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(54) **METHODS AND KITS FOR MONITORING BARRETT'S METAPLASIA**

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(58) **Field of Classification Search** ..... None
See application file for complete search history.

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(57) **ABSTRACT**

Disclosed are methods and kits for assessing risk of progression of Barrett's esophagus to adenocarcinoma.

7 Claims, 3 Drawing Sheets

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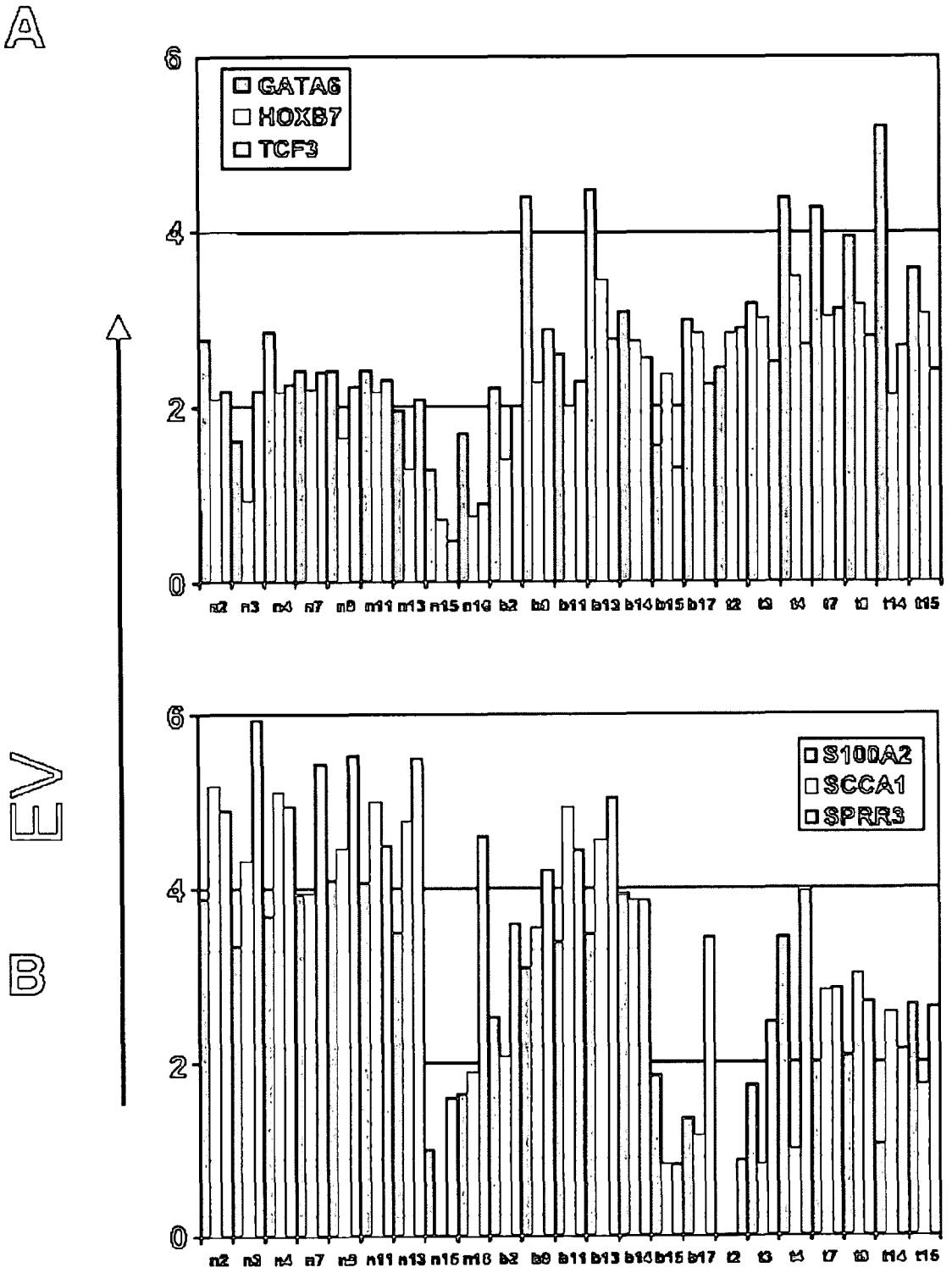
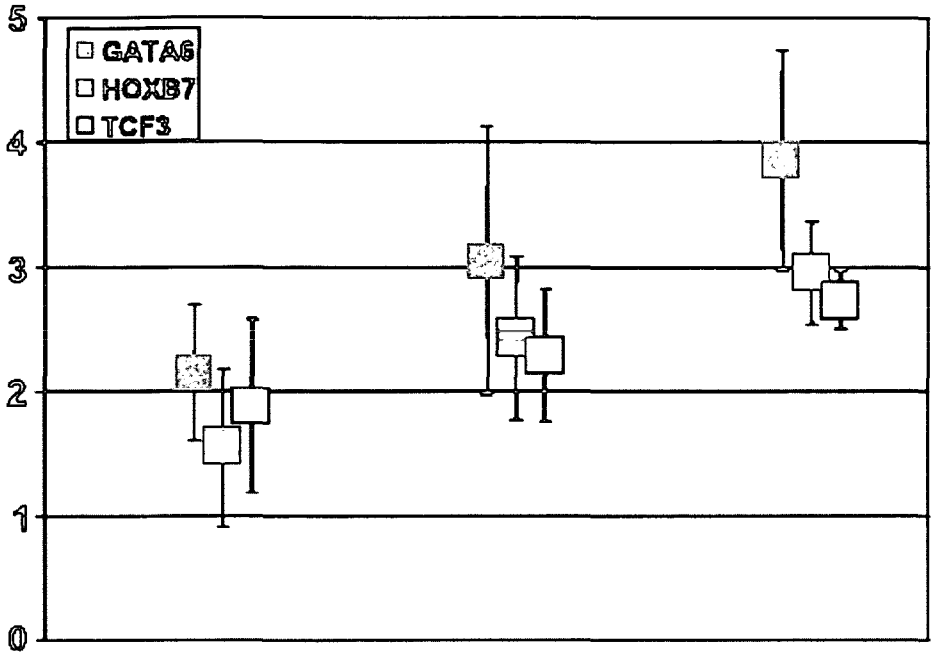
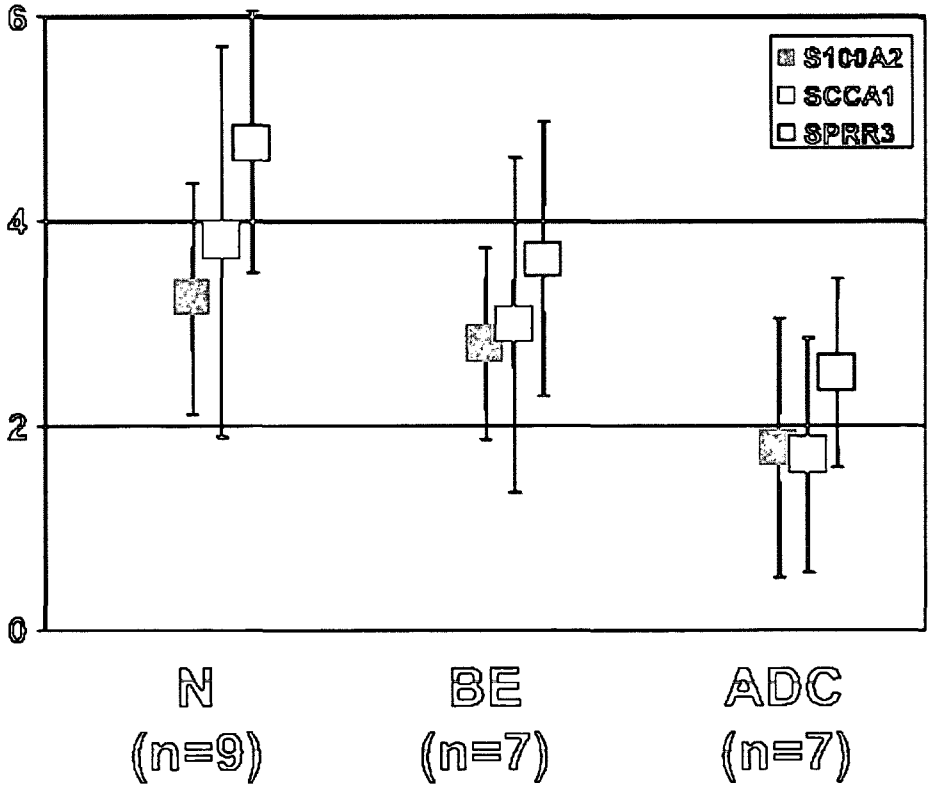


FIGS. 1A & B

C


GATA6
HOXB7
TCF3
5
4
3
2
1
0

D


S100A2
SCCA1
SPRR3
6
4
2
0
N
(n=9)
BE
(n=7)
ADC
(n=7)

FIGS. 1C & D

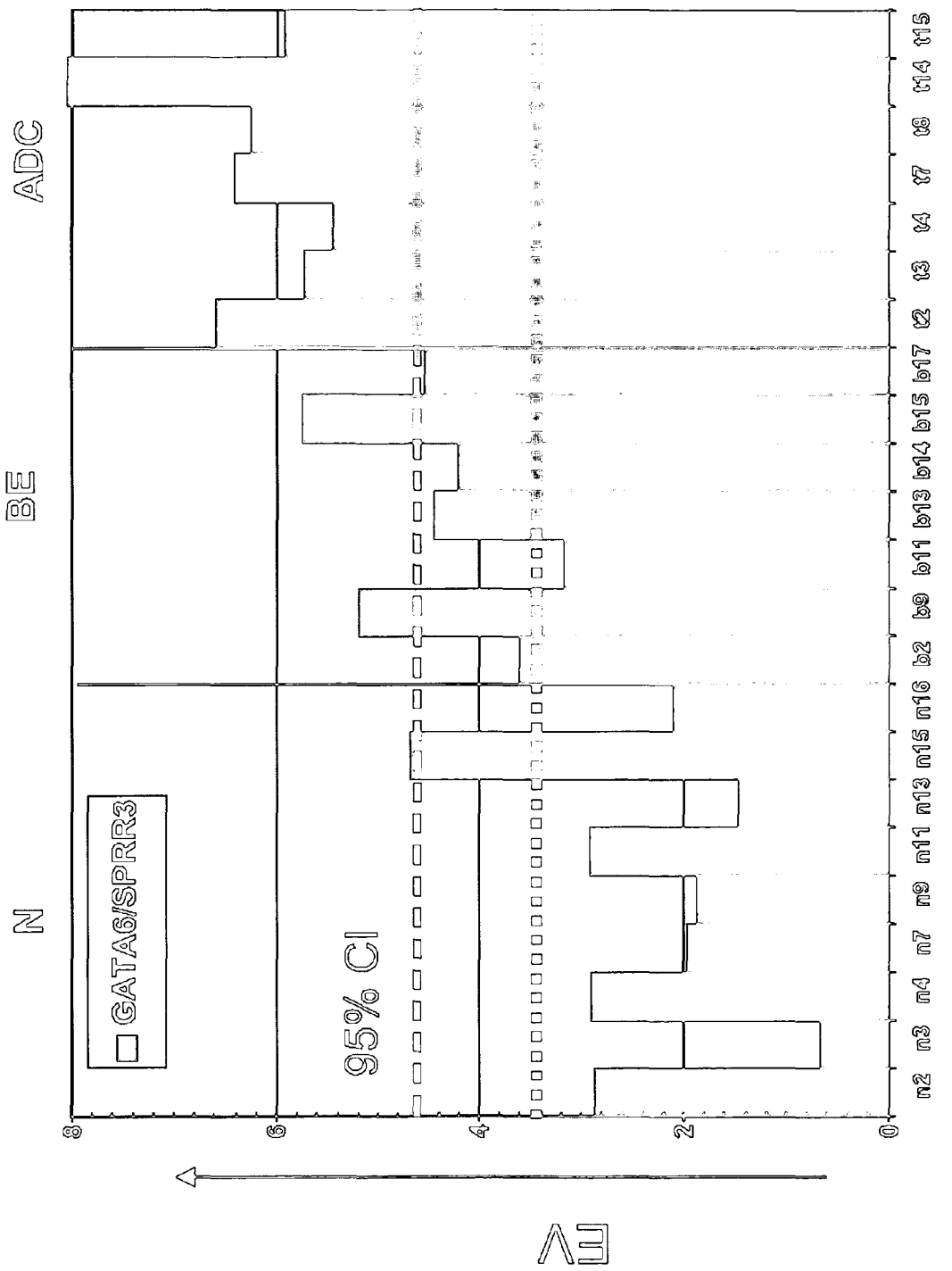

ADC
BE
N
GATA6/SPRR3
95% CI
EV
0
2
4
6
8
n2 n3 n4 n7 n9 n11 n13 n15 n16 b2 b9 b11 b13 b14 b15 b17 t2 t3 t4 t7 t8 t14 t15

FIG. 2

METHODS AND KITS FOR MONITORING BARRETT'S METAPLASIA

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/658,424, filed Mar. 2, 2005.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

The invention was made with U.S. Government support under Grant No. CA071933, awarded by The National Institutes of Health. The U.S. Government may have certain rights to this invention.

BACKGROUND OF THE INVENTION

Barrett's esophagus is a specialized intestinal metaplasia of normal squamous to columnar epithelium, which is thought to be a premalignant transformation and which is found in 80-100% of esophageal adenocarcinoma of the distal esophagus (1). The etiology of Barrett's esophagus is not well understood, but chronic gastroesophageal reflux is considered to be a major contributing factor (2). The presence of Barrett's esophagus increases the risk of developing adenocarcinoma 40 to 125-fold (3). The incidence of adenocarcinoma has increased 3.5-fold over the past 3 decades, which exceeds that of all other types of cancer (4, 5). Patients with adenocarcinomas of the esophagus present with advanced disease, and 5-year survival is approximately 25% (6). Currently, endoscopic surveillance is the only method of identifying patients with early-stage esophageal cancers arising in Barrett's esophagus.

Identification of biological markers of Barrett's esophagus progression may identify high risk patients for whom endoscopy would be indicated (8). Expressional profiling represents one method of identifying biological markers of Barrett's esophagus (9-12). However, no molecular markers that can be used to identify patients at higher risk for subsequent transformation of Barrett's esophagus to adenocarcinoma have been reported.

There exists a need in the art for new methods of evaluating the risk of progression of Barrett's esophagus to adenocarcinoma.

BRIEF SUMMARY OF THE INVENTION

In one aspect, the present invention provides methods of assessing risk of adenocarcinoma in a mammal with Barrett's esophagus. The method involves measuring the level of expression of at least two markers listed in Table 2 in a sample prepared from Barrett's esophageal cells. The level of expression in Barrett's esophageal cells is compared to that of a reference, a difference in the level of expression of a marker being indicative of increased risk of adenocarcinoma.

In another aspect, the invention provides kits for performing the methods of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**A-D shows plots of expression levels of markers as a function of sample type.

FIG. **2** shows a plot of the ratio of expression levels of two markers as a function of sample type.

DETAILED DESCRIPTION OF THE INVENTION

The Examples below describe the identification of molecular markers differentially expressed in normal esophageal epithelium, Barrett's esophagus, and esophageal adenocarcinoma. Measuring the level of expression of these markers allows discrimination between normal esophageal epithelium, Barrett's esophagus, and esophageal adenocarcinoma. Quantitation of these markers can be used to identify patients with Barrett's esophagus at increased risk for subsequent progression to adenocarcinoma.

DNA microarrays were used to evaluate differential gene expression patterns in resected esophageal specimens composed of normal esophageal epithelium, Barrett's esophagus, and adenocarcinoma obtained from the same individual patients. Based on this analysis, 96 genes that are differentially expressed in both Barrett's esophagus and adenocarcinoma were identified (Supplemental Table 2).

Of the 96 genes differentially expressed in Barrett's esophagus and adenocarcinoma, 21 genes (Table 2) were identified as being potentially useful for evaluating risk of progression from Barrett's esophagus to esophageal adenocarcinoma. These 21 genes were chosen because the change in expression is in the same direction (i.e., up-regulation or down-regulation) in both Barrett's esophagus and to esophageal adenocarcinoma, and because the change in expression is progressive from Barrett's esophagus to esophageal adenocarcinoma (i.e., the markers are up- or down-regulated to a greater degree in esophageal adenocarcinoma than in Barrett's esophagus). Because the change in expression from Barrett's esophagus to esophageal adenocarcinoma is progressive, is reasonably expected that the markers can be used to monitor progression from Barrett's esophagus to esophageal adenocarcinoma.

Of the 21 genes, six selected genes (GATA6, HOXB7, TCF3, S100A2, SCCA1 and SPRR3) were further evaluated. The level of expression of these genes, as measured by quantitative reverse transcription-PCR (QRT-PCR), discriminated between normal epithelium, Barrett's dysplasia and esophageal adenocarcinomas. It is possible to discriminate between normal epithelium and Barrett's esophagus or esophageal adenocarcinomas using any one of the 21 markers. Analysis of two or more markers permits discrimination Barrett's esophagus and esophageal adenocarcinomas. In the Examples, expression levels of GATA6/SPRR3, HOXB7/SPRR3, and GAT6/HOXB7/SPRR3 were evaluated and found to discriminate between Barrett's esophagus and esophageal adenocarcinomas. Additionally, it is specifically envisioned that any combination of two or more of the 21 markers provided in Table 2 will be useful in the methods of the invention. The markers may be analyzed individually or together in a multiplex.

In the methods of the invention, the level of gene expression was performed by indirectly measuring the mRNA by quantitative PCR, as described in the Examples. It is envisioned that mRNA, or cDNA prepared from mRNA, could be quantified through standard hybridization techniques using an oligonucleotide complementary to at least a portion of the mRNA or cDNA. Alternatively, the level of gene expression could be assayed using antibody detection methods and an antibody specific for an epitope of one of the gene products (i.e., mRNA or protein) of the 21 markers.

In the Examples, gene expression was evaluated by comparing expression levels of the 21 markers in normal esophageal epithelium, cells characteristic of Barrett's esophagus, and esophageal adenocarcinoma using resected esophagus samples. It is envisioned that any sample containing cells

characteristic of Barrett's esophagus could be used. For example, such cells may be obtained by an esophageal lavage, or scraping or biopsying a portion of the esophagus during endoscopy.

Marker expression levels in Barrett's esophagus can be evaluated by comparison to a reference. The reference may be normal esophageal epithelium obtained from the same individual, at the same time or at a different time. Alternatively, the reference may be marker expression levels in a sample comprising cells characteristic of Barrett's esophagus obtained from the same individual at a different time, which would permit changes in marker expression levels to be monitored over time. It is also envisioned that comparison of marker expression levels may be made with reference to a normal range established using normal cells from a population of individuals.

Differences in expression levels between Barrett's esophagus and a reference may be evaluated using any suitable statistical test. As one of skill in the art will appreciate, interpretation of results may be evaluated using different P values, depending on importance of minimizing false positives relative to the importance of minimizing false negatives in a particular application.

The methods of the invention may conveniently be performed using a kit. The kit may optionally comprise one or more probes for measuring expression at least one marker of Table 2. A probe may include, for example, a primer pair for performing quantitative PCR, an oligonucleotide that hybridizes to an mRNA or cDNA corresponding to one of the markers of Table 2, or an antibody specific for an epitope of an expression product (i.e., mRNA or protein) of a marker listed in Table 2. The kit may include instructions for performing a method according to the present invention.

EXAMPLES

The following non-limiting Examples are intended to be purely illustrative.

Clinical samples. Samples of normal, Barrett's, and adenocarcinoma were obtained from fresh pathological specimens of patients with known Barrett's esophagus and esophageal adenocarcinoma who had undergone esophagectomy. These specimens were processed by pathology within 15 minutes of resection. Samples representative of the various gross histologic types were obtained from experienced gastrointestinal pathologists. These samples were labeled and snap frozen in liquid nitrogen and stored at −80° C. for future RNA extraction.

Preparation of RNA and hybridizations. RNAs were purified by combination of column chromatography and TRizol (GIBCO BRL, MD) purification, as described previously (15). Preparation of labeled cRNA and hybridization with U133A chips was performed according to the manufacturer's instructions (Affymetrix, Calif.). Data were acquired using MAS 5.0 software (Affymetrix) and exported to MS Excel.

Submission of DNA array data. Data were submitted to the Microarray Analysis and Data Management System (MADAM) database of the University of Chicago, and constructed according to the Minimum Information about a Microarray Experiment (MIAME) recommendations. Data were also submitted to the GEO database (NCBI), with the accession number GSE1420.

Data analysis. Throughout this section, patients are denoted by the letter i=1, . . . 8, genes by the letter j, and tissue type by the letter k=1, 2, 3 (referring to normal (N), Barrett's esophagus (BE), and adenocarcinoma tumor (ADC).

For data normalization, the expression levels of each array were multiplied by $\overline{M}/M$, where M is the median expression of the array, and $\overline{M}$ is the overall median expression level. This resealing makes median expression levels equal across all arrays. For data filtration, genes were excluded based on present (P) or absent (A) calls as defined by MAS 5.0. Genes were excluded if $\Sigma_{i=1}{}^{8}A_i \geqq 3$ for all three tissue types, where A_i indicates whether a transcript is absent (A_i=1) or present (A_i=0). The genes were further filtered based on signal intensities using ROC analysis as previously described (16, 17). The total number of remaining genes was 8636.

Next, Significance Analysis of Microarrays (SAM) (18) was used to identify genes significantly over- and underexpressed in the three pairwise comparisons of Barrett's/normal, adenocarcinoma/normal, and Barrett's/adenocarcinoma. Significance analysis of microarrays identified genes with statistically significant differences between groups by assigning each gene a score on the basis of the difference in gene expression between two groups (e.g. normal and Barrett's) relative to the adjusted pooled standard deviation of the multiple measurements from both groups. Permutations of the measurements were then used to estimate the false discovery ratio (FDR), the percentage of genes identified by chance. As the cut-off point, a Δ-value was chosen such that the estimated median number of falsely discovered (called) genes was less than or equal to 1, and required at least a 2-fold expression ratio. In contrast to using a cut-off point of a fixed FDR level, this approach resulted in different cut-off Δs and FDR levels for the three comparisons: Barrett's/normal (Δ=1.270, FDR=0.33%), adenocarcinoma/normal, (Δ=1.555, FDR=0.121%) and Barrett's/adenocarcinoma (Δ=0.892, FDR=0.876%). Based on these criteria, 447 genes significantly expressed in adenocarcinoma compared with normal epithelium and 200 genes significantly expressed in Barrett's esophagus compared with normal epithelium were selected. A set of 85 genes was found to have significantly different expression between adenocarcinoma and Barrett's esophagus, of which 45 genes overlapped with genes significantly different in adenocarcinoma versus normal epithelium. Next, expression ratios of all genes between two tissue groups were compared to the reference "same-to-same" distribution in order to identify genes for which the ratios are larger than expected. In a simple case with two normal samples, the "same-to-same" distribution is the distribution of over all genes j (17). This concept was extended to a situation with more than two arrays by considering

$$L_j = \log_2\left[\left(\frac{N_{j1}\cdot N_{j2}\ \ldots\ \cdot N_{j,\frac{K}{2}}}{N_{j,\frac{K}{2}+1}\cdot N_{j,\frac{K}{2}+2}\ \ldots\ \cdot N_{jK}}\right)^{1/(K/2)}\right]$$

where K is an even number of normal samples, and N_{jk} represents the expression level of gene j. For every gene j, we consider the

$$C = \binom{K}{K/2}$$

possible ways the samples can be separated into two groups, obtaining C=70 possible combinations for each gene based on the 8 arrays, hybridized with RNA from normal tissues. For each of the 70 distributions quantiles $q_{0.005}$, $q_{0.0025}$,

$q_{0.0975}$, $q_{0.995}$, corresponding to nonparametric 95% and 99% confidence limits were computed. Averaging these over the 70 combinations provides cut-off points for where the bulk of the same-to-same log-ratios occur. For each gene j Barrett's/normal and adenocarcinoma/normal ("different-to-same") log-ratio

$$L_j = \log_2\left[\left(\frac{T_{j1}\cdot T_{j2}\ldots\cdot T_{jK}}{N_{j1}\cdot N_{j2}\ldots\cdot N_{jK}}\right)^{1/K}\right]$$

was then compared to the reference "same-to-same" distributions, and genes with expression ratios outside the cut-off limits were considered to be differentially expressed. Using the geometric mean rather than the non-standardized ratio allowed direct comparisons of the distributions of the "same-to-same" and "different-to-same" hybridizations, adjusting for the fact that the "different-to-same" ratios are based on K pairs of tumor and normal expression levels, and that the "same-to-same" ratios are based only on K normal expression levels. Thus, L_j can be naturally interpreted as the per-patient log-ratio.

Functional selection and prognosticators analysis. To select functionally significant groups of genes, OntoExpress software was used (19). Functional groups containing at least 3 genes were selected and analyzed using a binomial distribution with a significance level ≦0.05. Combining results of functional and expression-based selections, 214 genes were selected for further study. Two-dimensional hierarchical clustering of these genes was performed based on the estimation of the Euclidian distances by Ward's method using $\log_2 X_{ijk}/\overline{X}_j^{(N)}$, the log-transformed expression levels normalized to the average expression level in the normal tissues, $\overline{X}_j^{(N)}$. Samples T5 and N8 were removed as outliers. For clustering and data presentation, JMP and TreeView software was used as described previously (20).

To define genes that correlate with the progression of Barrett's esophagus to adenocarcinoma, the 96 genes expressed in both Barrett's esophagus and adenocarcinoma were considered. These genes were separated into two groups based on average between-patient expression: the first group contained genes which were up-regulated from normal to Barrett's esophagus and further from Barrett's esophagus to adenocarcinoma, and the second group was defined similarly for down-regulated genes. All other potential patterns were excluded from this analysis. The significance of the difference in expression from normal to Barrett's esophagus and from Barrett's esophagus to adenocarcinoma in each group was evaluated by a one-sided paired t-test using a p-value≦0.05 cut-off (taking into account that up- or down-regulated genes in each group had been pre-selected).

Quantitative reverse-transcription-PCR. cDNA was synthesized using Superscript II® reverse transcriptase (Invitrogen Life Technologies, Carlsbad, Calif., USA) following the manufacturer's instructions. cDNA was diluted 1:10 in sterile nuclease free water (Ambion, Tex.). Quantitative PCR was performed on an ABI 7700 system (Applied Biosystems, Foster City, Calif.) using SYBR Green PCR reagents in a 25 μl reaction mixture containing 2.5 μl 10×SYBR Green PCR buffer, 0.25 μl 10 mM primers, 2 μl dNTP mix, 3 μl 25 mM $MgCl_2$, 0.25 μl AmpErase, 0.125 μl Amplitaq Gold and 2.5 μl of the 1:10 diluted cDNA.

Primers for selected genes were designed based on UniGene reference sequences using PrimerExpress software (Applied Biosystems, Foster City, Calif.). For the internal control we used GAPDH. PCR was performed for 40 cycles at 95° C. for 15 seconds and 60° C. for one minute after initial incubations at 50° C. for 2 minutes and 95° C. for 10 min.

All samples were amplified in triplicate reactions. The expression of each individual gene was calculated based on the difference between amplification of the individual mRNA template and the internal control (GAPDH) mRNA template. These differences were measured by delta ct (dct) values as described in the manufacturer's instructions (Applied Biosystems, Foster City, Calif.). dct values were calculated as $(ct_x\text{-}ct_{GAPDH})$, where ct_x is the ct value of the specific gene X and ct_{GAPDH} is the amplification of the internal control. Fold induction was calculated as 2^{-dct} and therefore was equal to $2^{-(ct_x\text{-}ct_{GAPDH})}$ Ratios of gene X relative to gene Y in the same samples was calculated as: $R_{X/Y}=2^{-(ctX\text{-}ctY)}$. These ratios were multiplied by 100,000 to give a range greater than one. Finally, the data was converted to Log_{10} format to present them in linear scale. The final expressional value (EV) was calculated as:

$$EV_{x/y}=\mathrm{Log}_{10}[10^5\times\{2^{-(ctX\text{-}ctY)}\}]$$

Discrimination between normal esophageal epithelium, Barrett's metaplasia and adenocarcinomas based on expressional profiling. Genes differentially expressed (either up- or down-regulated) in Barrett's esophagus and adenocarcinoma were selected based on the results of the statistical analysis. Compared with normal esophageal epithelium, 200 genes differentially expressed in Barrett's esophagus tissue and 447 genes differentially expressed in the Barrett's esophagus-associated adenocarcinoma were identified. The comparison of genes differentially expressed in Barrett's esophagus and adenocarcinoma showed that 96 genes were commonly over-expressed in Barrett's esophagus and adenocarcinoma. In adenocarcinoma, 351 genes were found to be differentially expressed that are not differentially expressed in Barrett's esophagus; in Barrett's esophagus, 104 genes were found to be differentially expressed that are not differentially expressed in adenocarcinoma. These non-overlapping genes were used in subsequent selection of significant functional groups using OntoExpress software (Table 1). Genes were also selected by comparison of the "same-to-same" and "different-to-same" hybridizations as described above using 99% confidence intervals based on the non-parametric quantile analysis. Combining both approaches, 214 genes (Supplemental Table 1) were selected for two-dimensional hierarchical clustering to show the actual discrimination between normal samples, Barrett's esophagus, and adenocarcinoma. The data were separated into three expressional clusters: cluster 1 (80 genes) contains the genes up-regulated in adenocarcinoma compared with normal epithelium; cluster 2 (63 genes) contains the genes which are sequentially suppressed in Barrett's esophagus and adenocarcinoma compared with the normal epithelium; cluster 3 (71 genes) contains the genes most drastically suppressed in adenocarcinoma compared with normal epithelium and Barrett's esophagus (data not shown).

Expressional patterns of normal epithelium, Barrett's esophagus and adenocarcinoma include different functional groups of genes. The major functional groups associated with the three major expressional clusters were identified. Cluster 1 was found to contain functional groups of genes associated with immune response, cell-cell signaling and cell-ECM interactions, control of cell cycle/growth/proliferation, and regulation of transcription and receptor activity (see Table 1).

Cluster 2 was also found to include genes involved in regulating cell cycle/proliferation, as well as genes involved in intracellular transport, bile acid transport, and aldehyde

and lipid metabolism. Cluster 3 was found to contain functional groups of genes which may be specifically involved in the development of adenocarcinoma, including ectoderm development/epidermal differentiation, cytoskeleton, control of cell shape and cell-to-cell and cell-to-ECM interactions, Ca^{2+} binding and metabolism, and a group of proteases and protease inhibitors. Many of these genes are specifically associated with epidermal differentiation and malignant transformation.

Analysis of genes common to Barrett's and adenocarcinoma. Ninety-six genes were found to be differentially expressed (relative to normal esophageal endothelium) in both Barrett's esophagus and adenocarcinomas (Supplemental Table 2). Of those genes, a subset of 21 genes (Table 2) was chosen as prognostic or diagnostic markers because they are differentially expressed in the same direction (i.e., up- or down-regulated) in both Barrett's esophagus and adenocarcinoma, relative to normal esophageal epithelium, and the changes in expression are progressive from Barrett's esophagus to adenocarcinoma (i.e., expression is up- or down-regulated to a greater degree in adenocarcinoma relative than in Barrett's esophagus).

Analysis of expression by QRT-PCR. Differential expression as determined by DNA array-based analysis was confirmed for select markers within the group of markers shown in Table 2 using QRT-PCR. Briefly, RNA was purified from surgical samples, and QRT-PCR was performed, as described above, for GATA6, HOXB7, TCF3, S100A2, SCCA1 and SPRR3, with GAPDH as the internal control, using primer pairs having the sequences provided in Table 3. The results are shown in FIGS. **1** and **2**. With reference to FIG. **1**, panel A shows the expressional value (EV) calculated relative to GAPDH for three transcriptional factors (i.e., GATA6, HOXB7 and TCF3) for individual paired patient samples. Patient samples are identified by patient number and sample type, i.e., normal esophageal epithelium (n), Barrett's dysplasia (b), or esophageal adenocarcinoma (t). The results indicate that these genes are up-regulated in the progression from normal to Barrett's esophagus to adenocarcinoma. Panel B shows expressional value (EV) calculated relative to GAPDH for three genes related to keratinocyte differentiation (i.e., S100A2, SCCA1 and SPRR3) for individual paired patient samples. The results indicate that these genes are down-regulated in the progression from normal to Barrett's esophagus to adenocarcinoma. Four samples (n12, n14, nN17 and t17) failed to amplify specific gene products by PCR and were excluded. These data are consistent with the results from the entire set of tissue types in the microarray analysis, as shown in FIGS. **1**C and D, which show the corresponding average values, with the standard deviations indicated by the error bars.

To select expressional markers correlated with pre-malignant and malignant changes, p values and regression coefficients were calculated for six single genes and combinations of genes (Table 4). Each single marker can significantly discriminate normal esophageal epithelium from adenocarcinoma. However, only HOXB7 can discriminate normal tissues from Barrett's. None of the tested markers used alone can discriminate Barrett's from adenocarcinomas. However, as can be seen from Table 4, combinations of markers (GATA6/SPRR3, HOXB7/SPRR3 and GATA6+HOXB7/SPRR3) permit discrimination of Barrett's from adenocarcinomas. Additionally, mixed effects analysis of variance (ANOVA) models were used to determine whether there are differences in expression of GATA6/SPRR3, HOXB7/SPRR3 and GATA6+HOXB7/SPRR3 combinations between the three groups, accounting for the presence of intra-subject correlation due to the presence of several subjects with multiple samples. These analyses confirmed that the expression levels of these combination markers are significantly different between normal, Barrett and Tumor tissues types (data not shown). Also, for the combinations listed, the correlation between expression and tumor progression is higher than for either gene alone.

The GATA6/SPRR3 ratio was evaluated as marker of transformation (FIG. **2**). As can be seen in FIG. **2**, the ratio of GATA6 to SPRR3 progressively increases along the progression from normal epithelium to Barrett's dysplasia to adenocarcinomas. At the 95% confidence interval (dashed line, calculated as the mean of normal epithelium values+1.96 SD), the test has a specificity of 89% (8/9 negatives cases). For Barrett's esophagus, the sensitivity of the test, as measured by the percentage of positive cases, is 28.6% (2/9). For adenocarcinoma, the sensitivity is 100% (7/7). With a cut off level equal to 67% confidence interval (mean+1 SD), the specificity of the test is also equal to 89%, the sensitivity for Barrett's esophagus is 86% (6/7), and the sensitivity for adenocarcinoma is 100% (7/7).

Each reference cited herein is incorporated by reference in its entirety.

Table 1. Functional groups of genes selected for Barrett's and adenocarcinomas.

TABLE 1

Selected functional groups for Barrett's and adenocarcinomas

BARRETT		Adenocarcinomas			
		GO Biological process		GO Molecular function	
GO ID	Function name	GO ID	Function name	GO ID	Function name
GO Biological process		GO:0001558	regulation of cell growth	GO:0003700	transcription factor activity
GO:0000074	regulation of cell cycle	GO:0006081	aldehyde metabolism	GO:0003821	class II major histocompatibility complex
GO:0001501	skeletal development	GO:0006355	regulation of transcription, DNA-dependent	GO:0004029	aldehyde dehydrogenase (NAD) activity
GO:0006812	cation transport				
GO:0006915	apoptosis	GO:0006461	protein complex assembly		
GO:0006935	chemotaxis	GO:0006629	lipid metabolism	GO:0004263	chymotrypsin activity
GO:0006955	immune response	GO:0006886	intracellular protein transport	GO:0004295	trypsin activity
GO:0007160	cell-matrix adhesion	GO:0006899	nonselective vesicle transport	GO:0004601	peroxidase activity
GO:0007166	cell surface receptor linked signal transduction	GO:0006944	membrane fusion	GO:0004867	serine protease inhibitor activity
		GO:0006979	response to oxidative stress	GO:0004930	G-protein coupled receptor activity
GO:0007229	integrin-mediated signaling pathway	GO:0007048	oncogenesis	GO:0005152	interleukin-1 receptor antagonist activity
		GO:0007398	ectoderm development		

TABLE 1-continued

Selected functional groups for Barrett's and adenocarcinomas

BARRETT		Adenocarcinomas			
		GO Biological process		GO Molecular function	
GO ID	Function name	GO ID	Function name	GO ID	Function name
GO:0007267	cell-cell signaling	GO:0007417	central nervous system development	GO:0005198	structural molecule activity
GO:0008151	cell growth and/or maintenance	GO:0008284	positive regulation of cell proliferation	GO:0005200	structural constituent of cytoskeleton
GO:0008152	metabolism			GO:0005509	calcium ion binding
GO:0009653	morphogenesis	GO:0008544	epidermal differentiation	GO:0005524	ATP binding
GO Molecular function		GO:0016049	cell growth	GO:0005525	GTP binding
GO: 0004716	receptor signaling protein tyrosine kinase	GO:0019883	antigen presentation, endogenous antigen	GO:0008237	metallopeptidase activity
GO:0004872	receptor activity	GO:0019885	antigen processing via MHC I	GO:0016301	kinase activity
GO:0004895	cell adhesion receptor activity	GO:0045786	negative regulation of cell cycle	GO:0016853	isomerase activity
GO:0008201	heparin binding	null	cell shape and cell size control	GO:0030106	MHC class I receptor activity
		GO:0006470	protein amino acid dephosphorylation	GO:0045012	MHC class II receptor activity
				GO:0004033	aldo-keto reductase activity
		GO:0006805	xenobiotic metabolism	GO:0005488	binding
		GO:0006810	transport	GO:0008014	calcium-dependent cell adhesion
		GO:0006955	immune response	GO:0015125	bile acid transporter activity
		GO:0007155	cell adhesion	GO:0017017	MAP kinase phosphatase activity
		GO:0007156	homophilic cell adhesion	GO:0047115	trans-1,2-dihydrobenzene-1,2-diol dehydrogenase
		GO:0007267	cell-cell signaling		

Table 2. Genes progressively up- or down-regulated with the development of adenocarcinoma from Barrett's esophagus.

TABLE 2

Genes with progressive changes of expression in Barretts and adenocarcinomas

id	symbol	name	Ratio (B/N)	Ratio (T/N)
		up-regulated genes		
201301_s_at	ANXA4	ANNEXIN A4	2.28	3.13
201954_at	ARPC1B	ACTIN-RELATED PROTEIN 2/3 COMPLEX, SUBUNIT 1B	3.20	5.42
214439_x_at	BIN1	BRIDGING INTEGRATOR 1	2.23	3.26
202901_x_at	CTSS	CATHEPSIN S	3.08	5.26
210002_at	GATA6	GATA-BINDING PROTEIN 6	6.27	10.77
221875_x_at	HLA-F	MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I, F	2.28	3.35
204806_x_at	HLA-F	MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS I, F	2.17	3.20
204779_s_at	HOXB7	HOMEO BOX B7	3.56	5.82
216973_s_at	HOXB7	HOMEO BOX B7	2.71	4.42
201422_at	IFI30	INTERFERON-GAMMA-INDUCIBLE PROTEIN 30	2.23	4.11
212110_at	KIAA0062	SLC39A14: solute carrier family 39 (zinc transporter), member 14	5.26	7.86
203943_at	KIF3B	KINESIN FAMILY MEMBER 3B	2.27	3.43
218376_s_at	NICAL	NEDD9 interacting protein with calponin homology and LIM domains	2.03	3.12
219622_at	RAB20	RAB20, member RAS oncogene family	2.90	4.66
201206_s_at	RRBP1	RIBOSOME BINDING PROTEIN 1	4.02	5.80
201204_s_at	RRBP1	RIBOSOME BINDING PROTEIN 1	2.46	3.34
213811_x_at	TCF3	TRANSCRIPTION FACTOR 3	2.84	4.45
208998_at	UCP2	UNCOUPLING PROTEIN 2	3.52	6.57
		down-regulated genes		
210020_x_at	CALML3	CALMODULIN-LIKE 3	0.40	0.11
203585_at	ZNF185	ZINC FINGER PROTEIN 185	0.46	0.17
213005_s_at	KANK	KIDNEY ANKYRIN REPEAT-CONTAINING PROTEIN	0.49	0.24
211734_s_at	FCER1A	Fc FRAGMENT OF IgE, HIGH AFFINITY I, RECEPTOR FOR, ALPHA SUBUNIT	0.25	0.14
201848_s_at	BNIP3	BCL2/ADENOVIRUS E1B 19-KD PROTEIN-INTERACTING PROTEIN 3	0.43	0.26
219100_at	FLJ22559	hypothetical protein	0.48	0.29

TABLE 3

Primers for detection of genes progressively changing in Barrett's associated adenocarcinomas.

Gene	forward primer	reverse primer
gapdh	TGCACCACCAACTGCTTAGC SEQ ID NO: 1	GGCATGGACTGTGGTCATGAG SEQ ID NO: 2
gata6	AGCGCGTGCCTTCATCAC SEQ ID NO: 3	GCAAGTGGTCTGGGCACC SEQ ID NO: 4
hoxb7	GGATCTACCCCTGGATGCG SEQ ID NO: 5	GTCTTTCCGTGAGGCAGAGC SEQ ID NO: 6
s100a2	CTGTCTCTGCCACCTGGTCT SEQ ID NO: 7	CTCAAAGGCATCAACAGTCCT SEQ ID NO: 8
serpinb3 (SCCA1)	TTCATGTTCGACCTGTTCCA SEQ ID NO: 9	GCAGCTTTTCCTGTGGTGTT SEQ ID NO: 10
sprr3	ATCCCTGAGCAGCTGAAGAC SEQ ID NO: 11	CTGCTGTTGAAGCTGAGGTG SEQ ID NO: 12
tcf3	GTGACATCAACGAGGCCTTT SEQ ID NO: 13	CTGCTTTGGGATTCAGGTTC SEQ ID NO: 14

TABLE 4

p values and Pearson's correlation coefficients.

Gene symbol	p values N-ADENOCARCINOMA	p values N-BE	p values BE-ADENOCARCINOMA	R values
GATA6	0.0014	0.0797	0.1510	0.6909
HOXB7	0.0001	0.0183	0.1045	0.7657
TCF3	0.0063	0.2048	0.0769	0.5797
S100A2	0.0332	0.4123	0.1131	−0.4752
SCCA1	0.0171	0.3794	0.1184	−0.4551
SPRR3	0.0011	0.1116	0.1014	−0.6177
GATA6/SPRR3	1.4662E−06	0.0012	0.0013	0.8732
HOXB7/SPRR3	4.0369E−06	0.0092	0.0211	0.8176
GATA6 + HOXB7/SPRR3	1.6406E−06	0.0028	0.0034	0.8628

SUPPLEMENTAL TABLE 1

1 Probe set id	2 Gene symbol	3 Expressional cluster number	4 Gene number in FIG. 2	5 Expression in Barrett's relative to the normal epithelium [Log2 R (B/N)]	6 Expression in adenocarcinoma relative to normal epithelium [Log2 R (T/N)]
205927_s_at	CTSE	1	1	4.55	4.83
219580_s_at	TMC5	1	2	4.21	5.46
210143_at	ANXA10	1	3	5.38	4.62
203824_at	TM4SF3	1	4	2.73	3.07
203559_s_at	ABP1	1	5	3.28	3.64
208161_s_at	ABCC3	1	6	2.43	2.70
204714_s_at	F5	1	7	2.43	3.27
209301_at	CA2	1	8	2.56	1.94
219682_s_at	TBX3	1	9	2.93	1.99
64408_s_at	CLN6	1	10	3.03	2.82
201666_at	TIMP1	1	11	1.49	2.04
220974_x_at	BA108L7.2	1	12	1.34	1.34
219327_s_at	GPRC5C	1	13	1.14	2.05
210095_s_at	IGFBP3	1	14	1.55	2.57
219956_at	GALNT6	1	15	2.77	2.27
202910_s_at	CD97	1	16	1.57	2.08
209774_x_at	CXCL2	1	17	1.48	1.65
207522_s_at	ATP2A3	1	18	2.44	1.43
202267_at	LAMC2	1	19	2.11	3.01
210314_x_at	TNFSF13	1	20	2.42	3.18
219795_at	SLC6A14	1	21	3.03	3.49
202625_at	LYN	1	22	2.09	2.40
203058_s_at	PAPSS2	1	23	1.19	1.43
210754_s_at	LYN	1	24	1.56	1.54
222303_at	ETS2	1	25	1.74	0.96
220322_at	IL1F9	1	26	1.93	0.07
205668_at	LY75	1	27	1.16	1.80

SUPPLEMENTAL TABLE 1-continued

1 Probe set id	2 Gene symbol	3 Expressional cluster number	4 Gene number in FIG. 2	5 Expression in Barrett's relative to the normal epithelium [Log2 R (B/N)]	6 Expression in adenocarcinoma relative to normal epithelium [Log2 R (T/N)]
204363_at	F3	1	28	1.11	0.03
203510_at	MET	1	29	2.39	3.57
214235_at	CYP3A5	1	30	2.08	2.59
202820_at	AHR	1	31	1.86	2.59
210664_s_at	TFPI	1	32	1.17	1.33
205289_at	BMP2	1	33	1.62	2.05
201656_at	ITGA6	1	34	1.79	1.51
215177_s_at	ITGA6	1	35	1.40	1.02
221059_s_at	CHST6	1	36	2.43	3.02
205067_at	IL1B	1	37	2.42	0.84
210845_s_at	PLAUR	1	38	2.15	2.51
211924_s_at	PLAUR	1	39	1.99	2.27
206467_x_at	TNFRSF6B	1	40	1.83	2.44
39402_at	IL1B	1	41	2.84	1.04
209417_s_at	IFI35	1	42	1.28	2.04
201596_x_at	KRT18	1	43	1.62	1.83
204017_at	KDELR3	1	44	1.98	2.32
204989_s_at	ITGB4	1	45	1.18	1.16
207265_s_at	KDELR3	1	46	1.34	1.41
202831_at	GPX2	1	47	1.31	1.84
201189_s_at	ITPR3	1	48	1.36	1.71
202668_at	EFNB2	1	49	1.68	1.84
212282_at	MAC30	1	50	1.34	2.66
212281_s_at	MAC30	1	51	1.41	2.67
212279_at	MAC30	1	52	1.00	2.19
208829_at	TAPBP	1	53	0.89	1.69
211529_x_at	HLA-G	1	54	0.85	1.35
211911_x_at	HLA-B	1	55	0.93	1.34
208729_x_at	HLA-B	1	56	0.78	1.18
214459_x_at	HLA-C	1	57	0.86	1.23
203857_s_at	PDIR	1	58	0.89	1.09
211528_x_at	HLA-G	1	59	0.81	1.24
202737_s_at	LSM4	1	60	0.53	1.38
201063_at	RCN1	1	61	0.75	1.82
209762_x_at	SP110	1	62	0.63	1.31
205205_at	RELB	1	63	0.87	1.16
213258_at	TFPI	1	64	0.99	1.19
210927_x_at	JTB	1	65	0.46	1.14
218355_at	KIF4A	1	66	1.04	1.85
211048_s_at	ERP70	1	67	0.86	1.62
200699_at	KDELR2	1	68	0.94	1.69
212761_at	TCF7L2	1	69	0.77	1.24
201329_s_at	ETS2	1	70	1.01	0.17
200037_s_at	CBX3	1	71	0.34	1.47
211208_s_at	CASK	1	72	0.65	1.33
210052_s_at	TPX2	1	73	0.64	1.55
204641_at	NEK2	1	74	0.68	1.95
204670_x_at	HLA-DRB3	1	75	0.63	1.76
209312_x_at	HLA-DRB3	1	76	0.77	1.80
208306_x_at	HLA-DRB3	1	77	0.78	1.83
215193_x_at	HLA-DRB3	1	78	0.88	1.85
210982_s_at	HLA-DRA	1	79	0.73	1.53
208894_at	HLA-DRA	1	80	0.53	1.27
211126_s_at	CSRP2	2	81	−0.77	−1.84
207030_s_at	CSRP2	2	82	−0.80	−1.63
203659_s_at	RFP2	2	83	−0.81	−1.26
221960_s_at	RAB2	2	84	−0.71	−1.53
202582_s_at	RANBP9	2	85	−0.70	−1.61
209882_at	RIT1	2	86	−0.85	−1.85
201454_s_at	NPEPPS	2	87	−0.71	−1.55
204119_s_at	ADK	2	88	−0.75	−1.59
208771_s_at	LTA4H	2	89	−0.51	−1.40
200606_at	DSP	2	90	−0.32	−1.66
213572_s_at	SERPINB1	2	91	−0.39	−2.30
212268_at	SERPINB1	2	92	−0.38	−1.63
202814_s_at	HIS1	2	93	−0.46	−1.27
200697_at	HK1	2	94	−0.54	−1.47
208384_s_at	MID2	2	95	−0.66	−1.47
201192_s_at	PITPN	2	96	−0.60	−1.54
203081_at	CTNNBIP1	2	97	−0.58	−1.67
201161_s_at	CSDA	2	98	−0.54	−1.57

SUPPLEMENTAL TABLE 1-continued

1 Probe set id	2 Gene symbol	3 Expressional cluster number	4 Gene number in FIG. 2	5 Expression in Barrett's relative to the normal epithelium [Log2 R (B/N)]	6 Expression in adenocarcinoma relative to normal epithelium [Log2 R (T/N)]
211749_s_at	VAMP3	2	99	−0.39	−1.23
209157_at	DNAJA2	2	100	−0.60	−1.33
208951_at	ALDH7A1	2	101	−0.71	−1.40
208950_s_at	ALDH7A1	2	102	−0.80	−1.47
201337_s_at	VAMP3	2	103	−0.62	−1.70
201612_at	ALDH9A1	2	104	−0.85	−1.56
41644_at	SASH1	2	105	−0.65	−2.01
213236_at	SASH1	2	106	−1.08	−2.48
210094_s_at	PARD3	2	107	−0.44	−1.18
221526_x_at	PARD3	2	108	−0.63	−1.18
214040_s_at	GSN	2	109	−0.93	−2.37
202054_s_at	ALDH3A2	2	110	−1.05	−1.70
202053_s_at	ALDH3A2	2	111	−1.02	−2.19
209466_x_at	PTN	2	112	−1.43	−2.06
201041_s_at	DUSP1	2	113	0.22	−1.11
201044_x_at	DUSP1	2	114	−0.11	−1.59
202139_at	AKR7A2	2	115	−0.60	−1.20
209372_x_at	TUBB	2	116	−0.86	−1.77
215813_s_at	PTGS1	2	117	−0.42	−1.68
210186_s_at	FKBP1A	2	118	−0.74	−1.24
200678_x_at	GRN	2	119	−0.92	−1.46
216041_x_at	GRN	2	120	−0.96	−1.58
204246_s_at	DCTN3	2	121	−0.73	−1.63
200886_s_at	PGAM1	2	122	−0.45	−1.62
204029_at	CELSR2	2	123	−0.75	−2.04
36499_at	CELSR2	2	124	−0.68	−1.97
203586_s_at	ARF4L	2	125	−0.44	−2.37
213848_at	DUSP7	2	126	−0.28	−2.13
200844_s_at	PRDX6	2	127	−0.86	−1.39
208751_at	NAPA	2	128	−0.73	−1.20
202807_s_at	TOM1	2	129	−0.73	−1.39
214182_at	ARF6	2	130	−0.74	−1.68
209193_at	PIM1	2	131	−0.96	−1.95
205172_x_at	CLTB	2	132	−0.65	−1.78
211043_s_at	CLTB	2	133	−0.44	−1.95
206284_x_at	CLTB	2	134	−0.65	−1.95
200863_s_at	RAB11A	2	135	−0.51	−1.58
200752_s_at	CAPN1	2	136	−0.79	−1.65
204341_at	TRIM16	2	137	−0.82	−2.50
204151_x_at	AKR1C1	2	138	0.44	−0.96
211653_x_at	AKR1C2	2	139	0.37	−1.32
209699_x_at	AKR1C2	2	140	0.47	−1.03
216594_x_at	AKR1C1	2	141	0.40	−1.00
205403_at	IL1R2	2	142	1.24	0.13
206561_s_at	AKR1B10	2	143	0.58	−1.07
205549_at	PCP4	3	144	−1.55	−2.80
218559_s_at	MAFB	3	145	−0.99	−2.39
204379_s_at	FGFR3	3	146	−0.86	−2.28
205286_at	TFAP2C	3	147	−0.62	−2.58
203074_at	ANXA8	3	148	−0.89	−4.35
203407_at	PPL	3	149	−0.74	−3.93
202504_at	TRIM29	3	150	−0.65	−3.94
204942_s_at	ALDH3B2	3	151	−1.33	−5.08
202345_s_at	FABP5	3	152	−0.24	−2.58
201012_at	ANXA1	3	153	−0.22	−2.63
212657_s_at	IL1RN	3	154	−0.43	−3.01
218677_at	S100A14	3	155	−0.39	−2.56
201324_at	EMP1	3	156	−0.56	−2.97
201325_s_at	EMP1	3	157	−0.61	−3.85
219764_at	FZD10	3	158	−0.50	−2.69
209191_at	TUBB-5	3	159	−0.77	−2.28
201348_at	GPX3	3	160	−0.84	−2.71
205349_at	GNA15	3	161	−0.82	−2.96
209587_at	PITX1	3	162	−0.94	−3.92
213279_at	DHRS1	3	163	−1.28	−3.02
205863_at	S100A12	3	164	−0.59	−3.15
38158_at	ESPL1	3	165	−1.82	−2.87
205470_s_at	KLK11	3	166	−0.95	−3.79
217315_s_at	KLK13	3	167	−0.97	−4.60
205783_at	KLK13	3	168	−1.41	−4.41
216243_s_at	IL1RN	3	169	−1.12	−4.53

SUPPLEMENTAL TABLE 1-continued

1 Probe set id	2 Gene symbol	3 Expressional cluster number	4 Gene number in FIG. 2	5 Expression in Barrett's relative to the normal epithelium [Log2 R (B/N)]	6 Expression in adenocarcinoma relative to normal epithelium [Log2 R (T/N)]
204777_s_at	MAL	3	170	−0.74	−5.21
14599_at	IVL	3	171	−0.68	−4.91
214549_x_at	SPRR1A	3	172	−0.74	−4.05
204751_x_at	DSC2	3	173	−0.32	−2.35
204469_at	PTPRZ1	3	174	−0.03	−1.61
206032_at	DSC3	3	175	−0.74	−4.05
206166_s_at	CLCA2	3	176	−0.66	−4.29
210372_s_at	TPD52L1	3	177	−0.93	−2.87
203786_s_at	TPD52L1	3	178	−1.11	−3.28
213135_at	TIAM1	3	179	−0.67	−3.20
203797_at	VSNL1	3	180	−1.06	−2.72
207059_at	PAX9	3	181	−1.36	−3.68
204284_at	PPP1R3C	3	182	−0.68	−3.72
211726_s_at	FMO2	3	183	−1.01	−2.95
204614_at	SERPINB2	3	184	−0.77	−4.47
207602_at	HAT	3	185	−0.63	−3.87
205595_at	DSG3	3	186	−0.47	−3.58
209719_x_at	SERPINB3	3	187	−0.36	−3.79
211906_s_at	SERPINB4	3	188	−0.31	−4.07
205185_at	SPINK5	3	189	−0.63	−3.03
210413_x_at	SERPINB4	3	190	0.07	−4.32
204734_at	KRT15	3	191	−0.92	−7.56
220431_at	DESC1	3	192	−1.00	−5.02
220026_at	CLCA4	3	193	−0.61	−5.19
217528_at	CLCA2	3	194	−0.60	−4.31
206276_at	E48	3	195	−0.63	−4.18
209720_s_at	SERPINB3	3	196	−0.32	−3.72
208539_x_at	SPRR2B	3	197	−0.56	−3.89
213240_s_at	KRT4	3	198	−0.03	−4.73
213796_at	SPRR1A	3	199	−0.27	−3.30
219554_at	RHCG	3	200	−0.61	−6.05
205014_at	HBP17	3	201	−0.28	−4.24
203535_at	S100A9	3	202	−0.21	−3.46
39248_at	AQP3	3	203	−0.42	−3.75
204268_at	S100A2	3	204	−0.16	−3.42
202917_s_at	S100A8	3	205	0.00	−2.48
213680_at	KRT6B	3	206	−0.16	−2.92
218990_s_at	SPRR3	3	207	−0.07	−3.18
207935_s_at	KRT13	3	208	−0.19	−3.89
209126_x_at	KRT6B	3	209	−0.18	−3.52
201820_at	KRT5	3	210	−0.29	−3.89
209125_at	KRT6A	3	211	−0.24	−3.32
205064_at	SPRR1B	3	212	−0.23	−3.25
209351_at	KRT14	3	213	0.81	−2.17
220664_at	SPRR2C	3	214	−0.38	−4.25

SUPPLEMENTAL TABLE 2

Probe set ID	Gene name	Gene symbol	Log2 R (B/N)	Log2 R (T/N)
204272_at	galectin 4	LGALS4	4.90	4.83
211429_s_at	*Homo sapiens* PRO2275 mRNA	unknown	4.26	4.70
201839_s_at	tumor-associated calcium signal transducer 1	TACSTD1	3.27	3.76
209008_x_at	keratin 8	KRT8	2.88	3.00
209173_at	anterior gradient 2 homolog (*Xenopus laevis*)	AGR2	2.87	3.25
213059_at	old astrocyte specifically induced substance	OASIS	2.76	2.97
212444_at	retinoic acid induced 3	RAI3	2.68	2.92
213036_x_at	ATPase, Ca++ transporting, ubiquitous	ATP2A3	2.67	1.89
210002_at	GATA binding protein 6	GATA6	2.65	3.43
212314_at	KIAA0746 protein	KIAA0746	2.58	3.03
200644_at	MARCKS-like protein	MLP	2.52	3.00
212110_at	KIAA0062 protein	KIAA0062	2.40	2.98
205632_s_at	phosphatidylinositol-4-phosphate 5-kinase, type I, beta	PIP5K1B	2.32	2.52
209453_at	solute carrier family 9	SLC9A1	2.08	1.57
212311_at	KIAA0746 protein	KIAA0746	2.05	2.31
221766_s_at	chromosome 6 open reading frame 37	C6orf37	2.01	2.37

SUPPLEMENTAL TABLE 2-continued

Probe set ID	Gene name	Gene symbol	Log2 R (B/N)	Log2 R (T/N)
201206_s_at	ribosome binding protein 1 homolog 180 kDa (dog)	RRBP1	2.01	2.54
217989_at	retinal short-chain dehydrogenase/reductase 2	RETSDR2	1.97	2.23
208891_at	dual specificity phosphatase 6	DUSP6	1.96	1.49
212143_s_at	insulin-like growth factor binding protein 3	IGFBP3	1.89	2.51
220532_s_at	LR8 protein	LR8	1.88	2.49
218113_at	transmembrane protein 2	TMEM2	1.86	1.92
204779_s_at	homeo box B7	HOXB7	1.83	2.54
208998_at	uncoupling protein 2	UCP2	1.81	2.72
210264_at	G protein-coupled receptor 35	GPR35	1.70	1.91
201954_at	actin related protein 2/3 complex, subunit 1B, 41 kDa	ARPC1B	1.68	2.44
202901_x_at	cathepsin S	CTSS	1.62	2.40
219622_at	RAB20, member RAS oncogene family	RAB20	1.53	2.22
213811_x_at	transcription factor 3	TCF3	1.50	2.15
200972_at	tetraspan 3	TSPAN-3	1.49	1.50
218368_s_at	TNF receptor superfamily, member 12A	TNFRSF12A	1.49	1.41
203028_s_at	cytochrome b-245, alpha polypeptide	CYBA	1.47	2.05
208892_s_at	dual specificity phosphatase 6	DUSP6	1.47	1.20
216973_s_at	homeo box B7	HOXB7	1.44	2.14
212552_at	hippocalcin-like 1	HPCAL1	1.42	1.68
209270_at	laminin, beta 3	LAMB3	1.37	1.39
201204_s_at	ribosome binding protein 1 homolog	RRBP1	1.30	1.74
202180_s_at	major vault protein	MVP	1.30	1.48
201579_at	FAT tumor suppressor homolog 1	FAT	1.28	1.16
202369_s_at	translocation associated membrane protein 2	TRAM2	1.20	1.12
211799_x_at	major histocompatibility complex, class I, C	HLA-C	1.19	1.40
201301_s_at	annexin A4	ANXA4	1.19	1.65
221875_x_at	major histocompatibility complex, class I, F	HLA-F	1.19	1.74
203943_at	kinesin family member 3B	KIF3B	1.18	1.78
200599_s_at	tumor rejection antigen (gp96) 1	TRA1	1.17	1.52
201422_at	interferon, gamma-inducible protein 30	IFI30	1.16	2.04
214439_x_at	bridging integrator 1	BIN1	1.16	1.70
202838_at	fucosidase, alpha-L-1, tissue	FUCA1	1.14	1.30
204806_x_at	major histocompatibility complex, class I, F	HLA-F	1.12	1.68
209295_at	TNF receptor superfamily, member 10b	TNFRSF10B	1.09	1.43
209635_at	adaptor-related protein complex 1, sigma 1 subunit	AP1S1	1.06	1.53
203038_at	protein tyrosine phosphatase, receptor type, K	PTPRK	1.04	1.39
218376_s_at	NEDD9 interacting protein	NICAL	1.02	1.64
210776_x_at	transcription factor 3	TCF3	1.01	1.46
217741_s_at	zinc finger protein 216	ZNF216	−1.02	−1.49
213005_s_at	kidney ankyrin repeat-containing protein	KANK	−1.02	−2.06
201851_at	SH3-domain GRB2-like 1	SH3GL1	−1.05	−1.39
220942_x_at	growth and transformation-dependent protein	E2IG5	−1.07	−1.55
219100_at	hypothetical protein FLJ22559	FLJ22559	−1.07	−1.77
218205_s_at	MAP kinase-interacting serine/threonine kinase 2	MKNK2	−1.10	−1.29
220620_at	NICE-1 protein	NICE-1	−1.10	−2.37
218231_at	N-acetylglucosamine kinase	NAGK	−1.10	−1.76
203585_at	zinc finger protein 185 (LIM domain)	ZNF185	−1.12	−2.57
203771_s_at	biliverdin reductase A	BLVRA	−1.14	−1.79
219090_at	solute carrier family 24, member 3	SLC24A3	−1.15	−2.02
219597_s_at	dual oxidase 1	DUOX1	−1.16	−2.61
214279_s_at	NDRG family member 2	NDRG2	−1.18	−2.37
219104_at	ring finger protein 141	RNF141	−1.18	−2.12
209872_s_at	plakophilin 3	PKP3	−1.19	−1.90
201848_s_at	BCL2/adenovirus E1B 19 kDa interacting protein 3	BNIP3	−1.22	−1.92
55872_at	KIAA1196 protein	KIAA1196	−1.23	−1.45
57588_at	solute carrier family 24, member 3	SLC24A3	−1.23	−1.85
212659_s_at	interleukin 1 receptor antagonist	IL1RN	−1.26	−2.52
215440_s_at	hypothetical protein FLJ10097	FLJ10097	−1.28	−1.89
207469_s_at	Pirin	PIR	−1.29	−1.43
202575_at	cellular retinoic acid binding protein 2	CRABP2	−1.29	−2.84
218935_at	EH-domain containing 3	EHD3	−1.30	−2.39
210020_x_at	calmodulin-like 3	CALML3	−1.32	−3.13
203126_at	inositol(myo)-1(or 4)-monophosphatase 2	IMPA2	−1.32	−1.67
206004_at	transglutaminase 3	TGM3	−1.32	−2.61
217508_s_at	hypothetical protein MGC12909	MGC12909	−1.36	−1.94
209465_x_at	pleiotrophin	PTN	−1.37	−2.00
210096_at	cytochrome P450, family 4, subfamily B, polypeptide 1	CYP4B1	−1.38	−2.94
219983_at	HRAS-like suppressor	HRASLS	−1.39	−1.73
219165_at	PDZ and LIM domain 2 (mystique)	PDLIM2	−1.39	−2.27
206400_at	lectin, galactoside-binding, soluble, 7 (galectin 7)	LGALS7	−1.39	−2.58
204454_at	leucine zipper, down-regulated in cancer 1	LDOC1	−1.44	−1.22
221523_s_at	Ras-related GTP binding D	RRAGD	−1.44	−2.13
219529_at	chloride intracellular channel 3	CLIC3	−1.45	−2.61
208626_s_at	vesicle amine transport protein 1 homolog	VAT1	−1.55	−1.64
205623_at	aldehyde dehydrogenase 3 family, memberA1	ALDH3A1	−1.59	−2.64
211737_x_at	pleiotrophin	PTN	−1.63	−2.27

SUPPLEMENTAL TABLE 2-continued

Probe set ID	Gene name	Gene symbol	Log2 R (B/N)	Log2 R (T/N)
218484_at	NADH: ubiquinone oxidoreductase	LOC56901	−1.69	−2.66
221524_s_at	Ras-related GTP binding D	RRAGD	−1.72	−2.42
220016_at	hypothetical protein MGC5395	MGC5395	−1.74	−2.20
211734_s_at	Fc fragment of IgE, high affinity I, receptor	FCER1A	−2.03	−2.82

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The invention claimed is:

1. A method of assessing risk of adenocarcinoma in a mammal with Barrett's esophagus comprising:

(a) determining the ratio of the expression of GATA6 and SPRR3 in esophageal cells from the mammal; and

(b) comparing the ratio of step (a) to the ratio of expression of GATA6 and SPRR3 in a reference selected from the group consisting of normal esophageal epithelium obtained from the mammal at the same or different time, cells characteristic of Barrett's esophagus obtained from the mammal at a different time, and a normal range established using normal esophageal epithelium obtained from a population of individuals, an increase in the ratio of step (a) relative to the ratio of the reference being indicative of increased risk of adenocarcinoma.

2. The method of claim 1, wherein the level of expression is measured by quantitative reverse transcription-PCR.

3. The method of claim 2, wherein the level of expression is measured by real time PCR.

4. The method of claim 1, wherein the reference is normal esophageal epithelium obtained from the mammal at essentially the same time as the Barrett's esophageal cells.

5. The method of claim 1, wherein the reference is normal esophageal epithelium or second Barrett's esophageal cells obtained from the mammal prior to obtaining the Barrett's esophageal cells of step (a).

6. The method of claim 1, wherein the reference is a panel of normal esophageal epithelium obtained from a population of mammals.

7. The method of claim 1, further comprising determining the expression of a marker selected from the group consisting of HOXB7, TCF3, S100A2, and SCCA.

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