Patterns of expression of chromosome 17 genes in primary cultures of normal ovarian surface epithelia and epithelial ovarian cancer cell lines

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Oligonucleotide microarray analysis was applied to assess the expression profile of 332 probe sets representing 308 genes or expressed sequence tags (ESTs) that map to chromosome 17 in order to address epigenetic events that result in alterations in gene expression in epithelial ovarian cancer (EOC). Expression profiles were generated from 12 primary cultures derived from normal ovarian surface epithelium (NOSE) and four long-term cultures (TOV-81D, TOV-112D, TOV-21G and OV-90) derived from EOCs that have been previously characterized and shown to mimic features of the tumoral cells from which they were derived. The expression values of all 332 probe sets is highly correlated across the 12 NOSEs (89% correlation coefficients > 0.90). In two-way comparisons, differential patterns of gene expression were observed for 157 probe sets for which the expression value of at least one EOC cell line fell outside the limits of the range of expression of the 12 NOSEs. When compared to NOSEs, four genes displayed similar differential patterns of gene expression across all four EOC cell lines, and 26 genes displayed similar differential patterns of gene expression across the three EOC cell lines (TOV-112D, TOV-21G and OV-90) representing tumoral cells derived from the most aggressive disease. A total of 17 genes displayed differential patterns of gene expression greater than threefold in at least one EOC cell line in comparison to NOSE, and three genes were differentially expressed greater than threefold across all aggressive cell lines. The analysis of a selected number of genes by RT-PCR revealed patterns of gene expression comparable to those observed by microarray analysis in the majority of samples tested. Comparison of expression profiles of differentially expressed genes identified by microarray analysis in two-way comparisons of the EOC cell lines and the NOSEs with published reports revealed 10 genes previously implicated in ovarian tumorigenesis and 18 in

tumorigenesis of other types of cancer. The differential pattern of gene expression of genes that map to both the p and q arm of chromosome 17 is consistent with the hypothesis that a number of genes that map to this chromosome are implicated in the etiology of ovarian cancer.

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Epithelial ovarian cancer (EOC) is the second most common gynecological cancer and is responsible for almost half of deaths associated with gynecological pelvic malignancies. The molecular genetic analysis of EOC has revealed a significant role for chromosome 17 genes in the pathogenesis of ovarian malignancies through loss of heterozygosity (LOH) and karyotype studies (Eccles *et al.*, 1990; Lee *et al.*, 1990; Russell *et al.*, 1990; Sato *et al.*, 1991; Foulkes *et al.*, 1993; Phillips *et al.*, 1993; Tavassoli *et al.*, 1993; Mertens *et al.*, 1997; Dion *et al.*, 2000). The frequent reduction to hemizyosity implies that numerous genes on both arms of chromosome 17 are implicated in EOC. A number of ovarian tumors display loss of all informative polymorphic loci, strongly suggesting loss of an entire chromosome 17 (Foulkes *et al.*, 1993; Tavassoli *et al.*, 1993; Pieretti *et al.*, 1995; Papp *et al.*, 1996). The *TP53* tumor-suppressor gene (TSG) at 17p13.1 is one of the most frequently mutated genes in EOC (Okamoto *et al.*, 1991). However, LOH analysis suggests the location of novel TSGs on the short arm of chromosome 17 at or close to the *TP53* locus (Okamoto *et al.*, 1991; Tsao *et al.*, 1991; Eccles *et al.*, 1992; Cliby *et al.*, 1993; Foulkes *et al.*, 1993; Yang-Feng *et al.*, 1993) and at a more telomeric region, 17p13.3 (Phillips *et al.*, 1993; Konishi *et al.*, 1998; Phelan *et al.*, 1998; Hoff *et al.*, 2000). A number of genes on the long arm of chromosome 17 also have been shown to be implicated in EOC: the *ERBB2* oncogene at 17q21.1 (Slamon *et al.*, 1989), the hereditary breast/

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ovarian cancer TSG *BRCA1* at 17q21.1 (Miki *et al.*, 1994; Merajver *et al.*, 1995) and the *NME1* tumor metastasis marker gene at 17q21.3 (Mandai *et al.*, 1994; Leary *et al.*, 1995; Schneider *et al.*, 1996, 2000). In addition to the known genes implicated in ovarian tumorigenesis, LOH analysis and/or karyotype studies revealed the locations of novel TSGs on the long arm of chromosome 17 coincident with 17q21 (Tangir *et al.*, 1996), 17q21-23 (Jacobs *et al.*, 1993; Yang-Feng *et al.*, 1993; Godwin *et al.*, 1994), 17q23-qter (Mertens *et al.*, 1997) and 17q25 (Kalikin *et al.*, 1997; Dion *et al.*, 2000). Other mechanisms not detected by these methods such as alterations in methylation patterns and alterations in promoter activity may be invoked, which alter the expression of genes. Thus, alterations in patterns of gene expression may reveal novel loci that are implicated in tumorigenesis. These observations combined suggest that the dysregulation of a number of genes on chromosome 17, by both genetic and epigenetic events, are important to the etiology of EOC and thus could account for the high frequency of chromosomal anomalies associated with EOC.

Oligonucleotide expression microarray technology can be applied to the analysis of the genes on chromosome 17 implicated in the etiology of EOC in that it permits the simultaneous analysis of hundreds of genes (Golub *et al.*, 1999; Ross *et al.*, 2000; Welsh *et al.*, 2001). Recently, we have applied this technology to examine patterns of gene expression in an *in vitro* EOC cell model that is comprised of spontaneously transformed EOC cell lines (Tonin *et al.*, 2001). A comparison of the overall expression profiles of EOC cell lines with that representing a normal ovarian surface epithelium (NOSE) provided evidence for the usefulness of this approach, as the expression patterns were consistent with the phenotypes of the original tumors from which the EOC cell lines were derived (Tonin *et al.*, 2001). Given the importance of chromosome 17 genes in the etiology of ovarian cancer, we have applied oligonucleotide expression microarray analysis to identify specifically patterns of expression of genes that map unambiguously to this chromosome.

We identified 332 probe sets from the 6800 probe sets on the HuGeneFL Affymetrix GeneChip®, representing genes that map to chromosome 17. For comparative purposes, we established the expression profile of primary cultures of NOSE derived from 12 independently ascertained ovaries from women with no personal history of ovarian cancer. These cells were chosen because they reflect the cellular population from which EOC are derived (Auersperg *et al.*, 2001). Then, we compared the expression profile of each of the four EOC cell lines with the signature pattern of gene expression defined by the analysis of the panel of NOSEs.

As shown in Figure 1, the range of expression values of each probe set for the primary cultures derived from 12 NOSEs was compared (Figure 1). There were 95 (28.9%) probe sets that displayed expression values below 20 across all of the samples and were reassigned an expression value of 20. When the maximum and minimum expression values are compared within the set

of 12 NOSEs, 58 (17.5%) probe sets displayed expression values with a range of expression greater than or equal to threefold and 177 (53.6%) probe sets displayed values with a range of expression less than threefold (Figure 1) (the chromosome 17 data files are available on <http://www.genome.mcgill.ca/ovarian>). The expression values of all 332 probe sets are highly correlated across the 12 NOSEs, with the majority of the correlation coefficients being greater than 0.90 ($n=59$) and none smaller than 0.84 (data not shown). A schematic representation of the range of expression values of selected probe sets is shown in Figures 1 and 2. There appears to be no clustering of similar expression values reflecting either high or low expression in association with the map coordinates of the probe sets representing chromosome 17 genes (Figure 1).

The expression values of probe sets for each of the four EOC cell lines were compared to the maximum or minimum expression values for the 12 NOSEs. The expression values of all 332 probe sets is highly correlated across the four EOC cell lines with correlation coefficients greater than 0.81 and the highest correlation coefficients were observed with OV-90, TOV-21G and TOV-112D (each greater than 0.94) (data not shown). There were 104 (31.3%) of the 332 probe sets that displayed values below 20 across all of the four EOC cell lines and were reassigned a expression value of 20; 80 of these 104 (77%) probe sets also gave expression values at or below 20 in the panel of 12 NOSEs (Figure 3). Of the 332 (28.6%) probe sets, 95 displayed expression values for the four EOC cell lines that fell within the range of expression values of the 12 NOSEs. For at least one of the four EOC cell lines, 157 (47.3%) of the 332 probe sets displayed expression values that fell outside the range of expression of that observed in the 12 NOSEs (Figure 3). Six (1.8%) of these 157 probe sets displayed values that were discordant in two-way comparisons among the four EOC cell lines (data not shown). Only the probe sets for *UBTF* (X53390 and X56687) displayed values that were largely discordant when the expression profiles generated by these two probe sets were compared across all the four EOC cell lines. RT-PCR analysis of *UBTF* revealed similar patterns of gene expression across all samples tested and this pattern was not concordant with that displayed by the two probe sets represented on the GeneChip® (Figure 4). Thus, the discordance in expression results may also reflect specificity of the probe set. The distribution of the 157 probe sets displaying expression profiles that fell outside the range of expression of the 12 NOSEs for each EOC cell line is shown in Table 1. The EOC cell line TOV-81D showed the fewest number of probe sets displaying a difference in expression pattern compared to the panel of the 12 NOSEs (Table 1). These results are consistent with those of a previous analysis of 6416 probe sets by Affymetrix Hs6000 oligonucleotide microarray (Tonin *et al.*, 2001), and support the earlier findings that TOV-81D represents the most indolent form of the disease based on tumorigenicity assays and clinical features of the disease presentation of the patient from

which the primary tumor sample was derived (Provencher *et al.*, 2000).

The EOC cell line TOV-112D displayed the highest number of genes overexpressed among the four EOC cell lines in comparison to the 12 NOSEs (Table 1). This cell line has been shown to harbor trisomy 17 by karyotype analysis and LOH studies suggest that all three chromatids are derived from the same chromosome 17 homologue (unpublished data). Based on this karyotype, one may expect an increase in expression from three alleles in those genes that are expressed. This profile was reflected in the comparison of expression values of the four EOC cell lines, as TOV-112D has an excess of chromosome 17 mapped genes displaying increased expression values when compared with either the EOC cell lines or the 12 NOSEs (Table 1). In addition, TOV-112D harbored the largest number of probe sets ($n=52$) displaying the highest expression value when compared with all other EOC cell lines, TOV-21G ($n=23$), OV-90 ($n=23$) and TOV-81D ($n=13$), in the group of probe sets ($n=157$) displaying differential patterns of gene expression falling outside the range of expression of the 12 NOSEs (data not shown). However, the fold differences in gene expression were generally less than threefold when the expression profile of TOV-112D was compared with that of the 12 NOSEs. Previous studies from our group have also shown a correlation of expression profiles with allelic imbalance for genes that map to the chromosome 3 arm, where the magnitude of expression was less than threefold when the expression of the cell line harboring LOH of an entire chromosome 3p arm was compared with that of a NOSE (Manderson *et al.*, 2002). These results may reflect the sensitivity of Affymetrix GeneChip[®] microarray analysis. These results may be difficult to replicate with analyses involving solid tumor material because of the heterogeneity of the cell population. However, several studies reported a positive correlation between expression profiles generated by cDNA microarrays and DNA copy number in cancer-

derived cell lines and solid tumors (Pollack *et al.*, 1999; Lucito *et al.*, 2000; Kauraniemi *et al.*, 2001).

A similar differential expression pattern across all the four EOC cell lines compared to the 12 NOSEs was observed in four (2.5%) of the 157 probe sets (Figure 2). These probe sets represent *STAT5A*, *NPEPPS*, *UGTREL1* and *ARGHDIA*. Of the 157 probe sets, 26 (16.6%) had a similar pattern of expression only for the three most aggressive EOC cell lines, TOV-21G, OV-90 and TOV-112D (Figure 2). Seven genes, *NUP88*, *HOXB7*, *NME1*, *TOP2A*, *IGFBP4*, *PHB* and *BECN1* represented by this group of probe sets, have been previously implicated in ovarian cancer (Table 2). The expression profile of *NUP88*, *HOXB7* and *NME1* generated by microarray analysis are consistent with the expression reported for those genes in the literature. While the other genes in this group have also been studied in the context of ovarian cancer, direct comparisons with our data cannot be made as previous studies were not aimed at differentiating expression profiles between normal and tumor-derived tissues. Although the remaining genes in this group (19 out of 26 probe sets) have not been implicated in ovarian cancer studies, *PFN1*, *GPS2* and *COL1A1* have been studied in the context of other cancer types (Table 2). Although the remaining genes ($n=16$) in this group have not been associated with tumorigenesis, their gene function may invoke a possible role in tumorigenesis. For example, some of these genes encode products implicated in DNA repair such as *RPA1*, DNA replication such as *CDC6* and in the regulation of transcription such as *UBTF*. An interesting member of this group of genes, *BLMH*, has been implicated in the inactivation of the antitumor drug bleomycine.

Of the 157 probe sets, 17 (10.8%) displayed differential patterns of gene expression greater than or equal to threefold in at least one EOC cell line in comparison with the NOSE panel (Figure 2). Within this group, a similar pattern of gene expression was observed for 10 of the 17 probe sets for the most aggressive EOC cell lines

Figure 1 Distribution and expression profiles of selected chromosome 17 mapped probe sets for the panel of 12 NOSEs. Primary cultures were derived from NOSE from 12 independently ascertained ovaries (NOV-31, NOV-61, NOV-116D, NOV-220D, NOV-319, NOV-436G, NOV-504D, NOV-653G, NOV-821, NOV-848D, NOV-900 and NOV-910G) and established as described (Kruk *et al.*, 1990; Lounis *et al.*, 1994). The NOSE samples were derived from ovaries obtained from women with no prior history of ovarian cancer and who were undergoing prophylactic oophorectomy (mean age, 41 years) from surgeries performed at the Centre Hospitalier de l'Université de Montréal -Hôpital Notre-Dame, following informed consent. Cells were cultured in OSE medium consisting of 50:50 medium 199:105 (Sigma) (Kruk *et al.*, 1990) supplemented with 15% of fetal bovine serum (FBS) containing 2.5 µg/ml fungizone and 50 µg/ml gentamicin. Total RNA was extracted using TRIzol[™] reagent (Gibco/BRL, Life Technologies Inc., Grand Island, NY, USA) from the 12 primary cell cultures of NOSE grown to 80% confluence in 100 mm Petri dishes. Microarray analysis was performed with the Affymetrix Genechip[®] HuGeneFL microarray that contains oligonucleotide probe sets corresponding to the mRNA for 6800 full-length human genes or ESTs (Affymetrix, Santa Clara, CA, USA). Biotinylated hybridization target was prepared from total RNA as described (Tonin *et al.*, 2001). The complete protocol is available at <http://www.genome.mcgill.ca/ovarian>. Extractor[®] 2001 (Lypny and Tonin), a data filtering software application written using an XTalk scripting language (MetaCard Corporation) was used to retrieve information from probe sets representing genes or ESTs that map to chromosome 17 from UniGene database file dated 28 September 2001 on the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov>). This query identified 332 probe sets on the HuGeneFL Genechip[®] that map unambiguously to chromosome 17. The Human Genome Browser (<http://genome.ucsc.edu/goldenPath/hgTracks.html>) was used to retrieve map coordinates for those probe sets representing the chromosome 17 genes. The distribution of the probe sets according to cytoband coordinates on chromosome 17 is shown in the left most panel: distribution of the entire chromosome 17 probe sets ($n=332$) per cytoband (column A); distribution of probe sets displaying expression values less than 20 (column B) and distribution of probe sets displaying expression values greater than 20 for at least one NOSE (column C). The expression profiles of selected probe sets (represented as EST accession number followed by gene symbol) for each of the 12 NOSE samples based on a range of expression greater than 100 and with at least a threefold difference between the maximum and the minimum expression values is shown on the right-hand panel aligned according to chromosome 17 map coordinates

Gene symbol ¹	Accession no. ¹	12 NOSEs			EOC cell lines							
		Expression value			TOV-81D		OV-90		TOV-21G		TOV-112D	
		Maximum	Minimum	Fold difference								
NPEPPS	Y07701	20	20	1	25	1.3	29	1.5	47	2.4	29	1.5
UGTREL1	D87989	115	179	2	214	1.2	259	1.4	290	1.6	250	1.4
PFN1	J03191	673	1682	3	1067		2908	1.7	1930	1.1	2004	1.2
SLC25A11	X66114	20	63	3	40		98	1.6	117	1.9	87	1.4
NUP88	Y08612	20	46	2	20		48	1.0	50	1.1	86	1.9
GPS2	U28963	38	67	2	51		67	1.0	92	1.4	139	2.1
BLMH	X92106	20	34	2	25		50	1.5	72	2.1	78	2.3
CDC6	U77949	20	20	1	20		41	2.1	34	1.7	52	2.6
NMT1	M86707	29	65	2	35		80	1.2	87	1.3	125	1.9
HOXB7	M16937	20	33	2	20		62	1.9	62	1.9	41	1.2
PHB	S85655	52	146	3	143		227	1.6	252	1.7	267	1.8
NME1	X17620	177	410	2	225		510	1.2	481	1.2	475	1.2
COX11	U79270	20	51	3	41		63	1.2	123	2.4	81	1.6
AKAP1	X97335	20	20	1	20		27	1.4	27	1.4	33	1.7
KIAA0111	D21853	119	289	2	146		413	1.4	565	2.0	343	1.2
MAC30	L19183	20	23	1	20		174	7.5	229	9.9	133	5.8
DUSP3	L05147	20	22	1	37	1.7	20		68	3.1	69	3.1
RPA1	M63488	20	20	1	20		41	2.1	69	3.5	102	5.1
E2-EPF	M91670	55	260	5	154		994	3.8	356	1.4	455	1.8
TOP2A	J04088	20	49	2	20		141	2.9	105	2.1	207	4.2
UBTF	X56687	20	21	1	20		26	1.3	25	1.2	66	3.2
SEC14L1	D67029	34	84	2	75		53		344	4.1	106	1.3
CD7	M37271	20	37	2	20		137	3.7	63	1.7	23	
TBX2	U28049	20	33	2	20		20		20		170	5.1
STAT5A	U43185	29	51	2	25	-1.2	20	-1.5	24	-1.2	20	-1.5
ARHGDIA	X69550	510	914	2	454	-1.1	500	-1.0	270	-1.9	461	-1.1
ACADVL	D43682	245	476	2	308		146	-1.7	222	-1.1	166	-1.5
BECN1	L38932	149	233	2	198		146	-1.0	91	-1.6	128	-1.2
P4HB	J02783	946	1549	2	1223		774	-1.2	524	-1.8	428	-2.2
IGFBP4	M62403	765	2727	4	1889		122	-6.3	57	-13.4	20	-38.3
COL1A1	Z74615	563	2307	4	884		20	-28.1	132	-4.3	71	-7.9
TIP-1	U90913	308	607	2	375		100	-3.1	153	-2.0	73	-4.2
KIAA0864	D23673	258	579	2	510		186	-1.4	71	-3.6	240	-1.1
SC65	U47621	82	181	2	98		20	-4.1	48	-1.7	79	-1.0
MAP2K3	D87116	108	263	2	246		20	-5.4	158		64	-1.7
TIMP2	M32304	209	592	3	398		43	-4.9	296		87	-2.4
LGALS3BP	L13210	399	921	2	474		375	-1.1	668		22	-18.1

¹ Based on Unigene database on the NCBI web site (<http://www.ncbi.nlm.nih.gov>)

Fold-difference analysis between each EOC cell line and the 12 NOSEs:

Range of expression of the 12 NOSEs for each probe set

Over-expression Less than 3-fold difference
Greater or equal to 3-fold difference

Under-expression Less than 3-fold difference
Greater or equal to 3-fold difference

Within the range of expression of the 12 NOSEs

Figure 2 Two-way comparisons of expression profiles of the 12 NOSEs *versus* the four EOC cell lines of selected probe sets. The four EOC cell lines were established from ovarian malignant tumors (TOV-21G, TOV-81D, TOV-112D) and from an ovarian malignant ascites (OV-90) from chemotherapy naïve patients as described (Provencher *et al.*, 2000). They were derived from a grade 3 and stage III clear cell carcinoma, TOV-21G; a grade 1–2 and stage IIIc papillary serous adenocarcinoma, TOV-81D; a grade 3 and stage IIIc endometrioid carcinoma, TOV-112D; and an ascites fluid of a grade 3 and stage IIIc adenocarcinoma, OV-90. Cells were cultured in OSE medium consisting of 50:50 medium 199:105 (Sigma) (Kruk *et al.*, 1990) supplemented with 10% of FBS containing 2.5 µg/ml fungizone and 50 µg/ml gentamicin. The maximum and minimum values of expression across the 12 NOSEs of the selected probe sets are shown along with the fold differences in the expression values (maximum/minimum). The corresponding expression values of each EOC cell line for the same probe set is shown along with the fold difference in the expression value based on either the maximum (for increase in expression) or minimum expression value (for decrease in expression) of the 12 NOSEs (fold differences less than 0 reflect underexpression in comparison with the NOSEs)

Figure 3 Distribution and expression profiles of selected chromosome 17 mapped probe sets of the four EOC cell lines and comparison to NOSEs. The distribution of the probe sets according to cytoband coordinates on chromosome 17 is shown in the left most panel: the entire chromosome 17 probe sets ($n = 332$) per cytoband (column A); those displaying expression values less than 20 in all four EOC cell lines (column B); those displaying expression values within the range of expression of the 12 NOSEs (column C) and those displaying differential patterns of gene expression outside the range of expression of the 12 NOSEs in two-way comparisons between the 12 NOSEs and TOV-81D (column D), OV-90 (column E), TOV-21G (column F) and TOV-112D (column G). The expression profiles of selected probe sets (represented as EST accession number followed by gene symbol) for the maximum and minimum expression values of 12 NOSEs and the four EOC cell lines are shown on the right-hand panel aligned according to chromosome 17 map coordinates

UBTF

TIMP2

E2-EPF

TIP-1

COL1A1

MAP2K3

LGALS3BP

GAPDH

Table 1 Number of probe sets displaying differential patterns of gene expression in two-way comparisons between the 12 NOSEs and the EOC cell lines

Pattern of expression when compared with NOSE		Number of probe sets displaying expression values outside the range of expression of the 12 NOSEs			
		TOV-81D	OV-90	TOV-21G	TOV-112D
Over-expression	> 3	0	3	4	5
	< 3	22	42	59	70
	Total	22	45	63	75
Under-expression	> 3	0	6	3	4
	< 3	12	37	19	22
	Total	12	43	22	26

(Figure 2). Of these 17 genes, which showed differential patterns of gene expression greater than threefold in at least one EOC cell line when compared with the 12 NOSEs, eight have been previously implicated in tumorigenesis (Table 1). Two of these genes, *TIMP2* and *MAP2K3*, have been studied in the context of ovarian cancer. *TIMP2*, a member of a family of genes involved in the ability to control the activity of matrix metalloproteinases (MMPs), has recently been investigated in ovarian tumors for their association with disease outcome in advanced-stage ovarian carcinomas (Davidson *et al.*, 1999). Expression studies of *TIMP2* in ovarian tumors are controversial as reports have indicated an increased level of mRNA for *TIMP2* in association with *MMP2* increased expression and poor survival in ovarian tumors (Davidson *et al.*, 1999). Others reported a decreased level of expression of *TIMP2* in ovarian tumors (Kikkawa *et al.*, 1997) or in serous effusions of ovarian carcinoma (Davidson *et al.*, 2001), and these differences possibly mark the acquisition of a metastatic phenotype. It is worth noting is that the EOC cell line OV-90, which is derived from malignant ovarian ascites, displayed a decreased level of expression of *TIMP2* in concordance with what has been previously described by Davidson *et al.* (2001) in serous effusions of ovarian carcinomas. Although expression of *MAP2K3* has not previously been described in ovarian cancer, no mutations were found in an analysis of this gene in seven ovarian cancer-derived cell lines (Teng *et al.*, 2001).

We used semiquantitative RT-PCR with GAPDH as an internal control to evaluate the expression profiles assessed by microarray analysis of a selected panel of candidate genes (Figure 4). We choose genes under-expressed in at least one of the EOC cell lines (*LGALS3BP*, *TIMP2* and *MAP2K3*) or in the three most aggressive EOC cell lines (*TIP-1* and *COL1A1*), as well as genes overexpressed in at least one EOC cell line

(*E2-EPF*) or in the three most aggressive EOC cell lines (*UBTF*) when compared to the range of expression of the 12 NOSEs. Some of the genes were selected because their level of expression was greater than threefold when that of 12 NOSEs was compared to any (*LGALS3BP*, *TIMP2*, *MAP2K3*, *TIP-1*, *UBTF* and *E2-EPF*) or all of the three most aggressive EOC cell lines (*COL1A1*). The expression results based on RT-PCR analysis of *TIMP2*, *E2-EPF*, *COL1A1*, *MAP2K3* and *LGALS3BP* were comparable to those observed by microarray analyses (Figure 4). However, the RT-PCR analysis of *TIP-1* was slightly different compared to the microarray results. The high level of expression by RT-PCR of *TIP-1* observed for NOV-319, NOV-436, NOV-61 and TOV-81D and the low level of expression observed for TOV-112D is consistent with the microarray results observed for the probe set representing this gene. Discordance was only observed for TOV-21G and OV-90 in this comparative analysis. In contrast, the high level of expression of *UBTF* by RT-PCR analysis across all samples tested was not consistent with the microarray results of either probe set (X53390 and X56687) representing this gene (Figure 4). These results illustrate the need to validate expression profiles by alternative methods before large-scale studies are pursued assessing the significance of the differential patterns of gene expression in a large sample of tumors.

Microarray analysis of a small panel of well-characterized EOC cell lines and 12 NOSEs revealed genes that may be modulated in ovarian tumorigenesis that are located on chromosome 17. Our microarray analysis has identified differential patterns of gene expression of a number of genes that have been previously implicated in ovarian tumorigenesis where traditional methods of analysis were applied. The differential pattern of gene expression of genes that map to both the p and q arm of chromosome 17 is consistent with the hypothesis that a number of genes

Figure 4 Expression analysis of selected chromosome 17 genes. A measure of 2 µg of the total RNA from the four EOC cell lines and three NOSEs (NOV-61, NOV-436, and NOV-319) were reverse transcribed using the Ready-To-Go-Primed First Strand kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) according to the manufacturer's instructions. A total of 50–100 ng of the reverse transcribed cDNAs were used in a multiplex reaction with a pair of gene-specific primers and a pair of primers for GAPDH as a control. The product of the different amplification reactions were revealed by autoradiography after electrophoresis on a 5% polyacrylamide (BioRad; Hercules, CA, USA) denaturing gel. Sequences of the primers are available on <http://www.genome.mcgill.ca/ovarian>. Autoradiographs of RT-PCR products representing the analysis of selected genes (represented by gene symbol) alongside the expression values from Affymetrix HuGeneFL GeneChip® microarray analysis. Lanes 1-3: primary cultures of NOSEs, NOV-319, NOV-436 and NOV-61; Lane 4: TOV-21G; Lane 5: OV-90; Lane 6: TOV-112D and Lane 7: TOV-81D. Microarray analysis of *UBTF* is represented by the probe sets X53390 (a) and X56687 (b)

Table 2 Description of genes selected based on differential patterns of gene expression of the 12 NOSEs *versus* the EOC cell lines

Gene symbol ¹	Description ¹	Putative function ²	Reported in other studies in relation to	
			Ovarian cancer	Other type of cancer
Overexpressed genes ³				
<i>NPEPPS</i>	Aminopeptidase puromycin sensitive	Aminopeptidase with broad substrate specificity to several peptides. Involved in proteolytic events essential for cell growth and viability.	No	No
<i>UGTREL1</i>	UDP-galactose transporter related	Function unknown	No	No
<i>RPA1</i> ⁴	Replication protein A1(70 kDa)	Single-stranded DNA-binding protein, has roles in DNA replication, repair and recombination.	No	No
<i>PFN1</i>	Profilin 1	Regulates actin polymerization in response to extracellular signals.	No	Yes
<i>SLC25A11</i>	Solute carrier family 25 (mitochondrial carrier, oxoglutarate carrier). Member 11	Catalyzes the transport of 2-oxoglutarate across the inner mitochondrial membrane in an electroneutral exchange for malate or other dicarboxylic acids.	No	No
<i>NUP88</i>	Nucleoporin 88 kDa	Nuclear pore complex component implicated in transport.	Yes	Yes
<i>GPS2</i>	G protein pathway suppressor 2	Suppresses g-protein- and mitogen-activated protein kinase-mediated signal transduction.	No	Yes
<i>E2-EPF</i> ⁴	Ubiquitin carrier protein	Family member of enzymes known as ubiquitin carrier proteins, or E2s, which are an essential component of the ubiquitin–protein conjugation system.	No	No
<i>MAC30</i> ⁴	MAC30 hypothetical protein	Function unknown.	No	No
<i>TOP2A</i> ⁴	Topoisomerase (DNA) II alpha (170 kDa)	Control of topological states of DNA by transient breakage and subsequent rejoining of DNA strands. Topoisomerase II makes double-strand breaks.	Yes	Yes
<i>BLMH</i>	Bleomycin hydrolase	The normal physiological role of blm hydrolase is unknown, but it catalyzes the inactivation of the antitumour drug blm (a glycopeptide) by hydrolyzing the carboxamide bond of its b-aminoalaninamide moiety thus protecting normal and malignant cells from blm toxicity.	No	No
<i>CDC6</i>	CDC6 cell division cycle 6 homolog (<i>Saccharomyces cerevisiae</i>)	Regulator at the early steps of DNA replication.	No	No
<i>UBTF</i> ⁴	Upstream binding transcription factor, RNA polymerase 1	Recognizes the ribosomal RNA gene promoter and activates transcription mediated by RNA polymerase 1 through cooperative interactions with the species-specific factor S11.	No	No
<i>NMT1</i>	N-myristoyltransferase 1	Adds myristoyl group to N-terminal glycine residue of certain cellular and viral proteins.	No	No
<i>HOXB7</i>	Homeo box B7	Sequence-specific transcription factor, that is part of a developmental regulatory system, which provides cells with specific positional identities on the anterior-posterior axis.	Yes	Yes
<i>PHB</i>	Prohibitin	Prohibitin inhibits DNA synthesis and has a role in regulating proliferation.	Yes	Yes
<i>NME1</i>	Nonmetastatic cells 1	Transcription factor and nucleoside diphosphate kinase and has a role in the transcriptional regulation of <i>c-myc</i> expression.	Yes	Yes
<i>COX11</i>	COX11 homolog, cytochrome c oxidase assembly protein (yeast)	Exerts its effect at some terminal stage of cytochrome c oxidase synthesis, probably by being involved in the insertion of the copper b into subunit 1 (by similarity).	No	No
<i>AKAP1</i>	A kinase (PRKA) anchor protein 1	Anchors cAMP-dependent protein kinase near its physiological substrates, interacts with both the type I and type II regulatory subunits.	No	No
<i>KIAA0111</i>	KIAA0111 gene product	Function unknown.	No	No
<i>DUSP3</i> ⁴	Dual specificity phosphatase 3 (vaccinia virus phosphatase VHI related)	The protein shows both activity toward tyrosine-protein phosphate as well as with serine-protein phosphate. These phosphatases inactivate their target kinases by dephosphorylating both the phosphoserine/threonine and phosphotyrosine residues.	Yes	Yes

Table 2 (Continued)

Gene symbol ¹	Description ¹	Putative function ²	Reported in other studies in relation to	
			Ovarian cancer	Other type of cancer
<i>TBX2</i> ⁴	T-box 2	Involved in the transcriptional regulation of genes required for mesoderm differentiation.	No	Yes
<i>SEC14L1</i> ⁴	SEC14 (S. cerevisiae)-like 1	Belongs to the SEC14 cytosolic factor family. It has similarity to yeast SEC14 and to Japanese flying squid RALBP that suggests a possible role of the gene product in an intracellular transport system.	No	Yes
<i>CD7</i> ⁴	CD7 antigen (p41)	Function unknown.	No	Yes
Underexpressed genes ⁵				
<i>STAT5A</i>	Signal transducer and activator of transcription 5A	Carries out a dual function: signal transduction and activation of transcription.	No	Yes
<i>ARHGDIA</i>	Rho GDP dissociation inhibitor (GDI) alpha	Regulates the GDP/GTP exchange reaction of the rho proteins by inhibiting the dissociation of GDP from them, and the subsequent binding of GTP to them (by similarity).	No	No
<i>TIP-1</i> ⁴	Tax interaction protein 1	Function unknown.	No	No
<i>ACADVL</i>	Acyl-Coenzyme A dehydrogenase, very long chain	Active toward esters of long-chain and very-long chain fatty acids such as palmitoyl-CoA and stearoyl-CoA.	No	No
<i>KIAA0864</i> ⁴	KIAA0864 protein	Function unknown.	No	No
<i>IGFBP4</i> ⁴	Insulin-like growth factor-binding protein 4	IGF-binding proteins prolong the half-life of the IGFs and have been shown to either inhibit or stimulate the growth-promoting effects of the IGFs in cell culture. They alter the interaction of IGFs with their cell surface receptors.	Yes	Yes
<i>SC65</i> ⁴	Nucleolar autoantigen (55 kDa) similar to rat synaptonemal complex protein	Nucleolar protein associated with chromosomes during mitosis.	No	No
<i>BECN1</i>	Beclin 1 (coiled-coil, myosin-like BCL2 interacting protein)	May play a role in antiviral host defense. Interacts with bcl-2.	Yes	Yes
<i>COL1A1</i> ⁴	Collagen, type 1, alpha 1	Type 1 collagen is a member of group 1 collagen (fibrillar forming collagen).	No	Yes
<i>P4HB</i>	Procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), beta polypeptide	Has protein disulfide isomerase activity, catalyzes formation of 4-hydroxyproline in collagens.	No	No
<i>MAP2K3</i> ⁴	Mitogen-activated protein kinase kinase 3	Catalyzes the concomitant phosphorylation of a threonine and a tyrosine residue in the map kinase p38.	Yes	Yes
<i>TIMP2</i> ⁴	Tissue inhibitor of metalloproteinase 2	Complexes with metalloproteinases (such as collagenases) and irreversibly inactivates them.	Yes	Yes
<i>LGALS3BP</i> ⁴	Lectin, galactoside-binding, soluble, 3 binding	Family member of beta-galactoside-binding proteins known to be implicated in modulating cell-cell and cell-matrix interactions.	No	Yes

¹Based on UniGene database (NCBI web site: <http://www.ncbi.nlm.nih.gov>).

²Based on GeneCards (web site of the Weizmann Institute of Science. <http://bioinformatics.weizmann.ac.il/cards/index.html>) (Rehman *et al*, 1997).

³Genes or probe sets overexpressed in at least one EOC sample compared to the panel of 12 NOSEs.

⁴Genes identified as candidate genes based on a cutoff of greater than or equal to threefold difference between the EOC cell lines and the panel of 12 NOSEs.

⁵Genes or probe sets underexpressed in at least one EOC sample compared to the panel of 12 NOSEs.

A complete version of the table with the references is available on our web site at <http://www.genome.mcgill.ca/ovarian>.

that map to this chromosome are implicated in the etiology of ovarian cancer. New generation oligonucleotide microarrays with a comprehensive coverage of chromosome 17 will further elucidate epigenetic events important in ovarian tumorigenesis, as only a fraction (14%) (NCBI – Draft of the Human Genome web address: <http://www.ncbi.nlm.nih.gov>) of chromosome 17 genes are represented on the Affymetrix HuGeneFL GeneChip®.

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