# Differential gene expression in mesothelioma

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**Abstract To investigate the molecular events controlling malignant transformation of human pleural cells, we compared constitutive gene expression of mesothelioma cells to that of pleural cells. Using cDNA microarray and high-density filter array, we assessed expression levels of >6500 genes. Most of the highly expressed transcripts were common to both cell lines and included genes associated with stress response and DNA repair, outcomes consistent with the radio- and chemo-resistance of mesothelioma. Interestingly, of the fewer than 300 genes that differed between cell lines, most functioned in (i) macromolecule stability, (ii) cell adhesion and recognition, (iii) cell migration (invasiveness), and (iv) extended cell division. Expression levels of several of these genes were confirmed by RT-PCR and could be useful as diagnostic markers of human mesothelioma.** 

*Key words:* Mesothelioma; Mesothelial cell; cDNA microarray; High-density filter array; Cancer marker

## 1. Introduction

Although the use of asbestos fibers has been banned in most industrialized countries, fibers are still a major environmental and occupational health concern. Asbestos has been identified as a known human mutagen and carcinogen [1,2], producing several thousands of cancers each year. In France alone, asbestos is responsible for approximately 2000 new cases of bronchogenic carcinomas and mesotheliomas [3]. Mesothelioma is almost unique to asbestos exposure, yet the molecular mechanism controlling the transformation of pleural cells is largely unknown. To better understand the malignant conversion of mesothelial cells, we compared the expression of 6649 mRNAs in a control mesothelial cell line (Met-5A) to that of a mesothelioma cell line (MSTO-211H) using a cDNA microarray. These results were compared to 588 selected genes by differential display using high-density filters.

In this paper we found that genes with a high level of constitutive expression that are shared in both cell lines include genes that may be important in the resistance of these particular cancer cells to radiotherapy and chemotherapy. In addition, our findings from the hierarchy clustering analyses indicated that only a few specific subdivisions differ between mesothelioma and mesothelial cell lines. Common features about these subdivisions include their roles in macromolecule stability and metabolism, cell adhesion and molecular recognition, and cell growth and migration (invasion). Each of these functions could relate to the transformation processes of mesothelioma.

## 2. Materials and methods

### 2.1. Cell lines and mRNA purification

Two cell lines used in this study, Met-5A (CRL-9444) and MSTO-211H (CRL-2081, American Type Culture Collection (ATCC), Manassas, VA, USA), were grown respectively in M199 and RPMI 1640 supplemented with fetal calf serum (10%) according to the instructions of ATCC and as described previously [4]. When cells reached confluence, the medium was replaced and 18 h later, total RNA was harvested using Trizol™ (Gibco BRL, Grand Island, NY, USA). Polyadenylated mRNAs were isolated with Oligotex™ (Qiagen S.A., Courtaboeuf, France) and were fluorescently labeled or radiolabeled for microarray or high-density filter hybridization, respectively.

### 2.2. Microarrays

Target cDNA was generated from 1 μg polyadenylated mRNA that was reverse transcribed to produce cDNA and labeled either with Cy3 (MSTO-211H) or Cy5 (Met-5A) dUTP [5]. The average intensity of the Cy3 fluorescence divided by the average intensity of the Cy5 fluorescence equaled 0.97 (balance coefficient), indicating similar labeling efficiency for each set of target cDNAs. Target cDNA was hybridized on Incyte Pharmaceuticals™ arrays containing 6969 probes with sequences complementary to 3962 human genes and 3007 human expressed sequence tags (ESTs) (Unigem V™, Genome Systems Inc., St Louis, MO, USA). Following the hybridization and washing, the relative expression level of both cDNA populations was measured and compared by making the Cy3/Cy5 fluorescence ratio for each target cDNA satisfy the inclusion criteria. Inclusion criteria were derived from an image recognition algorithm for each cDNA in the analysis and included a fluorescent signal from the cDNA exceeding a signal to background ratio of 2.5 and the cDNA covering its grid location on the microarray for >40%. In this study, genes were considered differentially expressed (DE) if the change was >2.0-fold. The cDNAs corresponding to genes of known function were sorted by enzyme, function or pathway cluster analysis using a Gemtools software (v2.4.2, Incyte Pharmaceuticals, Freemont, CA, USA). The results from this study can be downloaded (0.7 Mb file) at website: http// www.inrs.fr/actualités/amiante/GeneMesoen.htm.

### 2.3. High-density filter arrays

Oligotex™ purified mRNAs (1 μg) of both cell populations were labeled with [$^{32}$P]αdATP using specific primers and MMLV polymerase (Atlas™ Cancer Human array, Clontech). The target mRNAs ($10^6$ Cerenkov cpm) were hybridized overnight (68°C) on separate filter arrays, washed (stringent, 68°C), and the β-radioactivity of each cDNA was counted with a PhosphorImager BAS2000™ (Fuji, Tokyo, Japan) following a 12 h exposure period. Results were expressed as the percent of total labeled cDNA (relative abundance). The ratio of

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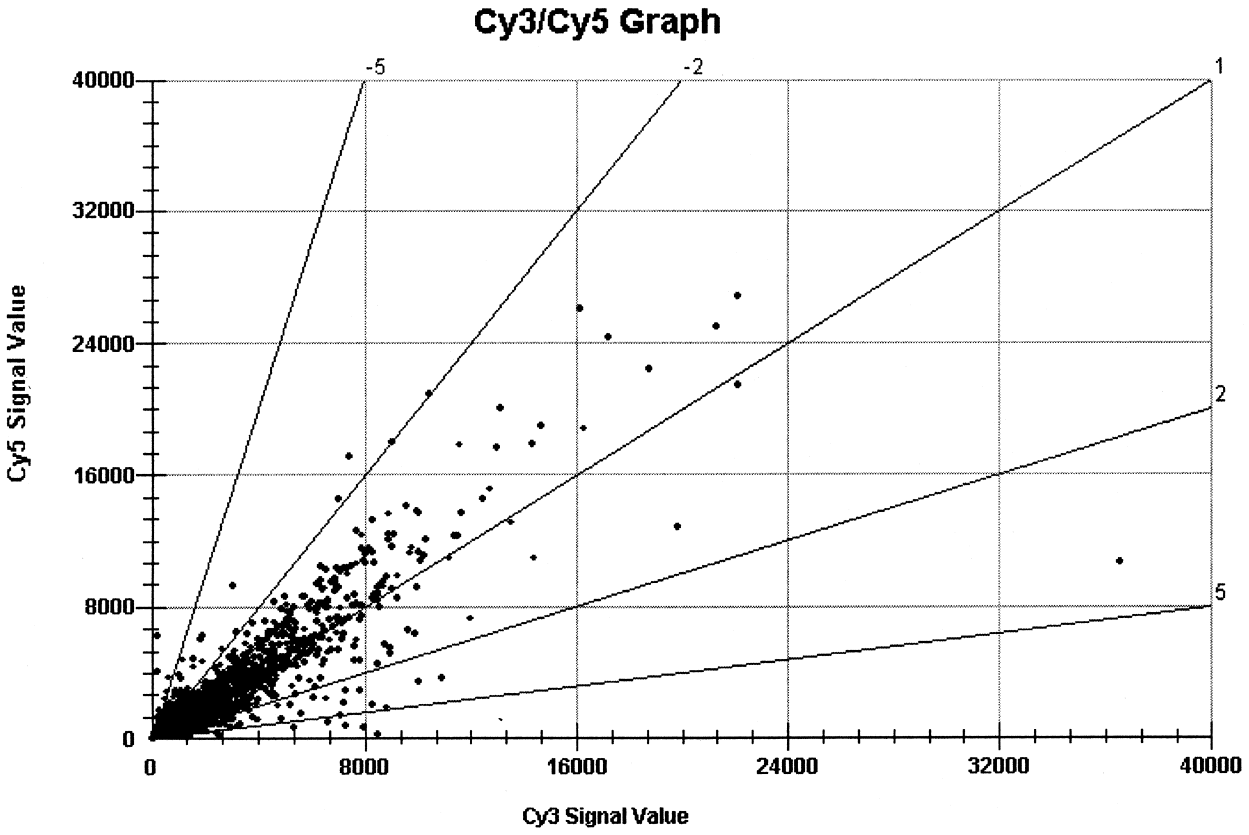


Fig. 1. Scatter plot of the DE in both cell lines. Lines correspond to the different DE levels. Each dot corresponds to a gene. The axes represent the fluorescence levels.

relative abundance of both cell populations corresponded to the DE of a given gene. The mean of at least three independent tests was calculated for each gene of interest.

*2.4. Confirmatory RT-PCR*

cDNAs of both cell lines were amplified in the presence of SYBR-Green™ (Master SYBR Green I™) in a Lightcycler™ (Roche Diagnostics, Mannheim, Germany). For each cDNA, the set of primers was chosen according to the clone sequence (Genome Systems Inc.). The $MgCl_2$ and the cycling parameters were optimized according the Lightcycler (v3, 1999, Roche). The copy ratio of each analyzed cDNA was determined as the mean of three experiments.

## 3. Results and discussion

*3.1. Microarrays*

Of the 6969 genes analyzed by microarray, 6649 (95.4%) satisfied the inclusion criteria. In mesothelioma cells, the signal intensity spanned over 10–$10^4$ fluorescent units from 65 (epithelial V-like antigen, AI819274) to 36 509 (ferritin, light polypeptide, AI608953) (Fig. 1). In mesothelial cells, the fluorescence signal had a similar range and varied from 56 (EST weakly similar to AA523426) to 27 694 (EST, Incyte number 3097582). These findings reflect nearly equivalent labeling efficiencies. The range of DE varied from +32.2 (plasminogen activator inhibitor-2, PAI-2, Y00630) to −27.1 (EGF-containing fibulin-like extracellular matrix protein 1, U03877). Respectively 0.1 and 0.3% of genes displayed a DE ranging from −39 to −5 and from +5 to +39.

Initial analysis of microarrays revealed that most genes (6356 or 95.6%) were not different in the mesothelioma cells as compared to the non-transformed mesothelial cells. However, 209 (3.1%) genes were increased ($>2$ fold) and 84 (1.3%) were decreased ($<2$-fold) in mesothelioma cells. Among these sequences in mesothelioma cells, 268 encoded known genes and 25 encoded ESTs with no homology to known genes. Inasmuch as we screened the expression of approximately 1/10 of the human genome, this finding suggests that at least 2500 known genes and 250 ESTs could be implicated in cancer transformation of mesothelial cells. Substantial efforts will be needed to elucidate these genes and their functions.

The distribution of the fluorescence signals of both cDNA populations was bimodal (Table 1). About 3000 cDNAs had fluorescence levels ranged from 49 to 499 units corresponding to low-level expression whereas 2000 cDNAs ranged from 1000 to 4999 units corresponding to moderately expressed genes. Interestingly, 20 genes had higher expression levels

Table 1
Distribution of relative expression levels in both cell lines

| Cell line | 50 000–10 000 | 9999–5000 | 4999–1000 | 999–500 | 499–49 |
|---|---|---|---|---|---|
| Mesothelioma | 31 (0.5) | 189 (2.8) | 2094 (31.5) | 1467 (22.1) | 2868 (43.1) |
| Mesothelial | 61 (0.9) | 178 (2.7) | 1965 (29.5) | 1494 (22.5) | 2951 (44.4) |

Percentage of analyzed genes in parentheses.

Table 2
Genes sorted by function clusters

| Function cluster | Similar expression | Increased in mesothelioma cells | Increased in mesothelial cells | Ratio mesothelioma/ mesothelial | Total screened genes |
|---|---|---|---|---|---|
| Signal transduction and regulation | 1173 | 25 | 9 | 2.7 | 1207 |
| Membrane transport | 164 | 2 | 2 | 1.0 | 168 |
| Protein modification and maintenance | 197 | 20 | 5 | 4.0 | 222 |
| Nucleic acid synthesis and modification | 118 | 6 | 3 | 2.0 | 127 |
| Adhesion and molecular recognition | 143 | 5 | 0 | – | 148 |
| Electron transfer | 69 | 4 | 2 | 2.0 | 75 |
| Structural and localized proteins | 2037 | 55 | 39 | 1.4 | 2131 |
| Extracellular membrane | 66 | 2 | 4 | 0.5 | 72 |
| Cytoskeleton | 161 | 3 | 4 | 0.7 | 168 |
| Total | 3901 | 117 | 63 | 1.9 | 4078 |

with a fluorescence level greater than 5000 units in mesothelioma cells. This compared to only six genes with high-level expression in mesothelial cells. Note also that about twice as many genes had a very high expression level ($>10\,000$ units) in mesothelial cells as compared to mesothelioma cells (Table 1).

The high similarity in gene expression ($>95\%$) between mesothelioma and non-transformed mesothelial cells is striking and suggests that only an additional 5% (or less) of the genome is activated during malignant transformation. This is remarkable since cell division and growth dysregulation is likely to require augmentation of numerous cellular pathways.

Categorizing genes by related function (hierarchical gene clustering) allowed assessment of the pathophysiological significance of differences in gene expression between the two cell lines. The ratios of the genes upregulated in mesothelioma compared to those in mesothelial cells were 2.8, 2.4, and 1.9 for the enzyme, pathway, and function clusters, respectively. Further subdivision of these gene clusters is shown in Tables 2 and 3. For enzyme cluster, 375 hydrolases, 372 transferases, 52 ligases, 50 isomerases and 49 lyases were screened. In mesothelioma cells, the ligase subdivision had six genes that increased as compared to only one gene in mesothelial cells. In the function cluster, the two subdivisions with the greatest differences involved genes important in 'protein modification and maintenance' and 'adhesion and molecular recognition' (Table 2). The pathway cluster had the greatest differences in the 'transmembrane trafficking', 'lipid metabolism', and 'cell division' subdivisions with ratios of the genes upregulated in mesothelioma of 6.0, 5.0, and 4.0, respectively, as compared to 2.4 (the average increase) for mesothelial cells (Table 3). In addition, the subdivision 'amino acid metabolism' contained four genes that were upregulated in mesothelioma cells that were unchanged in mesothelial cells.

### 3.2. High-density filter arrays

The largest changes in up- and downregulated genes in mesothelioma cells (mean result of three high-density filter arrays) are shown in Table 4. This analysis included several sequences contained on cDNA microarray results, but in general, most sequences were not included on the microarray. Thus, direct comparison of the results from the two methods

Table 3
Genes sorted by pathway clusters

| Cluster | Similar expression | Increased in mesothelioma cells | Increased in mesothelial cells | Ratio mesothelioma/ mesothelial[a] | Total screened genes |
|---|---|---|---|---|---|
| Metabolism | 1542 | 73 | 26 | 2.8 | 1641 |
| DNA repair | 29 | 1 | 0 | – | 30 |
| RNA metabolism | 517 | 14 | 9 | 1.5 | 540 |
| Transcription initiation | 401 | 7 | 6 | 1.2 | 414 |
| Lipid metabolism | 147 | 5 | 1 | 5.0 | 153 |
| Carbohydrate metabolism | 98 | 3 | 4 | 0.7 | 105 |
| Amino acid metabolism | 38 | 4 | 0 | – | 42 |
| Energy metabolism | 75 | 3 | 1 | 3.0 | 79 |
| Antioxidant defence | 11 | 1 | 0 | – | 12 |
| Xenobiotic metabolism | 39 | 2 | 4 | 0.5 | 45 |
| Hormone metabolism | 24 | 2 | 1 | 2.0 | 27 |
| Growth and development | 650 | 19 | 10 | 1.9 | 679 |
| Cell division | 128 | 8 | 2 | 4.0 | 138 |
| Proliferation and differentiation | 446 | 12 | 8 | 1.5 | 466 |
| Kinesis | 222 | 6 | 2 | 3.0 | 230 |
| Transmembrane trafficking | 162 | 6 | 1 | 6.0 | 169 |
| Environmental response | 111 | 9 | 5 | 1.8 | 125 |
| Inflammatory response | 70 | 2 | 2 | 1.0 | 74 |
| Total | 2595 | 109 | 45 | 2.4 | 2749 |

[a]Values correspond to the ratio of the number of genes overexpressed in mesothelioma to those overexpressed in mesothelial cells.

Table 4
Levels of common expressed genes in MSTO-211 and Met-5A cells analyzed with high-density filter hybridization

| | Ratio |
|---|---|
| *Genes upregulated in MSTO-211 cells* | |
| Rho8 protein (X95282) | 16.5 |
| Plasminogen activator inhibitor-2 (M18082) | 14.4 |
| c-*Myc* binding protein (D98667) | 10.0 |
| Nucleoside diphosphate kinase NM23 (Y07604) | 5.6 |
| Superoxide dismutase 1 (Cu/Zn) (K00065) | 3.8 |
| CD59 (M34671) | 3.6 |
| PCNA (cyclin) (M15796) | 3.4 |
| Integrin α3 (M59911) | 2.4 |
| *Genes with similar expression levels* | |
| Keratin, type ii cytoskeletal 8 (AI929696) | 1.1 |
| Proto-oncogene RhoA multidrug resistance protein (L25080) | −1.4 |
| *Genes upregulated in Met5A cells* | |
| Macrophage inhibition factor (M25639) | −2.3 |
| Basigin (L20471) | −20.5 |
| Fibronectin (X02761) | −32.4 |

The ratio was calculated as the mean of three independent experiments.

is difficult. Nonetheless, several of the genes measured by either method had comparable changes. For example, the expression level of PAI-2 was increased 14.4 times in mesothelioma cells in the high-density filter array (Table 4) and 32.2 times in the microarray. The fibronectin 1 gene that increased in mesothelial cells had a DE of −32.4 using the high-density filter array (Table 4) and −21.1 using microarrays. The level of cytokeratin 8 (AI929696), a proposed marker of mesothelial and mesothelioma cells [6], was similar in both cDNA populations (high-density filter array: +1.1, microarray: −1.4) though its expression level was high (12 356 in mesothelial cells).

### 3.3. Confirmatory RT-PCR

The expression levels of selected genes were confirmed by quantitative RT-PCR using cDNA neosynthesis quantitation by SYBRGreen™ incorporation on a Lightcycler™ fluorimeter (Roche Diagnostics). In general, several of the fold changes in high-level expression measured by microarray were similar to, although often somewhat less than, that measured by quantitative PCR. For example, in mesothelioma cells, the PAI-2 (Y00630) gene increased 33- and 90-fold using cDNA microarray and quantitative RT-PCR, respectively. Likewise, the iodothyronine deiodinase type II gene (U53506) displayed a 19.2-fold increase using cDNA microarray compared to a 40.0-fold increase using quantitative RT-PCR. At intermediate levels of expression, the results with each method were more comparable. For example, the sequence encoding the pentaxin-related gene increased in mesothelioma cells by 7.5-fold when analyzed with cDNA microarray and 7.0-fold with RT-PCR. Similarly, several downregulated genes like fibronectin 1 showed concordant results in both techniques. Genes with high DE confirmed by RT-PCR are good candidates for further validation studies as new mesothelioma markers.

### 3.4. Macromolecule stability and metabolism

Changes in macromolecular stability and metabolism were reflected by altered expression of the genes involved in protein metabolism and trafficking, nucleotide interactions, and lipid metabolism.

Protein metabolism was increased in mesothelioma cells as shown by the increased expression of four aminoacyl-tRNA synthetases (lysyl-, isoleucyl-, threonyl-, and arginyl-tRNA synthetases) the DE of which ranged from +3.7 to +2.1. In addition, proteins appeared more protected from hydrolysis as the DEs of the serine protease 11 (D87258) and cathepsin H (X16832) were respectively −3.8 and −4.0. Moreover, the correct folding and the macromolecular assembly of proteins were promoted through a variety of chaperonins. In our study the 60 kDa (M34664) and 90 kDa (M16600) heat shock proteins, CCT6A chaperonin containing TCP1 (L27706) and phosphoprotein 1 induced by stress (M86752) were overexpressed as their DEs varied from +2.9 to +4.7. Mesothelioma cells also display improved protein sorting and addressing properties. Four genes involved in protein import and addressing were overexpressed: karyopherin β-2 (U72069), preprotein translocase (X97544) and 72 kDa signal recognition particle (AF069765) which participate in respectively nuclear, mitochondrial and endoplasmic reticulum transport, and targetting of protein. In addition, transmembrane trafficking is facilitated through annexin A1 (X05908), a protein important for membrane fusion and exocytosis.

Similarly, proteins related to nucleotide interactions include genes products related to stabilization of mRNA and DNA. Degradation of mRNA, for example, may be less pronounced in mesothelioma cells as indicated by altered expression of genes encoding the ribonuclease L inhibitor (X76388, DE +4.8) and ribonuclease 4 (D37931, DE −3.6). In addition, the nibrin encoding gene (AF049895; DE +2.3), in association with Mre11 and Rad50, plays a role in double strand repair and telomere maintenance. Two components of the cyclin activating kinase, namely cyclin H (AA451817, DE +2.3) and CDK7 (X77743, DE +2.8), implicated in DNA transcription and repair are increased in mesothelioma cells. Also overexpressed in malignant cells, GADD45A (AI634658, high-density filter array DE +2.3) stimulates DNA excision and repair before the entry into S phase. In addition its protein product binds with proliferating cell nuclear antigen (PCNA; M15796, high-density filter array DE +3.4) (Table 4).

Lipid metabolism was also affected as FABP5 (AA972250), ATP citrate lyase (X64330) and 3-oxoacid-CoA transferases (U62961) were overexpressed. These genes play a central role in de novo lipid synthesis and FABP5 is involved in keratinocyte differentiation [7]. Type II iodothyronine deiodinase (U53506) was 19.2 times more expressed in MSTO-211A cells

than Met-5A cells. The enzyme encoded by this gene converts thyroxine to active T3 thyroid hormone. This process is critical in thyroid hormone economy and could increase metabolism in target cells. Thus, the macromolecule and the lipid metabolisms were changed in cancer cells compared to their non-transformed counterparts.

### 3.5. Adhesion and cell recognition

Integrins are known to play a role in extracellular matrix adhesion. Many integrin genes were upregulated in mesothelioma cells like integrin α3 (M59911), α4 (X16983), α6 (X53586) and β-like 1 (AF072752): the DEs were +2.4, +6.4, +7.5, and +6.2, respectively. The α6-β1 and α4-β1 heterodimers are receptors of laminins and V-CAM proteins. Indeed the laminin α4 and γ1 genes were overexpressed (DE +3.0 and +3.1, respectively) in mesothelioma cells also giving them increased efficiency in angiogenesis. Previously, seven human malignant mesothelioma cell lines have been found to overexpress the α3-β1 integrin heterodimer as measured by immunofluorescence and FACS [8]. In agreement with another previous study, we found two genes CD59 (M34671) and MIF (M25639), of which the DEs were respectively +3.6 and −2.3 using the high-density filter array (Table 4). These genes were shown to act by lowering the local inflammation and immune response. Indeed CD59 is a potent inhibitor of the complement attack complex action and protects malignant cells from C-mediated lysis. MIF, downregulated in mesothelioma cells, encodes a factor inhibiting macrophage migration and maintaining them activated at inflammatory loci.

### 3.6. Invasion

Human mesothelioma is characterized by local invasion, recurrence following surgery and a weak ability for metastasis [9]. The inhibition of fibronectin 1 (X02761) expression in mesothelioma cells, compared to mesothelial cells (DE −21.1), may partially explain the local proliferation of the cells. For example, Bourdoulos et al. [10] previously reported fibronectin has anti-migration and anti-proliferation properties, demonstrated by an inhibition of cdc 42. Using the same mesothelial (Met-5A) cell line, Kinnula et al. [11] found that fibronectin synthesis could be induced by transforming growth factor β, but not amosite, a variety of asbestos. Moreover, fibronectin concentrations were low or undetectable in five of six mesothelioma cell lines. In our study with the microarray, the fluorescence level of fibronectin cDNA was 196 (a very low level) and 4265 in mesothelioma and mesothelial cells, respectively. The localization of mesothelioma may also be related to secretion of V-CAM interacting integrins (discussed above) and BYSL (L36720; DE +2.4) which is involved in initial attachment of the blastocysts to the uterine epithelial cells.

In contrast to other lung tumors, mesothelioma rarely induces metastasis. PAI-2 (M31551) was markedly expressed in mesothelioma cells (DE +33.2). Interestingly, PAI-2 expression has been associated with the absence of nodal invasion of non-small cell lung carcinomas [12]. In addition, thrombospondin 2 (L12350) was overexpressed in mesothelioma cells (DE +4.2), and when overexpressed can have an inhibitory role in vascularization and progression in non-small cell lung cancers [13]. The isoform of this gene, thrombospondin 1, highly expressed in 74 of 78 malignant mesotheliomas (assessed by RT-PCR), is significantly lower in tumors with as compared to without lymph node metastasis [14].

Another gene, basigin (L20471, high-density filter DE −20.5), that decreased in mesothelioma cells encodes a glycoprotein that alters matrix metalloproteinase expression. Matrix metalloproteinases are important during tumor invasion and correlated with the metastasis phenotype of bronchopulmonary carcinomas [15]. In addition, we found tissue inhibitor of matrix metalloproteinase-3 (TIMP-3, D45917) was upregulated in mesothelioma cells (DE +3.4). The combined diminution of basigin and the presence of TIMP-3 gene products could act synergistically to delimit the metastatic potential of these cells.

Lei et al. [16] previously demonstrated that certain attributes of bronchopulmonary tumors (malignancy grade and the metastasis potential) were inversely correlated with nucleoside diphosphate kinase NM23-H4 (Y07604) expression levels. In agreement, our high-density filter array results showed an overexpression of this gene (DE +5.6, Table 4). Taken together, these data highlight our understanding of the low metastatic potential of mesotheliomas.

### 3.7. Genes involved in cell cycle and cell growth

Various genes implicated in cell cycle control were overexpressed (DE varying from +2.4 to +3.8) in mesothelioma as compared to mesothelial cells even though both mRNA populations were extracted from confluent cells. These included G1 to S phase transition 1 gene (U95742), proliferation-associated 2G4 (AF104670) encoding gene involved in metaphase, cell division cycle 27 (S78234) involved in anaphase entry, cyclin H (AA451817) and cdk-7 (X77743) involved in various phases of the cell cycle. Other gene products involved in cell proliferation were also upregulated in mesothelioma cells included the proto-oncogenes Ki-*ras* (AI740449; cDNA microarray DE +2.6) and c-*myc* (K01904; cDNA microarray 10 024 fluorescence level), and a regulatory protein, c-*myc* binding protein (D98667; high-density filter DE +10.0). Both proto-oncogenes are cell markers of the MSTO-211H cell line (ATCC technical data sheet) and c-*myc* was previously shown to be expressed in mesothelioma by immunoreactivity [17–19]. The mitogen-stimulated ornithine decarboxylase-1 (M81740), which increases in rat mesothelial cells following exposure to crocidolite fibers [20], also was overexpressed in mesothelioma cells (cDNA microarray DE +8.4). Finally, two sequences for the basic fibroblast growth factor (bFGF; J04513) and the serum-inducible kinase (AF059617) were increased in mesothelioma cells, and bFGF previously has been found in 92% of human mesotheliomas and correlates with tumor aggressiveness [21].

### 3.8. Xenobiotic resistance and oxidative stress response genes

Human malignant mesothelioma has long been known to be extremely resistant to chemotherapy but the cellular and subcellular mechanisms remain largely unknown. A battery of genes involved in drug resistance expressed in both cell lines may help to explain why mesothelial cells are intrinsically resistant. These genes included four glutathione *S*-transferase genes including the θ2 isoform (L38503), nine cytochrome P450 genes including the 2B subfamily (M29874), three drug transporter molecules including tetracycline transporter-like protein (L11669) and P glycoprotein 3/multiple drug resistance 3 (X06181). Xenobiotic intermediate metabolism and

detoxication genes included epoxide hydrolase 2, cytoplasmic (AI301066) and deoxyguanosine kinase (AA625191). These genes were moderately or highly expressed (fluorescence $>500$ units) in both cell lines. In addition, two genes involved in detoxification of the cytostatic drugs – 5-fluorouracil through dihydropyrimidine dehydrogenase (U20938) and bleomycin through bleomycin hydrolase (X92106) – were overexpressed (cDNA microarray DE +2.1 and +2.9, respectively).

In addition, several genes important in the response to oxidative stress were increased in mesothelioma cells. These included γ-glutamylcysteine synthetase (AL033397, cDNA microarray DE +2.4, previously found to be overexpressed in five human mesothelioma tumors by Ogretment et al. [22]), proliferation-associated gene A (L19184; cDNA microarray DE +3.3), superoxide dismutase Cu/Zn (K00065; high-density filter DE +3.8), annexin I (X05908; cDNA microarray DE +9.4, previously found to downregulate phospholipase A2), and thioredoxin (AI302890; cDNA microarray DE +2.5). Interestingly, the latter two genes were implicated by Sinha et al. [23] in chemoresistance of human stomach cancer cells.

## 4. Conclusion

We found that simultaneous analyses of thousands of genes revealed a complex phenotype in mesothelioma that was associated with changes in several major pathways. Examination of gene expression that is shared between mesothelial and mesothelioma cells revealed a battery of proteins allowing metabolization and detoxication of chemicals and increased DNA repair efficiency. Regulation of these pathways could confer functions related to the well-known chemo- and radioresistance properties of mesothelioma. Examination of gene expression that differs between mesothelial and mesothelioma cells revealed several pathways that may be important in cell transformation. In addition to the traits of cancer cells recently described by Weinberg and Hanahan [24], namely self-sufficiency in growth signal, tissue invasion, sustained angiogenesis and limitless replicative potential, we found altered regulation of several genes that control other attributes that distinguished mesothelioma cells from mesothelial cells. Gene expression linked to cell transformation appear to be related to (i) increased macromolecule stability, (ii) changes in cell adhesion and recognition, (iii) limited invasion properties, (iv) continuous cell division even at high cell density, (v) increased anabolism, and (vi) increased protein transmembrane transport. These properties may cooperate to explain the pathogenesis of mesothelial cell transformation to mesothelioma. Further studies comparing the gene expression patterns of both cultured cells to other cell lines, especially to cancer cells [25], could be useful. Such studies should foster the research of molecular markers (as many have suggested) allowing to better assess the phenotype of malignant mesothelioma. These strategies could improve the accuracy of its diagnosis, prognosis and therapy including by gene modulation.

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