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Review Article

Reprogramming of gastrointestinal cancer cells

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Cell reprogramming reverts cells to multipotent, preprogrammed states by re-establishing epigenetic markers. It can also induce considerable malignant phenotype modification. Because key events in cancer relapse and metastasis, including epithelial–mesenchymal transition phenotypes, are regulated primarily by reversible and transient epigenetic modifications rather than the accumulation of irreversible and stable genetic abnormalities, studying dynamic mechanisms regulating these biological processes is important. Transcription factors for induced pluripotent stem cells and non-coding microRNAs allow pluripotent phenotype induction. We present the current knowledge of the possible applications of cell reprogramming in reducing aggressive phenotype expression, which can induce tumor cell hibernation and maintain appropriate phenotypes, thereby minimizing relapse and metastasis after surgical resection of gastrointestinal cancer. (*Cancer Sci* 2012; 103: 393–399)

During cell reprogramming, mature cells revert to an immature, preprogrammed (undifferentiated) state, and usually acquire multidifferentiated characteristics following the loss and re-establishment of important epigenetic markers including DNA methylation.[1] This deregulation of important genomic and epigenomic factors is commonly associated with the abnormal cell differentiation characteristics of different cancers.[2–7] Emerging data suggest that epigenetic modifications and cell reprogramming-like processes are important for cellular transformation and the development of malignant cancer phenotypes.[8–12] Understanding the underlying process of epigenome reprogramming facilitates the use of regenerative medicine and cancer therapy. Here we discuss whether the reprogramming-like phenomenon observed in normal cells can be adapted for developing novel therapies.

Programming and Reprogramming of Cells

Mammalian tissues develop from a totipotent zygote. During cell differentiation, a less specialized cell (i.e. stem or progenitor cell) continuously produces more specialized cell types through cell division, and thus, a complex tissue system containing increasingly differentiated and specialized cells is established. Subsequently, pluripotent primitive ectodermal cells in the inner cell mass of blastocysts develop from the totipotent zygote.[13,14] Following blastocyst implantation, pluripotent epiblast cells differentiate into somatic cells. Repression of the somatic program and re-expression of pluripotency-specific genes through epigenetic modifications are necessary for germ cell development,[13,15] indicating that differentiated cells retain all the genetic information necessary to generate an entire organism. This was initially tested by cloning animals from differentiated cells by nuclear transfer. Mouse[16,17] and human ES cells[18] are derived from their respective blastocysts. The recently developed iPSCs[19,20] can produce derivatives of each germ layer. Differentiation occurs both during the developmental stages and in adults;[21] multipotent tissue stem cells produce completely differentiated daughter cells during normal cell turnover in adult tissues and during tissue repair.[22] Differentiation is associated with dynamic alterations in cell morphology, cell metabolism, and responsiveness to cell signaling,[21,23] which occur largely because of highly regulated gene expression through mRNA regulation[23] and non-coding miRNA expression.[24–26]

Defined factor-mediated reprogramming. Considering the ethical issues regarding the use of fertilized oocytes for establishing and producing ES cells, and the immunological compatibility that occurs in case of unrelated donors, a great breakthrough was reported by Takahashi and Yamanaka[19], who discovered that complete reprogramming can be achieved by introducing defined biological factors, such as Oct4 (also known as Pou5f1), Sox2, Klf4, and c-Myc, in mouse and human fibroblasts.[20] The initial experimental injection of Fbx15-selected iPSCs into mouse blastocysts revealed that iPSCs alone could not efficiently produce chimeric mice, presumably because of substantial methylation of immature gene (including Nanog and Oct4) promoters.[19] Subsequent studies indicated that modified selection methods of completely reprogrammed cells through expression of endogenous Nanog[27,28] and Oct4[28] allowed the successful generation of viable chimeras and detectable transmission into the germline.

Complete pluripotency. During stable Oct4 and Nanog selection, although the overall appearance of the colonies was similar,[27,28] quantitative differences existed between the two selection strategies. Oct4-selected ES-like colonies provided more stable and homogenous iPSC lines than Nanog-selected ES-like colonies.[28] Eventually, the fraction of ES-like colonies from Oct4-selected MEF cultures was two or threefold higher than that from Nanog-selected cultures, although initially fewer colonies existed with Oct4-selected MEF-derived iPSCs. This suggests that although the Nanog locus was more easily activated, a higher fraction of colonies from Oct4-selected MEF cells was reprogrammed to pluripotency.[28] These studies established that selection for Oct4 and Nanog expression results in germline-competent iPSCs with increased ES cell-like gene expression and DNA methylation patterns compared with Fbx15-iPSCs. Whereas one clone from seven Nanog-iPSC clones was transmitted through the germline to the next generation,[27] Oct4-iPSCs injected into tetraploid blastocysts can generate live late-term embryos.[28] The biological potency and epigenetic state of iPSCs and ES cells are the same. The overall estimated efficiency (0.05–0.10%) to establish iPSC lines from

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MEFs was similar between Oct4 and Nanog selection, despite the larger number of total Nanog-iPSC colonies[28] (Table 1).

Reprogramming barriers by tumor suppressors. Several factors can enhance the efficiency of iPSC generation, such as cell cycle checkpoints mediated by the cyclin-dependent kinase inhibitor family. The *CDKN2b–CDKN2a* locus on human chromosome 9p21 (mice chromosome 4) is frequently lost in cancer. The locus encodes three cyclin-dependent kinase inhibitors of the cell cycle: *p15INK4b*, *p16INK4a*, and *p14ARF* (*p19Arf* in mice) encoded by *CDKN2b*, *CDKN2a*, and an alternative reading frame of *CDKN2a*, respectively.[29] These inhibitors are endogenously expressed in differentiated cells and downregulated

Table 1. Summary of current studies of normal somatic cell reprogramming

Method for factor delivery	Factor	Starting material	Efficiency	Ref.
Retroviral vector	OSKM	Mouse embryonic and adult fibroblast	n.d.	(19)
Retroviral vector	OSKM	Human fibroblast	10 colonies/5×10^4	(20)
Lentiviral	OSNL	Human fetal fibroblast	198 colonies/0.9×10^6	(71)
Plasmid transfection	OSKM	MEF	Lower than the viral delivery method	(41)
Adenovirus (non-integrating vector)	OSKM	MEF and hepatocyte	n.d.	(72)
Retrovirus	OSKM	Adult mouse liver and stomach cells	n.d.	(73)
Retrovirus	OSKM OK OM	Mouse neural stem cell	3.6% 0.11% Slower than OK	(74)
Retrovirus	OSKM+5′Aza OSK+VPA/5′Aza	MEF	0.5% 11.8% 100 × higher than the OSKM method	(75)
Doxycycline-inducible lentiviruses	OSKM	Secondary somatic cells containing Dox-inducible OSKM expression (MEF, intestinal epithelium)	20–50 × higher than the direct infection method	(76)
Retroviral	OSKM	Adult human adipose stem cells	0.2%	(77)
Repeated protein transduction	OSKM + VPA OSK + VPA	MEF	Slower kinetic than the viral delivery method	(49)
Retrovirus	OK + BIX/BayK compound	Neural progenitor cells	12 colonies/3.5×10^4	(78)
Doxycycline-inducible transcription factors delivered by PiggyBac transposition	OSKM	Murine and human embryonic fibroblasts	n.d.	(43)
Retrovirus for OSK Transfection of miRNA mimics	OSK+miR-291-3p, miR-294, miR-295	MEF	0.1–0.3%	(79)
2A-peptide linked reprogramming cassette introduced by nucleofection	OSKM	MEF	2.5%	(42)
Sendai virus	OSKM	Human terminally differentiated circulating T cells	0.1%	(45)
Lentivirus	Oct4 + small compound (A-83-01, PD0325901, PS48, NaB)	Neonatal human epidermal keratinocytes, HUVECs, and amniotic fluid-derived cells	4–6 colonies/1 × 106 slower kinetic	(50)
Repeated transfection of synthetic modified messenger RNAs	OSKM	Primary human neonatal epidermal keratinocytes, BJ human neonatal foreskin fibroblasts, human fetal lung fibroblasts, and human fetal skin fibroblasts	1.4%, 36-fold higher than retrovirus	(80)
Lentivirus	miR-302a,b,c,d, miR-367 + VPA miR-302a,b,c,d, miR-367	MEF Human fibroblast	Faster kinetics efficiency 2 × higher compared with OSKM Efficiency 10000 × higher	(39)
Repeated transfection	miR-302s, miR-369-3p, miR-369-5p, miR-200c	Human and mouse adipose stromal cells, dermal fibroblast	5 colonies/5×10^4	(51)
Retroviral Repeated transfection of miRNA mimics	OSKM miR-106b, miR-93, miR-106a, miR-17	MEF	Addition of miRNA enhanced efficiency by 4–6-fold (miR-106b, 93), 3–4-fold (miR-106a,17)	(81)
Retrovirus Repeated transfection of miRNA mimics	OSKM OSK miR-302b, miR-372, miR-294	Human fibroblast	Addition of miRNA enhanced efficiency by promoting MET	(82)

BIX, the small molecule BIX-01294, an inhibitor of the G9a histone methyltransferase; K, Klf4; L, Lin28; M, c-Myc; MEF, mouse embryonic fibroblast; MET, mesenchymal–epithelial transition; miR, microRNA; N, Nanog; n.d.: not determined; O, Oct3/4; Ref., reference; S, Sox2; VPA, valproic acid, a HDAC inhibitor.

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by aberrant mitogenic signaling. The study of double KO (*Ink4ab*$^{-/-}$) and triple KO of all three ORFs (*Cdkn2ab*$^{-/-}$) showed that p15^{Ink4b} can act as a critical backup for p16^{Ink4a}, suggesting a rationale for frequent loss of the complete *CDKN2b–CDKN2a* locus in human tumors.[30] Endogenous p19^{Arf},[31] p16^{Ink4a},[32] and *Trp53* (also known as *p53*), all inactivated in several tumors,[33–35] can limit reprogramming and inhibit pathways leading to an increased level of iPSC generation. In mice, *Arf*, rather than *Ink4a*, blocks important reprogramming pathways through p53 and p21 (encoded by *Cdkn1a*) activation. However, in humans, *INK4a* is more important than *ARF*.[32] Loss of replicative potential may prevent cell reprogramming. The acquisition of cell immortality is a rate-limiting step for establishing pluripotency in somatic cells.[31,32] The transient inhibition of these proteins may significantly improve iPSC generation,[31–35] although the ability of the resultant iPSCs to become tumorigenic is not completely understood. During reprogramming, cells increase their intolerance to different types of DNA damage. A p53-mediated DNA damage response limits reprogramming to ensure iPSC genomic integrity and prevent genomic instability.[35] This phenomenon emphasizes the similarities between induced pluripotency and tumorigenesis. Eventually, approximately 20% of the offspring developed tumors attributable to c-Myc transgene reactivation. Retroviral c-Myc introduction should be avoided for clinical application.[27] Studies of the other barriers indicated that increased iPSC generation efficiency is observed after treating cells with butyrate[36] or vitamin C[37] or after exposing them to hypoxia.[38]

Reprogramming using miRNA. Considering the future application of reprogramming technology, two major non-mutually exclusive issues that should be solved are safety and efficiency. The introduction and addition of specific non-coding miRNA[25] can, for example, improve reprogramming efficiency.[9,39,40] Regarding safety, genomic modification, which is critical to carcinogenesis, is an important concern. The introduction of genes involved in reprogramming events is often facilitated by viral vector-mediated transduction, which can involve random insertions of exogenous sequences into the genome.[19,20] iPSCs can be obtained using virus-free, removable PiggyBac transposons or episomal systems,[41–44] but these approaches still use DNA constructs; thus, the possibility of genomic integration of introduced sequences is still a potential problem. Alternatively, the Sendai virus has been used; iPSCs were generated from human terminally differentiated circulating T cells[45] using Sendai RNA virus vectors.[46,47] Reprogramming events using just protein or mRNA has also been reported, but the protocols involved are technically challenging.[48–50]

Recently, two independent studies from the Morrisey group and our group have demonstrated that human and mouse somatic cells can be reprogrammed to iPSCs through forced miRNA expression, completely eliminating the need for ectopic protein expression.[39,51] Morrisey group[39] revealed that lentiviral-mediated transfection of immature miR-302/367 sequences generated reprogrammed cells (miR-302/367 iPSCs) displaying characteristics similar to those of Oct4/Sox2/Klf4/Myc-iPSCs, including pluripotency marker expression, teratoma formation, and chimera contribution and germline contribution for mouse cells. miR-367 expression is required for miR-302/367-mediated reprogramming, activation of *Oct4* expression, and Hdac2 suppression.[39] Conversely, direct transfection of direct mature double-stranded miRNAs (miR-200c + miR-302s + miR-369s) resulted in PSC generation from differentiated adipose-derived stem cells in humans and mice.[51] This reprogramming method does not require vector-based gene transfer, and thus holds significant potential in biomedical research and regenerative medicine.

Other reports have indicated that electroporation of the polycistronic cassette of hsa-miR-302a/b/c/d resulted in the reprogramming of human hair follicle cells.[40] This reprogramming mechanism functioned through miR-302-targeted cosuppression of four epigenetic regulators: AOF2 (also known as KDM1 or LSD1), AOF1, MECP1-p66, and MECP2.[40] Furthermore, retroviral expression of the polycistronic cassette of hsa-miR-302a/b/c/d allowed the development of iPSC-like phenotypes from human skin cancer cells.[9] Because these methods were carried out without transcription factors, the introduction of miRNAs may play critical roles in differentiated cell reprogramming in humans and mice.

The underlying mechanism of miRNA reprogramming is not completely understood. Generally, miRNAs are involved in translation inhibition, mRNA destabilization, and coding mRNA function suppression.[52,53] We hypothesize that miRNA expression fine-tunes cell reprogramming mainly by inhibiting mRNA signaling, although evidence also suggests that miRNAs may have other functions including translation stimulation through an unknown mechanism. For example, miR-369-3p, which was used for reprogramming,[51] acts as a unique switch for regulating translation repression and activation.[54] miR-302,[39,40,51,55] which targets TGFβ receptor 2 and antagonizes EMT,[55] was also reported to suppress AOF2, AOF1, MECP1-p66, and MECP2,[40] indicating that the miR-302 pathway is fundamental for reprogramming. Inhibition or reversion of EMT could be stimulated by miR-302,[39,40,55] miR-367,[39,55] and miR-200c.[51] TGFβ modulates reprogramming by EMT signaling, whereas Klf4 stimulated E-cadherin expression, a hallmark of MET, which is involved in the stimulation of important reprogramming events.[56] When mammary epithelial cells, which express endogenous Klf4 (MET expression is unnecessary), were used as the starting material, iPSCs were successfully developed only by introducing Sox2 and Oct4 without adding Klf4.[56] This suggests that the requirements needed for EMT inhibition may be dependent on cellular context.

Effect of reprogramming on cancer cells. Retrovirus-mediated gene transfer in gastrointestinal cancer cells resulted in the induction of ES-like gene and protein expression (patterns induced from the endoderm of the gastrointestinal tract to the mesoderm and ectoderm).[10] Interestingly, retrovirus-mediated exogenous expression of Oct4/Sox2/Klf4/Myc or Oct4/Sox2/Klf4 sensitized gastrointestinal cancer cells to vitamins and other chemotherapeutic agents.[10] *In vivo* experiments involving short-term cultured reprogrammed cells showed an inhibition of tumorigenicity in DLD-1 colorectal cancer cells.[10] The study also revealed changes in DNA methylation and histone modification and revealed that the epigenome of DLD-1 cells resembled that of ES cells. The promoter region of *p16Ink4a* was demethylated similar to the heavily demethylated state.[10] Long-term cultured reprogrammed cells with gain-of-function mutations, including *TP53*^{R175H} and *KRAS*^{G12D}, elicit a malignant transformation with c-Myc activation in *KRAS* and *TP53*-mutated HuCC-T1 cholangiocellular carcinoma cells, suggesting a role of such oncogenic mutations in malignant phenotype reactivation.[57] Recent studies have indicated that decreasing the p53 expression level enables the development of murine fibroblasts into iPSCs capable of generating germline-transmitting chimeric mice, indicating that p53 may not be necessary for reprogramming. Silencing p53 will significantly increase the reprogramming efficiency of human somatic cells.[33,34,58] Gain-of-function TP53 oncogenic mutations enhance defined factor-mediated cell reprogramming,[59] suggesting that the TP53 mutation context is influenced by the quality and quantity of reprogramming events. Reprogramming efficiency was increased in hypoxia,[38] an effect observed in cancer cells (Masaki Mori, unpublished data, 2011).

Transfection of miR-302 induces ES-like phenotypes of skin cancer.[9] MiR-302 also inhibits tumorigenecity by coordinating suppression of the Cdk2 and Cdk4/6 cell cycle pathways.[60] The study indicated that concurrent silencing of BMI-1, an

Table 2 Summary of current studies of cancer cell reprogramming

Method	Type of cancer	Malignant-related phenotype	Characterization	Ref.
Nuclear transfer	Medulloblastoma (primary culture, mouse, Ptc1 heterozygous)	Suppressed proliferation, restore normal differentiation, normal proliferation in cultured blastocyst	Cloned blastocyst can support postimplantation development, as the embryo appeared normal and showed extensive differentiation, although not viable after E8.5	(83)
Nuclear transfer (two-step cloning)	Melanoma *RAS+/Ink4a/Arf*$^{-/-}$	NT ES-cell chimeric mice developed various types of tumors with shorter latency and higher penetrance compared with the donor mouse model	NT ES cells could form teratoma and generate chimera. Injection into tetraploid blastocyst resulted in a normal embryo viable until E9.5	(84)
Nuclear transfer	EC	Dependent on donor ECs, one NT ES cell chimera suffered from head and neck EC and was inviable, and the other resulting NT ES cells showed a broad differentiation potential into teratomas and broad contribution to normal-appearing mid-gestation embryos	Nuclei from EC can direct preimplantation development, resulting in normal appearing blastocyst, higher efficiency of producing an ES cell line compared with the differentiated cells, although the degree of differentiation depends on the cell line character	(85)
Embryonic microenvironment	Metastatic melanoma, breast cancer	Reduced invasion, tumor growth, increased apoptosis Downregulated Nodal signaling through Lefty activation	n.d.	(86)
microRNA (miR-302a, b, c, d)	Melanoma (Colo), prostate cancer cell line (PC3)	Reduced migration ability, reduced expression of cell cycle-related genes (*CCND1*, *CCND2*, *CDK2*), and DNA methylation facilitator, MeCP2 MECP1-p66, and some melanoma oncogenes	Expression of pluripotency markers Nanog, Oct4, Sox2, SSEA3, SSEA4 Demethylation of *Oct4* Teratoma (+)	(9)
Defined transcription factor (OKM)	Melanoma (R545)	Chimeras were tumor-free at 5 months of age	Teratoma (+), chimera (+), ES cell marker expression, demethylation of *Nanog* and *Oct4*	(87)
Defined transcription factor (OSKM)	Gastrointestinal cancer (colon, liver, pancreatic cancer) DLD-1 completely characterized	Differentiated iPC (post-iPC) showed sensitivity to chemotherapy, reduced invasion, and reduced tumorigenicity, showed higher expression of p16 and p53 as compared to the parental cell	Expression of pluripotency marker, demethylation of *Nanog*, *in vitro* differentiation into adipocyte, epithelial, mesenchymal, and neural lineage, teratoma (–)	(10)
Defined transcription factor (OSKM)	KBM7 cells derived from blast crisis stage chronic myeloid leukemia (CML)	Completely resistant to imatinib, loss of *BCR-ABL*-dependent signaling	ES cell marker expression (+), demethylation of *Oct4* and *Nanog*, teratoma (+)	(88)
Defined transcription factor (OSLN)	A549 lung cancer	Increased tumorigenic properties when transplanted into a NOD/SCID mouse, more aggressive and invasive, teratoma (–)	Demethylation of *Oct4* promoter expressed endogenous Nanog and Oct4 although lower than HES cell ALP(+), teratoma (–) Reprogramming efficiency was higher compared with normal primary lung fibroblast	(89)
Oocyte extract	Breast cancer (MCF7 and HCC1945 cell lines)	Re-expression of tumor suppressor genes *RARB*, *CST6*, *CCND2*, *CDKN2A* through demethylation and remodeling of histone marks to a more euchromatic state No changes in DNA methylation at pluripotency gene promoters *Oct4/Nanog* Reduced colony formation	n.d.	(90)

ALP, alkaline phosphatase (staining); EC, embryonal carcinoma; HES, human embryonic stem; iPC, induced pluripotent stem (iPS)-like cancer cells; K, Klf4; L, Lin28; M, c-Myc; N, Nanog; n.d., not determined; NT ES, nuclear transfer-generated embryonic stem cells; O, Oct3/4; Ref., reference; S, Sox2.

miR-302-targeted CSC marker, further promoted tumor suppressor functions of p16Ink4a and p14/p19Arf directed against Cdk4/6-mediated cell proliferation. Also, miR-302 inhibits human pluripotent stem cell tumorigenicity by enhancing the multiple G_1 phase arrest pathways.[60] Another study of glioma indicated that the miR-302–367 cluster drastically affects the

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self-renewal and infiltration properties of glioma-initiating cells through Cxcr4 repression and consequent disruption of the Shh-Gli-Nanog network.[61] This indicates that the miR-302–367 cluster can efficiently trigger a cascade of inhibitory events leading to the disruption of CSC-like and tumorigenic properties.[61] Taken together, further study of novel reprogramming-based therapeutic approaches that could prove beneficial for treatment of tumors with p53 inactivation[33,34,58] and/or of CSCs, which can survive in a region of hypoxia,[38] is warranted (Table 2; Fig. 1).

Defined Factor-Induced Reprogramming and CSCs

The differential mechanisms between cancer cells, which undergo a mutated form of reprogramming, and naturally occurring CSCs remain unclear.

Gastrointestinal cancer cells. Recently, it has been proposed that two types of stem cells coexist in normal and cancer cells and that these stem cells are transiently regulated by epigenetic controls.[62–65] Emerging evidence indicates that quiescent and active stem cell subpopulations that are in lower metabolic and proliferative states, respectively, may coexist in several tissues.[62] It has been proposed that these stem cell populations have separate but cooperative functional roles, and these adult stem cells are crucial for physiological tissue renewal and regeneration after injury.[62] Generally, a stem cell divides asymmetrically into a new stem cell (self-renewal) and a committed progenitor (differentiation). Whereas the asymmetric architecture of the stem cell niche in *Drosophila* and *Caenorhabditis elegans* is apparent, mammalian adult stem cells are generally detected in a predominantly quiescent state.[63,64] Quiescent stem cells have been proposed to produce transit-amplifying cells in rapidly regenerating tissue, which differentiate into mature cells and provide tissue architecture. Considering that transit-amplifying cells have a short lifespan and limited self-renewal capabilities, recent studies suggest that stem cell populations that are long-lived yet constantly cycling are involved in the maintenance of tissue homeostasis.[62] A new model describes the coexistence of quiescent and active adult stem cell subpopulations in bone marrow, intestinal epithelium, and hair follicles.[62] In contrast to physiological tissues, serial transplantation experiments indicated that liver CSCs are composed of quiescent and active CSCs. This system plays a role in the exertion of resistance against chemoradiotherapy. During the study of CSCs, we identified $CD13^+$ CSCs as a subpopulation of quiescent stem cells of the liver.[65] Our study indicated that TGFβ induced the development of a $CD13^+$ CSC population (Masaki Mori, submitted). $CD13^+$ CSCs express immature genes often connected with a lower differentiation state, an observation that might explain why $CD13^+$ CSCs exhibit aggressive behaviors (Masaki Mori, unpublished data, 2011). Considering TGFβ signaling counteracts the induction of cell reprogramming from normal differentiated cells, the outcome of reprogramming-like stimulation should be investigated.

Reprogramming effect on CSCs. Endogenous expression levels of ES-like genes could be relevant to tumor cell malignancy.[66] The concept that a small population is contained in adult tissues may be relevant to CSCs in a tumor.[67] The involvement of a very small embryonic/epiblast-like stem cell population in carcinogenesis could support century-old concepts involving embryonic rest- or germline-origin hypotheses of cancer development;[67] however, this working hypothesis requires further direct experimental confirmation.[67] Further evidence indicates that tissues contain a unique population of mesenchymal stem cells or Muse cells,[68] and that Muse cells are a primary source of iPSCs in human fibroblasts.[69] By using immunocytochemistry to express Nanog, Oct3/4, and Sox2 and TRA-1–81 to assess reprogramming efficiency, the authors showed that iPSC

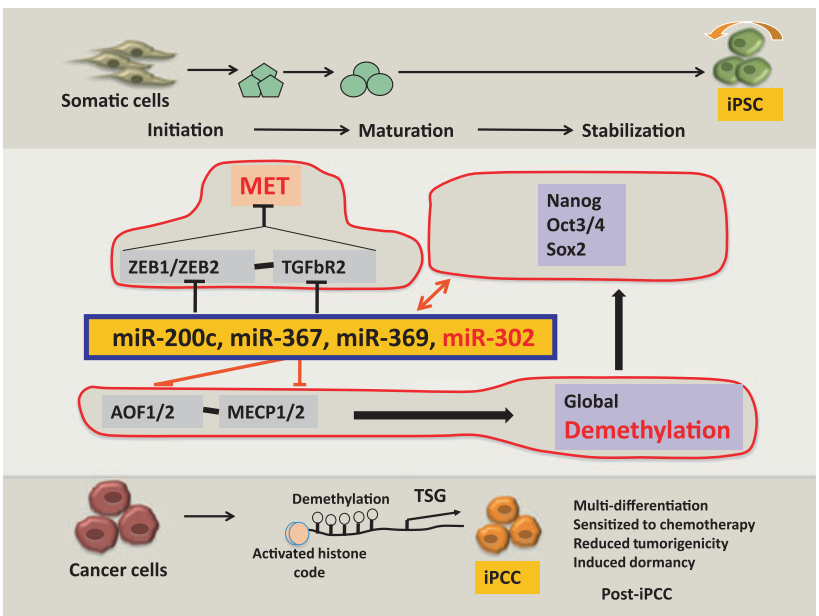

Fig. 1. Cellular reprogramming in normal and cancer cells. Cellular reprogramming in normal and cancer cells can be viewed globally as a mechanism of phenotype reversal of parental cells through the modulation of epigenetic status into a more undifferentiated state. Defined transcription factors (Yamanaka cocktail)-induced reprogramming is involved in the regulation of mesenchymal–epithelial transition (MET), which is controlled by a group of microRNAs (miR) through ZEB1/ZEB2 and TGFβR2. Those miRNAs play a role in global demethylation through AOF1/2 and MECP1/2. In contrast to normal cell reprogramming (upper panel), cancer cell reprogramming (lower panel) remains obscure. The reverse of MET, epithelial–mesenchymal transition (EMT), results in a chemotherapy-resistant phenotype. Thus, reprogramming is supposed to open the silent chromatin through DNA demethylation and activate histone codes, which would elicit re-expression of tumor suppressor genes, pushing cancer cells into a more benign phenotype. Further investigation would provide insight into how much of the tumor phenotype could be reversed through the contribution of reversible epigenetic and irreversible genetic changes in cancer. Reprogramming cancer cells might become a promising method for reversing or attenuating malignancy for therapeutic purposes. iPCC, induced pluripotent stem cell-like cancer cell; iPSC, induced pluripotent stem cell; TGF, transforming growth factor; TSG, tumor suppressor gene.

lines were generated with an efficiency of 0.001% from naive human skin fibroblasts, whereas Muse-iPSCs were formed with an efficiency of 0.03%, indicating that Muse cells generate iPSCs 30-fold more efficiently than naive fibroblasts.[69] This type of subpopulation study elicits a challenging notion that a subset of pre-existing adult stem cells in adult human tissues (or fibroblasts), which are somewhat similar to iPSCs, selectively become iPSCs, whereas the remaining cells make no contribution to iPSC generation.[69] Nevertheless, at least two issues should be considered. First, the efficiency of iPSC generation in this study is much lower than that reported in other studies (≥0.02%; Table 1).[20,70] Although the susceptibility to each cell reprogramming may be presumably based on pre-existing conditions of epigenetic and transcription factor networks, underestimation cannot be excluded without adjusting the complete reprogramming technology. Second, given that higher efficiencies of reprogramming have been reported (up to approximately 10%, see Table 1) than the pre-existing frequency of Muse cells in tissues, (1.1–1.3% of human fibroblasts or bone marrow stromal cells formed Muse cell-derived cell clusters in naive populations without long-term trypsin incubation), cells other than Muse cells may generate iPSCs. Taken together, it may be too early to conclude whether the defined factor-induced reprogramming fits the elite model,[69] rather than the stochastic model of iPSC generation.[70] To reconcile these issues, further investigation is necessary to improve the reprogramming efficiency and understand the mechanism by which cellular reprogramming functions, especially in subpopulations of susceptible clones subjected to defined factor-induced reprogramming. Considering that ES-like genes expressing CSCs and unique populations including very small embryonic/epiblast-like stem cells and Muse cells could be essential in cancer development, further research is necessary to determine the presence of these cell subpopulations in tumor tissues, relevancy to epithelial cancerous cells, and susceptibility of reprogramming events in these cell populations.

Perspective

Tissue homeostasis is a carefully balanced process controlled by epigenome regulation and efficient interplay between stem cells, their progeny, and the microenvironment (e.g. recently reviewed in intestinal stem cells[23]). Epigenome deregulation and malignant stem cell formation lead to tumor cell development. Reprogramming technology or epigenome modification through transfection of iPSC factors can lead to ES-like gene expression patterns and considerable malignant phenotype modification,[10,60] indicating that this technology could be used to create novel therapeutic targets against CSCs by combining small non-coding RNAs with efficient drug delivery systems.

Disclosure Statement

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Abbreviations

CSC	cancer stem cell
EMT	epithelial–mesenchymal transition
iPSC	induced pluripotent stem cell
MEF	mouse embryonic fibroblast
MET	mesenchymal–epithelial transition
miRNA	microRNA
Muse (cells)	multilineage-differentiating stress-enduring
TGF	transforming growth factor

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