Identification of Differentially Expressed Genes in Human Salivary Gland Tumors by DNA Microarrays[1]

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Abstract

Differentially expressed genes among different benign and malignant salivary gland tumors were identified by use of cDNA microarrays containing 19,000 human expressed sequence tags. Tumors were classified by using a subset of 486 genes. Benign Warthin's tumor and pleomorphic adenoma showed very distinctive gene expression patterns. One hundred and thirty-three genes differentiated the single malignant clear cell carcinoma from non-tumor salivary glands ($P < 0.01$), whereas only 16 genes separated it from the highly related benign pleomorphic adenoma ($P < 0.01$). Fifty-seven cDNAs were associated with mucoepidermoid carcinoma ($P < 0.01$). The identified genes might help to disclose the molecular mechanisms and processes underlying malignant salivary gland tumors.

Introduction

Pleomorphic adenoma is the most common benign tumor of major salivary glands. It shows a marked histological diversity with epithelial, myoepithelial, and mesenchymal components in a variety of patterns (1). Cytogenetic analysis have recognized, in addition to a subgroup with normal karyotypes, recurrent chromosomal translocations, with breakpoints at 8q12, 3p21, and 12q13–15 (2) and corresponding to *PLAG1* (3), *β-catenin* (*CTNNB1*; Ref. 4), and *HMGIC* (5) genes, respectively. The second most common benign lesion of the salivary glands is Warthin's tumor. It consists of abundant lymphoid stroma and bilayered oncocytic epithelium that forms cystic or papillary structures. The epithelial component represents the neoplastic proliferation of salivary ducts that have been entrapped within lymph nodes associated with the salivary gland.

Mucoepidermoid carcinoma is the most common malignant, locally invasive tumor of the salivary glands, especially of the parotid gland. Some markers of tumor progression, invasiveness, and prognosis are p21(Kip1), a cyclin-dependent kinase inhibitor, the oncoproteins Bcl-2 and Bax, the tumor suppressor gene product p53, terminal deoxynucleotidyltransferase-mediated nick end labeling staining, and the cell cycle antigen Ki-67 (6, 7). A rare form of malignant tumor, arising in pleomorphic adenoma of the salivary glands, is clear cell carcinoma. This tumor shows definite areas characteristic of pleomorphic adenoma combined with areas revealing evidence of malignancy.

The emerging technology of cDNA microarrays provides the ability to comparatively analyze mRNA expression of thousands of genes in parallel (8). Several studies have already demonstrated the usefulness of this technique for identifying novel cancer-related genes and for classifying human cancer at the molecular level (9–11). In this study, we have used a cDNA microarray containing 19,200 different human probes corresponding to different Unigene clusters to characterize benign and malignant salivary gland tumors. The main aim of the study was to identify gene expression alterations that are either common to most of the tumors studied or specific to clinical features of the tumors.

Materials and Methods

Patients and Tissue Specimens. Samples were derived from surgically resected parotid tissue from five patients with pleomorphic adenoma, four with Warthin's tumor, one with clear cell carcinoma, and two with mucoepidermoid carcinoma. Informed consent was obtained from patients to use their operated specimens for research purposes. Part of each sample was frozen in liquid nitrogen and stored at −80°C until RNA extraction, and the remaining part was used for histopathological analysis. Tumor paired control non-tumor tissues were obtained from a clinically unaffected site and were histologically normal.

Microarrays Assays. Total RNA was extracted from the biopsies by using RNAzol. Ten μg of total RNA were used for each sample. cDNA was synthesized by using Superscript II (Life Technologies, Inc.) and amino-allyl dUTP (Sigma). Monoreactive Cy3 and Cy5 esters (Amersham Pharmacia) were used for indirect cDNA labeling. A pool of eight non-tumor salivary glands RNA was labeled with Cy3 and used as control against the Cy5-labeled tumor cDNA. Human 19.2 K

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DNA microarrays, containing ESTs[3] corresponding to at least 15,448 different Unigene clusters, were used (Ontario Cancer Institute).[4] One hundred μl of the sample and control cDNA in DIG Easy hybridization solution (Roche) were used in a sandwich hybridization of the two slides constituting the 19.2 K set at 37°C overnight. Washing was performed three times for 10 min with 1× SSC, 0.1% SDS at 42°C and three times for 5 min with 0.1× SSC at room temperature. Slides were dried by centrifugation for 2 min at 2000 rpm. Hybridized slides were scanned using the GenePix 4000A scanner (Axon Instruments, Foster City, CA). Arabidopsis RNA was used as a reference for RNA labeling.

Images were analyzed by using GENEPIX PRO 3.0 (Axon Instruments). Spots showing no signal or obvious defects were accordingly flagged by GENEPIX PRO and excluded from the analysis. Local background was subtracted from the remaining spots, and then spots with fluorescence intensity in both channels of <1000 were stringently flagged as absent. The background level for a typical array was of 67.5 (SD, 145.2) for the Cy5 channel and of 182.7 (SD, 305.4) for Cy3 channel. Maximum fluorescence intensity is at about 65,500 in both channels, and arrays were scanned ensuring that the <1% of the total number of spots measured on the slide had median intensities close to maximal. Ratios of net fluorescence from the Cy5-specific channel to the net fluorescence from the Cy3-specific channel were calculated for each spot, representing tumor mRNA expression relative to the corresponding non-tumor salivary gland tissue. A normalization factor was estimated from ratios of median by GENEPIX PRO. Cy5:Cy3 expression ratios were then log-transformed (base 2) and normalized, for each different array, by adding the $\log_2$ of the respective normalization factor to the $\log_2$ of the ratio of medians for each spot within the array, so that the average log-transformed ratio equaled zero. The threshold for significant RNA expression changes (3.0-fold; *i.e.,* ~1.5 on the $\log_2$ scale) was established as three times the SD of an assay, where the same RNA sample was independently retrotranscribed and labeled with both cyanines. DNA spots present in at least 75% of the arrays (5,681 spotted cDNAs) and with expression ratios higher than the above-defined threshold, in at least one array, were selected for the clustering analysis (4,434 spotted cDNAs).

To select the probes that distinguish between two sample groups (*e.g.,* pleomorphic adenoma *versus* Warthin's tumor), we developed a program performing two statistical tests, *t* test and log likelihood. Log likelihood was defined as the difference between the mean of each probe in the two groups. To perform multiple *t* testing correction, the Benjamini and Hochberg procedure, as modified by Storey and Tibshirani (12), was applied. Briefly, it controls the pFDR, defined as the proportion of probes expected to be identified by chance (assuming probes are independent) relative to the total number of probes called significant. This procedure provides a good balance between discovery of significant spots and protection against false positives, because occurrence of the latter is held to a small proportion of the list. A balanced bootstrap analysis with 10,000 permutations was therefore applied to the tests to define the number of expected false positives by chance. Expression tables were analyzed by using hierarchical clustering, PCA, and similarity searches tools in JExpress v.2.1.[5] Variance normalization across the expression table was performed only prior to similarity search to identify probes with similar expression pattern and was obtained by normalizing the mean and then multiplying each vector by a scalar so that its variance becomes unity.

Results and Discussion

We compared the expression profiles of 12 salivary gland tumors and four normal tissue samples by hybridization to microarrays containing 19,200 cDNAs. Because the arrays shared a common normal tissue reference made of a pool of eight non-tumor salivary glands different from those used as test samples, the samples could be compared to identify gene expression patterns correlated with clinical features of the tumors.

Shared Expression Alterations in Salivary Gland Tumors. We used hierarchical clustering (13) to look at the variation in gene expression among tumors. A clustering analysis was performed by using the selected 4,434 spotted cDNAs, as detailed in "Materials and Methods," corresponding to 2,333 distinct Unigene clusters present in 75% of the arrays and with Cy5:Cy3 expression ratios that varied at least 3.0-fold in at least one array. By using the *t* test, we then sought to identify gene expression alterations differentiating the tumors from normal samples. *t* test selected 713 spotted cDNA probes ($P < 0.01$, average *t* test significant spots in bootstrap, 91.82; pFDR, 0.05) corresponding to 486 distinct Unigene clusters. The clustering based on the 486-probe set very well correlated with the tumors histotypes, with the sole exception of the mucoepidermoid carcinoma (Fig. 1). Warthin's tumors were the most distant branch, with the non-tumor salivary glands in a separate branch from that of the pleomorphic adenoma. The single case of cell carcinoma, arisen from pleomorphic tumor, correctly clustered within the pleomorphic adenoma group. Table 1 lists differentially expressed cancer-associated genes with the most highly significant *t* test values.[6] The list of up- and down-regulated genes in most salivary gland tumors include known genes involved in neoplasia, members of signal transduction pathways, transcription factors, oncogenes, and tumor suppressor genes, cell cycle regulator, and genes that encode protein involved in cellular metabolism and adhesion. Among up-regulated transcription factors are present forkhead box O1A (*FOXO1A*) and *PAX1.* Fusion of forkhead box O1A with *PAX3,* a *PAX1*-related gene, is associated with solid tumor rhabdomyosarcoma. Overexpression of the fusion protein

[3] The abbreviations used are: EST, expressed sequence tag; pFDR, positive false discovery rate.
[4] Internet address: http://www.uhnres.utoronto.ca/services/microarray/.
[5] Internet address: www.molmine.com.
[6] Additional tables regarding the differentially expressed genes in the different histotypes can be found at the http://biotecnologie.unife.it/microarrays/salivary website.

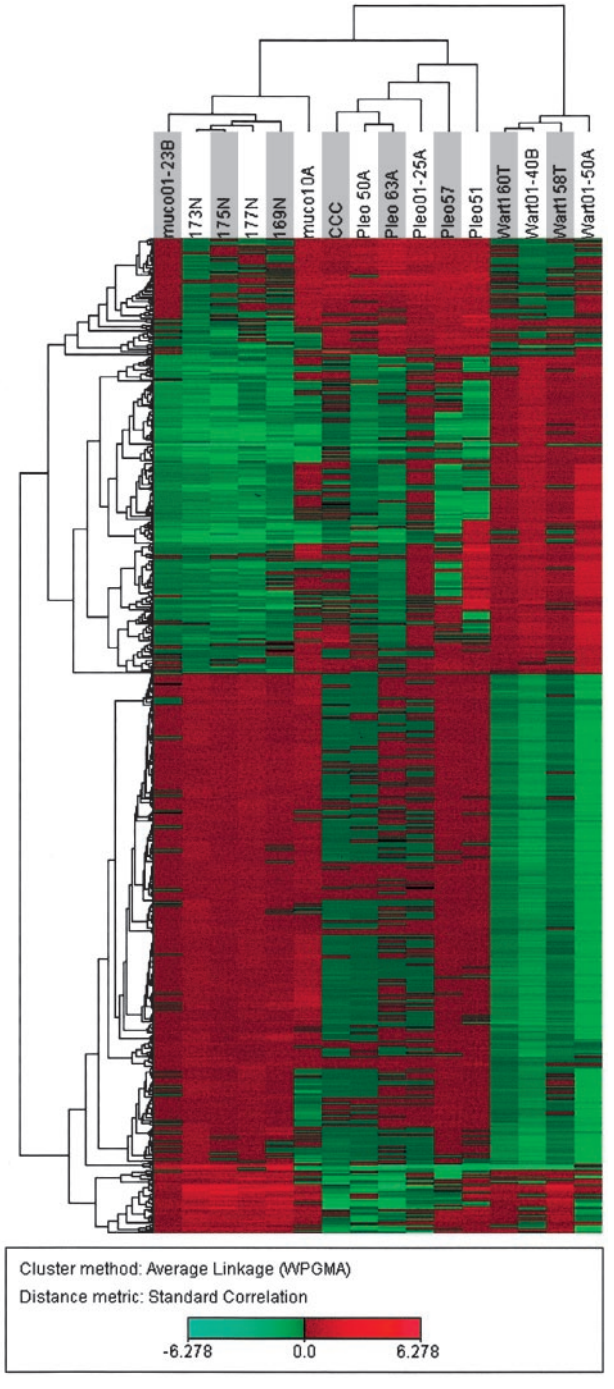

Fig. 1. Clustering of 713 spotted cDNA probes corresponding to 486 Unigene clusters, selected by *t* test, that were differentially expressed in salivary gland tumors ($P < 0.01$, average *t* test significant spots in bootstrap, 80.4; pFDR, 0.04). *Rows,* cDNA probes; *columns,* individual patient samples. *Red,* probe overexpression; *green,* underexpression.

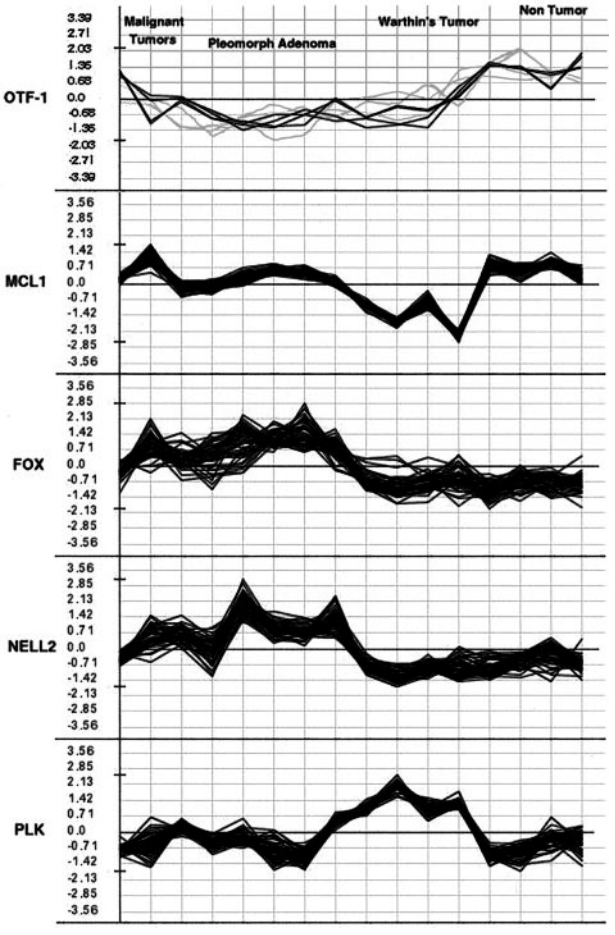

Fig. 2. Clones with expression patterns coherent to prominent tumor-associated genes, selected by using similarity search with Euclidean distances on the variance normalized expression patterns.

actives the transcription of antiapoptotic protein Bcl-XL (14), capable of suppressing cell death pathways by blocking the release of cytochrome *c* from mitochondria and the caspase protease cascade. By using a similarity search with Euclidean distances on the variance normalized expression table, as obtained by using the correspondent JExpress plugin, we identified genes with coherent expression patterns to those of some genes selected by the *t* test (Fig. 2). Interestingly, among gene subsets that display expression patterns coherent with *FOXO1A* there are the *PAX1*, homologous to the FOX fused gene above described, a novel Bcl-2 homologue (*MIL1* or *Bcl*-rambo), and the 7b and 7c subunits of cytochrome *c* oxidase (*COX7B*, *COX6C*). Cytochrome *c* oxidase down-regulation, alongside to cytochrome *c* release to the cytosol, was found to be present in Bax-induced growth arrest (15). Overrepresentation of this gene subset suggests that the activation of mechanisms that block apoptosis is a major characteristic of the tumor progression in salivary glands.

One of the very few genes consistently different in every tumor is *OTF1,* highly expressed in non-tumor salivary glands and underexpressed in all tumors. OTF1 protein binds specifically its POU domain to octamer DNA motifs present in the promoters of several genes and regulates their expression. In addition, OTF1 participates in the cellular response to DNA damage and is involved in the regulation of stress-inducible genes (16). By using semiquantitative reverse tran-

Table 1 Differentially expressed genes in salivary gland tumors

After *t* test selection, as explained in "Results and Discussion," the gene graphs were individually confirmed by using JExpress. In this table, a selection of genes with the most highly significant *t* test values is reported. The complete tables can be found at the web site.

Clone	Gene bank	Name	Sample groups[a]	*t* test
		Genes up-regulated in salivary gland tumors		
279762	N48348	*CEBPD*	pleo *vs.* wart	14.86
297460	W03635	*RER1*	pleo *vs.* wart	14.4
298792	W05091	*KIAA0105*	pleo *vs.* wart	12.72
259563	N41755	*MYO7A*	pleo *vs.* nt	11.96
320698	W32039	*DJ37E16.5*	pleo *vs.* nt	11.41
309842	N94626	*SNRPD2*	pleo *vs.* wart	11.15
280943	N47576	*TSLP*	pleo *vs.* wart	10.71
469566	AA027104	*SLC3A1*	pleo *vs.* wart	10.53
281588	N47992	*MTP*	pleo *vs.* wart	10.42
114869	T87395	*C5*	pleo *vs.* wart	10.37
154604	R55493	*PLVAP*	pleo *vs.* nt	10.2
322111	W37664	*SLC25A20*	pleo *vs.* wart	10.04
286970	N67453	*CDKN1A*	pleo *vs.* wart	9.92
285638	N66473	*HNRPA3*	pleo *vs.* nt	9.82
109931	T84450	*LOC51651*	wart *vs.* nt	9.59
112468	T85888	*DKFZP564O043*	pleo *vs.* nt	9.46
32194	R17332	*KIAA0564*	pleo *vs.* nt	9.07
297460	W03635	*RER1*	pleo *vs.* nt	8.89
322111	W37664	*SLC25A20*	pleo *vs.* nt	8.08
114869	T87395	*C5*	pleo *vs.* nt	7.95
503523	AA131279	*LMOD1*	pleo *vs.* nt	7.69
212698	H69644	*LOC51266*	wart *vs.* nt	7.49
27679	R13006	*GDA*	wart *vs.* nt	7.44
162251	H25971	*DKFZp434G171*	wart *vs.* nt	7.33
195560	R91819	*PIK3R2*	wart *vs.* nt	7.11
116851	T93608	*STAM2*	wart *vs.* nt	6.93
146355	R79518	*MCAM*	wart *vs.* nt	6.9
41067	R56098	*FREQ*	wart *vs.* nt	6.8
207669	H62271	*LGP1*	wart *vs.* nt	6.68
109863	T88721	*EMP2*	pleo *vs.* wart	5.74
271009	N42817	*COX6C*	pleo *vs.* nt	5.72
321607	W32908	*FOXO1A*	at *vs.* nt	5.6
308119	N92360	*NFATC3*	wart *vs.* nt	5.5
180257	R84726	*ADORA1*	pleo *vs.* wart	5.48
201990	R99333	*KIAA1733*	at *vs.* nt	5.23
501817	AA127953	*HHEX*	at *vs.* nt	5.18
116844	T93783	*FLJ13605*	wart *vs.* nt	5.04
278649	N66214	*NPEPPS*	at *vs.* nt	4.91
321269	W52940	*MIL1*	at *vs.* nt	4.75
446261	AA203732	*CHP*	at *vs.* nt	4.7
358848	W94646	*ITGAE*	wart *vs.* nt	4.24
109863	T88721	*EP2*	pleo *vs.* nt	4.16
		Genes down-regulated in salivary gland tumors		
42553	R61223	*CAMK2G*	wart *vs.* nt	−12.13
242058	H93330	*SLC9A3R1*	wart *vs.* nt	−11.59
152489	R62399	*MGC11352*	wart *vs.* nt	−9.43
33862	R19859	*EST*	pleo *vs.* wart	−8.84
46743	H10327	*RAP1B*	wart *vs.* nt	−8.74
132026	R24904	*PP1665*	wart *vs.* nt	−8.64
29961	R14696	*FLJ12438*	wart *vs.* nt	−8.52
111650	T84578	*CDC37*	wart *vs.* nt	−8.16
133326	R26844	*EST*	wart *vs.* nt	−8.13
265082	N30528	*PPARD*	wart *vs.* nt	−8.11
143341	R74281	*PPP4C*	wart *vs.* nt	−8.06
289002	N59818	*HRIHFB2436*	wart *vs.* nt	−8.04
33862	R19859	*EST*	pleo *vs.* nt	−7.98
148698	H12850	*EST*	pleo *vs.* nt	−7.97
129794	R16987	*SEC8*	wart *vs.* nt	−7.94
207992	H60458	*ACOX2*	pleo *vs.* wart	−7.64
488555	AA047136	*EST*	pleo *vs.* wart	−7.56
49961	H29294	*FLJ20364*	pleo *vs.* wart	−7.54

[a] at, all tumors; nt, non-tumor; pleo, pleomorphic adenoma; wart, Warth's tumor.

Table 1 Continued

After *t* test selection, as explained in "Results and Discussion," the gene graphs were individually confirmed by using JExpress. In this table, a selection of genes with the most highly significant *t* test values is reported. The complete tables can be found at the web site.

Clone	Gene bank	Name	Sample groups[a]	*t* test
		Genes up-regulated in salivary gland tumors		
36318	R21064	*KIAA0118*	pleo *vs.* nt	−7.52
241224	H91140	*FLJ10079*	pleo *vs.* wart	−7.32
66513	T66987	*EST*	pleo *vs.* wart	−7.28
39241	R51610	*EST*	pleo *vs.* nt	−7.14
136872	R36627	*11-Oct*	pleo *vs.* wart	−7.06
195560	R91819	*PIK3R2*	pleo *vs.* wart	−7.05
236338	H61357	*TP53*	pleo *vs.* wart	−7.01
132550	R25899	*HNRPD*	pleo *vs.* nt	−6.87
130426	R21806	*EST*	pleo *vs.* nt	−6.86
129794	R16987	*SEC8*	pleo *vs.* nt	−6.65
380794	AA054151	*OXA1L*	pleo *vs.* nt	−6.41
146858	R80974	*WEE1*	wart *vs.* nt	−6.26
178468	H47026	*MGAT3*	pleo *vs.* nt	−6.21
140878	R67235	*BAT1*	pleo *vs.* nt	−6.17
286050	N64281	*EST*	at *vs.* nt	−6.01
180257	R84726	*ADORA1*	wart *vs.* nt	−5.73
150673	H02088	*EST*	at *vs.* nt	−5.57
36318	R21064	*KIAA0118*	at *vs.* nt	−5.52
152489	R62399	*MGC11352*	at *vs.* nt	−5.49
46743	H10327	*RAP1B*	at *vs.* nt	−5.41
178468	H47026	*MGAT3*	at *vs.* nt	−5.36
380794	AA054151	*OXA1L*	at *vs.* nt	−5.35
375693	AA033736	*AD037*	at *vs.* nt	−5.28
179383	H50403	*FLJ20793*	at *vs.* nt	−5.19
134887	R32270	*EMP2*	wart *vs.* nt	−4.61
147839	R81839	*TXK*	at *vs.* nt	−3.16

[a] at, all tumors; nt, non-tumor; pleo, pleomorphic adenoma; wart, Warth's tumor.

scription, we confirmed the expression levels of *OTF1*, *Wee1*, and *EMP2* (data not shown). Additionally, we identified a number of up- and down-regulated ESTs, which suggest the involvement of a large number of genes that are not yet functionally characterized in salivary gland tumors.

Differential Expression between Warthin's Tumor and Pleomorphic Adenoma. Defined subsets of clones that most strongly defined the division of biopsies by histotype were identified by using the *t* test. When we applied this test to compare each tumor to non-tumor samples, we selected 599 cDNA elements differentially expressed ($P < 0.01$, average *t* test significant spots in bootstrap, 49.95; pFDR, 0.04) in pleomorphic adenoma and 2586 elements ($P < 0.01$, average *t* test significant spots in bootstrap, 5.73; pFDR, <0.001) specifically expressed in Warthin's tumors. The many clones associated with Warthin's tumor is probably related to the presence of lymphoid stroma in the affected glands, because some of the selected genes are T-cell specific (*i.e., NFATC3* and *ITGAE,* an integrin preferentially expressed on intraepithelial lymphocytes). One thousand and one hundred ninety-eight clones were differentially expressed ($P < 0.001$, average *t* test significant spots in bootstrap, 0.13; pFDR, <0.001) in pleomorphic adenoma when compared with Warthin's tumor, of which 479 are overexpressed in Warthin's, whereas 719 are overexpressed in pleomorphic adenoma. The specificity of clones expression is detailed in Table 1. *Plag1,* a gene that is often rearranged in pleomorphic tumor, is surprisingly significantly overexpressed in Warthin's tumor ($P < 0.01$). In the *Plag1* expression pattern group (Fig. 2), other genes present are: *PLK1,* progranulin, which elevated expression confers a transformed phenotype on epithelial cells, including anchorage independence *in vitro* and growth as tumors in nude mice (17); hypothetical protein FLJ13605 containing a tetratricopeptide repeat TPR domain, which forms scaffolds to mediate protein-protein interactions and often the assembly of multiprotein complexes and has a spatial arrangement of α-helices similar to those within 14-3-3 proteins; αL subunit of integrin leukocyte function antigen-1, a membrane glycoprotein that functions in cell-cell adhesion by heterophilic interaction with intercellular adhesion molecule 1; and polo-like serine/threonine kinase PLK1 and TXK, a member of the Tec nonreceptor subfamily of Src protein tyrosine kinases.

Expression Profiles of Malignant Tumors. Finally, we selected a subset of clones that most strongly defined the division between malignant and benign lesions. By using the log likelihood test, and as log2 threshold a value of 1.5, corresponding to 3× SD from the average, we identified 16 Unigene clusters differentiating this malignant clear cell carcinoma from its related benign pleomorphic adenoma ($P < 0.01$, as determined by bootstrap distribution). On the contrary, this clear cell carcinoma differed from the Warthin's tumors for a much larger number of cDNA elements (1,315), as expected for these two nonrelated tumors, whereas 133 elements were different from the non-tumor reference pool. Genes differentiating this clear cell carcinoma from its related

benign tumor pleomorphic adenoma include myelin transcription factor 1-like, huntingtin, ubiquitin specific protease 9, and *FLJ10355.*[7]

Because, as shown by hierarchical clustering (Fig. 1), Warthin's tumor was very divergent from the other biopsies, we then compared the mucoepidermoid carcinoma to all of the other samples after the exclusion of Warthin's tumor samples. By using the *t* test, we selected 57 cDNA probes ($P < 0.01$). Among relevant genes repressed in mucoepidermoid carcinoma, there is the *PSG11* member of the carcinoembryonic antigen family and two genes involved in apoptosis, *CIDEB* and *ADORA1*. *CIDEB* or cell death-inducing DFFA-like effector b, is involved in induction of apoptosis by DNA damage (18), and *ADORA1,* adenosine A1 receptor, is involved in induction of apoptosis by extracellular signals. Interestingly, these genes are repressed only in mucoepidermoid carcinoma. *FLJ10355,* which is overexpressed in the mucoepidermoid carcinomas, is also overexpressed in the other malignant tumor clear cell carcinoma and not expressed in the benign tumors. *FLJ10355* has some homology to *Bcl9,* a gene translocated in some B-cell malignancies with abnormalities of 1q21 (19) and with no recognizable protein motifs or significant homologies to any other known proteins.

In summary, the results show gene expression alterations common to all tumors and unique to each benign and malignant tumor. A larger epidemiological study is required to determine whether expression levels of the selected genes can be used as prognostic indicators or else additional genes need to be included in the analysis. Finally, the numerous *EST*s of unknown function we identified could also become useful as tumor markers and represent a set of novel tumor-associated genes.

References

1. Ellis, G., Auclar, P., and Gnepp, D. Surgical pathology of the salivary gland. Philadelphia: W. B. Saunders Co., 1991.

2. Bullerdiek, J., Wobst, G., Meyer-Bolte, K., Chilla, R., Haubrich, J., Thode, B., and Bartnitzke, S. Cytogenetic subtyping of 220 salivary gland pleomorphic adenomas: correlation to occurrence, histological subtype, and *in vitro* cellular behavior. Cancer Genet. Cytogenet., *65:* 27–31, 1993.

3. Astrom, A., Voz, M., Kas, K., Roijer, E., Wedell, B., Mandahl, N., Van de Ven, W., Mark, J., and Stenman, G. Conserved mechanism of PLAG1 activation in salivary gland tumors with and without chromosome 8q12 abnormalities: identification of SII as a new fusion partner gene. Cancer Res., *59:* 918–923, 1999.

4. Kas, K, Voz, M., Roijer E., Astrom, A., Meyen, E., Stenman, G., and Van de Ven, W. Promoter swapping between the genes for a novel zinc finger protein and B-catenin in pleiomorphic adenomas with t(3;8)(p21;q12) translocation. Nat. Genet., *15:* 170–174, 1997.

5. Schoenmakers, E. F., Wanschura, S., Mols, R., Bullerdiek, J., Van den Berghe. H., and Van de Ven, W. J. Recurrent rearrangements in the high mobility group protein gene, *HMGI-C,* in benign mesenchymal tumours. Nat. Genet., *10:* 436–444, 1995.

6. Choi, C. S., Choi, G., Jung, K. Y., Choi, J. O., and Chae, Y. S. Low expression of p27(Kip1) in advanced mucoepidermoid carcinomas of head and neck. Head Neck, *23:* 292–297, 2001.

7. Yin, H. F., Okada, N., and Takagi, M. Apoptosis and apoptotic-related factors in mucoepidermoid carcinoma of the oral minor salivary glands. Pathol. Int., *50:* 603–609, 2000.

8. Golub, T. R, Slonim, D. K., Tamayo, P., Huard, C., Gaasenbeek, M., Mesirov, J. P., Coller, H., Loh, M. L., Downing, J. R., Caligiuri, M. A., Bloomfield, C. D., and Lander, E. S. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science (Wash. DC), *286:* 531–537, 1999.

9. Bittner, M., Meltzer, P., Chen, Y., Jiang, Y., Seftor, E., Hendrix, M., Radmacher, M., Simon, R., Yakhini, Z., Ben-Dor, A., Sampas, N., Dougherty, E., Wang, E., Marincola, F., Gooden, C., Lueders, J., Glatfelter, A., Pollock, P., Carpten, J., Gillanders, E., Leja, D., Dietrich, K., Beaudry, C., Berens, M., Alberts, D., and Sondak, V. Molecular classification of cutaneous malignant melanoma by gene expression profiling. Nature (Lond.), *406:* 536–540, 2000.

10. Kahn, J., Simon, R., Bittner, M., Chen, Y., Leighton, S. B., Pohida, T., Smith, P. D., Jiang, Y., Gooden, G. C., Trent, J. M., and Meltzer, P. S. Gene expression profiling of alveolar rhabdomyosarcoma with cDNA microarrays. Cancer Res., *58:* 5009–5013, 1998.

11. Luo, J., Duggan, D. J., Chen, Y., Sauvageot, J., Ewing, C. M., Bittner, M. L., Trent, J. M., and Isaacs, W. B. Human prostate cancer and benign prostatic hyperplasia: molecular dissection by gene expression profiling. Cancer Res., *61:* 4683–4688, 2001.

12. Storey, J. D., and Tibshirani, R. Estimating the positive false discovery rate under dependence, with applications to DNA microarrays. Technical report number 2001–28, 2001. Stanford, CA: Department of Statistics, Stanford University, 2001.

13. Eisen, M. B., Spellman, P. T., Brown, P. O., and Botstein, D. Cluster analysis and display of genome-wide expression patterns. Proc. Natl. Acad. Sci. USA, *95:* 14863–14868, 1998.

14. Margue, C. M., Bernasconi, M., Barr, F. G., and Schafer, B. W. Transcriptional modulation of the anti-apoptotic protein BCL-XL by the paired box transcription factors PAX3 and PAX3/FKHR. Oncogene, *19:* 2921–2929, 2000.

15. Manon, S., Chaudhuri, B., and Guerin, M. Release of cytochrome *c* and decrease of cytochrome *c* oxidase in Bax-expressing yeast cells, and prevention of these effects by co-expression of Bcl-XL. FEBS Lett., *415:* 29–32, 1997.

16. Jin, S., Fan, F., Fan, W., Zhao, H., Tong, T., Blanck, P., Alomo, I., Rajasekaran, B., and Zhan, Q. Transcription factors Oct-1 and NF-YA regulate the p53-independent induction of the GADD45 following DNA damage. Oncogene, *20:* 2683–2690, 2001.

17. He, Z., and Bateman, A. Progranulin gene expression regulates epithelial cell growth and promotes tumor growth *in vivo*. Cancer Res., *59:* 3222–3229, 1999.

18. Chen, Z., Guo, K., Toh, S. Y., Zhou, Z., and Li, P. Mitochondria localization and dimerization are required for CIDE-B to induce apoptosis. J. Biol. Chem., *275:* 22619–22622, 2000.

19. Willis, T. G., Zalcberg, I. R., Coignet, L. J., Wlodarska, I., Stul, M., Jadayel, D. M., Bastard, C., Treleaven, J. G., Catovsky, D., Silva, M. L., and Dyer, M. J. Molecular cloning of translocation t(1;14)(q21;q32) defines a novel gene (*BCL9*) at chromosome 1q21. Blood, *91:* 1873–1881, 1998.

[7] See the http://biotecnologie.unife.it/microarrays/salivary website.