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ORIGINAL ARTICLE

Differential gene expression profiles of normal human parotid and submandibular glands

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BACKGROUND: Parotid and submandibular glands have different properties including characteristics of the secreted saliva and tumor incidences. The differences in properties of parotid and submandibular glands are not clear from a genetic viewpoint.

OBJECTIVE: To study differential gene expression profiles between normal human parotid and submandibular glands.

MATERIALS AND METHODS: Three pairs of normal parotid and submandibular glands were obtained. RNA was extracted from these samples. After reverse transcription, the cDNA was *in vitro*-transcribed to produce biotin-labeled cRNA. The purified biotin-labeled cRNA samples were hybridized to microarray chips.

RESULTS: Among the 54 675 tested transcripts, 47 transcripts were upregulated at least twofold in the parotid gland compared with the submandibular gland, including tumor-associated genes (pleiotrophin, WNT5A, ABCC1) and transport-associated genes (SLCO1A2, SLC13A5, KCNJ15). Ninety-eight transcripts were upregulated at least twofold in the submandibular gland compared with the parotid gland, including the chloride channel CFTR and mucin-associated genes that belong to the starch and sucrose metabolism pathway (GalNAc-T4, GalNAc-T7 and GalNAc-T13). Quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of nine differentially expressed genes confirmed the microarray results.

CONCLUSION: This study revealed the different gene expression profiles of normal human parotid and submandibular glands, providing a genetic basis for their differing properties.

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Keywords: salivary glands; microarray; tumor-associated genes; mucin-associated genes

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Introduction

Parotid and submandibular glands are the two major salivary glands, being larger in size than the other salivary glands. In adults, the parotid gland contains only serous acini that mainly secrete a watery substance, whereas the submandibular gland is composed of serous and mucosal acini that secrete a mixed substance. The contributions of the parotid and submandibular glands to total saliva secretion are 25% and 70%, respectively (Baum, 1981; Tylenda *et al*, 1988). Apart from differing in quality and quantity of secretion, parotid and submandibular glands also differ in tumor incidence. The parotid gland is the most common location for tumors of the salivary glands. The tumor incidence of the parotid gland is almost fourfold higher than that of the submandibular gland (Ito *et al*, 2005).

Gene chips have been used to identify differentially expressed genes associated with salivary gland tumors and to identify differentially expressed genes in salivary glands of male *vs* female mice (Kainuma *et al*, 2004; Treister *et al*, 2005). However, there are few reports of gene expression profiles and differentially expressed genes of human normal parotid and submandibular glands, which may be associated with the differential property between the two main salivary glands.

In this study, we employed the Human U133 Plus2.0 gene chip of Affymetrix Company (Santa Clara, CA, USA) to generate gene expression profiles and to screen differentially expressed genes of human normal parotid and submandibular glands. Real-time quantitative polymerase chain reaction (PCR) was employed to confirm the differential expression of the genes identified by microarray analysis. The purpose of this study was to provide a genetic basis for the different properties of parotid and submandibular glands.

Materials and methods

Samples and chemicals

The study was conducted with the consent of all family members and approved by the Ethics Committee of the Chinese National Human Genome Center (Beijing). Three normal pairs of parotid and submandibular

Table 1 Primer of differentially expressed genes selected to confirm by real-time quantitative PCR between normal parotid and submandibular gland

Gene symbol	*GenBank accession*	*Primer*	*Sequence (5′ -> 3′)*	*Amplicon size*
ABCC1	NM_019862	Forward	CTGAGTTCCTGCGTACCTATG	214
		Reverse	AGTTCTGCGGTGCTGTTGT	
CFTR	NM_000492	Forward	CAAGGAGGAACGCTCTATCG	262
		Reverse	GAAATGTGCCAATGCAAGTC	
UGT2B28	NM_053039	Forward	AGGCATTCCATTGTTTTGGG	200
		Reverse	GCAGGGGCTTTACTGGTTGA	
SLC13A5	NM_177550	Forward	TTGAAGTTACTCGGACAAAGACC	154
		Reverse	TCCAGAGCAGCACAAGAAGG	
SLCO1A2	NM_021094	Forward	TGGGCTTGTAGAAACAGGAG	135
		Reverse	CAACGAGTGTCAGTGGGAGT	
KCNJ15	NM_170736	Forward	TTGAGTTTGTGCCTGTGGTA	156
		Reverse	CATGTCCGTCCTCCTAGTCT	
WNT5A	NM_003392	Forward	TTCTGTCTTGCGTGATTTGT	172
		Reverse	CTATCCGTCATGGTTTCTCC	
PTN	NM_002825	Forward	ACCAGTGAGTCATCCGTCCA	251
		Reverse	TTCCCTGCTTCAGCAGTATC	
GALNT13	NM_052917	Forward	TTAATACGTGCCCGTCTTCG	133
		Reverse	CCGTTTTCCTGTCTTCCTTT	
ACTB	NM_001101	Forward	CATGTACGTTGCTATCCAGGC	250
		Reverse	CTCCTTAATGTCACGCACGAT	
GAPDH	NM_002046	Forward	TGTTGCCATCAATGACCCCTT	202
		Reverse	CTCCACGACGTACTCAGCG	

glands were obtained from donors who died due to traffic accidents. The samples were designated 1-1, 2-1, 1-2, 2-2, 1-3, and 2-3 (the former numbers, 1 and 2, representing the parotid and submandibular glands, respectively; the latter number referring to the donor). Upon the deaths of the donors, the parotid and submandibular glands were obtained with a routine RNase out procedure and stored in liquid nitrogen. All samples of parotid and submandibular glands were confirmed to be normal tissues by conventional hematoxylin–eosin staining. In this study, samples of the parotid gland were used as the test group, while samples of the submandibular gland were used as the control. All chemicals and reagents were recommended by the Affymetrix Company for the Human U133 Plus2.0 gene chip (GeneChip Human Genome U133 Plus2.0; Affymetrix). Equipment included the Affymetrix® Hybridization Oven 640, the Affymetrix® Fluidics Station 450, and the Affymetrix® GeneChip® Scanner 3000. The software used was Affymetrix® GeneChip® Operating Software Version 3.0.

Experimental procedures

RNA isolation

Total RNA generated from parotid and submandibular gland tissue was isolated using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and RNA samples were electrophoresed on RNA-grade agarose gels to assess possible degradation. The concentration and optical density (OD) 260/280 ratios were measured on a spectrophotometer (ND-1000; NanoDrop Technologies, Wilmington, DE, USA).

Microarray hybridization

The RNA samples of parotid and submandibular glands were hybridized to the Affymetrix GeneChip Human Genome U133 Plus2.0 microarray chips. Briefly, the first-strand synthesis was performed using the SuperScript Choice system (Life Technologies, Inc., Gaithersburg, MD, USA) and T7-(DT)24 primer. The second strand was synthesized by using DNA polymerase I and RNase H. Biotin-labeled cRNA was generated from the double-stranded complementary DNA *in vitro* transcription reaction using the Enzo BioArray High Yield RNA transcript labeling kit (Affymetrix) according to the manufacturer's instructions. The labeled cRNA was then fragmented and hybridized onto Affymetrix GeneChip Human Genome U133 Plus2.0 probe microarray chip. The probe arrays were washed and stained in the Affymetrix GeneChip Fluidics station using pre-programmed Affymetrix protocols. Finally, the probe arrays were scanned in the Affymetrix GeneChip Scanner 3000.

Gene expression

Analysis The six data files for parotid and submandibular gland samples were uploaded into Affymetrix MicroDB 3.0 software. This database file was then sorted and studied with the Affymetrix Data Mining

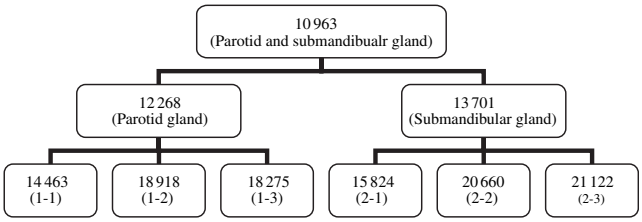

Figure 1 Whole gene expression profiles of human normal parotid and submandibular glands There were 12 268 expressed genes in the parotid gland and 13 701 expressed genes in the submandibular gland. There were 10 968 genes commonly expressed by both glands

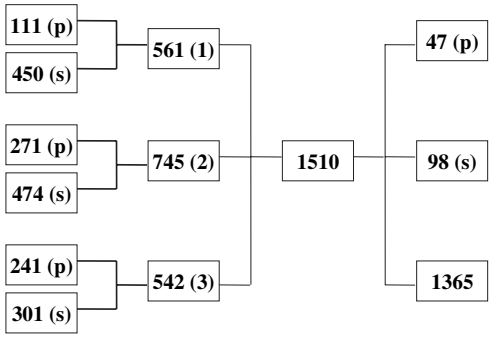

Figure 2 Differential gene expression profiles of human normal parotid and submandibular glands. There were 47 transcripts upregulated at least twofold in the parotid gland compared with the submandibular gland. There were 98 transcripts upregulated at least twofold in the submandibular gland compared with the parotid gland. P, parotid gland; S, submandibular gland. The number in bracket refers to the donor

Tool 1.2. The genes showing twofold changes between parotid and submandibular glands were organized into a list. Data from the samples were then queried using this gene list. There were an absolute call of 'Present' or 'Absent' in parotid and submandibular glands, and a difference call of 'Increased' or 'Decreased' between parotid and submandibular glands. The resulting list of genes was studied; further information on genes of interest was compiled, and the obtained data were finally analyzed by Gene Ontology (GO) on the http://fatigo.bioinfo.ochoa.fib.es website (Al-Shahrour *et al*, 2004). For each microarray, the submandibular and parotid glands were collected from one body so that there were no individual differences within the microarray.

Real-time quantitative RT-PCR

We used real-time reverse transcriptase PCR (RT-PCR) to verify the differential expression of selected genes. cDNA was transcribed from DNase-treated mRNA (5 μg) by SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) and oligo dT priming (Promega, Madison, WI, USA). We designed forward and reverse primers using Primer Express Software (Perkin-Elmer Life Sciences, Wellesley, MA, USA) (Table 1). First-strand synthesis was performed as described for the microarrays. Each 50-μl PCR reaction included 25 ng of double-stranded cDNA, 5 μl of 10x PCR buffer A [500 mM KCl, 100 mM Tris-HCl, 0.1 M ethylenediaminetetraacetic acid (EDTA), 600 nM passive reference dye, pH 8.3, at room temperature], 10 μl of 25 mM MgCl2, 1.5 μl each dNTP (10 mM dATP, dCTP, and dGTP, and 20 mM dUTP), 0.5 μl of forward and reverse primers (10 μM), and 0.25 μl of *Taq* supplied at 5 units/μl. PCR cycle parameters were 95°C for 15 min followed by 40 cycles at 95°C for 15 s and 59°C for 1 min. The primers and probes (Table 1) used in this study were designed using Primer Express software and synthesized by Shanghai Sangon Company (Shanghai, China).

Table 2 Molecular functions (level 3) GO of differential expressed genes between normal parotid and submandibular glands of human

Gene ontology: molecular function level: 3	*Parotid no. genes*	*Percentage*	*Submandibular no. genes*	*Percentage*	*Unadjusted* P-*value*	*Adjusted* P-*value FDR*
Nucleotide binding	1	4.17	7	19.44	1.284267e-01	1
Antigen binding	0	0	4	11.11	1.425882e-01	1
Phosphatase regulator activity	2	8.33	0	0	1.559322e-01	1
Ion binding	4	16.67	12	33.33	2.340794e-01	1
Protein binding	5	20.83	13	36.11	2.579138e-01	1
Receptor binding	3	12.50	1	2.78	2.920114e-01	1
Hydrolase activity	2	8.33	7	19.44	2.929821e-01	1
Amine transporter activity	1	4.17	0	0	4.000000e-01	1
Peroxidase activity	1	4.17	0	0	4.000000e-01	1
Translation factor activity, nucleic acid binding	1	4.17	0	0	4.000000e-01	1
Organic acid transporter activity	1	4.17	0	0	4.000000e-01	1
Selenium binding	1	4.17	0	0	4.000000e-01	1
Transferase activity	5	20.83	5	13.89	5.013804e-01	1
Lipid binding	2	8.33	1	2.78	5.581531e-01	1
Pattern binding	2	8.33	1	2.78	5.581531e-01	1
Nucleic acid binding	4	16.67	4	11.11	7.017800e-01	1
Carbohydrate binding	2	8.33	3	8.33	1	1
Carrier activity	0	0	1	2.78	1	1
Lipid transporter activity	1	4.17	1	2.78	1	1
Oxidoreductase activity	2	8.33	2	5.56	1	1
Receptor activity	3	12.50	4	11.11	1	1
Enzyme inhibitor activity	2	8.33	2	5.56	1	1
Transcription cofactor activity	0	0	1	2.78	1	1
Ion transporter activity	2	8.33	3	8.33	1	1
Channel or pore class transporter activity	1	4.17	1	2.78	1	1
ATPase activity, coupled to movement of substances	1	4.17	1	2.78	1	1
Extracellular matrix structural constituent	0	0	1	2.78	1	1
Structural constituent of muscle	0	0	1	2.78	1	1
Transcriptional activator activity	0	0	1	2.78	1	1
Transcription factor activity	2	8.33	2	5.56	1	1
Electron transporter activity	1	4.17	1	2.78	1	1

Table 3 Biological processes (level 3) GO of differential expressed genes between normal parotid and submandibular glands of humans

Gene ontology: biological process: level 3	*Parotid no. genes*	*Percentage*	*Submandibular no. genes*	*Percentage*	*Unadjusted* P-*value*	*Adjusted* P-*value*
Cell adhesion	0	0	5	14.29	6.264285e-02	1
Organismal physiological process	4	14.81	13	37.14	8.375522e-02	1
Cellular physiological process	21	77.78	20	57.14	1.098953e-01	1
Response to biotic stimulus	2	7.41	8	22.86	1.642188e-01	1
Response to abiotic stimulus	2	7.41	0	0	1.856161e-01	1
Localization	10	37.04	9	25.71	4.097042e-01	1
Extracellular structure organization and biogenesis	1	3.70	0	0	4.354839e-01	1
Regulation of enzyme activity	1	3.70	0	0	4.354839e-01	1
Pattern specification	1	3.70	0	0	4.354839e-01	1
coagulation	0	0	2	5.71	5.002644e-01	1
Positive regulation of biological process	2	7.41	1	2.86	5.752247e-01	1
System development	2	7.41	1	2.86	5.752247e-01	1
Response to external stimulus	1	3.70	3	8.57	6.256218e-01	1
Cell communication	8	29.63	9	25.71	7.795878e-01	1
Metabolism	12	44.44	14	40	7.981291e-01	1
Cell differentiation	0	0	1	2.86	1	1
Sexual reproduction	1	3.70	1	2.86	1	1
Regulation of physiological process	4	14.81	6	17.14	1	1
Negative regulation of biological process	0	0	1	2.86	1	1
Reproductive physiological process	0	0	1	2.86	1	1
Sensory perception	0	0	1	2.86	1	1
Interspecies interaction between organisms	0	0	1	2.86	1	1
Regulation of growth	0	0	1	2.86	1	1
Death	0	0	1	2.86	1	1
Morphogenesis	3	11.11	3	8.57	1	1
Physiological interaction between organisms	0	0	1	2.86	1	1
Regulation of cellular process	4	14.81	5	14.29	1	1
Cell growth	0	0	1	2.86	1	1
Response to stress	3	11.11	3	8.57	1	1
Organ development	1	3.70	2	5.71	1	1
Locomotion	1	3.70	1	2.86	1	1
Homeostasis	0	0	1	2.86	1	1

Table 4 Cellular components (level 3) GO of differential expressed genes between normal parotid and submandibular glands of human

Gene ontology: cellular component: level: 3	*Parotid no. genes*	*Percentage*	*Submandibular no. genes*	*Percentage*	*Unadjusted* P-*value*	*Adjusted* P-*value*
Extracellular space	4	16	0	0	3.202451e-02	1
Cell fraction	6	24	3	9.38	1.605634e-01	1
Extracellular matrix (sensu Metazoa)	0	0	3	9.38	2.481203e-01	1
Intracellular organelle	8	32	15	46.88	2.888675e-01	1
Membrane-bound organelle	8	32	14	43.75	4.202781e-01	1
Cell projection	1	4	0	0	4.385965e-01	1
Phosphoinositide 3-kinase complex	1	4	0	0	4.385965e-01	1
Membrane	13	52	18	56.25	7.936605e-01	1
Organelle lumen	0	0	1	3.12	1	1
Site of polarized growth	0	0	1	3.12	1	1
Non-membrane-bound organelle	0	0	1	3.12	1	1
Apical part of cell	0	0	1	3.12	1	1
MHC protein complex	0	0	1	3.12	1	1
Vesicle	0	0	1	3.12	1	1
Polarisome	0	0	1	3.12	1	1
Organelle envelope	0	0	1	3.12	1	1
Myosin	0	0	1	3.12	1	1
Hydrogen-translocating V-type ATPase complex	0	0	1	3.12	1	1
Intracellular	14	56	19	59.38	1	1
Proton-transporting two-sector ATPase complex	0	0	1	3.12	1	1
Golgi transport complex	0	0	1	3.12	1	1
Heterotrimeric G-protein complex	0	0	1	3.12	1	1

Results

Differential gene expression profile

Among the 54 675 tested transcripts of the U133 Plus2.0 chip, there were 12 268 expressed transcripts in the parotid gland and 13 701 expressed transcripts in the submandibular gland (Figure 1). There were 10 963 transcripts commonly expressed by both glands. Our results demonstrated significant ($P < 0.05$) differences in gene expression of the parotid and submandibular glands

Table 5 Forty-seven upregulated genes in the parotid glands

GenBank ID	*Gene title*	*Symbol*	*1-1/2-1*	*1-2/2-2*	*1-3/2-3*
BF059512	Delta-notch-like EGF	DNER	1	1.2	1.1
AI625747	Adrenergic, beta-1-receptor	ADRB1	1.4	1.2	1.1
AK021452	Zinc finger protein 521	ZNF521	1.5	1.4	1.1
AI766029	Fatty acid binding protein 4	FABP4	2	1.8	1.2
AB032261	Stearoyl-CoA desaturase	SCD	1	1.3	1.3
M24317	Alcohol dehydrogenase IB (class I)	ADH1B	1.8	1.4	1.3
M21692	Alcohol dehydrogenase IB (class I)	ADH1B	2	1.6	1.3
NM_002666	Perilipin	PLIN	1.2	1.7	1.3
NM_006741	Protein phosphatase 1, regulatory 1A	PPP1R1A	1.1	1.8	1.3
BF672975	Lipoprotein lipase	LPL	1.4	1.9	1.3
NM_000237	Lipoprotein lipase	LPL	1.6	1.9	1.3
NM_004797	Adiponectin, C1Q and collagen	ADIPOQ	1.8	1.9	1.3
AI968085	Wingless-type family, member 5A	WNT5A	1.6	2	1.3
AW149846	Glutathione peroxidase 3 (plasma)	GPX3	1.2	1.5	1.4
NM_006089	Sex comb on midleg-like 2	SCML2	1.3	1.6	1.4
NM_001442	Fatty acid binding protein 4, adipocyte	FABP4	1.5	1.7	1.4
AF230904	SH3-domain kinase binding protein 1	SH3KBP1	2.1	2	1.4
AA528080	Hypothetical protein LOC283070	LOC283070	2.3	2	1.4
NM_002084	Glutathione peroxidase 3 (plasma)	GPX3	1.6	1.4	1.5
AW272342	Thyroid hormone responsive	THRSP	1.7	2.2	1.5
NM_020365	Eukaryotic translation initiation factor – 58 kDa	EIF2B3	1.1	1.3	1.6
AJ000008	Phosphoinositide-3-kinase, class 2	PIK3C2 G	1.6	2	1.6
NM_003392	Wingless-type family, member 5A	WNT5A	2.3	2.2	1.6
U73191	Potassium inward-rectify-channel-15	KCNJ15	1.9	2.1	1.7
AI797218	Solute carrier family 13 member 5	SLC13A5	3	1	1.8
AW043602	KIAA1946	KIAA1946	1.4	1.4	1.8
NM_021083	Kell blood group precursor	XK	2.7	1.5	1.8
BC005916	Pleiotrophin	PTN	2.4	2.4	1.8
AW960707	Pyrophosphorylase	QPRT	2.8	2.4	1.8
BF690134	Shadow of prion protein homolog	SPRN	1.3	1.8	1.9
NM_003654	Carbohydrate	CHST1	2.8	2.1	2
AL565812	Pleiotrophin	PTN	2.5	2.2	2
M57399	Pleiotrophin	PTN	1.8	2.4	2
AW953794	Full-length cDNA clone	–	2.7	1.1	2.2
AK056897	CDNA FLJ32335 fis	–	2	1.7	2.3
NM_021094	Anion transporter family member 1A2	SLCO1A2	2	2.5	2.5
NM_005756	G protein-coupled receptor 64	GPR64	3.1	2.7	2.5
NM_001890	Casein alpha s1	CSN1S1	3.3	4	2.5
NM_030945	C1q and tumor necrosis factor related	C1QTNF3	4.7	1.9	2.6
AW471176	DMC	UNQ473	1.4	1.6	2.8
BC004863	Phosphoserine aminotransferase 1	PSAT1	1.6	2	3
AF085224	Anion transporter family member 1A2	SLCO1A2	1.4	3	3.4
NM_000325	Paired-like homeodomain	PITX2	2.3	4.6	3.4
AL355392	Chromosome 20 open reading frame	C20orf114	5	2.5	3.8
NM_004996	ATP-binding cassette sub-family C	ABCC1	2.4	3.6	4.4
AI539710	ATP-binding cassette, sub-family C	ABCC1	3.7	4.4	5.5
AI478172	Homogentisate 1,2-dioxygenase	HGD	2.7	4.2	5.7

(Figure 2). There were 145 transcripts that were upregulated or downregulated more than twofold between the parotid and submandibular glands. Forty-seven transcripts were upregulated at least twofold in the parotid gland compared with the submandibular gland, and 98 transcripts were upregulated at least twofold in the submandibular gland compared with the parotid gland.

Analysis of differential gene expression

Differential gene expression between normal human parotid and submandibular glands was analyzed on the http://david.niaid.nih.gov and http://fatigo.bioinfo.ochoa.fib.es websites (Dennis *et al*, 2003; Hosack *et al*, 2003; Al-Shahrour *et al*, 2004). The differentially expressed genes appear to be involved in a diverse array of molecular functions, biological processes, and cellular components (Tables 2–4). The 47 transcripts that were upregulated in the parotid gland compared with the submandibular included tumor-associated genes (pleiotrophin, WNT5A, and ABCC1) and transport-associated genes (SLCO1A2, SLC13A5, and KCNJ15) (Table 5). The 98 transcripts that were upregulated in the submandibular gland compared with the parotid gland included the chloride channel CFTR and mucin-associated genes that belong to the starch and sucrose metabolism pathway (GalNAc-T4, GalNAc-T7, GalNAc-T13, and UGT2B28) (Table 6).

Confirmed differential gene expression

To verify the microarray results, seven differentially expressed genes were selected for real-time PCR analysis: tumor-associated genes pleiotrophin, WNT5A, and ABCC1; transport-associated genes KCNJ15, chloride channel CFTR; and mucin-associated genes

Table 6 Ninety-eightupregulated genes in the submandibular glands

GenBank ID	*Gene title*	*Symbol*	*1-1/1-1*	*1-2/2-2*	*1-3/2-3*
AF238867	Lacritin	LACRT	−4.9	−5.5	−8.3
BF825703	Hypothetical LOC389429	LOC389429	−3	−9.2	−7.6
NM_001898	Cystatin SN	CST1	−7.1	−7	−7
NM_006061	Cysteine-rich secretory protein 3	CRISP3	−5.3	−5.8	−7
AI857688	Hypothetical LOC389429	LOC389429	−10	−8.6	−6.6
L13283	Mucin 7, salivary	MUC7	−3.2	−7	−6.6
AI922323	Anterior gradient 2 homolog	AGR2	−3.7	−5.7	−5.5
BF593509	Myosin binding protein C, slow type	MYBPC1	−4.6	−4.7	−5.5
AI697108	Mucin 5, subtype B, tracheobronchial	MUC5B	−2	−6.4	−4.8
NM_001899	Cystatin S	CST4	−5.2	−4.8	−4.7
AY180924	Salivary gland expression gene	RP11–49 G10.8	−2.4	−6.4	−4.1
NM_000492	ATP-binding-cassette sub-family-C 7	CFTR	−2.8	−2.1	−3.5
AF177272	UDP-glucuronosyltransferase2 B28	UGT2B28	−4	−6	−3.4
NM_005212	Casein kappa	CSN3	−4.4	−3.6	−3
NM_007350	Pleckstrin homology-like domain	PHLDA1	−3.1	−3.5	−2.9
AF088867	Anterior gradient 2 homolog	AGR2	−3.3	−4.7	−2.8
NM_017423	GalNAc-T7	GALNT7	−2.8	−2.9	−2.8
NM_007350	CDNA clone IMAGE:5531727	–	−3.2	−3.4	−2.7
AA651750	EPH receptor A7	EPHA7	−2.3	−1.7	−2.7
NM_000114	Endothelin 3	EDN3	−3.6	−3.8	−2.6
AC009227	GalNAc-T13	GALNT13	−2.6	−3.6	−2.6
NM_017855	APin protein	APIN	−3.9	−3	−2.6
AA584310	Collagen triple helix repeat	CTHRC1	−1.3	−1.5	−2.6
AI718421	Chromosome 4 open reading frame 7	C4orf7	−5	−3.3	−2.5
AL080065	DKFZP564 J102 protein	DKFZP564 J02	−1.1	−1.5	−2.5
NM_007350	Pleckstrin homology-like domain	PHLDA1	−5	−3.5	−2.4
M68874	Phospholipase A2, group IVA	PLA2G4A	−1.9	−3.3	−2.4
BF699855	GalNAc-T7	GALNT7	−5.1	−2.8	−2.4
BE326710	Hypothetical protein MGC10946	MGC10946	−2.5	−2.2	−2.4
AI795908	Pleckstrin homology-like domain	PHLDA1	−4	−3.3	−2.3
AA576961	Pleckstrin homology-like domain	PHLDA1	−4.1	−2.8	−2.3
R70320	SLIT NTRK-like family, member 6	SLITRK6	−2.8	−1.7	−2.3
L13283	Mucin 7, salivary	MUC7	−3.1	−4.1	−2.2
AK026181	CDNA clone IMAGE:5531727	–	−3.6	−2.1	−2.2
W60595	ATP-binding-cassette sub-family-C7	CFTR	−4.9	−1.7	−2.2
AL137517	SLIT NTRK-like family, member 6	SLITRK6	−3.3	−1.4	−2.2
BC005008	Carcinoembryonic	CEACAM6	−3.1	−4.7	−2
M18728	Carcinoembryonic	CEACAM6	−1.9	−4.5	−2
NM_016548	Golgi phosphoprotein 2	GOLPH2	−3.3	−4	−2
NM_012390	Submaxillary gland androgen regulated protein 3	SMR3A	−2.4	−2.3	−2
AI972498	Glycoprotein,galactosyltransferase 1	GGTA1	−1.9	−1.2	−1.9
AI680986	SLIT NTRK-like family, member 6	SLITRK6	−2.5	−2.2	−1.8
AA401492	GNAS complex locus	GNAS	−2.7	−2.1	−1.8
AA934610	CDNA FLJ37828 fis	–	−1.3	−1	−1.8
AL157377	Ectonucleotide 3	ENPP3	−1.5	−1.8	−1.7
AF235049	Inhibitor agammaglobulinaemia	IBTK	−2.6	−1.4	−1.7
AI689429	Protein kinase C, iota	PRKCI	−1.8	−1.4	−1.7
BF055462	Thrombospondin 1	THBS1	−1.3	−1.4	−1.7
L48516	Paraoxonase 3	PON3	−1.5	−1.2	−1.7
D88435	Cyclin G-associated kinase	GAK	−1.6	−1.6	−1.6
L18964	Protein kinase C, iota	PRKCI	−1.4	−1.5	−1.6
AL042588	Paternally expressed 3	PEG3	−2.1	−2	−1.5
NM_018013	Hypothetical protein FLJ10159	FLJ10159	−1.5	−1.9	−1.5
W79425	Hypothetical protein LOC147645	LOC147645	−1.9	−1.5	−1.5
NM_144583	ATPase H+ transporting, 42 kDa	ATP6V1C2	−1.8	−1.3	−1.5
D83043	Major histocompatibility complex	HLA-B	−1.5	−1	−1.5
NM_006348	Component of oligomeric golgi5	COG5	−1	−1	−1.5
BF439063	Transcribed locus	–	−2.9	−2.2	−1.4
AF208967	Paternally expressed 3	PEG3	−1	−2.2	−1.4
NM_017742	Zinc finger	ZCCHC2	−1.9	−1.7	−1.4
M24669	Immunoglobulin heavy constant mu	IGHM	−1.1	−1.7	−1.4
BG540628	Immunoglobulin kappa variable 1–5	IGKV1–5	−1.1	−1.4	−1.4
L14458	Immunoglobulin kappa constant	IGKC	−1	−1	−1.4
AB037784	Arylacetamide deacetylase-like 1	AADACL1	−1.4	−1.7	−1.3
BF033615	FUN14 domain containing 1	FUNDC1	−1.7	−1.5	−1.3
BG482805	Ig rearranged kappa-chain mRNA	–	−1.5	−1.5	−1.3
M85256	Immunoglobulin kappa light chain	–	−1.2	−1.4	−1.3
AI825926	Phospholipid scramblase 1	PLSCR1	−1.2	−1.3	−1.3
L07950	Major histocompatibility complex	HLA-B	−1.6	−1.1	−1.3

Table 6 Continued

GenBank ID	*Gene title*	*Symbol*	*1-1/1-1*	*1-2/2-2*	*1-3/2-3*
AI890347	GalNAc-T4	GALNT4	−1.4	−1.9	−1.2
AV707343	Transcribed locus	–	−1.4	−1.5	−1.2
AFFX-HUMRGE/M10098_3	Signal recognition particle 68 kDa	SRP68	−2.2	−1	−1.2
AL044570	GIPC PDZ domain containing family	GIPC2	−1.1	−1	−1.2
AF247704	NK3 transcription factor related	NKX3–1	−1.7	−3.2	−1.1
AF311912	Secreted frizzled-related protein 2	SFRP2	−1.5	−2.4	−1.1
BF515750	Chromosome 1 open reading frame	C1orf131	−1	−2	−1.1
NM_001873	Carboxypeptidase E	CPE	−1.3	−1.8	−1.1
AV700969	Hypothetical LOC401397	LOC401397	−1.3	−1.5	−1.1
BE676543	Zinc finger,CCHC containing 2	ZCCHC2	−2.2	−1.4	−1.1
BE550027	DKFZp761 N1114	DKFZp761 N114	−1.5	−1.4	−1.1
AA903473	Transcribed locus	–	−1.1	−1.2	−1.1
NM_015965	NADH dehydrogenase 1 alpha	NDUFA13	−1.4	−1.1	−1.1
AK025663	Zinc finger protein 291	ZNF291	−1.2	−1.1	−1.1
BC000181	G-protein-coupled receptor 160	GPR160	−1.1	−1.1	−1.1
L14457	Immunoglobulin kappa variable 1–5	IGKC	−1	−1.1	−1.1
NM_006235	POU domain, class 2	POU2AF1	−1.1	−1	−1.1
NM_020632	ATPase, H+ transporting	ATP6V0A4	−2.5	−1.7	−1
NM_000647	Chemokine (C-C motif) receptor 2	CCR2	−4.7	−1.5	−1
D87021	Immunoglobulin lambda joining 3	IGLC2	−1.3	−1.5	−1
X77598	Leupaxin	LPXN	−1.1	−1.5	−1
J03223	Proteoglycan 1, secretory granule	PRG1	−1	−1.4	−1
BC000893	Histone 1, H2bk	HIST1H2BK	−1.1	−1.3	−1
NM_025184	EF-hand domain	EFHC2	−1.1	−1.3	−1
BF940025	Choline phosphotransferase 1	CHPT1	−1.5	−1.2	−1
AW404894	Immunoglobulin kappa light chain	–	−1.1	−1.2	−1
NM_002727	Proteoglycan 1, secretory granule	PRG1	−1.1	−1.1	−1
BC018756	Monooxygenase, DBH-like 1	MOXD1	−1	−1.1	−1
AF103529	IG light chain V-region, VK gene	–	−1	−1.1	−1

Table 7 Change ratio of differential expressed gene between normal parotid and submandibular glands of human in microarray and real-time PCR, respectively

		1-1/1-2		*1-2/2-2*		*1-3/2-3*	
Gene symbol	*GenBank accession*	*Change ratio of microarray*	*Change ratio of real-time PCR*	*Change ratio of microarray*	*Change ratio of real-time PCR*	*Change ratio of microarray*	*change ratio of real-time PCR*
SLCO1A2	NM_021094	4	12.07	5.65	13.64	5.65	19.74
KCNJ15	NM_170736	3.73	3.99	4.28	10.44	3.24	5.01
ABCC1	NM_019862	12.99	15.53	21.11	18.83	45.25	36.49
WNT5A	NM_003392	3.03	3.76	3.73	5.04	3.48	4.53
PTN	NM_002825	5.65	5.43	4.59	9.95	4	6.67
SLC13A5	NM_177550	8	9.34	2	3.23	3.48	4.9
GalNAcT13	NM_052917	0.16	0.04	0.08	0.09	0.16	0.28
UGT2B28	NM_053039	0.06	0.007	0.01	0.3	0.09	0.01
CFTR	NM_000492	0.14	0.22	0.23	0.16	0.08	0.15

GalNAc-T13 and UGT2B28. The real-time PCR results confirmed the microarray data (Table 7, Figure 3).

Discussion

In the present study, we compared the gene expression profiles of normal human parotid and submandibular glands using microarrays. The tumor-associated gene ABCC1 was found to be upregulated at least twofold in the parotid gland compared with the submandibular gland, and this result was confirmed by real-time PCR. ABCC1 [ATP-binding cassette, sub-family C (CFTR/MRP), member 1] is a transporter gene associated with multidrug resistance of tumors (Cole *et al*, 1992). Pleiotrophin (PTN) and WNT5A were also found to be upregulated at least twofold in the parotid gland compared with the submandibular gland by both microarray and real-time PCR. The protein encoded by the PTN gene belongs to a recently described family of heparin-binding cytokines whose expression is temporally and spatially regulated during development. PTN protein induces growth, angiogenesis, differentiation, and transformation of cells (Milner *et al*, 1992; Kadomatsu and Muramatsu, 2004). PTN has the potential to regulate NIH 3T3 cell growth and may influence abnormal cell growth *in vivo*; thus, PTN is considered an oncogene (Chauhan *et al*, 1993). WNT5A is a member of the wingless-type MMTV integration site family, and WNT5A mRNA is highly expressed in the salivary gland, bladder, uterus, placenta, and fetal kidney. Upregulation of WNT5A mRNA was also detected in primary gastric cancer by cDNA-PCR.

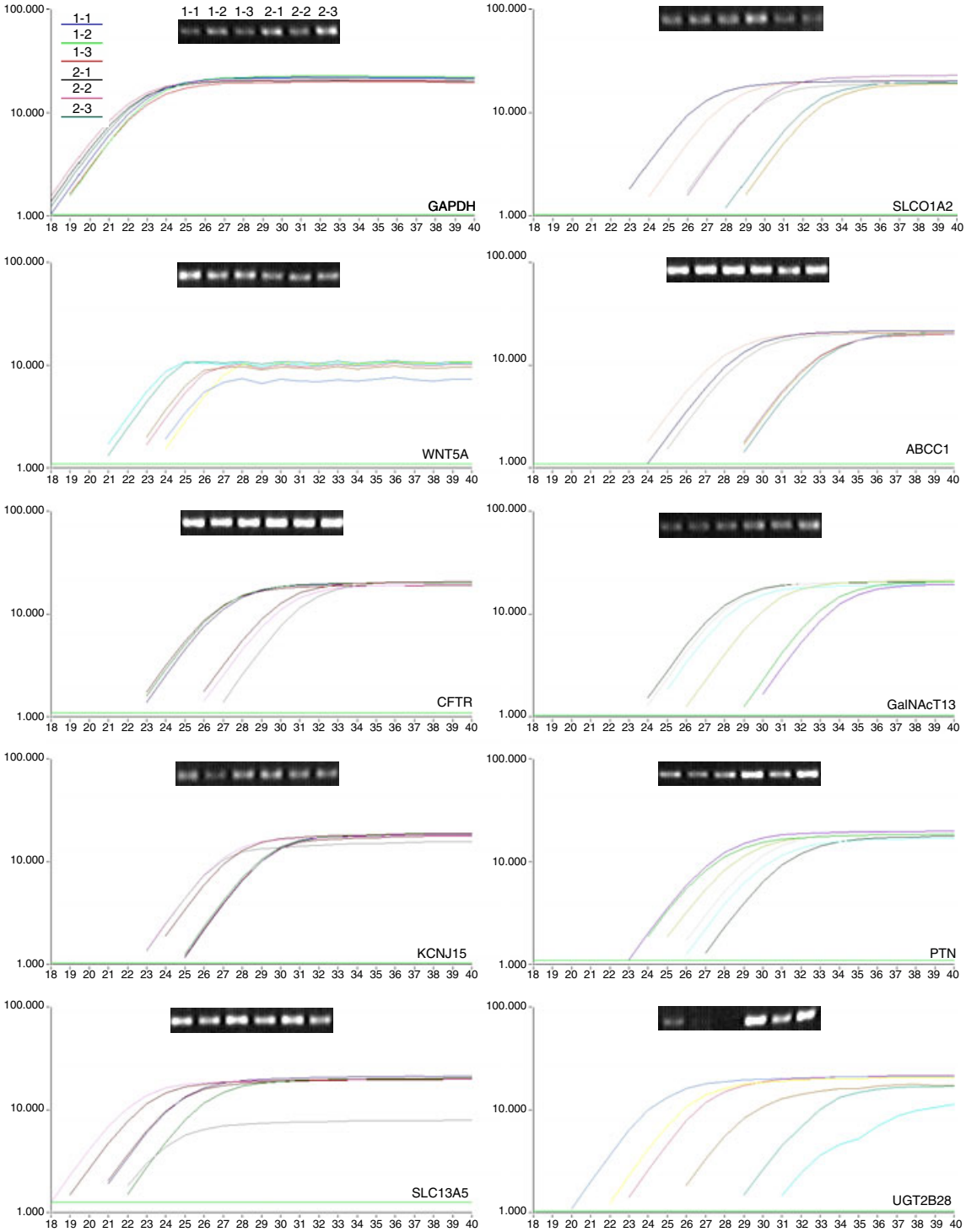

Figure 3 Real-timePCR of differentially expressed genes between normal human parotid and submandibular glands. Real-time PCR analyses of pleiotrophin, WNT5A, ABCC1, SLCO1A2, SLC13A5, KCNJ15, CFTR, GalNAc-T13, and UGT2B28 expression confirmed the microarray data, and the magnitudes of differential gene expression were similar in both approaches

Frequent upregulation of WNT5A mRNA in human primary gastric cancer might be due to cancer–stromal interactions (Saitoh *et al*, 2002). WNT5A was found to be highly expressed in malignant human neuroblasts. Furthermore, mutations in WNT5A that resulted in the inappropriate activation of the WNT signaling pathway were found in adenoid cystic carcinoma of salivary glands, leading to tumorigenesis (Daa *et al*, 2004; Blanc *et al*, 2005). The parotids are the salivary glands most often affected by tumors. Approximately 80% of

salivary tumors are diagnosed in a parotid gland, 10% are diagnosed in a submandibular gland, and the remaining 10% are diagnosed in other salivary glands (Ito *et al*, 2005). The findings of upregulated PTN and WNT5A in the parotid gland may be associated with the higher incidence of tumors in the parotid gland than in the submandibular gland.

This study showed that the mucins MUC7 and MUC5B were highly expressed in the submandibular gland compared with the parotid gland, in accordance with another report (Alos *et al*, 2005). We also found that mucin-associated genes that belong to the starch and sucrose metabolism pathway (GalNAc-T4, GalNAc-T7, GalNAc-T13, and UGT2B28) were highly expressed in the submandibular gland compared with the parotid gland. GalNAc-T13 and MUC5B were previously shown to be co-expressed in salivary glands, but there are few reports of expression of other genes, including the newly isolated and characterized gene UGT2B28 (Levesque *et al*, 2001), in salivary glands. Human saliva has been shown to contain at least two structurally and functionally distinct populations of mucins, the high-molecular-weight ($M_r > 10^6$ Da), oligomeric, gel-forming MG1 population and the lower-molecular-weight (M_r $1.2–1.5 \times 10^5$ Da), monomeric MG2 mucins (Wu *et al*, 1994; Van den Steen *et al*, 1998; Thornton *et al*, 1999). The polypeptide chain of the MG2 population has been identified as the product of the MUC7 gene (Bobek *et al*, 1993). Other data indicate that the MUC5B gene product is a major constituent of MG1 (Thornton *et al*, 1997). The submandibular glands containing mucous cells (producing MG1) and serous cells (producing MG2) secrete 30% of the salivary mucins (Nielsen *et al*, 1996). Human salivary mucins have O-linked (MG1 and MG2) and N-linked (MG2) oligosaccharide chains. Glycosylation of certain acceptor sites by one of the GalNAc polypeptide transferases is required before other sites can be glycosylated by another GalNAc polypeptide transferase, so there must be interactions between different GalNAc polypeptide transferases in the biosynthesis of human salivary mucins (Wu *et al*, 1994; Zhang *et al*, 2003). Further study of mucin-associated genes highly expressed in the submandibular gland is needed to identify the functions and interactions of those proteins in the biosynthesis of human salivary mucins.

Chloride ion transport through salivary gland cells was proposed to be the driving force of saliva secretion. Chloride ions are absorbed by the basal membranes of cells and then secreted into the lumen of the gland by acini, or reversely absorbed from lumen into cells in ducts. Chloride channels comprise an important chloride transporting system in the salivary glands, especially the CFTR chloride channel, which has been found to be expressed mainly in salivary gland ducts (Melvin, 1991; Zeng *et al*, 1997). In this study, we found that CFTR was more highly expressed in the submandibular gland than in the parotid gland, as determined by both microarray and real-time PCR analyses. CFTR may play an important role in the ductal modification of submandibular saliva, and there is differential ductal modification of saliva between parotid and submandibular glands. Potassium is another important electrolyte in saliva, and it is mainly secreted into saliva in ducts by potassium channels (Warth, 2003). An inward rectifier potassium channel gene KCNJ15 (Gosset *et al*, 1997) was found to be more highly expressed in the parotid gland than in the submandibular gland. Although the function of KCNJ15 in the parotid gland is unclear, the differential expression profile of KCNJ15 between the major salivary glands may indicate different modes of potassium transport in parotid and submandibular glands. Two members of the solute carrier organic anion transporter family, SLCO1A2 and SLC13A5, were more highly expressed in the parotid gland than in the submandibular gland. SLCO1A2 mediates the Na^+-independent transport of organic anions such as sulfobromophthalein and conjugated (taurocholate) and unconjugated (cholate) bile acids (Lee *et al*, 2005), whereas SLC13A5 is a sodium-coupled citrate transporter (NaCT) (Inoue *et al*, 2002). There are currently no reports regarding these proteins in parotid glands.

In summary, the present study demonstrates differential gene expression profiles between normal parotid and submandibular glands of human. These different gene expression profiles may provide a genetic basis for the different properties of parotid and submandibular glands.

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