University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Faculty Publications from the Department of Electrical and Computer Engineering

Electrical & Computer Engineering, Department of

2006

Human transcriptome corresponding to human oocytes and use of said genes or the corresponding polypeptides to trans-differentiate somatic cells

Jose Bernardo Cibelli

Arif Murat Kocabas

Bing Lim

Hasan H. Otu
University of Nebraska-Lincoln, hotu2@unl.edu

Follow this and additional works at: http://digitalcommons.unl.edu/electricalengineeringfacpub

Part of the Computer Engineering Commons, and the Electrical and Computer Engineering Commons

Human transcriptome corresponding to human oocytes and use of said genes or the corresponding polypeptides to trans-differentiate somatic cells

https://patents.google.com/patent/WO2008030610A2/

Abstract

The identification of 101 genes upregulated or differentially expressed by mature human oocytes is provided herein. These genes and the corresponding gene products will facilitate a greater understanding of oogenesis, folliculogenesis, fertilization, and embryonic development. In addition these genes and the corresponding gene products can be used to effect dedifferentiation and/or transdifferentiation of desired somatic cells. The resultant dedifferentiated cells and somatic cells derived therefrom can be used in cell therapies such as in the treatment of cancer, autoimmunity, and other diseases wherein specific types of cells such as hematopoietic cells may be depleted because of the underlying disease or the treatment of the disease. Also, a core group of 66 transcripts was identified by intersecting significantly up-regulated genes of the human oocyte with those from the mouse oocyte and from human and mouse embryonic stem cells. Within the up-regulated probe sets, the top overrepresented categories were related to RNA and protein metabolism, followed by DNA metabolism and chromatin modification. This invention therefore provides a comprehensive expression baseline of genes expressed in in vivo matured human oocytes. Further understanding of the biological role of these genes will also expand knowledge on meiotic cell cycle, fertilization, chromatin remodeling, lineage commitment, pluripotency, tissue regeneration, and morphogenesis.

Classifications

C12N15/8509 Vectors or expression systems specially adapted for eukaryotic hosts for animal cells for producing genetically modified animals, e.g. transgenic
View 4 more classifications

WO2008030610A2

WO Application

Find Prior Art Similar

Other languages
French

Inventor
Jose Bernardo Cibelli
Arif Murat Kocabas
Bing Lim
Hasan H. Otu

Original Assignee
Michigan State University

Priority date
2006-09-08

Family: US (1)WO (1)

Info

External links

Description

HUMAN TRANSCRIPTOME CORRESPONDING TO HUMAN OOCYTES AND USE OF SAID GENES OR THE CORRESPONDING POLYPEPTIDES TO TRANS- DIFFERENTIATE SOMATIC CELLS

RELATED APPLICATIONS

[0001] This application claims priority to US provisional Serial No. 60/842,990 filed on September 8, 2006. This application is incorporated by reference in its entirety herein.

FIELD OF THE INVENTION

[0002] The present invention relates to the identification of a set of genes which are expressed by in vivo matured human oocytes ("transcriptome" of human oocytes) and which are involved in oogenesis, folliculogenesis, fertilization, and embryonic development. These genes and the corresponding gene products are useful for dedifferentiation or transdifferentiation of somatic cells . Additionally, these genes are useful as markers of undifferentiated cell types and for assaying whether an ESC is capable of giving rise to an oocyte and for identifying pregnancy competent oocytes.

[0003] The identification of genes and deduced pathways from the mature human oocyte will also facilitate a greater understanding of oogenesis, folliculogenesis, fertilization, and ˙embryonic development. As described in detail infra, the present human oocyte transcriptome was derived using human metaphase II oocytes within minutes after removal from the ovary, and its transcriptome was compared with a reference sample consisting of a mixture of total RNA from 10 different normal human tissues not including the ovary. RNA amplification was performed by using a unique protocol. A core group of 66 transcripts was identified by intersecting significantly up-regulated genes of the human oocyte with those from the mouse oocyte and from human and mouse embryonic stem cells. In addition 101 human genes were identified as being differentially expressed by human oocytes that are potentially useful separately or in combination for effecting dedifferentiation of human somatic cells. Within the up-regulated probe sets, the top overrepresented categories were related to RNA and protein metabolism, followed by DNA metabolism and chromatin modification. This invention therefore provides a comprehensive expression baseline of genes expressed in in vivo matured human oocytes. Further understanding of the biological role of these genes will further expand the understanding of meiotic cell cycle, fertilization, chromatin remodeling, lineage commitment, pluripotency, tissue regeneration, and morphogenesis.

BACKGROUND OF THE INVENTION

[0004] The mammalian oocyte is responsible for a number of extraordinary biological processes. It has the ability to haploidize its DNA, to reprogram sperm chromatin into a functional pronucleus, to drive early embryonic development, and to give rise to pluripotent embryonic stem cells (ESCs). Identifying the genes in the oocyte that are essential for oogenesis, folliculogenesis, fertilization, and early embryonic development will provide a valuable genomic resource in reproductive and developmental biology. However, the oocyte transcriptome and its functional significance in the human are relatively unknown because of ethical and technical limitations.

[0005] Although extensive genomic studies of oocytes and preimplantation embryos have been conducted in mouse oocytes (ref 1-6)), in human the accessibility of mature oocytes i.e., metaphase II (MiI) oocytes, has been a major barrier to studying oocyte genomics using microarrays. Attempts have been made to address this problem by using candidate gene approaches employing RT-PCR and differential display (refs 7-22). In addition, serial analysis of gene expression (SAGE) and cDNA libraries was generated from human oocytes, and SAGE tags and expressed sequence tags were sequenced for rapid gene discovery and expression profiling in the oocytes (see reviews in refs. 23 and 24). However, these molecular approaches resulted in a small number of genes analyzed in each sample. Recently, four reports described initial transcriptome analyses of human oocytes using microarrays (refs. 25-28). Although they provided valuable information, these studies did not present a comprehensive picture of the human oocyte transcriptome because of a number of biological and technical constraints. Among the biological impediments are the use of discarded human oocytes that have failed to fertilize (refs 25, 28), limited coverage of the microarrays (refs. 25, 27), in some cases lack of sufficient biological replications (refs 26,28), and technical issues (ref. 27). Among the technical shortcomings, the most important is the use of a potentially unfaithful RNA amplification protocol. Li et al. (ref. 27) seem to have synthesized the first-strand cDNA using only a simple oligo(dT) primer, which makes target amplification unfeasible (ref 27); however, the actual procedure used for RNA amplification is unclear. Although we recognize this issue could have been a mistake on their described-published protocol, the actual procedure used for RNA amplification remains elusive.

[0006] The shortcomings of the prior art are disadvantageous as the identification of genes and deduced pathways from the mature human oocyte can help us better understand oogenesis, folliculogenesis, fertilization, and embryonic development.

BRIEF DESCRIPTION OF THE INVENTION AND EXEMPLARY EMBODIMENTS

[0007] This invention relates to a novel transcriptome or set of genes which are differentially expressed and/or upregulated by human oocytes.

[0008] This invention also relates to the identification of a discrete set of genes which are uipregulated by in vivo matured human oocytes and the use of these genes and the corresponding gene products for the dedifferentiation and transdifferentiation of somatic cells particular somatic cells derived from a subject that is to receive transplanted cells for treatment of a particular condition such as cancer or autoimmunity. The use of these genes and/or gene products produced using such donor transdifferentiated or dedifferentiated cells will allow for the production of desired immature and/or somatic cell types that are compatible for a desired donor and which therefore are suitable for human cell and gene therapy without the need for human nuclear transfer ("human therapeutic cloning") which has ethical concerns because such methods may result in the destruction of human embryos.

[0009] In addition, the present invention relates to the use of these genes and the corresponding gene products as markers of dedifferentiated cells such as human adult embryonic stem cells. Because these genes are expressed by in vivo matured human oocytes, it is anticipated that some of these genes will be expressed by other human immature cell types such as adult stem cells and cancer stem line cells.

[00010] Also, the invention relates to the use of these genes and gene products and probes specific thereto such as antibodies and oligonucleotides complementary thereto for the isolation and enrichment of such stem cells from heterogeneous cell samples such as by FACS, magnetic bead cell separations, and other cell separation methods.

[00011] Also, the present invention relates to the production of mammalian oocytes wherein one or more of these genes or an ortholog thereof in the case of non-human oocytes are

"knocked out" or expressed under regulatable conditions in order to study the effect of these genes on meiotic cell cycle, fertilization, chromatin remodeling, lineage commitment, pluripotency, tissue regeneration, morphogenesis, oogenesis, folliculogenesis, and embryonic development.

[00012] Further, the invention relates to dedifferentiated or transdifferentiated somatic cells produced using one or more of the genes and gene products disclosed herein that are upregulated on in vivo matured human oocytes.

[00013] ▪ In addition, the invention provides novel and improved cell and gene therapies using the transdifferentiated and dedifferentiated somatic cells produced by introduction of one or more of the disclosed genes and gene products comprised in the human oocyte transcriptome which is disclosed herein. In addition, the invention relates to culture medium containing one or more of these gene products corresponding to the disclosed human oocyte transcriptome or their non-human orthologs and variants and the use thereof for dedifferentiation and/or transdifferentiation of desired somatic or embryonic cell types.

[00014] Also, the invention relates to the use of these genes in order to establish a signature of normal human oocytes, i.e., human oocytes that are pregnancy competent and which when fertilized are capable of giving rise to a normal pregnancy.

BRIEF DESCRIPTION OF THE FIGURES

[00015] Fig. 1 : Summary of CRL RNA amplification protocol. (A) Flow Chart of the CRL amplification protocol. (B) Representative plot of gene intensities comparing the CRL and Ambion amplification methods using 20 ng and 1 μg of total RNA, respectively. [00016] Fig. 2. RT-PCR verification of the GeneChip array result. Loading orders of the gel were as following: M, 100 bp molecular weight standards with sizes as indicated on the left margin; OCT4, POU domain, class 5, transcription factor 1; STELLA, DPP A3, developmental pluripotency-associated 3; ESGI, embryonal stem cell-specific gene 1; VASAj DEAD box RNA helicase; GDF9, growth differentiation factor 9; ZPl, zona pellucida glycoprotein 1; HIFOO, Hl histone family, member O, oocyte-specific; CDH3, cadherin 3, type 1, P-cadherin (placental); TUBB4Q, β-tubulin; ACTB, β-actin; and negative control with no DNA template. [00017] Fig. 3. TGF-β signaling pathway. Genes shown in red are differentially up- regulated in human oocytes.

[00018] Fig.4. Venn diagrams showing the intersection between differentially up- regulated genes in the human (HU OC) and mouse oocytes (MO OC) (1,587 transcripts were found to be in common in both species) (A); HU OC and hESCs (388 transcripts were found to be common in both cell types) (B); MO OC and mESCs (591 transcripts were found to be common in both cell types) (Q; and HU OC/hESC and MO OC/mESC (78 transcripts were found to be common in all four cell types (D).

[00019] Fig. 5. Estrogen receptor signaling pathway. Genes shown in red are differentially up-regulated in the human and mouse oocytes.

[00020] Fig. 6. Digital RNA gel-like image showing the size distribution of the total RNA sample isolated from eight matured human oocytes run three times. The 28S and 18S ribosomal RNA bands are clearly visible in the intact RNA samples from the mature oocytes. L indicates the RNA 6000 ladder.

[00021] Fig. 7A-C. This Figure contains selected overrepresented Gene Ontology (GO) biological processes in oocytes identified by Expression Analysis Systematic Explorer (EASE) (EASE score less than 0.05).

[00022] Fig. 8. This Figure contains a listing of 101 upregulated genes expressed by human oocytes. The figure identifies these genes by gene nomenclature, gene symbol, NCBI Accession Number and further contains the nucleic acid sequence corresponding to each of the 101 genes. '∎

DETAILED DESCRIPTION OF THE INVENTION

[00023] Prior to discussing the invention in more detail, the following definitions are provided. Otherwise all words and phrases in this application are to be construed by their ordinary meaning, as they would be interpreted by an ordinary skilled artisan within the context of the invention.

"Transcriptome" refers to a set of genes expressed by a specific cell such as an oocyte or a somatic cell type. [00024] "Human Oocyte Transcriptome" herein refers to a set of genes upregulated by human oocytes that include genes that encode polypeptides that induce the transdifferentiation or dedifferentiation of somatic cells, preferably human somatic cells from a donor or recipient that is to undergo cell therapy.

[00025] "Pluripotent cell" refers to a cell that is capable of giving rise to all 3 cell lineages, i.e., ectoderm, endoderm and mesoderm cells.

[00026] "Multipotent" refers to a cell that is capable of giving rise to more than 1 cell lineage.

[00027] "Totipotent cell" is an undifferentiated cell such as embryonic cell such as an oocyte that is capable of giving rise to a viable offspring under appropriate conditions. [00028] "Embryonic Stem Cell or ESC" is a cell that is capable of giving rise to all 3 lineages. ESCs may be derived from early stage embryos, umbilical cord and other embryo tissue material as well as from nuclear transfer derived embryos.

[00029] "Adult stem cell" is a cell capable of giving rise to different somatic cells of a specific lineage, e.g., immune stem cells, hematopoietic stem cells, . neural stem cells, pancreatic stem cells and the like which cells are present in very few numbers in adult tissues and which cells unlike other adult somatic cells may be isolated and induced to differentiate resulting in the production of specific somatic cell lineages such as neural cells if the adult sten cell is a neural stem cell..

[00030] "Pregnancy-competent oocytes": refers to a female gamete or egg that when fertilized by natural or artificial means is capable of yielding a viable pregnancy when it is comprised in a suitable uterine environment.

[00031] "Viable-pregnancy": refers to the development of a fertilized oocyte when contained in a suitable uterine environment and its development into a viable fetus, which in turn develops into a viable offspring absent a procedure or event that terminates said pregnancy. "Cumulus cell" refers to a cell comprised in a mass of cells that surrounds an oocyte. These cells are believed to be involved in providing an oocyte nutritional and or other requirements that are necessary to yield an oocyte which upon fertilization is "pregnancy competent". [00032] "Differential gene expression" refer to genes the expression of which varies within a tissue of interest; herein preferably an oocyte. [00033] "Real Time RT-PCR": refers to a method or device used therein that allows for the simultaneous amplification and quantification of specific RNA transcripts in a sample. [00034] "SAGE" is an acronym for "Serial Analysis of Gene Expression".

"Microarray analysis": refers to the quantification of the expression levels of specific geiies in a particular sample, e.g., tissue or cell sample.

[00035] "Pregnancy signature": refers to a phrase coined by the inventors which refers to the characteristics levels of expression of a set of one or more genes, preferably at least 5, more preferably at least 10 to 20 genes, and still more preferably, at least 50 to 100 genes, that are expressed at characteristic levels in oocytes or oocyte associated cells, preferably cumulus cells, that surround "pregnancy competent" oocytes. This is intended to encompass the level at which the gene is expressed and the distribution of gene expression within cells analyzed. [00036] "Pregnancy signature gene": refers to a gene which is expressed at characteristic levels by a cell, e.g., cumulus cell, on a "pregnancy competent" oocyte. [00037] "IVF": refers to in vitro fertilization.

"Zona pellucida" refers to the outermost region of an oocyte.

[00038] "Method for detecting differential expressed genes" encompasses any known method for evaluating differential gene expression. Examples include indexing differential display reverse transcription polymerase chain reaction (DDRT-PCR); subtractive mRNA hybridization , the use of nucleic acid arrays or microarrays; SAGE (Serial Analysis of Gene Expression. (SAGE) and real time PCR (RT-PCR). For example, differential levels of a transcribed gene in an oocyte cell can be detected by use of Northern blotting, and/or RT-PCR. CRL amplification protocol refers to the novel total RNA amplification protocol that combines template-switching PCR and T7 based amplification methods. This protocol is well suited for samples wherein only a few cells or limited total RNA is available.

[00039] "EASE" is a gene ontology protocol that from a list of genes forms subgroups based on functional categories assigned to each gene based on the probability of seeing the number of subgroup genes within a category given the frequency of genes from that category appearing on the microarray.

[00040] "Dedifferentiated or transdifferentiated or reprogrammed somatic cell" refers to a somatic cell which is converted into a less mature cell, e.g., a stem or stem-like cell or a rejuvenated cell that has a longer lifespan than the parent somatic cell or is converted into a different somatic cell lineage. This is effected by incorporating into the cell one or more of the 101 genes disclosed herein that are differentially expressed by mature human oocytes, preferably expressed under the control of a regulatable promoter, or by contacting the cell or the nucleus or chromatin mass derived therefrom with a medium containing at least one gene product encoded by the 101 genes disclosed herein or a non-human ortholog thereof. In these methods the somatic cell, e.g., from a donor with a disease treatable by cell therapy is introduced (e.g., via electroporation, injection, infection) one or more human genes or a vector(s) containing, wherein said gene or genes are expressed by a mature human oocyte or said somatic cell or the nucleus thereof is cultured in a medium containing one or more gene products expressed by mature human oocytes that results in said somatic cell converting into a less differentiated cell, e.g., a stem cell or into a cell with an increased lifespan as evidenced by an increased telomere length or increased cell doublings or which somatic cell converts into a different somatic cell type. Dedifferentiation can be detected by screening for markers characteristic of pluripotent cell types, altered telomere length, increased number of cell doublings until senescence or by detecting for telomerase which is only expressed by pluripotent or immortal cells such as ESCs and cancer cells.

[00041] "Nuclear transfer embryo" refers to an embryo created by fusing or inserting a somatic cell or the nucleus or chromosomes thereof with an oocyte or other embryonic cell that is enucleated before, during or after fusion or insertion.

[00042] "Parthenogenic embryo" refers to an embryo that is produced using only male or female gametes. Typically, these parthenogenic embryos are incapable of giving rise to viable offspring.

[00043] "Gene Contained in Human Oocyte Transcriptome or an ortholog or variant thereof refers to one or more of the human oocyte expressed genes disclosed herein, i.e., the genes contained in Figure 8, or their non-human mammalian orthologs such as corresponding non-human primate and rodent genes or variants thereof which encode polypeptides possessing the same biological activity. Typically such variants will encode polypeptides at least 90% identical to polypeptides encoded by the genes contained

in Figure 8 or their orthologs. [00044] This invention provides a set of 101 genes which are differentially expressed and/or upregulated by normal mature human oocytes (transcriptome). These genes and the sequences which are contained in Figure 8 comprise a set of human genes that were identified as being differentially expressed or upregulated by in vivo matured human oocytes, according to the specific methods disclosed herein.

[00045] Particularly, these genes were identified using human metaphase II oocytes assayed within minutes after removal from the ovary, and its transcriptome was compared with a reference sample consisting of a mixture of total RNA from 10 different normal human tissues not including the ovary. RNA amplification was performed by using a unique protocol disclosed herein. Affymetrix Human Genome Ul 33 Plus 2.0 GeneChip arrays were used for hybridizations. Compared with reference samples, there were 5,331 transcripts significantly up- regulated and 7,074 transcripts significantly down-regulated in the oocyte. Of the oocyte up- regulated probe sets, 1,430 have unknown function. A core group of 66 transcripts was identified by intersecting significantly up-regulated genes of the human oocyte with those from the mouse oocyte and from human and mouse embryonic stem cells. GeneChip array results were validated using RT-PCR in a selected set of oocyte-specific genes. Within the up-regulated probe sets, the top overrepresented categories were related to RNA and protein metabolism, followed by DNA metabolism and chromatin modification. This invention therefore provides a comprehensive expression baseline of genes expressed in in vivo matured human oocytes. [00046J In addition, the present transcriptome was identified using young oocytes, as opposed to aged and fertilized ones, which could have quite significantly different expression profiles. Also, the inventors used the most comprehensive human microarray platform; and took advantage of publicly available gene expression databases to interpret our own data in a more meaningful way. Thus, the present invention was conducted using materials and methods that were designed to identify the gene transcripts present in young, untreated Mil oocytes within minutes after isolation from the ovary in three independent replicates and to compare these genes with a reference RNA (a mixture of total RNA from 10 different normal human tissues not including the ovary) by using Affymetrix GeneChip technology.

[00047] More specifically, to achieve this goal, a protocol that combined template- switching PCR and T7-based amplification methods was developed for the analysis of gene expression in samples of small quantity. The inventors amplified RNA from the oocyte and reference samples. Results were later compared with available transcriptome databases of mouse oocytes, and human ESCs (hESCs) and mouse ESCs (mESCs). W

[00048] Using these methods, the inventors provide herein the transcript profile of in vivo matured human Mil oocytes using the most recent Affymetrix human GeneChip array, interrogating >47,000 transcripts including 38,500 well characterized human genes. [00049] This invention particularly provides a transcriptome of 101 genes expressed by human oocytes. These genes and the orthologs of these genes are involved in the differentiation based on their specific expression in oocytes. Therefore, the introduction of one or more of these genes or the corresponding gene products should result in the transdifferentiation or dedifferentiation of a desired somatic cell into another cell type and/or lengthen the lifespan of said cell.

[00050] Particularly the introduction of at least one of said genes or gene products will result in the partial or complete dedifferentiation of a desired somatic pell into a pluripotent or multipotent cell., e.g., an adult stem cell or an embryonic cell type. Cells which transdifferentiate or dedifferentiate may be detected by screening by at least one marker that is specific for the particular cell type. For example, it is known that embryonic stem cells express certain genes such as Oct4, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81 and alkaline phosphatase. Also, dedifferentiated cells or cells having enhanced lifespan may be detected based on an analysis of telomerase expression or telomere length. Cells having longer telomeres relative to stating somatic cells correlate to an enhanced cell lifespan.

[00051] These transdifferentiated cells may be used e.g., for cell therapy or for study of the differentiation process. Diseases treatable by cell therapy include by way of example cancer, autoimmunity, allergy, inflammatory conditions, infection. Cancers treatable by use of cell therapy include solid and non-solid

tumor associated cancers and include by way of example hematological cancers such as myeloma, lymphoma, leukemia; sarcomas, melanomas, lung cancers, pancreatic, neurological cancers such as neuroblastomas, stomach, colon, liver, gall bladder, esophageal, tracheal, head and neck, cancers of the tongue and lip, ovarian, breast, cervical, prostate, testicular, bone and other cancers. In addition cell therapy is useful in alleviating the effects of specific treatments such as radiation and chemotherapy which may deplete specific cells such as bone marrow. Further the subject cell therapy may be used for treating infectious disease such as viral or bacterial or parasite associated diseases such as HIV. Also the subject cell therapy may be used in treating autoimmune conditions wherein the host W

autoimmune reaction may result in killing or depletion of host cells such as immune cells or other essential cell.

[00052] Reprogrammed or dedifferentiated or transdifferentiated cells generated from these methods may be used to replace cells in a mammal in need of a particular cell type. These methods may be used to either directly produce cells of the desired cell type or to produce undifferentiated cells which may be subsequently differentiated into the desired cell type. For example, stem cells may be differentiated in vitro by culturing them under the appropriate conditions or differentiated in vivo after administration to an appropriate region in a mammal. To optimize phenotypic and functional changes, reprogrammed cells can be transplanted into the organ (e.g., a heart) where they are intended to function in an animal model or in human patients shortly after dedifferentiation or transdifferentiation (e.g., after 1, 2, 3, 5, 7, or more days). The resultant cells implanted in an organ may be reprogrammed to a greater extent than cells grown in culture prior to transplantation. Cells implanted in an animal organ may be removed from the organ and transplanted into a recipient mammal such as a human, or the animal organ may be transplanted into the recipient.

[00053] To increase the length of time the cell, nuclei, or chromatin mass may be reprogrammed in vitro prior to administration to a mammal for the treatment of disease, the donor cell may be optionally modified by the transient transfection of a plasmid containing an oncogene flanked by loxP sites for the Cre recombinase and containing a nucleic acid encoding the Cre recombinase under the control of an inducible promoter (Cheng et al., Nucleic Acids Res. 28(24):E108, 2000). The insertion of this plasmid results in the controlled immortalization of the cell. After the cell is reprogrammed into the desired cell-type and is ready to be administered to a mammal, the loxP-oncogene-loxP cassette may be removed from the plasmid by the induction of the Cre recombinase which causes site-specific recombination and loss of the cassette from the plasmid. Due to the removal of the cassette containing the oncogene, the cell is no longer immortalized and may be administered to the mammal without causing the formation of a cancerous tumor.

[00054] Examples of medical applications for these cells include the administration of neuronal cells to an appropriate area in the human nervous system to treat, prevent, or stabilize a neurological disease such as Alzheimer's disease, Parkinson's disease, Huntington's disease, or ALS; or a spinal cord injury. In particular, degenerating or injured neuronal cells may be replaced by the corresponding cells from a mammal. This transplantation method may also be used to treat, prevent, or stabilize autoimmune diseases including, but not limited to, insulin dependent diabetes mellitus, rheumatoid arthritis, pemphigus vulgaris, multiple sclerosis, and myasthenia gravis. In these procedures, the cells that are attacked by the recipient's own immune system may be replaced by transplanted cells. In particular, insulin-producing cells may be administered to the mammal for the treatment or prevention of diabetes, or oligodendroglial precursor cells may be transplanted for the treatment or prevention of multiple sclerosis. For the treatment or prevention of endocrine conditions, reprogrammed cells that produce a hormone, such as a growth factor, thyroid hormone, thyroid-stimulating hormone, parathyroid hormone, . steroid, serotonin, epinephrine, or norepinephrine may be administered to a mammal. Additionally, reprogrammed epithelial cells may be administered to repair damage to the lining of a body cavity or organ, such as a lung, gut, exocrine gland, or urogenital tract. It is also contemplated that reprogrammed cells may be administered to a mammal to treat damage or deficiency of cells in an organ, muscle, or other body structure or to form an organ, muscle, or other body structure. Desirable organs include the bladder, brain, nervous tissue, esophagus, fallopian tube, heart, pancreas, intestines, gallbladder, kidney, liver, lung, ovaries, prostate,

spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, ureter, urethra, and uterus. Also, these cells may also be combined with a matrix to form a tissue or organ in vitro or in vivo that may be used to repair or replace a tissue or organ in a recipient mammal. For example, reprogrammed cells may be cultured in vitro in the presence of a matrix to produce a tissue or organ of the urogenital system, such as the bladder, clitoris, corpus cavermosum, kidney, testis, ureter, uretal valve, or urethra, which may then be transplanted into a mammal (Atala, Curr. Opin. Urol. 9(6):517-526, 1999). In another transplant application, synthetic blood vessels are formed in vitro by culturing reprogrammed cells in the presence of an appropriate matrix, and then the vessels are transplanted into a mammal for the treatment or prevention of a cardiovascular or circulatory condition. For the generation of donor cartilage or bone tissue, reprogrammed cells such as chondrocytes or osteocytes are cultured in vitro in the presence of a matrix under conditions that allow the formation of cartilage or bone, and then the matrix containing the donor tissue is administered to a mammal. Alternatively, a mixture of the cells and a matrix may be administered to a mammal for the formation of the desired tissue in vivo. Preferably, the cells are attached to the surface of the matrix or encapsulated by the matrix. Examples of matrices that may be used for the formation of donor tissues or organs include collagen matrices, carbon fibers, polyvinyl alcohol sponges, acrylateamide sponges, fib'rin- thrombin gels, hyaluronic acid-based polymers, and synthetic polymer matrices containing polyanhydride, polyorthoester, polyglycolic acid, or a combination thereof (see, for example, U.S. Pat. Nos. 4,846,835; 4,642,120; 5,786,217; and 5,041,138).

[00055] Additionally, these dedifferentiated somatic cells may be used to produce artificial tissues and organs by culturing said dedifferentiated cells in vitro e.g., in cell culture apparatus that are designed to facilitate the formation of desired cell structure and morphology. Additionally, these cells may be introduced into non-human animals as xenografted cells for example by injecting the dedifferentiated into desired organs. For example, dedifferentiated cells may be used to study the effect of dedifferentiated cardiac cells on damaged heart tissue to determine whether these cells promote the healing or regeneration process. Alternatively, dedifferentiated immune cells may be introduced into irnmunodeficient animals to assess whether this results in restoration of immune function.

[00056] in addition probes specific to the subject human genes or gene products may be used to identify dedifferentiated cells in a mixed cell population. For example, these probes such as labeled antibodies or oligos specific to the subject human genes or gene products differentially expressed by mature human oocytes may be used to identify and/or isolate adult stem cells contained in adipocyte tissue (fat) or other tissue that may contain adult stem cells. [00057] Also, these probes may be used to assess the viability and/or pregnancy competency of oocytes from donors that may have oocyte related pregnancy problems e.g., as a result of disease, genetics, age, or environmental insult. Since the subject transcriptome was derived from oocytes from young apparently healthy donors the expressed genes should include those which are required for the oocyte to be pregnancy competent upon natural or in vitro fertilization.

[00058] In order to describe the invention in greater detail the following experimental examples and results are provided below.

EXPERIMENTAL EXAMPLES [00059] The Materials and Methods below were used to derive the human transcriptome or set of genes upregulated by in vivo matured metaphase II human oocytes.

MATERIALS AND METHODS

[00060] Oocyte Collection Total RNA Extraction, and Reference RNA.

[00061] Human oocytes were obtained from three patients undergoing an assisted reproductive treatment (ART) at the Unit of Reproductive Medicine of Clinica Las Condes, Santiago, Chile. It is important to emphasize that the routine in vitro fertilization protocol at Clinica Las Condes calls for fertilizing only those oocytes that will be transferred into the uterus of the patient. Therefore, there is always a surplus of oocytes. We then had the opportunity to use specific criteria to select donors as follows: (i) <35 years old, (U) reproductively healthy with regular ovulatory cycles, («i) male factor as the only cause of infertility, and

(zv) considerable number of developing follicles that assured spared oocytes. The experimental protocol was reviewed and approved by a local independent Ethics Review Board. All donors signed informed consent. At the time this manuscript was submitted, all three donors had already conceived; two of them got pregnant during the ART cycle in which our samples were collected, and the third one got pregnant after a spontaneous cycle with artificial insemination using donated sperm. Ovarian stimulation, oocyte retrieval, and cell lysis were performed as described in Supporting Materials and Methods, which is published as supporting information (See Ref. 50). [00062] Three groups of 10 oocytes each were used. Total RNA was isolated following the guanidium thiocyanate method (Ref.45) by using the PicoPure RNA isolation kit (Arcturus, Sunnyvale, CA) following the manufacturer's instructions. However, only 6.5 µl of elution buffer (Arcturus) was used, and the elution was repeated at least three times by using the first eluate. All RNA samples within the purification column were treated with the RNase-Free'DNase (Qiagen, Valencia, CA). Extracted RNA was stored at -80^0C until used as template for cDNA synthesis. The quality and quantity of extracted total RNA from 8 matured oocytes (independent from the 30 oocytes used in this study) was evaluated on the Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA). Each mature oocyte was found to have ~330 pg of total RNA when the Arcturus RNA isolation kit was used. The quality of RNA was intact as shown in Fig. 6, which is published as supporting information on the PNAS web site. Reference RNA (100 µg) was prepared by mixing 10 µg of total RNA from each of 10 different normal human tissues, including skeletal muscle, kidney, lung, colon, liver, spleen, breast, brain, heart, and stomach (Ambion).

First Strand cDNA Synthesis and cDNA Purification

[00063] The following reagents were added to each 0.5 ml of RNase-free tube: 5 µl total

RNA (i.e., 3 ng for the reference and ~3 ng for the oocyte samples) and 300 ng of an anchored T7-01igo(dT)$_{24}$V promoter primer (Ambion). The reaction tubes were incubated in a preheated PCR machine at 70^0C for 2 min and transferred to ice. After denaturation, the following reagents were added to each reaction tube: 1.4 µl of SMART II A oligonucleotide (5'-AAGCAGTGGTATCAACGCAGAGTACGCGrGrGr-3') (Clontech), 4 µl of 5x first-strand buffer, 2 µl of 20 mM DTT, 0.6 µl of 5 mg/ml T4 Gene 32 Protein (Roche, Indianapolis, IN), 2 µl of 10 mM dNTPs, 20 units of RNase inhibitor (Ambion), and 1 µl of PowerScript Reverse Transcriptase (Clontech). The final first-strand reaction volume was 20 µl for all experiments. After gently mixing, reaction tubes were incubated at 42°C for 60 min in a hot-lid thermal cycler. The reaction was terminated by heating at 70^0C for 15 min and purified by NucleoSpin Extraction Kit (Clontech) following the manufacturer's instructions.

Double Stranded cDNA Synthesis by L.omg-Distance PCR and cDNA Purification PCR [00064] Advantage 2 mix (9 µl) was prepared as follows: 5 µl of 10* PCR Advantage buffer (Clontech), 1 µl of 10 mM dNTPs, 100 ng of 5^f SMART upper primer (S'- AAGCAGTGGTATCAACGCAGAGTA-3'), 100 ng of 3' SMART lower primer (5'- CGGTAATACGACTC ACTATAGGGAGAA-3'), and 1 µl of Polymerase Mix Advantage 2 (Clontech). This mix was added to 41 µl of the first-strand cDNA synthesis reaction product, and thermal cycling was carried out in the following conditions: 95°C for 1 min, followed by 15 cycles, each consisting of denaturation at 94°C for 30 s, annealing at 62°C for 30 s, and extension at 68^0C for 10 min. The cDNA was purified by the NucleoSpin Extraction Kit (Clontech).

[00065J For in vitro transcription, biotin-labeled aRNA purification and aRNA fragmentation, hybridization, washing, staining and imaging, and RT-PCR analysis, see Supporting Materials and Methods. Microarray Analysis

[00066] Transcriptional profile of each sample was probed by using Affymetrix Human

Genome UI 33 Plus 2.0 GeneChips. The raw data obtained after scanning the arrays were analyzed by dChip (ref. 46). A smoothing spline normalization method was applied before obtaining model-based gene expression indices, also known as signal values. There were no outliers identified by dChip so all samples were carried on for subsequent analysis. [00067] When comparing two groups of samples to

identify genes enriched in a given group, we used the lower confidence bound (LCB) of the fold change (FC) between the two groups as the cut-off criteria. If 90% LCB of FC between the two groups was >2, the corresponding gene was considered to be differentially expressed (DE). LCB is a stringent estimate of the FC and has been shown to be the better ranking statistic (Ref 46). Recently, dChip's LCB method for assessing DE genes has been shown to be superior to other commonly used approaches, such as MAS 5.0 and Robust Multiarray Average-based methods (Refs. 47 and 48).

[00068] Pathways analysis was performed by using Ingenuity Software Knowledge Base

(Redwood City, CA), which is a manually created database of previously published findings on mammalian biology from the public literature. We used the network analysis, using the knowledge base to identify interactions of input genes within the context of known biological pathways.

Gene Ontology Analysis

[00069] Gene Ontology was performed by using the EASE software package). Given a list of genes, EASE forms subgroups based on the functional categories assigned to each gene. EASE assigns a significance level (EASE score) to the functional category based on the probability of seeing the number of subgroup genes within a category given the frequency of genes from that category appearing on the microarray (Ref 29).

Comparison with External Data Sets.

[00070] Mouse Mil oocyte transcriptome data were obtained from Su et al. (Ref 33), who used custom designed Affymetrix chips to obtain gene expression profiles of oocytes and 60 other mouse tissue types. Using their expression database, we identified 3,617 differentially up- regulated transcripts in the mouse oocyte using the median expression value of the remaining 60 samples as the baseline (see Data Set 8, which is available in Ref 50. We selected transcripts with an expression value in oocyte samples that are 2-fold higher than the baseline. [00071] Human ESC data were derived from the work of Sato et al. (Ref 44), who profiled human stem cells and their differentiated counterparts using Affymetrix HG-UI 33 A representing -22,000 transcripts.

[00072] We analyzed the raw data using dChip and identified 1,626 hESC genes by selecting transcripts significantly up-regulated in human stem cells compared with their differentiated counterparts (see Data Set 9, which is available in Ref 50). [00073] Finally, for mESCs, we used a list of 1,687 differentially up-regulated mESC genes published by Fortunel et al. (49), which were identified by comparing mESCs to differentiated cells by using Affymetrix MG-U74Av2 chips representing «12,000 transcripts (see Data Set 10, which is available in Ref 50). We used commercially available Affymetrïx's NetAffx tool) for mapping genes across organisms and platforms used in the respective studies.

Ovarian Stimulation, Oocyte Retrieval, and Cell Lysis

[00074] Ovarian stimulation was performed under Gn-RH analog suppression [leuprolide acetate (Lupron); Abbott, Abbott Park, IL] in a daily s.c. dose of 0.5 mg. Recombinant FSH (rFSH, Gonal-f-Serono or Puregon; Organon, Roseland, NJ) was administered in daily doses that ranged between 200 and 300 units, starting the second day of the mense. Follicular growth and estradiol levels were monitored every 2 to 3 days until follicles had a mean diameter between 18 and 20 mm. Oocyte maturation was achieved by an injection of 10,000 units of hCG (Pregnyl; Organon). Oocytes were retrieved from the ovary by aspiration using guided transvaginal ultrasound 36 h after hCG administration. Three hours after retrieval, oocytes were denuded from surrounding corona and cumulus cells by a brief incubation (10-30 s) in 80 units/ml hyaluronidase solution (LifeGlobal, Guilford, CT) and subsequent pipetting to completely eliminate other cells. Oocytes were then observed at high magnification to confirm maturity (metaphase II stage) and to confirm the absence of other cells. Each oocyte was rinsed in sterile PBS and lysed in 100 μl of extraction buffer (XB, Arcturus, Mountain View, CA) in an RNase/DNase/Pyro gen-free 0.5 -ml

microcentrifuge tube. Each sample was incubated for 30 min at 42^0C, centrifuged at 3,000 x g for 2 min, and stored in liquid nitrogen until use.

In Vitro

Transcription (IVT), Biotin-Labeled Amplified RNA (aRNA) Purification, and aRNA

Fragmentation.

[00075] The purified double-stranded cDNA containing the T7 promoter sequence was used as a template for IVT-labeling assays in the presence of biotin-labeled ribonucleotides, using the BioArray High Yield RNA Transcript Labeling kit with T7 RNA polymerase (ENZO,

Farmingdale, NY) as described by the manufacturer. The biotin-labeled aRNA was purified using RNeasy mini columns (RNeasy Mini Kit; Qiagen, Valencia, CA). In vitro transcription of the cDNA for each replicate yielded 70-90 µg of biotinylated aRNA, and 15 µg of the labeled aRNA was fragmented at 94^0C for 35 min in Ix fragmentation buffer (40 mM Tris-acetate, pH

8.1/100 mM KOAc/30 mM MgOAc).

Hybridization, Washing, Staining, and Imaging.

[00076] The Affymetrix GeneChip system was used for hybridization, staining, and imaging of the arrays. Hybridization cocktails of 300 µl containing 15 µg of fragmented biotin- labeled aRNA and biotinylated exogenous hybridization controls (50 pM control Oligo B2, Eukaryotic hybridization controls) (BioB at 1.5 pM, BioC at 5 pM, BioD at 25 pM, and CreX at 100 pM), herring sperm DNA (0.1 mg/ml), BSA (0.5 mg/ml) in buffer (100 mM Mes/1M NaCl/20 mM EDTA/0.01% Tween 20) were hybridized to the GeneChip Human Genome Ul 33 plus 2.0 array (Affymetrix, Santa Clara, CA). Hybridizations were performed automatically, and each array was prehybridized with all components except the fragmented biotin-labeled aRNA in a chamber at 45°C for 15 min with rotation at 60 rpm. The prehybridized array was then hybridized with the aRNA mixture for 16 h under the prehybridization conditions. After hybridization, the mixture was removed from chip, and the array was filled with nonstringent wash buffer (6x SSPE and 0.01% Tween 20). The arrays were washed according to Affymetrix protocol on a Fluidics station using nonstringent and stringent (100 mM Mes/0.1 M NaCl/0.01% Tween 20) wash buffers. For the detection of hybridized fragments, the array was stained using SAPE (streptavidin-linked to phycoerythrin) stain and antibody solutions. SAPE stain solution (600 µl) contained 2 mg/ml BSA, 10 µg/rnl streptavidin phycoerythrin (SAPE) in 100 mM Mes, IM NaCl, and 0.05% Tween 20. The antibody solution (600 µl) also contained 2 mg/ml BSA, 0.1 mg/ml goat IgG, 3 µg/ml biotinylated anti-streptavidin antibodies in 100 mM Mes, IM NaCl, and 0.05% Tween 20. The order of staining is SAPE, antibody, and second SAPE. [00077] The arrays were scanned using Affymetrix's high density GeneArray Scanner

3000 and imaged using Affymetrix GeneChip Operating Software (GCOS). The GCOS expression data report was generated for each sample and was used to judge the quality of sample preparation and hybridization. The report included information about noise, background, and percentage of probe sets called present based on the manufacturer threshold and software settings. Information about performance of exogenously added prokaryotic hybridization control genes such as BioB, BioC, and BioD of the Escherichia coli biotin synthesis pathway and the ratio of intensities of 3' probes to 5' probes for housekeeping genes such as GAPDH and β-actin were also included in the expression report file (for independent oocyte and reference samples, see Expression Report Files 1-6 , which are available at www.canr.msu.edu/dep t/ans/community/people/cibelli_jose.html).

RT-PCR

[00078] . Equal amounts of the remaining long-distance PCR reactions diluted 1 : 1 with sterile H_2O were amplified by gene-specific primers (Table 4, which is published as supporting information on the PNAS web site). After an initial incubation at 94°C for 3 min, the RT-PCR was carried out at 94°C for 45 s, 60^{0}C for 30 s, 72°C for 40 s for 30 cycles. Upon the completion of PCR, the reaction was incubated at 72°C for an additional 10 min. RT-PCR products were electrophoresed on a 1.5% agarose gel and documented with a Gel Documentation System (Bio- Rad, Hercules, CA).

EXAMPLE 1: Validation of Amplification Fidelity (Amplified vs. Nonamplified RNA) [00079] A critical step in the analysis of gene expression on small samples is the faithful amplification of mRNA molecules present in the sample. We have designed a PCR-based amplification system using the combination of SMART II A oligonucleotide (Clontech, Mountain View, CA) and T7-Oligo(dT) promoter primers (CRL RNA amplification protocol) (Fig. 14). We isolated total RNA from a human cell line and 20, 3, and 1.5 ng input total RNA was amplified using the CRL amplification protocol. For each experiment, 15 µg of fragmented amplified RNA (aRNA) was hybridized to a single Affymetrix Human Genome U133 Plus 2.0 array. Nonamplified RNA from the same original sample (1 µg) was run in' parallel by using the MessageAmp II aRNA Kit (Ambion, Austin, TX). Gene expression results from both amplified vs. nonamplified RNA samples were compared, and the correlation coefficients were found to be 0.94 (Fig. IB). 0.93. and 0.91 for 20 ng, 3 ng, and 1.5 ng of total input RNA, respectively. The CRL Amplification protocol was repeated two times with 20 ng of initial total RNA from the same cell type, and the correlation between the two experiments was 0.99. These results show that our RNA amplification strategy faithfully and consistently amplifies small amounts of RNA to quantities required to perform microarray experiments. The CRL amplification protocol provides a practical approach to facilitate the analysis of gene expression in samples of small quantity while maintaining the relative gene expression profile throughout reactions.

EXAMPLE 2: Validation of Microarray Data

[00080] A selected list of genes was used to validate the microarray results by RT-PCR

(Fig. 2). These genes were found to be present in the oocyte sample and absent in the reference RNA. Figure 2, contains RT-PCR verification of the GeneChip array result. Loading orders of the gel were as following: M, 100 bp molecular weight standards with sizes as indicated on the left margin; OCT4, POU domain, class 5, transcription factor 1; STELLA, DPP A3, developmental pluripotency-associated 3; ESGI, embryonal stem cell-specific gene 1; VASA, DEAD box RNA helicase; GDF9, growth differentiation factor 9; ZPI, zona pellucida glycoprotein 1; HIFOO, HI histone family, member O, oocyte-specific; CDH3, cadherin 3, type 1, P-cadherin (placental); TUBB4Q, β-tubulin; ACTB, β-actin; and negative control with no DNA template.

EXAMPLE 3: Differentially Up-Regulated Genes in the Human Oocyte [00081] We generated a database of the human oocyte transcriptome by comparing the transcripts in the oocyte with the reference samples, which contain mRNA from several somatic tissues. A complete list of up- and down-regulated genes and functional, comparative, and correlation analyses are available (see Data Sets 1 and 2 in Ref. 50. In addition although the oocytes were thoroughly denuded manually from their surrounding cells, we were concerned over the risk for potential contamination of RNA belonging to cumulus cells. We then specifically checked for the levels of expression of cumulus cell-specific genes, such as GREMI, PTGS2 and PTX3, and found them absent or down-regulated in the oocyte RNA samples.

[00082] Compared with reference samples, there were 5,331 transcripts significantly up-regulated and 7,07 '4 transcripts significantly down-regulated in the oocyte. Genes up-regulated in oocyte samples included most of the well-known germ cell-specific genes, such as FIGLA, STELLA, VASA, DAZL$_5$ GDF9, ZPI$_5$ ZP2$_5$MOS, OCT4, NPM2, and HIFOO. Using Ingenuity Software Knowledge Base (Redwood City, CA), we confirmed the presence of pathways previously described in the mouse, in particular the TGF-β pathway (See Figure 3 which shows theTGF-β signaling pathway. Genes shown in red are differentially up-regulated in human oocytes). The number of genes expressed in young Mil human oocytes was 7,560 in our study (based on Unigene build 189; see Data Set 3, which is available in Ref 50) whereas the only

other study employing the same microarray Ref 28)) reported the gene number as 5,633 in aged human oocytes. As the complete list of genes is not available, a direct comparison of these data sets is not feasible. Although there is overlap between the two data sets, the difference in the number of genes detected could be because the oocytes assayed in the two studies were not equivalent to each other (young MiI oocytes vs. unfertilized and aged oocytes) or the effect of different RNA amplification protocols used.

EXAMPLE 4: Functional Annotation of Genes Overexpressed in the Human Oocyte [00083] To examine the biological processes performed by the oocyte, we implemented Expression Analysis Systematic Explorer (EASE) (Ref 29), contrasting the genes overexpressed in the oocyte with all of the genes present in the Affymetrix chip (Table 1, which is published as supporting information on the PNAS web site). One of the top overrepresented categories found in oocytes was related to RNA metabolism. This finding is in agreement with the fact that oocytes store RNA to support the events of fertilization and early embryonic development until the embryonic genome is activated (Ref 30, 31)). DNA metabolism and chromatin modification were also overrepresented categories, in agreement with the need of the oocyte to remodel the sperm chromatin upon fertilization. Detection of gametogenesis and reproduction as overrepresented categories further suggests the accuracy of this transcriptional profiling. An important category highly represented in the oocyte was related to nucleic acid metabolism and regulation of transcription. Although transcriptionally silent at the MiI stage, the oocyte is very active in transcription and translation throughout its growth phase and must be prepared to initiate transcription during embryonic genome activation at the four- to eight-cell stage in human (32). Many of the genes in this category represent Zinc-finger proteins that are not yet fully characterized, providing an opportunity to discover new transcriptional regulatory networks that operate during embryonic genome activation.

[00084] Chromatin remodeling genes are also represented in the human oocyte. Genes in this category included the following: DNA methyltransferases (DNMTI, DNMT3A and DNMT3B), histone acetyltransferases (NCOAI and -3, SRCAP, GCN5L2, and TADA2L), histone deacetylases (HDAC3, HDAC9, and SIRT7), methyl-CpG-binding proteins (MBD2 and MBD4), histone methyltransferases (EHMTI and SET8), ATP-dependent remodeling complexes (SMARCAI, SMARCA5, SMARCADI, SMARCC2, and SMARCDI), and other chromatin- modifying genes (ESRI, NCOA6, HMGB3, HMGNI, and HMGAI). These Gene Ontology results not only validate our transcriptome analysis when compared with candidate gene analysis already reported in other species but more importantly, shed new light into a large number of biological processes that take place in the human oocyte.

EXAMPLE 5: Intersection Between Human Oocyte and Mouse Oocyte Transcriptome [00085] Mouse has been the best model for genetic studies, and several groups have already reported the transcriptome analysis of mouse oocytes (Ref 4, 6). In an effort to find differences and similarities between the human and mouse oocyte, we compared our human oocyte transcriptome results with that of mouse oocyte transcriptome derived from data of Su et al. (33). We intersected differentially up-regulated genes in the human and the mouse oocyte transcriptome and found a set of 1,587 genes to be in common, indicating genes of conserved function in mammalian oocytes (Figure 4A) and Data Set 4, which is available in Ref 50. The functional characterization of 15 of the top 100 intersected genes that have their functions described in mouse oocytes is summarized in Table 1, which is published as supporting information on the PNAS web site. Many of these genes relate to oocyte maturation, from the first meiotic division to MiI arrest, encompassing various controls of cell cycle checkpoints and cellular machinery for DNA segregation and cell division. It was surprising to find at the intersection of these data sets the up-regulation of the estrogen receptor (ER) signaling pathway (Fig. 5, which is published as supporting information on the PNAS web site). Genes significantly up-regulated in this pathway were CTBP2, ESRI, GTF2H1, GTF2H2, MAP2K1, NCOAI, NCOA3, PCQAP, PHB2, POLR2C, POLR2J, RBM9, TAF3, TAF4 and 4B, TAF5, TAF6, TAFI 2, and TBP. Recent studies in knockout models for aromatase have shown that estrogen is not required for the generation of preimplantation embryos (Ref 34); our study, however, in agreement with previous reports Ref 35, 36) suggests that some genes associated with the ER pathways are indeed transcribed in the oocyte, perhaps in response to hormonal stimulation during folliculogenesis and oocyte maturation. It remains to be determined whether the ER pathway has a role during preimplantation development in human embryos. Considering the high degree of similarity in early

embryonic development between mouse and human, these 1,587 common genes deserve particular attention and must be considered for future candidate gene-approach studies related to fertility disorders, developmental defects, and assisted reproductive technologies. Furthermore, with the inherent ethical and technical difficulties of studying human oogenesis in the laboratory, the mouse model will continue to provide a platform for the functional characterization of other highly conserved genes that may bear significance in understanding human germ cell formation and maturation. Figure 4 contains Venn diagrams showing the intersection between differentially up-regulated genes in the human (HU OC) and mouse oocytes (MO OC) (1,587 transcripts were found to be in common in both species) (A); HU OC and hESCs (388 transcripts were found to be common in both cell types) (B); MO OC and mESCs (591 transcripts were found to be common in both cell types) (Q; and HU OC/hESC and MO OC/mESC (78 transcripts were found to be common in all four cell types (D).

EXAMPLE 7: Intersection Between Oocytes and ESC Transcriptomes [00086] The oocyte is derived from germ cell precursors believed to have segregated from pluripotent precursors before somatic tissue differentiation (Ref 37). Primordial germ cells (PGCs) undergo mitotic proliferation followed by meiosis. By the time the oocyte reaches the MiI stage, it is already a highly specialized cell capable of remodeling the sperm nucleus and restoring totipotency to the diploid zygote. In addition, somatic cell nuclear transfer (SCNT) into enucleated oocytes has shown that, when challenged with a somatic nucleus, the oocyte cytosol will attempt to completely erase the somatic epigenetic phenotype and transform the nucleus to a totipotent state. Although failures in this epigenetic reprogramming have been reported elsewhere, there are reported cases in which animals produced by SCNT have developed normally (Ref 38). Reinforcing the notion that, when SCNT is performed under ideal circumstances (yet to be described), the oocyte cytosol can turn a somatic nucleus into a totipotent one. Recent somatic cell-ESC fusion experiments suggest that ESCs retain similar as yet undefined components that can initiate the reprogramming of introduced somatic nucleus to confer pluripotency to the somatic nuclei (as measured by phenotypic and by transcriptional analyses). In this respect, the cytoplasmic environment of both ESCs and oocytes shares the capacity to reprogram a somatic nucleus (Ref 39-41). Furthermore, recent work suggests that mESCs can give rise to PGCs that can differentiate into cells similar to oocytes and sperm in a period significantly shorter when compared with in vivo gametogenesis (Ref 42,43). Taken together, this evidence indicates that there is a common set of genes between oocytes and ESCs responsible for reprogramming somatic cells. To identify these genes, differentially up-regulated transcripts in the oocyte were compared and intersected with recently published data for genes that are expressed preferentially in ESCs (Ref 44). Our analysis of the Sato et al.(Ref 44) data revealed 1,626 hESC differentially up-regulated genes. When these hESC genes were intersected with our human differentially up- regulated oocyte transcripts, we found an overlap of 388 transcripts (See Figure 4b) and Data Set 5, which is available in Ref 50). Our final intersection was drawn between these 388 human transcripts and a list of 591 genes common to mouse oocyte and mESCs (See Figure 4C) and Data Set 6, which is available in Ref 50). A final list of 66 unique genes (78 transcripts) common amongst mouse oocyte, mESCs, human oocyte, and hESCs was obtained; five of these genes have unknown functions (See Figure 4D); Table 3, which is published as supporting information in Ref 50 on the PNAS web site.. Among these 66 unique genes, there is a high abundance of chromatin and DNA modifying genes, suggesting that the genomes of both the ESCs and oocytes are maintained in a responsive or primitive-naive state, potentially primed for the activation of a whole repertoire of genes leading the generation of all tissue lineages. Whether any of these genes are involved in the ability to reprogram somatic nuclei should be further explored.

RESULTS

[00087] Table 1. Selected representations of genes common between human oocyte and mouse oocyte with homologues functionally characterized in the mouse oocyte (15 selected genes out of the top 100) Gene title Gene Reported function(s) in murine oocytes Reference symbol

Developmental DPPA3 Maternal effect gene required for oocyte (51) pluripotency development associated 3

Zona pellucida ZPi, ZP: Required for successful fertilization in (52) glycoprotein 1 which the sperm must bind and penetrate

(sperm receptor); the ZP; prevention of further sperm entry zona pellucida following fertilization; and prevents glycoprotein 3 blastocyst adherence to oviduct wall as it

(sperm receptor) travels down the uterus

v-mos Moloney MOS Activation of MAPK cascade that enables (53) murine sarcoma viral progression through oocyte maturation, oncogene homolog and subsequent maintenance of meiotic metaphase II arrest

Growth GDF9 Essential for ovarian follicular growth and (54) differentiation factor function (55)

9

Centromere protein CENPA, A component of meiotic kinetochores (56)

A, 17 kDa; CENPE required for meiosis I (57) centromere protein

E, 312 kDa

BUBl budding BUBIB Contribute to meiotic metaphase II arrest (58) uninhibited by independent of spindle checkpoint benzimidazoles 1 activation homolog β (yeast) Maternal embryonic MELK May play a role in regulatory signal (59) leucine zipper kinase transduction pathways in oocyte

BCL2-like 10 BCL2L10 A component of the Bcl2 protein family (60)

(apoptosis facilitator) important for the activation of a proapoptotic signaling pathway in ageing oocytes

Cyclin Bl CCNBI l Interacts with the cdc2 protein kinase to (61) form the maturation promoting factor essential of oocyte maturation and arrest

Cyclin A2 CCNA2 Distinct roles in both mitosis and meiosis (62) based on expression and localization changes in oocytes from embryonic stages to postnatal and adult ovaries

Aurora kinase B; AURKB, Meiotic prophase I reactivation via the (63) aurora kinase C AURKC polyadenylation of specific transcripts such as Mos serine/threonine kinase

DNA (cytosine-5-)- DNMT 1 , Maintenance methylation of imprinted (64) methyltransferase 1; DNMT3b* alleles in oocyte and preimplantation (65) DNA (cytosine-5-)- embryos (66) methyltransferase 3 β

*DNMT3b is ranked within the top 200 genes.

[00088] Table 2. List of 66 unique genes in common among human oocytes (hOoc), mouse oocytes, human embryonic stem cells (hESCs), and mouse ESCs (mESCs)

Gene Fold Fold symbol Gene title change change (hOoc) (hESC)

PDKl Pyruvate dehydrogenase kinase, isoenzyme 1 65.81 6.96

POU5F1 POU domain, class 5, transcription factor 1 6.75 5.67

JARID2 Jumonji, AT rich interactive domain 2 48.31 5.05

MSH2 mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli) 44.93 3.93

DNMT3B DNA (cytosine-5-)-methyltransferase 3 β 42.64 3.45

PTTGI Pituitary tumor-transforming 1 127.7 3.33

CDTI DNA replication factor 5.87 3.24

KJF2C Kinesin family member 2C 10.53 3.15

BUB 1 budding uninhibited by benzimidazoles 1 homolog BUBI (yeast) 39.85 3.05

ORCIL Origin recognition complex, subunit 1-like (yeast) 37.76 2.99

BIRC5 Baculoviral IAP repeat-containing 5 (survivin) 3.09 2.92

UNG Uracil-DNA glycosylase 20.81 2.88

CCNA2 Cyclin A2 224.87 2.77

STK6 Serine/threonine kinase 6 188.96 2.76

NASP Nuclear autoantigenic sperm protein (histone-binding) 7.4 2.68

CCNBI Cyclin BI 138.32 2.66

MCM7 minichromosome maintenance deficient 7 (Saccharomyces MCM7 cerevisiae) 5.13 2.63 CHKI checkpoint homolog (Schizosaccharomyces

CHEKI pombe) 48.59 2.52

CENPA Centromere protein A, 17 kDa 527.02 2.48

PRUVÏ2A Primase, polypeptide 2 A, 58 kDa 8.9 2.42

C10orf86 Chromosome 10 open reading frame 86 21.37 2.31

EXOSC7 Exosome component 7 15.45 2.26

AKAPI A kinase (PRKA) anchor protein 1 34.31 2.25

CSPG6 Chondroitin sulfate proteoglycan 6 (bamacan) 25.77 2.22

MAD2L1 MAD2 mitotic arrest deficient-like 1 (yeast) 30.33 2.2

CCNB2 Cyclin B2 204.76 2.19

USPIO Ubiquitin-specific peptidase 10 3.26 2.1

PIAS2 Protein inhibitor of activated STAT$_5$ 2 5.2 2.09

Asp (abnormal spindle)-like, microcephaly-associated

ASPM (Drosophila) 836.86 2.08

POLE2 Polymerase (DNA directed), ε 2 (ρ59 subunit) 31.24 2.07

TTK TTK protein kinase 194.33 2.07

DNA2L DNA2 DNA replication helicase 2-like (yeast) 18.76 2.01

ECT2 Epithelial cell-transforming sequence 2 oncogene 43.1 2

MCM2 minichromosome maintenance deficient 2, mitotin (S.

MCM2 cerevisiae) 71.22 1.98

SNRPDI Small nuclear ribonucleoprotein DI polypeptide 16 kDa 5.2 1.97 MCMIO minichromosome maintenance deficient 10 (S.

MCMIO cerevisiae) 9.91 1.96

NUPS8 Nucleoporin 88 kDa 9.41 1.96

NUP54 Nucleoporin 54 kDa 7.51 1.91

CKS IB CDC28 protein kinase regulatory subunit IB 27.18 1.88

SAP30 sin3-associated polypeptide, 30 kDa 9.71 1.87

FLJ20364 Hypothetical protein FLJ20364 25.52 1.82

SART3 Squamous cell carcinoma antigen recognized by T cells 3 15.78 1.82

HSPA14 Heat shock 70 kDa protein 14 13.69 1.82

HMGB2 High-mobility group box 2 6.08 1.81

DCLREIA DNA cross-link repair IA (PSO2 homolog, S. cerevisiae) 41.63 1.75

TCLIA T cell leukemia/lymphoma IA 118.95 1.75

RRM2 Ribonucleotide reductase M2 polypeptide 73.43 1.75

ASFIA ASFI anti-silencing function 1 homolog A (S. cerevisiae) 56.65 1.75

C 14orf94 Chromosome 14 open reading frame 94 27.15 1.75

GMNN Geminin, DNA replication inhibitor 14.18 1.74

PLKl Polo-like kinase 1 (Drosophila) 9.03 1.73

FLJI 1305 Hypothetical protein FLJI 1305 25.08 1.71

BLM Bloom syndrome 12.51 1.68

SMNI Survival of motor neuron 1, telomeric 7.99 1.66 CDCA3 Cell division cycle associated 3 27.59 1.65

NUP93 Nucleoporin 93 kDa 11.92 1.63

TRIP 13 Thyroid hormone receptor interactor 13 43.14 1.55

ORC6L Origin recognition complex, subunit 6 homolog-like (yeast) 59.73 1.52

NXIP 107 Nucleoporin 107 kDa 19.41 1.52

TCOFI Treacher Collins-Franceschetti syndrome 1 21.64 1.51

TCERGI Transcription elongation regulator 1 3.71 1.51

MCM4 minichromosome maintenance deficient 4 (S. MCM4 cerevisiae) 6.14 1.5

NAPILl Nucleosome assembly protein 1-like 1 3.18 1.48

MRPLI 8 Mitochondrial ribosomal protein LI 8 8.8 1.46

DHFR Dihydrofolate reductase 85.24 1.45

MAPKAPK5 Mitogen-activated protein kinase-activated protein kinase 5 3.43 1.45

[00089]

Table 3. Information of the primers and their sequences used for the validation of the microarray experiment by RT-PCR

Gene Accession no. Sequence Product size, bp

OCT4 NM 002701 S'-GAGTGAGAGGCAACCTGGAG-S' 274

5^l-GTGAAGTGAGGGCTCCCATA-3^l

STELLA AY317075 5 385

'-CTCAAATCTCCTCCGAGACG-S' ^{l}-TGAAGTGGCTTGGTGTCTTG-3^l

ESGI NM 001025290 5'-AGAGGTGTTCC AGGTCC AGA-3[1] 341

S'-GCTCTGGCCACAACCTAATC-S'

VASA AY004154 5'-TGGGACATTCAATTCGACAA-S' 364

5'-GAGAACTGGCCAACTTGGAG-3[i]

GDF9 NM 005260 5'-CTGCCTATCCTGTGGGAGAA-3[i] 301

S'-ACTGGAGAGCCATACCGATG-S'

ZPI NM 207341 5'-GGCTTCTGCAGAGGACAGAC-S' 344

5'-TCGTCGCTGTCTTGTAGTGC-3'

HlFOO NM 153833 S'-AGTCGGGAGAGGCTAGGAAG-S' 306

5'-GTACCACCTTGGACCCACTG-3[i]

CDH3 NM 001793 5'-GCTACCGCATCCTGAGAGAC-3' 376

5'-TCACCTTCCTCGTTGACCTC-S'

TUBB4Q U8311O 5'-CTGGTGTCTGCTACCGTGAG-3' 356

S'-GGGAGCCAGTCAGCAAAGTA-S'

ACTB NM 001101 5 496

˙-GGCATCCTCACCCTGAAGTA-3'

-CCATCTCTTGCTCGAAGTCC-S[1] [0090] The Tables referenced in the Examples are set forth supra. Li addition Figure 7 contains a compilation of selected overrepresented gene Ontology (GO) biological processes in oocytes identified by Expression Analysis Systematic Explorer (EASE) (EASE score less than 0.05). Figure 8 contains a listing of 101 genes identified according to the invention as being upregulated by in vivo matured human oocytes. As noted supra, these genes and the corresponding gene products are useful markers of undifferentiated cells and are useful in effecting the transdifferentiation and/or dedifferentiation of somatic cells e.g., into pluripotent or multipotent cells. These cells may be used in cell therapy and for the study of cell differentiation and embryogenesis.

CONCLUSIONS AND APPLICATIONS OF THE PRESENT INVENTION

[0091] This invention provides a comprehensive expression baseline of gene transcripts present in in vivo matured human Mil oocytes. Using the most recent Affymetrix Human GeneChip, we have identified 5,331 transcripts highly expressed in human oocytes, including well known genes such as FIGLA, STELLA, VASA, DAZL, GDF9, ZPI, ZP2, MOS, OCT4, NPM2, NALP5/MATER, ZARI, and HlFOO. More importantly, 1,430 of these up-regulated genes have unknown functions, arguing for the need for further studies aimed to elucidate the functional role of these genes in the human oocyte.

[0092] We have also identified a significant number of genes common between hESCs and MII oocytes. Such genes may provide the missing link between ESCs and Mil oocytes and may serve as genetic resources to identify ESCs that have full potential for differentiation into an oocyte. [0093] The results of the invention will facilitate a greater understanding as to the biological role of these genes and will expand enhance the understanding of the meiotic cell cycle, fertilization, chromatin remodeling, lineage commitment, pluripotency, tissue regeneration, and morphogenesis. The practical implications of compiling gene expression information on human gametes and embryos is enormous and will also bolster efforts to solve problems from infertility to degenerative diseases.

[0094] This invention in particular provides a transcriptome of 101 genes expressed by human oocytes. These genes and the orthologs of these genes are involved in the differentiation based on their specific expression in oocytes. Therefore, the introduction of one or more of these genes or the corresponding gene products should result in the transdifferentiation of a desired somatic cell into another cell type and/or lengthen the lifespan of said cell.

10095] Particularly the introduction of at least one of said genes or incubation of a somatic cell or the nucleus or chromatin mass derived therefrom with a medium containing at least one of said gene products will result in the partial or complete dedifferentiation or reprogramming of a desired somatic cell into a pluripotent or multipotent cell., e.g., an adult stem cell or an embryonic cell type. Cells which transdifferentiate or dedifferentiate may be detected by screening by at least one marker that is specific for the particular cell type. For example, it is known that embryonic stem cells express certain genes such as Oct4, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81 and alkaline phosphatase. Also, dedifferentiated cells or cells having enhanced lifespan may be detected based on an analysis of telomerase expression or telomere length. (Dedifferentiated cells will possess longer telomeres relative to parent differentiated cell). The subject methods may also be combined with the reprogramming procedures disclosed inUS2005014258 published January 20, 2005 and US Patent No. 7,253,334, issued on August 7, 2007, both of which are incorporated by reference in their entirety herein. In the present invention the cells or the nucleus or chromatin mass derived therefrom with initially be contacted with a cocktail containing a significant number of the subject human oocyte specific gene products, e.g. 50, 40, 30, 20, 10 or more, and the cocktails which result in dedifferentiation will then be modified by removal of different gene products one at a time in order to determine the gene products which are essential for dedifferentiation or reprogramming, as well as the cocktail of constituents that achieves optimal results, i.e., production of cell with a stem cell phenotype. The optimal ratios and concentrations of these constituents and incubation time for the desired dedifferentiation will also be determined. [0096] These transdifferentiated or dedifferentiated cells may be used e.g., for cell therapy or for study of the differentiation process. Additionally, these cells may be used to produce artificial tissues and organs by culturing said dedifferentiated cells in vitro e.g., in cell culture apparatus that are designed to facilitate the formation of desired cell structure and morphology. Additionally, these cells may be introduced into non-human animals as xenografted cells for example by injecting the dedifferentiated into desired organs. For example, dedifferentiated cells may be used to study the effect of dedifferentiated cardiac cells on damaged heart tissue to determine whether these cells promote the healing or regeneration process. Alternatively, dedifferentiated immune cells may be introduced into immunodeficient animals to assess whether this results in restoration of immune function.

LIST OFREFERENCES

[00095] The following references are cited in this application. The contents of these references is incorporated by reference herein.

(1) Brambrink T, Wabnitz P, Halter R, Klocke R_5 Carnwath J, Kues W, Wrenzycki C, Paul D, Niemann H. BioTechniques. 2002;33:376-378. 380, 382-385.

(2) Tanaka TS, Ko MS. EurJObstet Gynecol Reprod Biol. 2004;115(Suppl 1):S85-S91.

(3) Wang QT_5 Piotrowska K_5 Ciemerych MA_5 Milenkovic L, Scott MP_5 Davis RW_5Zernicka- Goetz M. Dev Cell. 2004;6: 133-144.

(4) Zeng F, Baldwin DA_5 Schultz RM. Dev Biol. 2004,272 :483-496

(5) Pan H_5 O'Brien MJ, Wigglesworth K_5 Eppig JJ, Schultz RM. Dev Biol. 2005,286:493- 506.

(6) Hamatani T, Carter MG, Sharov AA, Ko MS. Dev Cell. 2004;6: 117-131

(7) Pitera JE, Milla PJ, Scambler P, Adjaye J. Mech Dev. 2001,109:377-381.

(8) Verlinsky Y, Morozov G, Gindilis V, Strom CM, Freidin M, Rechitsky S, Verlinsky O, Ivakhnenko V, Zdanovsky V, Kuliev A. Mol Reprod Dev. 1995,41:127-132.

(9) Adjaye J, Daniels R, Monk M. J Assist Reprod Genet. 1998,15:344-348

(10) Adjaye J, Bolton V, Monk M. Gene. 1999,237:373-383. (11) Adjaye J, Monk M. Mol Hum Reprod. 2000;6:707-711.

(12) Daniels R, Lowell S, Bolton V, Monk M. Hum Reprod. 1997;12:2251-2256.

(13) Daniels R, Adjaye J, Bolton V, Monk M. Mol Hum Reprod. 199S;4:785-789.

(14) Hinkins M, Huntriss J, Miller D, Picton HM. Reproduction. 2005;130:883-888.

(15) Liu HC, He ZY, Tang YX, Mele CA, Veeck LL, Davis O, Rosenwaks Z. Fertil Steril. 1997;67:733-741.

(16) Salpekar A, Huntriss J, Bolton V, Monk M. Mol Hum Reprod. 2001;7: 839-844.

(17) Goto T, Adjaye J, Rodeck CH, Monk M. Mot Hum Reprod. 1999;5:851-860.

(18) Hsieh RH, Au HK, Yeh TS, Chang SJ, Cheng YF, Tzeng CR. Fertil Steril. 2004;81(Suppl 1):912-918.

(19) Huntriss J, Hinkins M, Oliver B, Harris SE, Beazley JC, Rutherford AJ, Gosden RG, Lanzendorf SE, Picton HM. Mol Reprod Dev. 2004;67:323-336

(20) Huntriss J, Gosden R, Hinkins M, Oliver B, Miller D, Rutherford AJ, Picton HM. Mol Hum Reprod. 2002;8:1087-1095.

(21) Monk M, Holding C, Goto T. Reprod Fertil Dev. 2001 ;13:51-57.

(22) Goto T, Jones GM, Lolatgis N, Pera MF, Trounson AO, Monk M. Mol Reprod Dev. 2002;62: 13-28.

(23) Ko MS. Trends Biotechnol. 2001 ;19:511-518 (24) Adjaye J. Reprod Fertil Dev. 2005;17:37-45

(25) Bermudez MG, Wells D, Malter H, Murine S, Cohen J, Steuerwald NM. Reprod Biomed Online. 2004,8:325-337.

(26) Dobson AT, Raja R, Abeyta MJ, Taylor T, Shen S, Haqq C, Pera RA. Hum Mol Genet. 2004,13:1461-1470.

(27) Li SS, Liu YH, Tseng CN, Singh S. Biochem Biophys Res Commun. 2006;340:48-53.

(28) Assou S, Anahory T, Pantesco V, Carrour TL, Pellestor F, Klein B, Reyftmann L, Dechaud H, Vos JD, Hamamah S. Hum Reprod. 2006;21:1705-1719.

(29) Hosack DA, Dennis G Jr, Sherman BT, Lane HC, Lempicki RA. Genome Biol. 2003;4:R70. '

(30) Bachvarova R. Dev .Sw/. 1985,1:453-524.

(31) Wassarman PM, Kinloch RA. Mutat Res. 1992,296:3-15.

(32) Braude P, Bolton V, Moore S. Nature. 1988,332 :459-46I .

(33) Su AI, Wiltshire T, Batalov S, Lapp H, Ching KA, Block D, Zhang J, Soden R, Hayakawa M, Kreiman G, et al. Proc Natl Acad Sd USA. 2004,101:6062-6067.

(34) Huynh K, Jones G, Thouas G, Britt KL, Simpson ER, Jones ME. Biol Reprod. 2004,70: 1263-1269.

(35) Sar M, Welsch F. Endocrinology. 1999;140:963-971. (36) Hiroi H, Momoeda M, Inoue S, Tsuchiya F, Matsumi H, Tsutsumi O, Muramatsu M, Taketani Y. EndocrJ. 1999;46:153-158.

(37) Cauffinan G_5 Van de Velde H, Liebaers I, Van Steirteghem A. MoI Hum Reprod. 2005;ll:405-411.]38.

(38) Lanza RP, Cibelli JB, Faber D, Sweeney RW, Henderson B, Nevala W, West MD, Wettstein PJ. Science. 2001;294:1893-1894.]

(39) Byrne JA, Simonsson S, Western PS, Gurdon JB. Curr Biol. 2003;13:1206-1213.

(40) Cowan CA, Atienza J, Melton DA, Eggan K. Science. 2005;309:1369-1373.

(41) Tada M, Takahama Y, Abe K, Nakatsuji N, Tada T. Curr Biol. 2001;ll:1553-1558.

(42) Hubner K, Fuhrmann G, Christenson LK, Kehler J, Reinbold R, De La Fuente R, Wood J, Strauss JF JJI, Boiani M, Scholer HR. Science. 2003;300:1251-1256.

(43) Geijsen N, Horoschak M, Kim K, Gribnau J, Eggan K, Daley GQ. Nature. 2004;427: 148-154.

(44) Sato N, Sanjuan IM, Heke M, Uchida M, Naef F, Brivanlou AH. Dev Biol. 2003;260:404-413.

(45) Chomczynski P, Sacchi N. Anal Biochem. 1987;162:156-159.

(46) Li C, Hung Wong W. Genome Biol. 2001 ;2 RESEARCH0032.

(47) Shedden K, Chen W, Kuick R, Ghosh D, Macdonald J, Cho KR, Giordano TJ, Gruber SB, Fearon ER, Taylor JM, Hanash S. BMC Bioinformatics. 2005;6:26.] (48) Irizarry RA, Bolstad BM_3 Collin F_5 Cope LM, Hobbs B, Speed TP. Nucleic Acids Res. 2003;31:el5.

(49) Fortunel NO, Otu HH, Ng HH, Chen J, Mu X, Chevassut T, Li X, Joseph M, Bailey C, Hatzfeld JA, et al. Science. 2003;302:393.

(50) Kocabas AF, Crosby J., Ross PJ, Out HH, Kaplan H., Leong TW, Rosa GJM, Halgren RG, Lim B, fernandez E., Cibelli JB, Proc Natl acad Sci.,USA, 2006, 104(38):14027-32. (51). Payer B, Saitou M, Barton SC, Thresher R, Dixon JP, Zahn D, Colledge WH, Carlton MB, Nakano T, Surani MA (2003) Curr Biol. 13:210-7.

(52). Acevedo N, Smith GD (2005) Front Biosci 10:2335-2345.

(53) . Kim MH, Yuan X, Okumura S, Ishikawa F (2002) Biochem Biophys Res Commun 296:1372-1377.

(54) . Drummond AE (2005) Cell Tissue Res 322: 107-115.

(55) . McNatty KP, Juengel JL, Reader KL, Lun S, Myllymaa S, Lawrence SB, Western A, Meerasahib MF, Mottershead DG, Groome NP, et al (2005) Reproduction 129:473-480.

(56) . Duesbery NS, Choi T, Brown KD, Wood KW, Resau J, Fukasawa K, Cleveland DW, Vande Woude GF (1997) Proc Natl Acad Sci USA 94:9165-9170.

(57) . Schatten G, Simerly C, Palmer DK, Margolis RL, Maul G, Andrews BS, Schatten H (1988) Chromosoma 96:341-352.

(58) . Brunet S, Pahlavan G, Taylor S, Maro B (2003) Reproduction 126:443-450.

(59) . Heyer BS, Warsowe J, Solter D, Knowles BB, Ackerman SL (1997) MoI Reprod Dev 47:148-156.

(60) . Tatone C, Carbone MC, Gallo R, Delle Monache S, Di Cola M, Alesse E, Amicarelli F (2006) Biol Reprod 74:395-402.

(61). Wu B, Ignotz G, Currie WB, Yang X (1997) Biol Reprod 56:253-259.

(62). Persson JL, Zhang Q, Wang XY, Ravnik SE, Muhlrad S, Wolgemuth DJ (2005)

Reproduction 130:411-422.

(63) . Crane R, Gadea B, Littlepage L, Wu H, Ruderman JV (2004) Biol Cell 96:215-229.

(64) . Lees-Murdock DJ, Shovlin TC, Gardiner T, De Felici M, Walsh CP (2005) Dev Dyn 232:992-1002.
(65) . Ratnam S, Mertineit C, Ding F, Howell CY3 Clarke HJ, Bestor TH, Chaillet JR, Trasler JM (2002) Dev Biol 245:304-314.

(66) . Kierszenbaum AL (2002) MoI Reprod Dev 63 :269-272.

Claims

CLAIMSWe claim:
1. A transcriptome comprising a set of genes expressed by a normal mature human oocyte, wherein said transcriptome comprises at least 10 of the 101 genes contained in the sequence listing herein or their non-human mammalian orthologs..
2. The transcriptome of claim 1 which comprises at least 20 genes.
3. The transcriptome of claim 1 which comprises from 20-50 of said 101 genes.
4. The transcriptome of claim 1 which comprises from 50-75 of said 101 genes.
5. The transcriptome of claim 1 which comprises from 75-101 of said 101 genes.
6. A human or non-human mammalian oocyte which has been genetically modified such that it does not express at least one of the genes contained in Figure 8 or an ortholog thereof.
7. A human or non-human mammalian oocyte which has been genetically modified such that it expresses at least one of the genes contained in Figure 8 or an ortholog thereof under the control of a heterologous regulatable promoter.
8. A method of dedifferentiating or transdifferentiating a somatic cell comprising introducing into a desired somatic cell or into a media which is in contact with said somatic cell at least one of the 101 genes or the polypeptides expressed thereby which are contained in Figure 8 or an ortholog thereof.

9. The method of claim 8 wherein at least 3 of said genes or the corresponding are introduced into said somatic cell or into a culture medium that contains said somatic cell..

10. The method of claim 8 wherein said method results in the dedifferentiation of said somatic cell.

11. The method of claim 8 wherein said method increases the lifespan of said cell.

12. The method of claim 11 wherein said method results in the conversion of said somatic cell into a pluripotent or multipotent cell.

13. The method of claim 8 wherein the cells are screened for pluripotency based on the expression of at least one gene that is selectively expressed by pluripotent cells.

14. The method of claim 13 wherein said polypeptide is selected from Oct4, telomerase and SSEA-3, SSEA-4, TRA- 1-60, TRA- 1-81 and alkaline phosphatase.

15. The method of claim 14 wherein said expression is detected using an antibody or other ligand that specifically binds to one of said polypeptides.

16. The method of claim 8 wherein the somatic cell is a human cell.

17. The method of claim 14 wherein said somatic cell is selected from a fibroblast, lymphocyte, endothelial cell, keratinocyte, bone cell, neural cell, heart cell, kidney cell, tooth cell, lung cell, skin cell, immune cell, stomach cell, esophageal cell, tracheal cell, liver cell, gall bladder cell, ovarian cell, urethral cell, testicular cell, red blood cell, diaphragm cell, muscle cell, a sensory cell involved in sight, hearing, taste, smell, or touch, and a pancreatic cell.

18. The method of claim 8 which converts said cell into an embryonic or adult stem cell type.

19. The method of claim 8 which converts said cell into an embryonic-like stem cell.

20. The method of claim 8 wherein the resultant transdifferentiated cell is itself suitable for cell therapy or is used to derive somatic cells which are suitable for cell therapy.

21. The method of claim 8 wherein said somatic cell is contained in a xenograft contained in a non-human mammal.

22. The method of claim 8 wherein said cells are contained in a plurality of microtiter wells which are each transfected or contacted with a different set of genes or a composition comprising polypeptides encoded thereby corresponding to the set of 101 genes contained in Figure 8.

23. The method of claim 22 wherein each of said microtiter wells containing a particular somatic cell is screened for the expression of at least one marker characteristic of a dedifferentiated cell type.

24. The method of claim 23 wherein said dedifferentiated cell type is a pluripotent or multipotent cell.

25. The method of claim 24 wherein said pluripotent or multipotent cell type is an embryonic stem cell or adult stem cell type.

26. The method of claim 22 wherein the somatic cells are selected from a fibroblast, endothelial cell, keratinocyte, bone cell, neural cell, heart cell, kidney cell, tooth cell, lung cell, skin cell, immune cell, stomach cell, liver cell, ovarian cell, urethral cell, testicular cell, red blood cell, diaphragm cell, muscle cell, sensory cell, and pancreatic cell.

27. A method of identifying, enriching or purifying dedifferentiated human cells from differentiated cells in a mixed cell sample comprising the use of a probe that specifically binds to one of the genes or gene products contained in Figure 8.

28. The method of claim 27 wherein the probe is an antibody or labeled oligonucleotide that specifically binds to one of said genes or the corresponding gene products.

29. The method of claim 27 which comprises a FACS or magnetic bead cell separation procedure.

30. A method of treating a subject that involves the introduction or transplantation of cells wherein the treatment method comprises introducing dedifferentiated or transdifferentiated cells produced according to claim 8.

31. The method of claim 30 wherein the somatic cells used for dedifferentiation or transdifferentiation are obtained from the treated subject or a genetically compatible donor.

32. The method of claim 30 which is used to treat a condition selected from cancer, autoimmunity, infection, inflammation disorder, and an allergic condition.

Patent Citations (1)

Publication numberPriority datePublication dateAssigneeTitle
US20030044976A1 *2001-08-272003-03-06Advanced Cell TechnologyDe-differentiation and re-differentiation of somatic cells and production of cells for cell therapies

Family To Family Citations
* Cited by examiner, † Cited by third party

Non-Patent Citations (9)

Title
ASSOU S. ET AL.: 'The Human Cumulus-Oocyte Complex Gene-Expression Profile' HUMAN REPRODUCTION vol. 21, no. 7, 2006, pages 1705 - 1719 *
BERMUDEZ M.G. ET AL.: 'Expression Profiles of Individual Human Oocyte Using Microarray Technology' REPRODUCTIVE BIOMEDICINE ONLINE vol. 8, no. 3, 21 January 2004, pages 325 - 337 *
BRANDEIS M. ET AL.: 'Cyclin B-2 Null Mice Develop Normally and are Fertile Wherease Cyclin-B-1 Null Mice Die In Utero' PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES vol. 95, April 1998, pages 4344 - 4349 *
CHAUDRY H.W. ET AL.: 'Cyclin A2 Mediates Cardiomyocyte Mitosis in the Postmitotic Myocardium' JOURNAL OF BIOLOGICAL CHEMISTRY vol. 279, no. 34, 20 August 2004, pages 35858 - 35866 *
DALBIES-TRAN R. ET AL.: 'Use of Heterologous Complementary DNA Array Screening to Analyze Bovine Oocyte Transcriptome and Its Evolution during In Vitro Maturation' BIOLOGY OF REPRODUCTION vol. 68, 2003, pages 252 - 261 *
DOBSON A.T. ET AL.: 'The Unique Transcriptome Through Day 3 of Human Preimplantation Development' HUMAN MOLECULAR GENETICS vol. 13, no. 14, 2004, pages 1461 - 1470 *
HENNEBOLD J.D.: 'Characterization of the Ovarian Transctiptome Through the Use of Differential Analysis of Gene Expression Methodologies' HUMAN REPRODUCTION UPDATE vol. 10, no. 3, 2004, pages 227 - 239 *
LI S. ET AL.: 'Analysis of Gene Expression in Single Human Oocytes and Preimplantation Embryos' BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS vol. 340, 2006, pages 48 - 53, XP024924009 *
NEILSON L. ET AL.: 'Molecular Phenotype of the Human Oocyte by PCR-SAGE' GENOMICS vol. 63, 2000, pages 13 - 24, XP004439452 *
* Cited by examiner, † Cited by third party

Cited By (8)

Publication numberPriority datePublication dateAssigneeTitle
US8048999B22005-12-132011-11-01Kyoto UniversityNuclear reprogramming factor
US8058065B22005-12-132011-11-15Kyoto UniversityOct3/4, Klf4, c-Myc and Sox2 produce induced pluripotent stem cells
US8129187B22005-12-132012-03-06Kyoto UniversitySomatic cell reprogramming by retroviral vectors encoding Oct3/4. Klf4, c-Myc and Sox2
US8211697B22007-06-152012-07-03Kyoto UniversityInduced pluripotent stem cells produced using reprogramming factors and a rho kinase inhibitor or a histone deacetylase inhibitor
US8791248B22007-12-102014-07-29Kyoto UniversityNuclear reprogramming factor comprising miRNA and a protein factor
US9213999B22007-06-152015-12-15Kyoto UniversityProviding iPSCs to a customer
US9499797B22008-05-022016-11-22Kyoto UniversityMethod of making induced pluripotent stem cells
US9683232B22007-12-102017-06-20Kyoto UniversityEfficient method for nuclear reprogramming
Family To Family Citations
* Cited by examiner, † Cited by third party, ‡ Family to family citation

Similar Documents

PublicationPublication DateTitle
Lengner et al.2010Derivation of pre-X inactivation human embryonic stem cells under physiological oxygen concentrations

Kurimoto et al.2007Global single-cell cDNA amplification to provide a template for representative high-density oligonucleotide microarray analysis
Hayashi et al.2011Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells
Braude et al.2002Preimplantation genetic diagnosis
Pellicer et al.1999In vitro fertilization plus preimplantation genetic diagnosis in patients with recurrent miscarriage: an analysis of chromosome abnormalities in human preimplantation embryos
Wrenzycki et al.2002In vitro production and nuclear transfer affect dosage compensation of the X-linked gene transcripts G6PD, PGK, and Xist in preimplantation bovine embryos
Wang et al.2014Programming and inheritance of parental DNA methylomes in mammals
Mertzanidou et al.2012Microarray analysis reveals abnormal chromosomal complements in over 70% of 14 normally developing human embryos
Shi et al.2004Methylation reprogramming and chromosomal aneuploidy in in vivo fertilized and cloned rabbit preimplantation embryos
Dyce et al.2004Stem cells with multilineage potential derived from porcine skin
Bettegowda et al.2008Identification of novel bovine cumulus cell molecular markers predictive of oocyte competence: functional and diagnostic implications
Nolen et al.2005X chromosome reactivation and regulation in cloned embryos
Staessen et al.2003PGD in 47, XXY Klinefelter's syndrome patients
Inoue et al.2006Inefficient reprogramming of the hematopoietic stem cell genome following nuclear transfer
Byrne et al.2007Producing primate embryonic stem cells by somatic cell nuclear transfer
Miyoshi et al.2003Improvements in cloning efficiencies may be possible by increasing uniformity in recipient oocytes and donor cells
US20020142397A12002-10-03Methods for altering cell fate
Shima et al.2004The murine testicular transcriptome: characterizing gene expression in the testis during the progression of spermatogenesis
Cauffman et al.2004Oct-4 mRNA and protein expression during human preimplantation development
Lequarre et al.2005Influence of antral follicle size on oocyte characteristics and embryo development in the bovine
Haston et al.2009Dazl functions in maintenance of pluripotency and genetic and epigenetic programs of differentiation in mouse primordial germ cells in vivo and in vitro
Tachibana et al.2013Human embryonic stem cells derived by somatic cell nuclear transfer
Park et al.2009Derivation of primordial germ cells from human embryonic and induced pluripotent stem cells is significantly improved by coculture with human fetal gonadal cells
Plaia et al.2006Characterization of a new NIH-registered variant human embryonic stem cell line, BG01V: A tool for human embryonic stem cell research
Hamatani et al.2006Global gene expression profiling of preimplantation embryos

Priority And Related Applications

Priority Applications (2)

ApplicationPriority dateFiling dateTitle
US842990062006-09-082006-09-08US Provisional Application
US60/842,9902006-09-08

Legal Events

DateCodeTitleDescription
2008-05-14121Ep: the epo has been informed by wipo that ep was designated in this application
Ref document number: 07811726

Country of ref document: EP

Kind code of ref document: A2

2009-03-10NENPNon-entry into the national phase in:
Ref country code: DE

2009-10-14122Ep: pct application non-entry in european phase
Ref document number: 07811726

Country of ref document: EP

Kind code of ref document: A2