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Cellular Differentiation Regulate Gene Expression Through DNA-Methylation

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ABSTRACT

Cellular differentiation is a transition of a cell from one cell to another and plays a very important role to regulate the gene expression through DNA methylation including small number of genes such as OCT4 and NANOG are methylated and their promoters repressed to prevent further expression.It is an epigenetic mechanism for the differentiation of pluoripotent stem cells and mammilain cells harbor small ncRNA,snoRNA,miRNA's, and dsRNA's associated with gene inactivation that cannot cause gene silencing denovo,but can also be acquired as a consequence of upstream gene downregulation. To find the transcription factor binding sites(TFBS),we fit a classification tree to the data using the CART software in R. The CART method selects a sequence of TFBS that optimally separates the gene classes.The deathbase database participate in extrinsic or intrinsic, pre-mitochondrial signaling events or post-mitochondrial caspase activation and apoptic cell clearance.

Introduction:

Cell differentiation involves a switch from one pattern of gene expression to another.Volvox carteri, a model organism for studying how unicellular organisms can evolve into multicellular organisms. Regulation of gene expression occurs through DNA methylation in which the DNA methyltransferase- mediated methylation of cytosine residues in CpG dinucleotide maintains heritable repression by controlling DNA accessibility.

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Immunoinformatics is using the basic bioinformatics tools such as ClustalW,BLAST and TreeView as well as specialized immunoinformatics tools such as EpiMatrix,IMGT/V-QUEST for IG and IR sequence analysis to study the complex protein-protein interactions and networks and allows a better understanding of immune responses.Epigenetic component is to be significant for the differentiation of pluoripotent stem cells into specific tissue lineages.DNA sequence-specific factors can provide the 'landing pad' for the recruitment of specialized enzymatic machineries that either deposit or remove the modification on the chromatin.Many miRNA are specially expressed during ESC differentiation and embroygenesis as well as during brain development,neuronal differentiation and hematopoietic lineage differentiation.Cell transformation is associated with cytoskeltal reorganization resulting from alterations in the expression of cytoskeltal protein components such as alpha-actinin,vinculin and alpha-tropomyosin. The CART transcription factor binding site analysis selects a sequence of TFBS that optimally separates the gene classes.Aim of the Deathbase database is to eliminate 'noise' in the field by focusing only on the proteins that have direct roles in cell death,rather than including protein whose overexpression induces apoptosis.

Materials and Methods:

Cellular differentiation and immunology:

Cell differentiation is a transition of a cell[1] from one cell type to another and it involves a switch from one pattern of gene expression to another. Cellular differentiation during development can be understood as the result of a gene regulatory network. A regulatory gene and its cis-regulatory modules are nodes in a gene regulatory network; they receive input and create output in the network[1]. Signal Transduction refers to cascades of signaling events, during which a cell or tissue signals to another cell or tissue to influence its developmental fate[2]. Yamamoto and Jeffer[3] investigated the role of the lens in eye formation in cave- and surface-dwelling fish, a striking example of induction[2]. Asymmetric cell divisions can occur because of asymmetrically expressed maternal cytoplasmic determinants or because of signaling[2]. The molecular and genetic basis of asymmetric cell divisions in green algae of the genus Volvox, a model system for studying how unicellular organisms can evolve into multicellular organisms[2]. In *Volvox carteri*, the 16 cells

in the anterior hemisphere of a 32-cell embryo divide asymmetrically, each producing one large and one small daughter cell. The size of the cell at the end of all cell divisions determines a specialized germ or somatic cell[2,4]. Female adipose cells, lung fibroblasts, and foreskin fibroblasts were reprogrammed into induced pluripotent state with the OCT4. SOX2. KLF4. and MYC genes. Patterns of DNA methylation in ESCs, iPSCs, somatic cells were compared. Regulation of gene expression occurs through DNA methylation, in which the DNA-methyltransferase mediated methylation of cytosine residues in CpG dinucleotides maintains heritable repression by controlling DNA accessibility[5]. The majority of CpG sites in embryonic stem cells are unmethylated and appear to be associated with H3K4me3-carrying nucleosomes[6]. Upon differentiation, a small number of genes, including OCT4 and NANOG[6], are methylated and their promoters repressed to prevent their further expression. DNA methylation-deficient embryonic stem cells rapidly enter apoptosis upon in vitro differentiation[5]. TGFs and FGFs have to sustain expression of OCT4, SOX2, and NANOG by downstream signaling to smad proteins[7]. Cytokine leukemia inhibitory factors are associated with the maintenance of mouse ESCs in an undifferentiated state. Immunoinformatics is using the basic bioinformatics tools such as ClustalW[8], BLAST[9], and TreeView, as well as specialized immunoinformatics tools, such as EpiMatrix[10,11], IMGT/V-QUEST for IG and TR sequence analysis, IMGT/ Collier-de-Perles and IMGT/StructuralQuery[11] for IGvariable domain structure analysis[12]. It aims to study the complex protein–protein interactions and networks and allows a better understanding of immune responses and their role during normal, diseased and reconstitution states. T cell epitopes are short linear peptides are cleaved from antigenic proteins, although T cell epitope generation by protein splicing[13]. Epitope presentation depends on both MHC-peptide binding and T cell receptor (TCR) interactions[14,15]. $CD4^+$ T cell epitopes are processed by antigen-presenting cells in membrane-bound vesicles, where they are degraded by proteases into the peptide fragments that bind to MHC class II proteins. Then they are delivered to the cell surface, where class II-peptide complexes can be recognized by the $CD4^+$ TCRs[16]. $CD8^+$ T cells recognize viral or self antigens expressed from within a cell[17], proteins that are cleaved into short peptides in the cytosol by the immunoproteasome[18] at the C-terminal end of the peptide[19]. The N terminus is later trimmed by proteases in endoplasmic reticulum[20]. After cleavage, peptides are translocated by the transporter associated with antigen processing (TAP) into the endoplasmic reticulum for loading onto HLA class I molecules[18,21,22]. The MHC class I-peptide complex is then presented on the cell surface, allowing recognition by epitope-specific TCRs on $CD8^+$ T cells[16,18].

Role of histone demethylases in embryonic stem cell epigenetics:

Epigenetic component is to be significant for the differentiation of pluripotent stem cells into specific tissue lineages. For example, an increase in the silencing-associated histone H3 Lys 9 dimethylation and trimethylation (Met2/Met3K9H3) marks on the chromatin and removal of MetK27H3. DNA sequence-specific factors can provide the 'landing pad' for the recruitment of specialized enzymatic machineries that either deposit[23] or remove the modification on the chromatin[44,45]. Most of histone modifications are reversible, methyl-groups could be enzymatically removed from lysine residues and enzymes that remove this modification have been identified[24,25,26]. The tri-met K27H3 can be actively removed by identifying two related jumonji-family proteins. JMJD3 and UTX. which specifically demethylate tri-met K27H3[27,28]. These demethylases are members of the mixed-lineage leukemia (MLL) protein complexes known to antagonize PcG-mediated gene silencing. JMJD3 is a direct gene target of Silencing Mediator of Retinoic Acid and Thyroid hormone receptors (SMRT), through its interaction with retinoic acid receptors (RARs) represses JMJD3 expression to maintain the neural stem cell state[29]. RA treatment of neural progenitors resulted in up-regulation of JMJD3 and a decrease in tri-met K27H3 levels on the promoter of the *Dlx5* gene, a marker of differentiated neurons.

Small non-protein-coding ribonucleic acids as epigenetic regulators of nuclear architecture:

A new integrated global regulatory network is based on the dynamic interplay of chromatin remodeling components i.e. TFs and small ncRNAs. These mechanisms synergize to choreograph stem cell self-renewal and the generation of cell diversity. Mammalian cells harbor numerous small ncRNAs, including small nucleolar RNAs (snoRNAs), microRNAs (miRNAs), short interfering RNAs (siRNAs) and small double-stranded RNAs, which regulate gene expression at many levels including chromatin architecture[30]. Although only 1.2% of the human genome encodes protein, a large fraction of it is transcribed. Whole chromosome-tiling chip arrays have detectable ncRNAs in human cells is much greater than mRNAs[31,32,33]. Many miRNAs are specifically expressed during ESC differentiation[34,35] and embryogenesis[36], as well as during brain development[37,38], neuronal differentiation[39], and hematopoietic lineage differentiation[34,40].Dicer-deficient mouse ESC are defective in differentiation and centromeric silencing[41]. Small RNAs also regulate chromosome dynamics, chromatin modification and epigenetic memory, including imprinting. DNA methylation and transcriptional gene silencing[30]. The RNAi pathway and non-coding RNAs have been central to the formation of silenced chromatin and chromosomal dynamics in animals, plants, fungi and protozoa.The RNAi machinery affects silencing and heterochromatin formation by reduction in histone H3 lysine-9 methylation and delocalization of the heterochromatin proteins HP1 and HP2. The localization of mammalian HP1 to heterochromatin involves its co-ordinate binding to methylated histone H3 and RNA, involving interactions in the hinge region between its chromodomains[42,43]. The chromodomains are present in many different types of chromatin-binding and chromatin-remodeling proteins, including the polycomb family, the histone methyltransferase and histone acetyltransferase families. RNA-interacting proteins are also components of the mammalian DNA methylation system[44,45].Non-coding RNA has the ability to induce either gene silencing[46] or gene activation[47] could be an integral component of epigenetic mechanisms dedicated to orchestrate gene transcription programs in ESCs[34]. This RNA, which occurs in the nucleus as a small 20 nt dsRNA,

controls the differentiation of adult neural stem cells and activates the transcription of genes containing NRSE/RE1 sequence, mediated through dsRNA–protein interactions, rather than through the classic function of siRNA or miRNA aimed to regulate mRNA production on the post-transcriptional level[47]. 'Functional junk' includes the example of a developmental-specific SINE B2 retrotransposon transcriptional unit providing the molecular boundary for separation of chromosomal domains of the differential transcriptional activity during cellular differentiation[48] in mammals.

ETS1 encoding a transcription factor involved in B-cell differentiation is recurrently deleted and down-regulated in classical Hodgkin's lymphoma

The neoplastic B-cell derived Hodgkin and Reed-Sternberg cells (HRS) in classical Hodgkin's lymphoma (cHL) undergo a substantial loss of B-cell identity. Epigenetic mechanisms such as DNA methylation are involved in this cHL-associated reprogramming[49]. DNA methylation is an epigenetic mechanism associated with gene inactivation that can not only cause gene silencing *de novo*, but can also be acquired as a consequence of upstream gene downregulation[50] and in cHL, a loss of a master transcription factor could lead to the methylation of genes involved in B-cell identity. The most significant enrichment for the transcription factors EBF1/OLF1 and E2A (E12/E47) to be involved in B-cell regulation and to be deregulated in cHL (Table)[51,52] . Binding sites for B-cell transcription factor. ETS1 among the group of genes hypermethylated exclusively in cHL. This led us to analyze the *ETS1* gene for mutations, copy number alterations and expression both on mRNA and protein level in cHL cell lines and primary biopsies.

Table 1. Top 10 Transcription factor motifs significantly over-represented in the group of gene hypermethylated in cH1

Sequence motif	Transcription factor	Enrichment within genes hypermethylated exclusively in cH4L	P*	Fold change downregulation of expression versus normal B-cells14**	Comment
V$EBF_Q6	EBF1/OLF1	1.43	5.70E-05	1.11	Down-reglulated in cH4L4
V$E12_Q6	E12(TCF3)	1.4	3.40E-04	1.45	Impaired function in cHL5
V$ETS1_B	ETS1	1.28	4.10E-04	7.23	Down-regulated in cHL
V$E2A_Q2	E2A(TCF3)	1.17	8.30E-04	1.45	Impaired function in cHL5
V$MTF1_Q4	MTF1	1.71	1.20E-03	1.2	
V$OLF1_01	EBF1/OLF1	1.59	3.00E-03	1.11	Down-regulated in cHL4
V$HAND1EA7_01	HAND1	1.17	4.40E-03	Not analyzed	
V$NERF_Q2	NERF(ELF2)	1.91	5.80E-03	1.36	
V$PAX9_B	PAX9	1.3	7.20E-03	0.46	
V$MINI20_B	MINI20(ELF2)	1.11	8.10E-03	1.36	

UTR's play a important role in cell differentiation:

Cell transformation is associated with cytoskeletal reorganization resulting from alterations in the expression of cytoskeletal protein components such as α-actinin, vinculin and α-tropomyosin (TM) [53]. These proteins are associated with actin in microfilaments and have a maior role in microfilament assembly and function. The levels of these structural proteins are decreased in transformed cells relative to normal cells[54-62]. Suppression of α-actinin and TM synthesis induces the transformed phenotype in fibroblasts indicating that these cytoskeletal proteins play a tumor suppressor role[53]. Loss of expression of TM isoforms is seen in human breast cancer cells[56,58]. In differentiating muscle cells, cytoskeletal proteins such as TM, troponin (TP) and α-cardian actin (AC) are expressed at high levels[62]. The 3′-untranslated regions (3′-UTRs) of the mRNAs encoding these proteins can induce differentiation in a differentiation-incompetent murine muscle cell line[62].

Neural Stem Cells:

Two cell clones were isolated by our colleague, Dr. Hedong Li, from v-myc-transformed E15 rat cortical cells[63]. Both expressed nestin, a marker of neural stem cells, and one (L2.3) also expressed a marker for radial glial cells (BLBP [FABP7]) and was selected based on radial-glial-like morphology. Upon withdrawal of bFGF, one clone (L2.2) differentiates into neuron-like ($TuJ1^+$) cells, eventually becoming electrically active GABA-ergic cells (Li et al., in preparation), and the other clone (L2.3) differentiates into a mixed neural phenotype, expressing neuronal (TuJ1), astrocytic (GFAP), and oligodendroglial (GalC) markers in individual cells. We prepared replicate cultures (n = 3) of cells at 0, 1, or 3 d following bFGF withdrawal for each of the two cell clones.

RNA Isolation, Labeling, and Hybridization:

RNA was prepared using the mirVana miRNA isolation kit (Ambion/Applied Biosystems), which produces both low-molecular-weight (LMW) RNA for microRNA

analysis and high-molecular-weight (HMW) RNA for mRNA analysis. Two micrograms of HMW RNA was labeled using the Chemiluminescent RT Labeling Kit (Applied Biosystems) and hybridized to AB1700 Rat Genomic Survey Arrays following the manufacturer's protocols.

Data Analysis:

Data extracted from the scanned arrays were processed using R/BioConductor scripts provided by Applied Biosystems. Raw data were quantile normalized and a linear model was fit to the data, estimating both cell line and differentiation effects: 1.337 probes exhibiting significant differences in either effect were selected, with an accepted false discovery rate of 5%. Significant probes were segregated into nine distinct clusters using a novel, model-based clustering method.

Probe Annotation:

Sequences for 26,857 probes from the AB1700 Rat Genome Survey Array were provided by Applied Biosystems. All available curated rat transcripts were obtained from the following sources: NCBI RefSeq (http://www.ncbi.nlm.nih.gov/projects/RefSeq/), NCBI Entrez Nucleotide DB (http://www.ncbi.nlm.nih.gov/entrez) (including dbEST, GenBank, and various other non-RefSeq rat transcripts), and Ensembl release 42(http://www.ensembl.org/Rattus_norvegicus/). Rat genomic sequences were obtained from both the reference genome assembly[63] and the Celera assembly from the public repository at NCBI. All sequences and associated annotation were stored locally on our LAMP model bioinformatics server. Each ~60-mer probe sequence was aligned to all available transcripts across all public sources using NCBI BLAST[64] running on a dedicated Linux server. Probes were aligned using a minimum expect-value of 1.0×10^{-6}.

CART Transcription Factor Binding-Site Analysis:

To interpret clusters using potential regulatory sequences, available 1-Kb regions upstream of the 1337 significant probes were searched for putative transcription-factor binding sites (TFBS) using the Match Algorithm, and associated vertebrate position weight matrices (PWM) included in the Transfac database.Cluster labels were treated as class labels for the purpose of detecting discriminating combinations of predicted TFBS. We explored different scoring mechanisms for the presence of a motif and the results were obtained using a measure that incorporates the number of hits as well as the score of a top hit. The top 10 scores for each motif were recorded, and a total score was obtained as the max(score)*range(10 scores). This measure is large if the top score is large and/or there are many moderate (multiple) hits in the promoter for this motif. Finally, to find the discriminating TFBS, we fit a classification tree to the data using the CART software in R[65]. The CART method selects a sequence of TFBS that optimally separates the gene classes. The first split in the tree is the single TFBS (TF1) that separates the gene classes. The next two splits find the two TFBS that improve the gene class separation: TF2(1+) separates the gene classes with TF1 present, and TF2(1–) best separates the gene classes with TF1 absent.

Deathbase Database:

DeathBase: A database on structure, evolution and function of proteins involved in apoptosis and other forms of cell death. The aims of this database is to eliminate 'noise' in the field by focusing only on the proteins that have direct roles in cell death, rather than including every protein whose overexpression induces apoptosis. We included in the DeathBase proteins that fit in one or more of the following categories: (i) which are part of the core evolutionarily conserved machinery of apoptosis (essentially, caspases, Bcl-2 family members, Apaf-1/FADD-like adapter proteins, IAPs, death receptors and ligands and proteins involved in phagocytosis of apoptotic corpses); (ii) proteins that directly regulate other proteins of the core machinery of apoptosis (e.g., Granzyme B or IAP antagonists); (iii) proteins that contain domains involved in apoptotic interactions such as CARDs or Death Domains; (iv) proteins that participate in non-apoptotic forms of cell death (for instance, RIP or *Drosophila* autophagic proteins); (v) that are homologous to central mediators of apoptosis[66]. We have classified many of the proteins in the DeathBase into a few cell death processes and pathways (Table) in which they participate: extrinsic or intrinsic; pre-mitochondrial signaling events or post-mitochondrial caspase activation; and apoptotic cell clearance. Proteins that participate in non-apoptotic forms of cell death have been assigned the descriptor 'cell death. A few examples of these non-apoptotic cell death processes are autophagic cell death during *Drosophila* development,[67] pyroptosis[68]&cornification[69].

Classification of proteins in processes, pathways and families

Protein	Species	Cell death process	Cell death pathway	Cell death pathway(secondary)	Protein Family
Bax	Mus musculus	Apoptosis	Intrinsic pathway	Pre-mitochondrial signaling events	Bcl-2 family,mutidomain Bcl-2
Caspase-8	Homo sapiens	Apoptosis	Extrinsic pathway	TNF/NF-kB signalling	Caspase
PARP-1	Mus musculus	Cell death(undefined)			Other
RIPK1	Homo sapiens	Necroptosis, apoptosis	Extrinsic pathway	TNF/NF-kB signaling	Other
Reaper	D. melanogaster	Apoptosis			IAP antagonist
Apaf-1	Homo sapiens	Apoptosis	Intrinsic pathway	Post-mitochondrial caspase activation	CARD-containing adapter protein

Discussion and Conclusion:

Cell differentiation is a process in which a generic cell develops into a specific type of cell in response to triggers from the body or the cell itself. This is the process which allows a single celled zygote to develop into a multicellular adult organism that can contain hundreds of different types of cells.Human pluripotent stem cells (hPSC), which have the potential to differentiate and eventually become any type of cell in the body. Their tests show that two specific proteins within stem cells—Oct4 and Sox2—can be used to track the four major early cell fate types that stem cells can turn into, allowing four screens to be performed at once. Regulation of gene expression occurs through DNA-methylation, in which the DNA-methyltransferase mediated methylation of cytosine residues in CpG dinucleotides mantains heritable repression by controlling DNA accessibility. Differentiation changes a cell,s size,shape,membrane potential,metabolic activity and responsiveness to signals.These changes are largely due to highly controlled modifications in gene expression.

Future work:

Cell differentiation has become a critical area of research, both in the context of adult and embryonic stem cells as well as a general and critical cellular process.Some of the most serious medical conditions, such as cancer and birth defects, are due to abnormal cell division and differentiation. It will used to identify how undifferentiated stem cells become the differentiated cells that form the tissues and organs. Turning genes on and off is central to this process. To increase the efficient of iPS directional differentiation choose the iPS-derives hepatocytes as a model cell. It will use the liver-specific miR-122 as a marker to distinguih normal differentiated liver cells from undifferentiated or cancerous cells by using enginerring regulatory pathway, the rigid standard for highly specific microRNA by applying several kinds of microRNA.

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