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Hypomethylation and Gene Regulation in Replicative Aging

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กระบวนการแก่ของเซลล์สามารถแบ่งออกเป็นสองแบบได้แก่ การแก่ของเซลล์ที่เกิดจากการแบ่งตัว เช่น ไฟโบรบลาสต์ และการแก่ของเซลล์ที่ไม่ได้เกิดจากการแบ่งตัว เช่นสมอง เป็นต้น การศึกษาก่อนหน้านี้พบว่าการลดลงของเมทิลเลชั่นสามารถพบได้ในกระบวนการแก่ของเซลล์ โดยที่ระดับของเมทิลเลชั่นบน *Alu* และ HERV-K ลดลงอย่างมีนัยสำคัญในตัวอย่างเม็ดเลือดขาวที่มีอายุมาก และพบว่าระดับเมทิลเลชั่นบน L1 เพิ่มขึ้นในบางช่วงอายุ ในการศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษาว่าการลดลงของเมทิลเลชั่นสามารถทำให้การแสดงออกของยีนเปลี่ยนแปลงหรือไม่ในกระบวนการแก่ของเซลล์ รวมทั้งยีนใดบ้างที่เป็นสาเหตุของการลดลงของเมทิลเลชั่นในกระบวนการแก่ของเซลล์ การศึกษานี้อาศัยข้อมูลไมโครอาเรย์ที่เกี่ยวข้องกับการแสดงออกของยีนในเซลล์ที่เกิดการแก่และเซลล์ที่มีการลดลงของเมทิลเลชั่น ผลการศึกษาพบว่าการลดลงเมทิลเลชั่นทำให้การแสดงออกของยีนในกระบวนการแก่ของเซลล์ที่เกิดจากการแบ่งเซลล์มีการเปลี่ยนแปลงอย่างมีนัยสำคัญ และพบยีนที่มีเกี่ยวข้องกับการลดลงของเมทิลเลชั่นจำนวน 82 ยีน หลังจากนั้นจึงได้ทำการเปรียบเทียบการแสดงออกของยีนกลุ่มนี้กับข้อมูลไมโครอาเรย์ที่เกี่ยวข้องกับการแสดงออกของยีนในเซลล์ที่ถูกยับยั้งการแสดงออกของยีนบางตัว จากการศึกษาทำให้ทราบว่ามียีนที่เกี่ยวข้องกับการแสดงออกของ 82 ยีนทั้งหมด 2 กลุ่ม คือยีนที่ทำหน้าที่ควบคุมการแก่ของเซลล์ และยีนที่ทำหน้าที่ในการชะลอการแก่ของเซลล์ เมื่อศึกษาหน้าที่ของยีน และการแสดงออกรวมทั้งโรคที่เกี่ยวข้องพบว่า ยีนทั้งสองกลุ่มทำหน้าที่เป็นทรานสคริปชั่นแฟคเตอร์, โปรตีนที่เกี่ยวข้องกับอาร์เอ็นเอ, เอนไซม์ต่างๆ และโปรตีนอื่นๆในเซลล์ นอกจากนั้นยังพบว่ามียีน 3 ตัวที่มีหน้าที่เกี่ยวข้องกับการเกิดเมทิลเลชั่น ได้แก่ *ELAV1*, *HDAC1* และ *EZH2* ที่น่าจะเกี่ยวข้องกับการลดลงของเมทิลเลชั่นในกระบวนการแก่ของเซลล์ที่เกิดจากการแบ่งเซลล์

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Cellular aging are divided to 2 groups, a replicative aging such as fibroblast and a non-replicative aging such as brain. Recently, hypomethylation in aging was reported. In aging cells of PBMC, hypomethylation of *Alu* and HERV-K was correlated to advancing age, while hypermethylation of L1 was found at specific age. In this study, we tested whether hypomethylation change gene expression in cellular and searched for genes that cause hypomethylation in replicative aging. We screened for expression profiles of cellular and demethylation to compare using statistical methods, Student's t-test and Pearson Chi-square test. The results showed that hypomethylation altered gene expression significantly in replicative aging. Moreover, 82 hypomethylated genes related replicative aging were extracted and compared with 516 expression profiles of gene knockdown experiment. Depending on their property, significant genes could be divided to 2 groups. There were 16 genes represented anti-aging function and 12 genes represented aging function also. For functional analysis, these 28 genes encoded various proteins such as transcription factor, RNA-binding protein, histone modification enzyme and other cytoplasmic proteins which related to many cellular phenotypes and age-associated disease. Furthermore,it was found that function of 3 genes, *ELAV1*, *HDAC1* and *EZH2*, correlate with DNA methylation implied that hypomethylation in replicative aging should be regulated by *ELAV1*, *HDAC1* and *EZH2*.

Field of Study : Medical Science Student's Signature Wachiraporn Wanichnopparat

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LIST OF ABBREVIATIONS

LINE (L1)	=	Long intersperse nuclear element 1
SINE	=	Short intersperse nuclear element
CU-DREAM	=	Connection Up- and Down-Regulation Expression Analysis of Microarrays
CU-DREAMX	=	Connection Up- and Down-Regulation Expression Analysis of Microarrays Extension
OR	=	Odd ratio

CHAPTER I
INTRODUCTION

Background and Rationale

Cellular aging can be divided into 2 groups, a replicative aging and a non-replicative aging. Replicative aging can be found in cells which proliferation rate of cells are limited[1]. Another type, non-replicative aging can be found when cells pass chronological time without cell division such as in brain[2].

The status that methylation in whole genome declined and became lower than normal condition called hypomethylaton which was generally discovered in repetitive sequence such as L1, *Alu* etc[3]. In aging, normal white blood cells of old subjects revealed hypomethylation status of *Alu* element and HERV-K compared with young counterparts, whereas methylation status of L1 didn't decline[4]. Moreover, the evidences of repetitive sequence hypomethylation were discovered in others biological processes including cancer and autoimmune disease. In many cancers, hypomethylation of L1 was detected as same as in systemic lupus erythematosus which was also detected L1 hypomethylation, but not *Alu*[5, 6]. Additionally, hypomethylation of repetitive sequence lead to genemic instability and gene expression alteration[7, 8]. However, the role on gene expression of hypomethylation was only demonstrated when hypomethylation occurs on L1 elements. There is no study the role of *Alu* and HERV-K hypomethylation and gene expression.

In this study, we investigated if hypomethylation alters gene expression in aging cells. Also we evaluated for genes that cause hypomethylation process in aging. We divided genes by expression change in aging cells and chemically induced hypomethylated cells. If there are regulated genes overlapped in significant numbers, hypomethylation should regulate gene expression in aging cells. Then, we compared the distribution of regulated both hypomethylated and aged cells with 516 expression profiles of siRNA treated cells. If there are significantly overlapped regulated genes, the genes of represented siRNA maybe play a role in hypomethylation process in aging cells.

We compared distribution of expression profiles by Connection Up- and Down-Regulation Expression Analysis of Microarrays (CU-DREAM), a program which help user to find a relationship between two microarray data[9]. The benefit of this study will be to understand the regulation of hypomethylation process which found in replicative aging.

Objective

To find candidate genes that causes hypomethylation process in replicative aging

Question

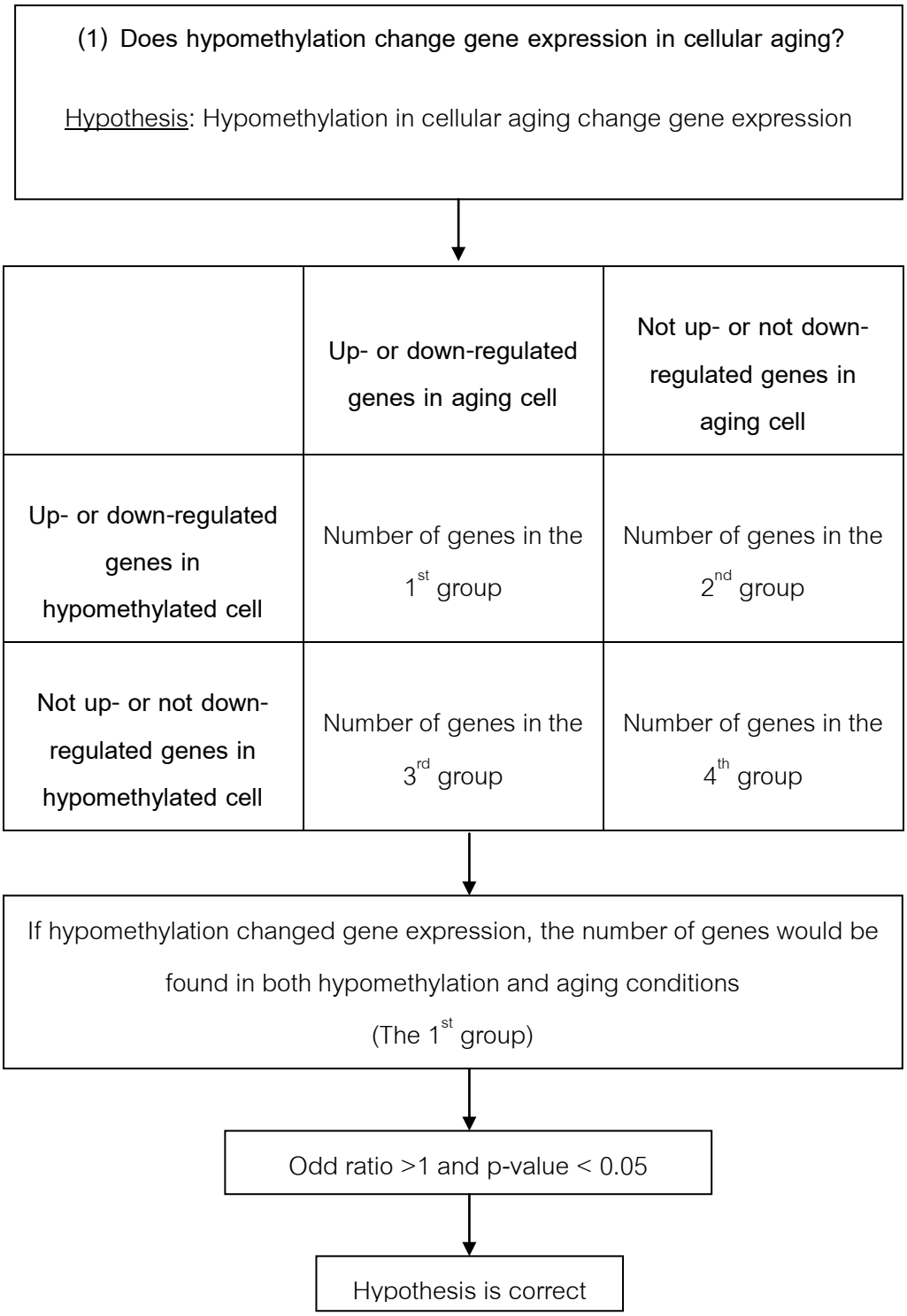
Primary question

- Does hypomethylation change gene expression in cellular aging?
- Which genes regulate hypomethylation process in replicative aging?

Hypothesis

- Methylation in cellular aging change gene expression
- Expression of siRNA knockdown cell significantly similar or significantly opposite to regulated hypomethylated aging genes, then the gene representing siRNA is the cause or consequence of hypomethylation associated aging

Conceptual Framework



Conceptual Framework (Continued)

(2) Which genes regulate hypomethylation process in replicative aging?

Hypothesis: Expression of siRNA knockdown cell significantly similar or significantly opposite to regulated hypomethylated aging genes, then the gene representing siRNA is the cause or consequence of hypomethylation associated aging

	Up- or down-regulated genes of siRNA transfected cell	**Not up- or not down-regulated genes of siRNA transfected cell**
Hypomethylated aging genes	Number of genes in the 1st group	Number of genes in the 2nd group
The rest	Number of genes in the 3rd group	Number of genes in the 4th group

↓

Expression of siRNA knockdown cell significantly similar or significantly opposite to regulated hypomethylated aging genes

↓

The cause or consequence of hypomethylation associated aging

Operation definition

Hypomethylation: methylation level in normal cells

Non-methylation: no methylation

Expected benefit

The benefit of this study will be to understand the regulation of hypomethylation process which found in replicative aging cells and genes associated in replicative aging process.

Research Methodology

1. Expression profiles from GEO datasets, NCBI
2. Statistical analysis by CU-DREAM and CU-DREAMX
3. Data analysis

CHAPTER II

REVIEW OF RELATED LITERATURES

1. Aging in mammals

1.1 Mechanism of aging in human

Cellular senescence or cell aged is a relevant biological process. This process is found in both eukaryote and prokaryote organisms[10, 11] and in several organs such as brain, skin fibroblast, liver and muscle etc[2, 12-15]. Cellular aging can be divided into 2 groups, a replicative aging and a non-replicative aging. Replicative aging can be found in cells which proliferation rate of cells are limited[1]. Another type, non-replicative aging can be found when cells pass chronological time without cell division such as in brain[2]. Hence, when cells reached senescence state, all type of senescent cells were altered several ways.

When cell aged, there are several physiological alterations of cells. Aged cells were limited their proliferation potential. So, notable aged cells are large flattened shape.[14] Moreover, gene expressions of cells going to senescent state were changed. Many genes in classes of immune system, inflammation, mitogen, regulation of proliferation, secretion factor and others were over expression.[2, 12-15] In addition, β-galactosidase was accumulated in senescent cells rather than normal cells[16]. Therefore, it was used as a marker to indicate cellular senescence termed as senescence-associated β-galactosidase[17]. On the other hand, some groups of genes were reduced their expressions.[18] For instance, EGFR mRNA was decreased by aging process. These changes were as a result of some regulation factors depended on the age of cell.[19]

It was putatively that there are many genes associated with cellular senescence. Importantly, p53 and Rb were strongly been an initial protein of this process[20, 21]. Downstream genes were regulated by these two protein led to cellular phenotypes alteration.

p53 is encoded by TP53 tumor suppressor gene[22]. In senescence process, p53 is activated by phosphorylation. Phosphorylated form of p53 may induce senescence

state or activated p21 leading to inhibition of Rb[20]. As well as to p53, Rb was believed to be an essential protein in cellular senescence. This protein is encoded by RB functioned as a transcription factor in many biological processes and atumor suppressor gene relative to Retinoblastoma[23]. In cellular senescence, hypohosphorylated form of Rb then inhibits E2F transcription factor. Therefore, target genes of E2F were arrested and senescence phenotypes were illustrated[20, 21].

2. DNA methylation in mammals

2.1 Mechanism of DNA methylation

DNA methylation is an epigenetic control found in many organisms and regulated genome without conversion in DNA sequences[24]. Methylation of DNA related to many proteins and some molecules containing in cells[24, 25]. Enzyme which performed crucial role in this mechanism is DNA methyltransferase (DNMT). During this mechanism, a methyl group of S-adenosylmethionine was transferred to the fifth carbon atom of cytosine. By strongly covalent bonds, cytosine of DNA sequences became 5-methylcytosine and methylation pattern was maintained. The reaction described before was manipulated by DNMT(Figure 2-2)[24, 26]. However, because of specific of DNMT functions, there were 3 types of these enzymes, DNMT1, DNMT3a and DNMT3b, played essential roles in human cells[27].

Three types of DNMT differentially methylate genome through various evidences. First, DNMT1, a maintenance methyltransferese was found a function to maintain methylation of whole genome. This enzyme acts in DNA replication and preferentially hemimethylate sister chromatid DNA[24]. The hemimethylated regions in sister chromatid then become to fully methylated at their CpG dinucleotide sites[28]. The two other types of DNMT are DNMT3a and DNMT3b. In contrast to DNMT1, target of methylation of DNMT3a and DNMT3b are a fully unmethylated region which found during embryogenesis in germ cells. Thus, their function were called de novo methylation enzyme[29].

2.2 DNA demethylation

Although DNA methylation seems relevant to biological process, DNA demethylation can be found in many evidences. Moreover, in cancer, global methylation was frequently found decreasing methylation level during tumor progression[6]. Recently, there were at least 3 mechanisms known that relative to DNA demethylion. In in vitro studies, some well-known reagents, 5'-azacytidine or 5'-deoxycytidine were used to inhibit methylation leading to lower level of methylation in cell culture[30]. As a function of nucleotide analog, 5'-azacytidine or 5'-deoxycytidine competitively bind to catalytic unit of DNMT. So, DNMT were temporary methylated DNA and methylation status of the cells was not maintained[31]. On the other hand, in vivo study found that demethylation of DNA in the genome was influenced by 3 factors. First, a defect of DNMT in the cells reduced enzyme activity and methylation level in the cells were declined[7]. Secondly, DNA glycosylase such as APOBEC and Aid were found the function as DNA demethylation enzyme via deamination follow by base excision repair process[32]. Furthermore, enzymes of Tet family alter 5-methylcytosine to be 5-hydroxymethylcytosine which continued to repair process as same as to DNA glycosylase[25]. Recently, oxidative stress was also proposed to be a cause of demethylation of DNA. In this process, relative oxygen species (ROS) induced DNA lesion which DNA methylation was inhibited by nucleotide conversion leading to CpG dinucleotide loss. Moreover, S-adenosylmethionine, a substrate of DNA methylation was declined from glutathione resynthesis during accumulation of oxidative stress. Therefore, methylation of DNA was reduced in oxidative stress condition[33].

2.3 Hypomethylation of genome

As described before, methylation of DNA was removed by many factors leading to whole genome methylation declined called hypomethylation. Because CpG islands spread widely in repetitive sequences[34, 35], hypomethylation was detected many times in the regions of them[4, 8]. Hypomethylation of genome was discovered in many evidences, for examples, cancers, autoimmune disease and aging. In many cancers, hypomethylation of L1 was detected as same as in systemic lupus erythematosus which was also detected L1 hypomethylation, but not *Alu*[5, 6]. In aging, normal white blood cells

of old subjects revealed hypomethylation status of *Alu* element and HERV-K compared with young counterparts, whereas methylation status of L1 didn't decline[4]. Additionally, hypomethylation of repetitive sequence lead to genemic instability and gene expression alteration[7, 8].

2.4 Mechanisms of gene suppression

Aberrant DNA methylation was reported to be mechanisms of gene suppression especially DNA hypermethylation. To suppress gene expression, there were many proteins played roles to inactivate gene. Methyl cytosine binding protein (MBP) and histone deacetylase (HDAC) were recruited to hypermethylation region on the promoter[36]. For silencing gene, transcritption factor, co-activator protein and histone acetylase were disturbed and could not bind to the promoter led to RNA polymerase could not transcribe mRNA of gene[37].

2.5 DNA methylation in aging cells

Methylation of DNA always effects on gene expression by changing DNA structure. In methylation modification, methyl group was modified at CpG dinucleotide on both strand of DNA[26]. Previous study reported that cells that were treated with 5' aza-deoxycytidine, a demethylation agent, would restore level of gene expressions. Loss of DNA methylation in vivo also correlated with tumor formation and metastasis[38, 39]. In contrast, promoter hypermethylation could reduce mRNA production of genes[40, 41]. In senescent process, the correlation of aging and DNA methylation also remained. It might be due to DNA methyltranferase (*DNMT*) gene family inactive or deficient by age. Thus, many age-related methylation diseases appeared in elder people such as autoimmune disease etc[42].

Methylation status of genome was reported the association with advancing age. Alteration of methylation level was found in many aged tissues. In $p21^{Waf1/Cip1}$, promoter methylation was increased during mid-age, but not in aging. Additionally, methylation level of $p16^{INK4a}$ was reduced during aging[43]. Furthermore, methylation related proteins were gradually declined such as DNMT, MBD and MeCP2 in many cell types which undergoing aging state[43-45]. Therefore, gene expression pattern was changed by

changing methylation level in cellular aging[46, 47]. Hypomethylation in aging was not reported only in specific genes, but in repetitive sequence was also discovered the alteration of methylation level.

Prior in vivo studies reported that methylation of *Alu* and HERV-K associated with age in normal white blood cell, but not LINE1. In addition, age-related hypomethylation was found at specific age both *Alu* and HERV-K. LINE1 also represented hypermethylation at certain age. Although, LINE1 was not direct correlated with age-related methylation, it was statistically correlated only with *Alu* methylation. That might indicate that *Alu* and HERV-K decrease methylation via different process[4]. As same as Bollati's which proposed that longitudinal and cross-sectional studies revealed the statistical correlation of advancing age and hypomethylation[48].

3. Connection Up- and Down- Regulation Expression Analysis of Microarrays (CU-DREAM)

The connection up- and down- regulation expression analysis of microarrays is program that finds an association between two interested experiments[9]. This program required the expression data from microarray which was publicly available on GEO datasets, a part of NCBI website[49]. Then, the template and parameter setting would be manipulated by users and the program would be operated them in command prompt and MS excel.

Depending on statistical base analysis, the program generated status of each gene by comparing mean of test group and control group using Student's t-test. The consequence was reported as up- or down-regulation of gene. Then, up- or down-regulation of genes of the experiment A were compared with up- or down-regulation of genes of the experiment B. The result of this step was shown the association between two experiments by odd-ratio and p-valued which measured by Pearson chi-square test[9].

As a figure below, the result of CU-DREAM illustrates 4 groups of association. In group A, gene members associated with experiment A and experiment B. Group B contained genes that associated with experiment B, but not A. Group C is opposite to group B which contained genes that associated with experiment A, but not B. The last

group, group D contained the rest which did not associate with both experiment A and experiment B (Figure 2-1).

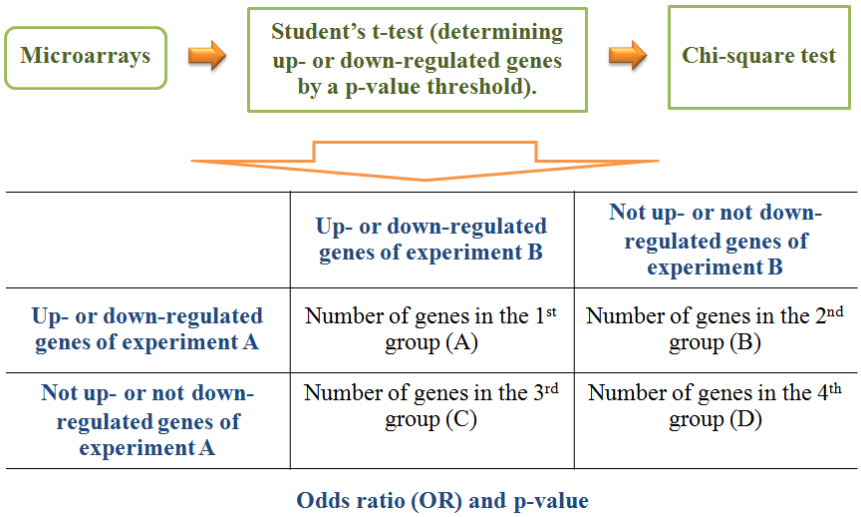


Figure 2-1 The CU-DREAM result illustrates 4 group of genes depending on the association between experiment A and experiment B. The odd ratio and p-value are reported also.

However, this program contained some limitations due to the number of gene in the experiment. Depending on sample sizes, a small sample size of experiment may not represent the association. Moreover, the inter-assay variation between the two conditions may appear because the program generate yield less significant statistical values, higher p-values and lower OR (when OR>1), than the actual values of significance[50].

CU-DREAM program also released the extent program, a CU-DREAM extension or CU-DREAMX, which statistical analysis was provided in the same principle of original program. Thus, CU-DREAMX revealed the association between an interest experiment and a certain condition, for instance, the association between lung cancer and intragenicL1[3].

CHAPTER III

MATERIALS AND METHODS

Materials

Expression profiles of related fields which publicly online on Gene Expression Omnibus were used in this study. Moreover, statistical analyses were measured using CU-DREAM and CU-DREAM extension.

Methods

Template preparation for aging

The expression profile of aging experiments were collected from Gene Expression Omnibus (GEO Datasets: http://www.ncbi.nlm.nih.gov/gds/) with their related platforms [61, 62] using keywords as “aging” and “senescence”. All expression profiles were available during August 2002 - February 2010. Data contained in related expression profiles were extracted to prepare templates which early passages or young samples were defined as control group and late passages or old samples were experiment group. Threshold parameter were adjusted to 95% confidential (level of significance = 0.05) in each direction.

Template preparation for siRNA

As same as to template preparation for aging, expression profiles published during March 2005 - October 2011which were results of searching by “siRNA”, “shRNA” and “gene knock down” were selected. Supplementary containing series matrix files and related platforms of them were downloaded as previous described. Moreover, samples of “scramble shRNA”, “mock experiment” or “shRNA or siRNA of reporter gene” should be control group whereas samples of “shRNA or siRNA of gene” should be experiment group. Threshold parameter should be set at level of significance equaled 0.01 in each regulation.

Hypomethylated library of replicative senescence

GSE5816[63] and GSE9764[64], expression profile of human bronchial epithelium cell and human mesenchymal stem cell treated with 5’-aza-deoxycytidine respectively, were compared with each expression profile of aging. Significant results from replicative

senescence experiment were combined and utilized as hypomethylated library to test our hypothesis.

Statistical analysis

First, microarray probes were converted to gene symbol. Then, expression level of each gene was tested a difference between control and experiment groups. Using templates, series matrix files and platforms, student's t-test of each gene was calculated to compare mean of control and experiment group. Dependent on a p-value threshold, regulation status of each gene was determined. After that, pearson's chi-squared test were calculated to find an association between two experiments. Result including odd ratio (OR), p-value, lower and upper 95%CI and genes in A-D groups were showed in MS excel format (Figure 2-1). All steps described previously were operated by CU-DREAM and CU-DREAM extension (http://pioneer.netserv.chula.ac.th/~achatcha/cu-dream/)[9].

Data analysis

The results of statistic would be displayed a relationship of related experiment by OR and p-value in MS excel format. After correlation analysis of gene knock down experiment, significant results were harvested at significant level equal 0.01 and separated by direction of regulation and OR respectively. However, non-significant results were grouped together and function of each gene was found out. After that siRNA genes which were statistically correlated with hypomethylated library were compared to genes which significant regulated in each direction of GSE11954[14] and GSE13330[12] also.

CHAPTER IV

RESULTS

Recently, it was found that hypomethylation of *Alu* associated with advancing age, while methylation of LINE1 were increased at certain age. So, hypomethylation should be a relevant process in aging. In this study, we searched for genes which regulated hypomethylation in aging. Firstly, we searched for expression profiles which related to cellular senescence process using keywords of “aging” and “senescence”. From GEO datasets, we found 8 experiments contained available information. They were also separated into 3 classes by their cellular property including non-replicative cell, replicative cell and stem cell. Thus, there were 4 non-replicative aging experiments, 2 replicative aging experiments and 2 stem cell agingexperiments. In addition, two expression profiles of demethylated experiments were collected (Table 4-1).

Table 4-1 Accession number and type of tissue of each experiment

Accession number	Type of tissue
Non-replicative aging experiments	
GSE80	Muscle cell
GSE1572	Brain; postmortem frontal cortex
GSE8764	Parotid gland
GSE15829	Fibroblast cell
Replicative aging experiments	
GSE11954	Hepatic stellate cell
GSE13330	Foreskin fibroblast cell
Stem cell aging experiments	
GSE12274	Mesenchymal stem cell
GSE13496	Hematopoietic stem cell
Demethylation experiments	
GSE5816	Human bronchial epithelium cell treated with 5’-aza-deoxycytidine
GSE9764	Human mesenchymal stem cell treated with 5’-aza-deoxycytidine

Hypomethylation altered gene expression in cellular aging

To prove that hypomethylation could alter gene expression in cellular aging, eight expression profiles of aging were compared with 5'-aza-deoxycytidines treatment in hBEC and hMSC by Student's t-test and Pearson chi-square test using CU-DREAM. The expected consequences should be significantly represented an odd ratio which was greater than 1. Here, there were 48 results derived from the comparison (Table 4-2). The results demonstrated that hypomethylation altered gene expression in cellular aging. Particularly, the comparison of down regulation of replicative aging experiments and down regulation of hypomethylation experiments showed significant results in every comparison (Bold letter).

Table 4-2 OR and P-value of each couple after comparison by CU-DREAM

Tissue/ Experiments*		GSE5816 (hBEC with 100 nm)		GSE5816 (hBEC with 1000 nm)		GSE9764 (hMSC)	
		Up	Dn	Up	Dn	Up	Dn
Non-replicative aging experiments							
Muscle cell	Up	0.810(0.769)	1.255(0.193)	1.128(0.648)	0.728(0.410)	1.260(0.261)	1.375(0.126)
	Dn	0.614(0.492)	1.202(0.236)	0.898(0.670)	0.883(0.691)	1.320(0.117)	0.967(0.872)
Brain	Up	1.123(0.707)	1.247(0.012)	1.009(0.948)	1.456(0.011)	1.279(0.017)	1.188(0.119)
	Dn	1.242(0.500)	1.305(0.004)	0.996(0.978)	0.872(0.474)	1.000(0.998)	1.217(0.098)
Parotid gland	Up	1.754(0.332)	1.697(0.002)	1.254(0.433)	2.272(0.001)	1.258(0.333)	1.474(0.093)
	Dn	1.540(0.458)	1.159(0.421)	1.185(0.541)	1.352(0.311)	1.215(0.389)	1.347(0.186)
Fibroblast cell	Up	0.840(0.862)	0.961(0.872)	$2.685(6.04 \times 10^{-5})$	1.479(0.283)	0.929(0.816)	0.634(0.239)
	Dn	0.569(0.260)	1.025(0.808)	1.159(0.332)	1.033(0.859)	1.229(0.084)	1.052(0.702)

Table 4-2 OR and P-value of each couple after comparison by CU-DREAM (continued)

Tissue/ Experiments*		GSE5816 (hBEC with 100 nm)		GSE5816 (hBEC with 1000 nm)		GSE9764 (hMSC)	
		Up	Dn	Up	Dn	Up	Dn
Replicative aging experiments							
Hepatic stellate cell	Up	1.200(0.577)	1.335(8.70×10^{-4})	1.847(7.66×10^{-8})	1.114(0.500)	2.674(1.80×10^{-31})	0.953(0.709)
	Dn	1.009(0.983)	**3.258(1.42×10^{-49})**	1.065(0.707)	**2.074(4.53×10^{-7})**	1.526(4.49×10^{-4})	**4.116(6.94×10^{-69})**
Foreskin fibroblast cell	Up	1.599(0.003)	1.462(1.78×10^{-17})	1.836(4.78×10^{-21})	1.089(0.299)	1.996(4.16×10^{-39})	1.988(1.53×10^{-35})
	Dn	1.035(0.852)	**2.273(1.09×10^{-79})**	0.931(0.361)	**2.462(5.74×10^{-36})**	1.681(2.80×10^{-20})	**1.776(5.93×10^{-23})**
Stem cell aging experiments							
Mesenchymal stem cell	Up	2.049(4.99×10^{-4})	1.631(3.25×10^{-14})	1.363(0.002)	1.455(9.05×10^{-4})	1.272(0.005)	1.930(3.84×10^{-17})
	Dn	0.000(0.414)	1.163(0.631)	0.980(0.968)	2.060(0.084)	0.960(0.924)	1.270(0.547)
Hematopoietic stem cell	Up	0.451(0.417)	1.175(0.460)	0.643(0.193)	0.917(0.800)	1.802(0.001)	0.930(0.771)
	Dn	1.733(0.130)	1.104(0.420)	1.161(0.411)	0.932(0.762)	1.209(0.192)	1.397(0.021)

*A number outside a parenthesis revealed OR, while an inside one revealed p-value.

Hypomethylated gene related replicative aging

After comparison of cellular aging and hypomethylation, it was found that hypomethylation altered gene expression. Moreover, it significantly correlated to replicative aging. Due to the purpose of this study which finding out gene that cause hypomethylation in aging, genes that their expression changed in this condition were harvested to use as a list of “hypomethylated gene related replicative aging”.

The distribution of hypomethylated gene related replicative aging was discovered when hepatic stellate cell, replicative senescence samples induced by Etoposide treatment, and replicative senescent foreskin fibroblast were compared with hMSC-demethylated experiments in down-down regulation direction. So, the consequence of comparison contained 82 candidate genes which hypomethylated in replicative aging and the candidate list was prepared for finding out regulatory gene by intersection with expression profiles of gene knockdown experiment in the next study (Figure 4-1 and Table 4-3).

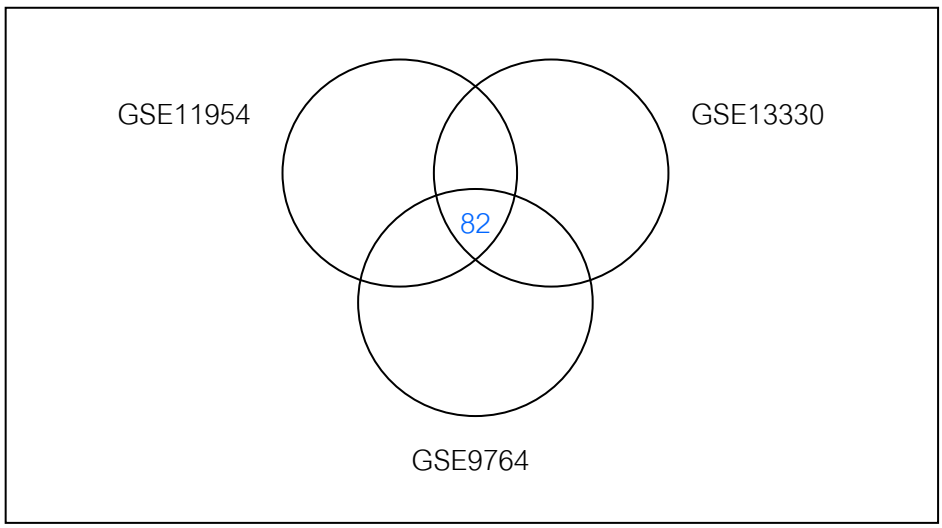


Figure 4-1 Diagram of intersection between 2 replicative senescent experiments (GSE11954 and GSE13330) and demethylated experiment (GSE9764)

Table 4-3 Hypomethylated replicative senescent genes, result of intersection between GSE11954, GSE13330 and GSE9764

	Gene name
Hypomethylated replicative senescence	*ADAMTS12, AK2, AKT3, ALDOC, ARHGAP5, ARNT, ASXL1, BNC2, C18orf10, C1RL, C5orf24, CAND1, CCNY, CDH11, CFL2, CHD2, COL12A1, CTDSPL2, CUGBP2, DIP2C, ENAH, FAM126A, FAM69A, FBLN7, FOXP1, G3BP1, GAB1, GNB4, GOLGA8A, GREM1, HFE, IFT80, IRF2BP2, ITGB5, ITGB8, JAZF1, KIAA1199, KIAA1715, LRRFIP1, MBNL1, MED28, MEIS1, MEIS2, MYLK, N4BP2L2, NEGR1, PALLD, PER3, PHF10, PHF17, PHF20L1, PLAGL1, PPP1R3B, PRKAA2, PSEN1, QKI, RAF1, RBM15, RBM7, RNASE4, RORA, SKI, SNX30, SNX4, SSPN, STAMBP, SYNCRIP, TAGLN, TBL1XR1, TCF4, TEK, TMPO, TNS1, USP34, VCAN, VGLL3, WAC, WSB1, YAP1, ZAK, ZMYM2, ZNF281*

Gene regulation of hypomethylation in replicative aging

The hypomethylated genes related replicative aging were combined to be a list as describing in the previous section. Here, the list and expression profiles of gene knockdown experiments were compared and performed statistical analysis as same as to CU-DREAM called CU-DREAM eXtension to determine whether some genes could be causes of hypomethylation in replicative aging. If there are significantly overlapped regulated genes in the comparison, the genes of represented siRNA maybe play a role in hypomethylation process in aging cells. Additionally, the statistically significant results could be found in many regulations including both down- and up-regulation, down-regulation and up-regulation. Moreover, OR of them were also greater than 1.

According to regulation of direction, there were 49 genes associated with hypomethylated genes related replicative aging both down and up regulation directions. Seventy-four and sixty-three genes associated with hypomethylated genes related replicative aging in down regulation and up regulation respectively. Moreover, the last groups contained 135 genes which did not significant correlate to hypomethylated

genes related replicative aging. Number of experiment, Number of gene and others information of each regulation were displayed in a table below (Table 4-4, Figure4-2).

Table 4-4 Number of experiment and gene with others information of each regulation

Regulation	No. of experiment	No. of gene	No. of cotransfection experiment	No of ncRNA experiment
Down & up > 1	63	49	2	3
Down > 1	106	74	4	6
Up > 1	76	63	3	1
Non-significant	263	135	9	7

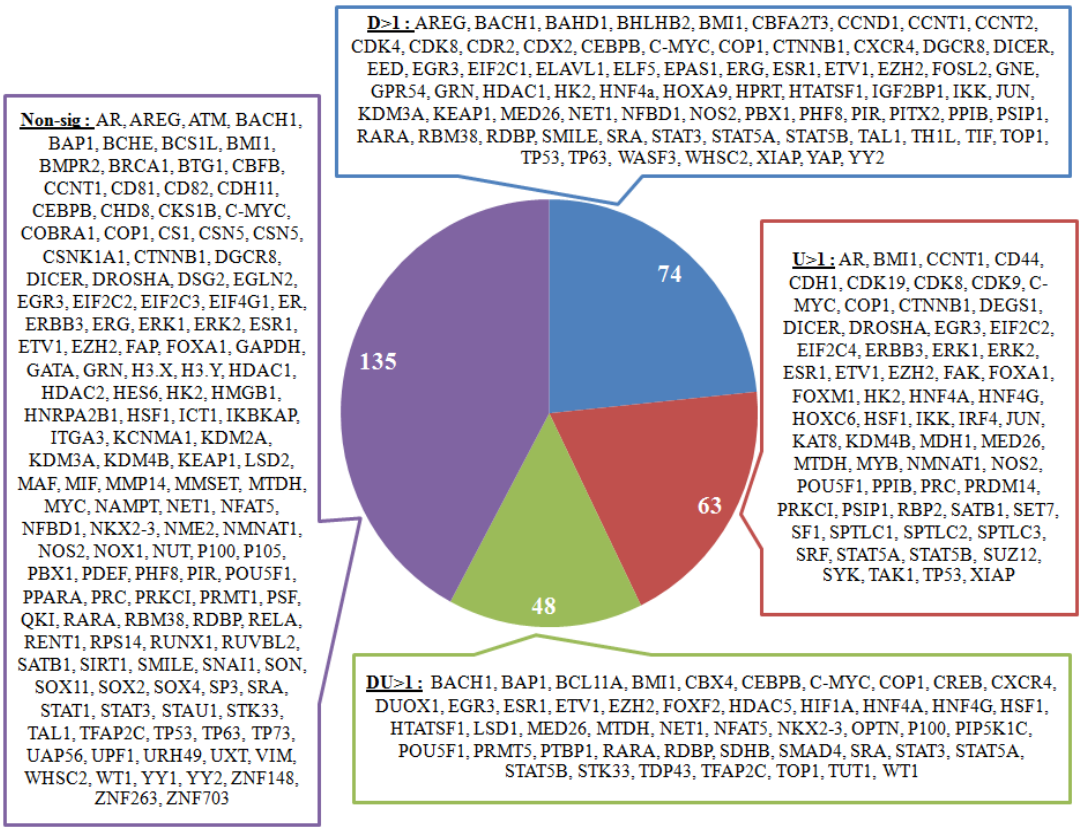


Figure 4-2 Pie chart illustrates siRNA genes in each direction

The significant siRNAs from previous study represented relative function with hypomethylated replicative aging. To examine genes correlated to replicative aging, siRNA genes were compared with expression profiles of replicative senescent cells depending on the directions. Therefore, the consequences were divided to 2 groups depending on their property. The result of down regulated siRNAs and down regulated genes of replicative aging represented 16 genes which function as down regulated gene promoting down regulation of hypomethylation in replicative aging. By contrast, the result of up regulated siRNAs compared with up regulated genes of replicative aging represented 12 genes which function as up regulated gene promoting up regulation of hypomethylation in replicative aging (Figure 4-3 and Table 4-5).

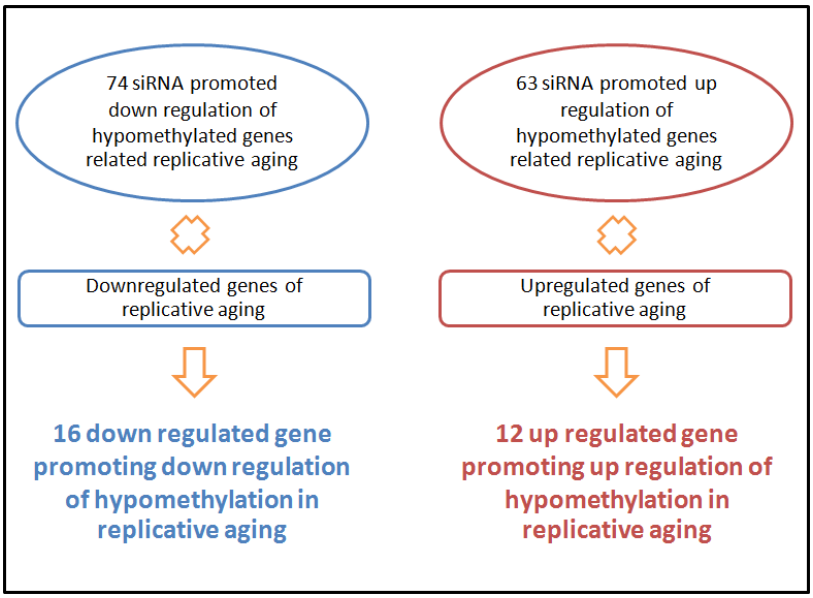


Figure 4-3 A flow chart illustrates intersections between siRNA and replicative aging genes in each direction.

Table 4-5 Genes with others information of each category

Type (Number of genes)	Genes (OR, P-value)
Down regulated gene promoting down regulation of hypomethylation in replicative aging (16)	*BACH1*(9.39, 4.11x10^{-23}), *CCNT2*(5.23, 3.54x10^{-4}), *DGCR8*(4.39, 6.39x10^{-3}), *EGR3*(4.28, 2.14x10^{-3}), *ELAVL1*(3.79, 6.90x10^{-9}), *FOSL2*(2.94, 3.51x10^{-5}), *HDAC1*(2.72, 4.50x10^{-2}), *PBX1*(5.26, 1.71x10^{-3}), *PITX2*(6.32, 4.86x10^{-5}), *PSIP1*(3.06, 1.76x10^{-4}), *RARA*(2.90, 3.06x10^{-2}), *RDBP*(2.50, 8.58x10^{-5}), *STAT5a* + *STAT5b* cotransfected(2.97, 7.69x10^{-4}), *TAL1*(3.05, 5.91x10^{-3}), *TH1L*(3.09, 9.67x10^{-8})
Up regulated gene promoting up regulation of hypomethylation in replicative aging (12)	*BMI1*(3.28, 1.75x10^{-3}), *CCNT1*(3.52, 3.38x10^{-4}), *CD44*(3.65, 7.24x10^{-3}), *DEGS1*(5.24, 8.16x10^{-7}), *ETV1*(3.71, 1.73x10^{-2}), *EZH2*(7.28, 2.39x10^{-2}), *FOXM1*(7.34, 1.03x10^{-4}), *HK2*(3.00, 2.07x10-3), *MTDH*(5.92,9.73x10^{-5}), *NMNAT1*(7.35, 8.05x10^{-6}), *PRKCI*(3.18, 3.91x10-2), *XIAP*(3.49, 9.84x10-3)

Functional analysis of related genes in hypomethylated replicative aging cells

Functional analysis of related genes in hypomethylated replicative aging cells was study to determine if these genes played role in methylation process or replicative aging. The molecular functions, cellular phenotypes and associated diseases were analyzed. The consequences display in a table below (Table 4-6 and table 4-7)

Table 4-6 Molecular functions, cellular phenotypes and associated diseases of down regulated gene promoting down regulation of hypomethylation in replicative aging

Genes	Functions	
	Molecular functions	Phenotypes/Associated Diseases
BACH1[65]	Helicase-like Protein	Interacts with BRCA1 for DNA break repair
CCNT2[66]	Cyclin T2, regulators of CDK kinases	Embryogenesis
DGCR8[67]	Double-stranded-RNA-binding protein	miRNA processing
EGR3[68]	Zinc finger protein	Muscle, brain and lymphocyte function, associated with Schizophrenia
ELAV1[69]	RNA-binding protein	-
FOSL2[70]	FOS-related antigen 2	Osteoclast control
HDAC1[71]	Histone deacetylase 1	Cell proliferation and differentiation
PBX1[72]	Transcription factor	Organ development, Diabetes mellitus
PITX2[73]	Transcription factor	Cell proliferation and differentiation
PSIP1[74]	Transcriptional co-activator and pre-mRNA splicing coordinator	Cellular cofactor for viral DNA integration
RARA[75]	Retinoic acid receptor, alpha	Development, differentiation, apoptosis, granulopoeisis, and transcription of clock genes.
RDBP[76]	RNA-binding protein	Negative elongation factor

Table 4-6 Molecular functions, cellular phenotypes and associated diseases of down regulated gene promoting down regulation of hypomethylation in replicative aging (continued)

Genes	Functions	
	Molecular functions	Phenotypes/Associated Diseases
STAT5A[77]	STAT protein family	Cytokine-mediated biological responses
STAT5B[77]	STAT protein family	Cytokine-mediated biological responses
TAL1[78]	Transcription factor	Cell differentiation
TH1L[76]	RNA-binding protein	Negative elongation factor

Table 4-7 Molecular functions, cellular phenotypes and associated diseases of up regulated gene promoting up regulation of hypomethylation in replicative aging

Genes	Functions	
	Molecular functions	Phenotypes/Associated Diseases
BMI1[79]	Polycomb protein	Hematopoietic differentiation
CCNT1[80]	Cyclin T1	HIV-1 Tat protein
CD44[81]	Cell-surface glycoprotein	Cell–cell interactions
DEGS1[82]	Delta(4)-desaturase, sphingolipid 1	EGFR Biosynthetic
ETV1[83]	Twenty-six family of transcription factor	Proto-oncogene and Androgen receptor-regulated gene
EZH2[84]	Histone methyltransferase, coactivator of transcription factor	Associate with myeloid leukemia and prostate cancer, cell differentiation

Table 4-7 Molecular functions, cellular phenotypes and associated diseases of up regulated gene promoting up regulation of hypomethylation in replicative aging (continued)

Genes	Functions	
	Molecular functions	Phenotypes/Associated Diseases
FOXM1[85]	Transcription factor	Cell cycle protein
HK2[86]	Hexokinase 2	Noninsulin-dependent diabetes mellitus
MTDH[87]	Interact with a variety of proteins	Oncoprotein, Development, Inflammation, Neurodegeneration, Migraine and Huntington disease
NMNAT1[88]	Nicotinamide nucleotide adenylyltransferase1	Axonopathy and Neurodegeneration
PRKCI[89]	Protein kinase C, Lambda/Iota	Glucose-induced insulin secretion
XIAP[90]	Apoptotic suppressor proteins	Cell apoptosis

CHAPTER V
DISCUSSION

Recently, hypomethylation of *Alu* was found associated with advancing age, while methylation of LINE1 was increased at certain age[4]. So, hypomethylation should be a relevant process in aging. In this study, we searched for the association between cellular aging and methylation process. Expression profiles of cellular aging were collected and determined aging types. Depending on proliferation potential of tissue samples in each experiment, there were divided to 3 types. First type was non-replicative cells aging experiments which included the collection of specific age samples including muscle, brain, parotid gland and fibroblast cells. Second type was replicative cells aging experiments. This type contained *in vitro* samples reached aging state after passed serial passages and DNA damaged-induced replicative aging including foreskin fibroblast and hepatic stellate cells respectively. Last type was stem cells aging experiments which the samples were stem cells, mesenchymal stem cells and hematopoietic stem cells (Table 4-1). After that, the selected expression profiles were intersected with demethylation experiments to examine if hypomethylation altered gene expression in cellular aging process.

Firstly, cellular aging profiles were intersected with methylation experiments. By treatment with 5' aza-deoxycytidine, the methylation levels of hBEC and hMSC were declined leading to hypomethylation status of the cells. It was found that dose of 5' aza-deoxycytidine affected gene expression in HBEC cell lead to different directions of gene regulation when compared with hepatic stellate cells. In HBEC cells, the significant results revealed in 6 tissues except muscle cell and hematopoietic stem cells. In addition to HBEC, the intersection results of hMSC treated with 5'aza-deoxycytidine and cellular aging was significant in brain, hepatic stellate cells, foreskin fibroblast, mesenchymal stem cells and hematopoietic stem cells (Table 4-2). Comparison between HBEC and hMSC demonstrated dose-dependent and cell type specific influenced gene expression in aging.

Interestingly, it was found that the results which showed the associations of cellular aging experiment and hypomethylation experiment markedly significant differed

between each type of cellular aging. Hypomethylation profiles demonstrated similar associations when compared with foreskin fibroblast profile and hepatic stellate cells profile, members of group of replicative aging, in down-down regulation. While hypomethylation process associated with replicative aging, other types of aging found positive results in some profiles of tissue sample such as brain, parotid gland, mesenchymal stem cells etc (Table 4-2). Besides, they did not showed any pattern in their groups comparable to replicative aging profiles. It should be proposed that hypomethylation was an evidence launch particularly in replicative aging.

Due to similar pattern of hypomethylation of replicative aging, 82 genes containing hypomethylation status were extracted from the intersection between hepatic stellate cells, foreskin fibroblast and hMSC treated with 5'aza-deoxycytidine (Figure 4-1). These 82 genes were used as a library of hypomethylated genes related replicative aging (Table 4-3). This library was intersected with gene knockdown experiments to illustrate genes which regulated hypomethylation in replicative aging.

The consequences of intersection between hypomethylated genes related replicative aging and gene knockdown experiments were divided to 4 groups by direction of regulation. The significant results comprised of 3 groups, down and up regulation, down regulation and up regulation, which displayed odd ratio more than 1. There were 49 genes associated with hypomethylated genes related replicative aging both down and up regulation directions. Seventy-four and sixty-three genes associated with hypomethylated genes related replicative aging in down regulation and up regulation respectively. Moreover, the last groups contained 135 genes which did not significant correlate to hypomethylated genes related replicative aging (Table 4-4).

Here, we propose a model of genes which should be a regulator of hypomethylation in replicative aging. Gene A should be defined as "Replicative aging gene" which plays roles in replicative aging without effects of methylation. Gene B should be defined as "Hypomethylated gene" which illustrates hypomethylation status in replicative aging and it is regulated by gene A. Thus, gene B will be 82 hypomethylated gene related replicative aging and regulate other downstream gene (Gene C) leading to cellular phenotypes of replicative aging expression (Figure 5-1).

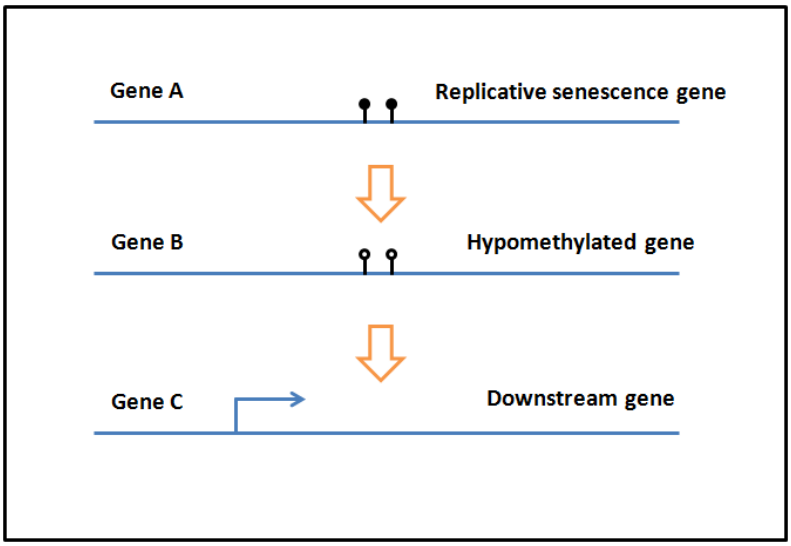


Figure 5-1 Schematic of a model describing gene regulation process in replicative aging

According to the proposed model, siRNA genes which showed significant results with hypomethylated gene related replicative aging in either down regulation or up regulation were considered. Seventy-six siRNA genes associated with hypomethylated genes related replicative aging in down regulation. The presence of these siRNAs in the cell should be called “siRNA promoted down regulation of hypomethylated genes related replicative aging”. By contrast, 63 siRNA genes associated with hypomethylated genes related replicative aging in up regulation. The presence of these siRNAs in the cells should be called “siRNA promoted up regulation of hypomethylated genes related replicative aging”.

Following the previous model, genes represented significant siRNA which would be determined as Replicative aging gene (gene A) should be found in replicative aging profiles in the same regulation direction. As a previous condition, the flow chart was suggested to illustrate a comparison between siRNA and replicative aging genes in each direction (Figure 4-3). In down regulation direction, the presence of siRNA promoted down regulation of hypomethylated genes related replicative aging or “down regulated gene promoting down regulation of hypomethylation in replicative aging” in the cells promoted aging phenotypes. Therefore, the cells should be prevented from aging by the expression of down regulated gene promoting down regulation of

hypomethylation in replicative aging of this group called “Anti-aging gene” which remained 16 from 76 genes after comparison. Additionally, the consequence of comparison of up regulation direction of repliactive aging profile and up regulated siRNA or “up regulated gene promoting up regulation of hypomethylation in replicative aging” consisted of 12 genes from 63 genes. In this case, siRNA promoted up regulation of hypomethylated genes related replicative aging prevented aging phenotypes. Thus, the expression of 12 genes should promote aging process and they were defined as “Aging gene” (Table 4-5). Furthermore, the functions of aging and anti-aging genes were studied.

Function analysis of each gene was reviewed included molecular function, cellular phenotype and associated diseases. Molecular functions of 16 aging genes and 12 anti-aging genes were determined as transcription factor, RNA-binding protein, histone modification enzyme and other cytoplasmic proteins. Cellular phenotypes of them associated with cell proliferation, differentiation and cell apoptosis. Moreover, age-associated disease should be inflammation, viral-associated disease, schizophrenia, neurodegenerative disease, cancer and diabetes mellitus (Table 4-6 and 4-7).

Surprisingly, among 28 genes associated hypomethylated in replicative senescence, some genes showed function particularly correlated to methylation process via DNMT stabilization and colocalization such as *HDAC1, ELAV1* and *EZH2*. HDAC1 is a histone deacetylase which found colocalization with DNMT3a[91], while ELAV1, a RNA-binding protein, related to DNMT3b mRNA stabilization[92]. EZH2, a histone methyltransferase, was reported that EZH2 binded to 3 types of DNMT, DNMT1, DNMT3a and DNMT3b. Thus, EZH2 should be a platform of DNMT recruitment to regulate methylation process in EZH2-targeted genes[93, 94]. Therefore, it should be implied that HDAC1 and ELAV1 maybe affect to de novo methylation process in replicative senescence, while EZH2 may needed in function of DNMTs during methylation process.

In conclusion, hypomethylation should be a common process which was found during replicative aging. There were many genes associated could be affect to replicative aging as aging gene or anti-aging gene. These genes functioned as

transcription factor, RNA-binding protein, histone modification enzyme and other cytoplasmic proteins led to many cellular phenotypes alteration. Some aging genes or anti-aging genes related to age-associated diseases such as inflammation, neurodegenerative diseases, cancer and diabetes. Furthermore, several genes such as *HDAC1, ELAV1* and *EZH2* related to methylation process and it could putatively regulated methylation status in replicative aging.

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APPENDICES

Appendix A

OR and P-value of intersections between aging and other experiments

Tissue/ Experiments*		Ago2 knockdown		Dicer knockdown		TSA treated	
		Up	Dn	Up	Dn	Up	Dn
Muscle	Up	1.200(0.403)	0.579(0.068)	1.840(0.022)	0.825(0.459)	0.471(0.189)	1.086(0.822)
	Dn	0.851(0.449)	1.664(0.005)	0.597(0.153)	1.329(0.131)	1.719(0.053)	1.074(0.820)
Brain	Up	1.174(0.151)	1.081(0.519)	1.000(0.998)	0.894(0.367)	0.911(0.662)	1.086(0.654)
	Dn	0.950(0.691)	1.139(0.315)	0.963(0.842)	1.153(0.260)	1.217(0.336)	0.785(0.272)
Parotid gland	Up	1.364(0.185)	0.995(0.985)	1.398(0.311)	1.145(0.587)	0.262(0.153)	2.101(0.032)
	Dn	1.200(0.490)	1.277(0.366)	1.061(0.879)	1.057(0.838)	1.434(0.434)	0.666(0.488)
Fibroblast	Up	1.593(0.162)	1.264(0.539)	1.465(0.413)	1.508(0.215)	0.000(0.138)	0.373(0.310)
	Dn	1.245(0.073)	1.169(0.236)	0.964(0.848)	0.951(0.705)	2.000(6.05×10^{-4})	0.956(0.856)

*A number outside a parenthesis revealed OR, while an inside one revealed p-value.

OR and P-value of intersections between aging and other experiments (continued)

Tissue/ Experiments*		Ago2 knockdown		Dicer knockdown		TSA treated	
		Up	Dn	Up	Dn	Up	Dn
Hepatic stellate cells	Up	1.166(0.135)	1.516(2.64×10^{-5})	0.739(0.082)	1.725(1.93×10^{-9})	1.543(0.035)	1.196(0.383)
	Dn	1.678(2.20×10^{-6})	1.182(0.189)	1.757(1.49×10^{-4})	0.804(0.115)	0.771(0.403)	1.694(0.009)
Foreskin fibroblast cells	Up	1.192(0.004)	1.426(1.34×10^{-8})	0.870(0.124)	1.422(2.44×10^{-9})	1.410(0.006)	1.429(0.001)
	Dn	1.399(7.58×10^{-8})	0.144(0.144)	1.788(8.04×10^{-12})	0.796(7.66×10^{-4})	1.082(0.560)	1.002(0.988)
Mesenchymal stem cells	Up	1.468(4.98×10^{-5})	1.138(0.227)	1.957(1.88×10^{-8})	0.823(0.082)	1.371(0.089)	0.713(0.113)
	Dn	0.000(0.093)	1.409(0.583)	3.011(0.065)	0.777(0.735)	0.752(0.778)	1.227(0.779)
Hematopoietic stem cells	Up	0.854(0.492)	1.321(0.179)	0.528(0.120)	1.485(0.039)	1.318(0.451)	1.036(0.923)
	Dn	1.279(0.089)	0.886(0.486)	1.567(0.018)	0.822(0.240)	1.418(0.183)	1.109(0.694)

*A number outside a parenthesis revealed OR, while an inside one revealed p-value.

OR and P-value of intersections between aging and other experiments (continued)

Tissue/ Experiments*		LINE1	Intronic Alu	Tissue/ experiments*		LINE1	Intronic Alu
Muscle	Up	0.611(0.128)	0.963(0.796)	Hepatic stellate cells	Up	0.874(0.339)	$1.609(1.20\times10^{-12})$
	Dn	0.916(0.717)	1.412(0.010)		Dn	$1.841(7.45\times10^{-7})$	$2.134(6.98\times10^{-21})$
Brain	Up	1.351(0.012)	1.206(0.011)	Foreskin fibroblast cells	Up	$1.355(1.27\times10^{-6})$	$1.492(4.09\times10^{-33})$
	Dn	1.224(0.128)	1.094(0.252)		Dn	1.140(0.056)	$1.528(1.57\times10^{-33})$
Parotid gland	Up	$2.328(2.20\times10^{-5})$	1.334(0.037)	Mesenchymal stem cells	Up	$1.658(5.61\times10^{-9})$	$1.268(4.20\times10^{-6})$
	Dn	1.634(0.025)	1.176(0.216)		Dn	1.698(0.154)	1.079(0.741)
Fibroblast	Up	$2.865(9.45\times10^{-7})$	1.022(0.894)	Hematopoietic stem cells	Up	0.687(0.224)	1.205(0.187)
	Dn	0.775(0.098)	1.068(0.327)		Dn	0.500(0.004)	1.023(0.814)

*A number outside a parenthesis revealed OR, while an inside one revealed p-value.

APPENDIX B

Odd ratios and P-values of gene knock down experiments

Down regulated siRNA

Accession Number	Genes	Down 0.01	
		OR	P-value
GSE28786	STAT3	13.21	1.88E-10
GSE20081	SRA	9.41	1.69E-29
GSE28053	BACH1	9.39	4.11E-23
GSE11618	XIAP	8.31	3.90E-09
GSE7700	YAP	7.41	3.45E-20
GSE7700	YAP	7.36	4.79E-09
GSE16962	miR-210	7.21	1.40E-21
GSE19114	STAT3 + CEBPb	7.21	2.22E-16
GSE26309	NET1	7.10	1.28E-05
GSE27275	PITX2	6.32	4.86E-05
GSE28786	HK2	6.09	4.49E-03
GSE10890	ESR1	6.04	1.13E-05
GSE21912	BMI1	5.71	8.33E-12
GSE28786	STAT3	5.51	1.26E-03
GSE21912	BMI1	5.31	2.63E-10
GSE25458	GPR54	5.31	3.03E-04
GSE28008	PBX1	5.26	1.71E-03
GSE20115	CBFA2T3	5.25	6.93E-05
GSE23973	lincRNA-ST8SIA3 targeting siRNA1	5.25	3.39E-04
GSE24182	lincRNA-ST8SIA3 targeting siRNA4	5.25	3.39E-04
GSE28339	Cyclin T2	5.23	3.54E-04
GSE8866	CCND1	5.23	1.30E-09
GSE7161	TOP1	5.17	2.67E-07
GSE26462	WASF3	5.14	4.74E-06
GSE29750	iNOS	5.14	2.10E-03

Accession Number	Genes	Down 0.01	
		OR	P-value
GSE19114	BHLHB2	5.10	2.14E-05
GSE21886	SMILE	5.03	2.66E-07
GSE19114	CEBPb	5.00	1.05E-12
GSE14801	ERG	4.98	3.06E-05
GSE5993	TP63	4.81	1.05E-07
GSE13763	CXCR4	4.63	7.91E-10
GSE17551	Pirin	4.58	2.69E-05
GSE23010	NFBD1	4.55	1.29E-03
GSE22363	ELF5	4.52	1.88E-09
GSE4246	DICER	4.42	2.07E-08
GSE13639	DGCR8	4.39	6.39E-03
GSE13640	Dicer	4.39	6.39E-03
GSE19114	STAT3	4.38	2.81E-07
GSE15991	HNF4	4.31	2.12E-09
GSE18913	EGR-3	4.28	2.14E-03
GSE8866	CDK4	4.13	8.67E-08
GSE31397	miR-101	4.08	3.57E-04
GSE18913	EGR-3	4.04	1.07E-02
GSE28448	TIF	3.91	4.42E-03
GSE32301	RBM38	3.88	1.34E-02
GSE19114	STAT3 + CEBPb	3.85	2.58E-04
GSE20115	CBFA2T3+IKK	3.84	1.80E-03
GSE20037	Cdr2	3.81	7.51E-04
GSE21575	IGF2BP1	3.81	1.32E-04
GSE23010	NFBD1 + TP53	3.79	1.55E-02
GSE29778	HuR	3.79	6.90E-09
GSE28786	STAT3	3.78	4.79E-02

Accession Number	Genes	Down 0.01	
		OR	P-value
GSE31772	GRN	3.78	8.71E-04
GSE32108	CDK8	3.66	4.69E-04
GSE6015	EED	3.66	7.54E-03
GSE14964	YY2	3.61	7.75E-03
GSE25458	GPR54	3.56	2.19E-02
GSE18956	Bcatenin	3.49	8.11E-04
GSE22572	CDX2	3.46	2.55E-02
GSE16097	BAHD1	3.45	2.00E-03
GSE13714	HOXA9	3.39	1.60E-07
GSE5823	C-MYC	3.39	1.18E-02
GSE30494	KDM3A	3.31	3.20E-02
GSE28448	TIF	3.30	3.26E-02
GSE23010	TP53	3.24	3.56E-03
GSE10234	Cyclin T1	3.23	3.62E-02
GSE19940	NELF-A	3.18	4.21E-05
GSE22336	GNE	3.18	8.45E-03
GSE29084	HNF4a	3.16	1.85E-02
GSE28786	HK2	3.13	4.00E-04
GSE28786	HK2	3.10	8.06E-04
GSE19940	NELF-C	3.09	9.67E-08
GSE3485	PSIP1	3.06	1.76E-04
GSE20546	TAL1	3.05	5.91E-03
GSE6015	EZH2	3.02	2.93E-04
GSE17482	STAT5a/b	2.97	7.69E-04
GSE19114	STAT3 + CEBPb	2.96	1.81E-04
GSE19114	FOSL2	2.94	3.51E-05
GSE16622	HIF2 alpha	2.90	8.89E-03

Accession Number	Genes	Down 0.01	
		OR	P-value
GSE22213	RARA	2.90	3.06E-02
GSE15505	CypB	2.84	1.06E-02
GSE22363	ELF5	2.84	1.49E-04
GSE28715	MED26	2.78	1.61E-03
GSE26079	AREG	2.74	1.35E-02
GSE22366	HDAC1	2.72	4.50E-02
GSE20753	PHF8	2.70	6.73E-04
GSE28053	BACH1	2.70	3.49E-06
GSE20381	EZH2	2.66	4.79E-02
GSE26298	ER	2.64	2.99E-02
GSE21955	COP1	2.63	7.53E-03
GSE28813	KEAP1	2.51	1.66E-02
GSE19940	NELF-E	2.50	8.58E-05
GSE24345	HPRT	2.50	1.13E-02
GSE13714	HOXA9	2.33	6.47E-04
GSE30670	EZH2	2.28	2.34E-02
GSE22937	ESRP	2.24	2.44E-02
GSE24522	miR16	2.24	2.66E-02
GSE19927	Tat-SF1	2.23	1.66E-03
GSE24522	miR16	2.23	3.82E-02
GSE4246	AGO1	2.21	1.85E-02
GSE27914	COP1	2.12	2.98E-02
GSE27914	COP1 + ETV1 + JUN	2.01	1.54E-02

Up regulated siRNA

Accession Number	Genes	Up 0.01	
		OR	P-value
GSE11428	AR	15.62	6.79E-15
GSE24592	ERK2	11.46	1.38E-10
GSE6015	SUZ12	8.08	8.64E-11
GSE18913	EGR3	7.60	3.18E-07
GSE13458	NMNAT1	7.35	8.05E-06
GSE2222	FOXM1	7.34	1.03E-04
GSE15890	EZH2	7.28	2.39E-02
GSE30622	FOXA1	6.65	2.25E-05
GSE26529	SF1	6.46	4.34E-06
GSE22085	MALAT	6.25	5.19E-05
GSE26134	MTDH	5.92	9.73E-05
GSE20193	MOF	5.91	7.18E-13
GSE19670	TP53i	5.86	1.07E-04
GSE22139	cMYC	5.47	7.48E-18
GSE4246	AGO2	5.38	3.09E-04
GSE11581	FAK	5.31	3.03E-04
GSE28059	DEGS1	5.24	8.16E-07
GSE23010	TP53	5.01	2.53E-03
GSE9182	HOXC6	4.98	1.42E-02
GSE18973	HNF4a(+HNF4g)	4.97	3.47E-11
GSE30361	SET7	4.93	2.83E-03
GSE27473	ESR1	4.82	4.42E-15
GSE16200	SYK	4.68	4.45E-03
GSE4246	AGO4	4.42	5.39E-04
GSE13639	Drosha	4.29	7.42E-03
GSE13640	AGO2	4.29	7.42E-03

Accession Number	Genes	Up 0.01	
		OR	P-value
GSE19921	ERBB3	4.19	8.56E-03
GSE5417	SATB1	4.04	3.69E-02
GSE16219	TAK1	4.03	3.49E-03
GSE29750	iNOS	3.94	1.24E-02
GSE21135	OCT4	3.92	1.25E-06
GSE28059	SPTLC123	3.79	5.56E-05
GSE15723	PTD-DRBD	3.78	4.74E-02
GSE18973	HNF4a+HNF4g	3.72	4.77E-07
GSE19396	ETV1	3.71	1.73E-02
GSE21371	MYB	3.70	1.82E-05
GSE16568	miR-22	3.69	1.03E-03
GSE32108	CDK8	3.67	2.01E-04
GSE20914	CD44	3.65	7.24E-03
GSE10233	Cyclin T1	3.52	3.38E-04
GSE28715	MED26	3.50	4.22E-05
GSE11618	XIAP	3.49	9.84E-03
GSE4246	DICER	3.48	4.68E-03
GSE12320	POU5F1	3.43	1.10E-02
GSE10738	Cyclin T1	3.37	2.91E-02
GSE22036	IRF4	3.30	3.26E-02
GSE6015	BMI1	3.28	1.75E-03
GSE32108	CDK19	3.27	2.14E-04
GSE15417	ERK1 + ERK2	3.22	2.83E-05
GSE25990	CIITA-BX648577	3.22	3.64E-02
GSE20115	IKK	3.21	3.70E-02
GSE23445	JMJD2B	3.19	1.07E-03
GSE26241	PKCiz	3.18	3.91E-02

Accession Number	Genes	Up 0.01	
		OR	P-value
GSE32108	CDK19	3.18	5.87E-04
GSE12762	HSF1	3.13	1.95E-02
GSE9187	MTDH	3.13	2.33E-06
GSE22606	SRF	3.03	1.23E-04
GSE22795	PRDM14	3.01	2.21E-04
GSE28786	HK2	3.00	2.07E-03
GSE17482	STAT5a/b	2.98	4.37E-04
GSE21751	CDK9	2.88	3.11E-03
GSE27914	COP1 + JUN	2.88	1.77E-04
GSE7508	PSIP1	2.78	3.80E-02
GSE24592	ERK2	2.72	4.52E-02
GSE16200	SYK	2.68	2.86E-02
GSE22606	SRF	2.67	2.56E-04
GSE9690	RBP2	2.67	4.86E-02
GSE15505	CypB	2.47	4.33E-02
GSE14428	PRC	2.30	3.11E-02
GSE9691	Ecadherin	2.23	8.42E-03
GSE3697	HSF1	1.97	6.08E-03
GSE9691	Ecadherin+Bcatenin	1.93	4.48E-02

Down and Up regulated siRNA

Accession Number	Genes	Down 0.01		Up 0.01	
		OR	P-value	OR	P-value
GSE10289	SDHB	2.07	1.79E-02	4.18	5.54E-10
GSE10890	ESR1	2.42	2.18E-02	3.25	1.28E-04
GSE12056	CREB	2.06	2.36E-02	3.48	1.12E-07
GSE12320	POU5F1	6.33	1.18E-07	4.06	3.28E-03
GSE12444	FOXF2	4.49	5.47E-03	6.06	1.65E-06
GSE12886	WT1	2.19	4.33E-02	3.14	2.10E-04
GSE13284	BCL11A	3.28	2.06E-04	3.36	1.26E-02
GSE13763	CXCR4	3.67	6.60E-07	2.65	2.84E-03
GSE15151	STK33	3.51	2.36E-02	4.10	9.75E-03
GSE15481	AP-2 gamma	2.34	3.96E-02	3.11	4.34E-02
GSE15499	HDAC5	3.45	1.05E-02	2.82	1.09E-02
GSE15792	PCARNEQ	1.86	3.16E-02	2.93	3.76E-05
GSE15991	HNF4	3.21	1.33E-05	1.79	3.66E-02
GSE17482	STAT3	3.24	2.42E-05	4.20	1.26E-07
GSE17482	STAT3	3.39	3.64E-05	3.67	4.55E-06
GSE17483	STAT5a/b	3.24	2.42E-05	4.20	1.26E-07
GSE18632	TDP43	3.05	7.65E-05	2.38	8.40E-03
GSE18913	EGR-3	3.83	5.09E-03	3.21	3.70E-02
GSE19114	STAT3 + CEBPb	3.40	1.15E-02	3.13	1.93E-02
GSE19114	CEBPb	3.72	3.35E-05	3.10	7.84E-04
GSE19114	STAT3	3.92	1.42E-05	2.43	3.09E-02
GSE19680	miR-125b	4.58	4.80E-03	3.18	3.86E-02
GSE19927	Tat-SF1	2.28	1.56E-03	3.22	8.23E-05
GSE19927	GFP	2.55	2.27E-02	2.92	1.54E-03
GSE19940	NELF-E	2.84	2.24E-06	1.74	4.42E-02
GSE20081	SRA	3.28	6.72E-03	2.63	3.03E-02

Accession Number	Genes	Down 0.01		Up 0.01	
		OR	P-value	OR	P-value
GSE20706	DUOX1	2.30	3.83E-03	1.84	4.83E-02
GSE21912	BMI1	3.63	5.80E-06	2.56	5.93E-03
GSE21912	BMI1	3.54	4.90E-06	2.85	4.88E-04
GSE21912	BMI1	3.53	3.55E-05	2.43	9.57E-03
GSE21912	BMI1	3.05	9.47E-04	2.89	1.74E-03
GSE21912	BMI1	3.94	1.14E-06	2.34	1.34E-02
GSE22852	ETV1	4.12	2.88E-03	3.19	3.82E-02
GSE22956	NFAT5	3.83	2.04E-05	2.39	2.37E-02
GSE23035	BAP1	3.68	1.91E-07	3.19	1.18E-05
GSE23035	BAP1	3.38	3.84E-05	2.70	4.61E-04
GSE23513	PTB	4.18	1.31E-10	1.79	3.00E-02
GSE23522	PTB	3.60	8.73E-09	1.76	4.02E-02
GSE23522	PTB	4.18	1.31E-10	1.79	3.00E-02
GSE23973	lincRNA-ST8SIA3 targeting siRNA4	4.35	1.88E-03	3.69	2.56E-03
GSE24020	P100	3.04	1.16E-02	2.62	3.07E-02
GSE24182	lincRNA-ST8SIA3 targeting siRNA1	4.35	1.88E-03	3.69	2.56E-03
GSE25725	PRMT5	3.95	1.22E-02	4.62	1.14E-03
GSE26298	RARalpha	4.65	7.65E-05	3.03	1.20E-02
GSE26309	NET1	4.08	1.07E-02	5.48	1.40E-03
GSE27914	COP1 + ETV1	3.10	7.98E-04	3.76	6.25E-05
GSE28053	BACH1-e	2.18	1.18E-03	1.99	1.22E-02
GSE28053	BACH1	3.62	1.07E-04	2.57	2.66E-03
GSE28053	BACH1-e	2.52	2.14E-04	2.37	1.60E-04
GSE28448	Smad4	5.79	1.98E-05	5.43	1.39E-03
GSE28656	NKX2-3	4.14	9.04E-03	3.96	1.19E-02

Accession Number	Genes	Down 0.01		Up 0.01	
		OR	P-value	OR	P-value
GSE28715	MED26	2.41	2.22E-02	5.38	8.35E-13
GSE30775	LSD1	2.45	3.21E-03	2.85	2.00E-04
GSE31093	Cbx4	2.18	3.49E-03	3.04	1.02E-06
GSE3697	HSF1	2.00	5.05E-03	1.70	3.84E-02
GSE5823	C-MYC	3.28	3.33E-02	3.61	2.03E-02
GSE6819	Optineurin	3.34	3.05E-02	3.00	2.51E-02
GSE7161	TOP1	2.25	8.04E-03	4.11	9.78E-08
GSE7835	HIF1 alpha	6.49	1.30E-15	1.99	2.05E-02
GSE8145	EZH2	2.19	2.51E-02	2.79	7.76E-03
GSE9187	MTDH	2.04	1.09E-02	2.27	2.41E-02
GSE9361	PIPKIa	2.57	8.84E-04	2.96	3.11E-05
GSE9361	StarPap	2.37	4.54E-03	4.18	1.87E-10

BIOGRAPHY

Miss Wachiraporn Wanichnopparat was born in Nakhonsawan in 1987. In 2008, she graduated from Department of Biology, Faculty of Science, Chiang Mai University in Genetics program and then attended to particulate in Medical Science program in Faculty of Medicine for her master degree. She plans to study in Ph.D. program.

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