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ORIGINAL PAPERS

Expression profile of genes coding for DNA repair in human oocytes using pangenomic microarrays, with a special focus on ROS linked decays

Yves Jr Menezo · GianLuigi Russo · Elisabetta Tosti · Said El Mouatassim · Moncef Benkhalifa

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Abstract

Purpose To determine the level of expression for mRNAs that regulate DNA repair activity in oocytes at the germinal vesicle (GV) stage. Reactive oxygen species (ROS) have been shown to play a major role in the appearance of deleterious DNA decays, and this study focuses on the repair of damage linked to decay caused by the action of ROS. The oocyte needs a mechanism for repairing DNA decays in the early preimplantation embryo before the onset of genomic activation, since in the absence of repair, residual DNA damage would lead to either apoptosis or tolerance. Tolerance of DNA damage is a source of potential mutations.

Method GV oocytes were selected for this study, both for the ethical reason that they are unsuitable for patient treatment, and because no transcription takes place during the period from GV to MII and then prior to genomic activation. The GV oocyte is therefore a good model for looking at DNA during the first cleavages of early preimplantation development. Six cohorts of GV oocytes were pooled for extraction of mRNA; the DNA was analysed using Affimetrix HG-UG133 Plus 2, containing 54,675 probe sets; spike and housekeeping genes were also added as internal controls.

Results In GV oocytes, DNA repair pathways for oxidized bases are redundant. One step repair procedure (OSR), BER (base excision repair), MMR (mismatch repair) and NER (Nucleotide excision repair) are present. All the recognition proteins are also present. The chromatin assembly factors necessary for the maintenance of genomic stability are highly expressed.

Conclusion Gene expression analysis shows that the oocyte does not allow a high level of tolerance for DNA decays. This regulatory mechanism should avoid transmitting mutations into the next generation.

Keywords Human oocyte · ROS · mRNA · DNA repair

Y. J. Menezo
UNILABS,
12 Place Cornavin,
Geneva, Switzerland

Y. J. Menezo
Laboratoire d'Eylau,
55 Rue St Didier,
75116 Paris, France

G. Russo
National Research Council,
83100 Avellino, Italy

E. Tosti
Stazione Zoologica,
Villa Communale 1,
Napoli, Italy

S. E. Mouatassim · M. Benkhalifa
ATL R&D. Reproductive Biology & Genetics Laboratory,
78320 La Verrière, France

Y. J. Menezo (✉)
UNILABS/Laboratoire d'Eylau,
55 rue St Didier,
75116 Paris, France
e-mail: Yves.menezo@eylau.fr

Introduction

The impact of male infertility on human reproduction is still a matter of debate. For many years, male fertility has been defined as the ability of sperm to fertilize oocytes that develop into early cleavage stage embryos. In human IVF, the development of two to four cell embryos was

considered to be a test for sperm fertilizing ability, with the assumption that all of the resulting embryos had the same developmental potential, irrespective of sperm and oocyte quality. However, it is now admitted that differences in male fertility are not related simply to sperm penetration failure, but also to the ability of the oocyte to support zygote development post fertilization. Attention has therefore been more carefully focused towards the quality of sperm DNA: a range of methods have been developed to evaluate paternal DNA integrity that are independent of all semen parameters including sperm morphology, concentration and motility [1]. The same conclusion is reached irrespective of the methodology used to examine DNA integrity: sperm DNA fragmentation and decays related to reactive oxygen species (ROS) lead to reduced fertility [2, 3], and ROS have a heavily deleterious impact on sperm DNA [4, 5]. A major end product of ROS damage to DNA is the formation of apurinic/apyrimidic (AP) sites [6, 7], but 8 oxo deoxyguanosine (8 OXO dG or 8 OH dG) can also be produced. We recently described the formation of ethenoadenosine and ethenoguanosine (1,N^2-etheno-2′-deoxyguanosine (εdGuo), and 1,N^6-etheno-2′-deoxyadenosine (εdAdo) in human sperm [8]. Shortened telomeres, in relation to age and sperm DNA fragmentation [9] may lead to inappropriate end to end fusion and the formation of chromosome bridges. On the other hand, paternal DNA always carries a varying level of DNA strand breaks [1–10]. These decays are the result of ROS and/or anomalies of topoisomerase activity during spermatogenesis. Other types of decays may arise, especially in sperm with high teratospermia. Demethylation mediated by DNA repair is also a potent promoter of epigenetic activation [11].

The worldwide use of ICSI raises questions regarding oocyte DNA repair activity. All DNA lesions have profound effects on cell viability, leading to apoptosis and then cell death, which is a recurrent feature in IVF. The most highly detrimental situation is probably when the cell tolerates these unrepaired decays—this leads sooner or later to mutations and oncogenic transformation. Fortunately, mammalian cells, including oocytes, have systems that allow DNA repair [12–15]. DNA repair is probably one of the most important processes taking place during and post-fertilization in the oocyte and zygote, in order to allow complete embryonic development and avoid developmental arrests. The quality of maternal DNA during oocyte maturation at the genetic and epigenetic level is a matter for conjecture, but there is indirect evidence showing a positive effect of antioxidants on oocyte quality [16]. GV oocytes collected during a stimulated IVF cycle are unsuitable for treatment and are therefore surplus; therefore, primarily for ethical reasons as the French law forbids any type of work destroying human embryos, these were selected to examine the expression profile of genes involved in DNA repair activity. However, the mRNA content in GV oocytes is the same as that of MII oocytes, since there is no transcription during the final stages of oocyte maturation, only a remodeling of mRNA that is basically related to polyadenylation [17]. The first steps of preimplantation embryo development, at least until maternal to zygotic transition (i.e. four to eight-cell stage), are carried out with the use of maternal stores of protein and mRNA [18–20].

Materials and methods

The oocytes were collected from two different IVF units, at the time of preparation for ICSI. The current protocol is semi long analog treatment followed by stimulation with recombinant FSH. The mean age of the patient was 33.1 years. After careful denudation of all oocytes retrieved, any GV oocytes present were used for this study, a total of 160 oocytes. The pooled oocytes were divided into six samples: the first four samples had ten oocytes per batch, and the remaining two samples were allocated sixty oocytes each, in order to increase the starting quantity of mRNA.

The analyses were performed with microarrays, using the Affymetrix HG U-133 plus 2.0 chips. Amplification was performed via double IVT (in vitro transcription, two-cycle cDNA synthesis kit, Affimetrix). Data were normalised using the algorithm MAS5, in order to generate a signal for each of the 54,613 transcripts of the chips (Probe set ID) that was proportional to the level of expression level. A call signal points out and specifies if the gene is expressed at a level above the background. The expression level is given as high, medium or low according to the signal. Housekeeping and spike controls were used for each determination. Expression was interpreted as low when the signal was above the background (Signal=15) and up to a signal of 200 in at least three of the samples. The expression was classified as medium for a signal between 200 and 1,000; it was considered as high for a signal repeatedly over 1,000. Our references for the human DNA repair genes were based on Wood et al. [12, 21] and Cline and Hanawalt [22].

Results

Out of 54,613 probes tested, the overall number detected was 15,615 (27.7%, range 23.7–33.7). The number of probes at the limit of detection was between 1.2 and 1.5%, with a maximum of 879 with no clear cut absence or presence. No one of the DNA repair probe was in this borderline zone of expression. Five housekeeping gene

probes and 17 spikes were used as internal controls. Absence of Amphiregulin (AREG), epiregulin (EREG) and betacellulin (BTC) that are normally present in cumulus cells, was checked in order to confirm that the GV oocytes were completely denuded.

The list of the genes involved in DNA repair activity [12, 21, 22], with their level of expression is listed in Table 1.

Discussion and conclusion

In all eukaryotic cells, DNA damage must be highlighted by DNA sensors that initiate a cellular response. Three options are then open in response to this signal: DNA repair, cell cycle arrest or apoptosis—events that occur frequently in human IVF. In order to maintain genomic stability, the early embryo must have the capacity to fully repair DNA, in particular damage linked to ROS-related decays in sperm DNA. DNA oxidation leads globally to three different oxidized molecules for each of the bases, the most important being 8 oxo guanine, 8 oxo adenine, FapyA and G (aA and G) and thymine glycol. Most of the genes with a proven or a suspected DNA repair activity for these decays are expressed in the oocyte.

Using BER, a multistep pathway involving several protein complexes, the removal of a single base is performed by a DNA glycosylase in the oocyte. The remaining AP site (apurinic/apyrimidic) site is hydrolyzed by an AP endonuclease. The single nucleotide gap is filled by polymerase beta (highly expressed in the oocyte—see Table 1–DNA polymerase, catalytic subunits) and ligated. BER pathways seem able to remove etheno-adducts and other oxidative products. APEX, capable of initiating the repair of apurinic/apyrimidic (AP) sites, the most common decay in damaged DNA, is highly expressed. APEX-1 also known to play an important role in response to oxidative stress, providing the reducing potential necessary for ref-1 activity in relation to thoredoxin:. [6, 23, 24]. The DNA-binding activity of APEX is modulated by a post translational mechanism involving reduction oxidation, and so is at least partly mediated by ROS: the RNAs coding for thiorexdoxin and Thioredoxin reductase and the transcripts for 6 members of the peroxiredoxin family are also present. This suggests that there is a very active complete pathway for the repair of ROS induced DNA decays. Surprisingly, OGG1, which removes 8 oxo DG, does not seem to have a high level of expression. The mRNA for 8-oxo-GTPase is highly expressed (see modulation of nucleotide pools): this enzyme hydrolyzes the oxidized nucleotide 8-oxo-dGTP to 8-oxo-dGMP, preventing incorporation of 8-oxo-dGTP into DNA.

After DNA replication, mismatch (MMR) repair is one of the pathways that is also associated with the repair of 8-oxoG lesions; MSH2–MSH6, highly expressed here, binds the 8-oxo containing DNA substrates, enhances ATPase and increases ADT—⟶ ADP exchange activity [25]. The oocyte seems to be well equipped for removal of DNA lesions linked to ROS, but not through the most common pathway, OGG1. We recently described the presence of etheno adducts in human sperm [8]. *N*-methyl purine DNA glycosylase (MPG) mRNA is highly expressed in the oocyte, and this is also true, to a lesser extent, for TDG (thymine DNA glycosylase): both enzymes remove the etheno adducts from DNA. TDP1 (tyrosyl–DNA phosphodiesterase 1), also highly expressed in the oocyte, removes glycolate from single-stranded DNA containing a 3′-phosphoglycolate, suggesting a role in the repair of free-radical mediated DNA double-strand breaks.

DNA methylation is a major epigenetic process, with a particular role in imprinting. SAM synthetase activity is required for DNA methylation activity through DMT (DNA methyl transferase), and has a high level of expression in both mouse and human oocytes and early preimplantation embryos [26]. The mechanism for direct reversal of damage, repairing alkylation damage in one step, is also expressed. This system is of major importance in the control of hypermethylation, i.e. the regulation of imprinting processes.

The oocyte has a full panel of mRNAs coding for Nucleotide excision repair (NER or global genome repair), a multistep pathway that involves more than 30 proteins. TCR, transcription-coupled-repair, is a more discrete pathway, responsible for removal of DNA insults that block transcription. Since there is no transcription before the 8-cell stage, this pathway is redundant at the earlier stages. However, depending on their mechanism of storage, these mRNAs can be translated at later stages post-fertilization. Other genes involved in this NER/TCR pathway are highly expressed: ERCC6(CSB), GTF2H1,2 and 5, and MMS19. Shortening of telomeres has a strong association with sperm DNA fragmentation [9], and this leads to aberrant DNA repair responses. The tumor suppressor gene TP53 plays a crucial role in controlling this inappropriate response to damage, by determining whether the DNA will be repaired, or whether the cell will undergo programmed cell death. Any decrease in TP53 activity will allow a permissive environment, leading to chromosome fusion and the formation of chromosome bridges. We found a surprisingly moderate level of TP53 transcription. TDP1 is a ubiquitous factor involved in repairing covalent topoisomerase I-DNA complexes (DNA-protein cross-links). DCLE1A and B, with DNA cross-link repair activity, are highly expressed (see Table 1: other identified genes with suspected DNA repair activity).

Table 1 Expression levels of mRNAs coding for DNA repair in human oocytes

Gene	Expression level	Role in DNA repair
Base excision repair (BER)		
UNG	High	Releases U
SMUG1	Low	Releases altered U
MBD4	Medium	Releases U or T opposite G at CpG
TDG	Low	Releases U, T or EthenoC opposite G
OGG1	Medium	Releases 8 oxo or FAPY G opposite C
MUTYH (MYH)	Medium	Releases A opposite 8 oxo
NTHL1 (NTH1)	Medium	Releases fragmented or ring saturated pyrimidines (Fapy), thymine glycol, 5 OHU, 6-OHC
MPG	High	Removes 3me-A, ethenoA, hypoxanthine
NEIL1	Low	Remove thymidine glycol, 8-oxoG, FapyG, FapyA, 5OH–U, 5OH–C
NEIL2	Low	Removes oxidative products of pyrimidines, 5OH–U, 5OH–C
APEX1	High	AP endonuclease
APEX2	Medium	AP endonuclease
LIG3	Low	Ligase III, DNA, ATP-dependent, main ligation function
XRCC1	High+	Ligase accessory factor, main ligation function
PNKP	Low	Convert DNA breaks to ligatable ends
PARP1 (ADPRT)	High	ADP-ribosyltransferase, protects against strand interruption
PARP2 (ADPRTL2)	High	Id
Direct reversal of damage		
MGMT	Medium	O-6-methylguanine–DNA methyltransferase
MGC90512 (ABH2)	High	Alkylation repair: 1-Me A dioxygenase
DEPC-1 (ABH3)	Medium	Alkylation repair: 1-Me A dioxygenase
Repair of DNA–protein cross-links		
TDP1	High	Tyrosyl–DNA phosphodiesterase: removes TOP1–DNA complex
Mismatch excision repair (MMR)		
MSH2	High	DNA mismatch repair protein, mismatch and loop recognition
MSH3	High	Id
MSH6	High	Id
MSH4	*Not expressed*	
MSH5	Medium	Id, specialized for meiosis
PMS1	Medium	DNA mismatch repair protein
MLH1	Medium	Mut forming heterodimers
PMS2	Medium	DNA mismatch repair protein
MLH3	*Not expressed*	Mut of unknown function
PMS2L3	Low	Id
PMS2L4 (PMS6)	*Not expressed*	
Nucleotide excision repair (NER)		
XPC	High	DNA repair protein, binds DNA damaged
RAD23B (HR23B)	High	Excision repair protein
CETN2	High	Binds damaged DNA as complex
RAD23A (HR23A)	Medium	Substitute for RAD 23B
XPA	High	Binds DNA damaged in preincision complex
RPA1	Very High	Replication protein, binds DNA in preincision complex
RPA2	Low	Replication protein, ID
RPA3	High	Replication protein, ID
TFIIH		Catalyses unwinding in preincision complex
ERCC3 (XPB)	*Not expressed*	« ‘to 5’ DNA helicase
ERCC2 (XPD)	*Not expressed*	5′ to 3′ DNA helicase
GTF2H1	High	General transcription factor
GTF2H2	High	ID
GTF2H3	Low	ID
GTF2H4	Low	ID

Table 1 (continued)

Gene	Expression level	Role in DNA repair
GTF2H5 (TTDA)	High	ID
CDK7	High	Kinase subunit of TFIIH
CCNH	High	Cyclin H, kinase subunit of TFIIH
MNAT1	*Not expressed*	Kinase subunit of TFIIH
ERCC5 (XPG)	High	3′ Incision
ERCC1	*Not expressed*	5′ Incision
ERCC4 (XPF)	*Not expressed*	5′ Incision
LIG1	Medium	DNA joining
NER-related		
CKN1 (CSA)	*Not expressed*	
ERCC6 (CSB)	High	Putative transcription-repair coupling factor
XAB2 (HCNP)	Medium	PA binding protein 2
DDB1	High	Damage-specific DNA binding protein
DDB2	Medium	Damage-specific DNA binding protein
MMS19L (MMS19)	Medium	Transcription and NER
Homologous recombination		
RAD51	Low	Homologous pairing, DNA repair protein
RAD51L1(RAD51B)	Medium	ID
RAD51C	High	ID
RAD51L3(RAD51D)	*Not expressed*	
DMC1	Low	Meiotic recombination protein
XRCC2	*Not expressed*	DNA break and cross-link repair
XRCC3	*Not expressed*	ID
RAD52	*Not expressed*	Accessory factor for recombination
RAD54L	Medium	DNA repair and recombination protein
RAD54B	Low	DNA repair and recombination protein
BRCA1	High	Accessory factor for transcription and recombination
BRCA2	Medium	Cooperation with RAD1 for homologous pairing
SHFM1 (DSS1)	Low	Associated with BRCA2
RAD50	Medium	ATPase
MRE11A	High	3′Exonuclease
EME1 (MMS4L)	High	Essential meiotic endonuclease
Non-homologous end-joining		
G22P1 (Ku70)	*Not expressed*	DNA end binding
XRCC5 (Ku80)	Medium	ATP dependant DNA helicase
PRKDC	*Not expressed*	Protein kinase, DNA-activated, catalytic polypeptide
LIG4	Medium	Ligase IV, DNA, ATP-dependent
XRCC4	High	Ligase accessory factor
DCLRE1C	Medium	DNA cross-link repair, nuclease
XLF NHEJ1)	Not expressed	XRCC4–LIG4 interacting factor
Modulation (sanitization) of nucleotide pools		
NUDT1 (MTH1)	Medium	8-OxoGTPase
DUT	High	DUTP pyrophosphatase
RRM2B (p53R2)	High	Ribonucleotide reductase (TP53 inducible)
DNA polymerases (catalytic subunits)		
POLB	High	Polymerase beta nuclear DNA directed
POLG	Medium	Polymerase delta, mitochondrial DNA directed
POLD1	Low	Polymerase DNA directed, NER and MMR
POLE	Low	Polymerase DNA directed, epsilon
PCNA	High+	Proliferating cell nuclear antigen, sliding clamp for POLD and POLE
REV3L (POLZ)	Medium	Catalytic subunit of DNA polymerase zeta
(REV7)MAD2L2	Medium	Catalytic subunit of DNA polymerase zeta
REV1L (REV1)	High	DCMP transferase
POLH	Medium	Polymerase DNA directed, eta
POLI (RAD30B)	Medium	Polymerase DNA directed iota, lesion bypass

Table 1 (continued)

Gene	Expression level	Role in DNA repair
POLQ	High	Polymerase, DNA directed, theta, DNA cross-link repair
POLK (DINB1)	*Not expressed*	Polymerase, DNA directed, kappa
POLL	*Not expressed*	Polymerase DNA directed, lambda
POLM	*Not expressed*	Polymerase (DNA directed), mu
POLN (POL4P)	*Not expressed*	Polymerase (DNA directed) nu
Editing and processing nucleases		
FEN1(DNaseIV)	High	Flap structure-specific endonuclease, 5′ nuclease
TREX2	*Not expressed*	3′ Repair exonuclease, 3 exo′ nuclease
EXO1(HEX1)	High	5′ Exonuclease
SPO11	*Not expressed*	Endonuclease
FLJ35220 (ENDOV)	Low	Incision 3′ of hypoxanthine and uracil
Rad6 pathway		
UBE2A (RAD6A)	*Not expressed*	Ubiquitin-conjugating enzyme
UBE2B (RAD6B)	*Not expressed*	Ubiquitin-conjugating enzyme
RAD18	Medium	E 3 ubiquitin ligase, assists repair or replication of damaged DNA
UBE2N (UBC13)	High	Ubiquitin-conjugating enzyme
Chromatin Structure		
H2AFX (H2AX)	High	H2A histone family, phosphorylated after DNA damage, DNA damage signalling
CHAF1A (CAF1)	High	Chromatin assembly in DNA replication and DNA repair
Other identified genes with a suspected DNA repair function		
DCLRE1A (SNM1)	High	DNA cross-link repair 1A
DCLRE1B(SNM1B)	High	DNA cross-link repair
RPA4	Medium	Replication protein, binds DNA in preincision complex
APTX (aprataxin)	Medium	Aprataxin: processing of DNA single strand interruption
NEIL3	Low	Endonuclease
RECQL (RECQ1)	Low	RecQ protein-like (DNA helicase Q1-like)
RECQL5	High	DNA helicase
HEL308	Medium	DNA helicase
RAD52B (RDM1)	*Not Expressed*	
Other conserved DNA damage response genes		
ATR	High	DNA damage sensor
RAD1	Low	Cell cycle checkpoint protein
RAD9A	High	Cell cycle checkpoint control protein
HUS1	Low	Checkpoint protein
RAD17 (RAD24)	Medium	Cell cycle checkpoint protein
CHEK2	High	CHK2 checkpoint homolog, effector kinase
TP53	Medium	Tumor suppressor 53, regulation of the cell cycle, apoptosis

Maintaining a correct three-dimensional chromatin structure is mandatory, as the 3D structure is critical during chromosome segregation. This is especially true in human IVF, where a high incidence of mosaicism is a common feature. Histones are a central feature of transcription regulation, DNA repair, DNA replication and chromosomal stability; BRCA1 and 2, highly expressed in the oocyte, play a central role in maintaining genomic stability (See also Wells et al. 2005 [20]). We also found a very high level of expression for H2AFX (H2A histone family member X) and CHAF1 (chromatin assembly factor 1), both involved in chromatin structure regulation during and after DNA repair. It must be pointed out that several types of regulation, in particular polyadenylation [17–27], will allow these mRNAs to be translated: portions of the mRNA can be lost both pre and post translation. Poly(A) binding proteins and the cytoplasmic polyadenylation elements are highly expressed in the oocyte (up to 500 times the background level). However, mRNA expression usually indicates that the corresponding proteins are being produced and further utilized, especially in the case of the early embryo prior to genomic activation [20].

In conclusion, high permissivity and tolerance for DNA decays is a highly detrimental situation for oocytes and

early preimplantation embryos. Although not all of the mRNAs detected here are fully translated, and we have not considered the level of functional protein repair, the pathways identified are highly expressed, and are somewhat redundant. In human IVF, developmental arrest and implantation failure may be linked to one of the classical responses to DNA decays, i.e. apoptosis [28]. However, when DNA decays reach a very high level in gametes, as is the case in heavy smokers [29], the limits of DNA repair activity might be overreached, with an increase in childhood cancer due to DNA mutations as a potential result. This aspect should not be overlooked in Assisted reproductive technology, especially for IVF/ICSI using poor quality sperm with high levels of ROS-related decays and a high degree of sperm DNA fragmentation [30].

On a final note, growth hormone (GH) up regulates DNA repair capacity in liver cells [31], human naked oocytes and cumulus cells have receptors for GH [32], and co-stimulation with GH has been shown to be efficient in human IVF, especially in patients over 40 years of age where mRNA expression is decreasing. An attempt to increase the DNA repair capacity of the oocyte, perhaps with the use of GH would be an interesting approach to examine.

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