Transcriptional changes in 4F-introduced mouse fibroblasts revealed three phases of reprogramming process termed initiation, maturation and stabilization.[34] The initiation phase is marked by the expression of genes that are involved in the mesenchymal-to-epithelial transition (MET), which is a critical event during reprogramming. In the maturation phase, the cells become independent of exogenous 4Fs and a subset of pluripotency-associated genes (e.g. *Nanog* and *Sall4*) are induced. In the stabilization phase, refinement of the cellular signature and the expressions of other pluripotent markers occur. Although these three phases had been revealed based on the transcriptional changes, each phase is accompanied by epigenetic changes as well. Events that occur during the initiation phase are so far well studied. The initiation phase involves MET via silencing of *Snail* genes, suppression of TGFβ signalling, and upregulation of *Cdh1* gene.[35] Demethylation of histone H3K79 is probably a necessary step for this phase because H3K79me2 modifications at mesenchymal marker genes, such as *SNAI1*, *SNAI2* and *TGFB2* are observed in human fibroblasts, but not in ES cells. Indeed, 4F-introduced fibroblasts decrease the H3K79me2 marks in the mesenchymal marker genes. Since methylated histone H3K79 indicates active transcriptional elongation, decreased level of H3K79me2 at mesenchymal genes implies suppression of mesenchymal property through transcriptional repressions. In agreement with this, inhibition of DOT1L, which is a methyltransferase for H3K79, enhances the reprogramming efficiency both in mouse and in human cells.[33] Maturation phase is characterized by high expression of a series of genes including *Nanog, Sall4* and *Esrrb*. Preceding the transcriptional induction of endogenous *Nanog* and *Esrrb*, Poly [ADP-ribose] polymerase 1 (Parp-1) and Tet2 are recruited to these pluripotency loci. Here, Parp-1 and Tet2 are involved in the histone modifications that typify an activated chromatin state, suggesting that Parp1 and Tet2 contribute to an epigenetic program that directs the transition into the maturation phase during the reprogramming process.[36] Towards the stabilization phase, genes including *Utf1*, *Lin28* and *Dppa4* gradually elevate their expression.[34] Throughout the maturation and stabilization phases, epigenetic alterations such as silencing of the transgenes and chromosome organization/segregation also proceed, resulting in the complete resetting of epigenome.[37]

At all events, successful establishment of the core transcriptional circuitry for pluripotency by the transient overexpression of 4Fs is accompanied by reorganization of epigenetic modifications. As mentioned, we might be able to utilize this novel reprogramming technology to understand the cellular behavior that is governed by epigenetic regulations. Given that epigenome plays an important role in altering cancer cell status, the iPSC technology can be applicable to understanding the nature of cancer cells by introducing epigenetic changes into cancer cells.

APPLICATION OF iPSC TECHNOLOGY TO CANCER RESEARCH

Cellular reprogramming technology is applicable to alter coordinated epigenetic modifications in somatic cells, and therefore it is useful to understand the mechanisms that maintain the epigenetic homeostasis. This approach might be the most promising in cancer research field, since epigenetic fluctuation is implied to contribute to the CSC emergence. Indeed, many groups have tried to reprogram cancer cells. Before the discovery of the transcription factor (TF)-mediated reprogramming method, experiments attempted to reprogram cancer cell nuclei were conducted with the nuclear transplantation (NT) method. NT is a method to reprogram nuclei of terminally differentiated cells into those of pluripotent embryonic cells that can direct development of organisms.[38,39] The first conclusive success of NT reprogramming was reported by J. B. Gurdon and coworkers in 1962. They successfully cloned a frog using intact nuclei from the intestinal epithelium cells of a *Xenopus* tadpole.[40–42] For his pioneering work, Gurdon was awarded a Nobel Prize in 2012. NT reprogramming of cancer cell nuclei was demonstrated several years after Gurdon's first cloning experiment. In 1969, one study using a frog model demonstrated that kidney carcinoma nuclei can be reprogrammed and that the resulting nuclei support the early development of frog embryos to the tadpole stage.[43] However, more recent study using a mouse model showed that the oocytes transplanted with the nuclei of leukemia, lymphoma and breast cancer cells did not give rise to ES cells, although they were able to support normal preimplantation development to the blastocyte stage.[44] One exception in this study is oocytes transplanted with nuclei of mouse melanoma cells that gave rise to ES cells capable of contributing to adult chimeric mice. However, it should be noted that the donor cells used here express oncogenic *RAS* only under a certain condition, namely the oncogenic *RAS* had not been expressed during the reprogramming process and the following embryonic developmental process[44] (Fig. 2a). Collectively, the study suggested that cancer cell nuclei are refractory to cellular reprogramming.

As soon as OCT3/4, SOX2, KLF4 and MYC were discovered as reprogramming factors, several groups tried to reprogram cancer cells by 4F introduction. Here again researchers faced difficulties. Although they have succeeded in generating iPSC-like cells from cancer cells including chronic myeloid leukemia (CML) cells[45,46] and gastrointestinal cancer cells,[47] the reprogramming efficiency was considerably lower. For example, reprogramming of human CML cell line shows the reprogramming efficiency of around 0.0002%,[45] which is about 100 times lower compared to non-cancer somatic cell reprogramming.[24] The oncogenic *RAS*-inducible melanoma cells which were the same cells as were used in above-mentioned NT experiment were also reprogrammed by 4F

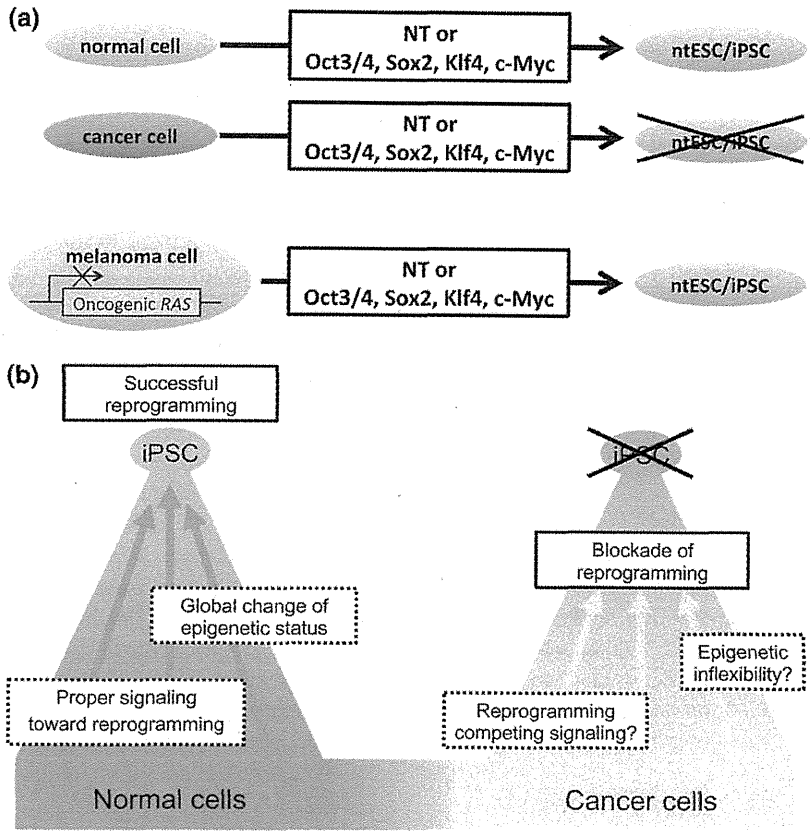

Figure 2 Cancer cells are resistant to be reprogrammed. (**a**) Although accumulating number of trials have succeeded in reprogramming of somatic cells, very few groups have succeeded in cancer cell reprogramming either by nuclear transfer (NT) method or by 4F introduction. Interestingly, reprogramming of oncogenic RAS-derived melanoma cells have been successfully performed under the condition that RAS was not expressed during the course of the reprogramming process. (**b**) Hypothesis to explain the mechanism of reprogramming resistance in cancer cells. Upon being exposed to external pressure which push the cells to reorganize their chromatin structure into the pro-reprogrammed composition, non-cancer cells rather easily change their epigenetic states, whereas cancer cells lack epigenetic flexibility possibly because of the abnormal accumulation of oncogenic transcription factors in the nuclei as well as aberrant chromatin modifications which affect the transcriptional profile, resulting in the reprogramming resistance.

introduction, but here again, the oncogene was not expressed during the reprogramming process (Fig. 2a).[48]

These studies give us some hints to understanding the epigenetic status of cancer cells. Cancer cell nuclei seem to be refractory against reprogramming regardless of the methods taken. Furthermore, the successful reprogramming of oncogenic *RAS*-inducible melanoma cells under the absence of the oncogene expression[44,48] raises the possibility that attenuation or blockade of oncogenic signalling enables cancer cell reprogramming. The reason why cancer cells are refractory to reprogramming is yet to be clarified, but one might be able to speculate that the epigenetic status that is unique to cancer cells competes with the reprogramming pressure, as successful reprogramming requires orchestrated changes in transcriptional and epigenetic signatures (Fig. 2b).

Recent studies have suggested that not only genetic alterations, but also epigenetic modifications play important roles in tumorigenesis. Abnormal patterns of genomic methylation in cancer cells are quite often observed. They are characterized by global losses of genomic methylation and increased methylation at specific loci, predominantly at CpG islands, that are often localized near transcription start sites.[49] DNA methylation is generally catalyzed by a family of enzymes called DNA methyltransferases (DNMTs). Human tumors often display an aberrant expression of DNMTs. Higher levels of DNMT1 and DNMT3A/3B are frequently observed in a wide variety of cancers.[50–52] On the other hand, DNA demethylation is suggested to be conducted, at least partially, by members of the ten–eleven translocation (TET) family. TET oxidizes 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC), and further oxidizes into 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC).[53] Although there has not yet been direct proof of the DNA demethylation function of TET family proteins, their contribution to the DNA demethylation is widely accepted based on the increasing amount of circumstantial evidence.[54] It is proposed that 'active' DNA demethylation is completed by the base excision repair (BER) following the elimination of the oxidation derivatives of 5mC that have been created by TET.[55] Besides the active demethylation mechanisms, TET is involved in the DNA demethylation process by 'passive' mechanisms as well. The oxidation derivatives of 5mC are not properly recognized by the methylation maintenance machinery, leading to the replication-dependent cytosine demethylation.[56,57] In tumor cells, TET functions are often aberrated. Levels of 5hmC are dramatically reduced in human breast, liver, lung, pancreatic and prostate cancers when compared with the matched surrounding normal tissues, being associated with the substantial reduction of the expression of all three TET-coding genes.[58] Since DNA

demethylation is assumedly carried out, at least in part, via 5hmC production by TET proteins, aberrant methylation in cancer cells are possibly caused not only via overexpression of DNMTs, but also via suppression of TET expressions. In glioma, glioma-CpG island methylator phenotype (G-CIMP) has been identified with distinct molecular and clinical characteristics.[59] Interestingly, G-CIMP is associated with somatic mutation of isocitrate dehydrogenase-1 gene (*IDH1*) and mutated IDH1 eventually inhibits TET activity by catalyzing the production of 2-hydroxyglutarate (2-HG).[60] *IDH* mutations are also found in acute myeloid leukemias (AML).[61] In a mouse model, conditional knock-in of mutated *IDH1* results in an increased number of early hematopoietic progenitors and causes splenomegaly and anemia, indicating that TET inactivation via *IDH1* mutation leads to leukemic DNA methylation signature by affecting the hematopoietic cell development.[62]

Deacetylation of histone protein is also involved in cancer development. Numerous clinical studies in cancer patients have shown that overexpression of HDAC is prevalent in tumor lesions. Increased expression levels of different HDAC family members have been reported for a wide variety of human malignancies including hematological malignancies, breast cancer, ovarian cancer, cancers of the digestive system, neuroblastoma, prostate cancer, and lung cancer.[63] Altogether, this evidence suggests that increased DNA methylating activity and increased histone deacetylating activity are closely associated with cancer development. Indeed, DNMT inhibitors and HDAC inhibitors are the first launched epigenetic targeted anti-cancer drugs.

Given that cellular reprogramming requires remodeling of epigenetic modifications, these epigenetic properties unique to cancer cells might be associated with refractoriness of cancer cells to successful reprogramming. TET proteins are suggested to play an important role in maintaining cellular pluripotency in ES/iPS cells by regulating the expression of pluripotency-related genes such as *Esrrb* and *Oct4* via interaction with NANOG protein.[64] Decreased expression of TET and/or inhibited TET function in cancer cells might impair the maintenance of pluripotency and hamper the reprogramming. Furthermore, in the case of non-cancer cell reprogramming, treatment with a HDAC inhibitor, trichostatin A (TSA)[65,66] and attenuation of DNMT1 activity by using hypomorphic allele[67] improve the reprogramming efficiency mediated by somatic cell nuclear transfer (SCNC). Similar improvements of reprogramming efficiency have also been demonstrated when cells are introduced with 4Fs, namely, treatment with a DNMT inhibitor, 5'-azacytidine (5'-azaC), as well as HDAC inhibitors, such as suberoylanilide hydroxamic acid (SAHA), valproic acid (VPA) and TSA, improve reprogramming efficiency by 4Fs introduction.[68] This evidence implies the possibility that the epigenetic properties of cancer cells, i.e., overexpression of DNMTs, low expression level of TETs and overexpression of HDACs, interfere with successful reprogramming processes. We might be able to perform successful reprogramming of cancer cells by precisely regulating the activity of such altered epigenetic modulators (Fig. 2b).

The difficulty of cancer cell reprogramming could be also attributable to the lack of epigenetic flexibility. Nuclear structure in cancer cell is quite different from that in normal cell. Cancer cells frequently display irregular chromatin morphology such as coarse heterochromatin aggregation and enlarged nucleoli,[69] possibly associated with dysregulated DNA methylation and histone modifications including H3K9 methylation in heterochromatin. Considering that epigenetic modifications are stably maintained over cell replications, it is expected that the abnormal nuclear structure is also stably maintained in cancer cells. Since the space coordinate of each gene in nucleus, i.e., chromosomal territory (CT), is critical for the transcriptional regulation,[70,71] aberrant nuclear structure with altered epigenetic regulations in cancer cells could affect the transcriptional profile via impeded accessibility of transcription factors to the target loci. This hypothetical inaccessibility of transcription factors leads to restricted response of cancer cells to transcription factors including the reprogramming 4Fs, which might render cancer cells transcriptionally inflexible, namely, state of 'lack of epigenetic flexibility', resulting in the refractory against reprogramming (Fig. 2b).

The fact that *RAS*-inducible melanoma cells were successfully reprogrammed under the absence of *RAS* expression[44,48] might be explained by the cancelation of the epigenetic stabilization brought by oncogene expressions. It has been suggested that transcriptional regulation of certain genes by a specific TF is involved in the maintenance of somatic cell identity which is related to the stable epigenetic modifications and that activation of such transcriptional machinery hampers somatic cell reprogramming.[72] This indicates that strong transcriptional activations caused by oncogenic signals might also maintain epigenetic stability in cancer cells, which eventually brings refractoriness to reprogramming.

The concept of epigenetic stability in cancer cells projects an important aspect of anti-cancer treatment, namely, there is a possibility that attenuation of oncogenic signals by anti-cancer drugs could increase the epigenetic flexibility in cancer cells, leading to the cell-state transition and eventually to the emergence of drug resistant cells and/or CSCs (Fig. 3). At the bedside, it is often observed that treatment of cancer patients with molecularly-targeted drugs that block oncogenic signals decrease the tumor cell mass shortly after the initiation of the treatment. However, in a long term, it is hardly possible to evade the tumor recurrence in most of the cases especially in solid tumor patients. Based on the hypothesis that oncogenic signal stabilizes epigenetic modifications, it is possible that blockade of oncogenic signal by molecularly-

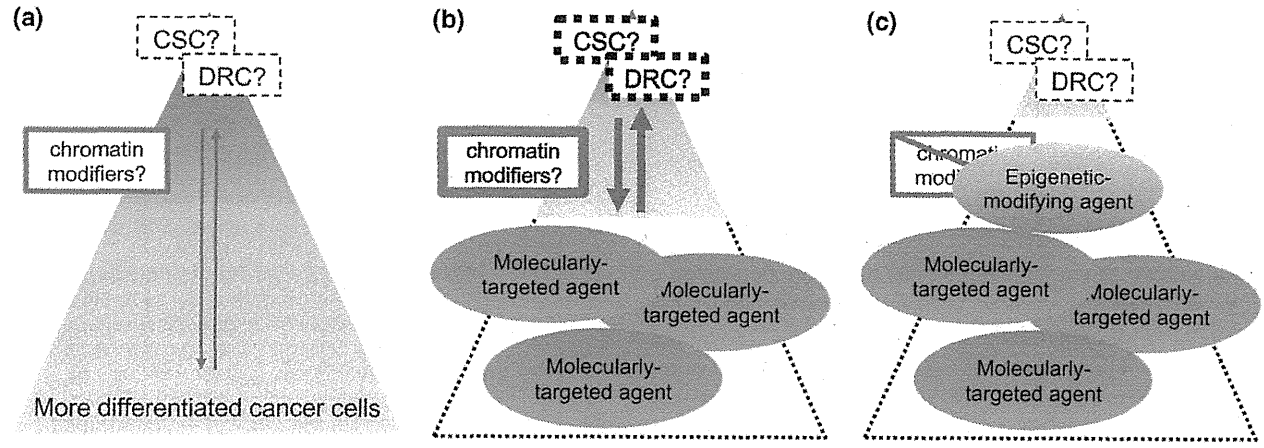

Figure 3 Possible strategy of cancer treatment to evade CSC and/or DRC emergence. (**a**) Cancer cells in tumor lesion are heterogeneous and their status is fluctuating possibly via epigenetic changes, which could lead to the emergence of cancer stem cells (CSCs) and/or drug resistant cells (DRCs). (**b**) Treatment with molecularly-targeted agent kills a large volume of tumor cell mass. Yet at the same time, blockade of the oncogenic signaling might increase the epigenetic flexibility in cancer cells, resulting in the promotion of CSC and/or DRC emergence. (**c**) Combination therapy with molecularly-targeted agent and epigenetic-modifying agent might yield better outcome in cancer therapy. In the presence of epigenetic-modifying agent that prevents cancer cells from obtaining epigenetic flexibility, the emergence of CSCs and/or DRCs might be diminished, which maximizes the efficacy of molecularly-targeted agent.

targeted drugs destabilizes the cancer cell epigenome, resulting in cell state conversion into drug resistant cancer cells. If this is the case, the underlying mechanisms for the recovery of the epigenetic flexibility in cancer cells triggered by the blockade of oncogenic signals could be a therapeutic target aiming for the prevention from the drug resistant cell emergence. Combination therapy of molecularly-targeted agents and chromatin-modifying agents (i.e., epigenetic-modifying agents) that block the conversion of epigenetic states in tumor cells might be one of the possible strategies to prevent the emergence of drug resistant cancer cells during the course of cancer therapies (Fig. 3).

It is noteworthy that the attempt to reprogram the cancer cell has already provided us a useful platform to uncover the epigenetic mechanisms for the emergence of drug resistant cancer cells. Successfully reprogrammed human chronic myeloid leukemia (CML) cells have lost their dependency on the signaling of BCR-ABL oncogene and become resistant to BCR-ABL inhibitor imatinib although they consistently express BCR-ABL,[45,46] indicating that epigenetic changes occurred during the reprogramming process endowed the CML cells drug resistance. Uncovering the epigenetic events that have altered drug sensitivity of the CML cells during the reprogramming process should provide us useful information to make strategy of curable cancer treatment. Application of the reprogramming technology to cancer research possibly broadens our understanding of the epigenetic status of cancer cells. Further accumulation of evidence might direct us to successful development of recurrence-free cancer therapies.

CONCLUSION

Reprogramming technology using defined transcription factors has brought a big impact on researchers in a wide variety of fields. Scientists have expected a great perspective with iPSCs. Functional somatic cells derived from iPSCs can be used for cell transplantation therapy in regenerative medicine, and patient-specific iPSCs are expected to be a powerful tool to understand pathogenesis and to discover drugs for many diseases such as diabetes and neurodegenerative diseases. These expectations will probably come true in near future. For example, research of retinal regeneration from iPSCs is being conducted intensively to put the clinical applications into practice.

Apart from these 'straightforward' applications of iPSC technology, we have presented here the idea of applying the reprogramming technology to cancer research. Accumulating evidence indicates the existence of epigenetic fluctuation in cancer cells. Depending on the epigenetic flexibility, a single cancer cell can give rise to various cells with different properties over the course of time. This epigenetic fluctuation might render cancer cells convert themselves from drug sensitive state to drug resistant state, which is closely related to the emergence of CSCs. By utilizing the reprogramming technology as a tool to introduce the 'pressure' to alter epigenetic regulations, we might be able to elucidate the epigenetic behavior that is unique to cancer cells. Eventually, cancer research field may get a lot of fruit from this new blanch of technology from the aspect of epigenetic regulations involving CSC emergence. Further studies will be

needed to broaden the possible applications of this novel technology and finally to develop novel cancer treatments that can evade tumor recurrence by targeting the drug resistant cancer stem cells.

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Review

Epigenetic regulation leading to induced pluripotency drives cancer development *in vivo*

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ABSTRACT

Somatic cells can be reprogrammed into induced pluripotent stem cells (iPSCs) by the transient expression of reprogramming factors. During the reprogramming process, somatic cells acquire the ability to undergo unlimited proliferation, which is also an important characteristic of cancer cells, while their underlying DNA sequence remains unchanged. Based on the characteristics shared between pluripotent stem cells and cancer cells, the potential involvement of the factors leading to reprogramming toward pluripotency in cancer development has been discussed. Recent *in vivo* reprogramming studies provided some clues to understanding the role of reprogramming-related epigenetic regulation in cancer development. It was shown that premature termination of the *in vivo* reprogramming result in the development of tumors that resemble pediatric cancers. Given that epigenetic modifications play a central role during reprogramming, failed reprogramming-associated cancer development may have provided a proof of concept for epigenetics-driven cancer development *in vivo*.

Contents

1. Introduction

During cellular reprogramming, multiple cellular processes act synergistically in a sequential manner [1–3]. However, the precise mechanism underlying the process of somatic cell reprogramming toward pluripotent stem cells remains unclear [4]. The dynamic reorganization of epigenetic modifications is considered to play a fundamental role in inducing pluripotency, leading to iPSC derivation [5]. Indeed, the functional importance of epigenetic regulation during the reprogramming process has been highlighted by recent studies showing that epigenetic modifications represented a bottleneck for iPSC derivation in both the early and late stages of cellular reprogramming [6,7]. The fact that the DNA methylation status at imprinted loci is associated with the quality of iPSCs supports the notion that proper remodeling of epigenetic modifications is essential to achieve successful reprogramming [8].

The efficiency of iPSC derivation is generally low, and only limited populations of intermediate cells give rise to iPSCs, which hampers the complete understanding of the reprogramming

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process [9]. It is known that non-iPSC like colonies often appear in culture dishes at the intermediate stage of cellular reprogramming *in vitro*. Although cells deviated from successful reprogramming contain metastable cells [10–12], the nature of such failed reprogramming state is unclear. Considering the fundamental role of epigenetic regulation in cell fate maintenance and conversion, it is expected that the failed reprogramming is attributable to the incomplete/unsuccessful reorganization of the epigenetic modifications.

It is noteworthy that the process of cell reprogramming toward a pluripotent state shares many characteristics with cancer development, although the process is not accompanied by the genetic alterations that are believed to be the causative abnormalities in most cancers. Somatic differentiated cells acquire self-renewing activities with unlimited proliferation properties during iPSC derivation, which is also a critical event during carcinogenesis. The metabolic switch to glycolysis that occurs during somatic cell reprogramming is similarly observed during cancer development [13,14]. Furthermore, Ben-Porath et al. demonstrated that a subgroup of transcriptional regulators that are highly expressed in embryonic stem cells (ESCs) are preferentially expressed in poorly differentiated aggressive tumors, suggesting that pluripotency-related transcriptional activity contributed to the development of particular types of cancer [15]. Such similarities suggest that the reprogramming process and cancer development may be at least partly promoted by overlapping mechanisms.

Indeed, previous studies demonstrated that a loss of tumor suppressor function is associated with the efficient induction of pluripotency [16,17]. Additionally, the currently used reprogramming factors generally include *Myc* and *Klf4*, both of which act as oncogenes in particular somatic cells. *Oct3/4*, a critical factor for somatic cell reprogramming, is overexpressed in germ cell tumors (GCTs) [18], and it is functionally involved in the self-renewal of GCT cells, possibly through the maintenance of an undifferentiated state [19]. Together, these findings may provide a link between transcription factor-mediated reprogramming and cancer development.

To investigate the relationship between cellular reprogramming and cancer development, it would be useful to analyze the reprogramming process in the *in vivo* environment. However, most of the previous studies investigating cellular reprogramming have been performed *in vitro*, and it has been difficult to adapt the *in vitro* phenotypes to uncover the association of reprogramming with cancer development *in vivo*.

2. *In vivo* expression of *Oct3/4* results in the expansion of dysplastic cells

Oct3/4 is highly expressed in pluripotent stem cells and is one of the essential transcription factors required for cellular reprogramming [1,2]. Hochedlinger et al. previously induced *Oct3/4* in various cell types in living mice *in vivo* [20]. Transgenic mice with a reverse tetracycline transactivator at *Rosa26*, together with a doxycycline (Dox)-responsive element, followed by the *Pou5f1* (*Oct3/4*) gene at *Collagen 1a1* were used in their study. The Dox-treated *Oct3/4*-induced mice developed dysplasia in many epithelial tissues, including the intestine, skin and forestomach [20]. Importantly, the dysplastic growth was dependent on transgenic *Oct3/4* expression, since withdrawal of Dox treatment resulted in a reversion of the dysplastic cells into normal-looking cells. These dysplastic cells revealed similar properties to tissue stem/progenitor cells, suggesting that the maintenance of the proliferating tissue stem/progenitor state caused the expansion of the dysplastic cells. The dysplastic cell expansion was accompanied by the upregulation of β-catenin, which is observed in a wide variety of cancers [21–23]. However, such dysplastic lesions did not show any evidence of metastasis, which is one of the hallmarks of cancer. Considering the critical role of *Oct3/4* in cellular reprogramming, this dysplasia model implied a possible association of reprogramming with cancer development. However, it should be noted that *Oct3/4* alone is not sufficient to reprogram most somatic cells. Therefore, the results did not provide direct evidence that somatic cells are reprogrammed toward a pluripotent state *in vivo*, nor that reprogramming itself is involved in cancer development.

3. *In vivo* reprogramming

In contrast to the results in *Oct3/4* single-induced mice, a later study by the Hochedlinger group using four reprogramming factors (*Oct3/4*, *Klf4*, *c-Myc*, and *Sox2*)-inducible mice suggested the possibility that somatic cells can be reprogrammed *in vivo* [24]. The reprogrammable mice frequently developed spontaneous teratomas, presumably because of leaky expression of the reprogramming factors. Given that pluripotent stem cells can form teratomas when inoculated into immunocompromised mice, the teratoma formation in the reprogrammable mice suggested that iPSCs arose *in vivo*, and these could give rise to teratomas. This notion has been further proven by recent studies with the controlled expression of reprogramming factors *in vivo* [25,26]. For example, Abad et al. showed that circulating cells in the four reprogramming factors-induced mice contain pluripotent cells capable of contributing to chimeric mice. We also demonstrated that iPSC-like cells could be established from teratomas in the four factors-induced mice, and such iPSC-like cells may indeed have pluripotency, with the ability to robustly contribute to chimeric mice. Collectively, these studies revealed that somatic cells can be reprogrammed by the transduction of four factors *in vivo*, and suggested that the *in vivo* reprogramming system could be a unique and powerful tool to study the *in vivo* behavior of failed reprogrammed cells, as well as fully reprogrammed cells. It is important that teratomas are benign tumors. Therefore, while these results demonstrated that *in vivo* reprogramming induces benign teratoma formation, they did not connect cellular reprogramming with malignant tumor development.

4. Premature termination of *in vivo* reprogramming causes malignant tumor development

Notably, a phenotype distinct from the teratoma formation was observed in mice with transient expression of the four reprogramming factors [26]. It was shown that the initial histological change after the induction of reprogramming factors *in vivo* is the expansion of dysplastic cells in many organs. Interestingly, the early dysplastic cells were able to revert to a non-dysplastic phenotype upon the withdrawal of reprogramming factor expression, which was similarly observed in the single *Oct3/4*-induced mice (Fig. 1). This is not surprising, because *Oct3/4* was one of the factors induced in these mice. However, in sharp contrast to the *Oct3/4*-induced dysplastic cells or the four factors-induced early dysplastic cells, the four factors-induced late dysplastic cells (Dox treatment for longer than 7 days) often did not revert to a non-dysplastic state, and many of these cells continued to grow even after the withdrawal of the transgene expression (Fig. 1). Tumor formation was also observed in some organs, such as the intestine, kidneys and pancreas. These tumors were able to form secondary tumors in the subcutaneous tissues of immunocompromised mice. Furthermore, the tumor cells revealed invasive growth into surrounding tissue and metastasis into lymph nodes, both of which are known to be a hallmarks of cancer [27]. Similar transgene-independent tumors were induced by three factors lacking the *Myc* transgene, but not by three factors without the *Oct3/4* transgene, a critical

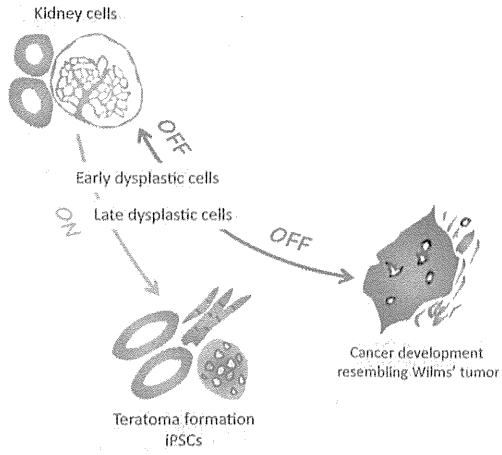

Fig. 1. *In vivo* induction of four reprogramming factors. Somatic cells can be reprogrammed into pluripotent stem cells *in vivo*, which is accompanied by teratoma formation. Dysplastic cell growth in somatic cells can be detectable in the intermediate stage of *in vivo* reprogramming. The early dysplastic cells revert to a non-dysplastic cells upon the withdrawal of the reprogramming factor expression. In contrast, the late dysplastic cells do not revert to a non-dysplastic state, and they continue to grow even after the withdrawal of the transgene expression, resulting in development of cancer, which resembles pediatric cancers, such as Wilms' tumors.

factor for reprogramming, demonstrating a tight connection between cancer development and reprogramming. Taken together, these findings indicated that premature termination of *in vivo* reprogramming results in cancer development, providing *in vivo* experimental evidence that failure in reprogramming into PSCs can drive cancer development.

5. Epigenetic regulation in failed reprogramming-associated cancer cells

The previous study dissected the ESC transcriptional program into three distinct modules; the Core, Myc, and Polycomb repressive complex (PRC) modules [28]. The ESC-Core module often includes genes related to the maintenance of pluripotency, while the ESC-Myc module contains a group of Myc target genes in ESCs. The ESC-PRC module is generally repressed in ESCs, and includes the genes that are bound by the Polycomb repressive complex in pluripotent stem cells. Of note, it was shown that the ESC-Core module and ESC-Myc module are similarly activated in both failed reprogramming-associated cancer cells and pluripotent stem cells when compared with those in normal kidney cells, indicating that there are shared characteristics between failed and successful reprogramming processes (Fig. 2). In contrast, many ESC-PRC module genes are aberrantly activated exclusively in failed reprogramming-associated cancer cells (Fig. 2). Since partially reprogrammed cells generated *in vitro* also reveal the failed repression of ESC-PRC module genes [12], the cancer cells in this model possess similar characteristics to the partially reprogrammed cells, suggesting that the partial reprogramming is associated with the failed reprogramming-associated cancer development.

However, aberrant activation of ESC-PRC target genes in cancer cells appears to contradict the current understanding of cancer epigenetics (Fig. 2). Previous studies found that ESC-PRC target genes are preferential targets for aberrant DNA hypermethylation in cancers [29–31]. Considering the repressive role of DNA hypermethylation at gene promoters, it would be expected that ESC-PRC targets are silenced in these cancers. Indeed, the previous study demonstrated that PRC2 target genes tend to be repressed in poorly differentiated cancers [15].

Altered DNA methylation patterns are the most extensively analyzed epigenetic abnormality in cancer cells [32]. Site-specific DNA hypermethylation and global DNA hypomethylation are shared alterations in the vast majority of cancers [33,34]. Indeed, in failed reprogramming-related cancers, a reduced representative bisulfite sequencing [35] analysis revealed global changes in the DNA methylation patterns (Fig. 2). However, in these failed reprogramming-related cancers, DNA hypermethylation at the proximal promoter regions was not evident, and the global DNA methylation level was comparable to that of normal cells, indicating a lack of both site-specific DNA hypermethylation and global DNA hypomethylation. Collectively, failed reprogramming-associated cancers showed distinguishable epigenetic properties from general cancers.

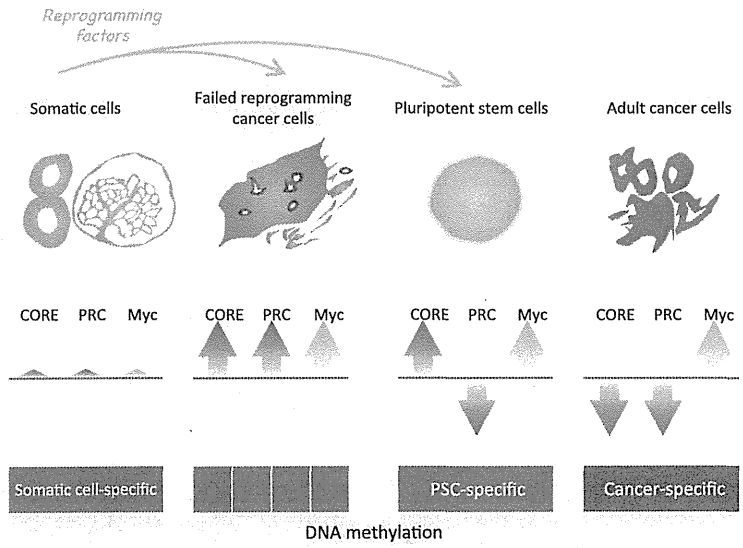

Fig. 2. Epigenetic regulation in failed reprogramming-associated cancer cells. The failed reprogramming-associated cancer cells reveal the different characteristics in epigenetic modifications from pluripotent stem cells or adult cancer cells. They show the activation of ESC-PRC module and the lack of cancer-specific DNA methylation alterations.

These discrepancies in epigenetic regulation raise an important concern regarding the application of this cancer model to investigate human cancer development. Given that failed reprogramming-associated cancers were induced through ectopic expression of four reprogramming factors that are not simultaneously expressed in somatic cells, these cancers are artificial, making the model less useful to uncover the mechanisms of human cancer development. However, it is noteworthy that failed reprogramming-associated cancers have a number of shared characteristics with pediatric cancers. Intriguingly, failed reprogramming-associated cancers in the kidney resembled Wilms' tumors, the most common pediatric kidney cancer. Additionally, the histology of liver tumors and pancreatic tumors showed similarities to those of hepatoblastomas and pancreatoblastomas, respectively. Of particular note, the ESC module activation in human Wilms' tumors shows a different pattern from that of adult cancers [26]. Wilms' tumors show the activation of all three modules (Core, Myc and PRC), recapitulating the findings in the mouse model. The failed reprogramming-associated cancer model could therefore be a model for pediatric cancers.

6. Genetic and epigenetic abnormalities in pediatric cancers

Cancer is considered to progress through multistep processes, which are associated with both genetic mutations and epigenetic abnormalities [27,32,36]. However, much attention has been paid to the role of epigenetic abnormalities in Wilms' tumors. Although some genetic abnormalities, such as *WT1*, *WTX* and *TP53* gene mutations [37,38] have been reported in Wilms' tumors, the incidence of these mutations is relatively low. In contrast, seminal studies identified that there are frequent epigenetic alterations in Wilms' tumors [39,40]. For example, the aberrant expression of *IGF2* with loss of imprinting, which is accompanied by increased DNA methylation at *IGF2/H19* locus, was identified in the majority of Wilms' tumors [40]. It is interesting to note that failed reprogramming-associated cancers in mice also showed abnormal DNA methylation at the *IGF2/H19* locus, with a number of shared molecular characteristics with Wilms' tumor. Similar types of epigenetic regulation between induced pluripotent cells and Wilms' tumor were further indicated by the previous findings that the chromatin profiles of Wilms' tumors resembled those of human ESCs [41]. Together, these findings indicated that Wilms' tumors harbor similar epigenetic changes as failed reprogramming-associated cancers induced by the transient expression of reprogramming factors. It is also noteworthy that some of epigenetics-related diseases such as imprinting disorders, Beckwith–Wiedemann syndrome and Angelman syndrome, are associated with pediatric blastomas [42,43]. Of note, the failed reprogramming-associated cancers in the liver and pancreas resembled hepatoblastomas and pancreatoblastomas, and the liver cancers were accompanied by the alteration of imprinting gene expressions. These findings provided a further connection between failed reprogramming-induced epigenetic regulations and pediatric blastoma development.

Recently, genome-wide sequencing studies have been performed in many types of cancers. These studies revealed that the rate of mutation is relatively low in most of the cancer-related genes [44]. A surprisingly low rate of genomic mutations was observed in pediatric cancers. Together with a previous finding regarding the close association between altered epigenetic modifications and the poor prognosis of pediatric cancers [45], the epigenetic changes associated with failed reprogramming might drive the development of particular types of pediatric cancers. Whole genome sequencing studies in ependymomas suggested that some ependymomas could occur without detectable driver mutations [46,47]. Notably, the rate of single nucleotide variants was very low in the ependymomas that occurred in the posterior fossa, while DNA hypermethylation was observed in progressive ependymomas, which support the notion that specific types of tumors might arise depending on the specific alterations in the epigenetic information.

7. Epigenetic reorganization toward pluripotency drives cancer development

Although altered epigenetic regulation can be detected in most cancer types, such epigenetic alterations may be attributable to the genetic mutations as a secondary effect. Consistent with this notion, somatic mutations at genes known to regulate the epigenetics have been often identified in a wide range of cancer types. For example, some glioblastomas harbor mutations in the H3.3-ATRX-DAXX pathway [48,49]. A mutation was detected in H3F3A, and was located at or just near the amino-terminal tail of the protein, which leads to post-transcriptional repression or activation.

It is of particular interest that failed reprogramming-associated cancer cells could be reprogrammed into iPSCs by the additional expression of reprogramming factors [26]. Notably, kidney cancer-derived iPSCs could contribute to adult chimeric mice that had normal-looking kidney tissues. Furthermore, tumor formation was not observed even in the elderly chimeric mice. Given that the DNA sequence remains unchanged during the reprogramming and re-differentiation of the reprogrammed cells, these findings indicated that the failed reprogramming-associated cancer genome is competent for normal development, and that genetic transformation is not a determinant of cancer development in this model. This further highlighted the primary and causative role of epigenetic regulations associated with induced pluripotency in the development of particular types of cancer.

However, it should be stressed that these findings do not provide evidence that the dedifferentiation process itself is involved in the development of human cancers, including Wilms' tumors. Further studies will be needed to identify the cancer types that are promoted by reprogramming-associated epigenetic changes. A better understanding would provide novel insights into effective cancer treatment targeting epigenetic regulation.

8. Conclusion

iPSC induction is accompanied by the acquisition of unlimited proliferative activity in somatic cells. Since epigenetic regulation plays a central role in the reprogramming process, it is suggested that epigenetic regulation leading to induced pluripotency confers self-renewing activity on somatic cells. Indeed, transient expression of reprogramming factors *in vivo* resulted in the development of cancer, independent of genetic transformation. A better understanding of the epigenetic regulation involved in induced pluripotency should be applicable to dissect the role of epigenetic regulation in cancer development, which eventually may contribute to the discovery of novel preventive or therapeutic strategies for cancers.

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Current Topics（カレントトピックス）

Ohnishi K, et al：Cell, 156：663-677, 2014

不完全な初期化によるエピゲノム発がん

大西紘太郎，蝉　克憲，山田泰広

がんは遺伝子変異の蓄積により生じると考えられている．われわれは，マウス生体内での体細胞の不完全な初期化によってがんが発生することを示した．それらの腫瘍細胞は，完全初期化により非腫瘍性の細胞に分化可能であることがわかった．これらの結果から“Genetic transformation”に依存しない発がん経路が存在することが強く示唆された．

発がんは遺伝子の変異の蓄積が主たる原因であると考えられてきた．近年エピゲノム制御にかかわる研究の進み，がんの発生・進展においてエピゲノム制御の異常が重要な役割を果たしていることが明らかになってきている．人工性多能性幹細胞（iPS細胞）は分化した体細胞に初期化因子（*Oct3/4*, *Sox2*, *Klf4*, *c-Myc*）を強制発現することで樹立することができる[1]．体細胞初期化過程において，細胞は広範に転写制御が変化し，最終的にがん細胞と同様の無限の増殖能を得る．一方で，体細胞初期化においては発がん過程とは異なり，特定の遺伝子配列に変化は必要なく，無限の細胞増殖能獲得にはエピゲノム制御の変化が重要な役割を果たしていることが示唆される．われわれは，細胞初期化過程にかかわるエピゲノム制御と発がんとの関連に着目し，生体内で細胞初期化因子を任意のタイミングで強制発現することができるマウスを作製し，一過性の初期化因子強制発現に続発する変化を観察した．

生体内での体細胞初期化の成功

われわれは既報をもとにドキシサイクリン誘導性に初期化因子を強制発現できる胎児性幹細胞（ES細胞）を作製した[2]．このES細胞由来のキメラマウスにドキシサイクリンを投与したところ，生体内のさまざまな組織で初期化因子を強制発現できることが確認できた．初期化因子を誘導し続け，28日目に解剖し観察したところ諸臓器に腫瘤形成を認めた．これらの腫瘤を組織学的に解析したところ，三胚葉各々への分化傾向を示す奇形腫であることが判明した．興味深いことに，この奇形腫を摘出し培養したところ，キメラマウスへの寄与能をもつiPS細胞を樹立することができた．このことから奇形腫中に多能性幹細胞が存在することがわかった．すなわち，初期化因子誘導可能マウスの生体内にて体細胞が初期化されたことが示された．

Premature termination of *in vivo* reprogramming reveals cancer development through epigenetic regulation

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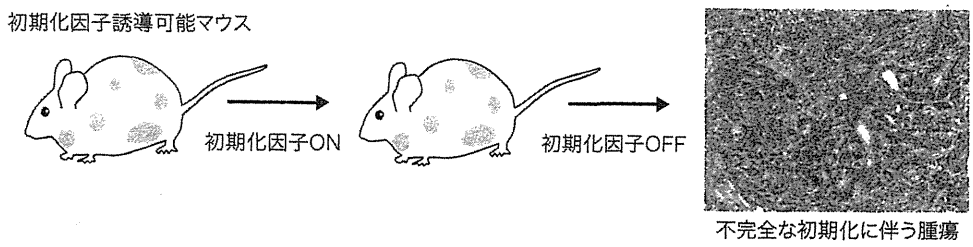

図1　不完全な初期化に伴う腫瘍の形成
ドキシサイクリン誘導性に初期化因子を生体内で強制発現させることができるマウスを作製し，初期化因子を一過性に発現することで腫瘍を形成する

生体内体細胞初期化を途中で停止すると腫瘍を形成する

次にわれわれは生体内で体細胞が初期化され，奇形腫が発生するまでの変化に興味をもった．生体内初期化過程の早期変化を同定するために，ドキシサイクリン投与後3～9日目のマウスを組織学的に解析した．早期変化として，上皮性組織を中心にがん細胞に類似した異型細胞※1が出現することがわかった．盛んに分裂増生する異型細胞に一致して初期化因子の発現を認め，初期化因子の発現が異型細胞の出現の原因となっていることが確認された．

次に，これら異型細胞の初期化因子依存性について検討した．初期化因子誘導可能マウスにドキシサイクリンを7日間投与し初期化因子を発現させた後，初期化因子発現を停止させ7日後に組織学的解析を行った．初期化因子の発現を停止しているにもかかわらず，一部のマウスにおいては増生し続ける腫瘍細胞が出現することがわかった．これらの腫瘍は奇形腫とは異なり未分化な異型細胞から構成され，周囲の組織への浸潤能，転移能，免疫不全マウス皮下組織での腫瘍形成能を認め，がんと同様の性質をもつことが確認された．初期化因子にはがん遺伝子である*c-Myc*が含まれるが，*c-Myc*を除く3因子を誘導できるマウスを作製し同じ実験を行ったところ，同様の腫瘍形成を認めた．しかしながら，体細胞初期化に必要とされる*Oct3/4*を除く3因子では腫瘍形成は認めなかった．以上より，初期化因子発現停止に伴う腫瘍は体細胞初期化に関連し発生する腫瘍であることが示唆された．

不完全な細胞初期化による腫瘍ではエピゲノム制御に変化を認める

次に，不完全な細胞初期化による発がんメカニズムの解明をめざした．初期化因子発現停止に伴う腎臓腫瘍において，マイクロアレイ法を用い遺伝子解析を行った．腎臓腫瘍では腎臓分化細胞に特異的に発現する遺伝子群の発現が低下していた．一方，ES細胞で特異的に発現している遺伝子群の発現が上昇していることが判明した．しかしながら，体細胞初期化のマーカーとなる*Nanog*や内在性*Oct3/4*の発現は多能性幹細胞に比して低く，腫瘍細胞は不完全な細胞初期化状態を反映していた．多能性幹細胞においてPolycomb抑制複合体にて発現が抑制されている遺伝子群が，腎臓腫瘍では発現が抑制されておらず，Polycombによる遺伝子発現抑制不全が細胞初期化失敗の原因の1つと考えられた．

次にわれわれは，不完全な細胞初期化に伴う腎臓腫瘍におけるDNAメチル化状態をRRBS（reduced representation bisulfite sequencing）法※2にて解析した[3]．腎臓腫瘍では正常腎臓細胞と比較して広範囲にDNAメチル化状態が変化していた．多能性幹細胞で

※1　異型細胞
正常組織細胞と比較し，異常な細胞構造を示すもの．この異常な細胞構造は細胞異型と定義され，構造異型とあわせて悪性腫瘍の組織学的特徴とされている．細胞異型の特徴としては，核の腫大・核の大小不同・核形不整・クロマチンの粗造化などがあげられる．

※2　RRBS（reduced representation bisulfite sequencing）法
プロモーター領域やCpGアイランド領域といったCpG配列に富むゲノムDNA領域を制限酵素により切断および回収し，バイサルファイト処理を行ってDNAメチル化状態を解析する手法．より少量のサンプルでも，網羅的にCpGアイランドを中心としたDNAメチル化状態の解析を行うことができる．

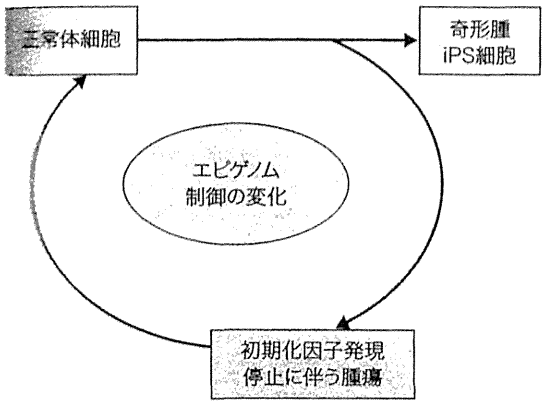

図2　本研究において示されたエピゲノム制御の変化に伴う生体内変化

に DNA がメチル化されている遺伝子群は，腎臓腫瘍ではすでにメチル化を獲得していた．一方で正常腎臓細胞でメチル化されている遺伝子群においては，腎臓腫瘍でもメチル化が維持されており，脱メチル化反応が不完全であることがわかった．一般にがん細胞では領域特異的に高い DNA メチル化状態を認める一方で，ゲノムワイドには低いメチル化状態を呈することが知られている．しかしながら，不完全な細胞初期化による腎臓腫瘍ではこの傾向は認めなかった．

不完全な細胞初期化による腎臓腫瘍は Wilms 腫瘍に類似する

初期化因子発現停止に伴う腎臓腫瘍は，組織学的特徴が小児腎腫瘍の1つである Wilms 腫瘍に類似していた．Wilms 腫瘍ではしばしばインプリンティング遺伝子の1つである *Igf2* の発現異常やその近傍の遺伝子座に存在する *H19* の DNA メチル化状態の異常が報告されるが，われわれの腎臓腫瘍でも同様の異常が観察された．また，遺伝子発現解析の結果，われわれの腎臓腫瘍で発現が上昇している遺伝子群は Wims 腫瘍でも発現が上昇していること，Wilms 腫瘍では ES 細胞で特異的に発現する遺伝子群が発現しているものの，Polycomb 抑制複合体の標的遺伝子群の発現が抑制されていないことが確認できた．以上より，体細胞初期化に関連するエピゲノム制御の変化が Wilms 腫瘍の発生および維持に関与する可能性が示唆された．

不完全な細胞初期化による腫瘍細胞から iPS 細胞を経て正常組織を誘導できた

われわれは初期化因子発現停止に伴う腎臓腫瘍細胞の初期化を試みた．まず，不完全な細胞初期化による腎臓腫瘍で *Lgr5* の発現が特異的に上昇していることを同定した．*Lgr5-EGFP* レポーター遺伝子を用いて *Lgr5*-EGFP 陽性の腎臓腫瘍細胞を FACS にて回収し，再度初期化因子を誘導し完全初期化をめざした．すると，正常腎臓細胞を初期化するよりも高効率および短期間で iPS 細胞様の細胞を得ることができた．これらの腎臓腫瘍由来 iPS 細胞は，初期胚へのインジェクションによってキメラマウスに寄与し，非腫瘍性の腎臓細胞へと分化した．この結果は，不完全な細胞初期化による発がんが，遺伝子配列変化に依存していないことを示唆するとともに，多能性幹細胞獲得に関連したエ

筆頭著者のつぶやき

私は M.D. であり，卒後5年目に現在の研究所に来て基礎研究をはじめました．卒後2年間は初期臨床研修，その後入局し2年間は自分の専門となる消化器内科の臨床経験を積むことで精一杯であり，正直なところ現在の研究所に来た当初は基礎研究についての知識も経験も全くない状態でした．そのなかで研究をはじめたので，論文アクセプトに至るすべての過程がはじめての経験でつまずくことの連続でした．失敗が続いていたときに気をつけていたのは，決して後ろ向きにならず前向きに物事を考えることでした．幸いにも私の周りの方々は優しく，根気よく指導してくれましたし家族も支えてくれました．すでに，あれほど忙しく日々過ごしていた卒後4年間の病院勤務と同期間基礎研究に従事していますが，それと同等以上の内容の濃い日々を過ごせていると思います．

（大西紘太郎）

ピゲノム制御が発がんの原因となりうることを示している．

おわりに

生体内での不完全な細胞初期化が小児がんに類似した発がんをひき起こすことが明らかとなった．この腫瘍細胞由来のiPS細胞は正常な体細胞へと分化可能であることを示した．これらの一連の流れにおいて中心的な役割を果たしているのはエピゲノム制御の変化であり，がんの発生・増生におけるエピゲノム制御の重要性が示唆された．

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● 筆頭著者プロフィール ●

大西紘太郎：2006年，岐阜大学医学部卒業．2年間の岐阜大学医学部附属病院での初期臨床研修後，同大学医学部消化器病態学講座に入局．高山赤十字病院での臨床経験を経て，'09年に岐阜大学大学院医学系研究科に入学，'10年より京都大学iPS細胞研究所初期化機構解析部門山田研究室にて特別研究生として従事している．興味のある研究分野はreprogramming，がんとepigenetics．

リプログラミング技術を用いたがんエピゲノム研究

蝉 克憲，山田泰広

2012年にノーベル生理学・医学賞を受賞したJohn Gurdon博士，山中伸弥博士によって報告された体細胞核および体細胞の初期化は再生医療の実現につながる技術基盤を確立した．近年，体細胞初期化技術はさまざまな生命科学分野の研究に応用されつつある．がんの研究分野においても，これらリプログラミング技術の応用がはじまっており，これまで技術的に解明困難であった問題を解決するための有効なツールとなっている．本稿では，体細胞リプログラミング技術を応用したがん研究への試みについて紹介するとともに，それらの解析から明らかとなってきたがんにおけるエピゲノムの重要性について解説する．

キーワード● *in vivo* リプログラミング，がんエピゲノム，エピゲノム発がん

はじめに

2006年に山中伸弥博士によって発表された体細胞リプログラミングは，線維芽細胞に対して，多能性幹細胞に高発現する4つの遺伝子（*Oct3/4*，*Sox2*，*Klf4*，*cMyc*）を一過性に強制発現させ，ゲノムの改変を必要とせずエピゲノムの変化を誘導することで細胞の運命を転換させる技術である[1]．この報告によって，細胞の運命はいくつかの転写因子（マスター因子）とそれらによって構築されるコアとなる転写ネットワークによって形成されること，他種細胞のマスター因子の強制発現によりエピゲノムの改変を伴うリプログラミングを誘導することが可能であることが示された．

一般にがんは遺伝子変異の蓄積により生じる疾患であると考えられている．がんの発生過程に生じる遺伝子変異は，がん発生において機能的に重要なドライバー変異と偶発的に生じたパッセンジャー変異の2つに分類することができる．ドライバー変異を有した遺伝子により制御される転写因子群をがんにおけるマスター因子と考えると，ドライバー変異を有する遺伝子により形成される転写ネットワークの活性化が，エピゲノム状態に改変を誘導し，正常細胞からがん細胞へのリプログラミングを誘導しているとも考えられる．がん細胞の理解にリプログラミングという概念を応用することで，新しいアプローチでのがん研究が展開されつつある．

1 がん研究におけるリプログラミング技術の利用

❶ リプログラミングによるがんエピゲノムの改変

iPS細胞の報告以前にも，細胞初期化技術によるがん細胞のリプログラミングが行われてきた．核移植による体細胞核の初期化は，1962年にJohn Gurdon博士によって確立された手法であり，脱核した未受精卵に正常体細胞の核を移植することで行われる．2004年にがん細胞核の核移植による初期化が報告されているが，大部分のがん細胞核では初期化が十分に誘導されず，

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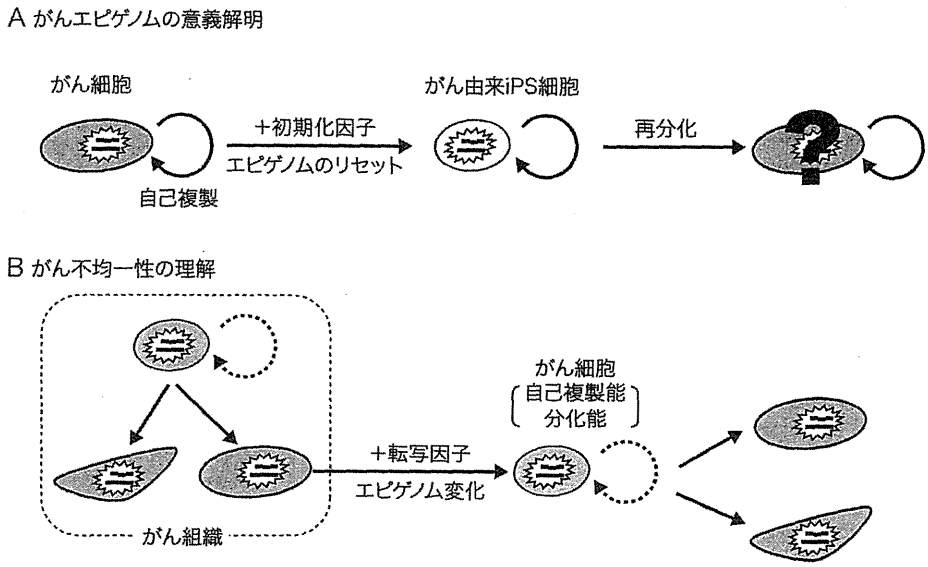

図1　がん研究における体細胞リプログラミング技術の利用
A）がん細胞のリプログラミングにより，がん特異的なエピゲノム異常をリセットする．その後，再分化の後に元のがん細胞との性質を比較する．B）腫瘍造成能の高いがん細胞に特徴的な転写因子を強制発現させ，がん細胞の性質を変化させることが示されている．

核移植後の胚からES細胞（nuclear transfer ES細胞：ntES細胞）の樹立が困難であることが報告されている．すなわち，がん細胞のエピゲノム状態は安定的に維持され，改変困難であることが示唆された．しかし，マウスメラノーマ細胞核では，がん遺伝子である*RAS*非存在下においてntES細胞の樹立およびキメラマウスの作製に成功していることから，がん細胞のもつ安定的なエピゲノム状態は，がん遺伝子発現により維持されていることが示唆されている[2]．このように，マウスにおいては核移植によるがん細胞の初期化が行われてきたが，ヒトがん細胞の初期化が行われたのは，iPS細胞登場以後である．これはiPS細胞が核移植と比べ簡便な技術である点が大きい．マウスおよびヒトにおいて，初期化4因子の導入によるがん細胞のリプログラミングの報告はいくつかなされている[3)~5)]が，その成功例は少なく，またがん由来iPS細胞の樹立効率が低いことが報告されている．その一方で，リプログラミングに成功した論文では，興味深い報告がなされている．がん遺伝子である*BCR-ABL*融合遺伝子を発現する慢性骨髄性白血病（chronic myelogenous leukemia：CML）患者から単離された白血病細胞を初期化したCML-iPS細胞，およびCML-iPS細胞から分化させた未分化な血球系細胞は，*BCR-ABL*融合遺伝子を発現しているにもかかわらず，抗がん剤であるimatinibに対する感受性を失うが，同iPS細胞から分化させた成熟血球系細胞は，imatinibに対する感受性を再び獲得する．これは，薬剤感受性に対して，がん細胞のエピゲノム状態が深く関与していることを示しており，エピゲノム制御によるがん治療の可能性を提示している（**図1A**）．

❷ リプログラミングによるがん不均一性の理解

リプログラミング技術はがん組織における不均一性（heterogeneity）の理解にも応用されている（**図1B**）．がん組織は一様な細胞集団ではなく，がんの発生およびその進展の過程で性質の異なる多様な細胞へと変化していくことが知られている．これらの不均一性は，がん進展過程での遺伝子変異の蓄積，または酸素条件や栄養条件，細胞傷害性のストレス，免疫応答などの環

境変化によって誘導されると考えられている．これまで，これらの不均一性がゲノム異常によるものか，エピゲノム制御変化によるものかについてのさかんな議論がなされてきた．がん幹細胞は，一般的に腫瘍組織に対して細胞を供給する組織幹細胞のようなものとして捉えられており，自己複製能および多分化能を有し，時にその細胞分裂速度はきわめて緩やかであることが知られている．これらの特性が，がんの不均一性を生み出す一端となっているとともに，化学療法や放射線治療に対する耐性獲得や治療後の再発の原因となりうることが示されている．がん細胞に転写因子を一過性に発現させ，エピゲノム変化を誘導することで，がん幹細胞の誘導を行った報告がいくつかなされている．

ヒト神経膠芽腫（グリオーマ）は，最も予後が悪いとされている固形腫瘍の1つであり，腫瘍組織中に自己複製能および多分化能を有するグリオーマ幹細胞の存在が確認されている．Bernsteinらのグループは，ヒト神経膠芽腫に存在する分化度の異なる2つの細胞集団を表面抗原マーカー（CD133）により単離し，High-throughput sequencerを用いた解析から，グリオーマ幹細胞特異的に発現する遺伝子群を同定し，それらのがん細胞への一過性強制発現を行った．その結果，*OLIG2*, *POU3F1*, *SALL2*, *SOX2*の4因子を強制発現させることで，がん幹細胞様の性質を有するiTPCs (induced tumor-propagating cells) の誘導に成功した[6]．さらに，これら4因子のノックダウンにより，CD133陽性のTPCsを移植したマウスの生存率が劇的に高まることを示し，ヒトグリオーマ組織におけるがん幹細胞様の性質をもつTPCsの出現には，4つの転写因子とその発現に伴うエピゲノム変化が重要であることが明らかとなった．さらに，4因子の存在下で発現が誘導されるヒストンH3K4の脱メチル化酵素であるLSD1，およびLSD1と複合体を形成するRCOR2が，未分化腫瘍治療のターゲットとなる可能性を示した[6]．この報告は，腫瘍組織中に存在するがん幹細胞の出現にエピゲノム変化が密接に関与している可能性を示唆するとともに，がん幹細胞の成立メカニズムの解明や，創薬スクリーニングに対して体細胞リプログラミング技術が有効なツールであることを示した．

2 生体内細胞初期化技術によるエピゲノム発がん

❶ 生体内細胞初期化システムの構築

前述のように，がんは遺伝子変異の蓄積によって生じる．通常，同一種類に分類されるがんでは，しばしば共通の遺伝子変異やゲノム異常が観察され，それらはドライバー遺伝子であると認識される．しかしその一方で，特に小児がんにおいては遺伝子変異頻度が少なく，共通の変異が確認できないがんも存在することが明らかとなってきた．われわれはこの点に着目し，エピゲノムの異常を主な原因とするがんが存在するのではないかと考えた．特に細胞初期化過程と発がん過程の類似性に着目し，細胞初期化に伴うエピゲノム変化により発がんが起こる可能性について検討を行った．まず生体内で細胞初期化にかかわるエピゲノム変化を誘導するために，ドキシサイクリン誘導性に初期化因子を強制発現させるマウスを作製した．このマウスにドキシサイクリンを投与したところ，生体内のさまざまな組織で初期化因子の発現が認められた．さらに，長期間の初期化因子発現により，諸臓器に三胚葉への分化が認められる奇形腫の発生が確認された．このことは，生体内で体細胞リプログラミングが誘導されたことを示している．事実，奇形腫から試験管内においてiPS細胞を樹立することができた．以上より生体内で体細胞を初期化可能であることが示された．

❷ 不完全な初期化が誘導するエピゲノム異常と発がん

作製した生体内細胞初期化システムを用いて，初期化因子発現に対する体細胞の振る舞いを組織学的に解析した．わずか数日の初期化因子発現誘導により上皮性組織を中心にがん細胞に類似した異型細胞の出現が認められた．しかし早期の異型細胞の異常増殖は初期化因子の発現に依存しており，初期化因子の発現を中断することにより非腫瘍性の由来細胞へと再分化することがわかった．この結果から，体細胞のアイデンティティーを維持するエピジェネティックメモリーが存在することが示唆された．しかしながら，初期化因子の発現を約1週間誘導すると，自立性に異常増殖を続ける腫瘍細胞の出現が観察された．これらの腫瘍は奇形

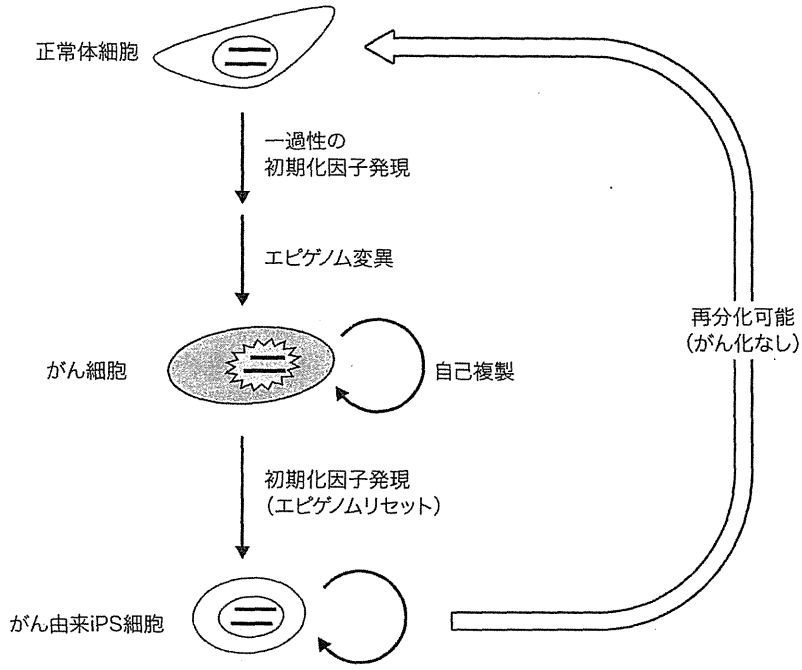

図2　エピゲノム発がんモデル
分化した体細胞に対して初期化因子の発現を一過性に行うことで，エピゲノムの変化を誘導することが可能である．エピゲノム変化を受けた細胞は，がん細胞と同様の性質を有している（増殖能，浸潤能）．これらがん細胞にリプログラミングを誘導し，完全初期化を誘導することでiPS細胞を樹立した．がん細胞由来iPS細胞は，非腫瘍性の体細胞へと分化することができた．このことは，ゲノム異常によらない発がんの可能性を示唆している．

腫とは異なり，未分化な異型細胞により構成され，さらに周囲組織への浸潤能や転移能，免疫不全マウス皮下組織中での腫瘍形成能を示したことから，がんと同様の性質を有していることが明らかとなった[7]．

次に，不完全な初期化により腎臓に形成された腫瘍に着目して発がんメカニズムの解明を行った．腫瘍細胞の遺伝子発現パターンはES細胞のそれに近づきつつあるものの，*Nanog*や*Oct3/4*といったES細胞のマーカーとなる遺伝子の発現は多能性幹細胞と比較して低く，初期化が不十分であることが示唆された．続いて，DNAメチル化に着目し，腫瘍細胞におけるエピゲノム変化をRRBS※（Reduced representation of bisulfite sequence）法により解析した．その結果，腫瘍細胞のDNAメチル化状態は，ES細胞におけるDNAメチル化状態に部分的に類似していることがわかった．特に，ES細胞で高度にDNAのメチル化を受けている遺伝子群については，腫瘍細胞においてもすでにDNAメチル化が獲得されていた．一方，正常体細胞で高度にメチル化されている遺伝子は腫瘍細胞においてもメチル化が保たれており，脱メチル化が十分に誘導されていないことが明らかとなった．以上から，このモデルにおける腫瘍細胞では初期化に向かう部分的なエピゲノム変化が誘導されていることが考えられた．

次にこの発がんがエピゲノム変化に依存しているかを検討するために，腎臓腫瘍細胞を単離し，そのリプログラミングを試みた．その結果，正常腎臓細胞よりも短期間，高効率でiPS細胞様のコロニー形態をもつ

※　RRBS
Reduced representation of bisulfite sequenceの略．High throughput sequencerを用いたゲノムワイドなDNAメチル化解析法の1つで，CpG richなプロモーター領域のDNAメチル化を1塩基の解像度で高精度かつ非常に少ないリード数で解析することが可能である．