Gene Expression Profiling of Human Salivary Gland Carcinogenesis with cDNA Microarray

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Abstract

인간 타액선 암발생에서 cDNA Microarray를 이용한 유전자발현 Profile연구

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종양발생에서 유전자 발현을 확인하고 profile 변화를 monitor하는 것은 병리학적 변화의 원인뿐 아니라 질병탐지와 진료의 새로운 목표를 확인하기 위한 새로운 기회를 제공해준다. cDNA microarray는 수천개의 유전자 발현을 동시에 연구할 수 있는 최신의 방법으로 피부, 유방, 간을 비롯한 다른 인체장기에서는 일부 이루어졌으나 array를 이용해 타액선 종양 연구에서는 전혀 이루어지지 않았다. 인간의 타액선 세포의 악성형질전환을 조절하는 분자적 상태를 연구하기 위해 본 연구는 약 2,000개의 유전자가 print된 cDNA microarray를 이용하여 인간 타액선 도관상피세포주(HSG)와 악하선에서 기원한 미분화 선암종(SGT)간에 비교연구를 하였다. Cy3와 Cy5 dye로 각각의 세포주에서 얻은 RNA와 reciprocal hybridize시키고 GenePix 4000 scanner로 스캔하고 GenePix Pro로 분석한 후 log2로 평균발현비율을 전환시켜 최소 2배이상의 발현을 보이는 유전자를 분석대상으로 하였다.

90%이상의 유전자가 비슷한 발현을 보였으며 2배이상의 발현을 보이는 경우 HSG가 SGT에 비해 72개 유전자가, SGT가 HSG에 비해 111개의 유전자 발현이 up-regulation되어 총 10%미만의 발현차이를 보였고 반복된 hybridization 으로부터 얻은 선택된 spot의 Pearson 상관계수는 -0.85이였다. HSG에서는 6번 p 염색체에서 과발현되는 유전자가 가장 많았고, SGT에서는 11번 q 염색체에서 가장 많았는데 HSG에서는 SGT에 비해 9, 13, 17, 18, 20, 21, 22염색체에서 과발현 되는 유전자 수가 많았고, SGT에서는 HSG에 비해 2, 7, 10, 14, 15 염색체에서 유전자 발현 증가가 관찰되었다.

HSG와 SGT간의 유전 발현을 기능별로 분석한 결과 몇 가지 주요 경로가 세포악성에 관련됨을 발견하였고, 타액선 도관상피세포에서 선암종을 구별하는데 기여하는 관련된 몇종의 과다 발현된 유전자를 찾았는데 전사인자, 성장인자 및 수용기, 세포골격 및 세포외기질 단백, 세포내 신호전달조절자 및 인자, 세포표면 항원등의 그룹으로 분류할 수 있었다. 따라서 이러한 microarray를 이용한 분자학적 표지자 연구가 악성 타액선 종양 발생과정에서 큰 도움을 줄 수 있을 뿐 아니라 유전자 조절에 의한 진단, 예후, 치료에서의 정확성을 개선시킬 수 있으리라 여겨진다.

Key words : 타액선, 암발생, Microarray, 유전자발현, Profiles

※이 논문은 2000년 원광대학교의 교비지원에 의해서 이루어짐.

Ⅰ. Introduction

Salivary gland tumors represent approximately 3% to 6% of all head-and-neck neoplasms and exhibits one of the most complex arrays of histopathology of any organ sytem. These characteristics have made the elucidation of cellular and subcellular events in carcinogenesis of salivary tissue problematic[1]. Reliable indicators of their biologic aggressiveness still are lacking and there, the clinical outcome is difficult to predict[2,3]. Studies to evaluate distribution of markers in normal salivary glands and their neoplastic lesions are appropriate to evaluate the normal and neoplastic lesions of salivary glands as biochemical and cytochemical changes associated with tumorigenesis are not poorly understood.

Cancers have been defined as a group of cells exhibiting an unrestrained proliferation phenotype. The developing of cancer is the result of a series of molecular changes that occurred in the cell. These events lead to expression changes of numerous genes, accompanied by different histologic and clinical classification of this abnormal cell growth[4,5]. Current methods of comparing global gene profile changes in different tissues or different pathological specimes include high density oligo arrays, differential display, serial analysis of gene expression(SAGE), differential cDNA screeing, large scale cDNA sequencing, expressed sequence tag(EST) database comparision and two dimentional gel electrophoresis of cellular proteins [6,7]. Approaches such as differential display and SAGE are suitable for the in-depth sampling of gene expression changes in a few samples of interest, but sue to their gel-based methodology they are laborious and limited to the number of samples that can be studied simultanoeusly. High-density oligo arays, on the other hand, are high through-output and provide identify and expression level changes of selected genes simultaneously[8]. However, this method can only be applied to known gene sequences, since those arrayed oliogos have to be synthesized from reported sequences.

Through the advancements of miniaturization of cDNA array fabrication and attachment chemistry between DNA molecules and the surface of solid supporting materials, a microarray with a density of over 1000 indenpendant cNDA clones per cm^2 on poly-L-lysine treated glass plate has been described recently[9]. This approach allows of both selected known genes and cDNAs representing unchracterized genes between numerous biological samples in a comparative, parallel fashion. cDNA microarray allows to monitor the expression of thousthand genes simultaneously and has been successfully to explore the gene expression of carcinoma and other disease[6-12].

However, it is still poorly understood how these factors induce the malignant transformation during salivary gland carcinogenesis. Although various gene abnormalities have been identified in different stages of human salivary gland tumor carcinogenesis[4,13-16], we still lack the understanding of how these genetic elements interact together during tumor development.

In the present study, we describe the application of cDNA microarray to measure the relative expression of 2000 genes across human submandibular gland duct cell line(HSG) and human salivary adenocarcinoma cells(SGT).

Ⅱ. Methods and Materials

1. cDNA microarrays. The 2000 human cDNAs used in this study were obtained from Research Genetics. The cDNA arrays were produced at Advanced Technology Center in National Cancer Institute.

2. Cell lines and preparation of RNA. The HSG cell line[5] was maintained in a complete medium of Dulbecco's modified Eagle's medium(DMEM)/ Ham's F-12(1 : 1), containing 5% fetal bovine serum(Biofluid, Rockville, MD), 100U/ml penicillin and 100㎍/ml streptomycin(Life Technologics, Gaithersburg, MD).

The cell line of salivary gland adenocarcinoma were a kind gift from Dr. C.H., Lee, Dankok University, Korea, where the primary cultures were obtained from a poorly differentiated adeno-

caricnoma of the human submandibular glands. The primary cultures were cloned using the colony cylinders technique and characterized in Dr. Mori's Lab[17]. One of the clones, SGT-1 which was used in the present study has been found to express cytokeratin 8, vimentin, glial fibrillary acidic protein and epidermal growth factor receptor and at an ultrastructual level, these cells were found to possess desomsomal communication and occasional bundles of fibrils running parallel to the cytoplasmic membrane, closely resembling the intercalated and basal cells of the salivary ducts. All the cells were maintained in Dulbecco's minimal essential medium(Gibco, Brulingto, Canada) supplemented with 10% fetal bovine serum (Gibco). Cell suspension for passaging and experiments were obtained by brief treatment with 0.05% trypsin and 0.5mM EDTA. The cells were maintanined at 37℃ in an humidified 5% CO^2/95% air atmosphere.

To minimize the contribution of variations in culture condition or cell density to differential gene expression, total RNAs were isolated with Trisol (Gibco-BRL) from the cells at about 80% confluence level in plates.

3. Microarray experiments. Isolated total RNA from cell cultures of human salivary adenocarcinoma cell line, SGT and human salivary gland ductal epithelial cell line, HSG (reference cell line) was used to synthesize fluorescently (Cy-5 or Cy-3) labeled cDNAs, and hybridized them to a microarray. We labeled 30㎍ (for Cy-3 labeling) or 60㎍ (for Cy-5 labeling) total RNA using the Superscript Ⅱ reverse transcription kit (Gibco-BRL). Briefly, total RNA was mixed with 4㎍ of oligo dT (20-mer) primer in total volume of 21㎕, denatured at 70℃ for 10min, primed while cooling to room temperature, and transferred on ice. To this primed total RNA, we added 2㎕ of 20X dNTP mix (10mM each of dATP, dGTP, dCTP and 4mM of dTTP), either 4㎕ of Cy3- or Cy5-dUTP (1mM), 8㎕ of 5X first strand buffer (250mM Tris-HCl, pH8.3, 375mM KCl, 15mM $MgCl_2$), 4㎕ of 0.1M DDT, 1㎕ of RNasin (10,000 unit, Promega), and 3㎕ of Superscript Ⅱ reverse transcriptase (200u/㎕). After 2 hr incubation at 42℃, the RNA was degraded by adding 10㎕ of 1N NaOH and incubating at 70℃ for 30min. The mixture was neutralized by adding 25㎕ of 1M Tris-HCl (pH7.5), and the volume brought to 500㎕ with TE buffer. The cDNA probes were washed and concentrated by a Centricon-30 micro-concentrator (Amicon). The separated probes were combined and the volume was adjusted to 15㎕. The hybridization mix consisted of human COT-1 DNA (0.56㎍/㎕, Boehringer Mannheim), poly-dA DNA (0.56 ㎍/㎕, Pharmacia), yeast tRNA (0.22㎍/㎕, Sigma), 3.5X SSC, 0.28% SDS, and cDNA probes was prepared in the volume of 23㎕. Before hybridization, the probes were denatured by heating for 2min at 100 ℃ and cooled to room temperature. Hybridization was carried out at 65℃ for over night in a water bath. Before scanning, slides were washed in 2X SSC with 0.1% SDS for 2min, then 1X SSC, 0.2X SSC, and 0.05X SSC, sequentially for 1min. Hybridized arrays were scanned at 10㎛ resolution on a GenePix 4000 scanner (Axon Instrument) at variable PMT voltage to obtain maximal signal intensities with less than 1% probe saturation. Resulting images were analyzed via GenePix Pro v3.0 (Axon Instrument) as described in manual. Each sample was examined at least twice by switching fluorescent dye Cy-5 and Cy-3. The variance in the duplicated fluorescence ratio measurements approached minimum when the fluorescence signal was greater than approximately 0.3% of the measurable total signal dynamic range above background in both channel of the hybridization. The subset of spots was selected by this criterion to identify well-measured spots. Average values of two experiments on each sample were obtained and values that do not agree in two experiments were excluded from further analysis.

4. Data analysis. Average expression ratios were transformed to log2 and genes with expression ratio that has at least 2-fold ratio difference relative to the reference cell line were further selected.

Ⅲ. Results

We characterizes gene expression profiles in SGT and HSG cell lines, by using cDNA microarrays (NCI-OncoChip, NCI, MD) containing 2000 sequence-verified cDNA elements (representing 1800 unique genes) on glass microscope slides. Total RNAs were purified from tumor cell cultures when cell growth was about 80% confluent, and used to prepare fluorescent cDNA probes labelled with the Cy-3 or Cy-5 dyes. A reference fluorescent cDNA probe was prepared from total RNA of human submandibular gland cell line, HSG. Each Cy-5 labelled experimental cDNA probe was combined with the Cy-3 labelled reference probe and the mixture was hybridized to the microarray. The fluorescence ratio of each gene was quantified and reflected the relative abundance of gene in each experimental RNA sample compared with common reference RNA. By comparing with common reference, variation in gene expression across 2 cell lines could be inferred from the observed variation in the normalized Cy-5/Cy-3 ratios across the hybridization. To exclude labelling biases, total RNAs from each cell line were labelled with the reciprocal fluorochrome in every other duplicated experiment. We selected the subset of spots as described in methods and materials to collect only well-measured spots for further analysis.

The expression level of each gene was represented by pseudo-colour in matrix format, with red representing expression greater than normal submandibular gland cell line HSG, green representing expressions less than HSG, and colour intensity representing the magnitude of expression ratio(Fig. 1).

Ⅲ-1. Microarray hybridization and scatter plots

Due to the fabrication and scanning protocols of the microarray, several locations without DNA samples were also dotted, probed and scanned. The average fluorescent signals of these bank spots were used as background to correct the hybridization signals for arrayed DNA samples. To determine the reliablility of hybridization signals and experimental variables unrelated to the differences in hybridization probes, the arrays was hybridized independantly three times with Cy3 and Cy5 labed HSG vs SGT cDNA probes simulatneously. The resluts show that on average, over 80% of the clones exhibit a Cy3 to Cy5 signal ratio, less than 1.5-fold intensity sifferences, and only about 10% of the clones have a ratio greater than 2.

A hierarchical clustering algorithm based on Pearson correlation coefficients was applied to group genes on the basis of similarity in the pat-

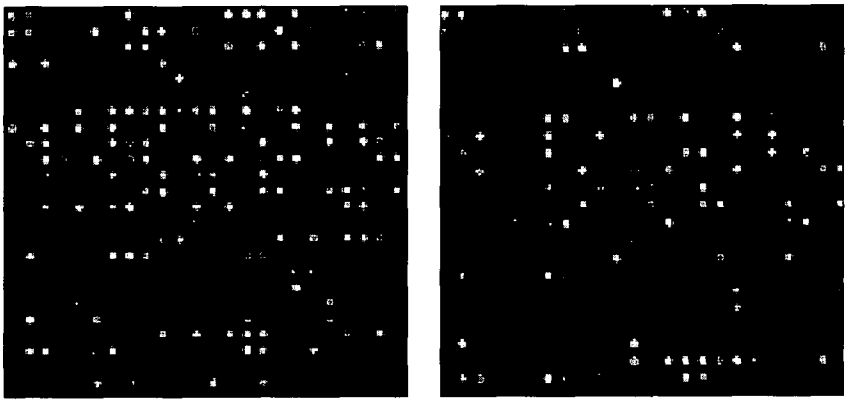

Fig. 1. A image from scanning a hybridized human array containing over 2000 genes. Each spot features a pool of identical single-stranded DNA molecules representing a single gene.

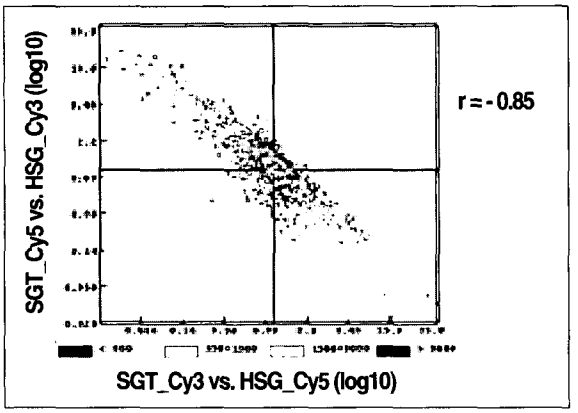

Fig. 2. Scatter plot of Log 10X array ratio vs log 10Y array ratio. Pearson correlation coefficients for the set of selected spots from duplicated hybridization was -0.85. The genes that showed dramatic changes in fluorescence ratio in duplicated experiments were further eliminated manually. Finally, average fluorescence ratios of each gene were calculated from duplicated experiments of each cell line, after inverting Cy-3/Cy-5 ratio values of reciprocal experiment

tern over all samples, and cell lines on the basis of similarity in the pattern over all genes.

Scatter plots with the values of Cy3 and Cy5 fluorescent signals also a tight distributiion pattern and clustered in an almost 45 degree diagonal line as expected. The scatter plot for one of the HSG/SGT cell line hybridization results is shown in Fig. 2.

A small number of clones exhibit greter than 2 fold signal differences in the HSG/SGT hybridization experiments. The arrayed cDNA s exhibits these signal differences are not consistent between identitical replications of these HSG/SGT Hybridization. This suggests that a small fraction of the observed signal variations in hybridization experiments may be contributed by minor differences in probe preparation, hybridization condition, data collection and the amounts of immoibilized DNA between fabricated arrays. However , these signal variations are unlikely to appear at the same locations in different hybridization experiments: these errors can be adressed by averaging the signal intensities from replicate experiments.

The changes in upregulated genes in HSG cells (mean result of 2 reciprocal relative abundance of genes) are shown in Table 1. For example, the expression level of S-100A4 was increased 30.1 times in HSG cells than in SGT.

Ⅲ-2 Clones exhibiting differential expression profiles (Table 1, 2)

To investigate and monitor the gene expression profile changes in salivary gland cancers, replicates of the faricated cDNA arrays were hybridized independantly with cDNA probes that were generated from poorly differentiated adenocarcinoma and immortalized human submandibular gland cells. The high similarity in gene expression(90%) between HSG and SGT cells is striking and suggests that only an additional 10% (or less)of the genome is activated during malignant transformation.

After analyzing salivary gland cells hybridization results, 183(119) such clones were select for further analysis. Of these, 111(87)clones cDNA exhibits greater than 2(2.5)-fold overexpression in SGT probes relative to nomal HSG probe, 72(32) cDNA s reveal greater 2(2.5) fold overexpression in normal HSG relative to tumor SGT probe. These changes in gene expression are highly reproducible and represent changes in the expression of a variety of molecular markers. For differential gene expression between HSG and SGT, we can to be divide to several fuctional categories: transciption factors, growth factors and therir receptors, cytoskeletal and extracellular matrix proteins, cell surface antigens, intracellular signal transduction modulators and effectors, and miscellaneous(Table 3).

Ⅲ-3 Discovering genomic imbalance with expression profiles

Structural alterations of chromosomes are critical part of tumorigenesis in human cancer. Aberrant regulation of gene expression in human cancer is frequently caused by gain or loss of chromosomal regions where the genes reside. Therefore, we hypothesized that genomic scale

Table 1. Genes With Expression Levels Higher in HSG than SGT

SGT-Cy3	SGT-Cy5	Mean	Well ID	Gene	Description
33.852	26.389	30.1205	17699	S100A4	S100 calcium binding protein A4
28.34	22.031	25.1855	16006	TXK	TXK tyrosine kinase
7.42	8.099	7.7595	17899	MADH2	Smad2=Madr2=Activated by TGF beta
8.013	5.043	6.528	15844	TGFBR3	TGF beta receptor type Ⅲ
6.777	4.667	5.722	16034	NK4	Natural killer cells protein-4
6.496	5.057	5.7765	16934	RTVP1	GliPR=glioma pathogenesis-related protein
6.335	6.096	6.2155	17057	PLAB	TGF-beta superfamily protein
4.34	6.064	5.202	16767	TIMP2	TIMP-2= metalloproteinase 2
6.014	4.612	5.313	16661	CDKN2C	p18-INK6=Cyclin-depen kinase 6 inhibitor
5.927	4.312	5.1195	15843	ETS2	ets-2=ets family transcription factor
5.773	3.336	4.5545	16424	PRKCL1	lipid-activated protein kinase PRK1
5.551	3.432	4.4915	17838	ENG	CD105
5.481	4.305	4.893	16444	PTPRM	receptor protein tyrosine phosphatase mu
5.202	3.695	4.4485	17862	ZNF220	monocytic leukaemia zinc finger protein
5.102	3.057	4.0795	16499	SIAT1	CD75=sialyltransferase
5.001	3.381	4.191	16494	NDR	Ndr protein kinase
4.74	3.559	4.1495	17524	BLMH	Bleomycin hydrolase
4.271	4.189	4.23	16061	PTP4A2	Prot tyros phosphatase type IVA mem 2
2.042	4.181	3.1115	17240	ESR1	Estrogen receptor
3.973	2.342	3.1575	16610	CD83	CD83=B-cell activation protein
3.901	3.78	3.8405	17726	FCGR3A	CD16=Fcgamma receptor IIIa
2.437	3.858	3.1475	16549	UCP2	UCP2=Mitochondrial uncoupling protein 2
3.37	3.82	3.595	16650	IL15	IL-15
3.674	3.133	3.4035	17600	MYB	myb proto-oncogene=c-myb
3.534	2.544	3.039	16925	AES	Homolog of Drosop enhancer of split m9/m10
3.05	3.48	3.265	17789	DAB2	mitogen-responsive phosphoprotein (DOC-2)
3.474	2.252	2.863	15841	MYC	c-myc
3.433	3.454	3.4435	16418	MADH7	Smad7=negative regulator of TGF beta signa
3.43	2.147	2.7885	17120	CREM	cAMP-respon modulator type1 alpha prot
3.318	2.993	3.1555	17276	TIMP3	Tissue inhibitor of metalloproteinase 3
3.262	2.489	2.8755	17521	FCER2	CD23A
3.242	2.808	3.025	16828	HU-K5	lysophospholipase homolog (HU-K5)
2.714	3.196	2.955	16830	MAP2K6	MEK6=MAP kinase kinase 6
3.172	2.474	2.823	16564	PPM1D	protein phosphatase Wip1
3.099	2.515	2.807	17625	DLEU1	leukemia associated gene 1
3.082	1.807	2.4445	17786	TSC22	TSC-22=TGF-beta-induced in mouse
3.053	2.521	2.787	16698	DLEU1	leukemia associated gene 1
2.991	2.372	2.6815	17509	BAG1	Bag-1=Bcl-2 interacting anti-apoptotic prot
2.808	2.967	2.8875	150018	POLA	UG5 polymerase (DNA directed), alpha
2.938	2.36	2.649	16616		fra-2=fos-related antigen 2
2.922	2.192	2.557	16495	EPHA3	Tyrosine kinase receptor ETK1
2.757	2.48	2.6185	17918	MADH4	Smad4=required for TGF beta signaling
2.714	2.213	2.4635	16115	FGF2	FGF-2=Basic fibroblast growth factor
2.636	2.684	2.66	15892	NR4A3	mitogen induced nuclear orphan receptor
2.679	2.199	2.439	16618	RBL2	p130 = RB related protein
2.627	2.211	2.419	16898	FANCC	FA-C=DNA crosslinking repair protein
2.196	2.574	2.385	16917	GPX3	Glutathione peroxidase 3 (plasma)
2.416	2.546	2.481	16560	FYN	fyn=Tyrosine protein kinase
2.514	1.981	2.2475	17430	FLJ20746	putative cyclin G1 interacting protein
1.812	2.495	2.1535	17435	MAP3K12	protein kinase (zpk)
2.44	1.989	2.2145	150017	POLR2B	UG3 polymerase II polypeptide B (140kD)
1.848	2.431	2.1395	17431	CDKN2A	p16-INK4a=Cyclin-depend kinase 4 inhibit A
2.428	1.993	2.2105	16072	GSTTLp28	glutathione-S-transferase homolog
2.426	1.867	2.1465	16088	MAPK14	p38 mitogen activated protein (MAP) kinase
2.378	2.092	2.235	16070	MAP4K1	HPK1=hematopoietic progenitor kinase
2.217	2.377	2.297	16459	AXL	axl=ufo=tyrosine kinase receptor
2.373	2.048	2.2105	17429	CLGN	Calmegin=putative testis-specific chaperon
2.031	2.319	2.175	16623	ADE2H1	ADE2H1 encoding SAICAR synthetase
2.313	1.908	2.1105	17640	PMAIP1	ATL-derived PMA-responsive peptide
2.309	1.905	2.107	17798	SMN2	spinal muscular atrophy gene
1.909	2.267	2.088	17227	IL16	Lymphocyte chemoattractant factor (LCF)
2.264	2.171	2.2175	17322	PBX3	pre-B cell leukemia transcription factor-3
2.226	1.866	2.046	16154	CAMK1	cam kinase I
2.205	2.045	2.125	17765	H2AFX	histone H2A.X
1.819	2.199	2.009	17356	MAD4	MAX-binding protein
1.908	2.187	2.0475	17540	PRKACG	cAMP-dependent protein kinase
2.184	2.07	2.127	16937	MRE11B	2 strand DNA break repair exonuclease
2.065	2.164	2.1145	17683	H2AFL	histone 2A-like protein (H2A/l)
2.141	1.953	2.047	16479	SRPK1	SRPK1=serine kinase
2.002	2.119	2.0605	16949	SLC20A1	leukemia virus receptor 1 (GLVR1)
2.113	1.996	2.0545	15881	NFATC3	NFAT4=NFATc3=NFATx
1.954	2.105	2.0295	17115	CBX5	HP1=heterochromatin protein homologue

Table 2. Genes With Expression Levels Higher in SGT than HSG

SGT-Cy3	SGT-Cy5	Mean	Well ID	Gene	Description
0.555	0.42	0.4875	17929	IL17R	IL-17 receptor
0.553	0.377	0.465	150005	KPNA2	UG5 karyopherin alpha 2
0.549	0.413	0.481	16020	ST13	progesterone receptor-associated p48 prot
0.542	0.4	0.471	16644	ZFP161	ZF5=POZ domain zinc finger protein
0.433	0.539	0.486	15967	CYP2C9	Cytochrome P450, IIC, polypeptide 9
0.537	0.415	0.476	17494		eIF-2 alpha=translation initiation factor
0.382	0.536	0.459	16107	EPHB3	tyrosine kinase receptor=large erk kinase
0.446	0.532	0.489	16909	GNLY	NKG5=natural killer cell and T cell gene
0.527	0.337	0.432	17499	KIAA0033	KIAA0033=putative kinase
0.526	0.335	0.4305	16755	RPS29	Ribosomal protein S29
0.526	0.423	0.4745	16149	HDAC3	HD3=histone deacetylase 3
0.526	0.366	0.446	17837	MAX	max=myc interacting HLH protein
0.413	0.526	0.4695	17526	LMNB1	lamin B1
0.367	0.521	0.444	16756	ACTN1	Alpha-Actinin 1
0.343	0.521	0.432	17095	CCNG2	Cyclin G2
0.521	0.511	0.516	16426	SCYA3L1	LD78 beta=chemokine
0.386	0.52	0.453	16064	MYLK	myosin light chain kinase
0.519	0.467	0.493	17579	EFNA1	EPH-related receptor tyrosine kinase ligand 1
0.333	0.518	0.4255	16067	MAP3K4	MEKK4=MAP kinase kinase kinase 4
0.515	0.41	0.4625	17704	CRIP2	Cysteine-rich protein 2=ESP1 protein
0.514	0.412	0.463	17042	GBP2	Interferon-induced guanylate-binding protein 2
0.512	0.418	0.465	17626	TNFRSF6	CD95=Fas
0.51	0.295	0.4025	17822	MET	Met proto-oncogene
0.477	0.508	0.4925	17659	RNASE6PL	ribonuclease 6 precursor
0.508	0.392	0.45	16632	SORL1	hybrid receptor gp250 precursor
0.507	0.475	0.491	17675	NUMA1	NuMA=coiled-coil nuclear protein
0.506	0.337	0.4215	16672	CSE1L	chromosome segregation gene homolog
0.503	0.359	0.431	17486	BAD	BAD=bbc6=proapoptotic Bcl-2 homolog
0.501	0.462	0.4815	15973	ARHGAP1	Cell division cycle 42 (GTP-binding protein)
0.501	0.321	0.411	17263	ISG15	Interferon-induced 17 KD protein
0.5	0.344	0.422	16289	BNIP1	NIP1=E1B 19K/Bcl-2-interacting protein
0.499	0.472	0.4855	17703	MYD88	myeloid different primary response prot
0.495	0.415	0.455	150026	RPL10A	UG3 ribosomal protein L10a
0.494	0.354	0.424	17467	EPO	Erythropoietin
0.494	0.377	0.4355	16971		Ser/Thr protein kinase receptor R4
0.457	0.492	0.4745	16939	SCYA14	HCC-1=chemokine linked to HCC-2
0.492	0.32	0.406	16591	MET	Met proto-oncogene
0.49	0.448	0.469	17901	PNOC	pre-pro-orphanin FQ=prepronociceptin
0.466	0.488	0.477	15911	GS3955	cancellous bone osteoblast GS3955
0.487	0.401	0.444	15870	IFIT1	Interferon-induced 56-KDa protein
0.392	0.486	0.439	17246	PPP2R5E	protein phosphatase 2A epsilon isoform
0.42	0.486	0.453	17303	HUMMAT1H	homolog of mouse MAT-1 oncogene
0.485	0.436	0.4605	15979	CD59	CD59
0.483	0.421	0.452	16816	CTSL	cathepsin L
0.482	0.464	0.473	16655	TRAF3	CD40- TNFR- and LMP1-associated protein
0.48	0.473	0.4765	16425	KAI1	CD82=suppressor of tumorgenicity-6
0.48	0.393	0.4365	16683	MARK3	p78=Putative SER/THR-protein kinase
0.47	0.48	0.475	17309	ID1	Id1=Inhibitor of DNA binding 1
0.44	0.479	0.4595	16902	BMPR2	Bone morphogenetic protein receptor, type II
0.479	0.432	0.4555	16000	ABCB2	TAP1=peptide transporter
0.472	0.478	0.475	17670	AHR	aryl hydrocarbon receptor (AhR)
0.475	0.409	0.442	16024	PTPRZ1	protein tyrosine phosphatase zeta
0.452	0.474	0.463	15955		FGFR2=Fibroblast growth factor receptor 2
0.472	0.365	0.4185	16600	F2RL2	PAR3=protease-activated receptor 3
0.471	0.381	0.426	17902	GSS	Glutathione synthetase
0.47	0.407	0.4385	16671	PIK3R1	PI3-kinase alpha regulatory subunit (p85)
0.466	0.395	0.4305	17272	PTPN6	SHP-1=protein-tyrosine phosphatase 1C
0.465	0.466	0.4655	16637	NEK6	NIMA-related protein kinase 2
0.464	0.356	0.41	16656	PTPN12	Protein tyrosine phosphatase, type 12
0.463	0.424	0.4435	17300	LY6E	retinoic acid induced RIG-E precursor (E)
0.461	0.418	0.4395	16544	SEC10L1	hSec10p=brain secretory protein
0.458	0.316	0.387	17692		PKA-RI beta
0.457	0.363	0.41	17696	FCGR1A	CD64=FC-gamma RI=IGG FC receptor I
0.457	0.426	0.4415	16473	DGKG	Diacylglycerol kinase, gamma (90kD)
0.447	0.456	0.4515	16373	DEK	dek=translocated in t(6;9) AML
0.455	0.408	0.4315	16982	PDHA1	pyruvate dehydrogenase alpha subunit
0.453	0.388	0.4205	17706	SRM300	KIAA0324
0.452	0.35	0.401	15866	NFKBIA	IkB alpha Yes
0.441	0.448	0.4445	17870	FAT	hFat=homologue of drosophila FAT gene
0.448	0.299	0.3735	150001	TP63	UG5 tumor protein 63 kDa
0.429	0.448	0.4385	17875	KIAA0303	KIAA0303
0.445	0.286	0.3655	17823	RUNX1	AML1 Proto-oncogene

SGT-Cy3	SGT-Cy5	Mean	Well ID	Gene	Description
0.42	0.443	0.4315	17522	DLEU2	leukemia associated gene 2
0.288	0.44	0.364	16946	CD59	CD59
0.439	0.406	0.4225	17443	LIMK1	LIMK1=LIM-kinase1
0.416	0.438	0.427	150059	XRCC3	XRCC3
0.435	0.367	0.401	17680	GSTT2	Glutathione S-transferase theta 2
0.433	0.383	0.408	16044	IL4R	IL-4 receptor alpha chain
0.433	0.4	0.4165	17890	TFAP4	Transcription factor AP-4
0.431	0.327	0.379	16140	CD44	Pgp-1=extracellular matrix receptor-III
0.429	0.33	0.3795	16660	ITGA3	CD49C=Integrin alpha 3
0.428	0.37	0.399	17614	PTPN12	Protein tyrosine phosphatase, type 12
0.426	0.362	0.394	17010		Evi-1 zinc finger protein fused to AML1
0.425	0.321	0.373	15942	IRF3	IRF-3=interferon regulatory factor-3
0.423	0.296	0.3595	15898	KIAA0303	KIAA0303
0.421	0.385	0.403	15856	KIAA0370	Protein-tyrosine kinase 7 and KIAA0370
0.418	0.318	0.368	17307	STK24	Ste20-like kinase 3 (mst-3)
0.418	0.34	0.379	17930	BRCA1	BRCA1=Mutated in breast and ovarian ca
0.417	0.355	0.386	17717	PAK1	Pak1=p21-activated protein kinase
0.41	0.384	0.397	17442	CSNK2A1	Casein kinase II alpha chain
0.409	0.328	0.3685	16580	TINF2	TIN2=TRF1-interacting nuclear factor 2
0.409	0.247	0.328	17909	PPP1CA	Protein phosphatase 1, catalytic subunit
0.408	0.345	0.3765	16709	PIG8	Pig8=p53 inducible gene
0.396	0.38	0.388	17214	PPP2R5C	Protein phosphatase 2A, regulatory subunit
0.385	0.388	0.3865	17107	LDHB	Lactate dehydrogenase B
0.368	0.387	0.3775	16429	IFI41	HNPP=nuclear phosphoprotein
0.381	0.371	0.376	17011	HSPCA	Heat shock 90kD protein 1 alpha
0.378	0.335	0.3565	16438	EIF2B1	Eukaryotic translation initiation factor
0.376	0.27	0.323	15910	PPP1CA	Protein phosphatase 1, catalytic subunit
0.375	0.285	0.33	16926	BAG1	Bcl-2 interactanti-apoptotic prot
0.373	0.335	0.354	17888	GUK1	guanylate kinase (GUK1)
0.366	0.314	0.34	150014	JUN	avian sarcoma virus 17 oncogene
0.365	0.346	0.3555	17934	LYN	lyn=tyrosine kinase
0.364	0.336	0.35	16613	UBE2V1	Paired box homeotic gene 3
0.358	0.331	0.3445	16975	KPNB1	importin beta subunit
0.358	0.353	0.3555	17441	ITGA9	Integrin alpha 9
0.342	0.356	0.349	15961	DGUOK	Deoxyguanosine kinase
0.355	0.326	0.3405	17731	PIG8	Pig8=p53 inducible gene
0.353	0.35	0.3515	16915	HMG17	non-histone chromosomal prot
0.301	0.35	0.3255	16759	IL1B	IL-1 beta
0.238	0.348	0.293	17577	SDC1	Syndecan-1
0.348	0.187	0.2675	15990	XPC	XP-C repair complementing prote
0.319	0.345	0.332	15983	CD36	CD36
0.312	0.34	0.326	16173	LYN	lyn=tyrosine kinase
0.33	0.27	0.3	17126	TSG101	tumor susceptiblity protein
0.328	0.315	0.3215	16592	TIEG	EGR alpha
0.321	0.238	0.2795	17802	SC5DL	thymosin beta-4
0.316	0.278	0.297	16718	PTPRA	receptor prot tyroe phospha a
0.242	0.314	0.278	17727	GRO2	macrophage infla protein-2 alpha
0.309	0.286	0.2975	17216	BNIP2	Bcl-2-interacting protein
0.244	0.307	0.2755	17318	HRAS	H-ras
0.185	0.299	0.242	17877	IFI27	Interferon-alpha induced protein
0.295	0.235	0.265	16048	IRF1	interferon regulatory factor-1
0.292	0.224	0.258	17293	BNIP3	pro-apoptotic mitochonl protein
0.283	0.196	0.2395	15950	GSTM4	Gluta S-transferase M1 or M2
0.266	0.225	0.2455	16770	TUBB	tubulin-beta
0.217	0.259	0.238	16159	LOC56851	fos-related antigen 1
0.255	0.188	0.2215	16945	S100A4	S100 calcium binding protein A4
0.254	0.25	0.252	16083	ARHGDIB	LyGDI=RHO GDI 2
0.248	0.211	0.2295	16512	HLA-E	MHC Class I=HLA-E
0.248	0.217	0.2325	16169	LOC56851	fos-related antigen 1
0.242	0.232	0.237	16129	CCND1	Cyclin D1=BCL1=PRAD1
0.236	0.166	0.201	16506	GNG10	G protein gamma-10 subunit
0.235	0.228	0.2315	150004	FOSL1	UG5 FOS-like antigen-1
0.233	0.216	0.2245	16492	KRT14	Keratin type I cytoskelatin 14
0.232	0.2	0.216	17590	HLA-E	MHC Class I=HLA-E
0.231	0.168	0.1995	15992	SHC1	SHC signaling adaptor protein
0.225	0.198	0.2115	17611	SORL1	hybrid receptor gp250 precursor
0.201	0.199	0.2	16673	UGT2B10	udp glucuronosyltransferase
0.201	0.184	0.1925	16927	CASP4	CASPASE-4
0.195	0.167	0.181	16156	TIP30	CC3=metastasis suppresor gene
0.188	0.165	0.1765	16040	CDC25B	M-phase inducer phosphatase 2
0.179	0.158	0.1685	17780	SDC1	Syndecan-1
0.174	0.159	0.1665	16732	MAPK12	p38 gamma MAP Kinase
0.119	0.155	0.137	16754	MMP2	Matrix metalloproteinase 2

SGT-Cy3	SGT-Cy5	Mean	Well ID	Gene	Description
0.155	0.153	0.154	17350	ITGB4	CD104=Integrin beta4
0.153	0.116	0.1345	17644	SERPINB1	Monocyte/neutrophil elast inhib
0.126	0.143	0.1345	16050		interferon-gamma IEF SSP 5111
0.143	0.137	0.14	16933	TNFRSF10C	TRAIL receptor 3=DcR1= (LIT)
0.131	0.101	0.116	17794	ITGA6	CD49F=Integrin alpha 6
0.129	0.116	0.1225	17842	SDC4	Syndecan-4
0.125	0.118	0.1215	17148	PRKG1	cGMP-depend prot kinase
0.124	0.101	0.1125	16068	IFI16	interferon-induc myel trans acti
0.076	0.115	0.0955	15968	CYP2C8	Cytochrome P450, subfamily IIC
0.114	0.098	0.106	16687	CTSB	cathepsin B
0.097	0.107	0.102	17410	THBS1	Thrombospondin 1
0.048	0.093	0.0705	17310	GSTP1	Glutathione-S-transferase pi-1
0.053	0.079	0.066	17298	DDR1	
0.073	0.077	0.075	16858	CCND2	Cyclin D2
0.058	0.063	0.0605	17791	FN1	Fibronectin 1

Table 3. Gene group and Average Expressed Ratio that are up-regulated in SGT and HSG

	HSG		SGT	
1. Transcriptional factor and DNA binding protein	*ETS2	5.11	*IRF3	0.37
	*Fra-2	2.64	*TF-AP4	0.41
	*Pre-B cell leukemia transcription factor3	2.21	*NFKBIA	0.40
			*Aryl hydrocarbon receptor	0.47
	*MAD4	2.00	*Max(myc interacting protein)	0.44
	*NFATC3	2.05	*Histone deacetylase 3	0.47
2. Growth factor and cytokine and chemokine	*Smad2	7.75		
	*TGFBR3	6.52	*SCYA14(HCC-1, chemokine)	0.47
	*PLAB(TGF-b superfamily)	6.21	*FGF receptor2	0.46
	*FGF2	2.46	*LD78 beta(chemokine)	0.51
	*IL16	2.08		
3. Cell cycle regulator	*S-100A4	30.12	*PAK1	0.38
	*Cyclin-depend kinase 6 inhibitor	5.31	*BRCA1(mutated in breast & ovarian Ca)	0.37
	*PPM1D(protien protein phosphatase Wip1)	2.82	*Cell division cycle42(GTP-binding protein)	0.48
	*p130	2.43		
	*FA-C	2.41		
	*FLJ20746	2.24	*cyclin G2	0.43
4. Growth and develpment	*GliPR(glioma pathogenesis related protein)	5.77	*Lyn(oncogene)	0.35
	*ESR1(estrogen receptor)	3.11	*JUN(oncogene)	0.34
	*Mitogen-reponsive phosphopotein(DOC-2)	3.26	*HUMMAT1H(MAT-1, oncogene)	0.45
			*protein phosphatase1 CA	0.32
	*CD23A(oncogene and supressor gene)	2.87	*Met(protooncogene)	0.40
			*RUNX1(leukemia proto-oncogene)	0.36
	*ZNF220(proto-oncogene: monocytic leukemia ZFP)	4.44	*TP63(oncogene)	0.37
			*Met(protooncogene)	0.40
	*C-myb(proto-oncogene)	3.40	*RNAse 6 precusor(growth factor receptor)	0.49
	*C-myc(proto-oncogene)	2.86		
	*Leukemia associated gene1(suppressor)	2.80	*MYD88(myeloid differentiation)	0.48
			*Protein tyrosine phosphatase molecule	0.44
	*TSC-22(suppressor)	2.44		
5. Cell surface and adhesion molecule	*NK4	5.72	*Syndecan-1	0.29
	*Leukemia virus receptor 1(GLVR1)	2.06	*IntegrinA9	0.35
			*Integrin alpha3	0.37
			*CD44	0.37
			*IL4R	0.40
			*FAT	0.44
			*CD64(affinity)	0.41
			*Lamin B1	0.46

	HSG		SGT	
6. Signal transduction modulator and effectors	*PTPRM	4.89	*LIM-kinase 1	0.42
	*IL15	3.59	*CD59	0.36
	*Tyrosine kinase receptor ETK1	2.55	*PPP2R5E(protein phosphatase)	0.45
	*Smad4	2.61	*CD59	0.46
	*AES	3.03	*TRAF3	0.47
	*MAP2K6	2.95	*KAI1(CD82)	0.47
	*MAPK14	2.14	*MARK3(SER/THR protokinase)	0.43
	*MAP4K1	2.23	*Protein tyrosine phoaphatase 1C	0.43
	*AXL(tyrosine kinase receptor)	2.29	*Tyrosine kinase receptor(EPHB3)	0.45
7. Apoptosis proteins	*Bag-1	2.68	*BAG1	0.33
			*CD95	0.46
			*MAP3K4	0.42
			*CSE1L(chromosome segregation gene)	0.42
			*BAD(proapotic bcl-2)	0.43
			*BNIP1	0.42
8. Protein kinase	*TXK(tyrosine kinase)	25.18	*casein kinase II alpha chain	0.39
	*PRKCL1	4.55	*protein-tyrosine kinase 7	0.40
	*NDR	4.19	*protein tyrosine kinase 12	0.39
	*Tyrosine protein kinase	2.48	*EFNA1	0.49
	*MAP3K12	2.15	*P13-kinase alpha regulatory subunit	0.43
	*PRKACG	2.04	*protein tyrosine phosphatase type12	0.41
9. Miscellaneous	*TIMP2	5.20	*PNOC(neurotransmitter)	0.46
	*TIMP3	3.15	*Cathepsin L	0.45
	*CD105(Glycoprotein)	4.49	*GS3955(cancellous bone osteoblast)	0.47
	*CD75(Glycoprotein)	4.19	*BMP receptor II	0.45
	*CD83(lymphocyte antigen)	3.15	*NK cell & T cell gene(GNLY)	0.48
	*PTP4A2(hydrolase)	4.23	*EIF-2 alpha(translation initiation)	0.47
	*HU-K5(Hydrolase/lipid metabolism)	3.02	*Cytochrome P450, IIC, Poly(peptide 9(oxidization)	0.48
	*FCGR3A(Virus infected immune response)	3.84	*ZFP161	0.47
			*ST13(progesterone receptor-associated p48)	0.48
			*KPNA2(Dptor binding)	0.46
	*UCP2(Transport molecule)	3.14	*IL-17 receptor	0.48
	*UG5 polymerase, alpha(DNA polymerase)	2.88	*ABCB2(peptide transporter)	0.45
			*Protease activated receptor 3	0.41
	*Glutathione peroxidase 3(oxidative protection)	2.38	*Gluathione synthetase	0.42
			*RA induced RIG-E precusor	0.44
	*UG3 polymerase II polypeptide B(RNA polymerase)	2.21	*SEC10L1(brain secretory protein)	0.43
			*PKA-R1 beta	0.38
			*Diacylgylcerol kinase, gamma	0.44
	*GSTTLp28(peroxidase)	2.21	*DEK(trasnlocated in AML)	0.45
	*Calmegin(chaperon)	2.21	*Myosin light chain kinase	0.45
	*ADE2H1(polypeptide)	2.17	*RIBOSOMAL PROTEIN s29	0.49
	*PMAIP1(ATL derived PMA induced gene)	2.11	*KIAA0033(kinase)	0.43
			*IL-1 beta(inflammatory response)	0.32
	*SMN2(spinal muscular atrophy gene)	2.10	*HMG17(nucleosomal DNA)	0.35
			*PIG8(p53 inducible gene)	0.34
	*MRE11B (repair)	2.12	*PIG8	0.37
	*H2AFL(histone)	2.12	*DUGOK(deoxyguanosine kinase)	0.34
			*Importin beta subunit(nuclear protein)	0.34
			*Guanylate kinase	0.35

* Number is average fluorescence ratios of each gene, were calculated from duplicated experiments of each cell line, after inverting Cy-3/Cy-5 ratio values.

analysis of expression profiles might be able to detect the chromosomal aberration in human salivary gland carcinogenesis. In order to test this hypothesis, we reclustered expression pattern of genes based on their location in chromosomes (Table 4). Analysis of the reclustered gene expression patterns revealed that region-wide expression bias within chromosomes of human salivary gland caricnoma is evident.

In HSG Chromosome 6p, and chromosome 11q

Table 4. Chromosome Number and percentage of Highly expressed gene in HSG and SGT

Chromosome No	Highly expressed gene(2 fold) Number in HSG	Highly expressed gene Number(2 fold) in SGT
1	5(6.9%)	13(11.7%)
2	2(2.7%)	(8.1%)
3	4(5.4%)	11(9.9%)
4	6(8.3%)	4(3.6%)
5	3(4.1%)	8(7.2%)
6	8(10.8%)	10(9.0%)
7	0(0%)	10(9.0%)
8	3(4.1%)	7(6.3%)
9	7(9.7%)	4(3.6%)
10	2(2.7%)	6(5.4%)
11	2(2.7%)	22(19.8%)
12	3(4.1%)	6(5.4%)
13	3(4.1%)	2(1.8%)
14	0(0%)	13(11.7%)
15	1(1.4%)	4(3.6%)
16	3(4.1%)	4(3.6%)
17	4(5.4%)	0(0%)
18	5(6.9%)	1(0.9%)
19	6(8.3%)	1(0.9%)
20	1(1.4%)	8(7.2%)
21	1(1.4%)	1(0.9%)
22	2(2.7%)	3(2.7%)
X	1(1.4%)	2(1.8%)

in SGT showed the most percentage of higher level of expression than other chromosome, suggesting that region-wide disruption of regulation of gene expression in specific chromosome. Especially 0% chromosome 7, and chromosome 14 of highly expressed gene in HSG, which highly increased chromosome percentage in each chromosome of SGT increased at 9.0%, 11.7%, respectively.

Some genes in the chromosomes showed dramatic change in expression ratios. For example, gene expression in chromosome 2, 7, 10, 14, 15 was highly increased in SGT than HSG cell lines, while gene expression in chromosome 9, 13, 17, 18, 19, 20, 21, 22 in HSG was highly elevated than SGT.

Ⅳ. Discussion

Most salivary gland tumors have low malignant potential and are non-invasive and encapsulated. However, as many as 15% of tumors are initially highly invasive, potentially malignant carcinomas, which frequently exhibits metastasis to regional lymph nodes and/or distant organs[18]. This is the main cause of treatment failure. Although the mechanisms responsible for the metastatic behavior of tumor cells are not fully understood, accumulated evidence indicates that the invasive and metastatic dissemination of many kind of human cancers is angiogensis, cell cycle and growth and miscellanous[2,3].

Adenocarcinoma of the salivary gland is a devastating neoplasm that is usually associated with a poor prognosis. Although the reasons for the aggressiveness of this disorder are not known, some alterations of oncogenic molecules have been demonstrated in malignancy, and such alterations may give adenocarcinoma cells a growth advantage[13]. In addition, increased exprssion of EGF and its receptor has been demonstrated in HSG cells that are responsive to EGF in vitro[14], suggesting that excessive growth -factor expression may contribute to neoplastic cell growth. Since Osaki et al.[15] have been demonstrated that HSY human salivary gland adenocaricnoma cells can proliferate in serum-free culture without FGF and Myoken[16] et. al. disclose that HSY cells express FGF-1, FGF-2, and FGFR-1 in serum-free culture.

Maintenance of the acinar phenotype in primary salivary gland cell cultures has been proved difficult. So, there are no well established cell models to study salivary acinar cell differentiation

of function and little is known about factors responsible for the initiation and maintainance of these process despite the number of immortalized salivary gland cell lines described[6,15,17]. The immortalized human submandibular gland cell line(HSG) is derived from intercalalted duct cells[5]. During salivary gland development , intercalated ductal cells are belieived pluripotent stem cells that give rise to acinar and myoepithelial cells[3]. But there is no comprative gene expression study between HSG and SGT. Therefore, in the present study, we examined the application of cDNA microarray to measure the relative expression of 2000 genes across cell lines human submandibular gland duct cell line(HSG) and human salivary adenocarcinoma cells(SGT).

One of the principal applications of cDNA microarray technology is to perform comparative gene expression analyses between cells that have differences of a histological, pathological, pharmacological or regulatory nature[10]. Such analyses will ultimately lead to a comprehensive understanding of the molecular basis for many biologically significant conditions. Microarray system has proven sensitive enough to detect gene expression differences between two transformed cell lines from distinctly different biological sources[6-9]. This array was able to achieve a high degree of reproducibility and accuracy based on the degree of reproducibility, accuracy, and sensitivity of the system reported here. And it was powerful tool for comparative analysis of gene expression that will aid in the quest to understand the molecular basis of initiation and progression of cancer[19].

In this study, expression patterns of some known tumor-related genes have suggested that cell proliferation, immune reaction and other processes change in initiation and progression of adenocarcinoma and multiple related genes expressed abnormally. Using cDNA microarrays containing approximately 2,000 cDNAs, we conducted a systematic characterization of gene expression in HSG and SGT cell line.

The highest expressed gene was S-100A4 in HSG(30.1times than SGT). Calcium binding S-100 proteins are low molecular wight EF-hand proteins. Recently, S-100 genes, renamed S-100A1-A13 were localized to the chromosomal region 1q21[20]. Calcium ion play important role in cell regulation, such as secretion, protein synthesis and membrane permeability[21], and S-100 protiens may be involved in secretion from granular cells[22]. Immunohistochemical studies of S-100 calcium binding proteins, with the new classification of S-100A1, A2, and A66, have been reported in human salivary gland tumors[23]. Human salivary gland cell line HSG and the adenoid cystic carcinoma cell have expressed S-100 protien in vitro[24,25]. The expression pattern of S-100A4 in human HSG cells suggest an involvement on Ca^{++} regulated differentiation and important implications for the histogenesis of salivary gland tumors[26].

For differential gene expression between HSG and SGT, we can to be divide to several fuctional categories: transciption factors, growth factors and therir receptors, cytoskeletal and extracellular matrix proteins, cell surface antigens, intracellular signal transduction modulators and effectors, and miscellaneous.

Ⅳ-1. Transcription factor

The TXK tyrosine kinase gene that 25.1 times increased in HSG rather than in SGT. The Tec family is a recently emerging subfamily of non-receptor protein-tyrosine kinases (PTKs) represented by its first member, Tec. This family is composed of five members, namely Tec, Btk, Itk/Emt/Tsk, Bmx and Txk/Rlk. Tec family kinases have been shown to be involved in the intracellular signaling mechanisms of cytokine receptors, lymphocyte surface antigens, heterotrimeric G-protein-coupled receptors and integrin molecules[27].

Fos-related antigen(FRA-2) is a member of the Fos family. Evidence suggests FRA-2 may contributre to transforming growth factor alpha signaling events. This gene was also found to be strongly upregulated in a cAMP-induced apoptic rat leukemia cell line[28]. But fra-2(2.64 times) is upregualted in HSG, and its induction in our

HSG reflects its involvement in the TGF signaling transduction pathways.

Small molecules that modulate specific protein functions are valuable tools for dissecting complex signaling pathways. A small molecule that induces the assembly of the interferon-beta (IFN-beta) enhance by stimulating all the enhancer-binding activator proteins: ATF2/c-JUN, IRF3, and p50/p65 of NF-kappaB. Interferon regulatory factor 3 (IRF3) is known to participate in the transcriptional induction of interferon (IFN) alpha and IFN beta genes, as well as of a number of interferon-stimulated genes (ISGs)[29], is upregulated in SGT rather than HSG.

Overall, several transcriptional factors in SGT expressed at highly levels, such as TF-AP4, NFK-BIA, Aryl hydrocarbon receptor, Max, and Histone deacetylase 3. This may reflect the fact that the function of transcription factor genes is strongly regulated at the trascription level while this control is like to require argumentation on numerous pathways.

Ⅳ-2. Cell cycle and Cell growth

On the other hand, we observed no increase in the HSG cell levels of cyclin D1, and those of no metalloproteases(MMP-7, MMP-10, MMP-14), which reflects the no-invasive behavior of this tumor type. Furthermore, we observed a remarkable increase in the levels of many growth and angiogenic factors in HSG including TFGβR3, PLAB(TGFβ superfamily), FGF2. In HSG, also cell cycle regulator such as FA-C(2.41), p130(2.43), PPM1D(protein phosphatase Wip1, 2.82), CDK6 inhibitor(5.31) were upregulated.

Various genes implicated in cell cycle control were overexpressed in SGT as compared to HSG cells. These in SGT included cell division cycle 42(0.48), cyclin G2(0.43), BRCA1(0.37), PAK1 (0.38) involved in various phases of the cell cycle. This supports the conclusion that this adenocarcinoma cell type secretes factors that are likely to induce epithelial cell growth in an autocrine fashion in addition to promoting the growth of stromal cells and the process of neovascularization[30].

Furthermore, these malignant salivary gland tumor cells also overexpress several proapoptotic molecules, including caspase precursors, only Bag-1(2.68) in HSG, varius gene such as Bag-1 (0.33), CD95(0.46), MAP3K4(0.42) BAD(0.43), BNIP1(0.42) in SGT cell line.

Although several studies have shown that genes that are associated with rapid cell proliferation are frequently up-regulated in neoplastic cells [31,32], there was a notable paucity of such genes in our study. We attribute this difference to the fact that the growth rate of the HSG and SGT cells in tissue culture is similar.

Ⅳ-3. Macromolecule stability and metabolism

Protein metabolism was increased in HSG cells as shown by the increased expression of UG3 polymerase Ⅱ polypeptide B(RNA polymerase), UG5 polymerase, and alpha(DNA polymerase).

Moreover, the correct folding and the macromolecular assemble of proteins were promoted through Calmegin(chaperon). In our study the 90 kDa (M16600) heat shock proteins(HSP-CA, 0.37) in SGT induced by stress were overexpressed.

Proteins appeared more protected from hydrolysis as cathepsin L in SGT. And hydrolase as PTP4A2, RIU-K5 were increased in HSG rather than SGT, 4.2, 3.0 folds, respectively.

Similarly, proteins related to nucleotied interactions inclued genes products related to stabilization of mRNA and DNA. Degradation of mRNA, for example, may be more pronounced in SGT cells as indicated by altered expression of genes encoding the ribonuclease(RNA-SE6PL, 0.44) .

In addition, repair gene as XRCC3 was highly expressed in SGT. XRCC3 was originally identified as a human gene able to complement the DNA damage sensitivity, chromosomal instability and impaired growth of the mutant hamster cell line irs1SF. The phenotype of irs1SF and the identification of XRCC3 as a member of the RAD51 gene family have suggested a role for XRCC3 in repair of DNA damage by homologous recombination[33].

Ⅳ-4. Adhesion and Cell recognition

Integrins are known to play a role in extracellular matrix adhesion. Many integrin genes were upregulated in SGT cells like integrin α3 (0.37), α integrin A9(0.35). Integrin alpha 3 beta 1 mediates epidermal intercellular adhesion as well as cell-substrate adhesion and, alterations in the expression of the alpha 3 beta 1 and alpha 6 beta 4 integrins may thus allow human prostate carcinoma cell to become more invasive, and lead to an increased propensity for metastasis[34]

The ability of maturing salivary epithelial cells to attain their differentiated state has been shown to depend, in part, on interactions between extracellular matrix (ECM) proteins and their integrin receptors. In a search for key regulators of salivary cell lineage, Menko[35]et al, have studied alpha 3 beta 1 integrin, a receptor for the basement membrane protein. They revealed that laminin alpha 3 beta 1 is required for normal salivary cell differentiation and that its absence affects multiple components of adhesive complexes and their associated signalling pathways. Indeed the laminin B1 gene was overexpressed (0.46) in SGT cells also giving them increased efficiency in angiogenesis.

Carcinogenesis of epithelial cells involves alterations of the adhesive properties of the cells to each other and to the basement membrane. We have found upregulation of several genes involved in the interaction of the cell with its external milieu. Laminin-B1(0.46) in SGT helps anchor the cell to the basement membrane, and immunohistochemical localization of type Ⅳ collagen and laminin in normal salivary glands and in salivary gland tumours[36], herefore laminin and collagen Ⅳ are involved in the process of malignant transformation of salivary gland tumor and their biological progression.

Ⅳ-5 Signal transduction factor

We found CD59 (0.369) gene was highly expressed in SGT, which shown to act by lowering the local inflammation and immune response. Indeed CD59 is a potent inhibitor of the complement attack complex action and protects malignant cells from C-mediated lysis.

Expression of CD44, a transmembrane molecule involved in cell-matrix interactions, confers metastatic potential on carcinoma cells in animal models and might also be important in the clinical progression of some human tumors[37,38]. CD44v3 and v6 in myoepithelium of normal salivary glands may argue in favour of the role of these molecules in the regulation of growth and renewal of normal tissues and, potentially, on the morphogenesis of salivary gland neoplasia[39]. Malignancies with different degrees of aggressiveness may express different levels and patterns of Hyaluronan and CD44. CD44 expression was seen only in tumor cells (not stroma) of malignancies, and was of similar intensity in both low and high grade salivary gland tumors[35]. In our case, CD44 was highly expressed in SGT than HSG, SGT cell was metastasizing tumor, so CD44 are also likely associated with tumor invasion and metastasis.

Cancer cells overexpressed signaling molecules participating in the MAP kinase pathway, including MARK3(0.43) in SGT, which is likely to contribute to the enhanced growth stimulation in these cells.

Ⅳ-6. Tumor progression and transformation

Several genes identified in this study have been reported to be differentially expressed in cancers and to affect cell proliferation and tumor progression. The MMP genes are thought to be involved in tumor invasion, since their action is to degrade elements of the extracellular matrix. Acquisition of metastatic ability by human salivary-gland tumor cells is closely associated with increased secretion of several metalloproteinases as well as decreased or altered TIMP-1 expression. In addition, we found tissue inhibitor of matrix metalloproteinase (TIMP-3, 3.15) and TIMP 2(5.20) were upregulated in HSG cells. And similar results from non-metastasizing to metastasizing adenocarcinoma cells was performed by means of enzyme immunoassay, zymogram, or immunoblot-

ing[40]. The combined presence of TIMP-3 and TIMP-2 gene products could act synergistically to delimit the metastatic potential of these cells.

The role of S-100A4, a member of the S-100 family of calcium-binding proteins, in carcinogenesis was originally postulated to be as a tumor suppressor. Recent work has shown it to be regulated by epidermal growth factor receptor activation, overexpressed in epidermal hyperplasia and squamous cell carcinoma, and, often concomitantly overexpressed with K6[41,42]. We show its extremely specific overexpression in adenocarcinoma cell line as compared with our panel of epithelial ductal cell line.

Cellular proto-oncogenes encode proteins that propagate growth, differentiation or apoptosis signals from cell membrane to nucleus. The proliferation and differentiation of normal eukaryotic cells are precisely controlled. Tumor cells usually are characterized both by the continuous growth signal and by the block of cell differentiation. Gene products involved in cell proliferation were also upregulated in HSG cells included the proto-oncogenes C-myc(2.86), C-myb(3.40) .

The c-Myb and v-Myb proteins are transcription factors that regulate cell proliferation and differentiation. Both Myb proteins have been shown to interact with a number of cellular proteins, some of which are transcription factors that cooperate to activate specific promoters, while others regulate the transcriptional activity of Myb in specific contexts. Hematopoietic tumors in both humans and mice frequently up-regulate expression of the c-myb gene[43].

The c-myc oncogene is commonly amplified in breast cancer and is known to interact synergistically with transforming growth factor alpha (TGF alpha) in vitro to promote phenotypic transformation of mammary epithelial cells. An immunoblotting assay clearly demonstrated the expression of c-myc and p53 gene products in both the benign and malignant forms of the pleomorphic adenoma [4], and strong c-myc mRNA expression was observed on epithelial cells of labial salivary glands from patients with primary Sjogren syndrome[44]. Highly expressed protooncogne in HSG, explained that oncognes are highly homologous to cellular genes in normal growth control(proto-oncogenes). Cellular studies using normal human cells in which the complexity of the system can be carefully controlled by the addition of one, two, or even more genes associated with cancer development may provide valuable information about how the products of the genes interact with each other.

The expression of laminin (0.45), protein tyrosine phosphatase(CL100) (0.46) have been reported to be altered in tumors[45,46]. It is not known whether any genes identified in this study can be causally linked to the carcinogenesis, but several genes, such as KIAA0033(0.43) that are preferentially expressed in adenocarcinoma cell lines represent potential candidates.

Ⅳ-7 Oxidative stress response gene

In combined chemotherapy for head-and-neck cancer (HNC), salivary gland-cell adenocarcinoma (SGA) shows insufficient clinical outcome, and it has been suggested that the sensitivity and/or the mechanism of resistance to anti-cancer drugs are different between SGA and oral squamous-cell carcinoma[47]. A battery of genes involved in durg resistance expressed in both cell lines may help to explain why salivary gland cells are intrinsically resistant. These genes included glutathione S-transferase (GST-T2, 0.40), cytochrome P450 genes including the 2C subfamily (0.48). Human salivary adenocarcioma has long been known to be extremely resistant to chemotherapy but the cellular and subcellular mechanisms remain largely unknown, but these gene expression may explain that point.

Ⅳ-8 Miscellaneous

Bone morphogenetic proteins (BMPs) belong to the transforming growth factor-beta (TGF-beta) family and have been identified as factors that stimulate bone formation in vivo. They turned out to be multifunctional molecules regulating the growth, differentiation, and apoptosis in various target cells. Some BMPs and their receptors

(BMPRs) are expressed on epithelial cancer cells including oral keratinocyte[48-50]. Jin et al.[48] results indicate that there was an overexpression of BMP-2/4, BMP-5, bBMP-McAb and BMPR-IA in the high-risk premalignant and malignant lesions of oral epithelium. These findings suggest that BMP-2/4 and BMP-5 but not BMPR-IA might be involved in the metastasis of oral carcinoma cells [49]. Hatakeyama[50] disclosed that BMP-1, BMP-6, BMP-7 and BMP-2 was expressed in HSG cell. On the other hand, among these carcinoma cells, HSG induced bone in nude mouse tumors[50]. In additionally, our SGT cells more expressed BMP receptor Ⅱ and GS3955(cancellous bone osteoblast, 0.47) rather than HSG, these findings indicated that the neoplastic epithelial cells possessed a rather great potency to express BMP receptor Ⅱ.

Thus, collectively these results demonstrate that adenocarcinoma cells exhibit a distinct pattern of gene expression, which might help explain many of the cellular abnormalities described in this tumor type.

Understanding global overview of the causes of cancer in molecular level is the key to develop the better diagnosis and treatment for patients, since the outcome of the cancer phenotype is the result of many interactive pathways. Several independent studies have proved that previously unrecognized distinct subtypes of cancers could be identified by examining the genome-wide transcriptional profiles of clinical or experimental samples[51-52]. Although the gene expression profile study with cell lines was not able to to address the correlation between the expression pattern and clinical features, the gene expression profiles measured in HSG and SGT cell lines will provide a good baseline that helps to interpret data generated from clinical salivary gland tumor or salivary specimens.

Our results show that the cDNA microarray is extremely useful not only to discover differentially expressed genes in cancer, but also to identify previously undetectable subtypes of cancer that are different ion transcriptional modules composed of hundred of genes. Thus, the total genomic view of gene expression and genetic alteration in salivary adenocarcinoma may provide insights into the molecular salivary carcinogenesis and identify novel targets for cancer theraphy.

Ⅴ. Conclusion

We characterizes gene expression profiles in SGT and HSG cell lines, by using cDNA microarrays containing 2000 sequence-verified cDNA elements. Examination of gene expression that is shared(90%) between HSG and SGT cells. We identified 111(87)clones cDNA exhibits greater than 2(2.5) -fold overexpression in SGT probes relative to nomal HSG probe, 72(32) cDNA s reveal 2(2.5) fold overexpression in normal HSG relative to tumor SGT probe. Pearson correlation coefficients for the set of selected spots from duplicated hybridization was -0.85. In HSG, chromosome 6p in HSG, and chromosome 11q in SGT showed higher level of 2 fold overexpression gene percentage than other chromosome. Examination of gene expression that differs between HSG and SGT cells revealded several pathways that may be important in cell transformation. Gene expression linked to cell transformation apprear to be related to : transciption factors, growth factors and therir receptors, cytoskeletal and extracellular matrix proteins, cell surface antigens, and intracellular signal transduction modulators and effectors. Such studies should foster the research of molecular markers allowing to better assess the phenotype of malignant salivary gland tumor. These strategies could improve the accuracy of its diagnosis, prognosis and therapy including by gene modulation.

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원고 접수일 2001년 05월 10일
게재 확정일 2001년 06월 20일

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Paper received 10 May 2001
Paper accepted 20 July 2001