Article

Identifying new human oocyte marker genes: a microarray approach

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Abstract

The efficacy of classical IVF techniques is still impaired by poor implantation and pregnancy rates after embryo transfer. This is mainly due to a lack of reliable criteria for the selection of embryos with sufficient development potential. Several studies have provided evidence that some gene expression levels could be used as objective markers of oocyte and embryo competence and capacity to sustain a successful pregnancy. These analyses usually use reverse transcription–polymerase chain reaction to look at small sets of pre-selected genes. However, microarray approaches allow the identification of a wider range of cellular marker genes which could include additional and perhaps more suitable genes that could serve as embryo selection markers. Microarray screenings of around 30,000 genes on U133P Affymetrix™ gene chips made it possible to establish the expression profile of these genes as well as other related genes in human oocytes and cumulus cells. This study identifies new potential regulators and marker genes such as *BARD1*, *RBL2*, *RBBP7*, *BUB3* or *BUB1B*, which are involved in oocyte maturation.

Keywords: *cell cycle, cumulus–oocyte complex, marker genes, microarray*

Introduction

The quality of oocytes obtained under ovarian stimulation for classical IVF varies considerably. Whilst most oocytes are capable of being fertilized, only half of those fertilized complete preimplantation development and fewer still implant.

After oocyte retrieval, several layers of cumulus oophorus cells still surround mature oocytes (metaphase II, MII) and immature oocytes [germinal vesicle (GV) and MI]. Granulosa cell-derived cumulus cells surround the oocyte in the antral follicle, and play an important role in regulating oocyte maturation (Dekel *et al.*, 1980; Larsen *et al.*, 1986). Ebner *et al.* (2006) demonstrated that, *in vitro*, the culture of human oocytes with attached cumulus cells may improve preimplantation embryo development.

Gene expression alterations in oocytes and their supporting cells can be related to defects or variations in the ovulation or maturation processes. Gene expression in granulosa cells is altered in patients with empty follicle syndrome (Inan *et al.*, 2006). A number of studies suggest that changes in gene expression, such as *GDF9* or *bone morphogenic protein-15* (*BMP15*) in oocytes, or *pentraxin 3* (*PTX3*) in cumulus cells, can be monitored for selecting oocytes for fertilization and embryos for implantation (Elvin *et al.*, 1999; Yan *et al.*, 2001; Zhang *et al.*, 2005).

Therefore, gene expression studies in human oocyte and cumulus cells could contribute not only to the identification of factors involved in the oocyte maturation pathway, but could also provide valuable molecular markers of abnormal gene expression in oocytes with reduced competence.

Specific gene expression screenings for caspase and cell death proteins (Spanos *et al.*, 2002), FSH receptor and luteinizing hormone/choriogonadotropin receptor (Patsoula *et al.*, 2003) or cell adhesion molecules (Bloor *et al.*, 2002) have also been attempted to determine the status of embryos. More recently, Wells *et al.* (2005a,b) analysed by quantitative polymerase chain reaction (Q-PCR) a panel of cell division and DNA damage marker genes (*BRCA1* and *2*, *ATM*, *TP53*, *RB1*, *BUB1*, *MAD2* and *APC*) to establish a correlation between their expression levels and the quality of preimplantation embryos (Wells *et al.*, 2005b). The aim of the present study, based in part on data obtained by Assou *et al.* (2006), was to apply a microarray approach to identify new potential regulators and marker genes involved in human oocyte maturation and in cumulus cell function.

Materials and methods

Oocytes and cumulus cells

Oocytes and cumulus cells were collected from patients consulting in the authors' centre for classical IVF or for intracytoplasmic sperm injection (ICSI). This study has received institutional review board approval. Patients were stimulated with a combination of gonadotrophin-releasing hormone agonist (GnRHa) (Decapeptyl PL 3; Ipsen, France) and recombinant FSH (Puregon; Organon, France or GONAL-f; Serono, France) or human menopausal gonadotrophin (HMG, Menopur; Ferring Pharmaceuticals Inc.). Ovarian response was evaluated by serum oestradiol concentration and daily ultrasound examination to observe follicle development. Retrieval of oocytes occurred 36 h after HCG administration and was performed under ultrasound guidance. Cumulus cells were removed from one or two mature (MII) oocytes 21 h post-insemination. Immature (GV and MI) oocytes and unfertilized MII oocytes were collected 21 h or 44 h post-insemination or post-microinjection by ICSI. Cumulus cells and oocytes were frozen at –80°C in RLT buffer (guanidine isothiocyanate buffer plus β-mercaptoethanol: RNeasy kit; Qiagen, Valencia, CA, USA) before RNA extraction. Two pools of immature oocytes (20 GV: seven patients, mean age 30 years ± 4.6 years; 20 MI: six patients, mean age 30.1 ± 6.7 years), two pools of MII oocytes (16: six patients, mean age 34 ± 4.5 years; 21: eight patients, mean age 33.2 ± 6.4 years) and two pools of cumulus cells (two patients, age 31 and 37 years) were separately analysed on six Affymetrix DNA microarrays. All these oocytes or cumulus cells were from couples referred to the centre for classical IVF (tubal infertility) or for ICSI (male infertility).

Complementary RNA (cRNA) preparation and microarray hybridization

RNA was extracted using the micro RNeasy Kit (Qiagen) and the total RNA quantity was measured with a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies Inc., DE, USA). RNA integrity was assessed with an Agilent 2100 Bioanalyser (Agilent, Palo Alto, CA, USA). cRNA was prepared with two rounds of amplification according to the manufacturer's protocol 'small sample protocol II', starting from total RNA (ranging from ~4 ng for pooled oocytes to 100 ng for cumulus cells), and hybridized to HG-U133 plus 2.0 GeneChip pangenomic oligonucleotide arrays (Affymetrix™, Santa Clara, CA, USA). HG-U133 plus 2.0 arrays contain 54,675 sets of oligonucleotide probes ('probeset'), which correspond to ≈30,000 unique human genes or predicted genes. Primary image analysis of the arrays was performed with the GeneChip Operating Software 1.2 (GCOS) (Affymetrix), resulting in a single value for each probe set ('signal'). Data from each different array experiment were scaled to a target value of 100 by GCOS using the 'global scaling' method. This algorithm determines whether or not a gene is expressed with a defined confidence level ('detection call'). This 'call' can either be 'present' (when the perfect match probes are significantly more hybridized than the mismatch probes, P-value < 0.04), 'marginal' (for P-values > 0.04 and < 0.06) or 'absent' (P-value > 0.06). A gene was denoted as 'absent' in a sample when all of its probe set displayed an 'absent' or 'marginal' detection call for this sample. The dataset was floored to 2, i.e. each signal value under 2 was given the value 2.

Statistical analysis

Samples were analysed by pairwise comparison using the GCOS 1.2 software (Affymetrix).

For hierarchical clustering, the probe sets included in **Table 1** were used (for a gene, the probe set with the highest signal in one of the samples). Signal values lower than 2 were arbitrarily floored to the value of 2. Data were log transformed, mean centred, and processed with the CLUSTER and TREEVIEW software packages with the average linkage method and un-centred correlation (Eisen *et al.*, 1998).

Gene search

Gene annotation lists (Unigene Build 190) were searched to identify related genes based on their description. The gene annotation lists included the following terms: gene symbol; gene name; the Gene Ontology 'biological process', 'cellular component' and 'molecular function'; and genetic pathway. The genes were filtered with the following criteria: lists comprising the terms 'retinoblastoma' (for *RB1*), 'bub' for *BUB1*, 'atm' OR 'atr' for *ATM*, 'tp53' for *TP53*, 'brca' for *BRCA1* and *2*, 'mad' for *MAD2L1*, and 'adenomatosis' for *APC* identified the genes presented in this study.

Database

The expression, including signal values, of all genes cited in **Table 1** can be examined on the authors' website as online supplemental data: expression of these genes in various normal tissues transcriptome datasets, including ovarian and testis samples, is provided through the 'Amazonia!' database web page: http://amazonia.montp.inserm.fr/.

Table 1. Affymetrix™ GeneChip signal values of 46 genes expressed in oocytes and cumulus cells.

Reference[a]	*Symbol name*	*Gene name*	*Cum*[b]	*GV*	*MI*	*MII*[b]	*Highest fold increase*[c]	
203132_at	RB1	retinoblastoma 1 (including osteosarcoma)	**920.2**	48.7	39.2	*9.0*	×23.5	in Cum/MI
212781_at	RBBP6	retinoblastoma binding protein 6	**327.7**	39.1	13.1	55.9	×25.0	in Cum/MII
205296_at	RBL1	retinoblastoma-like 1 (p107)	**30.9**	*9*	*2*	*9.7*	nd	Cum only
226696_at	RBBP9	retinoblastoma binding protein 9	**129.4**	27.3	34.9	*49.0*	×4.7	in Cum/GV
205169_at	RBBP5	retinoblastoma binding protein 5	40.9	94.1	165.5	**616.9**	×15.1	in MII/Cum
210371_s_at	RBBP4	retinoblastoma binding protein 4	315.9	1131.8	**2914.8**	1679.2	×9.2	in MI/Cum
201092_at	RBBP7	retinoblastoma binding protein 7	1765.1	3678.4	6372.3	**9743.3**	×5.5	in MII/Cum
209284_s_at	RAP140	retinoblastoma-associated protein 140	549.0	2344.6	**2874.2**	2054.3	×5.2	in MI/Cum
203344_s_at	RBBP8	retinoblastoma binding protein 8	356.4	**1904.8**	873.3	152.5	×12.5	in GV/MII
211950_at	RBAF600	retinoblastoma-associated factor 600	**529.1**	494.9	122.7	*246.8*	×4.3	in Cum/MI
212331_at	RBL2	retinoblastoma-like 2 (p130)	529.6	279.5	238.1	**657.7**	—	
209642_at	BUB1	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	42.5	821	**887.9**	251.4	×20.9	in MI/Cum
203755_at	BUB1B	BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast)	33.0	3802	**6299.9**	1033.1	×190.9	in MI/Cum
212350_at	TBC1D1	TBC1 (tre-2/USP6, BUB2, cdc16) domain family, member 1	117.5	527.3	**649.8**	621.4	×5.5	in MI/Cum
209974_s_at	BUB3	BUB3 budding uninhibited by benzimidazoles 3 homolog (yeast)	1220.8	2362	**2624.7**	629.2	×4.2	in MI/MII
200801_x_at	ACTB	actin, beta	**5347.3**	705.8	1809.2	4294.6	×7.6	in Cum/GV
210858_x_at	ATM	ataxia telangiectasia mutated (comp. groups A, C and D)	101.6	98.6	165	**562.0**	×5.7	in MII/GV
209903_s_at	ATR	ataxia telangiectasia and Rad3 related	449.4	**781.7**	511.6	205.4	×3.8	in GV/MII
201855_s_at	ASCIZ	ATM/ATR-Substrate Chk2-interacting $Zn2^{+}$-finger protein	392.6	**1369.3**	712.4	*117.4*	×3.5	in GV/Cum
201746_at	TP53	tumour protein p53 (Li-Fraumeni syndrome)	63.2	27.5	*29.7*	**163.8**	×6.0	in MII/GV
210609_s_at	TP53I3	tumour protein p53 inducible protein 3	**363.6**	*51.6*	*6.2*	*33.9*	nd	Cum only
222392_x_at	PERP	PERP, TP53 apoptosis effector	**131.3**	*12.1*	*2.5*	*42.4*	nd	Cum only
203050_at	TP53BP1	tumour protein p53 binding protein, 1	**445.4**	132.1	208.8	*8.0*	×3.4	in Cum/GV
225402_at	TP53RK	TP53 regulating kinase	**112.5**	*7.2*	*2.4*	*22.5*	nd	Cum only
203120_at	TP53BP2	tumour protein p53 binding protein, 2	**611.4**	158.8	200.6	*101.7*	×3.8	in Cum/GV
220167_s_at	TP53TG3	TP53TG3 protein	118.0	**571.3**	457.7	247.4	×4.8	in GV/Cum
225912_at	TP53INP1	tumour protein p53 inducible nuclear protein 1	707.4	438	553.6	**1770.8**	×4.0	in MII/GV
219370_at	RPRM	reprimo, TP53 dependant G2 arrest mediator candidate	*49.1*	*34.5*	73.3	*200.6*	nd	MI only
210886_x_at	TP53AP1	TP53 activated protein 1	**69.7**	33.9	44.8	*65.5*	×2.1	in Cum/GV
223342_at	RRM2B	ribonucleotide reductase M2 B (TP53 inducible)	789.8	848.4	1429.7	**2028.9**	—	
224836_at	TP53INP2	tumour protein p53 inducible nuclear protein 2	**430.6**	362.8	394.4	183.2	—	
204531_s_at	BRCA1	breast cancer 1, early onset	*11.4*	**988.2**	226.9	106.0	×9.3	in GV/MII
205345_at	BARD1	BRCA1 associated RING domain 1	61.0	1214.7	2897.5	**4351.0**	×71.3	in MII/Cum
235609_at	BRIP1	BRCA1 interacting protein C-terminal helicase 1	*10.6*	80.6	**127.3**	*75.0*	—	
213473_at	BRAP	BRCA1 associated protein	125.7	521.9	**687.2**	151.2	×5.5	in MI/Cum
230922_x_at	BRCC3	BRCA1/BRCA2-containing complex, subunit 3	**115.9**	27.5	4.4	*32.7*	×26.3	in Cum/MI

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Reference[a]	*Symbol name*	*Gene name*	*Cum*[b]	*GV*	*MI*	*MII*[b]	*Highest fold increase*[c]	
201419_at	BAP1	BRCA1 associated protein-1 (ubiquitin carboxy-terminal hydrolase)	**131.8**	*95.6*	*30.3*	*30.6*	nd	Cum only
202757_at	COBRA1	cofactor of BRCA1	**210.8**	199	175.5	177.4	—	
208368_s_at	BRCA2	breast cancer 2, early onset	5.9	270.9	246.3	**575**.2	×97.5	in MII/Cum
227322_s_at	BCCIP	BRCA2 and CDKN1A interacting protein	541.6	1239.8	**1594.8**	343.1	×4.6	in MI/MII
203362_s_at	MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)	81.7	3217	4035.1	**9559.6**	×117.1	in MII/Cum
203094_at	MAD2L1BP	MAD2L1 binding protein	140.7	**405.1**	310.7	44.8	×9.0	in GV/MII
204857_at	MAD1L1	MAD1 mitotic arrest deficient-like 1 (yeast)	*11.1*	**78.4**	32.1	*8.2*	×2.4	in GV/MI
223234_at	MAD2L2	MAD2 mitotic arrest deficient-like 2 (yeast)	130.7	100.4	110.7	**294.6**	—	
203525_s_at	APC	adenomatosis polyposis coli	210.8	**841.5**	590.3	131.3	×6.4	in GV/MII
227965_at	APC2	adenomatosis polyposis coli 2	18.6	*8.1*	*2*	*30.5*	nd	Cum only

Genes undetected in cumulus cells and oocytes: TP53I11, tumour protein p53 inducible protein 11; TP53I13, tumour protein p53 inducible protein 13; APCDD1, adenomatosis polyposis coli down-regulated 1.
[a]Affymetrix probe set reference; [b]Values represent the average value derived from independent samples hybridized to two DNA chip arrays; [c]Best fold increase (>2 and $P < 0.001$) found for the pairwise comparison given, or not determined (nd) between 'absent' genes or when not significant (—). Bold type = sample with highest signal; italic type = 'absent'. Cum = cumulus cells, GV = germinal vesicle, MI and MII = metaphase I and II oocytes respectively.

Results

Analysis of 'marker gene' expression in human oocytes and cumulus cells

The gene expression level of *BRCA1* and *2*, *ATM*, *TP53*, *RB1*, *BUB1*, *MAD2*, *APC* and *ACTB* was evaluated in cumulus cells, in unfertilized MII oocytes and in immature (GV and MI) oocytes. These genes are presented in bold type at the top of each section in **Table 1**. For cumulus cells and MII oocytes, the presented average signal values were calculated from two independent sample chip hybridization experiments for each. All genes were detected in cumulus cell and oocyte samples with the following exceptions: *BRCA1* was absent in cumulus cells, *TP53* was absent in MI oocytes, and *RB1* was absent in MII oocytes. In addition, *ACTB* and *MAD2L1* were present in all samples and presented the highest signal levels (circa 20-fold higher on average). The signal fold increase between cumulus cell average signal and all oocyte average signals (**Figure 1**) indicates that *RB1* is down-regulated in oocytes. On the other hand, *BUB1*, *BRCA1* and *2* and MAD2L1 are down-regulated in cumulus cells, whereas *ATM and APC* are slightly up-regulated in oocytes. Although weaker in GV and MI oocytes, *ACTB* expression varied little between samples.

Expression profile of new marker genes

The 'marker gene' names or symbol names were used as a keyword list to search the GeneNote annotations associated with each probe set present on the chip, in order to identify eight groups of genes related to the marker genes cited above (β-*Actin* was not included in the search). Thus, 149 probe sets were retrieved, of which one for each of the 40 corresponding genes is listed in **Table 1**. The signal values of the probe sets for each sample are indicated (highest sample signal in bold type, value in italic when absent). The highest fold change between sample pairs is also indicated (**Table 1**). Three of these genes (*TP53I-11, TP53I-13* and *APCDD1*: full names can be found in **Table 1**) were never detected in the samples, and are listed at the bottom of the table. In general, retrieved genes corresponded to proteins belonging to the regulatory pathway, to interacting partners or to paralogous proteins of the 'marker genes'.

The hierarchical clustering analysis of the probe set signal values from **Table 1** across all samples showed that oocytes cluster together, and suggested that some gene expression levels could be specific to the degree of oocyte nuclear maturation. As expected, the cumulus cell lineage is the most distant from oocytes. The main expression groups are for genes over-expressed in cumulus cells or in oocytes. For the latter, sub-groups of genes specific to MII oocytes, or to GV and MI oocytes, are also apparent (**Figure 2**). A lesser distinction was observed between GV and MI oocytes.

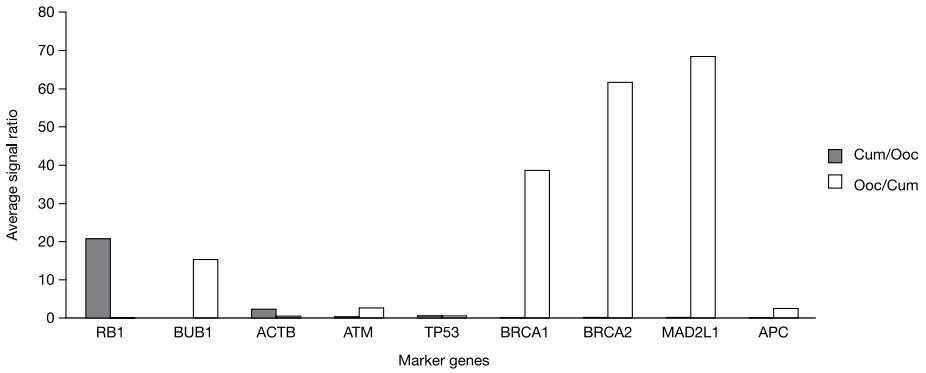

Figure 1. Marker genes up- or down-regulated in cumulus cells and oocytes. Histogram representation of the fold increase in the signal between cumulus cell and oocyte average signals for nine genes (cumulus versus oocyte in grey bars and oocyte versus cumulus in white bars). Probe set values are from **Table 1**.

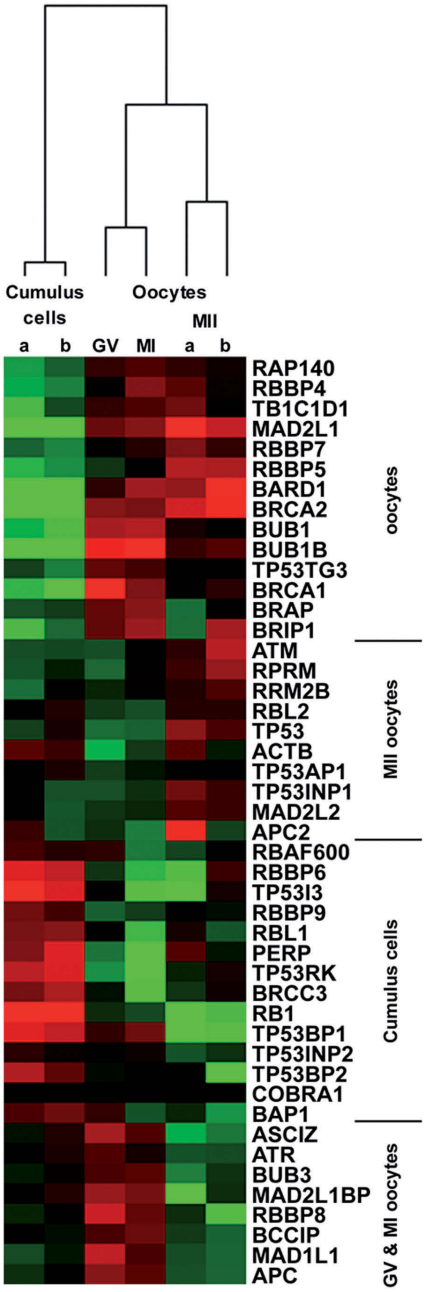

Figure 2. Hierarchical clustering of 46 genes signal values across cumulus cell and oocyte samples. Signal values were floored (minimal signal value = 2), log transformed and mean centred. Average linkage with un-centred correlation was evaluated using the CLUSTER software (Eisen *et al.* 1998). On the right side, genes are clustered by their preferential expression in the four samples. Red and green mark over- and under-expression respectively and black colour represents mean values. GV = germinal vesicle; M = metaphase.

RB1 group

The *RB1* profile was also found for *RBL1*, *RBBP6* and *RBBP9*, which were absent in MII oocytes. However, the *RB1* pathway was not completely down-regulated in oocytes. In contrast, the highest expression levels were detected with *RBBP7*, *RBBP4*, *RAP140*, and *RBBP8* in oocyte samples. *RBBP8* gene was highest in GV oocytes, *RBBP4* in MI oocytes and *RBBP7* and *RBL2* in MII oocytes.

BUB1 group

TBC1D1, BUB1B and *BUB3* displayed patterns similar to that of *BUB1*. *BUB1B* and *BUB3* mimicked *BUB1* expression but at much higher levels (6- and 3-fold respectively), although *BUB3* was high in cumulus cells as well.

ATM group

ATR and the *ATM*/*ATR* substrate *ASCIZ* differed from *ATM* in that their expression was high in cumulus and strongest in immature oocytes, particularly in GV oocytes.

TP53 group

Unlike *TP53*, for which the highest expression levels were found in MII oocytes, many targets or *TP53* partners were not expressed in MII oocytes. Two targets of *TP53* (*TP53TG3*, *TP53INP1*) were found at higher levels in oocytes whereas four partners (*TP53RK*, *PERP*, *TP53BP1* and *2*) and one target (*TP53I3*) were specific or over-expressed in cumulus cells. Among the genes expressed in oocytes, *RPRM*, *RRM2B* and *TP53INP2* were evenly expressed across the four samples (no significant change). *TP53TG3* and *TP53INP1* were up-regulated in GV and MII oocytes respectively.

BRCA1 and *BRCA2* group

BRCA1 and *BRCA2* partners remained mostly confined to oocytes with the strongest expression generally found in immature oocytes or cumulus cells. *BRCA1* was stronger in GV oocytes and *BRCA2* in MII oocytes. The cofactor *COBRA1* was highest in cumulus cells, but was actually not significantly different between all samples, whereas *BRCC3* and *BAP1* low expression was turned down in oocytes compared with cumulus cells. *BRIP1* was only found in GV and MI oocytes. Expression of *BARD1* represented the strongest signals, increasing from GV to metaphase MI and MII oocytes. *BRAP* and *BCCIP* were similar, but with reduced expression in MII oocytes.

MAD2 group

Genes related to *MAD2L1* did not match its high expression levels. *MAD2L2* is similar, but found at much lower levels. *MAD2L1BP* and *MAD1L1* appeared GV specific, and were not found or low in MII.

APC group

Following the search criteria, only one gene related to *APC, APC2,* was expressed in the sample, although at low levels and only in cumulus cells: no expression of the *APCDD1* gene was found.

Discussion

Marker gene expression in cumulus cells and immature oocytes

Recently, the expression of circa 30,000 human genes was reported in cumulus–oocyte complex gene expression profiling studies (Assou *et al.*, 2006). Some of them have previously been described as potential markers for the evaluation of human oocyte or embryo quality, based on their expression pattern in preimplantation embryos (Wells *et al.*, 2005a,b). These genes were *BRCA1* and *2*, *ATM*, *TP53*, *RB1*, *BUB1*, *MAD2*, *APC* and *ACTB*, which are involved in cell cycle checkpoint and DNA repair control.

The analysis of cumulus cell expression provided additional information on the gene expression profile of oocyte supporting cells. Overall, similar expression patterns were observed in all oocyte stages for these genes, but differences were nevertheless observed. Wells *et al.* (2005a,b) reported the strongest signal for *APC* in a 'typical' oocyte and used this value as a 100% scale reference for all the genes tested. In this study, *ACTB* and *MAD2L1* were present in all samples and showed the highest signal levels (circa 20-fold higher on average). Apart from these two genes, the strongest signals came from *RB1* in cumulus cells (signal value = 920), *BRCA1* in GV oocytes (988), *BUB1* in MI oocytes (888) and *BRCA2* in MII oocytes (575). The variations in levels of expression (e.g. for the *MAD2L1* and *APC* genes) could be due to the detection methods (microarray versus quantitative RT-PCR). The use of specific marker genes to normalize expression data should help in the comparison of expression values measured in different laboratories. The genes for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) or beta-2-microglobulin (*B2M*) are commonly used as ubiquitously expressed reference markers. The IkappaB kinase alpha gene (*CHUK*) was also recently proposed as a better internal standard for oocytes and pre-embryo cells (Falco *et al.*, 2006). *MAD2L1* was already observed at very high levels in single oocyte microarray analyses (Bermudez *et al.*, 2004). High *MAD2L1* expression in the MII oocytes could also reflect that these unfertilized oocytes are blocked in pro-metaphase II (Wassmann *et al.*, 2003). However, these variations do not interfere with the quality of these genes as oocyte and embryo fitness markers. Thus, for this set of genes, expression was similar to that of previous reports with the additional detection of *RB1* in cumulus cells.

The analysis of expression in immature (GV and MI) oocytes and in unfertilized MII oocytes provided supplemental insights into the *BRCA1* and *BRCA2* expression profiles: the two genes are co-expressed, but expression of the former is down-regulated, whereas the latter increased slightly during oocyte maturation.

Identification of new marker genes

Transcriptional control

The restriction of *RB1* expression to cumulus cells was intriguing for a gene usually found in most tissues. Expression of other

factors interacting with RB1, in particular *RBL1*, which is tightly restricted to cumulus cells, further suggests that regulation by *RB1* is involved in these cells. Although it is absent from oocytes and preimplantation embryos (Wells *et al.*, 2005a), its expression was detected in hatching blastocysts (Wells *et al.*, 2005b). Finding high *RBBP8* expression in GV oocytes is consistent with its binding to and modulation of *BRCA1* expression (Yu *et al.*, 2000; Yu and Chen, 2004). The high expression levels of *RBBP7*, *RBBP4* and *RBL2* in MI and MII oocytes suggested that this regulation pathway could be active during oocyte maturation. For *TP53* genes, the strongest signals were observed in MII oocytes for *RRM2B* and *TP53INP1*, which are both induced by *TP53*, with *TP53INP1* having the same positive action as *TP53* on catalases and proteasome endopeptidases (Tomasini *et al.*, 2005). *TP73*, a potential *TP53INP1* activator and TP53 homologue, was not expressed in the microarrays.

DNA repair markers

ATM and *ATR* both phosphorylate *BRCA1* (Gatei *et al.*, 2001) and were differentially expressed during maturation, with *ATR* appearing mostly in immature oocyte.

Expression of *BARD1* is interesting, because it displayed the strongest signals in its group and was co-expressed in oocytes with both *BRCA1* and *BRCA2*. BARD1 is an important regulator of BRCA1 activity: binding of BARD1 with BRCA1 maintains both proteins in the nucleus, thus preventing apoptosis (Fabbro *et al.*, 2004b). BARD1 is very similar to BRCA1 and both proteins induce apoptosis when they are confined to the cytoplasm (Schuchner *et al.*, 2005). BARD1 is also a key factor in DNA repair (reviewed by Henderson, 2005). The BRCA1–BARD1 complex is also required for ATM/ATR (*ataxia-telangiectasia-mutated/Rad3-related*)-mediated phosphorylation of P53 (Ser-15) (Fabbro *et al.*, 2004a).

Cell cycle checkpoint markers

The expression profile and the interaction networks of *BUB* and *MAD2* genes suggest that they could also provide new marker genes. The proteins BUB3 and BUB1B interact with CDC20 at checkpoint activation (Tang *et al.*, 2001). MAD2L2 negatively regulates the CDC20/anaphase promoting complex APC (Chen and Fang, 2001). MAD2L1 together with BUB1B inhibits CDC20/APC to prevent premature separation of sister chromatids (Fang, 2002). MAD2L1BP and MAD1L1 bind MAD2L1 and are crucial for localization of MAD2L1 to kinetochores, where it binds to CDC20 (Sironi *et al.*, 2001).

Identifying new oocyte or embryo marker genes

Gene expression is a first step in the identification of potential marker genes, and it has been undertaken by different research groups using microarray approaches (Bermudez *et al.*, 2004; Richards *et al.*, 2005; Assou *et al.*, 2006). The Affymetrix GeneChip is a reliable microarray system (www.Affymetrix.com/community/publications/index.affx), presenting little inter-laboratory variability (Irizarry *et al.*, 2005). Different criteria can be used to preselect candidate marker genes. Stronger expression may be easier to detect but variation in expression could be less visible or less relevant. Genes that are more specific reflect tighter regulation and could provide better reporter genes because variations may be more readily detectable. However, after their identification on the basis of gene expression profiles and the verification of their patterns by Q-PCR, understanding the function of factors in regulating pathways will be the next validation step to select marker genes. In the end, experimental data linking their expression levels with oocyte or embryo quality status will determine their practical value. The list of genes presented here was filtered on the basis of keywords and was not pathway oriented. In the case of *APC*-related genes, the search criteria were clearly not appropriate. Analysing other genes such as *CTNNB1* (highly expressed in oocytes in Bermudez *et al.*, 2004), *AXIN2*, *WNT1* or *WNT8A*, which are partners of *APC* in the WNT signalling pathway could be more relevant. Probably other *ATM*/*ATR* targets, not initially reported with the search criteria, may represent alternate markers to *ATM*: *H2AFX*'s and *CHEK1*'s profiles are similar to that of *ATM* but with stronger signals, and *CHEK2* was only detected in GV and MI oocytes (data not shown). The fact that some genes analysed in this study were not detected at all, or only in some samples, raises different interpretations. They may be truly absent, their expression levels could be below the detection threshold of the microarray approach or the lack of detection could reflect sample or experimental discrepancies. The two former possibilities seem most likely, because only a couple of signal values are sporadic and could result from the latter explanation. Indeed, most absent signals were either duplicated in separate experiments (cumulus cells and MII samples) or were concomitantly observed within groups of related samples such as immature oocytes or all oocytes. Therefore, absent signals reported here should be viewed as very low or absent transcripts.

With the present study, some factors are clearly put in perspective as potential markers of oocyte competence (**Figure 3**). Their expression profiles suggest different roles in supporting cumulus cells or in oocytes during successive maturation stages. *RBL1*

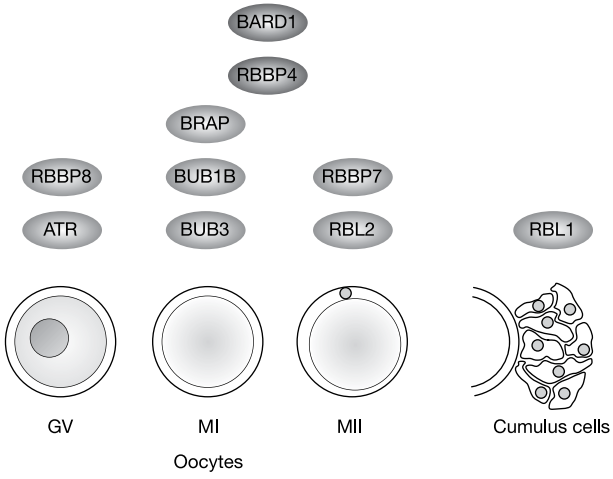

Figure 3. New marker genes involved in oocyte maturation. Factors identified in this study with restricted expression in immature germinal vesicle (GV) and metaphase I (MI) oocytes, in unfertilized metaphase II (MII) oocytes and in cumulus cells. Factors in darker shade are shared by MI and MII oocytes.

appears as a very specific marker in cumulus cells. However, most of the genes presented here are preferentially expressed in oocytes and different criteria should be used to identify cumulus cell markers that could reflect oocyte quality. A number of oocyte factors interact within the *BRCA1* regulation pathway and are co-expressed in MII oocytes. RBBP8, BRAP and ATR bind and modulate BRCA1 activity (Li *et al*., 1999) and are co-expressed with BRCA1. RBBP8 also binds RB1 and BARD1 (Yu and Baer, 2000). RBBP4 and RBBP7 bind BRCA1 like RB1 (Yarden *et al*., 1999). The interaction of BUB1B and BUB3 with RBL2 (Cam *et al*., 2004) also link them to the RB1 and BRCA1 regulation pathways. *BUB1B* or *RBBP7* were already observed as highly expressed genes in previous studies (Bermudez *et al*., 2004; Assou *et al*., 2006). Thus, genes were identified with relevant expression patterns to serve as a resource for potential new oocyte markers. Interestingly, the factors encoded by these genes intersect in common regulatory pathways.

The aim was to show the relevance of the microarray approach to identify and bring forward new potential regulators and marker genes. Such study is qualitative and partly quantitative within the limits of the microarray approach. The adjunction of additional independent series could strengthen these results further. However, once a discrete number of genes has been selected, the validation of differential expression by Q-PCR is more reliable, faster and more cost-effective. Thus, Q-PCR analyses on specific genes presented herein will be the focus of future studies. Finally, the account of oocyte and cumulus cell gene expression profiles should be strengthened by functional analyses, since protein activity often depends on post-translational modification and interactions with other partners. Proteomics and interactome analyses may have the last word in determining the real activity of genes and proteins.

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