Molecular classification of green tea catechin-sensitive and green tea catechin-resistant prostate cancer in the TRAMP mice model by quantitative real-time PCR gene profiling

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We previously found that human prostate cancer (CaP) progression is accompanied by differential expression of a panel of 8 informative genes, some of which are metabolically related. Gene profiling focused on this 8-gene pack by northern blot analysis in combination with standard clinical information provided reliable prognostic prediction of human CaP. For a better insight into the potential of this 8-gene signature in tumor detection/classification and therapeutic response, we determined, by qPCR, the expression of these informative genes in the TRAMP mice model of CaP progression. The 8-genes signature resulted effective in discriminating, by linear discriminant analysis (LDA), the prostate of wild type mice from transgenic TRAMP mice developing CaP (P < 0.0002). Since it is known that Green Tea Catechin (GTC) administration to TRAMP mice results in a substantial delay of CaP progression in 80% of the animals, while 20% remain unresponsive, we determined the 8-gene signature in the prostates of GTC-sensitive and GTC-resistant mice. LDA discriminated benign tissue from CaP (i.e. wild-type + chemoprevented, GTC-sensitive TRAMP mice, in which CaP progression was delayed, was discriminated from TRAMP mice + GTC-resistant TRAMP mice, in which CaP developed irrespective of GTC administration; P < 0.01). Moreover, GTC-sensitive TRAMP mice bearing CaP were discriminated from GTC-resistant ones, (P = 0.0001). These results show that qPCR gene profiling, based on the signature of the 8-genes selected by us, could represent an appropriate means for studying the biological behavior of CaP, which may lead to identifying new tools of potential prognostic value, in that a molecular classification for the presence/absence of cancer and for discriminating GTCs-responsive from GTC-resistant CaP is provided.

Introduction

It is widely believed that, in the near future, prostate cancer (CaP) will be the most relevant cause of cancer death in the Western Countries. In fact, CaP incidence is steadily increasing in these populations in strict relation to aging. Due to the fact that prognosis is generally unfavorable when CaP spread to other tissues, becoming androgen-independent and refractory to hormonal therapy, detection of CaP at early curable stage is highly desirable. Unfortunately, screening methods actually available present limitations, especially if based on determination of total serum prostate specific antigen (PSA) when the prostate gland is still on site. Thus, new molecular markers for early screening and a better clinical management of CaP are urgently needed. Many efforts have been recently made using gene expression analysis to increase our knowledge about the biology of CaP (1).

Molecular or gene signatures are used to model clinically relevant information (e.g. prognosis, survival time, sensitivity to drugs, etc.) as a function of gene expression data obtained at the mRNA level, but instead of individual genes, the signature components, also defined ‘metagenes’, are used as predictors. We previously identified a gene signature, comprising a selected group of 8 informative genes, which provided reliable prognostic prediction of human CaP. The prediction was further enhanced when molecular data were used in combination with standard clinical information (2). By this finding, we showed that molecular characterization of CaP progression is indeed possible. The gene signature was obtained by assessing the expression level of a subset (or ‘metagene’) of metabolically related genes, such as ornithine decarboxylase (ODC), ornithine decarboxylase antizyme (OAZ), adenosylmethionine decarboxylase (AdoMetDC), spermidine/spermine N1-acetyltransferase (SSAT) (the regulatory genes of the polyamine metabolism), another subset of genes (metagene) related to cell cycle progression, such as histone H3 and growth arrest specific gene 1 (Gas1) [specific markers of S- and G0-phases, respectively, (3) and (4)], gyceraldehyde 3-phosphate dehydrogenase (GAPDH) (involved in the glycolitic pathway) and clusterin (CLU). CLU (also known as SGP-2, TRPM-2 or ApoJ) is a gene highly over expressed during prostate gland involution and remodeling (5,6) and down-regulated during CaP progression (7–9). In our hands, all the genes studied exhibited strictly related and highly significant coexpression during the progression of CaP (7). For instance, specific alterations of the levels of transcripts belonging to the polyamine metabolism metagene were found to be one class of major events in the metabolic derangement leading to neoplastic transformation (7). Recently, a meta-analysis study (10) validated the ODC, OAZ, AdoMetDC and SSAT metagene in CaP, and differential gene expressions of CLU and Gas1 were also confirmed (11) (http://genome-www.stanford.edu/microarray).

In this paper we proceeded further by studying the same 8-gene signature previously identified (2,7), in the

Abbreviations: ADO or AdoMetDC, adenosylmethionine decarboxylase; CaP, prostate cancer; CLU, clusterin; GAPDH, gyceraldehyde 3-phosphate dehydrogenase; Gas1, growth arrest specific gene 1; GTCs, green tea catechins; LDA, Linear Discriminant Analysis; OAZ, ornithine decarboxylase antizyme; ODC, ornithine decarboxylase; PIN, prostate intraepithelial neoplasia; qPCR, quantitative PCR; SSAT, spermidine/spermine N1-acetyltransferase.
pre-clinical TRAMP mouse model of CaP progression. The TRAMP model of prostate carcinogenesis is a well known autochthonous transgenic animal model (12) that was developed as an important tool for understanding the progression of CaP. The TRAMP model displays in situ and invasive carcinoma of the prostate, mimicking the whole spectrum of human CaP progression from prostate intraepithelial neoplasia (PIN) to androgen-independent disease (13). TRAMP mice express SV-40 TAg antigen under the control of the prostate-specific minimal rat probasin promoter. Recent studies have demonstrated that administration of Green Tea Extracts (GTCs) can effectively inhibit CaP development in this model (9,14), and recently we showed that histone Gas1 mRNAs were differentially regulated in the TRAMP mice with respect to wild-type controls. In the same animals, we also validated CLU (one gene of the 8-gene signature) at mRNA and protein level, showing that it is down-regulated during CaP onset and progression, while maintained to high levels in the prostate of TRAMP mice in which CaP development was strongly inhibited by administration of GTCs. In the latter animals, induction of pro-apoptotic nuclear CLU was detected at an early stage of treatment. On the basis of these (9) and other experimental evidence (8,15–18) we have suggested a possible role for CLU as a novel tumor-suppressor gene in the prostate gland.

Gene expression signatures are important for diagnostic purposes by displaying genes that are related to a particular pathological condition only at transcriptional level on the basis of their coexpression. Thus, the aim of this study was to check whether the 8-gene signature alone, obtained by Quantitative Real-Time PCR (qPCR), without integrating other pathological or clinical data, could generate enough information for molecular classification of tissue specimens on the basis of the presence or absence of tumor and the response to application of a chemoprevention protocol in this well-defined model of CaP progression. It is worth highlighting that, due to the fact that our experimental data concerns only the levels of mRNA coded by these genes, and considering that most of the genes studied are known to be regulated at post-transcriptional, translational and post-translational level, a functional cause and effect interpretation of such data will not be systematically attempted in this work. The conclusions drawn here are entirely based on determination of the 8-gene signature for molecular classification of prostate tissue specimens in the TRAMP model. The qPCR experimental data have been used as such for classification using appropriate statistical analysis tools, with the aim of discriminating presence/absence of CaP in prostate specimens (molecular diagnosis) and tumor susceptibility to growth inhibition following GTCs administration (molecular prognosis, based on final outcome of treatment at 24 weeks of age) working with a previously established and well-defined experimental protocol that we have already described in detail (9).

Methods

Animals

TRAMP mice, heterozygous for the PB-Tag transgene, were maintained in a C57BL/6 background. Transgenic males for the studies were routinely obtained as [TRAMP × C57BL/6]F offspring. Purification of mouse-tail DNA and PCR screening were performed routinely as described previously (9,12). Parental C57BL/6 animals were used as ‘wild-type’ controls.

Chemoprevention with GTCs

Chemoprevention by GTC administration in drinking water was carried out as previously described (9). GTCs were extracted from green tea leaves and their content and purity were assessed by HPLC (EGC 5.5%; EC 12.2%; EC3G 51.9%; ECG 6.1%; total GTCs 75.7%, caffeine <1.0%) (9). Mice were given 0.3% GTC extract in tap water, made fresh every day.

In this study, 4 different experimental groups of mice were considered: (i) wild-type (C57BL/6; WT); (ii) TRAMP (transgenic mice not treated with GTCs spontaneously developing CaP; TRAMP/CaP); (iii) GTC-sensitive TRAMP (transgenic mice, genetically identical and not distinguishable from the previous ones, successfully chemoprevented with GTCs and showing no fully developed CaP at 24 weeks of age; TRAMP/GTCs); and (iv) GTC-resistant TRAMP (transgenic mice, genetically identical and not distinguishable from the previous ones, in which GTCs chemoprevention failed and CaP fully developed at 24 weeks of age; TRAMP/GTCs/CaP). For groups i–iii, 9 animals were sacrificed at 12, 17 and 24 weeks of age to study CaP progression in a time-course fashion (total: 27 animals/group), while for GTC-resistant TRAMP, 9 mice were sacrificed at 24 weeks of age, thus representing the final outcome and definitive failure of chemoprevention treatment. Three tissue pools were made from each of the 4 experimental groups by pooling three glands together and used for qPCR.

Mice were killed by cervical dislocation and the whole prostate gland (all lobes) were quickly removed. Hematoxylin and eosin stained slides were examined in parallel for the presence of CaP, and classified according to the grading system previously described (13). Metastases were searched thoroughly in the animal body by autopic observation and the India ink staining method as previously reported (14).

Reverse transcription-real-time PCR

Total RNA was extracted from frozen TRAMP mice prostate as previously described (9). Retro-transcription and qPCR were performed individually three times (for each experimental condition) on three different prostate pools, each one containing three single glands. Five µg aliquots from each RNA preparation was electrophoresed to check for RNA quality, then 2 µg of total RNA from each experimental condition was primed with 0.75 µg of random primers (Gibco, Grand Island, NY, USA) and incubated at 42°C for 60 min in a 30 µl (final volume) reaction mixture containing 50 mM tris HCl pH 8.3, 75 mM KCl, 3 mM MgCl2, 10 mM dithiothreitol, 0.5 mM dNTPs and 300 units of Superscript II Reverse Transcriptase (Gibco). One µl of each cDNA preparation was PCR-amplified using the set of primers described below. PCR conditions were: 96°C for 30 s, 55°C for 30 s and 72°C for 60 s.

For Real-time PCR analyses of the mouse prostate glands the following primers have been used.

- **ODC**: Forward (FW): 0 - GGA ATT TGA ATC ACG TTT GT-3 ; Reverse (REV): 0 - TCA ACA GGA CTC TAG TTA TCA TCA GAG GAA GA-3 .
- **AdoMetDC**: Forward (FW): 0 - TCT CTT TGT TTG ACT GC-3 ; Reverse (REV): 0 - AGT ACT ACC TTC GGG TCT CC-3 .
- **CSAT**: Forward (FW): 0 - AGT ACT ACC TTC GGG TCT CC-3 ; Reverse (REV): 0 - AGT ACT ACC TTC GGG TCT CC-3 .
- **Gas1**: Forward (FW): 0 - GTC CAG TAC ACC ATC TTA AA-3 .
- **Hypoxanthine guanine phosphoribosyltransferase (HPRT)** as housekeeping gene.

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- **Gas1**: Forward (FW): 0 - GTC CAG TAC ACC ATC TTA AA-3 .
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The present study was conducted on a well-established in vivo animal model of CaP onset and progression, the TRAMP mouse model (12,13). Animals were sacrificed, the TRAMP/GTCs-resistant TRAMP tumors excised from the TRAMP/GTCs/CaP mice were organ-confined and no metastasis were allowed to form. Palpable tumors from both TRAMP/CaP and TRAMP/GTC-resistant TRAMP mice were obtained in a time interval allowing full development of the disease (i.e., 12, 17 and 24 weeks as done previously (9)).

The data shown are the mean value of 2^{ΔΔCT} ± SD determined in the prostate glands of mice sacrificed at 12, 17 and 24 weeks of age: wild-type controls (WT 12w, WT 17w and WT 24w, respectively), TRAMP spontaneously developing CaP (TRAMP 12w CaP, TRAMP 17w CaP and TRAMP 24w CaP, respectively), GTC-treated TRAMP in which tumor progression was inhibited (TR + GTCs 12w, TR + GTCs 17w and TR + GTCs 24w, respectively; i.e. GTC-responsive tumors) and GTC-resistant tumors collected at 24 weeks of age (TR + GTCs 24w CaP). Significant differences by t-test analysis are reported (\(P < 0.05\); \(P < 0.01\); statistical significance for each single gene is versus WT 12 w).

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Table I. qPCR data, expressed as \(2^{-ΔΔCT} \pm SD\)

<table>
<thead>
<tr>
<th></th>
<th>CLU</th>
<th>ODC</th>
<th>OAZ</th>
<th>ADOMET</th>
<th>SSAT</th>
<th>H3</th>
<th>Gas-1</th>
<th>GAPDH</th>
</tr>
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<tr>
<td>WT 12w</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
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</tr>
<tr>
<td>WT 17w</td>
<td>1.06 ± 0.023</td>
<td>0.56 ± 0.070(5)</td>
<td>0.65 ± 0.12(8)</td>
<td>1.47 ± 0.088(9)</td>
<td>1.15 ± 0.091</td>
<td>3.61 ± 0.244(3)</td>
<td>0.59 ± 0.019(5)</td>
<td>1.46 ± 0.076(3)</td>
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<tr>
<td>WT 24w</td>
<td>0.20 ± 0.008(5)</td>
<td>0.80 ± 0.010(3)</td>
<td>0.54 ± 0.017(5)</td>
<td>3.65 ± 0.818(9)</td>
<td>0.92 ± 0.093</td>
<td>1.33 ± 0.043(3)</td>
<td>0.31 ± 0.015(3)</td>
<td>0.83 ± 0.052(3)</td>
</tr>
<tr>
<td>TRAMP 12w CaP</td>
<td>0.52 ± 0.192(3)</td>
<td>0.56 ± 0.024(5)</td>
<td>0.42 ± 0.141(5)</td>
<td>1.70 ± 0.407(9)</td>
<td>0.85 ± 0.203</td>
<td>5.43 ± 0.181(3)</td>
<td>0.26 ± 0.042(3)</td>
<td>0.68 ± 0.193</td>
</tr>
<tr>
<td>TRAMP 17w CaP</td>
<td>0.87 ± 0.273</td>
<td>0.26 ± 0.067(5)</td>
<td>0.32 ± 0.110(3)</td>
<td>1.25 ± 0.461</td>
<td>0.39 ± 0.167(3)</td>
<td>5.99 ± 1.974(3)</td>
<td>0.24 ± 0.073(3)</td>
<td>0.71 ± 0.242</td>
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<tr>
<td>TRAMP 24w CaP</td>
<td>0.04 ± 0.003(5)</td>
<td>0.19 ± 0.041(3)</td>
<td>0.63 ± 0.267</td>
<td>0.46 ± 0.061(3)</td>
<td>0.11 ± 0.011(5)</td>
<td>10.36 ± 2.373(3)</td>
<td>0.13 ± 0.013(3)</td>
<td>0.97 ± 0.293</td>
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<tr>
<td>TR + GTCs 12w</td>
<td>0.62 ± 0.106(3)</td>
<td>0.54 ± 0.094(3)</td>
<td>0.17 ± 0.306(3)</td>
<td>2.46 ± 0.836(5)</td>
<td>0.89 ± 0.274</td>
<td>6.34 ± 1.483(3)</td>
<td>0.22 ± 0.041(3)</td>
<td>1.16 ± 0.080</td>
</tr>
<tr>
<td>TR + GTCs 17w</td>
<td>0.68 ± 0.279</td>
<td>0.27 ± 0.050(5)</td>
<td>0.26 ± 0.103(3)</td>
<td>1.19 ± 0.211(5)</td>
<td>0.54 ± 0.193(3)</td>
<td>2.26 ± 0.617(3)</td>
<td>0.32 ± 0.103(3)</td>
<td>0.73 ± 0.214</td>
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<tr>
<td>TR + GTCs 24w</td>
<td>1.22 ± 0.469</td>
<td>0.41 ± 0.427</td>
<td>0.19 ± 0.045(5)</td>
<td>0.74 ± 0.232</td>
<td>0.54 ± 0.141(3)</td>
<td>7.06 ± 2.592(3)</td>
<td>0.19 ± 0.072(3)</td>
<td>0.41 ± 0.146(3)</td>
</tr>
<tr>
<td>TR + GTCs 24w CaP</td>
<td>0.03 ± 0.009(5)</td>
<td>0.32 ± 0.081(3)</td>
<td>2.20 ± 0.314(3)</td>
<td>0.65 ± 0.186(3)</td>
<td>0.82 ± 0.693</td>
<td>26.20 ± 9.65(3)</td>
<td>0.42 ± 0.158(3)</td>
<td>1.96 ± 0.313 (3)</td>
</tr>
</tbody>
</table>

The data shown are the mean value of 2^{ΔΔCT} ± SD determined in the prostate glands of mice sacrificed at 12, 17 and 24 weeks of age: wild-type controls (WT 12w, WT 17w and WT 24w, respectively), TRAMP spontaneously developing CaP (TRAMP 12w CaP, TRAMP 17w CaP and TRAMP 24w CaP, respectively), GTC-treated TRAMP in which tumor progression was inhibited (TR + GTCs 12w, TR + GTCs 17w and TR + GTCs 24w, respectively; i.e. GTC-responsive tumors) and GTC-resistant tumors collected at 24 weeks of age (TR + GTCs 24w CaP). Significant differences by t-test analysis are reported (\(P < 0.05\); \(P < 0.01\); statistical significance for each single gene is versus WT 12 w).
The expression level of CLU can be considered an internal validation of the experimental protocol applied. In fact, in Table I and Figure 1, down-regulation of CLU transcription was again confirmed in the TRAMP animals at 12w and 24w, while CLU expression was re-established in the GTCs chemoprevented TRAMP at 24w, as previously found (9). The lowest level of CLU transcription was detected in 24w GTC-resistant CaP, a finding that is again consistent with our previous report (9).

Validation of the mRNA level of expression data was carried out by northern blot on the same RNA preparations used for qPCR. We focused on 24w TRAMP mice treated or not with GTCs and GTC-sensitive/GTC-resistant specimens. The results obtained by northern blot were consistent with qPCR data (Figure 2).

ΔCt qPCR data were used for LDA to check whether discrimination of benign tissue from its cancer counterpart could be achieved on the basis of gene signatures alone, different from what we have shown to occur with human CaP, where the best score was reached when also including clinical data (2).

LDA results are shown in Table II. In panel A it is shown that the gene profiling of prostates obtained from wild-type mice (controls, C57BL/6) is significantly different (P < 0.0002) from that obtained from all other experimental groups comprising TRAMP animals, irrespective of the presence of cancer tissue. This can be considered as expected, since the prostate tissue from TRAMP mice express high levels of SV-40 T/t antigens, an event that causes a profound and general alteration of gene expression (22). More importantly, gene signature of benign prostate tissue from WT controls and TRAMP/GTC mice grouped together (no fully developed cancer in both groups), was significantly different from that of untreated TRAMP mice bearing cancer (TRAMP/CaP) and TRAMP animals treated with GTCs but refractory to chemoprevention (TRAMP/GTC/CaP), also grouped together, both animal groups bearing organ-confined prostate carcinoma (Table IIB; P < 0.01). The above is an important confirmation, in the well-defined TRAMP mice model, of the discrimination power of our 8-gene signature that we have previously reported with human CaP (2). Even in the case of a heterogeneous tumor-like prostate carcinoma, and in different biological models such as mouse and human prostate, changes in the expression of the 8 informative genes are involved in cancer progression, probably because prostate cell transformation is accompanied by disruption of the coordinate expression of a particular set of genes (metagenes) controlling the levels of important specific metabolites in a tissue-specific manner. This result is further confirmed in the context of the same genetic background and in the presence of SV-40 T/t antigens expression (i.e. TRAMP mice). In fact, in Table IIC it is shown that specimens from animals responsive to treatment [TRAMP/GTCs, i.e. GTC chemoprevented TRAMP mice without fully developed CaP at 12, 17 and 24 weeks of age, but only showing pre-neoplastic lesions such as high-grade prostate intraepithelial neoplasia (HG-PIN) and hyperplasia] were significantly (P < 0.0001) discriminated from GTC-resistant TRAMP CaPs (TRAMP/GTCs/CaP, i.e. TRAMP mice bearing fully developed tumors at 24 weeks of age in spite of GTC treatment starting immediately after weaning). Most importantly, Table IID shows that the 8-gene signature of CaP that spontaneously developed in TRAMP mice not receiving GTCs (TRAMP/CaP) was significantly different (P < 0.0001) from that of CaP that developed in 24-week-old TRAMP mice receiving the catechins (TRAMP/GTC/CaP, i.e. GTC-resistant TRAMP tumors). CaP tissue specimens obtained from mice belonging to the two above experimental
The result shown is representative of three independent experiments.

The specific radioactive probes used for the hybridization are indicated.

Ten µg/lane of the same total RNA preparations on which qPCR was performed was loaded on each lane and resolved by electrophoresis.

A particular, 24w GTC-resistant CaPs show lower levels of high discrimination capacity of this molecular approach. In groups are hard to discriminate by morphological observation; however, they were significantly discriminated by LDA based on the 8-genes signature described here, showing a high discrimination capacity of this molecular approach. In particular, 24w GTC-resistant CaPs show lower levels of CLU and higher levels of OAZ, H3 and GAPDH transcripts when compared with spontaneously developing 24w TRAMP CaPs, a pattern very similar to that already reported in high-grade human CaP (7).

Altogether, application of LDA showed the high classification power of the 8-gene signature, which was further strengthened by the 0% misclassification ratio detected in the 12w, 17w and 24w datasets (data not shown). Especially important, in our opinion, is the result obtained with 12 week-old mice, among which, those prone to develop CaP could be discriminated on the basis of gene expression alone long before CaP fully develop, while histological and phenotypical analyses only reveal microscopic hyperplastic lesions (9,12,13).

What seems important in our study is that 12w and 17w TRAMP/CaP mice (exhibiting signs of tumor transformation at initial or intermediate stages) have been fully discriminated from TRAMP/GTCs mice of all ages (also exhibiting initial stages of transformation but not fully developed cancer). At the same time, 24w TRAMP/CaP have been fully discriminated from 24w TRAMP/GTCs/CaP. These latter biological entities look virtually identical at pathological examination. Thus, molecular classification and LDA analysis enabled us to discriminate biological entities that are virtually impossible to distinguish by simple morphological analysis.

In the light of such results, we tried to reduce the number of genes necessary for molecular classification. Thus, LDA was performed again onto the 12, 17 and 24 weeks datasets, but only different combinations of two genes at a time, extracted from the whole set of eight genes (with a total of 28 different possible combinations), were taken into consideration. Among these combinations, only the best performing ones, in terms of misclassification ratio, were chosen. At 24 weeks, the best performing combinations were CLU-GAPDH and OAZ-ADO (see Figure 3, panel A for a graphical representation of the results), with a 0% misclassification ratio over the four classes studied (datasets), while, at 17 weeks, classification with the CLU-SSAT combination produced only a 5.5% misclassification error (1 case misclassified out of 18; data not shown). Also at 12 weeks, only a 5.5% misclassification was obtained with the ADO-GAS1 and H3-GAS1 combinations (Figure 3, panel B). Interestingly, because of the fact that H3 and GAS1 are specific markers of S and G0 phases of the cell cycle, respectively, the high power of classification exhibited by this combination (metagene) at 12 weeks of age suggests that GTC administration is already affecting the proliferation rate of TRAMP mice prostate tissue at this age.

It is also worth noting that, at 24 weeks of age, the combination CLU-GAPDH can fully discriminate all four different groups (datasets). In particular, we succeeded in discriminating GTC-resistant (cyan dots) from GTC-sensitive (green dots) CaPs (Figure 3, panel A). This result is of potential prognostic relevance, because failure of GTC treatment occurred in animals that are still vital and tumor development is not at final stage. In principle, a second line treatment, based on a different agent, could be ideally hypothesized, suggesting the possibility of the use of this molecular classification and the metagene CLU-GAPDH to guide clinical management of GTC-refractory tumors.

Table II. Summary of Fisher’s linear discriminant function analysis output

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cases for Group A (WT)</th>
<th>No. of cases for Group B (TRAMP/CaP + TRAMP/GTCs)</th>
<th>No. of independent variables</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>No of cases for Group A (WT) = 9</td>
<td>No cases for Group B (TRAMP/CaP + TRAMP/GTCs) = 21</td>
<td>No of independent variables = 8</td>
<td>P &lt; 0.0002</td>
</tr>
<tr>
<td>B</td>
<td>No of cases for Group A (wt + TRAMP/GTCs) = 18</td>
<td>No cases for Group B (TRAMP/CaP + TRAMP/GTCs) = 12</td>
<td>No of independent variables = 8</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>C</td>
<td>No of cases for Group A (TRAMP/GTCs) = 18</td>
<td>No cases for Group B (TRAMP/GTCs/CaP) = 3</td>
<td>No of independent variables = 8</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>D</td>
<td>No of cases for Group A (TRAMP/CaP) = 9</td>
<td>No cases for Group B (TRAMP/GTCs/CaP) = 3</td>
<td>No of independent variables = 8</td>
<td>P &lt; 0.0001</td>
</tr>
</tbody>
</table>

Fig. 2. Northern blot autoradiogram for validation of the data obtained by qPCR. Ten µg/lane of the same total RNA preparations on which qPCR was performed was loaded on each lane and resolved by electrophoresis. The specific radioactive probes used for the hybridization are indicated. (A) 24w TRAMP/CaP; (B) 24w TRAMP/GTCs; (C) 24w TRAMP/GTCs/CaP. The result shown is representative of three independent experiments.
is that it could be exploited to explore the molecular basis of drug resistance. In fact, while the efficacy of GTCs in cancer growth inhibition has been well documented in several experimental models (25–27), little is known at the present time about the possible mechanisms by which cancer cells can escape and acquire resistance to GTC administration. Further studies are currently ongoing in our laboratory determining the 8-gene signature in prostate biopsies obtained from human CaP. If confirmed, the application of this approach to human prostate biopsies could possibly provide useful molecular information for diagnosis and prognosis of CaP (i.e. molecular classification of CaP aggressiveness and outcome). Moreover, individual response to GTCs could be studied on the same type of material collected before prostatectomy. Molecular classification of GTC-sensitive and GTC-resistant CaP by means of the 8-gene signature can take on a particular meaning for guiding the clinical management of this disease in the light of our recent result (23) demonstrating that, although CaP progression can be inhibited with 90% efficacy in HG-PIN subjects, patients unaffected by the treatment indicate a different biological behavior that needs to be further studied.

We believe that, in the near future, the molecular picture rendered by this approach based on the 8-gene signature might be of help in taking rational decisions on different therapeutic options for CaP.

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References


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