Gene-expression Profiling in a Patient with Esophageal Squamous Cell Carcinoma

Nan-Yung Hsu, I-Ping Chiang[1], Chih-Shiung Shih, Pin-Ru Chen, Heng-Chien Ho[2]

Division of Chest Surgery, [1]Department of Pathology, [2]Department of Medical Research, China Medical University Hospital, Taichung, Taiwan, R.O.C.

A 32-year-old man who has been an active tobacco smoker, alcohol drinker and betel nut chewer for more than 10 years was diagnosed as having esophageal squamous cell carcinoma (ESCC) after undergoing transhiatal esophagectomy and reconstruction with gastric graft. The pathology report indicated that the tumor had invaded the superficial muscle layer but had not metastasized to the adjacent lymph nodes. Gene-expression in the ESCC tissue was profiled by the cDNA microarray method. A total of 8354 genes were screened. Among them, 26 genes whose expression levels (fluorescence intensity) in esophageal carcinoma were more than 5 times (up regulated) those expressed in normal esophageal epithelium were selected; in addition, 28 genes whose expression levels in esophageal carcinoma were less than 0.2 times (down regulated) those expressed in the normal esophageal epithelium were selected. A search on MEDLINE of papers published from 1965 to the present revealed that only 7 of the 26 up-regulated genes and only 8 of the 28 down-regulated genes had been reported. We suggest that these characteristic genes will provide useful information for understanding the malignant nature of ESCC occurring in a young patient who has actively consumed tobacco, alcohol, and betel nut. **(Mid Taiwan J Med 2005;10:43-8)**

Key words

esophageal squamous cell carcinoma, microarray

INTRODUCTION

The cDNA microarray technique has been used to profile gene-expression in esophageal squamous cell carcinoma (ESCC); the results have helped uncover the molecular mechanisms of carcinogenesis [1-3], tumor progression [4,5], and chemotherapy sensitivity [6] in ESCC patients. We encountered ESCC in a young patient who actively chewed betel nut, drank alcohol, and smoked tobacco. The purpose of this study was to profile the genes expressed in this young patient with ESCC by cDNA microarray.

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Address reprint requests to : Heng-Chien Ho, Department of Medical Research, China Medical University Hospital, 2 Yuh-Der Road, Taichung 404, Taiwan, R.O.C.

CASE REPORT

A 32-year-old man with a 10-year history of smoking, alcohol drinking, and betel nut chewing presented with acute abdomen. At admission, his serum amylase was 431 U/L and his r-GT was 102 IU/L. Alcoholic pancreatitis was suspected. Subsequent endoscopy revealed an esophageal carcinoma 1 cm above the esophagogastric junction. The patient underwent transhiatal esophagectomy and the esophagus was reconstructed by gastric tube. The postoperative course was uneventful. Subsequent histopathological studies showed a poorly differentiated squamous cell carcinoma without adjacent lymph node metastasis. The tumor, measuring 0.7 cm × 0.5 cm × 0.5 cm, had

Table 1. Gene-expression profiling (fluorescence intensity) in esophageal carcinoma compared with the normal esophageal epithelium

Gene name	Accession*	Ratio of mean†	Genetic locus
Up regulation			
Metabolism/Redox enzymes			
Ornithine decarboxylase 1	AA460115	13.420	2p25
Aldehyde dehydrogenase	AA664101	7.680	9q21.13
Glutathione peroxidase	AA135152	5.721	14q24.1
Argininosuccinate synthetase	AA676466	5.707	9q34
Oligoadenylate synthetase	AA146772	5.675	12q24.1
Arylsulfatase B	AA128825	5.140	5p11
ESTs	AA143342	12.037	21q22.1
Parathyroid hormone-like hormone	AA845432	5.166	12p12.1
Lactotransferrin	AA677706	5.132	3q21
Cell Proliferation/Inflammation			
Gamma-aminobutyric acid A receptor-epsilone	H63934	6.250	Xq28
Tryptophanyl-tRNA synthetase	AA664040	8.003	14q32.31
Replication factor C	H54751	6.414	7q11.23
Chromosome 10 open reading frame 3	AA131908	5.979	10q23.33
Cell adhesion protein			
Integrin-alpha 6	R17993	5.469	2q31
Pleckstrin homology-Sec7	AA480859	5.054	17q25
Cell Cycle regulator			
Insulin-like growth factor binding protein 3	AA598601	5.347	7p13
Retinol binding protein 1-cellular	AA700832	5.225	3q23
Fas-associated via death domain	AA430751	13.114	11q13.3
Jun D proto-oncogene	AA131585	5.688	19p13.2
2`-5`-oligoadenylate synthetase 2-69/71kDa	AF074990	5.227	12q24.2
Intracellular signalling			
Myelin basic protein	N62730	18.838	18q23
Interferon-alpha-inducible protein	AA406019	8.548	11p13
Cell membrane receptor			
Hypothetical protein FLJ10261	AA400086	18.097	11q13.2
Others			
Collagen-type I-alpha 1	R48843	6.645	17q21.3
Cytoskeleton-associated protein 1	AA504477	5.244	19q13.11
Hypothetical protein LOC90850	AA620404	18.996	16p13.3
Down regulation			
Metabolism/Redox enzymes			
Hydroxyprostaglandin dehydrogenase 15-(NAD)	AA775223	0.175	4q34
Transglutaminase	N90882	0.186	20q11.2
Myosin, light polypeptide 9, regulatory	AA877166	0.137	20q11.23
DKFZP566F084 protein	AA773458	0.139	10q24.32
Deiodinase-iodothyronine-type II	AA864322	0.174	14q24.2
Arachidonate 15-lipoxygenase	AI474406	0.198	17p13
Cell Proliferation			
Eukaryotic translation initiation factor 4 gamma-1	R12349	0.161	3q27
Hypothetical protein LOC253827	N91811	0.138	12q14.1
Hypothetical protein MGC2668	AA424545	0.174	9q34.13
Cell Cycle regulator			
Synaptopodin 2	W52186	0.034	4q27
Retinoic acid binding protein 2	AA598508	0.197	1q21.3
Mitogen-activated protein kinase	H01340	0.148	19q13.2
Four and a half LIM domains 1	AA456394	0.149	Xq26
Intracellular signaling			
Mal-T-cell differentiation protein	AA227594	0.172	2cen-q13
p55-GAMMA	AI394701	0.181	1p34.1
BMP-2 inducible kinase	R98008	0.184	4q21.23

*Genbank ID. †Wavelength intensity of tumor (635 nm) and normal tissue (532 nm).

Table 1. Continued

Gene name	Accession*	Ratio of mean†	Genetic locus
Cell adhesion protein			
Sorbin and SH3 domain containing 1	AA459944	0.055	10q23.3
Phosphoglucomutase 5	AA706788	0.064	9p12
Desmuslin	AA877815	0.076	15q26.3
Alpha-actinin-2-associated LIM protein	AA972352	0.177	4q35
Cell membrane receptor			
Brain cell membrane protein 1	N52772	0.183	Xp11.4
Endothelin receptor type B	H28710	0.189	13q22
Others			
Cytokeratin 4	AA629189	0.140	12q12
Collagen-type VIII	AA872420	0.170	3q12.3
Cytokeratin 1	AA706022	0.174	12q12
Serine protease inhibitor	Hs.331555	0.148	5q32
Caldesmon 1	AA402898	0.173	7q33
Alpha-fetoprotein	T56880	0.174	4q11

Table 2. A search of MEDLINE for papers published from 1965 to the present revealed that 7 of the 26 up-regulated genes and 8 of the 28 down-regulated genes had been reported

Number	Up-regulated genes	Down-regulated genes
1	Ornithine decarboxylase	Transglutaminase
2	Aldehyde dehydrogenase	Serine protease inhibitor
3	Glutathione peroxidase	Retinoic acid binding protein
4	Parathyroid hormone-like hormone	Cytokeratin 4
5	Lactotransferrin	Mitogen-activated protein kinase
6	Integrin-alpha 6	Arachidonate 15-lipoxygenase
7	Fas-associated via death domain	Cytokeratin 1
8		Alpha-fetoprotein

infiltrated the superficial muscle layer. The pathological stage was T2N0M0 (IIA). The patient has been well for one year after the surgery without any sign of recurrence.

Gene expression in the ESCC tissue was profiled by the cDNA microarray method. In brief, total RNA was extracted from the normal and tumor tissues by TRIzol reagent. The isolated RNA was qualified by gel analysis and the ratio of absorbance was determined at 260 nm and 280 nm (> 1.7). Thirty micrograms of total RNA from normal and tumor tissues were Cy3- and Cy5-labeled, respectively. PCR products were arrayed by a Microgrid II pro arrayer (Biorobotics Ltd., Cambridgeshire, United Kingdom). The labeled RNA was then hybridized at 62°C for 15.5 h. Arrays were scanned and each spot was defined by automatic positioning of a grid using image-analysis spot-tracking software (patent US 10/173,672 June 19, 2002; CA 2,389,901 June 20, 2002). We focused first on a set of 1416 genes (about 17.0% of the 8354 genes analyzed, which comprised 922 up-regulated genes and 494 down-regulated genes), whose transcripts varied in abundance by at least twofold expression levels (fluorescence intensity) from their mean abundance in esophageal carcinoma compared with the normal esophageal epithelium. Among the 922 up-regulated genes, 26 (2.8%) genes whose expression levels in esophageal carcinoma were more than 5 times the levels expressed in normal esophageal epithelium were selected. In addition, 28 (5.7%) of the 494 down-regulated genes whose expression levels in esophageal carcinoma were less than 0.2 times the levels expressed in normal esophageal epithelium were selected. These genes were classified according to their biologic roles, such as metabolism, cell proliferation, cell adhesion protein, cell cycle regulator, intracellular signaling and transmembrane protein (Table 1). A search of MEDLINE for papers published from 1965 to

the present revealed that only 7 of the 26 up-regulated genes and only 8 of the 28 down-regulated genes have been reported to be associated with gene expression in ESCC (Table 2).

DISCUSSION

The major methods for screening and identifying specific gene(s) involved in carcinogenesis, disease progression and resistance of treatment of ESCC comprise microarray, cDNA subtraction, serial analysis of gene expression (SAGE), differential display and proteomics [6-8]. Although cDNA microarray technology has limitations, such as chip to chip variation, unanswered biological relevance of fold change, cross-hybridization of sequences with high identity, and difficulty of mRNA levels reflecting protein levels [9], it has enabled the expression profiles of thousands of genes to be analyzed simultaneously. In addition, although the correlation between clinicopathological phenotypes and gene expression status has not been completely proven, this technique can provide a powerful means to stratify tumors into classes with distinct molecular pathophysiologies [5].

Several of the genes expressed in ESCC tissue identified in the literature were also found in this study. For example, ornithine decarboxylase was the most highly (13.4-fold) expressed up regulated gene in this study [10]. In addition, ornithine decarboxylase has also been reported to be one of the transcriptional genes expressed in esophageal adenocarcinoma [11]. The Fas-assocated via death domain (FADD), which was abundantly expressed in our study, has been reported to be associated with an increased risk of developing ESCC [12,13]. Interestingly, it has been reported that both transglutaminase and cytokeratin were down-regulated genes in ESCC in Chinese [14]. The clinical relevance of those two genes needs to be clarified.

It is still unclear whether the disease process in young patients with ESCC is more aggressive and has a worse long-term outcome than in older patients [15,16]. It has been reported that esophageal carcinogenesis differs between the young and the elderly [17]. Whether there is any significant difference in gene-expression profiling between young patients and older patients with ESCC needs to be explored in the future.

In this study, tobacco smoking was defined as one pack or more per month; alcohol consumption was defined as consumption of at least one alcoholic beverage (equivalent to 125 mL of normal strength beer) per week; betel nut chewing was defined as consumption of at least 10 betel nuts per week [18]. The patient in this study was defined as an active tobacco smoker, alcohol drinker and betel nut chewer. A study of Taiwanese who smoke, drink alcohol and chew betel nut revealed that 89% of betel nut chewers also were tobacco smokers, and that 66% of male and 61% of female betel nut chewers also drank alcohol [18]. Clinically, it has been reported that the synergetic effect of betel nut and tobacco induces a higher risk of cancer of the oral cavity, pharynx, and esophagus [19,20]; however, relatively limited information is available on gene-expression profiling regarding the synergetic effect of these three habits on ESCC.

We were able to profile the genes expressed in ESCC tissue occurring in one patient. In the near future, we will try to confirm the validity of the microarray results by qualitative reverse transcription-PCR and immunohistochemical evaluation of the gene products whose levels were assessed to be differentially expressed in normal and tumor cells in this patient.

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發生在一位年輕食道癌病人的基因表現資料

許南榮 江宜平[1] 施志勳 陳品儒 何恆堅[2]

中國醫藥大學附設醫院 胸腔外科 病理部[1] 醫學研究部[2]

一位32歲男性病人有抽煙，喝酒和嚼檳榔10年以上的病史，因診斷在食道下段有鱗狀細胞癌而接受經食道裂孔切除和胃重建。根據病理檢查，腫瘤侵犯至表皮肌肉層，而無周圍淋巴腺轉移。於是，食道鱗狀細胞癌組織以cDNA微陣列方法，進行基因表現資料分析。一共篩選8354個基因，其中比正常組織多5倍螢光強度的基因表現有26個基因，而少5倍的有28個基因。經由資料搜查，發現26個基因中，僅有7個；而28個基因中，僅有8個已發表在文獻中。我們建議這些基因的特質可以針對具有抽煙，喝酒和嚼檳榔病史的年輕食道癌病人，提供食道鱗狀細胞癌惡化本質有用的資料。（中台灣醫誌 **2005;10:43-8**）

關鍵詞

食道鱗狀細胞癌，微陣列

聯絡作者：何恆堅

地　　址：404台中市北區育德路2號

中國醫藥大學附設醫院　醫學研究部

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