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APPLICATION OF TWO-DIMENSIONAL ELECTROPHORESIS AND NIH 3T3 CELL TRANSFECTION ASSAY IN THE STUDY OF TUMOR-ASSOCIATED PROTEINS AND GENOMIC DNA TUMOROGENICITY IN MALIGNANT HUMAN ESOPHAGEAL SPECIMENS

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Abstract

Total protein and DNA extracted from histologically diagnosed normal nonmalignant and esophageal tumor tissues were used for analysis of polypeptides pattern by two-dimensional gel electrophoresis and DNA transforming activity in NIH 3T3 cell transfection assay, respectively. In comparison to normal tissues, eight polypeptides underwent down-regulation or disappeared, while seven polypeptides were subjected to up-regulation or appeared for the first time in tumor tissues. We suggest that the first eight polypeptides are necessary for maintenance of normal phenotype and the other seven polypeptides are involved in the development of malignant phenotype. However, results obtained from NIH 3T3 cell transfection with esophageal tumorous DNA showed that such DNAs lack transforming efficiency. These data may agree with the published reports about the absence of activated transforming oncogenes such as the *ras* family in esophageal cancer specimens.

Introduction

Cancer cells represent a range of genetic changes which result in a change in cellular composition. It is well known that such cells have the ability to produce new proteins and that some proteins, which are normally present, may be modified, augmented, diminished or even eliminated [1,2]. Two-dimensional gel electrophoresis has been utilized for direct, simultaneous detection of a large number of cellular proteins and is being considered as the most powerful direct analytical technique for the detection of protein patterns [1,2]. Positional, slight differences in molecular weights and quantitative variations may represent expression or repression of different functional genes [3]. By this technique, several proteins were detected that appeared to be associated with cancer

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phenotype. These observations have provided helpful tools which can be used for diagnostic and therapeutic treatments [3,4].

Cellular DNA transfection into the mouse cell line NIH 3T3, and the resultant morphological transformation of these cells, is a commonly used method to detect potential cellular oncogenes in human cancer [5]. Members of genes from the *ras* family, the most involved human transforming genes, have been detected by transfection of NIH 3T3 cells with tumorous DNA [6]. The use of this technique has led to the detection and identification of a new oncogene, *hst*-1, in some stomach carcinoma specimens [7]. Amplification of this gene and *int*-2 were detected in 19 out of 36 (52%) esophageal carcinomas in Japan [8]. In addition, altered *myc*, *myb* and H-*ras* genes were frequent and demonstrate clinical correlation [9].

Esophageal cancer is well demonstrated to be the sixth most common cancer in the world. In the developing countries, however, it ranks fourth and occurs with high frequencies in certain regions of the world such as China, Iran, South Africa and France [10], with five-year survival rates as low as 5% [11].

The present study has focused on the characterization of tumor markers and indicators of transforming activity of tumor-derived DNAs for diagnostic and therapeutic applications.

Materials and Methods

Patients and Tissue Sampling

Cancerous samples and histologically normal tissues adjacent to malignant tumors were collected from 25 patients with esophageal cancer who were admitted and underwent surgery at several clinics and hospitals in the city of Tehran. Following the collection of specimens from the surgeon in the operating room, the samples were packed in aluminum foil and snap frozen in liquid nitrogen, then transferred to the laboratory. The esophageal tumors (dissected from necrotic tissue) and the freed surrounding normal tissues (removed from tumor samples as completely as possible) were segregated and processed. The specimens, if not processed immediately, were stored frozen in liquid nitrogen for further processing. The age of the patients at the time of diagnosis ranged from 27 to 86 years (63% males, 37% females) with a mean of 55 years.

Protein Preparation

For protein sample preparation, tissues were thawed, sliced on ice then pulverized to a powder under liquid nitrogen freezing conditions using a microdismemberator (Braun, Germany). All chemicals were purchased from Sigma (Dorset, England) unless otherwise specified. Proteins were extracted from the ground tumorous and normal tissues by the homogenization buffer (10 mM Tris-HCl, 5 mM $MgCl_2$ pH 7.4) according to the previously reported methods with slight modification and adaptation [12,13,14]. Briefly, 150-200 mg of each type of tissue (normal and tumor) were pulverized and diluted in 600 µl of homogenization buffer, and 10 µl of the following protease inhibitors: pepestatin (at 1 mg/ml in isopropanol), benzamidine (at 16 mg/ml in H_2O) and phenyl-methylsulphonyl fluoride (PMSF at 25 mg/ml in isopropanol) were added. To this homogenate mixture, 10 µl of RNase-A (at 10 mg/ml in homogenization buffer) and DNase I (at 1 mg/ml in homogenization buffer) were added and left on ice for 20 minutes to eliminate nucleic acids. Urea, β-mercaptoethanol and SDS (at 9 M, 5%, and 0.5% final concentration, respectively) were gradually added and adjusted to 1.5 ml with the homogenization buffer. The extracted samples were transferred to 1.5 ml Eppendorf tubes, centrifuged at high speed to remove insoluble particles and frozen at -70°C until they were electrophorsed.

Electrophoresis

Samples were employed for the first dimensional isoelectrofocusing (IEF) separation following adaptation and slight modifications [15]. Briefly, 5 µl of each extracted protein sample were used for protein estimation according to the Bradford method [16]. First dimension isoelectric focusing separation and 2-DE techniques were carried out following the preparation of the gel mixture containing 4.2% acrylamide, 0.22% N,N' methylene bisacrylamide, 8.5 M urea, 0.27% v/v Nonidet P-40 (NP-40), 5% sucrose, and 6% ampholytes (pH 4-6, 5-7 and 6-8 in a ratio 2:1:2, respectively) and 0.05% TEMED. Gel solution was degassed and 0.04% ammonium persulfate was added, mixed and casted into a glass tube to a height of 100 mm of the cylindrical tube (1.5 mm width) and left to polymerize. A volume of sample equal to 75 µg of total protein was mixed with 0.33 volume of neutralizing buffer (9 M urea, 8% NP-40 and 5% ampholytes pH 3.5-10) and then loaded on IEF gel and overlayered with 10 µl sample overlay buffer (8 M urea, 1% ampholyte pH 3.5-10) and filled with catholyte. The upper chamber was filled with extensively degassed catholyte (0.02 M NaOH) and the lower chamber with anolyte (0.01 M phosphoric acid). Isoelectric focusing was applied, without prefocusing, at 300 V for 1 h, then at 600 V for 10h and 800 V for 1 h. Gels were removed and allowed to equilibrate for 20 min at room temperature in equilibration solution (60 mM Tris-HCl, pH 6.8, 2% SDS, 5% β-mercaptoethanol (v/v), 10% glycerol (v/v), and 0.002% bromophenol blue). Second dimensional SDS separation gel consisted of 33.3 ml of 30% stock solution of acrylamide and N, N' methylene bisacrylamide (29.2% and 0.8% w/w), 41.7 ml deionized water and 25 ml separation gel buffer (1.5 M Tris-HCl, pH

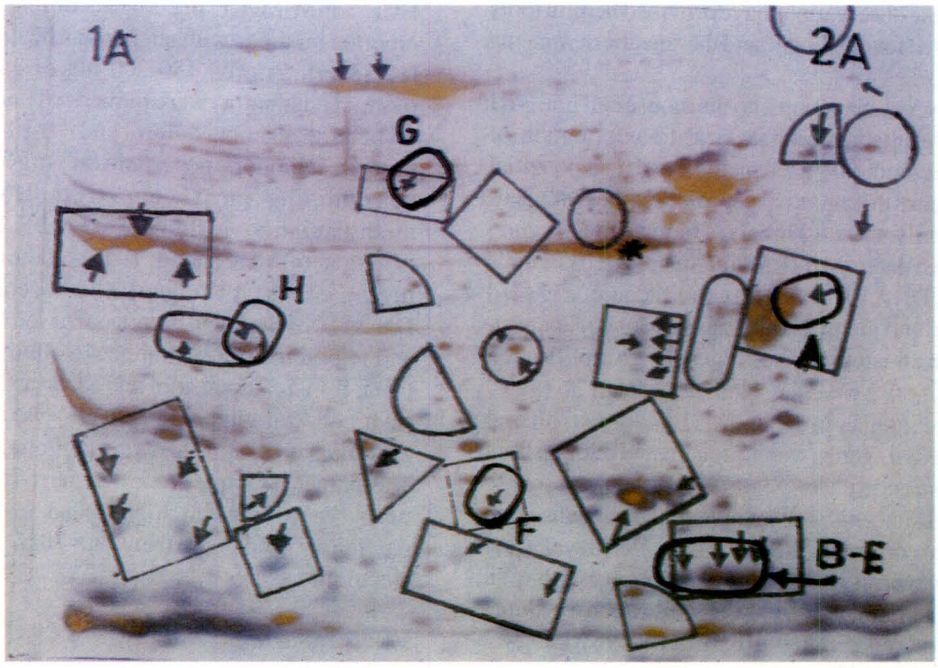

Figure 1. A. A representative polypepticle pattern of the normal nonmalignant tissue of a patient with esophageal cancer. Polypeptides outlined by areas and indicated by arrows are expressed in normal tissue but are lost or down regulated in mulignant tissue. Star indicates the location of actin which is immunologically detected.
B. Polypeptide pattern of the corresponding malignant tissue of the same patient. Polypeptides outlined by areas and indicated by arrows are upregulated or newly appeared in tumor. Star indicates the location of actin.

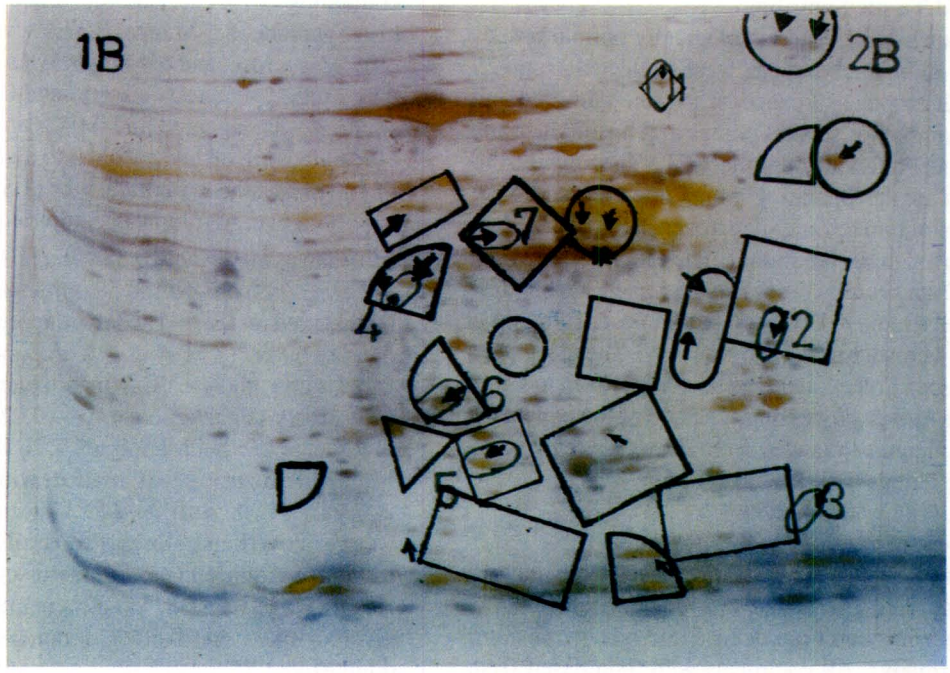

Figure 2. A. A presentation of the eight most frequent spots in an electrogram of the normal tissue which were observed in at least 70% of cases studied and nominated alphabetically as A through H.
B. A presentation of the seven most frequent spots in the electrogram of the corresponding tumor tissue of Figure 2A which were observed in at least 70% of tumors and numbered from 1 to 7.

8.8, 0.4% SDS), 0.034% w/v ammonium persulfate and 0.05% TEMED. The first dimensional equilibrated gels were layered over second dimensional SDS gel and fixed with 1% agarose and electrophoresis was done at 30 mA/plate at constant temperature (10°C) by the application of a cooling system.

Protein Detection

The proteins were detected using a slightly modified version of the previously reported method [15] as follows: The slab gel was transferred into a tray containing approximately 400 ml of methanol, water, acetic acid and formaldehyde (50/38/12/0.05 per volume) as a fixative solution for at least 1h, followed by 3×20 minute washes in a 50% ethanol solution. The gel was later pretreated with $Na_2S_2O_3.5H_2O$(0.2 g/L) for 1 min, and washed three times (each for 20 seconds) with distilled water. Impregnation of the gel was performed using a solution of $AgNO_3$(1.9 g/L), 0.075% (v/v) of 37% formaldehyde. The residual of $AgNO_3$ was removed from the gel by 2×20 second successive washes with deionized water. Color development process was carried out by soaking the gel in the developing solution containing Na_2CO_3 (60 g/L), 0.05% (v/v) of 37% formaldehyde and $Na_2S_2O_3$. $5H_2O$ (4 mg/L) for 10 min up to the appearance of yellowish brown spots. The gel was then rinsed twice (each time for 2 min) and the reaction was stopped by rinsing the gel in stop solution containing 50% methanol and 12% acetic acid. The gel was finally stored in 30% ethanol at 4°C until photography.

Determination of Molecular Weight in the Gel

Protein standards (Sigma, Dorset, England) were applied for molecular weight and pI determination [43]. The protein mixture was directly loaded in IEF gel and the same gel was used for molecular weight determination in SDS-PAGE gels.

Analysis of Protein Pattern in 2-DE Gels

For protein patterns analysis, pairs of tumor and normal gels were compared. The location, size, form and intensity of each spot was considered for identity evaluation (quantitative variants). The relative position of each spot to other spots was used for this identification [17]. Visual intensity differences between each spot of a pair, as well as the appearance and disappearance of spots (qualitative variants) in one of the two tumor and normal gels were recorded. The protein variants of each pair gels (tumor and normal) were registered, then the most common changes (70% and higher) in all 2-DE gels were used as a reference for comparison between the tumor and normal gels.

Immunological Detection and Localization of Actin

After 2-DE as explained above, immunological detection of actin was carried out with some modification [18]. Gels were equilibrated for 30 min in transfer buffer (25 mM Tris-base or HCl, 192 mM glycine, 20% methanol and 0.1% SDS) and polypeptides were electophoretically transferred to nitrocellulose membranes at 14 volts overnight. Membranes were blocked for two hours in blocking solution (TBST: 100 mM Tris-base or HCl, pH=7.5, 0.9% NaCl and 0.05% Tween 20) and exposed to biotinylated anti actin antibody at 1/3000 dilution. After one hour incubation with shaking, the solution was removed and membranes were washed three times with TBST and incubated with strptavidin conjugated alkaline phosphatase in TBST at 1/4000 dilution and further incubated for another hour with constant shaking. Membranes were washed two times with TBST and once with TBS (TBST without Tween 20) and were exposed to five milliliters of color solution (33 µL NBT from 50 mg/ml stock solution in 70% dimethylformamide and 17 µl BCIP from 50 mg/ml stock solution in 100% dimethylformamide, respectively). Excess color formation was stopped with distilled water.

DNA Isolation

High-molecular weight genomic DNA was isolated from the frozen tumors and surrounding noncancerous tissues belonging to44 patients according to the previously reported methods [18]. Briefly, frozen tissue specimens were thawed to 4°C and dissected on ice (approximately 1 mm^3) then ground to a powder at -80°C by applying a microdismemberator with the aid of liquid nitrogen. The resulted fine frozen powder was transferred to a 15 ml polypropylene falcon tube, suspended in 4 ml of SDS-Proteinase-K digestion solution (0.5% SDS, 50 µg/ml Proteinase-K, 100 mM sodium chloride, Tris–HCl pH 7.5-8.0 and 10 mM EDTA) then incubated overnight at 65°C. Contamination with RNA was relatively overcome following the digestion with 5 µg/ml RNase-A at 37°C for 3-4 h. Extraction with an equal volume of phenol followed by an equal volume of chloroform-isoamylalcohol (24:1) was used to remove proteins and followed by centrifugation each time. DNA was precipitated with sodium acetate (0.3 M final concentration) and two-fold of cold absolute ethanol. The DNA was pooled with a sterilized plastic stick then washed twice with cold 70% ethanol followed by centrifugation at 2500 rpm for 10 min. The pelleted DNA was dried and resuspended in sterilized TE buffer (10 mM Tris-HCl pH 7.5-8.0, 1 mM EDTA) and rotated for an overnight period, then quantified spectrophotometrically at 260 nm and stored in a 1.5 ml Eppendorf tube at 4°C until use.

Cell Culture and Maintenance

The NIH 3T3 cell transformation assay was designed

to allow expression of transformed foci on a monolayer of nontransformed contact-inhibited NIH 3T3 cells.

A continuous NIH 3T3 cell line, a gift from Professor Sydney Shall (Sussex University, Brighton, England), was subcultured and grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% Fetal Calf Serum (FCS), 2 mM L-glutamine and penicillin and streptomycin at 50 Iu/ml and 50 ug/ml, respectively, before reaching 80% confluence under a humidified atmosphere of 5% CO_2 at 37°C and were used as recipient in the DNA transfection assay.

Transfection Protocol

Transfection assay was carried out using the basic technique of Graham and Van der Eb [19] and Wigler and coworkers [20,21], with a slight modification. Briefly, cells were plated at 2×10^5 cells/10 cm Petri dish 36 hours before transfection with the donor DNAs. During this period, the medium was changed twice at the 12th and 36th hour after the initial seeding. Approximately 30 µg [22] of high molecular weight DNA extracted from histologically confirmed malignant patient specimens was sheared two-three times through a 20 gauge syringe needle and diluted in 1 ml of 0.5 M $CaCl_2$. To this solution, slowly and with constant shaking, an equal volume of 2 × HBS (280 mM NaCl, 50 mM HEPES and 1.5 mM Na_2PHO_4 (pH 7.1)) was added. After formation of the calcium phosphate precipitate, this DNA mixture was added to a 10 cm Petri dish in which cells were at the semiconfluent growth stage. After incubation for 2 h at 37°C, the precipitate was removed and replaced by 15 ml of complete medium (DMEM + 10% FCS) and reincubated for a further 6 h. Cells had medium removed, were washed twice with prewarmed Phosphate Buffer Saline (PBS), dislodged by trypsinization (0.25%), dispersed then resuspended in complete medium and centrifuged at 1000 rpm for 5 min. The cell pellets were resuspended in complete medium and seeded. For each transfection assay, one 10 cm Petri dish of NIH 3T3 recipient cells was used and then divided into five 6 cm Petri dishes following the trypsinization and washing steps. Seven milliliters of DMEM medium containing 5% FCS were added to each 6 cm Petri dish. The medium was changed the next day, and then all the dishes were re-fed with DMEM supplemented with 5% FCS approximately every three days for two-three weeks until foci developed. DNA from the NIH 3T3 cells was used as a negative control, while DNA from a1-1 cell line (a secondary transformant of NIH 3T3 cells induced by transfection with DNA from a human T24 bladder carcinoma) kindly donated by Professor Takashi Sugimura (National Cancer Center Research Insitute, Tokyo, Japan) was applied as positive control. In order to discover whether tissue processing, DNA extraction assay and transfection protocol have artifactual influences on colony formation, normal DNAs from nonmalignant tissues of eight patients were randomly selected and assayed simultaneously as secondary controls with other donor DNAs. The DNA of NIH 3T3 and a1-1 cell lines was extracted as mentioned above. Colonies were scored following 95% ethanol fixation and staining with Giemsa. Each transfection assay for each donor DNA was done twice and the average of the scored foci of the two separate experiments was considered for foci formation analysis. The transfection efficiency is defined as the number of formed foci/8×10^5 cells/30 µg of foreign DNA.

Results

For constructing a reference map, well resolved polypeptides were recorded for each 2-DE gel. The achieved map contained about 400 spots ranging in molecular mass from 13 to 220 kDa and pI from 4.8 to 8.0. We could identify approximately 92 certain spots from 25 normal tissues belonging to 25 patients that disappeared or underwent quantitative changes in the matched tumor samples. On the other hand, nearly 88 spots which appeared for the first time in the analyzed tumors underwent variations with respect to their expression compared to the matched normal tissues. To establish which polypeptides are tumor-associated, we compared all normal tissues with their matched tumors. To eliminate the possibility of artifactual interference and blood protein contamination, we considered those spot variants which occurred commonly or at 70% in either tumorous or normal esophageal tissues. These spots were 8 of 92 and 7 of 88 of the normal and malignant samples, respectively. Figures 1 and 2 represent the pattern of proteins in normal and tumor tissues from a patient and are indicated by arrows. The control variant spots were named A, B, C, D, E, F, G and H, while the tumor variant spots were numbered 1, 2, 3, 4, 5, 6, and 7. Normal tissues showed the polypeptide spots A-D and H which were subjected to various alterations in the tumor specimens. The apparent molecular weight and approximate isoelectric point characterization of these spots are presented in Table 1. We found that spots A and H were completely lost in two-thirds of the investigated tumor samples. However, spots B, C and D were drastically down regulated in the tumors, while spots E, F and G underwent observable down regulation.

The state and characteristics of protein spots in the tumorous tissues are shown in Table 2. In this study, we found that spots 1-4 were newly expressed, whereas spot 5 was expressed at high levels in tumor compared with normal specimens. Spots 1,3 and 4 were found in more than two-thirds of the tumors, and spots 2 and 5 were commonly detected in the malignant specimens. We should mention that some spots were not always visible under the

Table 1. Apparent molecular weight and pI of eight polypeptides in normal tissue disappeared or down regulated in human esophageal cancer identified by two–dimensional electrophoresis (2-DE)

Esophageal cancer				
Spot designation	Apparent mol. wt. k Da	Estimated pI	Disappeared in tumor	Down regulated in tumor
A	40.7	5.5	×	
B	17.7	5.54	×	
C	17.7	5.70	×	
D	17.7	5.90	×	
E	17.7	6.1		×
F	26	6.7		×
G	65.5	7.3		×
H	35	7.4	×	

Table 2. Apparent molecular weight and pI of seven polypeptides appeared in human esophageal cancer identified by two–dimensional electrophoresis (2-DE)

Spot designation	Apparent mol. wt.	Estimated pI	Appeared in tumor	Overexpressed in tumor
1	164	6.1	×	
2	32.7	5.6	×	
3	20	5.4	×	
4	42.5	6.8	×	
5	27	6.2		×
6	30	6.6	×	
7	50	6.50		×

staining condition for some 2-DE gels during this study. Also, some spots showed a range of variation in either their molecular weight or pI values. Because of this we could not consider these spots in our analysis. On the other hand, several intense spots escaped detection and/or quantitation either because they were not well focused (streaking) or because they were comigrated with a cluster of polypeptides. With respect to proliferation rate and behavior, since most tumors consist of heterogeneous populations of cells, such as contamination with normal esophagus cells, some similarities are expected in the pattern of some proteins in both tumor and normal tissues.

As demonstrated in Table 3, none of the DNA samples prepared from 44 surgically removed human esophageal tumors could transform NIH 3T3 cells. These cells were also not transformed when transfected with their own DNA as negative control. However, DNA from al-1 cells (an NIH 3T3 transformant containing high copy number of the transforming human H-*ras* gene of T24 bladder carcinoma cells) as positive control induced foci of morphologically altered NIH 3T3 cells (Fig. 3). Cells dispersed from such foci were efficiently able to form many colonies when replated in Petri dishes. Colony formation in NIH 3T3 cells exposed to al-1 DNA varied from 1 to 12.5 foci per 10 cm Petri dish used for each one of the separate experiments. The maximum frequency of transformation observed was 12,5 colonies per approximately 800000 cells. However, DNA prepared from normal tissues of eight patients did not transform NIH 3T3 cells (data not shown). These results indicated

that the application of the described procedures had no artifactual effects on the DNA transfection assay. However, some colonies were induced by a few of the donor malignant DNAs, but cells resulting from dispersing such colonies could not form new colonies when replated in Petri dishes. This observation confirms that colonies formed such may result from clumps formed during the trypsinization step.

Discussion

Several studies have described the application of 2-DE

Table 3. Transforming assay of genomic DNA from human esophageal tumors

Donor DNA	Formed foci in five 6 cm Petri dishes										Average foci per 10 cm Petri dish	Transforming efficiency per 8×10^5 per 30μg DNA
	Expet. 1 Plate No.					Expet. 2 Plate No.						
	1	2	3	4	5	1	2	3	4	5		
Group A												
Patient No. 1-5*	0	0	0	0	0	0	0	0	0	0	0	0.0
a1-1 Cell	0	3	1	0	0	0	4	3	0	0	5.5	0.0000067
Group B												
Patient No. 6-11	0	0	0	0	0	0	0	0	0	0	0	0.0
a1-1 Cell	5	2	0	0	0	3	0	3	3	1	8.5	0.000106
Group C												
Patient No. 12*-15	0	0	0	0	0	0	0	0	0	0	0	0.0
a1-1 Cell	0	1	0	0	1	4	0	0	1	0	3.5	0.000044
Group D												
Patient No. 16-19	0	0	0	0	0	0	0	0	0	0	0	0.0
a1-1 Cell	0	0	0	0	0	3	0	0	0	1	2	0.0000025
Group E												
Patient No. 20-24*	0	0	0	0	0	0	0	0	0	0	0	0.0
a1-1 Cell	3	2	0	0	0	3	0	0	0	0	4	0.000005
Group F												
Patient No. 25-29*	0	0	0	0	0	0	0	0	0	0	0	0.0
a1-1 Cell	4	2	4	0	0	1	0	0	0	0	5.5	0.0000069
Group G												
Patient No. 30-35*	0	0	0	0	0	0	0	0	0	0	0	0.0
a1-1 Cell	3	0	0	0	0	2	2	0	0	0	3.5	0.0000044
Group H												
Patient No. 36-44	0	0	0	0	0	0	0	0	0	0	0	0.0
a1-1 Cell	7	2	3	5	0	3	0	0	5	0	12.5	0.0000156
NIH 3T3 Cell**	0	0	0	0	0	0	0	0	0	0	0	0.0
No DNA**	0	0	0	0	0	0	0	0	0	0	0	0.0

*In some tumor donor DNAs (DNA from patients numbered 3, 12, 24, 29 and 35), colonies were formed but following their dispersal no foci were developed, therefore such formed colonies were interpreted as clusters formed during trypsinization and washing steps.
**In each set of experiments, DNA from NIH 3T3 and plates without external DNA were also included as negative controls and as shown in the table colony formation was not observed.

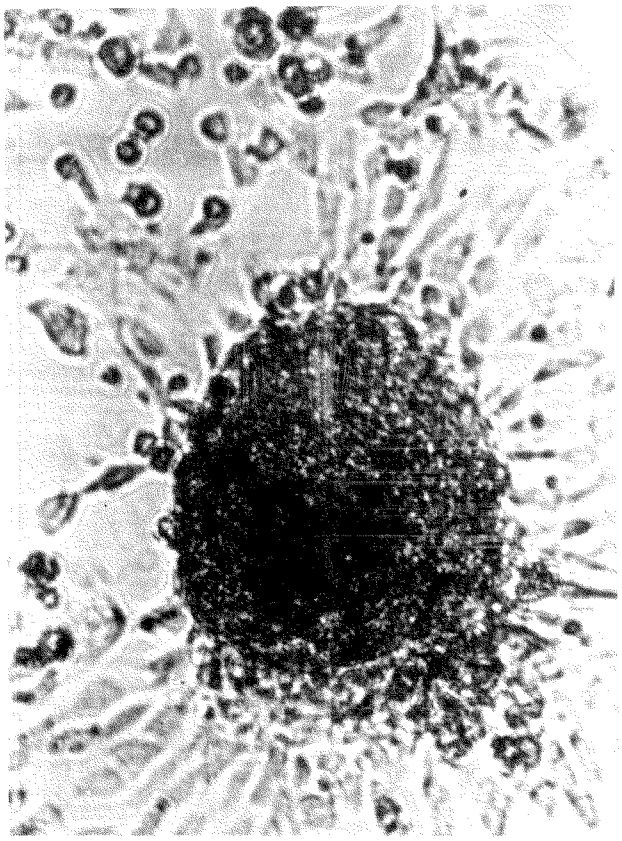

Figure 3. Colony formed from positive control DNA (extracted from a1-1 cells) when applied against NIH 3T3 cells in transfection assay

in a wide variety of cancers such as breast [4, 23], lung [4, 24], liver [25, 26], brain [27, 28, 29] and colon [30, 31], in which a number of variant proteins of tumor differ from the normal pattern. In the present 2-DE study, several polypeptides were identified and appeared to be tumor-associated. We were able to localize many spots in normal esophageal tissue which underwent considerable quantitative variations including: down regulation and disappearance in tumor compared with normal specimens, or the appearance of new spots or the overexpression of others in malignant specimens. A significant number of 2-DE studies have suggested using the changes in the protein maps of normal and malignant specimens as tumor-associated markers for diagnostic application in certain types of cancers when histological criteria are inconclusive or the amount of specimen is limited and not sufficient [24, 28, 32, 33]. However, there is only one study which has reported the identification of five variant polypeptides in normal and tumor samples in patients with esophageal carcinomas [14]. None of these reported spots was included in our study, although we have observed the variation in their pattern as previously reported [14]. In other words, to the best of our knowledge our reported spots are the first ones to be introduced for specimens obtained from patients with esophageal cancer. The presence of special proteins in the normal tissue, which were subjected to several types of changes in tumors, suggests that such polypeptides are either directly or indirectly involved in the maintenance of the normal phenotype and may be essential for transformation suppressor activity. This notion has been supported by some other previous studies concerning different tumor cell types [34, 35]. This suggestion can also be supported by the recent study in which a resumption of many protein activities was observed following transfection of p53 null non-small cell lung carcinoma cell line with the wild type of p53 tumor suppressor gene [36]. Although the main part of our study is descriptive in nature and only involves the identification of protein pattern differences between normal and malignant esophagus samples, previous reports have shown that several tumor suppressor genes are affected in the tumor tissue of esophagus [37,38,39,40]. On the other hand, those spots which appeared for the first time and underwent overexpression may be induced and associated with the malignant status of esophagus tissue. In other words, the expression or modulation of some of these polypeptides may reflect the synthesis of novel proteins which are associated with the development of neoplastic phenotype, since a similar suggestion has been proposed for describing the differences between high and low metastatic cell clones [33].

Some of our detected spots show quite a resemblance both in pI and molecular weight to those shown by other investigators in normal, polyp, and carcinoma [41] and nuclear matrix proteins of human colon cancer [31] respectively. These are spot A and the area which comprised spots B to E from the normal tissue, along with spots 1,2,6 and 7 of the tumor tissue. Such similarity between 2-DE data of esophagus and colon tumors may be due to the common origin of the two organs during the developmental differentiation events and possibly because they are subjected to similar mechanisms and pathways which lead their affected cells to the phenomena of carcinogenesis.

Our results show that the extracted DNAs from esophageal tumor specimens were unable to transform the NIH 3T3 cells. This observation may corroborate studies which thus far have concluded that *ras* family genes have no role in the development of this type of malignancy [42]. This is because no report has indicated the alteration of *ras* oncogene in esophageal cancer, and it is well known that *ras* oncogene can transform NIH 3T3 cells in the culture. On the other hand, Sakamoto *et al.* were the first to report the discovery of *hst*-1 oncogene following the transformation of NIH 3T3 cells by tumorous stomach DNA [22]. Also these investigators reported a high ratio of

hst-1 activated oncogene in esophagus tumors [8]. However, since one normal stomach tissue and only two tumors from a large number of stomach tumors could transform NIH 3T3 cells in the study of Sakamoto *et al.*, these authors attributed the observed transforming activity of the tumorous stomach DNA in their previously reported data to artifactual effects caused by *hst*-1 rearrangement during transfection assay. This interpretation may validate our observation of the lack of potential transforming activity of DNAs extracted from a high number of esophagus tumors. Although we did not investigate the status of *hst*-1 in our study, we do not exclude the possibility that this oncogene was altered in the esophagus tumors we examined. In other words, we suggest that the oncogenicity of *hst*-1 oncogene, which leads to it becoming involved in the development of esophageal cancer, does not necessarily mean that this gene has the ability to transform NIH 3T3 cells.

In conclusion and comparing our results with those of other laboratories, we were able to confirm the presence of 15 variant polypeptides in esophagus tumor tissues. This observation encouraged us to consider them as potentially useful tumor-associated markers for the esophagus cancer diagnosis strategy. Our next attempt will be the identification of these proteins and determination of their possible roles in the process of tumor invasion. We are presently in the process of developing monoclonal antibodies against these polypeptides after their recovery from prepared gels.

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Correction

Regrettably, the following error occurred during the process of printing of the previous issue (Vol. 9, No. 2, Spring 1998, Bahar 1377, *J. Sci. I.R. Iran*) beyond the journal management's control which is hereby corrected:

The legend for Figure 2 on page 139 was misplaced with the legend for Figure 6 on page 141.