Characterization of a Putative Ovarian Oncogene, Elongation Factor 1α, Isolated by Panning a Synthetic Phage Display Single-Chain Variable Fragment Library with Cultured Human Ovarian Cancer Cells

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Abstract

Purpose: In an effort to identify cell surface targets and single short-chain antibody (scFv) for ovarian cancer therapy, we used a phage display approach to isolate an antibody with high reactivity against ovarian cancer.

Experimental Design: A phage scFv library was subjected to panning against human SK-OV-3 ovarian cancer cells. A clone with high reactivity was selected and tested in immunoperoxidase staining on a panel of normal tissues and ovarian carcinoma. Using immunoprecipitation, a differentially expressed band was analyzed by mass spectrometry. The antigen subclass was characterized with reverse transcription-PCR on cDNA library of normal tissues, and 91 ovarian cancer specimens, and correlated with clinicohistopathologic characteristics.

Results: Ninety-six individual scFv clones were screened in ELISA following panning. scFv F7 revealed high reactivity with ovarian cancer cell lines and showed intense staining of 15 fresh ovarian cancer specimens and no staining of a panel of normal tissues. A 40-kDa protein was identified to be translation elongation factor 1α1 (EEF1A1; $P < 0.05$). The expression of EEF1A2, a highly homologous and functionally similar oncogene, was found to be restricted only to the normal tissues of the heart, brain, and skeletal muscle. Aberrant EEF1A2 mRNA expression was found in 21 of 91 (23%) of ovarian cancer specimens and significantly correlated with increased likelihood of recurrence ($P = 0.021$).

Conclusions: scFv F7 may represent an ovarian cancer–specific antibody against translation EEF1A family of translational factors. We propose that EEF1A2 may be a useful target for therapy of human ovarian cancer.

Epithelial ovarian cancer (EOC) is the leading cause of gynecologic cancer deaths in the United States (1). Although it is clear that most patients with EOC will respond to platinum- and paclitaxel-based chemotherapy, including complete responses, the relapse rate within 2 years is ~85% (2). Once relapse occurs, there is no known curative therapy and management becomes primarily palliative. Thus, there is a need to develop additional therapeutic approaches for the management of this disease. A proposed strategy for minimizing the risk of recurrent disease is immunotherapy. Patients who show complete response to frontline surgery and chemotherapy could be considered for immunotherapy, with the presumption that the majority do in fact have micrometastases. The development of successful immunotherapeutic or targeted therapy strategies requires the identification and characterization of ovarian tumor–associated antigens that will be recognized by the host immune system, leading to tumor rejection. Therefore, there is a need to identify and characterize novel antigens that may be useful for immunotherapy approach against this cancer (3).

Antibody therapy has long been sought after as a treatment strategy in cancer care. The classic approach to obtain tumor-specific antibodies has been to immunize mice with tumor cells and to screen the mouse serum for antibodies for their binding specificity. Inevitably, this also results in cross-reactivity with normal cells and restricts clinical application. The advent of antibody phage display technology (4) through the development of large (>6×10^9 clones) phage display libraries is an effective rapid method for identifying and creating antibodies to purified selected antigen targets (5–8). In contrast, panning cellular targets has been more difficult given lower antigen concentrations, antigen complexity, and the tendency of phage

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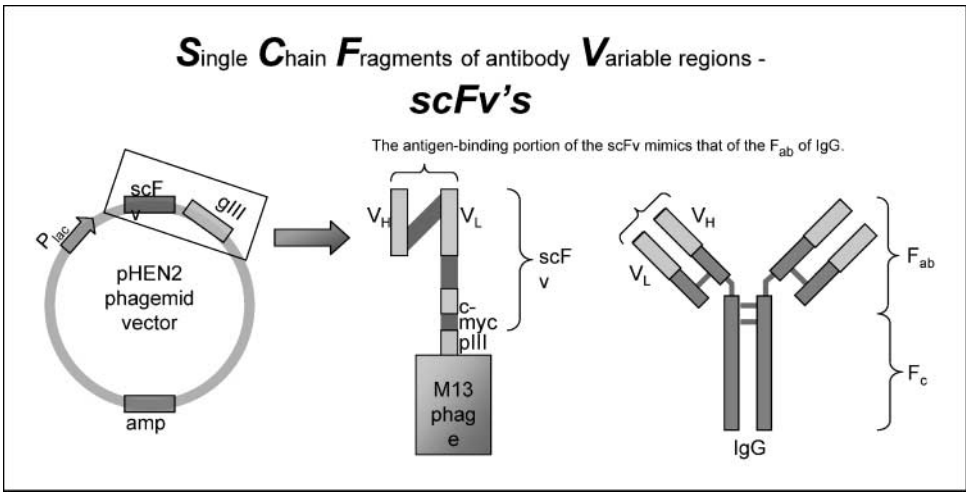

Fig. 1. A synthetic scFv phage library is initially constructed from naive human B cells and contains up to a billion different specificities. Each specific, unique single-chain fragment of an antibody variable region is composed of a random combination of one variable region of immunoglobulin light chain (*VL*) mimicking the Fab of IgG. This schematic represents the phagemid vector containing the scFv-gIII fusion gene. This encodes a scFv-pIII chimeric protein, which is expressed on the surface of phage containing the modified vector. This chimeric protein interacts with antigens in a similar fashion as the Fab fragment of a normal antibody, allowing selection for binding specificities.

to bind nonspecifically with cells (9). However, considerable progress has been made in this area (10–16), with most studies focusing on melanoma (14–17). Here, we report the isolation of a single-chain variable fragment (scFv) F7 with high reactivity against ovarian cancer cell lines and tumors and restricted reactivity against normal tissues. Further, we show that the antigenic target of scFv F7 belongs to the family of elongation factor 1α (EEF1A). The protein elongation machinery of elongation factor, EEF1A, has been implicated in cell transformation (18). EEF1A1 and EEF1A2 have been described as putative oncogenes and they are highly homologous (19). The functional significance of each specific factor and their interrelationship with another is not fully understood. Whereas EEF1A1 is relatively ubiquitous, EEF1A2 is a tissue- and tumor-restricted putative oncogene (19). These findings suggest that scFv F7 may represent an EOC-specific antibody against the translational factors and that specifically EEF1A2 may be a useful target for therapy of human ovarian cancer.

Materials and Methods

Synthetic phage scFv library. The synthetic phage scFv library (a gift from Dr. Greg Winter, Medical Research Council Center for Protein Engineering, Cambridge, United Kingdom) was constructed as described previously (8). Figure 1 represents a schematic of the phagemid vector containing the scFv-gIII fusion gene.

Cell lines. The human EOC cell lines SK-OV-3 and OV-CA-3, the human B lymphoid cells LG-2, the immortalized normal human ovarian surface epithelial cell line HOSE, the colon carcinoma cell line HT-29, the melanoma cell line SK-MEL-31, and the breast cancer cell lines MDA-MB-435 and MDA-MB-231 were grown in DMEM (Life Technologies) supplemented with 10% fetal bovine serum (BioWhittaker). All cell lines were maintained at 37°C in a 5% CO_2 atmosphere. Cell lysates were prepared by solubilizing the cells (1×10^7) that had been washed with HBSS (Life Technologies) in 1.2 mL of lysis buffer (10 mmol/L Tris, 1 mmol/L EDTA, 150 mmol/L NaCl, 1% NP40 containing 1 mmol/L phenylmethylsulfonyl fluoride). Silver-coated microtiter plates were prepared as described previously (20).

Panning of synthetic phage scFv library with ovarian cancer cells. The synthetic phage scFv library ($\sim 1 \times 10^{13}$ transducing units/2 mL PBS) was added to 2 mL of an SK-OV-3 ovarian cancer cell suspension (5×10^6 cells/mL of 4% dry milk-PBS). The cell suspension was incubated for 90 min at room temperature on a turntable and then stood at room temperature for 30 min. Following six washings with PBS, bound phages were eluted by incubating the cells with 200 μL of 76 mmol/L citric acid (pH 2.8) in PBS (10). After neutralization with 200 μL of 1 mol/L Tris-HCl (pH 7.4), eluted phages were used to infect exponentially growing *Escherichia coli* TG1 cells. Bacteria were plated on trypticase-yeast extract medium (21) containing ampicillin (100 μg/mL; Boehringer Mannheim) and 1% (w/v) glucose (TYE-amp-glu). Phages were rescued using the helper phage VCS-M13 (Stratagene) and used for the next round of panning. A total of 2 μL of a phage suspension ($\sim 1 \times 10^9$ transducing units) that eluted after the third round of panning was used to infect *E. coli* TG1 cells that were subsequently grown on TYE-amp-glu plates for screening.

Preparation of phage-displayed and soluble scFv fragments. Phage-displayed scFv fragments were produced from single, ampicillin-resistant colonies by rescue with helper phage VCS-M13 as described previously (22). Soluble scFv fragments in supernatant were produced from individual ampicillin-resistant colonies by induction with isopropyl-β-D-thiogalactopyranoside (23). Alternatively, scFv fragments were prepared from the periplasmic space of individual colonies (24).

Binding assays. To assess the reactivity of soluble scFv in supernatant and/or periplasmic space with target cells, cell ELISA was done as described (25).

Table 1. ELISA screening of cell lines using scFv F7

Cell line	scFv F7	Control (scFv 119)	
LG-2	0.068	Negative (0.030)	Positive (3.59)
SK-OV-3	2.253	Negative (0.015)	Positive (1.856)
HOSE	0.558	Negative (0.039)	Positive (3.751)
OV-CA-3	1.920	Negative (0.030)	Positive (0.953)
HT-29	0.511	Negative (0.117)	Positive (2.387)
SK-MEL-31	0.195	Negative (0.047)	Positive (2.097)
MDA-MB-435	1.457	Negative (0.440)	Positive (2.317)
MDA-MB-231	0.175	Negative (0.320)	Positive (3.835)

NOTE: Reactivity of scFv F7 and scFv 119 (irrelevant control antibody) against a panel of cell lines. scFv F7, initially identified by panning against the SK-OV-3 ovarian cancer cell line, also showed high reactivity against another ovarian cancer cell line (OV-CA-3) but low reactivity to normal ovarian cell line (HOSE), B-cell line (LG-2), and other carcinoma cell line: colon cancer (HT-29), melanoma (SK-MEL-31), and breast cancer (MDA-MB-435 and MDA-MB-231). Positive and negative controls are shown for reference. The results are expressed as absorbance at 450 nm.

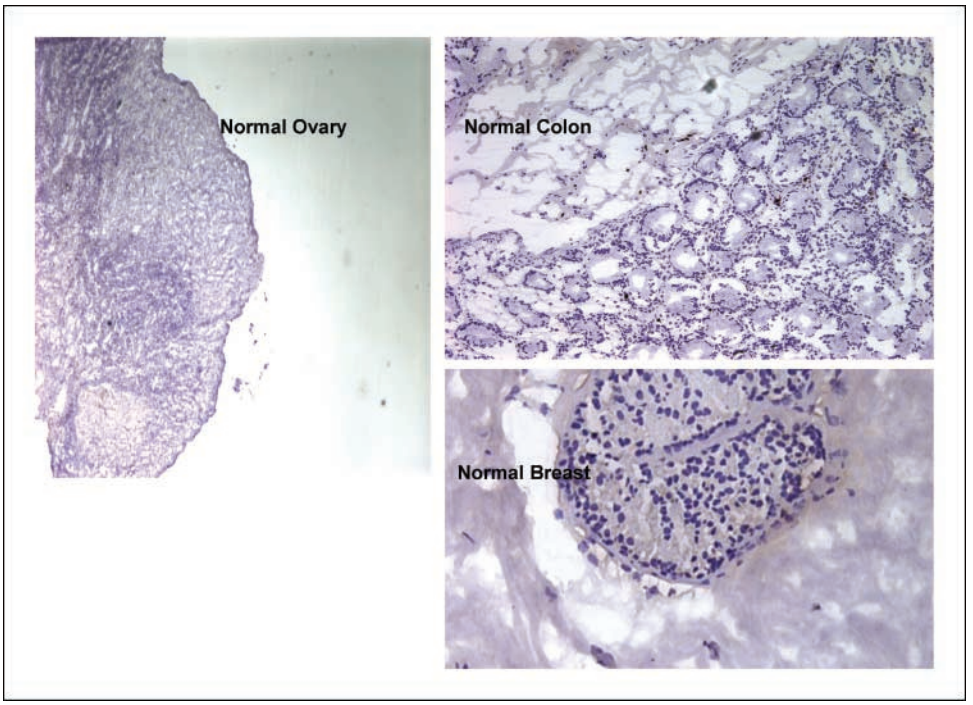

Fig. 2. Immunohistochemistry on fresh tissue using a periplasmic preparation of the candidate scFv F7 confirmed the ELISA findings: no staining of a panel of normal tissues, including ovary, colon, and breast specimens, as shown in this slide.

Determination of the nucleotide sequence of V_H and V_L segments and V_H gene family of isolated scFvs. Phagemid DNA for sequencing was extracted using QIAprep Spin Miniprep kit as per the manufacturer's instructions (Qiagen, Inc.). Plasmid DNA was sequenced using an automatic DNA sequencer (ABI Prism Model 377, PE Biosystem) using primers LMB3 and Fdseq1 to establish V_H and V_L gene identity.

Preparation of cell lysate and immunoprecipitation by F7 (scFv fragment isolated and scFv 119, control scFv fragment). The human EOC cell line SK-OV-3 was used. Two separate cell lysates were prepared. The first cell lysate (lysate 1) was prepared by solubilizing the cells (2×10^7) that had been washed with HBSS. The cells were resuspended in DMEM short-term labeling medium (methionine and cysteine free; Life Technologies) at 37°C for 30 min. The cells were then incubated with [^{35}S]L-methionine (1,000 Ci/mmol; Amersham) for 3 h at 37°C, 5% CO_2 incubator. The radiolabeled cells were then solubilized in 12 mL of lysis buffer (50 mmol/L Tris, 4 mmol/L EDTA, 150 mmol/L NaCl, 0.5% NP40 containing 1 mmol/L phenylmethylsulfonyl fluoride). The dish was placed on ice for 30 min, with occasional mixing. The cell lysate was spun at 13,000 × *g* for 30 min at 4°C. The supernatant (5 mL) was collected and transferred to a clean tube, labeled radiolabeled cell lysate 1. The second cell lysate (lysate 2) was prepared by solubilizing the SK-OV-3 cells (10×10^7) that had been washed with HBSS in 12 mL of lysis buffer (50 mmol/L Tris, 4 mmol/L EDTA, 150 mmol/L NaCl, 0.5% NP40 containing 1 mmol/L

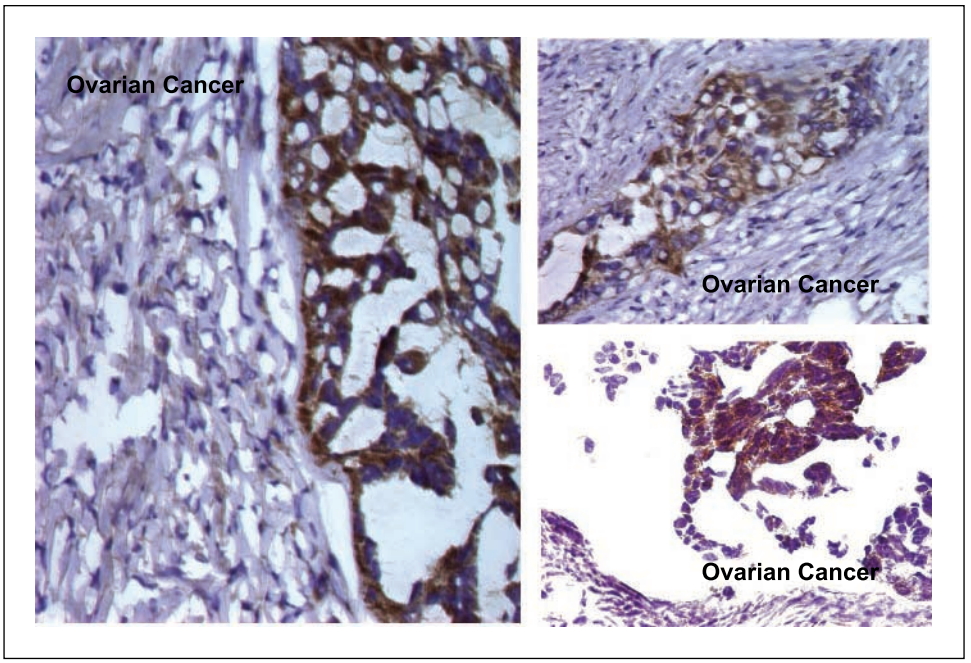

Fig. 3. There was positive staining of all 15 ovarian cancer tissue specimens tested as shown in these examples. Ca-125, used as a positive control (data not shown), had a distinctly separate pattern of staining of ovarian carcinoma tissue compared with that of F7.

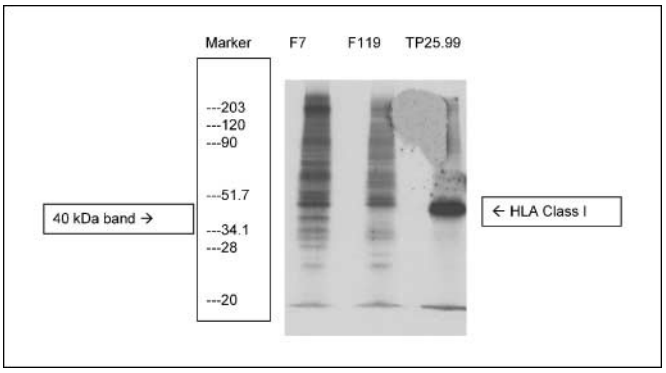

Fig. 4. Immunoprecipitation of radiolabeled SK-OV-3 cell lysate with study scFv antibody (F7), control scFv antibody (F119), and anti-HLA class I antibody (TP 25.99). The 40-kDa band was unique to the F7 precipitate.

phenylmethylsulfonyl fluoride). The dish was placed on ice for 30 min, with occasional mixing. The cell lysate was spun at 13,000 × *g* for 30 min at 4°C. The supernatant (5 mL) was collected and transferred to a clean tube, labeled cell lysate 2. Both cell lysates was precleared with an irrelevant normal rabbit serum and protein A-Sepharose mixture (Sigma-Aldrich) six times. Rabbit anti-mouse IgG (Jackson ImmunoResearch Laboratories, Inc.) and protein A-Sepharose mixture was prepared and divided into two equal sets. Monoclonal antibody 9E10 (26) was also used to bridge rabbit anti-mouse and scFv because scFv cannot bind to rabbit anti-mouse directly. Thereafter, each lysate supernatant was immunoprecipitated twice, on each occasion with protein A-Sepharose – rabbit anti-mouse IgG complex and periplasmic preparation of F7 (single-chain antibody) and F119 (control single-chain antibody). The immunoprecipitates were washed with low-salt buffer (150 mmol/L NaCl, 1 mmol/L EDTA, 10 mmol/L Tris, 0.1% bovine serum albumin, 1% NP40), high-salt buffer (350 mmol/L EDTA, 1 mmol/L EDTA, 10 mmol/L Tris, 0.1% bovine serum albumin, 1% NP40), and lysis buffer. The proteins from the radiolabeled cell lysate 1 were then resolved on a reducing 10% Tris-HCl SDS-polyacrylamide gel. The radioactive protein that was immunoprecipitated was detected by autoradiography by exposing dry gel to film for 24 h at -80°C (27).

Mass spectrometry. Proteins in the precipitates of cell lysate 2 (F7) single-chain antibody and a control antibody (F119) were resolved on a reducing 10% Tris-HCl SDS-polyacrylamide gel and stained with Coomassie blue. A band distinct to the F7 precipitate at ~40 kDa was excised from the SDS gel and then incubated in 20 μg/mL trypsin solution (Promega) in 40 mmol/L NH_4HCO_3/10% acetonitrile for 16 h at 37°C. Peptide extracts were analyzed by nano-liquid chromatography – nano-electrospray tandem mass spectrometry (LC-NanoESI MS/MS) using an Agilent 1100 series capillary LC system with a flow splitter operating at a flow rate of 400 nL/min coupled to a New Objective PicoView 200 NanoESI source and a Bruker Daltonic Esquire quadrupole ion trap mass spectrometer. A linear gradient of 0.2% formic acid/water-0.2% formic acid/acetonitrile was generated to elute the samples over 120 min. The nano-electrospray tandem mass spectrometry analysis was done by searching the NCBInr protein database with the tandem mass spectra using the MASCOT algorithm (Matrix Science).

Patients and specimens. Flash-frozen tissue specimens were obtained from patients undergoing debulking surgery for EOC at the Roswell Park Cancer Institute (Buffalo, NY) between 1995 and 2003. All tissue specimens were collected under an approved protocol from the Institutional Review Board. All pathology specimens were reviewed in our institution, and tumors were classified according to WHO criteria (28). The medical records of the patients were also retrospectively reviewed under an approved Institutional Review Board protocol. The review included outpatient and inpatient treatment, including surgery and chemotherapy. Study outcomes included overall survival and time to progression, each measured from the time of definitive surgery. Progression was defined as objective evidence of recurrence because all therapy was given in the adjuvant setting. The duration of overall survival was the interval between diagnosis and death. Observation time was the interval between diagnosis and last contact (death or last follow-up). Data were censored at the last follow-up for patients with no evidence of recurrence or progression.

Total tissue RNA isolation. Total tissue RNA was isolated from frozen tumor tissues and from ovarian cancer cell lines by use of the

A

m/z Observed	Charge	Mr(expt)	Mr(calc)	Δ	Sequence
514.01	2+	1026.01	1024.60	1.40	IGGIGTVPVGR
703.61	2+	1405.21	1403.72	1.49	YYVTIIDAPGHR
839.43	3+	2515.27	2514.38	0.89	VETGVLKPGMVVTFAPVNVTTEVK

B

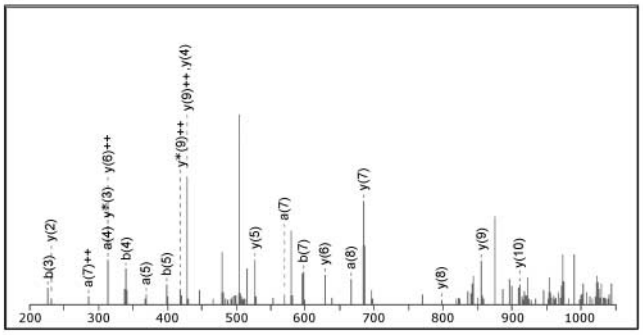

Fig. 5. Identification of translation EEF1A1 by tandem mass spectrometry. *A,* three peptides of translation EEF1A1 identified by tandem mass spectrometry. *B,* tandem mass spectrometry spectrum of IGGIGTVPVGR indicating the product ions for this peptide (*solid line*). *Dotted line*, product ions that correlate with theoretical b ions and y ions.

Table 2. Patient characteristics

No. evaluable patients	91	
Age, y, median (range)	62 (29-89)	
Follow-up, mo, median (range)	23 (0.46-119)	
	n (*N* = 91)	%
FIGO stage		
I	8	9
II	5	5
III	68	75
IV	10	11
Histology		
Serous	64	70
Mucinous	2	2
Endometrioid	7	8
Clear cell	6	7
Mixed	3	3
Carcinosarcoma	4	4
Undifferentiated	4	4
Grade		
1	5	5
2	7	8
3	77	85
Site		
Ovarian	76	84
Primary peritoneal	15	16
Response to frontline chemotherapy		
Complete response	43	47
Partial response	38	42
Unknown	10	11
Residual tumor at surgery		
Optimal cytoreduction	48	53
Suboptimal cytoreduction	41	45
Unknown	2	2
Recurrence		
Yes	26	29
No	18	20
Persistent disease	37	40
Unknown	10	11
Current status		
Alive no evidence of disease	25	27
Alive with disease	18	20
Dead of disease	48	53
Dead without disease	0	0
EEF1A2 status		
Positive	21	23
Negative	70	77

TriReagent (Molecular Research Center, Inc.) according to the manufacturer's protocol. Potentially contaminating DNA was removed by treating with RNase-free DNase I (Boehringer Mannheim). After phenol treatment and drying, RNA was dissolved in RNase-free H_2O. The resulting RNA concentration was measured spectrophotometrically (GeneQuant; Amersham Pharmacia Biotech Ltd.), and the quality of the RNAs was checked by electrophoresis on 1% agarose gel.

Reverse transcription-PCR analysis of EEF1A2 expression. Two micrograms of each RNA sample were subjected to cDNA synthesis using the Ready-To-Go First-Strand Synthesis kit (Pharmacia). PCR was subsequently done to analyze expression of EEF1A2. Glyceraldehyde-3-phosphate dehydrogenase expression was used as control. The primers for EEF1A2 were 5′-GCCTACATCAAGAAGATCG-3′ (sense) and 5′-TTGTACACGTCCTGCAGC-3′ (antisense). The primers for glyceraldehyde-3-phosphate dehydrogenase were 5′-TGAAGGTCGGAGTCAACGGATTTGGT-3′ (sense) and 5′-CATGTGGGCCATGAGGTCCACCAC-3′ (antisense). Amplification for EEF1A2 and glyceraldehyde-3-phosphate dehydrogenase gene products was 1 min at 94°C, 1 min at 66°C, and 1.5 min at 72°C for 30 cycles. A panel of normal tissue cDNA was analyzed (BD Biosciences). Heart tissue was used as a positive control. The PCR products were 239 bp for EEF1A2 and were visualized by ethidium bromide staining after separation over a 1.5% agarose gel.

Statistical analysis. All statistical analyses were done with the Statistical Package for the Social Sciences software (29). The χ^2 test was used to analyze the distribution of EEF1A2 expression and clinicopathologic variables (stage, residual tumor, recurrence, disease-free interval, and overall survival). Survival probabilities were estimated by Kaplan-Meier method (30), and statistical significance was determined by the log-rank test (31).

Results

ELISA screening. Three rounds of panning of the synthetic phage scFv library with SK-OV-3 cells resulted in a 1.9×10^2–fold enrichment of ovarian cancer cell–binding scFv fragments. Individual phage-displayed scFv fragments were then tested for reactivity with SK-OV-3 cells and LG-2 cells (negative control) in a cell ELISA. Of the 96 scFv fragments screened, 7 candidate scFv clones were selected with strong reactivity to SK-OV-3 cell line and low reactivity to LG-2 cell line. These clones were selected for further screening against the remaining cell lines. One candidate, named F7, showed high reactivity to both SK-OV-3 and OV-CA-3 ovarian cancer cell lines and weak reactivity to the normal ovarian cell line (HOSE) and LG-2 and to the breast, colon, and melanoma cell lines. This clone has characteristics most consistent with a tumor-specific antibody. These findings are summarized in Table 1.

Immunohistochemistry. A periplasmic preparation of the candidate scFv F7 on fresh tissue confirmed the ELISA findings. There was no staining on a panel of normal tissues, including ovary, colon, lungs, brain, and breast specimens (Fig. 2). However, there was positive staining of all 15 serous ovarian carcinoma tissue specimens tested (Fig. 3). Ca-125, used as a positive control, had a distinctly separate pattern of staining of ovarian carcinoma tissue compared with that of F7 (data not shown).

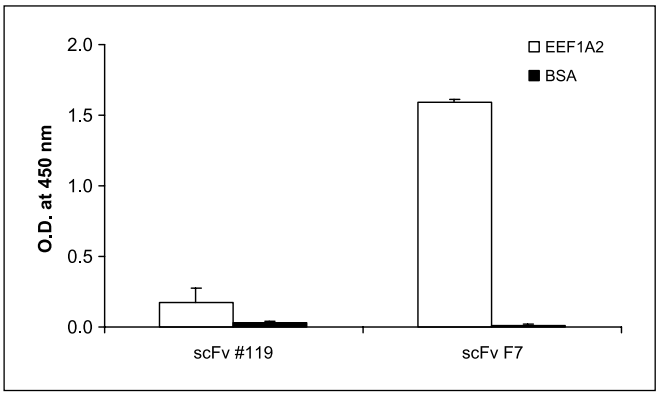

Fig. 6. Reactivity in ELISA of scFv F7 with recombinant human EEF1A2 full-length protein. Ninety-six–well plates were coated overnight at 40°C with recombinant protein EEF1A2 (1 μg/well; GenWay Biotech, Inc.) in PBS. Following blocking with PBS-2% bovine serum albumin (*BSA*), wells were incubated for 2 h at room temperature with 50 μL of supernatant scFv F7 and 50 μL of monoclonal antibody 9E10 (1 μg/well 2% bovine serum albumin-PBS). Binding of scFv antibody to recombinant protein was detected by addition of horseradish peroxidase–conjugated anti-mouse IgG Fc antibodies (Jackson ImmunoResearch Laboratories). Results are expressed as absorbance at 450 nm. The scFv 119 and bovine serum albumin were used as specificity controls.

Fig. 7. Reverse transcription-PCR results for EEF1A2 expression. cDNA from normal cell tissues. Lane 1, heart; lane 2, brain; lane 3, skeletal muscle; lane 4, placenta; lane 5, lung; lane 6, liver; lane 7, kidney; lane 8, pancreas; lane 9, spleen; lane 10, thymus; lane 11, prostate; lane 12, testis; lane 13, ovary; lane 14, small intestine; lane 15, colon; lane 16, leukocyte; lane 17, human control. M, marker. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Sequencing. The nucleotide and amino acid sequence of F7 showed that the scFv uses a V segment of the V_H3 and V_L1 families using the antibody database VBASE.

Immunoprecipitation of radiolabeled cell lysate and identification of EEF1A1 by tandem mass spectrometry. Cell lysate 2 of SK-OV-3 was immunoprecipitated with either the single-chain antibody (F7) or a control scFv antibody that recognizes an irrelevant antigen (scFv 119; ref. 27). The anti-HLA class I antibody (TP 25.99; ref. 32) was used as a positive control antibody. The resulting precipitates were resolved on SDS-PAGE and visualized by staining with Coomassie blue (data not shown). There were multiple bands that appeared in both precipitates (F7 and F119) and were therefore considered as nonspecific binding. However, one band at 40 kDa was unique to the (F7) precipitate (Fig. 4) and was excised and in-gel digested with trypsin. The resulting tryptic peptides were analyzed by LC-NanoESI MS/MS and the corresponding proteins were identified by searching the tandem mass spectra against the NCBInr protein database using MASCOT. The human proteins identified in the 40-kDa band included keratins types I and II, which are common contaminants, and translation EEF1A1. The data supporting the identification of EEF1A1 are shown in Fig. 5.

Study population. The characteristics of the study population are presented in Table 2. The median age of the patient group was 62 (29-89) years, and the median duration of follow-up was 23 (0.46-119) months. The majority of patients had advanced-stage disease (86%) and serous histology (70%). A complete response to therapy was achieved in 43 of the 91 patients (47%); a partial response was achieved in 38 patients (42%), whereas the remaining 1 patient had progression of disease. The median survival for all patients was 45 months (95% confidence interval, 35-65 months).

Expression of EEF1A2 mRNA in normal tissues and EOC. EEF1A1 has previously been shown to be relatively ubiquitous (19). EEF1A2 is a tissue- and tumor-restricted putative oncogene with 95% homology to EEF1A1. Because of our interest in identifying a potential therapeutic target, we tested reactivity of scFv F7 to recombinant human EEF1A2 full-length protein and also focused on the analysis of EEF1A2 in a large panel of ovarian cancer tissues. As anticipated based on the high homology between EEF1A1 and EEF1A2, our results indicate reactivity of scFv F7 to full-length human EEF1A2 protein by ELISA (Fig. 6). EEF1A2 expression was restricted only to the normal tissues of the heart, brain, and skeletal muscle (Fig. 7). EEF1A2 mRNA expression was shown in 21 of 91 (23%) of the ovarian cancer specimens by reverse transcription-PCR (Fig. 8). Using flow cytometry, we confirmed reactivity of scFv F7 with human ovarian SK-OV-3 cells, although we also noted binding of the human B lymphoblastoid LG-2 cells to a much lesser extent in this assay (Supplementary Fig. S1). Although the latter result is not in full agreement with that obtained from cell ELISA, it may be a reflection of less sensitivity of cell ELISA compared with flow cytometry. These data indicate that EEF1A1 and/or EEF1A2 may be also expressed on a variety of other tumor cells, although EEF1A1 and/or EEF1A2 are mostly expressed on human ovarian cells based on the present study.

Correlation of EEF1A2 expression with clinical outcome. The analysis of EEF1A2 mRNA expression and clinicopathologic characteristics is presented in Table 3. The data indicated that although EEF1A2 expression significantly correlated with increased likelihood of recurrence risk in univariate and multivariate analysis (P = 0.021), Kaplan-Meier analysis indicated EEF1A2 expression to be a nonsignificant predictor of overall survival.

Discussion

The development of strategies for actively stimulating immunologic rejection of tumors, previously an elusive goal, has been accelerated by recent improved understanding of the molecular basis of immune recognition and immunoregulation of cancer cells. It is now well known that the immune system has the ability to recognize tumor-associated antigens displayed on human malignancies and to direct humoral and cytotoxic responses to these targets (33). However, progress in harnessing immune therapies for ovarian cancer has been hampered by the lack of tumor-specific antigens and by the potential for toxicity due to cross-reaction of antibodies or CTL with normal tissues. In the current study, we have applied phage display technology to isolate a scFv antibody that stains ovarian tumor tissues but not a panel of normal tissues. We have also shown that the target antigen for scFv F7 is in the family of translation EEF1A.

Phage display technology allows detection and isolation of a scFv-specific antigen by repeated panning against a specific tissue or cell or protein of interest. This approach can be used to pan against a known purified protein to isolate a specific scFv antibody that ultimately can be humanized and used for treatment. Alternatively, as in this study, the phage library can be subjected against a specific cell type in a "shotgun" approach to isolate a specific scFv antibody to that specific tumor tissue. The power of this technique lies in the fact that conformational relationships are in place when using live cells, which will most

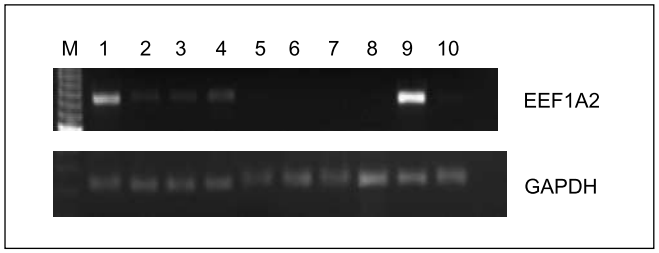

Fig. 8. Reverse transcription-PCR results for EEF1A2 m-RNA expression. mRNA from EOC specimens. Lanes 1 to 4, positive; lanes 5 to 8, negative; lane 9, skeletal muscle (positive control); lane 10, normal ovary (negative control).

Table 3. Patient characteristics by EEF1A2 status

No. evaluable patients	91		
	Positive	Negative	*P*
EEF1A2	21 (23%)	70 (77%)	
Age, y (median)	60.4	62.5	
FIGO stage			0.182
I	2 (10%)	6 (19%)	
II	3 (14%)	2 (3%)	
III	15 (71%)	53 (76%)	
IV	1 (5%)	9 (13%)	
Histology			0.337
Serous	14 (67%)	50 (71%)	
Mucinous	1 (5%)	1 (1%)	
Endometrioid	1 (5%)	6 (9%)	
Clear cell	3 (14%)	3 (4%)	
Mixed	1 (5%)	2 (3%)	
Carcinosarcoma	1 (5%)	3 (4%)	
Undifferentiated	0	4 (6%)	
Grade			0.581
1	2 (10%)	3 (4%)	
2	1 (5%)	6 (9%)	
3	18 (86%)	59 (84%)	
Site			0.327
Ovarian	19 (90%)	57 (81%)	
Primary peritoneal	2 (10%)	13 (19%)	
Response to frontline chemotherapy			0.957
Complete response	12 (57%)	31 (44%)	
Partial response	6 (29%)	32 (46%)	
Unknown	3 (14%)	7 (10%)	
Residual tumor at surgery			0.870
Optimal cytoreduction	10 (48%)	31 (44%)	
Suboptimal cytoreduction	11 (52%)	37 (53%)	
Unknown	0	2 (3%)	
Recurrence			0.021
Yes	12 (57%)	14 (20%)	
No	4 (19%)	14 (20%)	
Persistent disease	2 (9%)	35 (50%)	
Unknown	3 (15%)	7 (10%)	

Abbreviation: FIGO, International Federation of Gynecology and Obstetrics.

closely resemble the *in vivo* state. Even more importantly, the very nature of this technique will select for that scFv antibody against the most frequent antigen and the antigen with the strongest bond expressed on the cell. Our data indicate that the scFv F7 may represent an EOC antibody that has strong immunoreactivity to a cell surface ovarian cancer antigen. Based on the pattern of immunoreactivity, and molecular weight, F7 does not seem to recognize Ca-125 and therefore may recognize a newer, relatively unknown ovarian tumor–specific antigen group, translation elongation factor.

In the current study, we isolated translation EEF1A1 using mass spectrometry. EEF1A1 and EEF1A2 are similar proteins with 95% homology. EEF1A1 has been implicated in prostate cancer, with potential for inducing tumorigenesis in nude mice (34). The translation elongation factor EEF1A2 was identified as a variant of EEF1A1 in the early 1990s (35, 36). The two forms of EEF1A are encoded by separate loci, but the resulting proteins are 95% identical. Whereas EEF1A1 is widely expressed, EEF1A2 is restricted to the heart, brain, and skeletal muscle (18). The fact that EEF1A1 is expressed ubiquitously whereas EEF1A2 expression is restricted and has transforming potential would suggest that EEF1A2 is also an important oncogene (19). In support of this notion, EEF1A2 maps to the genomic region of 20q13, a region known to be amplified in a high percentage (50%) of ovarian cancers (19, 37). 20q13 amplifications in ovarian tumors are associated with worse survival and a more aggressive tumor pathology than those with normal 20q copy number (38, 39). Therefore, in our current study, after we identified translational elongation factor as a possible antigen for scFv F7, we focused on EEF1A2, rather than EEF1A1, as it seems to be more promising in ovarian cancer tumorigenesis. Furthermore, the functional significance of the tissue-restricted expression pattern of EEF1A2 is not clearly understood. Additionally, the relative interrelationship of these two putative oncogenes has not been clearly defined in ovarian cancer. Consistent with our data indicating aberrant expression of EEF1A2 in 23% of ovarian carcinomas, a previous report (19) indicated EEF1A2 expression in 30% of ovarian tumors but not in normal ovary.

Our data show that EEF1A2 expression significantly correlated with increased likelihood of recurrence of EOC, although there were no differences in overall survival. This raises the important question of the role of EEF1A2 in ovarian tumorigenesis, invasion, and metastasis. The elevated apoptosis in the EEF1A2-deficient wasted mice (40) implies that it might be an inhibitor of apoptosis. Furthermore, EEF1A2 expression has been implicated in cadmium-induced apoptosis in various tumors, including ovarian cancers (41). There is also evidence indicating that acquisition of cisplatin resistance in a human head and neck cancer cell line was associated with increased EEF1A2 expression (42). Taken together with the high tumor-specific expression of EEF1A2, it is possible that expression of EEF1A2 might contribute to chemoresistance in ovarian cancer, leading to increased likelihood of recurrence.

In summary, we identified highly reactive scFv antibody against ovarian cancer that recognizes EEF1A as the antigenic target. Our data suggest that approximately a quarter of ovarian carcinomas aberrantly express the antigen EEF1A2, and tumor expression is associated with a higher risk of recurrence. Based on these findings, future studies are warranted to determine the mechanistic role of EEF1A1 and EEF1A2, in ovarian tumor progression, and their potential for targeted therapy. Toward this end, we plan on studying the relative expression of EEF1A1 and EEF1A2 in ovarian tumors and specifically determine mechanisms of tumorigenesis.

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Characterization of a Putative Ovarian Oncogene, Elongation Factor 1 α, Isolated by Panning a Synthetic Phage Display Single-Chain Variable Fragment Library with Cultured Human Ovarian Cancer Cells

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