Separation and identification of differentially expressed nuclear matrix proteins between human esophageal immortalized and carcinomatous cell lines

Xing-Dong Xiong, En-Min Li, Li-Yan Xu, Hai-Bin Chen, Ling Chen, Wei-Jia Cai, Ya-Li Han, Zhong-Ying Shen, Yi Zeng

CONCLUSION: These differentially expressed NMPs may play an important role during malignant transformation from SHEE to SHEEC. Their separation and identification will contribute to searching for specific markers and probing into the pathogenesis of esophageal carcinoma.

Xiong XD, Li EM, Xu LY, Chen HB, Chen L, Cai WJ, Han YL, Shen ZY, Zeng Y. Separation and identification of differentially expressed nuclear matrix proteins between human esophageal immortalized and carcinomatous cell lines. World J Gastroenterol 2003; 9(10): 2143-2148
http://www.wjgnet.com/1007-9327/9/2143.asp

INTRODUCTION

Nuclear matrix (NM) represents the insoluble structural framework of nucleus which removes membrane lipid, soluble protein and chromatin. According to many investigations, nuclear matrix has been shown to play an important role not only in maintaining the structure of nucleus, but also in chromatin/chromosome construction, DNA replication, gene expression and regulation (RNA synthesis, RNA splicing and RNA transportation)\(^1\). Recently changes of the composition, structure and function of NMPs in the generation and development of tumors have been more and more concerned. Separation and identification of tumor associated NMPs have been a new way to search for tumor specific markers and to study tumor pathogenesis. Nowadays several tumor specific NMPs have been separated and identified from hepatocellular carcinoma\(^2\), colon cancer\(^3\) and prostate cancer\(^4\), etc. Some of them (such as NMP22) have been applied to clinical diagnosis and therapy\(^5\), but studies of the separation and identification of esophageal carcinoma specific NMPs have not been carried out.

Esophageal carcinoma is one of the most common malignant tumors in China\(^6\)-\(^7\). In recent years, it has been increasingly concerned about the roles of human papilloma virus (HPV) in esophageal carcinogenesis\(^8\)-\(^13\). In our previous work, we transfected human embryonic esophageal mucosal cells with HPV18 E6E7 genes, and established an immortalized epithelial cell line SHEEC\(^13\),\(^14\). The SHEE cells were further exposed to the tumor promoter (12-O-tetradecanoyl-phorbol-13-acetate, TPA) to induce malignant transformation from which a human embryonic esophageal epithelial carcinoma cell line SHEEC was then established\(^15\),\(^16\). These studies not only provided the evidence for the close relationship between HPV and esophageal carcinogenesis, but also established a reliable model for studying the molecular mechanisms of esophageal carcinogenesis. In the present study, the differentially expressed NMPs between SHEE and SHEEC were investigated by Western blot, 2-DE and MALDI-TOF-MS, and three esophageal carcinoma associated NMPs were preliminarily identified. The separation and identification of these proteins may contribute to searching for specific markers and studying the pathogenesis of esophageal carcinoma.
MATERIALS AND METHODS

Cell culture

SHEE and SHEEC were cultured in MEM medium (Gibco) supplemented with 100 mL/L fetal bovine serum (100 u/mL penicillin, 100 u/mL streptomycin) and incubated at 37 °C in humidified atmosphere of 50 mL/L CO2. Cells were harvested when they grew into a full monolayer and kept at -70 °C until use.

Extraction of NMPs

The method used was modified from Fey et al[37]. Cultured cells were extracted by cytoskeleton (CSK) buffer (100 mM KCl, 3 mM MgCl2, 1 mM EGTA, 10 mM PIPES pH 6.8, 300 mM sucrose, 0.5 % triton X-100, 1.2 mM PMSF) and dialyzed overnight at 4 °C for 20 min. After centrifugation at 1000 g for 10 min, the pellets were resuspended in digestion buffer (same as CSK buffer except with 50 mM NaCl instead of KCl) containing 400 μg/mL DNase I and 100 μg/mL RNase A. Enzyme digestion was carried out for 20 min at room temperature and terminated by adding cold ammonium sulfate to a final concentration of 0.25 M. After centrifugation at 1000 g for 10 min, the pellets were then solubilized in disassembly buffer (8 M urea, 20 mM Mes pH 6.2, 2 mM β-mercaptoethanol, 1 mM PMSF) and dialyzed overnight at 4 °C against 1000 volumes of assembly buffer (150 mM KCl, 5 mM MgCl2, 0.125 mM EGTA, 25 mM imidazole hydrochloride pH 7.1, 2 mM dithiothreitol, 0.2 mM PMSF). The samples were centrifuged at 200000 g for 100 min. The protein concentration of supernatants containing NMPs was determined by Bradford method and then precipitated in 5 volumes of absolute ethanol. The dried pellets were resuspended in electrophoresis sample buffer. The sample aliquots were stored at -70 °C until use.

Western blot

The experimental procedures were referred to Molecular cloning[38]. Briefly, the proteins were separated by SDS-PAGE and then transferred onto nitrocellulose membranes. Nonspecific reactivity was blocked by incubation overnight at 4 °C in buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 2 % Tween-20, 4 % bovine serum albumin). The membrane was then incubated with primary antibody including mouse anti-human histone (Roche), mouse anti-human DNA topoisomerase IIα (Roche) and mouse anti-human PCNA (Dako). The secondary antibody (Zymed) was used to detect bound primary antibody. Reactive protein was detected by Western blot luminol reagent (Santa Cruz) and exposed to X-films (Kodak). The X-films were scanned and analyzed with Kodak 1-D 3.5 software (Kodak).

Two-dimensional electrophoresis

To separate NMPs, the 2-DE procedures were referred to Xiong et al[37]. Briefly, 2-DE was carried out by using the Mini-PROTEAN II 2-D apparatus (Bio-Rad). 90 μg of the NMPs was mixed with the rehydration solution to a total volume of 125 μL. After rehydration, the isoelectric focusing (IEF) and equilibration, and the IPG strips (pH 4-7, 7 cm) were placed on a 1.0 mm thick, 10 % SDS-PAGE gel. On electrophoresis, the SDS-PAGE gels were stained with PlusOne™ silver staining kit (Phamacia).

Image acquisition and analysis

Image scanning for the silver-stained 2-D gels was performed with EDAS290 digital camera system (Kodak) and image analysis with the PDQuest 6.2 software (Bio-Rad). To obtain reliable results, three gels were employed for each cell line. After the background subtraction, spot detection and match, one standard gel for each cell line was obtained. These standard gels were then matched to yield information about the spots of differentially expressed NMPs.

Protein identification by MALDI-TOF-MS

Three spots in which the differentially expressed protein was more obvious in each cell line were cut out from the gel. The gel pieces were treated by a series of steps including silver-removal, reduction, alkylation and in-gel digestion with trypsin. The peptide mass maps were generated by Applied Biosystems Voyager System 6192 MALDI-TOF-mass spectrometry (ABI, USA). Peptide masses were analyzed using the MS-Fit search program (http://prospector.ucsf.edu/ucsfhtm4.0u/msfit.htm).

RESULTS

Evaluation of NMPs quality

The NMPs extracted from SHEE and SHEEC were evaluated by Western blot, and the aim of evaluation was to monitor whether some NMPs were lost and whether other non-NMPs components were mixed. In recent years, many studies proved that DNA topoisomerase IIα and PCNA were the major components of NMPs[40,41], the existence of histone in nucleosome was the major soluble protein of nucleus. In our study, 170 KD DNA topoisomerase IIα was detected in the nuclear protein fraction of SHEE and SHEEC while the same position of NMPs fraction, but the density in NMPs fraction was weaker than that in the nuclear protein fraction (Figure 1). This agreed with other reports[40]. PCNA (36kD) was detected in the NMPs fraction of SHEE and SHEEC, and this showed PCNA was the major protein of NMPs (Figure 2). Moreover, histone was seen at 23 kD and 12-15 kD in the nuclear protein fraction (Mw of histone: H1:23 kD, H2A:14.5 kD, H2B:13.8 kD, H3:15 kD, H4:11.8 kD) and almost no histone was detected at the same position of NMPs (Figure 3). This showed majority of histone was removed during extraction and there was almost no histone in the NMPs fraction of SHEE and SHEEC. According to these results, the NMPs fractions extracted from SHEE and SHEEC almost deleted the histone and retained the major NMPs components including DNA topoisomerase IIα and PCNA, so the NMPs were pure in high quality.

Figure 1 Western blot analysis of DNA topoisomerase IIα. Lane 1, nuclear matrix fraction of SHEE. Lane 2, total crude nuclear protein fraction of SHEE. Lane 3, total crude nuclear protein fraction of SHEEC. Lane 4, cytoplasmic protein fraction of SHEE. Lane 5, cytoplasmic protein fraction of SHEEC. M, molecular weight standard.

Figure 2 Western blot analysis of PCNA. Lane 1 to Lane 3, nuclear matrix fraction of SHEE. Lane 4 to Lane 6, nuclear matrix protein of SHEE. M, molecular weight standard.

Figure 3 Two-dimensional electrophoresis of SHEE and SHEEC. M, molecular weight standard.
Figure 3 Western blot analysis of histone. Lane 1, nuclear matrix fraction of SHEEC. Lane 2, nuclear matrix protein of SHEE. Lane 3, cytoplasmic protein fraction of SHEEC. Lane 4, cytoplasmic protein fraction of SHEE. Lane 5, total crude nuclear protein fraction of SHEEC. Lane 6, total crude nuclear protein fraction of SHEE. M, molecular weight standard.

2-D map and image analysis
To obtain a higher electrophoretic resolution, IPG strips (pH 4-7) were selected to separate the NMPs of SHEE and SHEEC in our study because the pI range of NMPs was mainly in acid pH. Three pairs of gels from different batches of SHEE and SHEEC were analyzed by using the software PDQuest6.2. There were 106±7.1 and 132±5.0 protein spots observed in SHEE and SHEEC respectively. Most of them were matched one another (r=0.72), only 16 protein spots were found differing in intensity. These 16 protein spots belonged to 4 types. Namely A: one protein spot was detected in SHEE (No.2). B: Seven protein spots were detected only in SHEEC (No.3, 4, 9, 11, 12, 14, 16), C: One protein spot was expressed higher in SHEE (No.1), D: Seven protein spots were expressed higher in SHEEC (No.5, 6, 7, 8, 10, 13, 15). According to the standard molecular weight and pH gradient of IPG strips, the positions of these 16 protein spots were estimated with the software PDQuest6.2 (Table 1). Three protein spots (No.4, 14, 16) which belonged to type B and had a higher intensity were selected and analyzed with MALDI-TOF-MS (Figure 4).

MALDI-TOF-MS analysis and protein identification
These three protein spots (No.4, 14, 16) were cut out from the gels and analyzed with MALDI-TOF-MS. Peptide mass fingerprint (PMF) of each protein spot was then generated (Figure 5). By searching the NCBI protein database with the MS-Fit search program, we identified these three proteins combined with the searching results. The characteristics of the protein, the number and intensity of peptide matching peak, the sequence coverage of matching peptide, as well as the theoretical and approximate values of Mr and pI, the identified protein names, accession numbers, as well as the sequence coverages, the theoretical Mr and pI values for each protein spot are listed in Table 2.

Figure 4 Differentially expressed NMP spots observed in SHEE (left) and SHEEC (right) two-dimensional gels (IPG dry strips: pH 4-7, 7 cm). The arrows show differentially expressed protein spots. Three protein spots (No.4, 14, 16) were selected and analyzed with MALDI-TOF-MS.

Table 1 Differentially expressed protein spots between SHEE and SHEEC

| Spot No. | Type | Experimental Mr | Experimental pI | Spot No. | Type | Experimental Mr | Experimental pI |
|----------|------|-----------------|-----------------|----------|------|-----------------|-----------------|
| 1        | C    | 73.0            | 5.55            | 9        | B    | 38.2            | 5.79            |
| 2        | A    | 45.1            | 6.60            | 10       | D    | 35.8            | 5.96            |
| 3        | B    | 34.0            | 4.10            | 11       | B    | 33.5            | 5.83            |
| 4        | B    | 30.0            | 4.60            | 12       | B    | 23.1            | 5.88            |
| 5        | D    | 62.5            | 4.50            | 13       | D    | 44.6            | 6.32            |
| 6        | D    | 21.0            | 5.03            | 14       | B    | 37.6            | 6.66            |
| 7        | D    | 26.2            | 5.45            | 15       | D    | 24.6            | 6.33            |
| 8        | D    | 22.2            | 5.53            | 16       | B    | 22.7            | 6.54            |

Table 2 Proteins identified by MALDI-TOF-MS

| Spot No. | Accession No.(gi) | Theoretical Mr | Theoretical pI | Intensity matched | Length (aa) | Name of protein                      |
|----------|-------------------|----------------|---------------|------------------|-------------|--------------------------------------|
| 4        | 37424             | 27975          | 4.8           | 44 %             | 239         | Cytoskeletal tropomyosin              |
| 14       | 17149849          | 37227          | 6.9           | 29 %             | 327         | FK506-binding protein 6              |
| 16       | 13647876          | 26775          | 5.7           | 54 %             | 230         | Similar to retinoblastoma binding protein 8 |
study, cytoskeletal tropomyosin (TPM3) showed overexpression in the NMPs fraction of SHEEC cells, indicating that cytoskeletal tropomyosin is related to the generation and development of colon carcinoma. Furthermore, Martin-Zanca and his associates have proved that cytoskeletal tropomyosin is related to the chromosome scaffold, and dispersively distributed in the interphase nuclei and metaphase chromosomes. Many studies found that tropomyosin was localized in the nuclear matrix and endoplasmic reticulum, and FKBP25 in the nucleus. Like other FKBP family members, FKBP6 was overexpressed in the NMPs fraction of SHEEC. This indicated that FKBP6 as an active NMP might play a role during malignant transformation of the immortalized human esophageal epithelial cells.

Cytoskeletal tropomyosin (tropomyosin 3, cytoskeletal (TPM3)) belongs to the tropomyosin family. There were at least 5 isoforms in tropomyosins including TPM1, TPM2, TPM3, TPM4, TPM5 which were produced by a tissue-specific alternative mRNA splicing mechanism. The tropomyosins were a group of actin-binding proteins which served to mediate the effect of Ca2+ on the actin-myosin interaction. Actin is one of the major components of NMPs. Owing to active cell division during the course of malignant transformation, cytoskeletal tropomyosin connected with actin in the nucleus becomes overexpressed. Zeng and his associates found that tropomyosin was localized in the nuclear matrix and chromosome scaffold, and dispersively distributed in the interphase nuclei and metaphase chromosomes. Many studies have proved that tropomyosin is related to the generation and development of tumors. Martin-Zanca and his associates found the oncogene Onc D (named later "trk") in colon carcinoma. Sequence analysis for the oncogene Onc D found that it was generated by a somatic rearrangement of two genes, one of which coded for cytoskeletal tropomyosin (TPM3) and the other for a tyrosine-specific protein kinase. The amino end of TPM3 has been found to be fused with tyrosine protein kinase domain and becomes a new oncogenic protein. These results indicate that cytoskeletal tropomyosin is related to the generation and development of colon carcinoma. Furthermore, Lawrence and his associates reported that TPM3-ALK (anaplastic lymphoma kinase, ALK) and TPM4-ALK fusion genes occurred in inflammatory myofibroblastic tumors. In our study, cytoskeletal tropomyosin (TPM3) showed overexpression in the NMPs fraction of SHEEC cells, indicating that cytoskeletal tropomyosin as a oncoprotein might be related to esophageal carcinoma. But its characteristics and functions in esophageal carcinoma remain to be further studied.

Figure 5 MALDI-TOF mass spectrum map of protein spot 16.

DISCUSSION

In the present study, we studied the differentially expressed NMPs extracted from SHEE and SHEEC by Western blot, 2-DE and MALDI-TOF-MS, and preliminarily identified three differentially expressed NMPs. According to their characteristics, three of these proteins were connected with diseases such as colon carcinoma and myofibroblastic tumor, etc., but they were not related to esophageal carcinoma.

The tropomyosins (tropomyosin 3, cytoskeletal (TPM3)) belongs to the tropomyosin family. There were at least 5 isoforms in tropomyosins including TPM1, TPM2, TPM3, TPM4, TPM5 which were produced by a tissue-specific alternative mRNA splicing mechanism. The tropomyosins were a group of actin-binding proteins which served to mediate the effect of Ca2+ on the actin-myosin interaction. Actin is one of the major components of NMPs. Owing to active cell division during the course of malignant transformation, cytoskeletal tropomyosin connected with actin in the nucleus becomes overexpressed. Zeng and his associates found that tropomyosin was localized in the nuclear matrix and chromosome scaffold, and dispersively distributed in the interphase nuclei and metaphase chromosomes. Many studies have proved that tropomyosin is related to the generation and development of tumors. Martin-Zanca and his associates found the oncogene Onc D (named later "trk") in colon carcinoma. Sequence analysis for the oncogene Onc D found that it was generated by a somatic rearrangement of two genes, one of which coded for cytoskeletal tropomyosin (TPM3) and the other for a tyrosine-specific protein kinase. The amino end of TPM3 has been found to be fused with tyrosine protein kinase domain and becomes a new oncogenic protein. These results indicate that cytoskeletal tropomyosin is related to the generation and development of colon carcinoma. Furthermore, Lawrence and his associates reported that TPM3-ALK (anaplastic lymphoma kinase, ALK) and TPM4-ALK fusion genes occurred in inflammatory myofibroblastic tumors. In our study, cytoskeletal tropomyosin (TPM3) showed overexpression in the NMPs fraction of SHEEC cells, indicating that cytoskeletal tropomyosin as a oncoprotein might be related to esophageal carcinoma. But its characteristics and functions in esophageal carcinoma remain to be further studied.

FK506-binding protein 6 (FKBP6) is one member of FKBP family. The FKBP family proteins were those directly binding to the immunosuppressant drug FK506. So far there are several FKBP family members, namely FKBP6, FKBP12, FKBP13, FKBP25 and FKBP52. These FKBP family members differ in their subcellular localization. FKBP12 and FKBP25 are localized in the cytosol, FKBP13 in the endoplasmic reticulum, and FKBP25 in the nucleus. Like other FKBP members, FKBP6 had some peptidyl-prolyl cis-trans isomerase activity and a drug binding site. Owing to its possession of cis-trans isomerase activity, FKBP6 are thought to be essential for protein folding during protein synthesis. FKBP6 gene is localized in chromosome 11 and consists of nine exons. Meng and his associates found FKBP6 gene was deleted in Williams syndrome (WS), and this might contribute to certain defects such as hypercalcemia and growth delay in WS. In our study, FKBP6 was overexpressed in the NMPs fraction of SHEEC. This indicated that FKBP6 as an active NMP might play a role during malignant transformation of the immortalized human esophageal epithelial cells.

Similar to retinoblastoma binding protein 8 (STRBP8), it contains 230 amino acid residues. Its sequences are the same as residues 668-897 of retinoblastoma binding protein 8 (RBP8), and so it is named STRBP8. So far there are not any reports about the function of STRBP8. Moreover, RBP8 is localized in the nucleus. It might interact with some tumor suppressive factors including CtBP1, Rb1 and BRCA1, and was involved in transcription regulation and tumor suppression. The interaction with CtBP1 requires a short cis-trans isomerase activity, and FKBP6 are thought to be essential for protein folding during protein synthesis. FKBP6 gene is localized in chromosome 11 and consists of nine exons. Meng and his associates found FKBP6 gene was deleted in Williams syndrome (WS), and this might contribute to certain defects such as hypercalcemia and growth delay in WS. In our study, FKBP6 was overexpressed in the NMPs fraction of SHEEC. This indicated that FKBP6 as an active NMP might play a role during malignant transformation of the immortalized human esophageal epithelial cells.
be involved in the malignant transformation of SHEE.

Although three NMPs have been found to be associated with esophageal carcinoma in the present study, but whether they would become specific markers of esophageal carcinoma needs to be further studied. In addition, the generation and development of tumors are the extraordinary pathological complex phenomena, and the process of coordinated interaction and programmed development of many proteins. It will help elucidate the process of tumor generation and development to study the network relationship of these tumor associated proteins. Therefore, the network functional relationship of these differentially expressed NMPs needs to be further studied.

REFERENCES
1. Pederson T. Thinking about a nuclear matrix. J Mol Biol 1998; 277: 147-159
2. Yoon GS, Lee H, Jung Y, Yu E, Moon HB, Song K, Lee I. Nuclear matrix of calreticulin in hepatocellular carcinoma. Cancer Res 2000; 60: 1117-120
3. Brunagel G, Vietmeier BN, Bauer AJ, Schoen RE, Getzenberg RH. Identification of nuclear matrix protein alterations associated with human colon cancer. Cancer Res 2002; 62: 2437-2442
4. Lakshmanan Y, Subong EN, Partin AW. Differential nuclear matrix protein expression in prostate cancers: correlation with pathological stage. Urol 1998; 159: 1235-1239
5. Oge G, Atsu N, Kendi S, Ozen H. Evaluation of nuclear matrix protein 22 (NMP22) as a tumor marker in the detection of bladder cancer. Int Urol Nephrol 2001; 32: 367-370
6. Xiong XD, Xu LY, Shen ZY, Cai WJ, Luo JM, Han YL, Li EM. Identification of differentially expressed proteins between human esophageal immortalized and carcinomatous cell lines by two-dimensional electrophoresis and MALDI-TOF-mass spectrometry. World J Gastroenterol 2002; 8: 777-783
7. Hou J, Lin PZ, Shen FS, Guo LP, He YT, Qiao CY, Guo CL, Duan JP, Wen DG. Field population-based blocking treatment of esophageal epithelium dysplasia. World J Gastroenterol 2002; 8: 418-422
8. Hao MW, Liang YR, Liu YF, Liu L, Wu MY, Yang HX. Transcription factor EGR-1 inhibits growth of hepatocellular carcinoma and esophageal carcinoma cells lines. World J Gastroenterol 2002; 8: 209-217
9. Shen ZY, Shen WY, Chen MH, Shen J, Cai WJ, Zeng Y. Mitochondria, calcium and nitric oxide in the apoptotic pathway of esophageal carcinoma cells induced by As2O3. Int J Mol Med 2002; 9: 385-390
10. Xu M, Jin YL, Fu J, Huang H, Chen SZ, Qu P, Tian HM, Liu ZY, Zhang W. The abnormal expression of retinoic acid receptor-β, p53 and Ki67 protein in normal, premalignant and malignant esophageal tissues. World J Gastroenterol 2002; 8: 200-202
11. Deng LY, Zhang YH, Xu P, Yang SM, Yuan XB. Expression of IL-10 converting enzyme in 5-FU induced apoptosis in esophageal carcinoma cells. World J Gastroenterol 1999; 5: 50-52
12. Wang AH, Sun CS, Li LS, Huang YJ, Chen QS. Relationship of tobacco smoking, CYP1A1, GSTM1 gene polymorphism and esophageal cancer in Xi’an. World J Gastroenterol 2002; 8: 49-53
13. Shen ZY, Xu LY, Li EM, Cai WJ, Chen MH, Shen J, Zeng Y. Telomere and telomerase in the initial stage of immortalization of esophageal epithelial cell. World J Gastroenterol 2002; 8: 357-362
14. Shen ZY, Shen WY, Chen MH, Shen J, Cai WJ, Yi Z. Nitric oxide and calcium ions in apoptotic esophageal carcinoma cells induced by arsenite. World J Gastroenterol 2002; 8: 40-43
15. Shen ZY, Shen J, Li QS, Chen CY, Chen JY, Zeng Y. Morphological and functional changes of mitochondria in apoptotic esophageal carcinoma cells induced by arsenic trioxide. World J Gastroenterol 2002; 8: 357-362
16. Shen ZY, Xu LY, Li C, Cai WJ, Shen J, Chen JY, Zeng Y. Comparative study of telomerase activity and malignant phenotype in multi-step immortalization of oral squamous cell carcinoma cells induced by human papillomavirus. Int J Mol Med 2001; 8: 633-638
17. Yu GQ, Zhou Q, Ivan D, Gao SS, Zheng ZY, Zou JX, Li YX, Wang LD. Changes of p53 protein blood level in esophageal cancer patients and normal subjects from a high incidence area in Henan, China. World J Gastroenterol 1998; 4: 305-306
18. Li J, Feng CW, Zhao ZG, Zhou Q, Wang LD. A preliminary study on ras protein expression in human esophageal cancer and pre-cancerous lesions. World J Gastroenterol 2000; 6: 278-280
19. Liu XL, Xiao B, Yu ZC, Guo JC, Zhao QC, Xu L, Shi YQ, Fan DM. Down-regulation of Hsp90 could change cell cycle distribution and increase drug sensitivity of tumor cells. World J Gastroenterol 1999; 5: 199-208
20. Zhang LJ, Chen KN, Xu GW, Xing HP, Shi XT. Congenital expression of mdr-1 gene in tissues of carcinoma and its relation with pathomorphology and prognosis. World J Gastroenterol 1999; 5: 53-56
21. Gao SS, Zhou Q, Li YX, Bai YM, Zheng ZY, Zou JX, Liu G, Fan ZM, Qi YJ, Zhao X, Wang LD. Comparative studies on epithelial lesions at gastric cardia and pyloric antrum in subjects from a high incidence area for esophageal cancer in Henan, China. World J Gastroenterol 1998; 4: 332-333
22. Shen ZY, Tan L, Cai WJ, Shen J, Cai WJ, Tang XM, Zheng MH. Arsenic trioxide induces apoptosis of esophageal carcinoma in vitro. Int J Mol Med 1999; 4: 33-37
23. Shen ZY, Shen J, Cai WJ, Hong C, Zheng MH. The alteration of mitochondria is an early event of arsenic trioxide induced apoptosis in esophageal carcinoma cells. Int J Mol Med 2000; 5: 75-78
24. Gu ZP, Wang YJ, Li JG, Zhou Y. VEGF165 antisense RNA suppresses oncogenic properties of human esophageal squamous cell carcinoma. World J Gastroenterol 2002; 8: 44-48
25. Su M, Lu SM, Tian DP, Zhao H, Li LY, Li DR, Zheng ZC. Relationship between ABO blood groups and carcinoma of esophagus and cardia in Chaoshan inhabitants of China. World J Gastroenterol 2001; 7: 657-661
26. Wu MY, Chen MH, Liang YR, Meng GZ, Yang HX, Zhuang CX. Experimental and clinicopathologic study on the relationship between transcription factor Egr-1 and esophageal carcinoma. World J Gastroenterol 2001; 7: 490-495
27. Xiao ZF, Yang ZY, Zhou ZM, Yin WB, Gu XZ. Radiotherapy of doubleprimary esophageal carcinoma. World J Gastroenterol 2000; 6: 145-146
28. Shen HB, Chen L, Zhang JK, Shen ZY, Su ZJ, Cheng SB, Chew EC. Human papillomavirus 16 E6 is associated with the nuclear matrix of esophageal carcinoma cells. World J Gastroenterol 2001; 7: 788-791
29. Lavergne D, de-Villiers EM. Papillomavirus in esophageal papillomas and carcinomas. Int J Cancer 1999; 80: 681-684
30. Xu LY, Li EM, Xiong HQ, Cai WJ, Shen ZY. Study of neutrophil gelatinase associated lipocalin (NGAL) gene overexpression in the progression of malignant transformation of human immortalized esophageal epithelial cell. Shengwu Huaxue Yu Shengwu Wuli Jinzhan 2002; 8: 839-843
31. Ma QF, Jiang H, Feng YQ, Wang XP, Zhou YA, Liu K, Jia ZL. Detection of human papillomavirus DNA in squamous cell carcinoma of the esophagus. Shi jie Huaren Xiaohua Zazhi 2000; 8: 1218-1224
32. Zhang J, Yan XJ, Yan QJ, Duan J, Hou Y, Su CZ. Cloning and expression of the HPV16 L2 DNA from esophageal carcinoma in E. coli. Shi jie Huaren Xiaohua Zazhi 2001; 9: 273-278
33. Shen ZY, Xu LY, Chen KH, Cai WJ, Shen J, Chen JY, Huang TH, Zeng Y. The genetic events of HPV-immortalized esophageal epithelial cells. Int J Mol Med 2001; 8: 537-542
34. Shen ZY, Chen S, Cai WJ, Teng ZP, Shen J, Hu Z, Zeng Y. Immortalization of human fetal esophageal epithelial cells induced by E6 and E7 genes of human papillomavirus 18. Zhanghua Shiyan HeLinchuan Binguangxue Zazhi 1999; 13: 121-124
35. Shen ZY, Cai WJ, Shen J, Xu JJ, Cen S, Teng ZP, Hu Z, Zeng Y. Human papilloma virus 18E6E7 in synergy with TPA induced malignant transformation of human embryonic esophageal epithelial cells. Bingdou Xuebao 1999; 15: 1-5
36. Shen Z, Cen S, Shen J, Cai W, Xu J, Teng Z, Hu Z, Zeng Y. Study of immortalization and malignant transformation of human embryonic esophageal epithelial cells induced by HPV18 E6E7. J Cancer Res Clin Oncol 2000; 126: 599-594
37. Fey EG, Penman S. Nuclear matrix proteins reflect cell type of origin in cultured human cells. Proc Natl Acad Sci U S A 1988; 85: 121-125
38 Sambrook J, Fritsch EF, Maniatis T. Molecular cloning A Laboratory Manual, 3rd ed. Jin DY, Li MF, trans-ed, Beijing, China: Sci Pub 1998: 888-898
39 Xiong XD, Xu LY, Shen ZY, Han M, Niu YD, Han YL, Li EM. An optimized protocol for two-dimensional gel electrophoresis. Shantou Daxue Xuebao 2002; 17: 5-9
40 Fernandes DJ, Qiuj, Catapano CV. DNA topoisomerase II isozymes involved in anticancer drug action and resistance. Adv Enzyme Regul 1995; 35: 265-281
41 Balajee AS, May A, Bohr VA. Fine structural analysis of DNA repair in mammalian cells. Mutat Res 1998; 404: 3-11
42 Reinach FC, MacLeod AR. Tissue-specific expression of the human tropomyosin gene involved in the generation of the trk oncogene. Nature 1998; 322: 648-650
43 MacLeod AR, Houlker C, Reinach FC, Talbot K. THEMRNA and RNA-copy pseudogenes encoding TM 30 nm, a human cytoskeletal tropomyosin. Nucleic Acids Res 1986; 14: 8413-8426
44 Zeng XL, Jiao MD, Xing M, Wang XG, Hao S. Tropomyosin is localized in the nuclear matrix and chromosome scaffold of Physarum polycephalum. Cell Res 1999; 9: 61-69
45 Martin-Zanca D, Hughes SH, Barbacid M. A human oncogene formed by the fusion of truncated tropomyosin and protein tyrosine kinase sequences. Nature 1986; 319: 743-748
46 Lawrence B, Perez-Atayde A, Hibbard MK, Rubin BP, Dal Cin P, Pinkus JL, Pinkus GS, Xiao S, Yi ES, Fletcher CD, Fletcher JA. TPM 3-ALK and TPM 4-ALK oncogenes in inflammatory myofibroblastic tumors. Am J Pathol 2000; 157: 377-384
47 Fruman DA, Blierer BE, Benes JE, Burakoff SJ, Austen KF, Katz HR. The complex of FK506-binding protein 12 and FK506 inhibits calcineurin phosphatase activity and IgE activation-induced cytokine transcripts, but not exocytosis, in mouse mast cells. J Immunol 1995; 154: 1846-1851
48 Jin YJ, Burakoff SJ. The 25-kDa FK506-binding protein is localized in the nucleus and associates with casein kinase II and nucodolin. Proc Natl Acad Sci U S A 1993; 90: 7769-7773
49 Meng X, Lu X, Morris CA, Keating MT. A novel human gene FKBP6 is deleted in Williams syndrome. Genomics 1998; 52: 130-137
50 Schaeper U, Subramanian T, Lim L, Boyd JM, Chinnadurai G. Interaction between a cellular protein that binds to the C-terminal region of adenovirus E1A (CtBP) and a novel cellular protein is disrupted by E1A through a conserved PLDLS motif. J Biol Chem 1998; 273: 8549-8552
51 Yu X, Baer R. Nuclear localization and cell cycle-specific expression of CtBP, a protein that associates with the BRCA1 tumor suppressor. J Biol Chem 2000; 275: 18541-18549

Edited by Wu XN and Wang XL