytoplasmic localization in subpopulations of cells with coexpression of the CTA MAGE-A3. Antibodies to recombinant ECSA/DPPA2 protein were detected in the sera of 4 of 104 patients with NSCLC but not in healthy controls.

Conclusions: The restricted expression in normal tissues, expression in tumors with coexpression of CTAs, and spontaneous immunogenicity indicate that ECSA/DPPA2 is a promising target for antigen-specific immunotherapy in NSCLC.

Despite improvements in the treatment of non–small cell lung cancer (NSCLC), current approaches such as chemotherapy and radiotherapy have had little effect on survival for patients with advanced disease (1–3). An improvement in the understanding of molecular processes involved in pulmonary carcinogenesis has led to new treatment options with targeted small molecules and vaccines demonstrating encouraging potential (4–8). The heterogeneity of clinical outcome in lung cancer patients with similar stage disease, spontaneous regression of tumors and improved survival among patients with tumor-infiltrating lymphocytes, and those who develop empyemas provide evidence that immune responses may influence outcome in NSCLC patients (9–11). Utilizing the immune system to target lung carcinoma is an approach that has yet to reach its full potential; however, it is a modality clearly warranting further exploration.

Cancer testis antigens (CTA) are a group of antigens, many of which are immunogenic (12), characterized by restricted expression in testis but aberrant expression in a variety of cancer types including NSCLC (13). These properties render them attractive candidates for cancer vaccines, although their function is largely unknown. Indeed, vaccination with either the full-length recombinant CTA NY-ESO-1 protein, or CD4- or CD8-restricted peptide epitopes enhances anti–NY-ESO-1 reactivity (14, 15) such that tumor regression has been achieved in isolated cases (16).

Embryogenesis involves a deprogramming or erasure of the epigenetic information governing differentiated cell behavior, thus returning the cell to the proliferative, undifferentiated, stem cell state (17–19). By the blastocyst inner cell mass and the primordial germ cell stages, the embryonic cells are totipotent stem cells capable of giving rise to immortal cell lines in vitro and teratomas in vivo. Embryonic genes that are active at this stage may be associated with similar properties of deprogramming, maintenance of the undifferentiated cell state, proliferation, invasiveness, and indefinite growth of cancer
cells. The identification of several embryo-cancer transcripts that are expressed in human preimplantation embryos, absent in normal differentiated somatic tissues, but re-expressed in tumor tissue supports this hypothesis (20). To search for novel molecules that may be immunotherapeutic targets, we explored genes known to be expressed in the preimplantation embryo but not expressed in differentiated somatic tissues. Of these transcripts, embryo-cancer sequence A (ECSA) subsequently entered into gene databases as developmental pluripotency associated-2 (DPPA2) has been the focus of this current study. This gene maps to chromosome 3q13 over 8 exons and encodes a protein product of 297 amino acids. The primary protein structure contains a SAP motif and localizes to the nucleus.

In this study, we show specific expression of this novel embryo-cancer antigen in a subpopulation of putative stem cells in NSCLC and show its ability to invoke spontaneous immune responses in vivo. We also show that ECSA/DPPA2 is coexpressed with many CTAs.

### Materials and Methods

**Patients and clinical specimens.** Two hundred tumor samples representing a panel of malignancies at various clinical stages snap frozen in liquid nitrogen and stored at -80°C within the Ludwig/Austin tissue bank under Institutional Review Board–approved protocols were used for the extraction of total RNA. A further 110 tumor specimens for which 104 paired sera were collected and stored under Institutional Review Board–approved protocols from patients with NSCLC who underwent surgery between 1991 and 2004 at the Department of Cardio-Thoracic Surgery, Weill Cornell Medical College, New York were used for screening and serologic investigations.

Total RNA from normal tissues including brain, placenta, liver, heart, kidney, lung, bone marrow, colon, small intestine, spleen, stomach, and thymus were obtained from Clontech Laboratories.

Normal cadaveric tissues including brain, liver, heart, kidney, colon, stomach, and lung from five individual donors obtained under Institutional Review Board–approved protocols were investigated for ECSA/DPPA2 protein using immunohistochemistry.

**RNA extraction and cDNA synthesis.** Total RNA was isolated from frozen tumor tissue using RNeasy kits (Qiagen) or Ambion kits (Invitrogen) for 60 min at 42°C. Reverse transcriptase was omitted from each PCR reaction with a final concentration of 2 mmol/L magnesium chloride.

**PCR.** One microliter of cDNA (100 ng of total RNA) was used in each PCR reaction with a final concentration of 2 mmol/L magnesium chloride, 0.02 mmol/L deoxyxynucleoside triphosphate (Applied Biosystems), 40 units of RNase inhibitor, and 10 units Moloney murine leukemia virus reverse transcriptase (Invitrogen) for 60 min at 42°C. Reverse transcriptase was omitted for negative controls.

**Quantitative real-time PCR.** Introns-spanning multiplex assays were designed for quantitative real-time PCR using the Universal Probe Library assay design center4 (Roche). All reactions were carried out in duplicate using the ABI 7700 Prism Sequence Detector (Applied Biosystems). Primers used were ECSA-L, 5'-AGACCAGATTACAGCGATGT, which amplified a gene fragment of 430 bp in the Cornell Champions Of Disease database (CDOE) and thymus were obtained from Clontech Laboratories. Normal cadaveric tissues including brain, liver, heart, kidney, colon, stomach, and lung from five individual donors obtained under Institutional Review Board–approved protocols were investigated for ECSA/DPPA2 protein using immunohistochemistry.

**Table 1. Primers and conditions used for conventional reverse transcription-PCR reactions**

<table>
<thead>
<tr>
<th>Designation</th>
<th>Sequence (5' to 3’)</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NY-ESO-1 F</td>
<td>CAGGGCTGTAATGATGTCGAGA</td>
<td>322</td>
</tr>
<tr>
<td>NY-ESO-1 R</td>
<td>GCCCTCTGCGTGAGGAGG</td>
<td>338</td>
</tr>
<tr>
<td>LAGE-1 F</td>
<td>CTGCTCCAGGAGAAGGTGCC</td>
<td>421</td>
</tr>
<tr>
<td>LAGE-1 R</td>
<td>GCCTCCTTGCGCTTGGAGGAGC</td>
<td>423</td>
</tr>
<tr>
<td>MAGE-A1 F</td>
<td>GCTGGAAACCCTACTGGTGGGCC</td>
<td>446</td>
</tr>
<tr>
<td>MAGE-A1 R</td>
<td>GCCGCCAGAAACCTGCTGACC</td>
<td>446</td>
</tr>
<tr>
<td>MAGE-A3 F</td>
<td>GAAGCCGCCGCAGGTCCTG</td>
<td>92, 410, 500</td>
</tr>
<tr>
<td>MAGE-A3 R</td>
<td>GAGTCTCTCATGGATTGTCGCT</td>
<td>434</td>
</tr>
</tbody>
</table>

**NOTE:** Shaded primers detect CTAs not located on the X chromosome.

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3 http://www.roche-applied-science.com/sis/rtpcr/upl/adc.jsp
of 94°C for 20 sec, and 60°C for 45 sec. All results were normalized to 18S amplification (Applied Biosystems). Relative expression was calculated using the target threshold value for testis or normal lung as a calibrator (21).

Generation of recombinant ECSA/DPPA2 protein and antibodies. For prokaryotic expression construction of ECSA/DPPA2, the template vector pBluescriptR containing the cDNA clone BC018070 was PCR amplified with the following primers: 5'-TTTT GCA GCC ATG TTA TCG GGT GTG CTA CTA CTT TTC TCT CTC CAC TTG CAT TAA (italics indicating the restriction sites), corresponding to nucleotides 126 to 1,022. PCR-amplified products were inserted into the Escherichia coli expression vector PGE-4T, between BamH1 and XhoI restriction sites. The fusion protein (amino acids 1-298) was translated in vitro from the start codon of the vector. After sequence verification, the prokaryotic expression vector pGEX-4T-DPPA2 was introduced into Escherichia coli, between BamH1 and XhoI restriction sites. The fusion protein (amino acids 1-298) was translated in-frame from the start codon of the vector. After sequence verification, the prokaryotic expression vector pGEX-4T-DPPA2 was introduced into the bacteria host E. coli following standard protocol, and the expression of fusion protein was induced by adding isopropyl-1-thio-β-D-galactopyranoside. The fusion protein was purified using the glutathione S-transferase tag. After 10% SDS-PAGE analysis, a band of 60 kDa was found from the sample of fusion proteins purified by glutathione-sepharose beads.

For antibody production, New Zealand rabbits were injected s.c. with 50 µg of the ECSA/DPPA2 antigen described above. For the first immunization, the antigen was admixed 1:1 with complete Freund’s adjuvant; for the next four boosts (on days 28, 42, 60, and 78), incomplete Freund’s adjuvant was used. Anti-ECSA/DPPA2 antibody production was determined by testing in an ELISA.

Affinity purification was achieved by coupling 1 mg of fusion protein to activated Sepharose 4B beads (Pharmacia) in accordance with the manufacturers’ instruction. After conjugation, the beads were washed thrice with PBS and 25 mL of rabbit antiserum was added to the beads. The 5-mL column was washed once with 1 mol/L Tris-HCl (pH 8.0) and once with 1 mol/L Tris HCl (pH 5.0). The specific antibody was then eluted in 1 mol/L Tris HCl (pH 2.5).

ELISAs. Detection of specific serum antibody to ECSA/DPPA2 and NY-ESO-1 were done by indirect ELISA. Briefly, FluoroNunc Maxisorp ELISA plates were coated with 1.5 µg/mL of recombinant ECSA/DPPA2 or 3 µg/mL NY-ESO-1 protein (50 µL per well) and incubated overnight at 4°C. After washing with 0.2% Tween 20 and blocking with 0.1% human serum albumin (blocking buffer), sera diluted in blocking buffer at a 1:400 dilution were incubated at room temperature for 1 h. As the recombinant proteins were produced in E. coli bacteria, all sera were preadsorbed with E. coli lysates for 1 h at 4°C to remove potential contamination of serum E. coli antibodies that are often found in normal sera. After further washing, alkaline phosphatase–conjugated affinity-purified sheep anti-human IgG (Chemicon) diluted in blocking buffer at 1:1,000 dilution was added for 1 h at room temperature. P-nitro-phenyl phosphate substrate, carbonate buffer, and 2% magnesium chloride was added for 30 min at room temperature, and development stopped using 3 mol/L sodium hydroxide. Excitation at 450/50 and emission 580/50 with gain of 25 was read using an ELISA plate reader ( Molecular Devices). For detection of ECSA/DPPA2 protein, 5-µm sections from stored-frozen tissues were cut and fixed by immersing in cool acetone for 30 min. For paraffin sections, 4-µm formalin-fixed sections were prepared and dried overnight at 37°C. These were dewaxed in xylene and rehydrated through alcohols followed by water bath antigen retrieval for 20 min using EDTA buffer (pH 8.0; NeoMarkers). Endogenous peroxidase activity was quenched with 0.3% hydrogen peroxide for 10 min. After 10 min blocking with Maxtags protein-blocking agent (Immunon; Thermo Shandon), sections were incubated with affinity-purified ECSA/DPPA2-specific rabbit polyclonal antibody diluted in PBS at 1:400 dilution for 1 h at room temperature. Negative controls omitting the primary antibody and with a rabbit polyclonal antibody control (Vector Laboratories) were also incubated in parallel. Dako Envision+ horseradish peroxidase–labeled polymer (DakoCytomation) was added and incubated at room temperature for 30 min. Immunodetection was achieved by incubating the slides in 3-aminophenylcarbazole (Sigma-Aldrich) and counterstaining with Mayer’s hematoxylin (Amber Scientific) and Scott’s tap water before coverslipping.

NY-ESO-1 (E978), MAGE-A3 (GC1), and MAGE-C1 (CT7-33) antibodies were obtained from the Ludwig Institute for Cancer Research and used at a concentration of 2.5 µg/mL for E978 and a 1:40,000 dilution for CT7-33. Antigen retrieval was done for 20 min using EDTA buffer (pH 8.0; NeoMarkers) for E978 and citrate buffer (pH 6.0; NeoMarkers) for CT7-33. Dako Envision+ horseradish peroxidase–labeled polymer (DakoCytomation) was used as the secondary antibody and immunodetection, done as described above. A mouse IgG1 isotype control (Southern Biotech) was incubated in parallel. For double staining, sections were treated as described. ECSA/DPPA2-specific rabbit polyclonal antibody was diluted 1:800 and stained using Envision+ horseradish peroxidase–labeled polymer (DakoCytomation) as the secondary antibody and 3-3 Diaminobenzidine (Sigma-Aldrich) as the chromagen. NY-ESO-1 (E978) was used at
the concentration described previously, and stained using the Envision+ AP-labeled polymer (DakoCytomation) and visualized using Fuchsin (DakoCytomation) as the chromogen.

Images were acquired using an Axioskop 2 microscope, Axiocam HRc camera, and Axiovision v3.1 software (CarlZeiss Vision).

Results

Normal and malignant tissue expression of ECSA/DPPA2.

Among normal tissues, ECSA/DPPA2 transcripts were primarily found in testis, although low-level expression was also found in placenta, bone marrow, thymus, and kidney (Fig. 1). Expression in the kidney was not confirmed by immunohistochemistry of normal kidney sections (data not shown). The transcript was present in 8 of 27 (30%) NSCLC from the Ludwig/Austin data set and 33 of 110 (30%) of tumors screened from the Cornell University data set, as well as in a small percentage of melanomas, colorectal cancers, and lymphomas (Fig. 2A). There was no obvious correlation between ECSA/DPPA2 expression and histologic classification of the different lung cancers (Fig. 2B). The ECSA/DPPA2 gene transcript was present in squamous cell, adenocarcinoma, and large cell tumors, although squamous cell carcinomas expressed the gene at higher levels than the other histologic subtypes. The degree of differentiation did not seem to correlate with ECSA/DPPA2 expression.

Fig 2. Distribution of ECSA/DPPA2 transcripts in a panel of tumor tissue using conventional PCR (A). Given 30% expression in NSCLC, quantitative real-time PCR was done to determine expression levels using samples from both the Ludwig/Austin and Cornell University data set (B). NTC, no template control; SqCC, squamous cell carcinoma; Adenoca, adenocarcinoma; N lung, normal lung.
expressed in lung cancer MAGE-A3, NY-ESO-1, and MAGE-C1 (Fig. 3D-F). These panels show that compared with ECSA/DPPA2, MAGE-A3, NY-ESO-1, and MAGE-C1 all stain many more cells within the tumor, although still within defined areas, and in the case of MAGE-A3, it is clear that the ECSA/DPPA2-staining cells are a subset of the MAGE-A3-positive population.

**ECSA/DPPA2 is coexpressed with CTAs.** In view of the immunohistochemical findings of a possible relationship between the expression of ECSA/DPPA2 and the CTAs, we sought to further characterize any correlation with CTA expression by PCR. Figure 4 shows that the ECSA/DPPA2-positive tumors expressed those CTAs located on the X chromosome, such as the MAGE family, LAGE, and NY-ESO-1, at a much higher rate than the ECSA/DPPA2-negative tumors ($\chi^2$ test, $P = 0.001$). MAGE-A4 in particular was expressed in 85% of ECSA/DPPA2-positive tumors compared with only 15% of ECSA/DPPA2-negative tumors. BORIS, a CTA not located on the X-chromosome, was also more likely to be expressed in ECSA/DPPA2-positive tumors (Fig. 4).

**ECSA/DPPA2 is immunogenic.** Given the coordinate expression of ECSA/DPPA2 transcripts with CTAs, we investigated whether ECSA/DPPA2 was spontaneously immunogenic in NSCLC patients. High-affinity IgG antibodies were detected in 2 of 26 patients whose tumors expressed ECSA/DPPA2, 2 of 78 whose tumors were negative for ECSA/DPPA2 but in none of the 18 normal sera screened (Fig. 5). Positive ECSA/DPPA2 ELISA results were confirmed by Western blot, which showed immunoglobulin binding to a 60-kDa recombinant ECSA/DPPA2 protein band (data not shown).

Because both recombinant proteins were produced in *E. coli*, NY-ESO-1 protein was used as a specificity control, as it was possible that responses to the ECSA/DPPA2 protein were against contaminating bacterial protein. Thirteen patients including those known to be seropositive for ECSA/DPPA2 antibody and NY-ESO-1 antigen were screened for NY-ESO-1 antibodies using an ELISA based on NY-ESO-1 protein, which was similarly produced in an *E. coli* expression system. Figure 5 shows that ECSA/DPPA2-positive sera were negative for NY-ESO-1. Two serum samples contained high-affinity antibodies to NY-ESO-1, but there was no overlap with ECSA/DPPA2-positive samples, indicating that these were likely true NY-ESO-1-specific antibody responses.

**Discussion**

We have described an embryo-derived gene, ECSA/DPPA2, as a human cancer antigen that is predominantly expressed in NSCLC but also in other malignancies including colorectal cancer, lymphoma, and melanoma. It is expressed in a subpopulation of putative stem cells, seems to induce spontaneous immune responses in lung cancer patients, and shows a unique association with the majority of the tested CTAs. These characteristics suggest its potential as a therapeutic target.

ECSA was initially described in human preimplantation embryos, primordial germ cells, and several somatic tumors by Monk and Holding (20). Using a bioinformatics approach in the mouse, a homologous gene, *Dppa2*, was independently

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**Fig. 3.** Immunohistochemistry staining using ECSA/DPPA2 antibody (brown). In the testis, ECSA/DPPA2 stains the nucleus of spermatogonia located on the basement membrane of the seminiferous tubules, but staining is lost in the more differentiated cells closer to the tubular lumen (A). In a squamous cell NSCLC, ECSA/DPPA2 stains both the nucleus and cytoplasm of basally located tumor cells abutting stroma (B). Staining in a different squamous cell NSCLC with ECSA/DPPA2 (C) and MAGE A3 (D). MAGE A3 positively stains the nucleus and cytoplasm in a larger area of the tumor, whereas ECSA/DPPA2 is coexpressed in a smaller cluster of cells. NY-ESO-1 (E) and MAGE C1 (F) staining in the same NSCLC (B), again demonstrating a larger area of staining compared with the basal subpopulation of cells seen with ECSA/DPPA2. Bar, 100 $\mu$m.
identified to have an expression pattern similar to Oct3/4 (23); one of several molecules including NANOG and SOX2 (24), which are characteristically present in pluripotent stem cells. Another molecule that shares this expression pattern in the mouse, Dppa4, lies 16-kb upstream of Dppa2 and encodes a closely related SAP domain (23, 25). These domains are thought to be involved in RNA metabolism and in the organization of nuclear architecture, suggesting that both may be involved in controlling cellular plasticity (26). Dppa2 and Dppa4 are both found exclusively in pluripotent cells; however, a recent study in murine embryonic stem cells suggests that Dppa4 may not be critical in maintenance of the pluripotent phenotype as its overexpression did not inhibit cellular differentiation (27). Further characterization is therefore necessary to document the role of these molecules in pluripotent cells.

Germ cells also express a number of these molecules, so it is not surprising that NANOG, OCT3/4, GDF3, and STELLAR have been found in germ cell tumors (28–30). In contrast, few publications, limited to breast (31–34) and bladder cancer (35), implicate these molecules in tumors arising from somatic cells. Their expression in somatic tumors may underpin biological functions that cancers share with embryonic cells, primordial germ cells, and possibly adult stem cells, such as self-renewal and proliferative potential. Suppression of differentiation is a hallmark of pluripotent embryonic cells, and although cancer cells may not necessarily have the capacity to proliferate along multiple differentiated lineages, loss of differentiation is a common feature.

The relationship between the embryo-associated antigen ECSA/DPPA2 and the germ cell–associated CTAs requires further study. ECSA/DPPA2-positive tumors were highly enriched for the CTAs (Fig. 4), and we have shown coexpression of the CTA MAGE-A3 with ECSA/DPPA2 in lung cancer cells. The CTAs are not known to be markers of pluripotent stem cells, although their expression in germ cells and various cancers suggests a role in primitive cell populations. A recent review of the CTAs by Simpson et al. (12) provides a conceptual framework that links cancer with germ cell development. The authors suggest that the epigenetic dysregulation and subsequent derepression of germ cell programs that would normally be silenced in somatic cells may contribute to dedifferentiation and the subsequent malignant phenotype in cancer cells. The interaction we have

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**Fig. 4.** Enrichment for CTAs in ECSA/DPPA2-positive tumors. Primers for a panel of CTAs were used to investigate gene transcripts in resected NSCLC using conventional PCR. Tumors were grouped according to expression of ECSA/DPPA2 and then analyzed using a \( \chi^2 \) test to determine if the frequencies of CTA expression were significantly different between ECSA/DPPA2+ and ECSA/DPPA2- samples. Significant enrichment of X chromosome antigens and BORIS was evident in ECSA/DPPA2+ tumors.

**Fig. 5.** Detection of ECSA/DPPA2 antibody in the sera of NSCLC patients by ELISA. Three SDs above the mean absorbance in the normal sera was used as a cutoff for a positive result (horizontal dotted line). Positive samples were confirmed by Western blot. The sera are grouped according to expression of respective antigen (Ag) in the resected tumor as typed by PCR. The same sera were also incubated in parallel with NY-ESO-1 protein. Samples positive for ECSA/DPPA2 antibody were negative for NY-ESO-1 antibody (dashed arrows), and those positive for NY-ESO-1 antibody were negative for ECSA/DPPA2 antibody, demonstrating the specificity of the assay.
shown may be the result of the same epigenetic mechanisms that unmask gene expression programs in the malignant cell. In this case, the programs would be those also associated with stem cell function.

In grouping these molecules, we propose that ECSA/DPPA2 is best classified as an "Embryo-Cancer Antigen," as reflected by its association with embryogenesis rather than gametogenesis. Supporting this view is, first, that ECSA/DPPA2 is expressed in pluripotent embryonic cells, human embryonic stem cell lines, as well as primordial germ cells. In contrast, CTAs have not been reported to be expressed in human embryonic stem cells or preimplantation embryos. Second, ECSA/DPPA2 is coexpressed with other markers of pluripotent stem cells including OCT3/4 and NANOG (20, 25). The association between these molecules and ECSA/DPPA2 is closely linked, whereas a similar relationship with the CTAs has not been shown. Thus ECSA/DPPA2 seems to be a molecule that has unique associations because it belongs to a group of molecules that is implicated in pluripotent stem cells on the one hand and shares expression patterns in common with CTAs on the other.

Immunogenicity of these molecules in cancer patients has previously only been investigated for SOX2. Serum antibodies have been reported against SOX2 in meningioma and small cell lung cancer (36, 37), although it is unclear how these responses arose because SOX2 expression was not documented in the meningiomas, and expression in the small cell lung cancer patients was not investigated. A recent publication has also shown SOX2 antibodies in patients with monoclonal gammopathy of undetermined significance in which the SOX2-defined the clonogenic cells (38). In this study, the presence of serum antibody was also associated with T-cell responses and improved clinical outcome, although the numbers reported were small. It is intriguing that the pluripotent marker SOX2 marked the clonogenic cells and was immunogenic in monoclonal gammopathy of undetermined significance, a finding that parallels our own.

Spontaneous immune responses to pluripotent markers have not been previously described in NSCLC. Our data suggest that such responses do exist in a minority of patients, although their significance is as yet undefined. It is noteworthy that all of the sera tested were from resected early stage NSCLC, which potentially limited the exposure of tumor antigens to the immune system. It is unclear whether the two patients who were ECSA/DPPA2 antibody–positive but whose tumors did not seem to express ECSA/DPPA2 represent nonspecific antibody binding or whether their antibody responses reflect immune activation and "immunoediting" which could down-regulate tumor antigen expression (39).

Further studies will define the function and role of this antigen in NSCLC, stem cells, and gametogenesis and promise to provide important insight into pulmonary carcinogenesis. We believe ECSA/DPPA2 is a novel and promising therapeutic target for antigen-specific immunotherapy in lung cancer because it shows restricted expression in normal tissues, aberrant expression in a subpopulation of lung cancer cells, coordinate expression with X chromosome antigens and BORIS, and has the ability to generate spontaneous immune responses in a limited number of patients.
34. Ezeh UI, Turek PJ, Rejo RA, Clark AT. Human embryonic stem cell genes OCT4, NANOG, STELLAR, and GDF3 are expressed in both seminoma and breast carcinoma. Cancer 2005;104:2255–65.
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