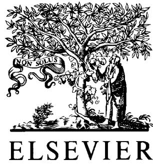

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Differentially expressed genes in gastric tumors identified by cDNA array

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Abstract

Using cDNA fragments from the FAPESP/lICR Cancer Genome Project, we constructed a cDNA array having 4512 elements and determined gene expression in six normal and six tumor gastric tissues. Using *t*-statistics, we identified 80 cDNAs whose expression in normal and tumor samples differed more than 3.5 sample standard deviations. Using Self-Organizing Map, the expression profile of these cDNAs allowed perfect separation of malignant and non-malignant samples. Using the supervised learning procedure Support Vector Machine, we identified trios of cDNAs that could be used to classify samples as normal or tumor, based on single-array analysis. Finally, we identified genes with altered linear correlation when their expression in normal and tumor samples were compared. Further investigation concerning the function of these genes could contribute to the understanding of gastric carcinogenesis and may prove useful in molecular diagnostics.
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Keywords: Gene expression; Gastric cancer; cDNA array; Molecular marker

1. Introduction

During the last 10 years, the incidence of gastric cancer is declining worldwide but, nevertheless, it is still a tumor of high incidence [1]. Worldwide, tumors of the stomach are the fourth in incidence and second in cancer-related deaths (IARC home page: http://www.dep.iarc.fr).

At the molecular level, little is known about the mechanisms involved in gastric carcinogenesis. As established for tumors in general, it was proposed that, for gastric adenocarcinomas, accumulation of genetic alterations in a multistep fashion would correlate with disease progression and differences between diffuse and intestinal type adenocarcinomas would be linked to distinct mutation pathways [2]. These genetic

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alterations can be either chromosomal aberrations or confined to mutations in one or more genes.

For chromosomal aberrations, several studies applying comparative genomic hybridization identified the 20q region as the most frequent gain. Other frequent gains were observed at 6p, 7q, 8q, and 17q and losses were at 4q, 5q, 9p, and 18q [3–5]. A high level amplification of the region 17q12-21 was observed in the intestinal type of tumors [4] and fluorescent in situ hybridization analyses using probes for either gastrin or ERBB2 revealed that both genes were simultaneously amplified [6]. Using at least two highly polymorphic microsatellite markers for each nonacrocentric chromosomal arm, an exhaustive scanning for loss of heterozygosity (LOH) revealed significant LOH at several loci such as 3p, 4p, 5q, 8p, 9p, 13q, 17p, and 18q, suggesting the presence of potential tumor suppressor genes [7].

Altered expression of genes known to play a role in oncogenic transformation has also been detected in gastric cancer, either in freshly isolated tissue or in cell lines. It is well documented that mutations in the p53 gene is a frequent event in gastric cancer and detected in as much as 50% of advanced cases [8,9]. Interestingly, p53 knockout mice, carrying either one or two mutated alleles appear to be more sensitive to experimental *Helicobacter* infection [10]. Other genes with altered expression or frequently amplified in gastric cancer are cErbB2 and c-met [11], TGF-βII receptor [12], e-Cadherin [13,14], β-Catenin [15], among others.

Another tumor type of gastric cancer that accounts for 2% of the cases is designated GIST (gastrointestinal stromal tumor) and comprises the majority of gastrointestinal mesenchymal tumors (reviewed by Miettinen and colleagues [16]). At the molecular levels, GIST is commonly associated with losses in chromosomes 14 and 22 whereas gain or high-level amplification is observed in 3q, 8q, 5p, and Xp [17,18]. Mutations in the c-Kit gene have been frequently associated with GIST [19,20] and these tumors showed a remarkably homogeneous gene expression profile [21].

More recently, several groups described the utilization of high throughput methodology in order to identify genes differentially expressed in gastric cancer [3,22–24].

The FAPESP/lICR Human Genome Cancer Project finished a major effort in sequencing over 1 100 000 ORESTES (open reading frame ESTs) derived from various tumor types and a significant proportion of yet unknown sequences were generated [25]. Taking advantage of the clone collection generated by this project, we constructed a cDNA array and searched for genes differentially expressed in normal versus tumor gastric mucosa and searched for differentially expressed genes that could distinguish between normal and tumor tissues. Detailed analysis of the genes could help in understanding the molecular events related to gastric carcinogens and also, could bring some improvement towards diagnostics and prognostics of gastric cancer.

2. Materials and methods

2.1. Tissue specimens and RNA extraction

Fresh tissues from surgically resected gastric cancers were collected by the Gastric Surgery Department from Hospital do Câncer AC Camargo, São Paulo. All patients signed an informed consent and the project was approved by the in-house ethics committee. Six gastric tumors (four adenocarcinomas and two gastrointestinal stromal tumors) and six, not paired, disease-free gastric mucosa were used. Disease-free tissue from tumor margins or obtained from radical gastrectomy was considered as normal tissue. At the time of RNA extraction, histological confirmation of normal or tumor status was performed by hematoxylin–eosin staining of frozen sections. The frozen sections were also used for dissection of samples in order to enrich for tumor cells (see Fig. 1, upper panels). Only samples with at least 70% of tumor tissue and negative for infiltrating inflammatory cells were further processed. In the case of normal samples, only gastric mucosa was used. Total RNA was extracted using TRIzol Reagent (Life Technologies, Grand Island, NY) following the procedure recommended by manufacturer.

2.2. Production of cDNA arrays

A collection of 4512 ORESTES fragments derived from the FAPESP/LICR Human Cancer Genome Project [25] was immobilized in nylon membranes.

As positive control for labeling and hybridization, we spotted, in serial dilutions, a cDNA corresponding to a fragment of the lambda phage Q gene. Bacterial clones were grown in LB medium containing 7.5% glycerol and, from each clone, the cDNA insert was amplified by polymerase chain reaction (PCR), using M13 reverse and forward primers in a final volume of 100 μl. From all 4512 PCR products, 5 μl were fractionated through a 1% agarose gel in order to quality control DNA products and the remaining 95 μl were purified with QIAquick 96 PCR purification kit (Qiagen) or Sephadex G50 (Amershan Pharmacia). Purified DNA was printed onto nylon membranes by Flexys robot (Genomic Solutions, UK) using a 96 flat pinhead in 96 blocks of 7 × 7 elements.

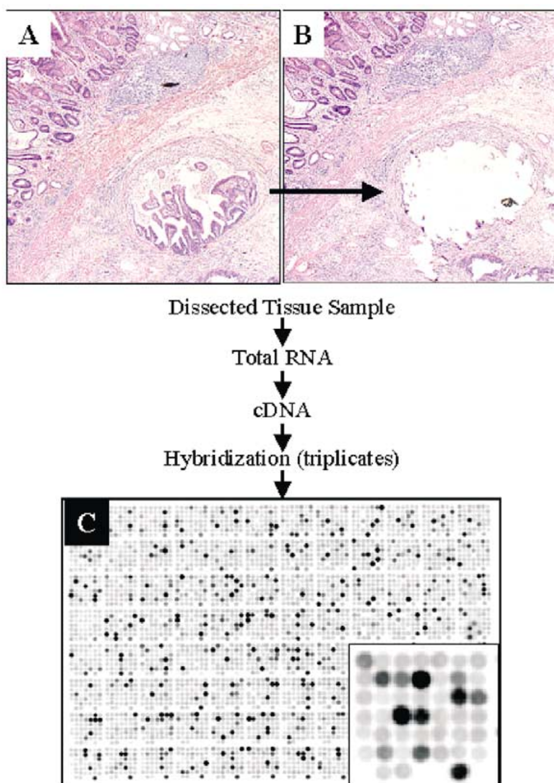

Fig. 1. Identification of genes differentially expressed in gastric tumors. Schematic representation of our experimental design. (A,B) Representation of the dissecting procedure were only the invasive portion of an adenocarcinoma was excised and processed for RNA extraction. Each RNA sample was hybridized with three identical arrays. (C) Representative image of our nylon array after hybridization with [α-^{33}P]dCTP-labeled cDNA. Signals were captured on a phosphorimager and data was acquired by the ArrayVision software using.gel files.

2.3. Labeling, hybridization, and scanning of arrays

Thirty micrograms of total RNA were contaminated with a defined concentration of synthetic, polyadenylated RNA corresponding to the lambda phage Q gene. To this mix, we added 2.0 μg $(dT)_{15}$ in a final volume of 11 μl of water, and the mix was heated to 70 °C for 10 min and subsequently cooled to 43 °C. Reverse transcription was performed in a total volume of 50 μl using Superscript II reverse transcriptase (Life Technologies Inc.) for 2 h at 43 °C in the presence of 0.25 mM each of dATP, dGTP and dTTP, 1.66 μM-dCTP and 30 μCi of [α-^{33}P]dCTP (3000 Ci/mmol; Amersham, UK). Subsequently, 1.5 μl 1% SDS, 1.5 μl 0.5 M EDTA and 3 μl 3 M NaOH were added and the RNA was hydrolyzed for 30 min at 65 °C and 15 min at room temperature. The solution was then neutralized with 1.5 μl 1 M Tris–HCl (pH 8) and 4.5 μl 2 M HCl. Probes were purified by gel chromatography (BioSpin 6; Bio-Rad). Prior to hybridization, the solution was boiled for 2 min, and then cooled on ice. Arrays were prehybridized for at least 1 h in 0.25 M Na_2HPO_4 (pH 7.2), SDS 7%, BSA 1%, 1 mM EDTA. Hybridization was conducted in the same buffer at 65 °C overnight [26]. For each cDNA sample, three identical membranes were hybridized simultaneously (normal samples correspond to membranes 1–18 and tumor samples correspond to membranes 19–36). The filters were then washed for 30 min in 0.5 M Na_2HPO_4 (pH 7.2), SDS 1%, 1 mM EDTA and image acquired by a phosphorimager (Molecular Dynamics Storm Imager, Molecular Dynamics, USA).

2.4. Data acquisition

Data acquisition was performed with the ArrayVision software (Amersham, UK), using.gel files. To quantify signal intensities of the hybridized spots, a template composed by equal-sized ellipses were drawn around all spots. Following the identification of the spots, the software calculated the spot-intensity value and array background intensity.

2.5. Data normalization

The background from a given array was subtracted from all 4512 spot-intensity values and we considered in our analysis only the 4388 genes with positive background-corrected values across all 36 arrays. Next we normalized the data in all arrays using total energy (4388 spots) on each given array.

2.6. Statistical analysis

Single genes with difference in expression when comparing normal and tumor samples were identified by their t-values, denoted by t_{nc}, which is the difference between Normal and Tumor sample-mean log-transformed gene expressions, standardized by the corresponding sample standard deviation. For a given gene, say gene k, and $j = 1, \ldots, 18$, let N_j^k denote its log-transformed gene expression on the jth normal sample and T_j^k its log-transformed gene expression on the jth tumor sample. The t_{nc} value for gene k is computed as follows:

$$t_{nc}^k = \frac{\overline{N^k} - \overline{T^k}}{\sqrt{\frac{S_{N^k}^2}{18} + \frac{S_{T^k}^2}{18}}}$$

where $\overline{N^k}$ (respectively $\overline{T^k}$) denotes gene k sample mean expression value in normal (tumor) arrays, S_{N^k} (respectively S_{T^k}) denotes its sample standard deviation in normal (tumor) arrays. We choose the normalization term for t_{nc} as in Ref. [27] even though our experimental setting is different because it penalises strongly replica measurement errors and therefore provides a simple and yet stringent statistics to evaluate differences in gene expression.

Elements with t_{nc} values equal or higher than 3.5 in absolute values were considered as differentially expressed. This set of cDNAs was then analyzed by Self-Organizing Map (SOM) and hierarchical clustering algorithms, both implemented in Matlab (MathWorks) neural networks and statistics toolboxes.

To find pairs and trios of genes that would allow perfect linear separation of Normal and Tumor samples we used a supervised learning technique known as Support Vector Machines, also implemented in Matlab (Cawley, G.C., Support Vector MachineToolbox v0.50, http://theoval.sys.uea.ac.uk/ ~ gcc/svm/toolbox, Support Vector Machine toolbox for Matlab Version 2.4, August, 2001, copyright Anton Schwaighofer (2001) mailto: anton.schwaighofer@gmx.net).

Write $(N^k = N_1^k, N_2^k, \ldots N_{18)}^k$ and $(T^k = T_1^k, T_2^k, \ldots T_{18)}^k$ for the vectors of expressions of gene k, respectively, among normal and tumor samples. To look for pairs of genes whose coordinated patterns of expression would change in comparing the two conditions we computed, for each pair of genes k and l, their Pearson linear correlation coefficient among normals, $\mathrm{corr}(N^k, N^l)$, and among tumors, $\mathrm{corr}(T^k, T^l)$.

3. Results

In order to determine the profile of gene expression in gastric tissues, we isolated total RNA from six tumor samples and from six samples of disease free gastric mucosa. For each sample, three identical nylon arrays were simultaneously hybridized, giving 18 membranes corresponding to normal tissue and 18 membranes corresponding to tumor tissues. Fig. 1 represents a scheme of our experimental design.

3.1. Identification of 80 cDNAs differentially expressed in gastric cancer

The data obtained from all 36 membranes were normalized by total energy as described in Section 2. Therefore, after normalization, all our 36 arrays have the same total expression values and one can meaningfully compare gene expressions from different arrays [27,28].

With normalized data, we computed the t-statistic, t_{nc}, for each single cDNA. In Fig. 2A, we plotted data from all 4388 cDNA clones based on their t_{nc} value. Fig. 2B represents the histogram with the t_{nc} values and in Fig. 2C we represent a quantile–quantile plot of this data versus theoretical quantiles from a normal distribution. The heavy tails of the empirical distribution of t_{nc} indicate the presence of several genes whose expression levels differ between normal and tumor samples.

Before the application of more elaborated, but also more computer-intensive, exploratory methods, it is quite natural to first select a smaller subset of genes to

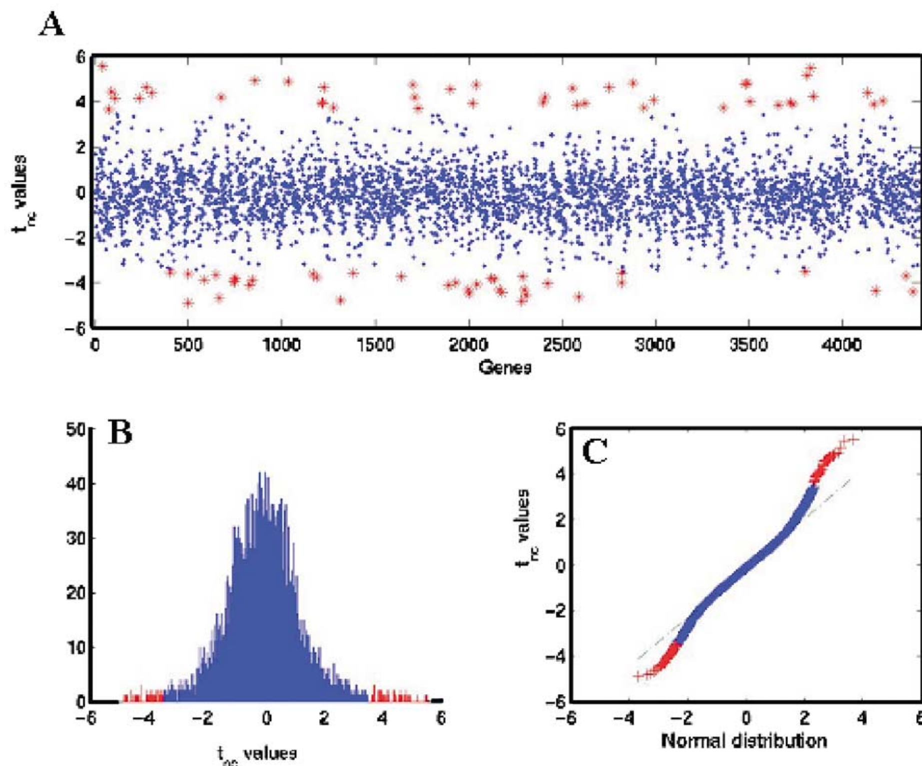

Fig. 2. Distribution of t_{nc} values for 4388 cDNAs tested in gastric tumors: Normalized data from 36 arrays hybridized with complex cDNA probes derived from normal or tumor samples were used to compute the t_{nc} value for each of the 4388 cDNAs. (A) Representation of the t_{nc} value for each single cDNA. In red, we represent 80 cDNAs with t_{nc} greater than 3.5 in absolute values. (B) Histogram representing data from (A). (C) Quantile–quantile plot of data represented in (A) against the expected value from a normal distribution.

deal with. This important step, sometimes called feature selection [29,30], was done here with the help of t_{nc} as genes with larger t_{nc}, in absolute value, are good candidates for playing a role in carcinogenesis as well as in the discrimination among normal and tumor tissues. We arbitrarily choose a threshold of 3.5 for t_{nc} we found a set of 80 cDNAs, 43 with t_{nc} larger than 3.5 (indicated in green in Fig. 3) and 37 with t_{nc} smaller than -3.5 (indicated in red in Fig. 3). All these 80 cDNAs were sequence verified.

In Fig. 3, we have a graphic representation of all 80 differentially expressed cDNAs with their respective t_{nc} value. As can be observed, five genes are represented by two or more distinct cDNA fragments. Ribosomal protein L 10 (RPL10) is represented by five cDNA clones, α2-glycoprotein 1 is represented by three clones, and metallothionein IG, Elongation Factor 1-α1, and lactate dehydrogenase A are represented by two clones. Clones representing the same gene showed very similar t_{nc} values and appeared together in the same side of Fig. 3, confirming the reproducibility of our experimental conditions and the consistence of our statistical analysis. From these 80 cDNAs, we identified 35 known genes, 31 ESTs with no functional annotation and three ORESTES sequences not yet submitted to GenBank. If a more relaxed threshold for t_{nc} is used, namely 3 instead of 3.5, 61 extra cDNAs are identified and a list with these 141 cDNAs can be visualized in our web page (http://www.array.ludwig.org.br/gastriccancer/canlettersmeireles). The sequence of all 141 cDNAs was verified experimentally.

We selected ten genes in order to experimentally confirm their differential expression in 26 new RNA samples (13 from normal tissue and 13 from tumor tissue). The levels of mRNA were estimated by RT–

PCR followed by Southern blot and phosphorimager analysis. For normalization, we used three distinct housekeeping genes (β-actin, α-tubulin, and TBP) and, for each gene, we determined its arbitrary expression unit (ratio of signal for gene/normalizing gene). A gene was considered as confirmed when the

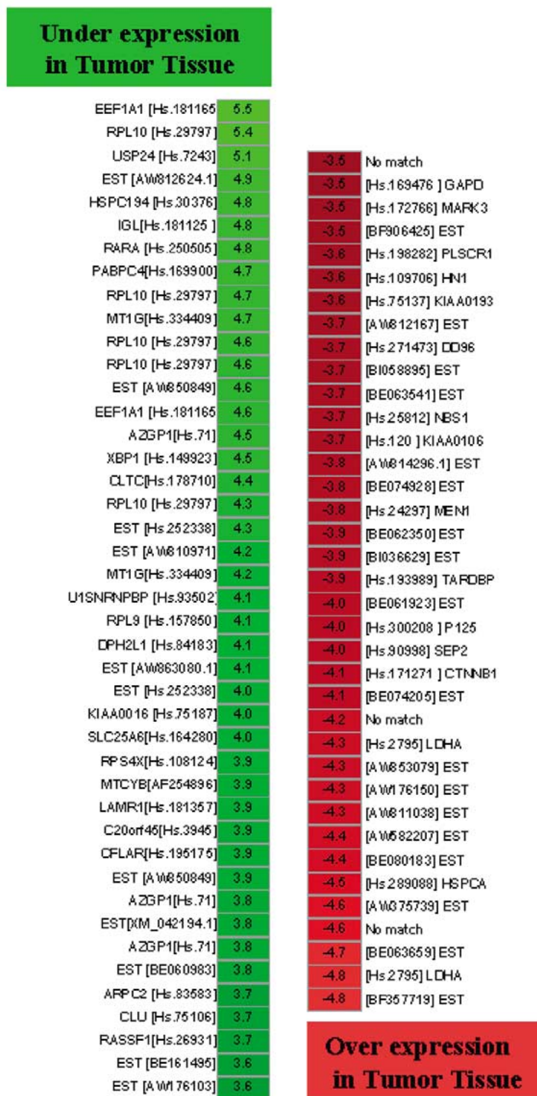

Fig. 3. Genes with differential expression in gastric tumors. Based on data presented in Fig. 2A, we list genes (with respective accession numbers indicated within brackets) and indicate their t_{nc} values. In green, genes with lower expression in tumor tissue (positive t_{nc} values); in red, genes with higher expression in tumor tissue (negative t_{nc} values).

ratio of its average expression units (normal/tumor) followed its t_{nc} value. Seven of the ten genes (RPL10, CLTC, EEF1A1, TARDBP, HSPCA, NBS1, Est AW812624) could be experimentally confirmed. Nevertheless, validation of array data by RT–PCR must take in consideration the tremendous variability of housekeeping genes [31] and, more importantly, that in our case, a gene can have a high t_{nc} value even if its fold change in rather small. Similar observations were published by [32]. For instance, in our array data, β-Catenin differs only 1.3-fold between normal and tumor samples but its t_{nc} value is 4.32 due to its small SD.

3.2. Clustering algorithms: SOM and hierarchical

After selecting the 80 cDNA clones with absolute t_{nc} value higher than 3.5, we applied a SOM algorithm [33] to identify clusters of expression profiles according to samples. Two clusters were identified and they represented a precise separation of normal and tumor samples (data not shown). When we applied a hierarchical cluster algorithm, we observed that all replicas from a given patient are grouped together, further confirming reproducibility of our data (data not shown). Next, we applied again the SOM algorithm, now to separate genes according to their expression across all 36 membranes, into six clusters. In Fig. 4, we represent these clusters and, within each cluster, we further ordered genes according to their hierarchical distance, as indicated by each dendrogram.

3.3. Genes with coordinated pattern of expression

Next we used a supervised computer learning method called Support Vector Machine (SVM) to search for trios of genes with a coordinated pattern of expression. We searched the dataset corresponding to the 80 cDNAs with t_{nc}, in absolute value, larger than 3.5 to find trios of genes whose pattern of expression in individual membranes would be such that, when plotted on three dimensional space, a plane could be found separating perfectly the 36 data points into two groups, one with 18 normal samples and another with the remaining 18 tumor samples. We found several interesting trios with this property. One trio is composed of β-Catenin, Clathrin, and Retinoic Acid

Receptor-α (Fig. 5A) and represents genes that can be mapped into a common biochemical pathway known to be implicated in gastric carcinogenesis. Another trio is composed of Ribosomal Protein L10, Humanin, and β-Catenin (Fig. 5B).

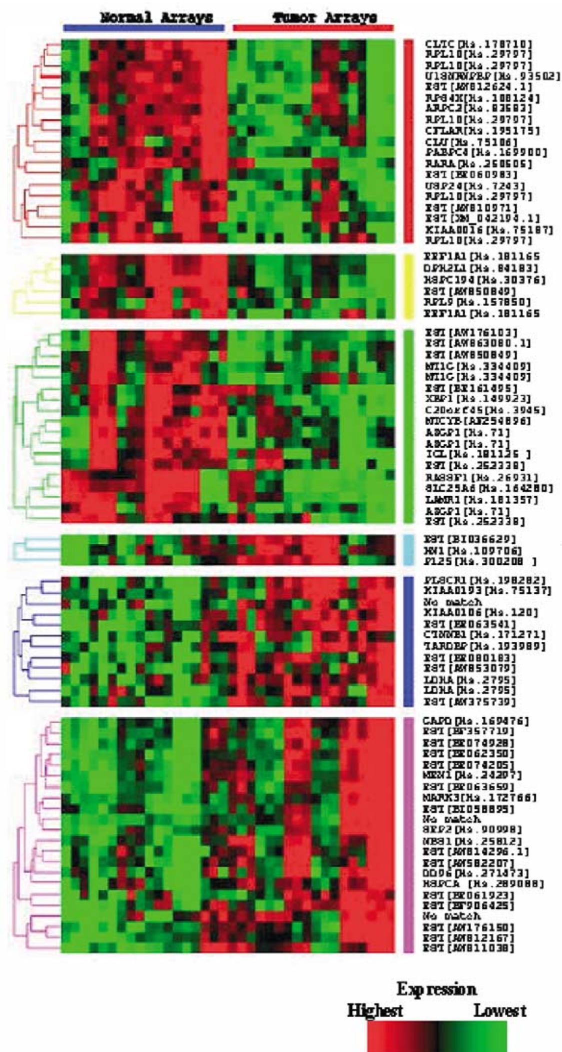

Fig. 4. Schematic representation of samples and genes clustered by Self-Organizing Map (SOM). Using the 80 cDNAs with t_{nc} higher than 3.5 in absolute values we applied SOM to cluster samples based on the expression profile of the 80 cDNAs. The resulting two clusters are represented at the top of the figure by the blue and red bars. Next, cDNAs were grouped into six clusters based on their log-transformed normalized signal intensity. For each cDNA, a maximum value is represented in bright red, minimum value in bright green and the intermediate value in black. At the left side of each cluster is a dendrogram representing hierarchical distances.

3.4. Genes with changes in their Pearson linear correlation

We also looked for pairs of genes whose pattern of expression would show changes in their Pearson linear correlation when normal and tumor samples

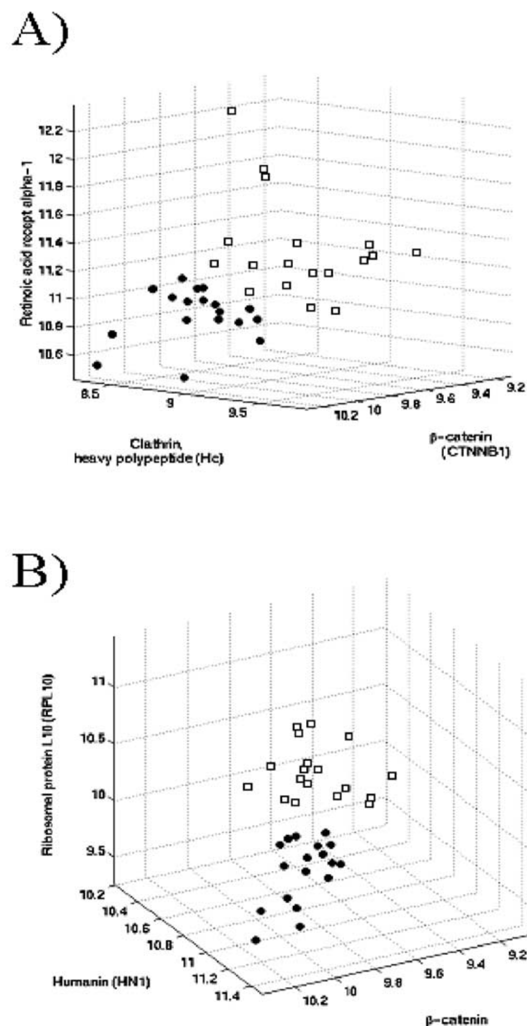

Fig. 5. Trios of genes that allow sample classification. From a gene list having cDNAs with absolute values of t_{nc} greater than 2, we applied the SVM algorithm and identified trios of genes that allowed perfect separation of all 18 normal and 18 tumor arrays. (A,B) Three-dimensional space where the log-transformed normalized signal intensity for each cDNA is plotted. Each data point in space represents one individual array; data from normal samples are represented in open squares and tumor samples are represented by dots.

were compared. For this search, we use a list of 432 cDNAs that showed t_{nc} value greater than 2.0 (in absolute value). We constructed a scatter plot where, to each pair of genes, we associate a point on the plane with coordinates given by their log-transformed normalized signal intensity on normal samples and in tumor samples. As one would expect, we found that all five clones corresponding to different cDNA fragments of Ribosomal Protein L10 had a strong positive correlation among themselves, both in normal and tumor samples. However, all five RPL10 clones showed strong positive linear correlation with MARK3 on normal samples but negative correlation on tumor samples (Fig. 6). Another gene group exhibiting this sort of correlation change is composed by Ras association domain family 1 (RASSF1), α2-glycoprotein 1 (AZGP1) and Metallothionein 1G. RASSF1 has strong positive correlation with the expressions patterns of two cDNA segments representing Metallothionein 1G in normal samples but very small correlation on tumor samples. Moreover, Metallothionein 1G has strong positive correlation with α2-glycoprotein 1 in normal samples but small correlation in tumor samples (data not shown).

4. Discussion

Gastric cancer is the second cause of cancer-related death worldwide. This observation can be explained, at least in part, by the fact that gastric cancer does not respond well to chemotherapy and/or radiotherapy, leaving surgery as the treatment of choice [34,35]. Efforts towards early diagnosis of gastric cancer are regarded as high priority since it would allow more conservative procedures, improving survival and quality of life. And as in the case of many other tumors, the molecular events related to oncogenesis of gastric cancer are not well understood. Thus, identification of genes with differential expression in gastric cancer will certainly have a positive impact in this field [36]. Such genes would be prime suspects in sharing some responsibility on the onset, development, or behavior of gastric cancer and good

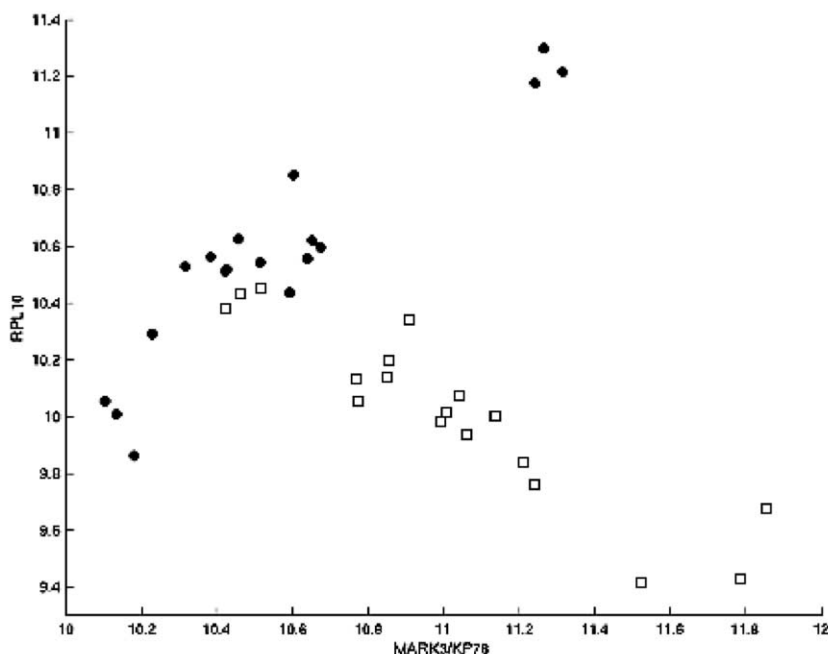

Fig. 6. Linear Pearson correlation coefficient between RPL10 and MARK3 expressions. The figure represents a scatter plot where, to each array, we associate a point on the plane representing the log-transformed normalized signal intensity for RPL10 and MARK3 on normal samples (dots) and on tumor samples (open squares).

candidates as markers for diagnosis [37]. Using cDNA arrays, we searched for genes modulated in gastric tumors and applied several statistical tools to identify correlations in their expression pattern.

We first identify single genes, whose expression would be different in normal and cancer samples. Instead of considering differences in fold expression, as is usual in the literature, we computed the *t*-statistic for each cDNA. The well-known problem in evaluating differences in expression simply by fold change is that one does not know whether a fixed value, for a given gene, is sufficiently large to characterize that gene as differently expressed without taking into account its variation of expression across all samples. By using the *t*-statistic this variation is taken into account and genes that can be considered differentially expressed would be those with larger t_{nc}, in absolute value. Among the genes with differential expression, we identified well-known tumor suppressor genes and proto-oncogenes, known to play a role in gastric cancer. We found that RASSF1, known as tumor suppressor gene [38], was underexpressed in tumor samples ($t_{nc} = 3.7$). Loss or abnormal down-regulation of RASSF1 is observed in a considerable proportion of lung, breast, ovarian, bladder, nasopharyngeal [39–44] and, more relevant, in gastric adenocarcinomas [45].

We also detected overexpression of known oncogenes. Of notice, β-Catenin was overexpressed in tumor samples ($t_{nc} = -4.1$). The role of the WNT pathway in development and oncogenesis has been widely investigated [46–48]. In the case of gastric tumors, e-Cadherins and β-Catenin are of particular importance [13,49,50]. Indeed, mutations in e-Cadherin gene has been associated with familial cases of gastric cancer [51,52].

Interestingly, two other genes involved in the WNT pathway were also found as differentially expressed in our samples. Clathrin and Retinoic Acid Receptor α were both underexpressed in tumor samples ($t_{nc} = 4.4$ and 4.8, respectively). Reduced levels of Clathrin leads to reduced recycling e-Cadherin, lowering its level at cell surface and, as consequence, more β-Catenin would be available in the cytoplasm for signaling via interaction with LEF/TCF [53,54]. Recently, it was shown that retinoic acid (RA) decreases the activity of the β-Catenin-TCF/lEF signaling pathway by inducing ubiquitin-dependent degradation of cytoplasmic β-Catenin as well as by competition with TCF for β-Catenin binding [55,56]. Thus reduced levels of Clathrin and RAR might also contribute to increased WNT signaling.

Two other genes identified as overexpressed in tumor samples might have important implications in the oncogenesis of gastric cancer, Nibrin ($t_{nc} = -3.7$) and Humanin mRNA ($t_{nc} = -3.6$). Nibrin is a member of the Mre11/Rad50/Nbs1 complex, implicated in numerous aspects of double-strand break repair, and considered as a typical tumor suppressor gene (reviewed by Wang [57]). In agreement with our data, Nibrin mRNA was also found to be augmented in GIST [21]. This is also confirmed by SAGE analysis (http://cgap.nci.nih.gov/Pathways). It is possible that, based on the findings by Paull and co-workers [58], Nibrin overexpression might favor the nucleolytic activity of the Mre11/Rad50/Nbs1 complex. Humanin was recently described as a small polypeptide that could rescue neuronal cells from specific death signals [59,60]. To the best of our knowledge, this is the first report of augmented expression of Humanin in tumor tissues and its overexpression by cancer cells could represent yet another survival signal, favoring tumor development.

Based on published observations, it is clear that molecular classification of cancer is not only feasible but also, might prove to be the method of choice to identify sub groups of a given tumor [61–65]. Having identified these 80 cDNAs, we applied other statistical tools to classify our normal and tumor samples. It has been suggested that the SOM has some important advantages for interpreting gene expression patterns, when compared to other clustering algorithms [66]. When we applied SOM to group samples, two predominant clusters of expression profile were identified and they could precisely separate normal and tumor samples (Fig. 4). Using a support vector machine algorithm, recently described as a tool to build classifiers for cancer samples [63], we performed an exhaustive search for trios of cDNAs that would allow precise separation between normal and tumor samples. We identified several trios composed by the 80 genes from Fig. 3 that, when plotted on a three-dimensional space, normal and tumor samples could be precisely separated by a plane (Fig. 5). It is possible that combination of various trios with the

properties described here might have an added accuracy for molecular classification when compared to a list of differentially expressed genes as currently suggested [62,65,67]

The identification of these trios was based on their simultaneous expression levels on each given array and one could use this information to investigate whether, in such trios, the genes would fall into a common biochemical pathway or whether they belong to distinct pathways that, together, would point to some metabolic advantage for tumor cells. Indeed, the three genes from Fig. 5A, Clathrin, β-Catenin, and Retinoic Acid Receptor can all be mapped into a common pathway, as discussed earlier. In other trio (Fig. 5B), the three genes cannot be directly linked to a single pathway. RPL10, might have tumor suppressor activities and negatively regulate c-Jun activity [68]. Thus, reduced RPL10 and augmented β-Catenin in tumor samples would favor mitogenic signals, whereas elevated Humanin could provide a survival advantage for tumor cells, as mentioned above.

We also searched for genes with change in their linear Pearson correlation. This kind of analysis would allow the identification of genes whose expression occurs in a coordinated fashion in one group of samples but either are not correlated or, perhaps more interestingly, with inverse correlation in the other group. Importantly, it could be that, genes with this behavior might have low t_{nc} values in absolute numbers and thus not identified as differentially expressed. In Fig. 6, we represent the changes in linear correlation between RPL10 and MARK3. This pair of genes has a positive linear correlation in normal samples that changes to a negative linear correlation in tumor samples. As we discussed before RPL10 might function as a negative regulator c-Jun-mediated mitogenic pathway. In contrast, overexpression of β-Catenin and consequent activation of the WNT pathway activates c-Jun gene expression [69] and, possibly, MARK3 [24,70]. Hence, in tumor cells, a negative linear correlation, would favor a mitogenic signaling pathways.

Finally, it is clear that gastric adenocarcinomas and gastrointestinal stromal tumors are consequences of the transformation of different cell lineages and hence, we made no efforts neither in distinguishing nor in comparing these two tumor types. Intentionally, we simply looked for genes with conserved alterations in all tumor samples. It is not surprising that a common set of genes can be identified in two distinct tumor types. As discussed above RASSF1, Clusterin, β-Catenin, and many others genes are commonly altered in a variety of tumors. Specifically, NBS1 that we identified as overexpressed in tumor samples was also found augmented in GIST by Allander and co-workers [21]. As expected, we did identify differences in the expression profile of the two tumor types, especially in genes from cluster 1 (uppermost cluster, second and third last triplicates from the right). However, based on our findings (Figs. 5 and 6) we can suggest that, as for gastric adenocarcinomas, the WNT pathway might also be altered in GIST. We can also conclude that classifiers based on genes commonly altered in adenocarcinomas and GIST can precisely distinguish both tumor types from normal gastric mucosa (Fig. 5) and this would imply that, regardless of differences in oncogenesis, a single classifier could be applied for gastric tumors.

Taken together, the information extracted from our dataset can contribute to the better understanding of oncogenesis of gastric cancer as well as to the development of molecular-based diagnostic tools.

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