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Expression profiles during dedifferentiation in newt lens regeneration revealed by expressed sequence tags

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Purpose: The adult newt can regenerate lens from pigmented epithelial cells (PECs) of the dorsal iris via dedifferentiation. The purpose of this research is to obtain sequence resources for a newt lens regeneration study and to obtain insights of dedifferentiation at the molecular level.
Methods: mRNA was purified from iris during dedifferentiation and its cDNA library was constructed. From the cDNA library 10,449 clones were sequenced and analyzed.
Results: From 10,449 reads, 780 contigs and 1,666 singlets were annotated. The presence of several cancer- and apoptosis-related genes during newt dedifferentiation was revealed. Moreover, several candidate genes, which might participate in reprogramming during dedifferentiation, were also found.
Conclusions: The expression of cancer- and apoptosis-related genes could be hallmarks during dedifferentiation. The expression sequence tag (EST) resource is useful for the future study of newt dedifferentiation, and the sequence information is available in GenBank (accession numbers; FS290155-FS300559).

Some species are endowed with unique physiology and provide new paradigms for both basic and applied biology. For example, adult newts can regenerate body parts, including lens, limb, tail, jaw, small intestine, brain, and heart. This regenerative ability is the highest even among amphibians, including axolotls and frogs [1,2]. It is well known that lens regeneration is mediated by dedifferentiation and transdifferentiation of terminally differentiated pigmented epithelial cells (PECs). After lens removal, PECs in dorsal irises undergo dedifferentiation where PECs exclude pigment granules and lose their cellular identity, proliferate, and then differentiate into lens cells. Although lens regeneration never occurs from the ventral iris, dedifferentiation events, depigmentation, proliferation, and gene expression are observed in ventral iris PECs [2]. Transdifferentiation of PECs has been directly demonstrated by clonal culture experiments [3,4].

For cells to change their identity and assume a new fate, a considerable degree of gene regulation must take place. It has been shown that nucleostemin, a stem cell-specific nucleolar protein found in mammals [5], accumulates in nucleoli as PECs dedifferentiate during lens regeneration [6]. We have also recently reported that mammalian stem cell pluripotency-maintaining factors cellular myelocytomatosis oncogene, sex determining region Y box 2, and kruppel-like factor 4 are expressed and regulated during lens regeneration as well [7]. These data suggest that a PEC is reprogrammed to a stem cell-like cell during dedifferentiation. However, information about molecular events during dedifferentiation is not well elucidated.

To understand the process of dedifferentiation, analysis of global gene expression during dedifferentiation is needed. Although more than 34,000 cDNA sequences for the axolotl *Ambystoma mexicanum*, 677,000 cDNAs for the African clawed frog *Xenopus laevis*, and 1,271,000 cDNAs for the western clawed frog *Xenopus tropicalis* are available [8-13], cDNA resources are lacking in the newt field. Here, we generated expression sequence tags (ESTs; 1,368 contigs and 3,357 singlets) from the iris undergoing dedifferentiation during the process of lens regeneration and analyzed their expression profiles.

METHODS

Animals: One hundred Japanese newts, *Cynops pyrrhogaster*, were collected in the northern part of Okayama prefecture. All animal procedures were approved by animal care board in Center for Developmental Biology, Riken Kobe. Newts were euthanized by anesthesia (soaking in 0.1% of MS-222 [Sigma-Aldrich, Tokyo, Japan] for 15 min) followed by decapitation.

mRNA extraction: Both dorsal and ventral irises 8 days after lentectomy (when dedifferentiation events of PECs are

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Table 1. Process of lens-regenerating iris ests assembly into contigs.

Assembly	Number of sequences	Number of contigs	Number of singlets	Average contig length (bp)	Average singlet length (bp)	Total length of sequences (Mbp)
Initial reads after Phred analysis	10,449				943	9.85
After trimming 20 bases from ends and vector screening	10,405				899	9.35
After cap3 assembly	4,725	1,368	3,357	1,279	915	4.82

ongoing, i.e., initiation of depigmentation and proliferation [6]) were collected for the cDNA library of lens-regenerating iris. mRNA was purified using Dynabeads Oligo(dT)$_{25}$ (Dynal Biotech, Oslo, Norway) according to the manufacturer's instructions. Briefly, Dynabeads Oligo (dT) was added to homogenized iris sample. mRNA hybridized to Dynabeads Oligo(dT) was isolated by magnetic separation, washed, and eluted by incubation with 10 mM Tris (2-amino-2-hydroxymethylpropane-1,3-diol)-HCl buffer, pH 7.5 at 75 ºC for 2 min.

Construction of the cDNA library: The cDNA library was constructed using the ZAP-cDNA synthesis kit (Stratagene Japan, Kunitachi, Japan). Reverse transcription reaction was performed using oligo-dT primer, and the synthesized cDNAs were directionally inserted into a Uni-ZAP XR Vector (Stratagene Japan). The vector-containing cDNA was packaged in a lambda phage, using a Gigapack III gold cloning kit (Stratagene Japan). pBluescript phagemid was prepared by in vivo excision using ExAssist helper phage (Stratagene Japan) and XLI-Blue MRF strain (Stratagene Japan). XLI-Blue MRF strain cells were transformed with the phagemid and plated on L-Broth-ampicilin plate.

Sequencing of cDNA: Each colony was picked using an automated colony picker Qpix (Genetix K.K., Toyo, Japan), and template DNA for sequencing was amplified using the TempliPhi DNA sequencing template amplification kit (GE Healthcare Life Sciences, Piscataway, NJ). The sequencing reaction was performed using the BigDye terminator v. 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA). The primer used in the sequence reaction was the T3 primer (Stratagene Japan). The sequence reaction products were analyzed using a 3730xl DNA analyzer (Applied Biosystems). The GenBank accession numbers for ESTs are FS290155-FS300559.

Assembly of sequence data: To remove possible low-quality fragments at both edges of individual reads, 20 bases were trimmed off each end of all sequences. Then, potential vector contamination was removed or masked. Individual reads from all tissues were assembled using CAP3 software with default settings [14].

Functional annotation: Functional annotation of the assembled sequences was performed using the Blast2GO program with default settings [15]. Putative "gene names" and functions are also assigned to sequences with GO annotations as part of the annotation process. The annotations were augmented by the Annotation Expander (ANNEX) software [16].

RESULTS AND DISCUSSION

Sequencing, assembly, and functional annotation of newt expression sequence tags: As mentioned previously dedifferentiation events occur in the ventral iris as well as the dorsal iris. Therefore dorsal and ventral irises 8 days after lentectomy, when dedifferentiation of PECs is ongoing, were collected, cDNA libraries were constructed using mRNA from the regenerating iris, and ESTs from the iris libraries were obtained. The initial set of cDNA sequence reads were 10,449 (Table 1). The total length of these sequences was 9.85 Mb, which was reduced to 4.82 Mb by assembling overlapping reads, using CAP3 software. This assembly was composed of 4,725 unique sequences (1,368 contigs and 3,357 singlets). Of 1,219 contigs and 3,357 singlets, 780 contigs and 1,666 singlets were annotated by Blast2GO.

Expression profiles of iris ongoing dedifferentiation: Functional annotation of the contigs and singlets was performed using the Blast2GO program. The 15 most frequently counted biologic process terms, cellular component terms, and molecular function terms are shown in Figure 1.

Tyrosinase, a possible marker for differentiated PECs, was not found in the EST. No detection of tyrosinase in this EST might reflect changing of the original PEC gene expression 8 days after lentectomy. Nucleostemin, a nucleolar protein mainly expressed in stem cell populations in mammals [5], was found in the ESTs (Table 2). This result was consistent with a previous report in which nucleostemin emerged in nucleoli during newt dedifferentiation [6]. Crystallins, which are known to be lens differentiation markers, were not found in the EST list, suggesting that these ESTs consist of cDNAs involved in the dedifferentiation stage before lens differentiation.

A close examination of the list revealed that several members of the bone morphogenetic protein and transforming growth factor pathways were present in our ESTs. We were unable to find any from the wingless-type mouse mammary tumor virus integration site family, fibroblast growth factor, or sonic hedgehog pathway. The reason for the presence or

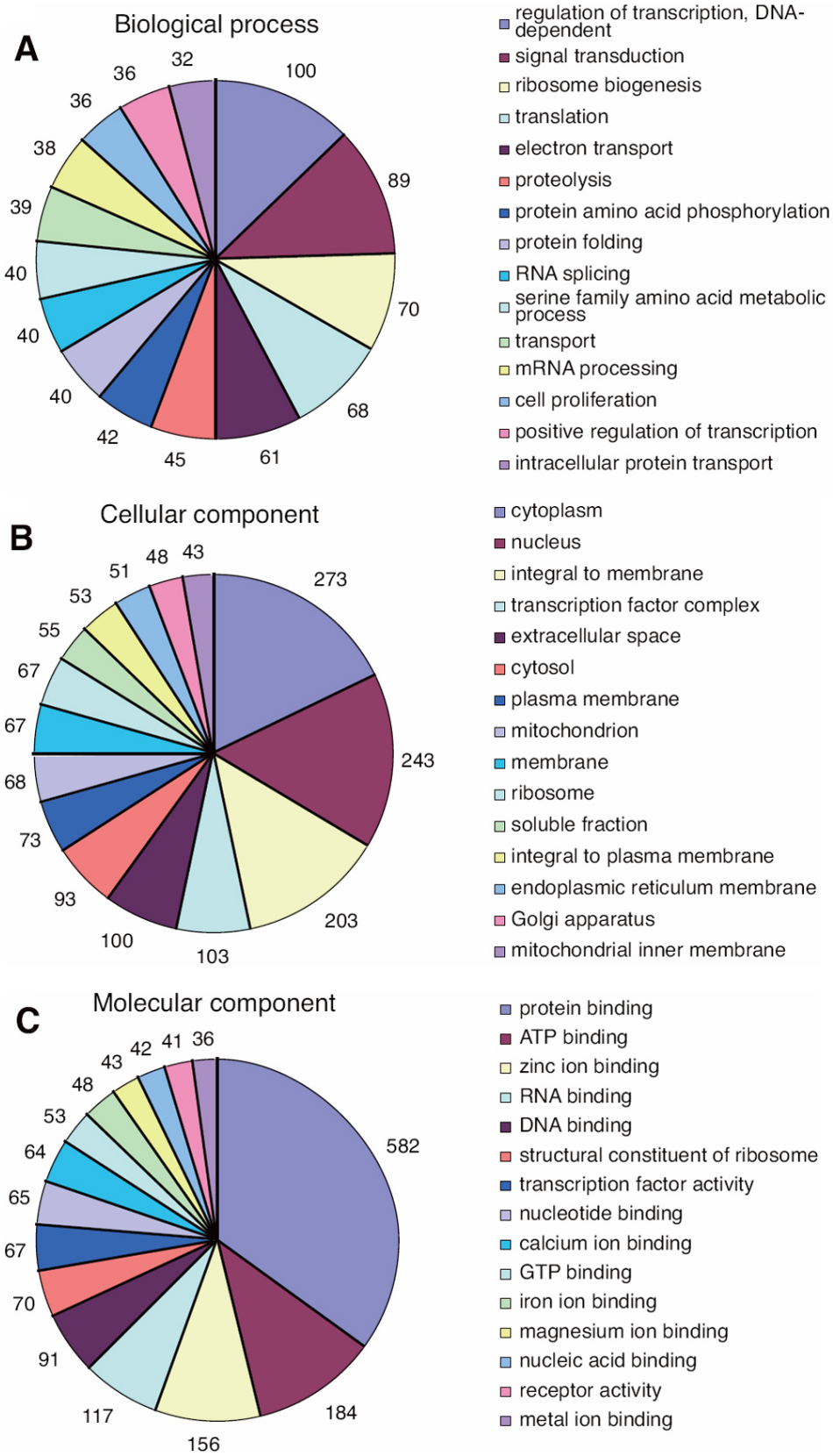

Figure 1. Most frequently counted terms in the lens-regenerating iris. The fifteen most frequently counted terms of biologic process (**A**), cellular component (**B**), and molecular function (**C**) are shown. The total number of these most frequently counted terms was 5,356, 3,259, and 4,152, respectively. In the figure, ATP is adenosine triphosphate and GTP is guanosine triphosphate

Table 2. Apoptosis related genes expressed during dedifferentiation.

Annotation of sequence
Apoptosis-inducing mitochondrion-associated 1
BCL2-like 13
BCL2-associated agonist of cell death
Programmed cell death 2
Programmed cell death 4
Programmed cell death 5
Programmed cell death 6
Programmed cell death 6 interacting protein

All the sequences shown have e-value less than 3.5E-17.

Table 3. Cancer-related genes found in iris during dedifferentiation.

Annotation of sequence	Annotation of sequence
Ras	**Tumor necrosis factor**
Kras	Tumor necrosis factor alpha
Ras associated protein RAB1	Tumor necrosis factor alpha-induced protein 6
Ras homolog gene family, member T1	Tumor necrosis factor receptor associated factor 2
Ras homolog gene member a	Tumor necrosis factor receptor member 1b
Ras p21 protein activator 4 isoform 1	Tumor necrosis factor receptor member 14
Ras related GTP-binding protein B	
Ras related nuclear protein	**Tumor protein**
	Tumor protein d52
Retinoblastoma	Tumor protein d52-like 2
Retinoblastoma binding protein 4 isoform 1	
	Others
Jun	Fat tumor supressor, homolog 1
c-jun	Feline sarcoma oncogene
Jun-B oncogene	Fyn oncogene related to yes*
	Glioma tumor suppressor candidate region gene 2
p53	Large tumor supressor, homolog 1
Tumor protein p53	Leydig cell tumor 10 kDa protein
p53-associated parkin-like cytoplasmic protein	Tumor translationally-controlled 1

All the sequences shown have e-value less than 8.2E-16.

absence could be due to the stage of the iris that was isolated. The iris was at the dedifferentiation stage. It is possible that pathways that are important for lens differentiation, such as fibroblast growth factor or sonic hedgehog, are simply not active at that stage [17,18]. Alternatively, it is possible that certain factors were not cloned in our experiment.

It appears that a wide variety of cancer-related genes were expressed during dedifferentiation (Table 3). A total of 27 cancer-related genes were found in the ESTs, especially a large number of Ras-related genes and tumor necrosis factor-related genes. The expression of cancer-related genes might be related to the initiation of proliferation during dedifferentiation. Additionally, as c-Myc is one of four factors (cellular myelocytomatosis oncogene, octamer-binding transcription factor 4, sex determining region Y box 2 and kruppel-like factor 4) to reprogram somatic cells to pluripotent stem cells [19], there is a possibility that cancer-related genes play a role in reprogramming during dedifferentiation. In addition to cancer-related genes, eight apoptosis-related genes were found in the EST list (Table 2). The expression of cancer- and apoptosis-related genes could be one of the hallmarks during dedifferentiation in newt regeneration.

Candidate genes that regulate reprogramming during dedifferentiation: From our list of the lens-regenerating iris ESTs, possible candidate genes for participating in nuclear regulation during newt dedifferentiation were identified (Table 4). Epigenetic regulation, a range of heritable chromatin modifications, including histone modifications, DNA methylation, and chromatin remodeling, play a pivotal role in the control of differentiation and maintenance of cellular identity. It is therefore expected that epigenetic regulation plays an important role during newt dedifferentiation.

Histone acetylation is generally related to transcriptional activation and mediated by histone acetyltransferase. In the

Table 4. Candidate genes which regulate reprogramming during dedifferentiation.

Annotation of sequence	Annotation of sequence
Histone acetyltransferase	**Non-histone chromosomal protein**
CREB binding protein / p300*	High-mobility group protein box 2*
Histone acetyltransferase MYST3*	High-mobility group protein box 3
Histone deacetylase	**Nucleolar protein**
Histone deacetylase 2*	Nucleostemin*
Histone deacetylase 5*	
	Transcriptional factor/repressor
Histone demethylase	Nf-kappaB*
Jumonji domain containing 1b	COUP transcription factor 1*
Jumonji domain containing 2a*	REST/RE1-silencing transcription factor*
DNA methyltransferase	
DNA (cytosine-5-)-methyltransferase 1*	
Williams beuren syndrome chromosome region 22*	

All the sequences shown have e-value less than 1.0E-35. The asterisk indicates genes commonly found in ovary ESTs but not in intact limb ESTs [unpublished data].

ESTs, there were two types of histone acetyltransferases, cyclic adenosine monophosphate response element-biding protein binding protein/p300 [20] and MYST3 [21], as well as two deacetylases, histone deacetylase 2 and 5 (HDAC2, HDAC5; Table 4) [22]. The balance between histone acetyltransferase and deacetylase might be strictly regulated during newt dedifferentiation.

To form heterochromatin, heterochromatin protein 1 is recruited to methylated histone H3K9 [23,24]. It has been demonstrated that the Jumonji domain-containing 2a (JMJD2A) is a histone demethylase against histone H3K9 and H3K36 and antagonizes heterochromatin formation via histone H3K9 [25]. It is thought that JMJD1B is a H3K9 histone demethylase because JMJD1B has a JmjC domain [26]. Interestingly, JMJD2A and JMJD1B were found in the ESTs (Table 4). This might indicate that these histone demethylases eliminate heterochromatin during dedifferentiation.

DNA methylation is a covalent modification of DNA and confers a heritable gene repression during and after development [27,28]. DNA (cytosine-5-)-methyltransferase 1 [28] and putative DNA methyltransferase Williams Beuren syndrome chromosome region 22 [29] were found in the ESTs (Table 4).

The high mobility group protein is a nonhistone chromatin protein. In vitro experiments have demonstrated that high mobility group protein box 2 (HMGB2) nonspecifically binds and bends DNA. It is suggested that HMGB2 facilitates cooperative interactions between cis-acting proteins by promoting DNA flexibility [30]. Like HMGB2, HMGB3 contains DNA-binding HMG box domains and is thought to be able to alter DNA structure [31]. Thus, HMGB2 and HMGB3 might promote genome-wide DNA flexing, which allows new sets of gene expression during dedifferentiation.

Transcriptional factors, nuclear factor-κB [32], and chicken ovalbumin upstream promoter transcription factor 1 [33] were found. Repressor element 1 silencing transcriptional factor (REST) binds to repressor element 1 and recruits a wide variety of chromatin modification enzymes, such as the histone deacetylases HDAC1 and HDAC2, histone H3K9 methylases G9a and SUV39H1, and a histone H3K4 demethylase lysine-specific demethylase 1 (LSD1) directly or indirectly with the CoREST complex or the mammalian switch independent 3 (mSin3) complex [34]. Interestingly, REST/RE1-silencing transcription factor and HDAC2 were found in the ESTs, suggesting cooperation of these molecules during dedifferentiation.

The oocyte has an ability to reprogram the somatic nucleus, which was demonstrated by the nuclear transfer into an oocyte [35]. Interestingly, most of the nuclear genes identified as candidates to regulate newt dedifferentiation were found in ovary ESTs as well (14,429 reads) but not in intact limb ESTs (1,098 reads; Table 4). Functional analysis of these genes might provide an advanced understanding of cellular plasticity, with possible future applications in regenerative therapies.

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REFERENCES

1. Del Rio-Tsonis K, Tsonis PA. Eye regeneration at the molecular age. Dev Dyn 2003; 226:211-24. [PMID: 12557200]
2. Sanchez Alvarado A, Tsonis PA. Bridging the regeneration gap: genetic insights from diverse animal models. Nat Rev Genet 2006; 7:873-84. [PMID: 17047686]
3. Eguchi G, Okada TS. Differentiation of lens tissue from the progeny of chick retinal pigment cells cultured in vitro: a demonstration of a switch of cell types in clonal cell culture. Proc Natl Acad Sci USA 1973; 70:1495-9. [PMID: 4576021]
4. Abe S, Eguchi G. An analysis of differentiative capacity of pigmented epithelial cells of adult newt iris in clonal cell culture. Dev Growth Differ 1977; 19:309-17.
5. Tsai RY, McKay RD. A nucleolar mechanism controlling cell proliferation in stem cells and cancer cells. Genes Dev 2002; 16:2991-3003. [PMID: 12464630]
6. Maki N, Takechi K, Sano S, Tarui H, Sasai Y, Agata K. Rapid accumulation of nucleostemin in nucleolus during newt regeneration. Dev Dyn 2007; 236:941-50. [PMID: 17133523]
7. Maki N, Suetsugu-Maki R, Tarui H, Agata K, Del Rio-Tsonis K, Tsonis PA. Expression of stem cell pluripotency factors during regeneration in newts. Dev Dyn 2009; 238:1613-6. [PMID: 19384853]
8. Habermann B, Bebin AG, Herklotz S, Volkmer M, Eckelt K, Pehlke K, Epperlein HH, Schackert HK, Wiebe G, Tanaka EM. An Ambystoma mexicanum EST sequencing project: analysis of 17,352 expressed sequence tags from embryonic and regenerating blastema cDNA libraries. Genome Biol 2004; 5:R67. [PMID: 15345051]
9. Sczyrba A, Beckstette M, Brivanlou AH, Giegerich R, Altmann CR. XenDB: full length cDNA prediction and cross species mapping in Xenopus laevis. BMC Genomics 2005; 6:123. [PMID: 16162280]
10. Shin Y, Kitayama A, Koide T, Peiffer DA, Mochii M, Liao A, Ueno N, Cho KW. Identification of neural genes using Xenopus DNA microarrays. Dev Dyn 2005; 232:432-44. [PMID: 15614765]
11. Putta S, Smith JJ, Walker JA, Rondet M, Weisrock DW, Monaghan J, Samuels AK, Kump K, King DC, Maness NJ, Habermann B, Tanaka E, Bryant SV, Gardiner DM, Parichy DM, Voss SR. From biomedicine to natural history research: EST resources for ambystomatid salamanders. BMC Genomics 2004; 5:54. [PMID: 15310388]
12. Gilchrist MJ, Zorn AM, Voigt J, Smith JC, Papalopulu N, Amaya E. Defining a large set of full-length clones from a Xenopus tropicalis EST project. Dev Biol 2004; 271:498-516. [PMID: 15223350]
13. Malloch EL, Perry KJ, Fukui L, Johnson VR, Wever J, Beck CW, King MW, Henry JJ. Gene expression profiles of lens regeneration and development in Xenopus laevis. Dev Dyn 2009; 238:2340-56. [PMID: 19681139]
14. Huang X, Madan A. CAP3: A DNA sequence assembly program. Genome Res 1999; 9:868-77. [PMID: 10508846]
15. Conesa A, Gotz S, Garcia-Gomez JM, Terol J, Talon M, Robles M. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 2005; 21:3674-6. [PMID: 16081474]
16. Myhre S, Tveit H, Mollestad T, Laegreid A. Additional gene ontology structure for improved biological reasoning. Bioinformatics 2006; 22:2020-7. [PMID: 16787968]
17. Del Rio-Tsonis K, Trombley MT, McMahon G, Tsonis PA. Regulation of lens regeneration by fibroblast growth factor receptor 1. Dev Dyn 1998; 213:140-6. [PMID: 9733109]
18. Tsonis PA, Vergara MN, Spence JR, Madhavan M, Kramer EL, Call MK, Santiago WG, Vallance JE, Robbins DJ, Del Rio-Tsonis K. A novel role of the hedgehog pathway in lens regeneration. Dev Biol 2004; 267:450-61. [PMID: 15013805]
19. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126:663-76. [PMID: 16904174]
20. Spiegelman BM, Heinrich R. Biological control through regulated transcriptional coactivators. Cell 2004; 119:157-67. [PMID: 15479634]
21. Champagne N, Pelletier N, Yang XJ. The monocytic leukemia zinc finger protein MOZ is a histone acetyltransferase. Oncogene 2001; 20:404-9. [PMID: 11313971]
22. Wade PA. Transcriptional control at regulatory checkpoints by histone deacetylases: molecular connections between cancer and chromatin. Hum Mol Genet 2001; 10:693-8. [PMID: 11257101]
23. Lachner M, O'Carroll D, Rea S, Mechtler K, Jenuwein T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature 2001; 410:116-20. [PMID: 11242053]
24. Nakayama J, Rice JC, Strahl BD, Allis CD, Grewal SI. Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. Science 2001; 292:110-3. [PMID: 11283354]
25. Klose RJ, Yamane K, Bae Y, Zhang D, Erdjument-Bromage H, Tempst P, Wong J, Zhang Y. The transcriptional repressor JHDM3A demethylates trimethyl histone H3 lysine 9 and lysine 36. Nature 2006; 442:312-6. [PMID: 16732292]
26. Katoh M, Katoh M. Comparative integromics on JMJD1C gene encoding histone demethylase: conserved POU5F1 binding site elucidating mechanism of JMJD1C expression in undifferentiated ES cells and diffuse-type gastric cancer. Int J Oncol 2007; 31:219-23. [PMID: 17549425]
27. Jones PA, Takai D. The role of DNA methylation in mammalian epigenetics. Science 2001; 293:1068-70. [PMID: 11498573]
28. Reik W. Stability and flexibility of epigenetic gene regulation in mammalian development. Nature 2007; 447:425-32. [PMID: 17522676]
29. Doll A, Grzeschik KH. Characterization of two novel genes, WBSCR20 and WBSCR22, deleted in Williams-Beuren syndrome. Cytogenet Cell Genet 2001; 95:20-7. [PMID: 11978965]
30. Paull TT, Haykinson MJ, Johnson RC. The nonspecific DNA-binding and -bending proteins HMG1 and HMG2 promote the assembly of complex nucleoprotein structures. Genes Dev 1993; 7:1521-34. [PMID: 8339930]
31. Nemeth MJ, Curtis DJ, Kirby MR, Garrett-Beal LJ, Seidel NE, Cline AP, Bodine DM. Hmgb3: an HMG-box family member expressed in primitive hematopoietic cells that inhibits myeloid and B-cell differentiation. Blood 2003; 102:1298-306. [PMID: 12714519]

32. Baud V, Karin M. Is NF-kappaB a good target for cancer therapy? Hopes and pitfalls. Nat Rev Drug Discov 2009; 8:33-40. [PMID: 19116625]
33. Nagasaki S, Suzuki T, Miki Y, Akahira JI, Shibata H, Ishida T, Ohuchi N, Sasano H. Chicken ovalbumin upstream promoter transcription factor II in human breast carcinoma: Possible regulator of lymphangiogenesis via vascular endothelial growth factor-C expression. Cancer Sci 2009; 100:639-45. [PMID: 19154418]
34. Ooi L, Wood IC. Chromatin crosstalk in development and disease: lessons from REST. Nature Rev Genet 2007; 8:544-54. [PMID: 17572692]
35. Gurdon JB, Melton DA. Nuclear reprogramming in cells. Science 2008; 322:1811-5. [PMID: 19095934]

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