Special Issue: Direct Reprogramming

Mini Review

Direct reprogramming based on transcriptional regulatory network analysis

Takahiro Suzuki and Harukazu Suzuki*

RIKEN Center for Life Science Technologies (CLST), Division of Genomic Technologies, RIKEN Yokohama Campus, Yokohama, Japan

Trans-differentiation of cells through direct reprogramming offers significant potential for regenerative medicine and drug screen, but currently it requires exhaustive trials and errors before the desired target cells can be created. Based on the comprehensive transcriptome analyses by the FANTOM consortium, in particular the recently published transcriptional regulatory network analysis, we have developed a systematic method for direct reprogramming and succeeded in creating monocyte-like cells with several monocyte-specific functions. Further analysis of the created cells reveals that the transcriptional regulatory networks and the epigenomes of the original terminally differentiated cells act as strong barriers that prevent easy reprogramming by extra stimuli. These biological insights should be carefully considered for efficient and complete direct reprogramming.

Rec.6/30/2014, Acc.9/30/2014, pp224-232

*Correspondence should be addressed to:
Harukazu Suzuki, PhD, RIKEN Center for Life Science Technologies (CLST), Division of Genomic Technologies, RIKEN Yokohama Campus, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama City, Kanagawa, 230-0045, Japan. Phone: +81-45-503-9222, Fax: +81-45-503-9216, E-mail: harukazu@gsc.riken.jp

Key words direct reprogramming, epigenome, FANTOM, transcription factor, transcriptional regulatory network

Introduction

A human body consists of approximately 200 types of cells[1] which are all created from pluripotent embryonic stem cells through differentiation and proliferation. In mammals, it has been long believed that differentiation from the pluripotent cells is a one-way process, making it impossible for terminally differentiated cells to be reprogrammed back to the pluripotent state. Somatic cell nuclei can be reprogrammed to enter pluripotent state by nuclear transplantation into fertilized eggs[2] but it had been thought that the mechanism of reprogramming is too complicated to be reproduced artificially. In 2006, Yamanaka and Takahashi succeeded in generating embryonic stem-like cells, designated as induced pluripotent stem cells (iPSC), from dermal fibroblasts simply by ectopic expression of four transcription factors, *Oct4, Sox2, Klf4* and *cMyc*[3]. This epoch making achievement allowed the possibility of direct cell reprogramming of fibroblasts into many

types of cells, such as neurons, pancreatic β-cells, and cardiomyocytes[4], where specific sets of transcription factors were simultaneously overexpressed in order to elicit cellular function and achieve trans-differentiation. One of the most important steps in these trials is the selection of the key transcription factors critical for direct cell reprogramming, but due to the lack of any systematic methods, it has involved exhaustive trial-and-error approaches. Furthermore, researchers are still exploring the completeness of the achieved target cell reprogramming in many cases.

In this mini-review, we briefly introduce our comprehensive transcriptome analysis through the FANTOM consortium, and then explain our approach for direct cell reprogramming based on our knowledge of transcriptional regulatory networks.

Transcriptional regulation analysis in FANTOM

The FANTOM (Functional ANnoTation Of Mammalian genome) is an international scientific consortium that focuses on mammalian transcriptome analysis. The goal of the FANTOM consortium has been shifting from element analysis to system analysis; mouse full-length cDNAs were sequenced and annotated in the early FANTOMs 1 to 3 resulting in the discovery of a large number of long non-coding RNAs[5-7], while transcriptional regulatory networks and transcriptional regulatory elements were analyzed to understand gene expression regulation in the recent FANTOMs 4 and 5[8, 9]. In the transcriptome analysis in FANTOMs 4 and 5, we have used our unique technology CAGE (Capped Analysis of Gene Expression)[10, 11]. CAGE is a method to capture capped transcripts followed by the 5'-end sequencing as CAGE tags, providing us with the exact, genome-wide locations of the transcription start sites that are tightly linked with the proximal promoters of the transcripts. In addition, CAGE analysis produces promoter-based expression profiles (promoter activity profiles) since the frequency of a given CAGE tag corresponds to the expression level of the transcript (and the promoter activity) at the identified transcription start site.

In FANTOM4, we developed a systematic pipeline to produce the promoter-based dynamic transcriptional regulatory network during cell differentiation[8], in which the CAGE technology was combined with next generation sequencers (deepCAGE). We found that cellular status and functional changes are strictly regulated by concerted actions of transcription factors, with the transcriptional regulatory network being restructured following cell differentiation. In the latest FANTOM5 project, we comprehensively analyzed human and mouse transcripts and their promoters and enhancers[9], utilizing the further improved non-amplified deepCAGE technology, which removes any bias from sequencing sample construction[10]. These large-scale data provide us with deep biological insights of many different cell types, including enriched and active key transcription factors.

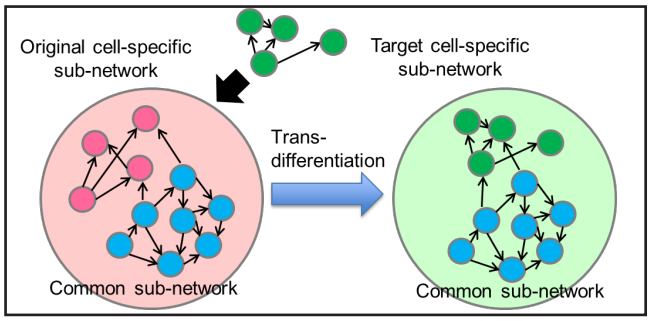

Fig.1 Direct cell reprogramming by reconstitution of target cell-specific sub-networks

Concept of transcriptional regulatory network reconstitution

For a given cell, its cellular status, type and function are determined by its overall gene expression levels, which are regulated by the concerted actions of transcription factors forming a robust transcriptional regulatory network. Various transcriptional regulatory networks would contain the shared “common sub-network”, responsible for controlling the expression levels of genes involved in common cellular features such as maintenance of structures, small organs, metabolic pathways, transcription and translation systems and repair system etc. For functions specific to each cell type, “cell type specific sub-networks” are involved. Therefore, direct cell reprograming is considered as the reconstitution of target cell-specific sub-networks into the original cell (Fig.1); it is expected that transduction of selected key transcription factors into target cell would induce target cell-specific sub-networks in the original cell while the original cell type-specific sub-networks decline, resulting in the cell reprogrammed with target cell-specific functions and without original cell-specific functions.

Table 1 The hepatocyte core transcriptional regulatory network elements

Symbol	IS	Rank (Rfc)	Rank (Rco)
FOXA2	1.200	1	5
MSC	1.011	87	1
FOXA1	0.548	2	21
EGR3	0.502	663	2
EGR3	0.501	1388	2
CEBPA	0.349	3	65
HNF4A	0.338	223	3
HNF4A	0.337	263	3
HNF4A	0.336	451	3
NR2E3	0.334	1282	3
NR2E3	0.334	1313	3
NR2E3	0.334	1523	3
CITED4	0.265	4	65
FOXA3	0.215	5	65
FOXA2	0.202	434	5
FOXD3	0.202	511	5
FOXA2	0.201	939	5
NR1H4	0.182	6	65
NOTCH2	0.146	293	7
ESR1	0.145	407	7

Top 20 transcription factor elements were selected according to our method (reference 12). IS, Rfc and Rfo stand for importance score, differential expression fold change rank and co-occurrence rank, respectively. FOXA2, EGR3, HNF4A and NR2E3 appear multiple times because we treat isoforms as different transcription factors.

Development of the systematic target cell-specific sub-network reconstitution method

The basic concept of target cell-specific sub-network reconstitution is intuitive. Generation of iPS cells using the four Yamanaka factors may be the most representative, successful example of the concept. However, in actual practice, it is not easy to select the minimum essential key transcription factors for the given target cell-specific sub-network reconstitution because the transcriptional regulatory role of a single transcription factor *versus* the combinatorial role of multiple transcription factors remain largely unexplored. Thus, key factor selection for direct reprogramming has been mainly through trial-and-error approaches. The four Yamanaka factors were also selected from iPS cell creation assays involving more than 20 transcription factors. To address this difficulty, we have developed a systematic method to reconstitute target cell-specific sub-networks in terminally differentiated cells, using fibroblast to monocyte cell reprograming as a model[12].

The method consists of three steps: 1) selection of key factor candidates, 2) identification of the minimum set of essential transcription factors for the reconstitution of target cell-specific sub-networks, and 3) combinatorial transfection of the selected key factors and validation of the converted cells. In step 1, we calculate the Importance Score (IS) for transcription factors, which are then ranked and the top twenty are selected. The IS is obtained by combining two pieces of information: 1) differences in expression levels of transcription factor genes between the original cells (fibroblasts) and the target cells (monocytes), and 2) literature search of the MEDLINE database for the co-occurrence of the terms “monocyte” and the key biological-processes such as “differentiation”, “reprogramming” and “transformation”. In step 2, the selected transcription factors are individually over-expressed in the original cell to examine whether other selected transcription factor genes are endogenously induced/up-regulated. We used the lentivirus over-expression system followed by quantitative RT-PCR since this system is applicable to most of cell types. Using the results, the transcriptional regulatory network consisting of the selected 20 transcription factors is constructed to identify the most important (upstream) transcription factors. In our model, we determined that the four transcription factors SPI1, CEBPA, MNDA and IRF8 are essential for inducing/up-regulating all of the selected transcription factors from the first step. Finally, in step 3, the most important transcription factors are transduced into the original cell in a combinatorial manner.

Fibroblast cells, transducing the four most important key factors, are isolated by drug selection and FACS sorting followed by evaluation of monocyte-specific phenotypes. The four factor-transduced fibroblast cells show morphologies similar to monocyte cells. We successfully evaluated that the reprogrammed cells exhibit phagocytosis, a specific type of endocytosis by monocytes and macrophage cells to remove pathogens and cell debris, and induction of cytokine genes upon LPS treatment. These evidences suggest that the series of genes involved in the monocyte-specific phenotypes are expressed, and we conclude that we have successfully created monocyte-like cells.

Next, in order to evaluate the generality of our method, we used in-house gene expression data with our method to select the important transcription factors for reprogramming of fibroblasts into hepatocytes (Table 1). Several groups have reported direct reprogramming into mouse and human hepatocytes/hepatoblasts, although distinct sets of transcription factors are used for the reprogramming[13-17].

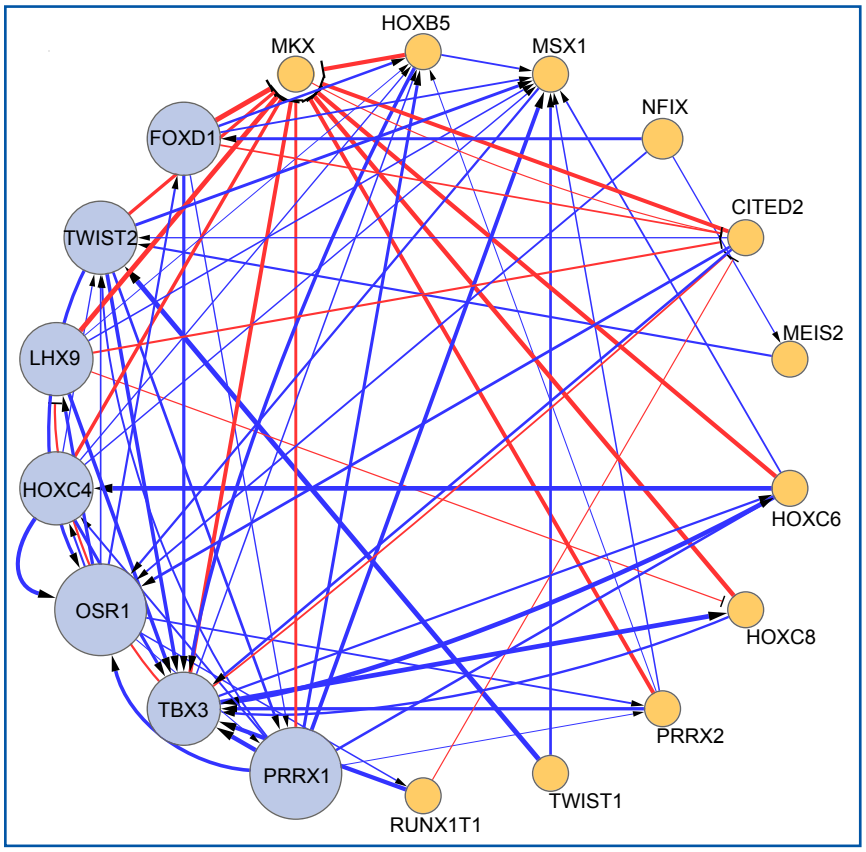

Fig.2 Transcriptional regulatory network among fibroblast-enrich transcription factors

Arrowhead and bar head in the edges indicate direction of regulation. Blue and red lines indicate positive and negative regulations. Thicker line indicates greater differential expression as compared to siRNA control. Seven transcription factors, shown by blue nodes, are most frequently connected by activating edges. Re-produced from reference 18).

Our method successfully selects the most commonly used transcription factor, FOXA3, and other transcription factors used for the reprogramming (FOXA1, FOXA2, HNF4A and CEBPA) as the top 20 important transcription factors. HNF1A is not included in the top 20 (ranked 34), which may be due to their low expression in cultured hepatocytes compared with that of hepatocytes in liver tissue, suggesting the importance of reference sample selection. On the other hand, we successfully select HNF4A because of its high rank in the literature score.

Finally, we compared our method with other well-used methods for direct reprogramming. Both methods consist of three steps, “selection”, “screen” and “evaluation”, the most distinct step being the “screen” step. Our screen method is based on the construction of transcriptional regulatory networks, whereas other methods depend on phenotype and/or bio-marker expression assays. Furthermore, our method does not rely on any particular prior knowledge in the “selection” step because key transcription factor candidates are automatically calculated using gene expression and literature information. However, this may indicate a limitation of our method when applied to target cell types without much annotation.

Knockdown-mediated direct reprogramming

While the reconstitution of monocyte-specific sub-networks in fibroblast cells resulted in monocyte-like phenotypic features, gene expression profiles of the created cells did not perfectly match those of monocytes. We found that 40% of the fibroblast-enriched genes do not change their expression levels in the created cells. Fibroblast-enriched transcription factors, such as TWIST2 and PRRX1, are still highly expressed, suggesting that reconstitution of

target cell-specific sub-networks does not always actively diminish original cell-specific sub-networks entirely and some of them may remain. While fibroblast cells are the most frequently used cell type for direct reprograming, little is known about its specific transcriptional regulatory sub-networks, prompting us to explore the fibroblast-specific sub-network in more detail[18].

We first selected eighteen fibroblast-enriched transcription factors by comparing the expression profiles of 49 fibroblast cells originating from various tissues with those of other cell types in the FANTOM5 dataset. We then examined their regulation with the MatrixRNAi approach using human dermal fibroblast cells. The MatrixRNAi is a method for exploring transcription factor regulation by knocking down one transcription factor at a time followed by qRT-PCR to measure the expression changes of other transcription factor genes. We successfully created a fibroblast-specific sub-network consisting of 53 activating and 19 inhibiting edges, denoted by lines with arrowheads and bar heads, respectively (Fig.2). Each edge represents the regulatory relationship between the source transcription factor and its target gene. Seven out of 18 transcription factors (blue nodes) are most frequently connected by activating edges, suggesting that these transcription factors compose core of the fibroblast-specific transcriptional regulatory sub-network. Interestingly, TWIST2 and PRRX1, the transcription factors that are still expressed in the monocyte-like cells, are included in those seven transcription factors.

Because the original cell-specific sub-networks may act as barriers to direct cell reprograming, we hypothesized that we may be able to achieve direct cell reprograming if we weaken the robustness of original cell-specific sub-networks by knocking down the key transcription factors. We targeted adipocyte cells because of their close lineage to fibroblast cells, as they both derive from mesenchymal stem cells. In addition, the chemical differentiation method for converting mesenchymal stem cells to adipocyte cells using the adipocyte differentiation medium has been well-established[19, 20]. When we knocked down each of the above-mentioned 7 key transcription factors in the fibroblast culturing medium, we could not observe any phenotypic changes. Use of the adipocyte differentiation medium did not show any phenotypic changes in fibroblast cells either. Surprisingly, when we knocked down each of the key transcription factors together with the adipocyte differentiation medium, accumulation of Orange-Red-stained lipid droplet, a typical feature in adipocyte cells, was observed for four out of the 7 key transcription factors, OSR1, PRRX1, TWIST2 and LHX9. The lipid droplet accumulation was enhanced when we combinatorially knocked down those 4 key transcription factors. When we analyzed the 4 key transcription factor knocked-down cells using qRT-PCR and microarrays, we found that the well-known adipocyte-specific genes such as *PPARG2, CEBPA* and *MXLIPL* were drastically induced. Principal component analysis revealed the similarity of expression profiles between knocked-down cells and mature adipocyte cells.

Taken together, our results indicate that original cell-specific sub-networks can act as barriers for direct cell reprogramming, and disrupting them together with adequate cell culture medium (or certain drugs) makes it possible to convert original cells to other types of cells. Thus, our results point to a novel, alternative method for direct cell reprogramming that is different from the established method of transducing key transcription factors of target cells into original cells. It has been reported in literature that it is possible to reprogram fibroblasts to hematopoietic progenitor cells by overexpression of Oct4[21], to neural stem cells by overexpression of Oct4 and use of certain drugs[22] and to iPS cells by overexpression of ES-specific miRNAs[23]. Oct4 expression is not essential for hematopoietic stem cells or neuronal cells. Overexpression of ES-specific miRNAs also does not directly induce important transcription factors for target cells. Their effects are thought original cells being “plastic”, although how original cells become plastic has not been clearly elucidated. We speculate that Oct4 and miRNAs may convert the original cells into plastic states by disrupting these sub-networks, as well as we showed in this section.

Epigenome as another barrier for direct reprogramming

Epigenomic modification such as histone modification and DNA methylation is strictly controlled in a spatio-temporal manner to regulate gene expression. DNA methylation of cytosine in CpG di-nucleotides at gene promoter regions is considered to be one of the most robust epigenetic marks for gene silencing in terminally differentiated cells[24]. In our primary direct cell reprograming experiment from fibroblasts to monocyte cells, we found that one of the most important key transcription factors, SPI1, was expressed from exogenously transduced construct, but not endogenously expressed in the created monocyte-like cells. Because it has been known that proximal and distal regulatory regions

for *SPI1* gene is de-methylated during differentiation to hematopoietic stem cells[25], we explored DNA methylation status of those regions in our monocyte-like cell by chromatin-immunoprecipitation using DNA methylation binding protein (MBP) followed by DNA sequencing (MBP-seq). We found that those regions are heavily methylated in fibroblast cells, but significantly de-methylated in monocyte cells. On the other hand, the created monocyte-like cells using four key transcription factors showed DNA methylation status quite similar to that of fibroblasts and did not show any DNA demethylation.

Our results show that, in addition to core key transcription factors for proper reconstitution of target cell-specific sub-networks, there need to be other factors for the epigenome regulation toward complete direct cell reprogramming. Actually, DNA demethylation of hematopoietic stem cells at *SPI1* regulatory regions occurs between hemangioblasts and hematopoietic stem cells[25], suggesting that the molecule(s), responsible for the demethylation may be temporally activated/up-regulated at this differentiation stage. Because the status changes of DNA methylation occur in a genomic loci-specific manner, the most plausible candidate molecules are transcription factors that can recognize specific DNA sequence patterns. In fact, loci-specific DNA demethylation by transcription factors NANOG, EBF1, PPARγ and SPI1 has been recently reported[26-29], although the detailed mechanism is still not well understood.

Future perspective

In this mini-review, we described the concept of direct cell reprogramming based on transcriptional regulatory network and our systematic direct reprograming method that should be applicable for any pairs of cell types in any organisms. Although our approach is still not perfect, we hope that further developments will eventually allow us to achieve complete direct cell reprogramming at will. Through the direct cell reprogramming study, we have obtained many biological insights on how genes are positively and negatively regulated by transcriptional regulatory networks and epigenomes. We also found that there are robust mechanisms in terminally differentiated cells that make them not easily reprogrammable by extra stimuli. We believe that the full understanding of these mechanisms will contribute not only to reprogramming, but also to better definition of cell types. Insights into loci-specific DNA methylation modification will also prove to be valuable for medical research, since aberrant DNA methylation of certain genes is one of the major triggers of cancer[30].

Regenerative medicine using iPS-derived retinal pigment epithelium cells has entered in the clinical study phase, which is the first such case[31]. In the near future, we expect the application of iPS-derived cells for regenerative medicine and drug screens will become more prevalent. Nonetheless, there are still enough niches for direct cell reprogramming research. For instance, many types of cells have not been able to be differentiated efficiently from iPS/ES cells yet, and for these cases, direct reprogramming may provide a novel, efficient differentiation method. In order to obtain the reprogrammed cells with high quality in future, it is essential that we continue to improve the direct cell reprograming technologies.

Acknowledgement

We thank all member of RIKEN CLST Division of Genomic Technologies and previous RIKEN Omics Science Center, especially Shin, J.W., Tomaru Y. and Kubosaki A., for their contribution to the direct reprogramming study and Kwon A. T. for his English editing.

Source of funding

This research was supported by the grant for the RIKEN Center for Life Science Technologies from the MEXT, Japan.

Conflict of interests

Authors declare that there are no conflicts of interests in our research described in this article.

References

1) Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD: Differentiated cells and the maintenance of tissues; Molecular Biology of the Cell 3rd edition. Garland Publishing, New York and London, pp1139-1193, 1994.
2) Yang X, Smith SL, Tian XC, Lewin HA, Renard JP, Wakayama T: Nuclear reprogramming of cloned embryos and its implications for therapeutic cloning. Nat Genet. 2007; 39: 295-302.
3) Takahashi K, Yamanaka S: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006; 126: 663-676.
4) Ladewig J, Koch P, Brustle O: Leveling Waddington: the emergence of direct programming and the loss of cell fate hierarchies. Nat Rev Mol Cell Biol. 2013; 14: 225-236.
5) Kawai J, Shinagawa A, Shibata K, Yoshino M, Itoh M,

Ishii Y, Arakawa T, Hara A, Fukunishi Y, Konno H, Adachi J, Fukuda S, Aizawa K, Izawa M, Nishi K, Kiyosawa H, Kondo S, Yamanaka I, Saito T, Okazaki Y, Gojobori T, Bono H, Kasukawa T, Saito R, Kadota K, Matsuda H, Ashburner M, Batalov S, Casavant T, Fleischmann W, Gaasterland T, Gissi C, King B, Kochiwa H, Kuehl P, Lewis S, Matsuo Y, Nikaido I, Pesole G, Quackenbush J, Schriml LM, Staubli F, Suzuki R, Tomita M, Wagner L, Washio T, Sakai K, Okido T, Furuno M, Aono H, Baldarelli R, Barsh G, Blake J, Boffelli D, Bojunga N, Carninci P, de Bonaldo MF, Brownstein MJ, Bult C, Fletcher C, Fujita M, Gariboldi M, Gustincich S, Hill D, Hofmann M, Hume DA, Kamiya M, Lee NH, Lyons P, Marchionni L, Mashima J, Mazzarelli J, Mombaerts P, Nordone P, Ring B, Ringwald M, Rodriguez I, Sakamoto N, Sasaki H, Sato K, Schonbach C, Seya T, Shibata Y, Storch KF, Suzuki H, Toyo-oka K, Wang KH, Weitz C, Whittaker C, Wilming L, Wynshaw-Boris A, Yoshida K, Hasegawa Y, Kawaji H, Kohtsuki S, Hayashizaki Y; RGER Group II Team and the FANTOM Consortium: Functional annotation of a full-length mouse cDNA collection. Nature. 2001; 409: 685-690.

6) Okazaki Y, Furuno M, Kasukawa T, Adachi J, Bono H, Kondo S, Nikaido I, Osato N, Saito R, Suzuki H, Yamanaka I, Kiyosawa H, Yagi K, Tomaru Y, Hasegawa Y, Nogami A, Schonbach C, Gojobori T, Baldarelli R, Hill DP, Bult C, Hume DA, Quackenbush J, Schriml LM, Kanapin A, Matsuda H, Batalov S, Beisel KW, Blake JA, Bradt D, Brusic V, Chothia C, Corbani LE, Cousins S, Dalla E, Dragani TA, Fletcher CF, Forrest A, Frazer KS, Gaasterland T, Gariboldi M, Gissi C, Godzik A, Gough J, Grimmond S, Gustincich S, Hirokawa N, Jackson IJ, Jarvis ED, Kanai A, Kawaji H, Kawasawa Y, Kedzierski RM, King BL, Konagaya A, Kurochkin IV, Lee Y, Lenhard B, Lyons PA, Maglott DR, Maltais L, Marchionni L, McKenzie L, Miki H, Nagashima T, Numata K, Okido T, Pavan WJ, Pertea G, Pesole G, Petrovsky N, Pillai R, Pontius JU, Qi D, Ramachandran S, Ravasi T, Reed JC, Reed DJ, Reid J, Ring BZ, Ringwald M, Sandelin A, Schneider C, Semple CA, Setou M, Shimada K, Sultana R, Takenaka Y, Taylor MS, Teasdale RD, Tomita M, Verardo R, Wagner L, Wahlestedt C, Wang Y, Watanabe Y, Wells C, Wilming LG, Wynshaw-Boris A, Yanagisawa M, Yang I, Yang L, Yuan Z, Zavolan M, Zhu Y, Zimmer A, Carninci P, Hayatsu N, Hirozane-Kishikawa T, Konno H, Nakamura M, Sakazume N, Sato K, Shiraki T, Waki K, Kawai J, Aizawa K, Arakawa T, Fukuda S, Hara A, Hashizume W, Imotani K, Ishii Y, Itoh M, Kagawa I, Miyazaki A, Sakai K, Sasaki D, Shibata K, Shinagawa A, Yasunishi A, Yoshino M, Waterston R, Lander ES, Rogers J, Birney E, Hayashizaki Y; FANTOM Consortium; RGER Group I & II Team: Analysis of the mouse transcriptome based on functional annotation of 60,770 full-length cDNAs. Nature. 2002; 420: 563-573.

7) Carninci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, Oyama R, Ravasi T, Lenhard B, Wells C, Kodzius R, Shimokawa K, Bajic VB, Brenner SE, Batalov S, Forrest AR, Zavolan M, Davis MJ, Wilming LG, Aidinis V, Allen JE, Ambesi-Impiombato A, Apweiler R, Aturaliya RN, Bailey TL, Bansal M, Baxter L, Beisel KW, Bersano T, Bono H, Chalk AM, Chiu KP, Choudhary V, Christoffels A, Clutterbuck DR, Crowe ML, Dalla E, Dalrymple BP, de Bono B, Della Gatta G, di Bernardo D, Down T, Engstrom P, Fagiolini M, Faulkner G, Fletcher CF, Fukushima T, Furuno M, Futaki S, Gariboldi M, Georgii-Hemming P, Gingeras TR, Gojobori T, Green RE, Gustincich S, Harbers M, Hayashi Y, Hensch TK, Hirokawa N, Hill D, Huminiecki L, Iacono M, Ikeo K, Iwama A, Ishikawa T, Jakt M, Kanapin A, Katoh M, Kawasawa Y, Kelso J, Kitamura H, Kitano H, Kollias G, Krishnan SP, Kruger A, Kummerfeld SK, Kurochkin IV, Lareau LF, Lazarevic D, Lipovich L, Liu J, Liuni S, McWilliam S, Madan Babu M, Madera M, Marchionni L, Matsuda H, Matsuzawa S, Miki H, Mignone F, Miyake S, Morris K, Mottagui-Tabar S, Mulder N, Nakano N, Nakauchi H, Ng P, Nilsson R, Nishiguchi S, Nishikawa S, Nori F, Ohara O, Okazaki Y, Orlando V, Pang KC, Pavan WJ, Pavesi G, Pesole G, Petrovsky N, Piazza S, Reed J, Reid JF, Ring BZ, Ringwald M, Rost B, Ruan Y, Salzberg SL, Sandelin A, Schneider C, Schonbach C, Sekiguchi K, Semple CA, Seno S, Sessa L, Sheng Y, Shibata Y, Shimada H, Shimada K, Silva D, Sinclair B, Sperling S, Stupka E, Sugiura K, Sultana R, Takenaka Y, Taki K, Tammoja K, Tan SL, Tang S, Taylor MS, Tegner J, Teichmann SA, Ueda HR, van Nimwegen E, Verardo R, Wei CL, Yagi K, Yamanishi H, Zabarovsky E, Zhu S, Zimmer A, Hide W, Bult C, Grimmond SM, Teasdale RD, Liu ET, Brusic V, Quackenbush J, Wahlestedt C, Mattick JS, Hume DA, Kai C, Sasaki D, Tomaru Y, Fukuda S, Kanamori-Katayama M, Suzuki M, Aoki J, Arakawa T, Iida J, Imamura K, Itoh M, Kato T, Kawaji H, Kawagashira N, Kawashima T, Kojima M, Kondo S, Konno H, Nakano K, Ninomiya N, Nishio T, Okada

M, Plessy C, Shibata K, Shiraki T, Suzuki S, Tagami M, Waki K, Watahiki A, Okamura-Oho Y, Suzuki H, Kawai J, Hayashizaki Y; FANTOM Consortium; RGER Group and Genome Science Group: The transcriptional landscape of the mammalian genome. Science. 2005; 309: 1559-1563.

8) Fantom Consortium, Suzuki H, Forrest AR, van Nimwegen E, Daub CO, Balwierz PJ, Irvine KM, Lassmann T, Ravasi T, Hasegawa Y, de Hoon MJ, Katayama S, Schroder K, Carninci P, Tomaru Y, Kanamori-Katayama M, Kubosaki A, Akalin A, Ando Y, Arner E, Asada M, Asahara H, Bailey T, Bajic VB, Bauer D, Beckhouse AG, Bertin N, Bjorkegren J, Brombacher F, Bulger E, Chalk AM, Chiba J, Cloonan N, Dawe A, Dostie J, Engstrom PG, Essack M, Faulkner GJ, Fink JL, Fredman D, Fujimori K, Furuno M, Gojobori T, Gough J, Grimmond SM, Gustafsson M, Hashimoto M, Hashimoto T, Hatakeyama M, Heinzel S, Hide W, Hofmann O, Hornquist M, Huminiecki L, Ikeo K, Imamoto N, Inoue S, Inoue Y, Ishihara R, Iwayanagi T, Jacobsen A, Kaur M, Kawaji H, Kerr MC, Kimura R, Kimura S, Kimura Y, Kitano H, Koga H, Kojima T, Kondo S, Konno T, Krogh A, Kruger A, Kumar A, Lenhard B, Lennartsson A, Lindow M, Lizio M, Macpherson C, Maeda N, Maher CA, Maqungo M, Mar J, Matigian NA, Matsuda H, Mattick JS, Meier S, Miyamoto S, Miyamoto-Sato E, Nakabayashi K, Nakachi Y, Nakano M, Nygaard S, Okayama T, Okazaki Y, Okuda-Yabukami H, Orlando V, Otomo J, Pachkov M, Petrovsky N, Plessy C, Quackenbush J, Radovanovic A, Rehli M, Saito R, Sandelin A, Schmeier S, Schonbach C, Schwartz AS, Semple CA, Sera M, Severin J, Shirahige K, Simons C, St Laurent G, Suzuki M, Suzuki T, Sweet MJ, Taft RJ, Takeda S, Takenaka Y, Tan K, Taylor MS, Teasdale RD, Tegner J, Teichmann S, Valen E, Wahlestedt C, Waki K, Waterhouse A, Wells CA, Winther O, Wu L, Yamaguchi K, Yanagawa H, Yasuda J, Zavolan M, Hume DA, Riken Omics Science C, Arakawa T, Fukuda S, Imamura K, Kai C, Kaiho A, Kawashima T, Kawazu C, Kitazume Y, Kojima M, Miura H, Murakami K, Murata M, Ninomiya N, Nishiyori H, Noma S, Ogawa C, Sano T, Simon C, Tagami M, Takahashi Y, Kawai J, Hayashizaki Y: The transcriptional network that controls growth arrest and differentiation in a human myeloid leukemia cell line. Nat Genet. 2009; 41: 553-562.

9) Fantom Consortium, the RP, Clst, Forrest AR, Kawaji H, Rehli M, Baillie JK, de Hoon MJ, Lassmann T, Itoh M, Summers KM, Suzuki H, Daub CO, Kawai J, Heutink P, Hide W, Freeman TC, Lenhard B, Bajic VB, Taylor MS, Makeev VJ, Sandelin A, Hume DA, Carninci P, Hayashizaki Y: A promoter-level mammalian expression atlas. Nature. 2014; 507: 462-470.

10) Kanamori-Katayama M, Itoh M, Kawaji H, Lassmann T, Katayama S, Kojima M, Bertin N, Kaiho A, Ninomiya N, Daub CO, Carninci P, Forrest AR, Hayashizaki Y: Unamplified cap analysis of gene expression on a single-molecule sequencer. Genome Res. 2011; 21: 1150-1159.

11) de Hoon M, Hayashizaki Y: Deep cap analysis gene expression (CAGE): genome-wide identification of promoters, quantification of their expression, and network inference. Biotechniques. 2008; 44: 627-628, 630, 632.

12) Suzuki T, Nakano-Ikegaya M, Yabukami-Okuda H, de Hoon M, Severin J, Saga-Hatano S, Shin JW, Kubosaki A, Simon C, Hasegawa Y, Hayashizaki Y, Suzuki H: Reconstruction of monocyte transcriptional regulatory network accompanies monocytic functions in human fibroblasts. PLoS One. 2012; 7: e33474.

13) Sekiya S, Suzuki A: Direct conversion of mouse fibroblasts to hepatocyte-like cells by defined factors. Nature. 2011; 475: 390-393.

14) Huang P, He Z, Ji S, Sun H, Xiang D, Liu C, Hu Y, Wang X, Hui L: Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. Nature. 2011; 475: 386-389.

15) Huang P, Zhang L, Gao Y, He Z, Yao D, Wu Z, Cen J, Chen X, Liu C, Hu Y, Lai D, Hu Z, Chen L, Zhang Y, Cheng X, Ma X, Pan G, Wang X, Hui L: Direct reprogramming of human fibroblasts to functional and expandable hepatocytes. Cell Stem Cell. 2014; 14: 370-384.

16) Yu B, He ZY, You P, Han QW, Xiang D, Chen F, Wang MJ, Liu CC, Lin XW, Borjigin U, Zi XY, Li JX, Zhu HY, Li WL, Han CS, Wangensteen KJ, Shi Y, Hui LJ, Wang X and Hu YP: Reprogramming fibroblasts into bipotential hepatic stem cells by defined factors. Cell Stem Cell. 2013; 13: 328-340.

17) Du Y, Wang J, Jia J, Song N, Xiang C, Xu J, Hou Z, Su X, Liu B, Jiang T, Zhao D, Sun Y, Shu J, Guo Q, Yin M, Sun D, Lu S, Shi Y, Deng H: Human hepatocytes with drug metabolic function induced from fibroblasts by lineage reprogramming. Cell Stem Cell. 2014; 14: 394-403.

18) Tomaru Y, Hasegawa R, Suzuki T, Sato T, Kubosaki A, Suzuki M, Kawaji H, Forrest AR, Hayashizaki Y, Consortium F, Shin JW, Suzuki H: A transient disruption of fibroblastic transcriptional regulatory network facilitates trans-differentiation. Nucleic Acids Res. 2014; 42: 8905-8913.
19) Teboul L, Gaillard D, Staccini L, Inadera H, Amri EZ, Grimaldi PA: Thiazolidinediones and fatty acids convert myogenic cells into adipose-like cells. J Biol Chem. 1995; 270: 28183-28187.
20) Shahdadfar A, Fronsdal K, Haug T, Reinholt FP, Brinchmann JE: In vitro expansion of human mesenchymal stem cells: choice of serum is a determinant of cell proliferation, differentiation, gene expression, and transcriptome stability. Stem Cells. 2005; 23: 1357-1366.
21) Szabo E, Rampalli S, Risueno RM, Schnerch A, Mitchell R, Fiebig-Comyn A, Levadoux-Martin M, Bhatia M: Direct conversion of human fibroblasts to multilineage blood progenitors. Nature. 2010; 468: 521-526.
22) Zhu S, Ambasudhan R, Sun W, Kim HJ, Talantova M, Wang X, Zhang M, Zhang Y, Laurent T, Parker J, Kim HS, Zaremba JD, Saleem S, Sanz-Blasco S, Masliah E, McKercher SR, Cho YS, Lipton SA, Kim J, Ding S: Small molecules enable OCT4-mediated direct reprogramming into expandable human neural stem cells. Cell Res. 2014; 24: 126-129.
23) Anokye-Danso F, Trivedi CM, Juhr D, Gupta M, Cui Z, Tian Y, Zhang Y, Yang W, Gruber PJ, Epstein JA, Morrisey EE: Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell. 2011; 8: 376-388.
24) Guibert S, Weber M: Functions of DNA methylation and hydroxymethylation in mammalian development. Curr Top Dev Biol. 2013; 104: 47-83.
25) Hoogenkamp M, Lichtinger M, Krysinska H, Lancrin C, Clarke D, Williamson A, Mazzarella L, Ingram R, Jorgensen H, Fisher A, Tenen DG, Kouskoff V, Lacaud G, Bonifer C: Early chromatin unfolding by RUNX1: a molecular explanation for differential requirements during specification versus maintenance of the hematopoietic gene expression program. Blood. 2009; 114: 299-309.
26) Costa Y, Ding J, Theunissen TW, Faiola F, Hore TA, Shliaha PV, Fidalgo M, Saunders A, Lawrence M, Dietmann S, Das S, Levasseur DN, Li Z, Xu M, Reik W, Silva JC, Wang J: NANOG-dependent function of TET1 and TET2 in establishment of pluripotency. Nature. 2013; 495: 370-374.
27) de la Rica L, Rodriguez-Ubreva J, Garcia M, Islam AB, Urquiza JM, Hernando H, Christensen J, Helin K, Gomez-Vaquero C, Ballestar E: PU.1 target genes undergo Tet2-coupled demethylation and DNMT3b-mediated methylation in monocyte-to-osteoclast differentiation. Genome Biol. 2013; 14: R99.
28) Fujiki K, Shinoda A, Kano F, Sato R, Shirahige K, Murata M: PPARgamma-induced PARylation promotes local DNA demethylation by production of 5-hydroxymethylcytosine. Nat Commun. 2013; 4: 2262.
29) Guilhamon P, Eskandarpour M, Halai D, Wilson GA, Feber A, Teschendorff AE, Gomez V, Hergovich A, Tirabosco R, Fernanda Amary M, Baumhoer D, Jundt G, Ross MT, Flanagan AM, Beck S: Meta-analysis of IDH-mutant cancers identifies EBF1 as an interaction partner for TET2. Nat Commun. 2013; 4: 2166.
30) Bergman Y, Cedar H: DNA methylation dynamics in health and disease. Nat Struct Mol Biol. 2013; 20: 274-281.
31) David Cyranoski: Japanese woman is first recipient of next-generation stem cells. Nature News. 2014; doi:10.1038/nature.2014.15915.