The emerging roles of Oct4 in tumor-initiating cells

Ying-Jie Wang1,2* and Meenhard Herlyn2

1State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang, China; and 2Molecular and Cellular Oncogenesis Program, The Wistar Institute, Philadelphia, Pennsylvania

ALTHOUGH THE CANCER STEM CELL hypothesis was put forth decades ago, it remains controversial and is still evolving (44, 47, 83). According to this hypothesis, tumor cells are heterogeneous and only a subpopulation of them has the ability to proliferate extensively, give rise to differentiated cells, and form new tumors. The terminologies for such cell populations have not been unified, with the “cancer stem cells” (CSCs) and the “tumor-initiating cells” (TICs) being the most commonly coined ones. The former terminology indicates their ability to self-renew and generate differentiated progeny, while the latter one emphasizes their ability to initiate a tumor upon transplantation (44, 47). In theory, the CSC is a more inclusive terminology than the TIC. However, since it is experimentally more definitive to validate the tumor-initiating potency than to determine the self-renewal and multilineage differentiation capacity, the TIC term is adopted in this review. To isolate TICs from various tumors, researchers usually employ fluorescent-conjugated antibodies to cell surface markers that are specifically expressed or enriched in TIC populations, combined with fluorescence-activated cell sorting (FACS). However, to date, none of the identified surface markers are universal among all the TICs, and some non-cell surface markers have also been used successfully to enrich for TICs (41, 98).

Octamer-binding transcription factor 4 (Oct4), a homeodomain transcription factor, is well established as one of the most important transcription factors that control the self-renewal and pluripotency of pluripotent stem cells (72), and is increasingly appreciated as an important non-cell surface marker for TICs (59, 81). Oct4 proteins are encoded by the POU5F1 gene that can generate at least three transcripts (Oct4A, Oct4B, and Oct4B1) and four protein isoforms (Oct4A, Oct4B-190, Oct4B-265, and Oct4B-164) by alternative splicing and alternative translation initiation (35). Human Oct4A (often referred to as Oct4) has 360 amino acids and consists of an N-transactivation domain (137-amino acids) which is unique to Oct4A, a POU domain (152-amino acids), and a C-transactivation domain (71-amino acids). The POU domain contains two structurally independent DNA binding domains (an NH2-terminal 75 amino acid POUS domain and a COOH-terminal 60 amino acid POUH domain) that are connected by a linker of 17 amino acids (Fig. 1A). POUS and POUH domains can independently and flexibly bind half-sites of the characteristic octamer motif (ATGCA/TAAT) through which Oct4 recognizes the enhancer or promoter regions of its target genes (Fig. 1B). This flexibility allows Oct4 to form heterodimers with other transcription factors (such as Sox2, Fig. 1C) and to form homodimers in PORE motif (ATTTGAAAAT/GGCAAAT)- or MORE motif (ATG-
CATATGCAT)-binding conformation, depending on the positioning of POU5 and POU1 domains relative to each other (35). So far, neither the physiological stimuli nor the molecular mechanisms that modulate Oct4 oligomerization and its various conformations is clear. Purified Oct4 proteins can bind nucleosomes in vitro, and in vivo they preferentially target silent sites enriched for nucleosomes thereby acting as a pioneer transcription factor during cell reprogramming (90, 91).

A large body of research has documented the detection of Oct4 in tumor cells and tissues, its requirement for tumor initiation and propagation. In a few studies, efforts have been made to identify its target genes in TICs of different sources. Based on such information and other in-depth analyses, Oct4 is considered to play an important role in the self-renewal, epithelial-mesenchymal transition (EMT), and drug resistance development of TICs.

Glossary

- CRISPR: Clustered regularly interspaced short palindromic repeat
- CSC: Cancer stem cell
- ECC: Embryonal carcinoma cell
- EMT: Epithelial-mesenchymal transition
- ESC: Embryonic stem cell
- GBM: Glioblastoma multiforme
- HCC: Hepatocellular carcinoma
- LAC: Lung adenocarcinoma
- MORE: More PORE
- Oct4: Octamer-binding transcription factor 4
- PORE: Palindromic Oct factor recognition element
- PTM: Posttranslational modification
- ROS: Reactive oxygen species
- RT-PCR: Reverse transcription-polymerase chain reaction
- TALEN: Transcription activator-like effector nuclease
- TIC: Tumor-initiating cell

Detection of Oct4 in Cancer Tissues and Cells

It was shown that, during gastrulation, a series of coordinated processes led to the methylation of the promoter and enhancers of the POU5F1 gene, and hence its irreversible silencing in progenitor cells and differentiated somatic cells (24). The conclusion that Oct4 is dispensable for the maintenance of adult stem cells was drawn from a study in which an inducible Cre-mediated recombination was used for tissue-specific POU5F1 gene ablation. In the absence of Oct4, none of the investigated tissues known to contain a population of adult stem cells exhibited any sign of dysfunction (49). Therefore, the prevailing view among stem cell biologists and developmental biologists is that there is virtually no expression of Oct4 in somatic cells. Although numerous reports have documented the detection of Oct4 mRNA by RT-PCR and/or the detection of Oct4 protein by immunohistochemistry and Western blotting in cancerous adult tissues/cells and cultured cancer cell lines (1, 32, 45, 51, 62, 65, 79, 99), the claims remained controversial, mainly because the RT-PCR primers and anti-Oct4 antibodies employed did not distinguish Oct4A from other Oct4 isoforms and its pseudogene products (9, 50, 55, 68).

Atlasi et al. (2) designed isoform-specific primers and found that Oct4A and Oct4B1 expression was mainly restricted to pluripotent stem cells including embryonic stem cells (ESCs) and embryonal carcinoma cells (ECCs), whereas Oct4B was expressed in various nonpluripotent cell types. By employing Oct4A-specific primers and Oct4A-specific restriction digestion of PCR fragments, and by confirming the PCR products with DNA sequencing, Jez et al. (36) found that Oct4A transcription was undetectable in normal adult human dermal fibroblasts but was significantly induced when the cells were treated with hypoxia and FGF2, leading to a so-called “regeneration-competent” state. Thus, it is possible that Oct4A gene in differentiated cells may be reactivated under certain conditions. Using the Oct4A-specific RT-PCR primers designed by Atlasi et al. (2) and the primers amplifying Oct4A and selected Oct4 pseudogenes, combined with DNA sequencing of the amplified PCR products, Zhao et al. (106) provided evidence that the transcripts of both Oct4A and its three pseudogenes (Oct4-pg1, Oct4-pg3, and Oct4-pg4) were present in a variety of cultured cancer cell lines. More recently, with further optimized RT-PCR primers which were highly specific for Oct4A, Xu et al. (102) provided the most convincing evidence to date that Oct4A transcripts were truly present in all the tested human adult stem cells and differentiated somatic cells albeit at much lower levels compared with those present in human pluripotent stem cells. Also, in differentiated cells, the tran-
script levels of Oct4A were significantly lower than those of its pseudogenes.

In comparison, the supporting evidence at the protein level is relatively weaker because so far there is no commercially available Oct4A-specific antibody. There are three commercial anti-Oct4 antibodies claimed to be able to specifically detect Oct4A, which in fact can bind to the NH2-termini of Oct4A, Oct4-pg1, Oct4-pg3, and Oct4-pg4 proteins (102). Based on the predicted molecular masses, it is possible to distinguish by Western blotting the Oct4A proteins with the Oct4-pg3 or Oct4-pg4 proteins, but not with the Oct4-pg1 proteins. Xu et al. (102) detected a 45 kDa major band in most tested human somatic cells with all the three anti-Oct4 antibodies which most likely represented Oct4A and/or Oct4-pg1. By one of these antibodies (sc-5279), Zhao et al. (106) detected two bands (of 43 and 47 kDa) in a variety of human cancer cell lines in Western blots that can be significantly reduced by an shRNA targeting POU5F1 and Oct4-pg1, indicating they were highly associated with the Oct4A protein. Detection of the upper band (47 kDa) by the antibody that can specifically recognize a phosphorylated Oct4A in ECCs (57) further indicated its close association with Oct4A. However, given the extremely high degree (95%) of sequence homology between Oct4A and Oct4-pg1 proteins, it was difficult to distinguish them at the protein level using routine approaches. Thus, in future studies, it will be important to purify the corresponding proteins and unequivocally identify them using protein chemistry approaches such as amino acid sequencing.

Most of the above mentioned studies detected Oct4 in bulk-cultured cells or tumor tissues, and only a few of them investigated the isolated TICs where Oct4 was always expressed at a higher level than in the rest of the cancer cells (62, 79). Recently, Zhu et al. (107) showed that the CD13+CD133+ liver TICs sorted from primary hepatocellular carcinoma (HCC) cells possessed a 2- to 17-fold higher level of Oct4 mRNA than that of the CD13-CD133- non-TICs, and the tumorspheres derived from those primary cells which were enriched in TICs also exhibited an up to eight-fold increased Oct4 mRNA level over the nonsphere cells. To allow for more efficient identification and isolation of TICs from the bulk of tumor cells or tissues, Tang et al. (93) designed a modular lentiviral reporter construct in which six concatenated repeats of a composite SOX2/OCT4 response element (SORE6) were coupled to a minimal CMV promoter and used to drive the expression of a destabilized GFP reporter gene. After being introduced into the cells, this reporter identified a subpopulation in human breast cancer cell lines and primary human tumor samples that had the expected characteristics of TICs, such as the upregulated Oct4 and Sox2 expression, the potentiated tumor-initiating ability, and the increased resistance to chemotherapeutics in vitro and in vivo (93). Despite the lack of immunoblotting data, this study represents one of the most compelling demonstrations so far for the functionality of endogenous Oct4 proteins in TICs.

Requirement of Oct4 for Tumor Initiation and Propagation

Knocking down endogenous Oct4 or overexpressing ectopic Oct4 in different types of cancer cells implicated the requirement of Oct4 for maintaining the tumor-initiating and tumor-propagating capabilities of the TICs. Using a doxycycline-dependent inducible expression system, Hochedlinger et al. (30) first reported that ectopic expression of Oct4 blocked progenitor cell differentiation and caused dysplasia in epithelial tissues. Du et al. (21) reported that Oct4 was highly expressed in human gliomas and glioma cell lines, and its expression levels were positively correlated with glioma grades. Oct4 was only detected in cultured rat C6 glioma cells and rat neural stem cells but not in differentiated rat brain cells. Reducing endogenous Oct4 level in C6 cells by RNA interference (RNAi) led to attenuated cell proliferation and colony formation. Further analysis revealed that Oct4 could upregulate the phosphorylation of Stat3 to promote tumor cell proliferation.

The acquisition of stemness is a hallmark of aggressive human HCC, and the expression of Oct4 in HCCs correlated with those of putative TIC markers and TIC properties (69, 97, 103). Sex determining region Y (Sry) appeared to be an important regulator for Oct4 expression and TIC maintenance in HCCs (69). Ectopic expression of Sry increased Oct4 levels and promoted TIC phenotypes such as self-renewal, chemoresistance, and tumorigenicity while Sry knockdown decreased Oct4 expression and the TIC phenotypes. Furthermore, Sry was found to be highly expressed in some HCC patients. Sry-binding sites were identified at the Oct4 promoter region, supporting a direct regulation of Oct4 transcription by Sry (69).

Ample evidence showed that glioblastoma multiforme (GBM) originates from TICs (19, 28, 47). Oct4 was highly expressed in primary gliomas, and its expression levels increased in parallel with pathological grades (89). By overexpressing Oct4, Sox2, and Nanog, Olmez et al. (75) differentiated two patient-derived GBM cell lines into induced glioma stem cells (iGSCs). Compared with parental GBM cells, the iGSCs formed large neurospheres even in the absence of exogenous mitogens and exhibited significant sensitivity to salinomycin and chemoresistance to temozolomide. Furthermore, key signaling pathways including the Notch1 and Wnt/β-catenin pathways were upregulated and the expression of other TIC markers (CD133, CD44, and ALDH1A1) were significantly induced in iGSCs (75).

In human metastatic melanoma tissues and cell lines, high Oct4 levels were found in a subset of cells characterized by rhodamine 123 low retention (95). This cell population was also positive for other stem cell markers, more likely to form tumorspheres, and more resistant to chemotherapy. Overexpression of Oct4 in melanoma cells led to a more dedifferentiated phenotype, loss of melanocyte-specific markers, and increase in cell motility (5, 46). In the subcutaneous xenograft and tail vein injection assays, these Oct4-overexpressing cells exhibited significantly increased tumorigenic capacities. The dedifferentiated melanoma cells acquired features associated with TICs such as multipotent differentiation capacity and expression of melanoma TIC markers such as ABCB5 and CD271. Mechanistically, Oct4-induced dedifferentiation was associated with increased expression of endogenous Oct4, Nanog, and Klf4, and global gene expression changes that enriched for transcription factors. RNAi-mediated knockdown of Oct4 in dedifferentiated cells led to diminished TIC phenotypes. Oct4 expression was also detected in a subpopulation of melanoma cells isolated from clinical samples. These results suggest that TIC phenotype is dynamic and may be acquired through dedifferentiation, and Oct4-mediated tumor cell dedi-
fferentiation could play an important role during tumor progression (46). Microphthalmia-associated transcription factor, MITF, a master regulator of melanocytic differentiation, appeared to repress Oct4 (12). Accordingly, inhibition of MITF upregulated Oct4 and other stem cell markers and led to a slow-cycling cell population with high tumorigenic potential in murine B16 melanoma cells (12). It remains to be determined whether the JARID1B-enriched slow-cycling melanoma cells that are required for continuous tumor growth (84) express a higher level of Oct4.

To address the issue of whether Oct4 expression is important for oncogene-driven spontaneous tumors, Schreiber et al. (87) generated knock-in transgenic mice that express GFP under the control of the endogenous Oct4 promoter and crossed them either with MT/ret mice that develop melanocytic tumors in response to transgenic expression of the Ret oncogene, or with MMTV-PyMT transgenic mice that develop polyomavirus middle T oncogene-driven multifocal mammary adenocarcinomas, or with MMTV-Neu mice that develop polyclonal mammary adenocarcinomas due to expression of the activated Neu oncogene in the mammary epithelium. Tumors developing in these compound transgenic mice were analyzed for expression of the GFP reporter indicative of Oct4 transcription. They concluded that no discernible GFP signals could be detected in the tumors and tumor cells derived from the animals, suggesting that Oct4 transcription is unlikely to be a key determinant of TIC properties in these autochthonous tumor models. However, the authors did note that for the MMTV-PyMT tumors, a three-fold significant increase in GFP expression was observed in the Oct4-GFP* tumors compared with GFP negative tumors (87). Based on the conclusions drawn from the above section that the endogenous Oct4 expression level in somatic cells is much lower than that in pluripotent stem cells, it is not too surprising that the Oct4-representing GFP signals in the tumors might be too weak to be detected by fluorescence microscopy. Also, Oct4 expression may be more relevant in other tumor models and such possibility awaits further testing in future studies. Taken together, the requirement of Oct4 for tumor initiation and propagation has been circumstantially established in various cultured cell models, which needs to be further validated in appropriate in vivo systems.

Regulation of Oct4 Expression in TICs

There is increasing evidence that TIC properties are plastic and can be gained or lost, for example, in response to microenvironmental cues including but not limited to hypoxia, low pH, nutrition deprivation, and oxidative stress (17, 25, 67).

Although methylation of the POU5F1 promoter and enhancer regions has been proposed as the primary mechanism in shutting down Oct4 expression in differentiated cells (24), substantial evidence indicated the epigenetic-based silencing is most likely to be partially reversible and additional factors may also contribute to the silencing. For instance, bisulfite sequencing analysis showed that the methylation levels of the POU5F1 promoter and exon were significantly reduced in primary gliomas over normal brain tissues, which were associated with increased Oct4 gene expression. In vitro, Oct4 transcription was upregulated following treatment with a demethylation reagent in glioma cell lines (89). During differentiation of mouse ESCs, transcription of the Oct4 pseudogene Oct4P4 yielded a long noncoding RNA that formed a complex with the histone methyltransferase SUV39H1 to direct the imposition of H3K9me3 and HP1α to the Oct4 promoter, contributing to the silencing of Oct4. Remarkably, knocking down Oct4P4 in differentiated mouse embryonic fibroblasts led to increased Oct4 transcription and partial reactivation of the stemness (86). In some tumor cell lines, Oct4B was also reported to modulate Oct4A expression as a noncoding RNA, mimicking the way that microRNAs function (52). In fact, it has been firmly established that certain microRNAs (miR-145, miR-302 cluster) can regulate Oct4 expression in pluripotent stem cells at the posttranscriptional level (96), and their regulatory roles in cancer cells and TICs are being investigated.

Multiple studies indicated that the hypoxic microenvironment facilitates the initiation and maintenance of the TICs partially by enhancing the expression and activity of pluripotency factors including Oct4 and Nanog (14, 28, 46, 54, 85). The effects of hypoxia on TICs seem to be primarily mediated by hypoxia-inducible factors (HIFs), particularly HIF2α. HIF2α is highly expressed in TICs and its loss led to significant decrease in TIC proliferation and self-renewal (54). Covello at al. (18) showed that HIF2α, but not HIF1α, bound to the POU5F1 promoter and induced Oct4 expression and transcriptional activity, thereby contributing to defective hematopoietic stem cell differentiation in embryoid bodies, and large ESC-derived tumors characterized by altered cellular differentiation. Furthermore, loss of HIF2α severely reduced the number of embryonic primordial germ cells, which required Oct4 expression for survival and maintenance. This study identified Oct4 as one of the HIF2α target genes and indicated that HIF2α can regulate stem cell function via activation of Oct4, which in turn contributes to HIF2α’s tumor-promoting activity. Hjelmeland et al. (29) showed that acidic conditions, independent of hypoxia, promoted the self-renewal of glioma TICs and tumor growth. Culturing cells at low pH (pH 6.5) conditions augmented the induction of HIF2α, which was associated with the upregulation of TIC markers including Oct4 and Nanog, but not Sox2. Similarly, Martinez-Outschoorn et al. (64) found that lactate increased the stemness of breast cancer cells and promoted their metastasis.

Overconsumption or deprivation of nutrition such as tryptophan represents one of the key features of the tumor microenvironment, and the consequent accumulation of the tryptophan metabolite kynurenine (Kyn) was found to be associated with tumor progression (76, 80). The tumor-promoting effect of Kyn was exerted through the binding of Kyn to its receptor, aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor originally identified and characterized as a key factor responding to environmental toxins, which is now gaining increasing attention for its critical roles in immune responses and carcinogenesis (70, 74). Cheng et al. (14) found that ITE, another endogenous derivative of tryptophan, at its physiologically achievable concentrations, can be engaged with high affinity to the AhR and facilitate its binding to and suppression of the POU5F1 promoter in vivo. Reduction of endogenous ITE levels in cancer cells by tryptophan deprivation or hypoxia led to Oct4 elevation and augmented cancer cell stemness, indicating that the silenced POU5F1 gene in differentiated cancer cells is still partially activatable and that stemness is a rather general and flexible quality of tumor cells. Thus, endogenous tryptophan metabolites may serve as prim-
ing factors for reversible stemness. In human ESCs and ECCs, the extremely low levels of AhR were associated with very high levels of Oct4, consistent with the hypothesis that relieving from the transcriptional suppression by AhR may account for the upregulated Oct4 levels during differentiation. Interestingly, recent evidence was provided in mouse ESCs that Oct4/Sox2/Nanog complexes can bind to the AhR distal promoter region to repress AhR expression (43), raising the possibility of a reciprocal regulatory mechanism between AhR and Oct4 (39). Thus, it is important to investigate in future studies the exact causal relationship between AhR and Oct4 and their differential regulatory modes in pluripotent stem cells versus TICs.

Reactive oxygen species (ROS), the highly chemically reactive byproducts of aerobic metabolism, are now recognized as important mediators in stem cell biology. A consensus is being reached that low levels of ROS help to maintain stemness, whereas higher levels of ROS promote stem cell differentiation (82). Although high ROS levels are generally detrimental to cancer cells, they can promote tumor formation by inducing DNA mutations and pro-oncogenic signaling pathways (105). Mahalingaiah et al. (63) found that chronic oxidative stress led to increased growth and neoplastic transformation in normal kidney epithelial cells at nontoxic doses and increased adaptation to cytotoxic levels. Antioxidant N-acetyl-cysteine (NAC) did not reverse the chronic oxidative stress-induced growth and adaptation, indicating that the altered biological functions in those cells were irreversible. Partial reversal of oxidative stress-induced growth and adaptation was achieved by silencing of Oct4 and Snail1, respectively, suggesting that these changes were mediated by acquisition of stem cell and EMT characteristics. Thus, it was concluded that chronic exposure to elevated levels of oxidative stress is sufficient to induce malignant transformation in kidney epithelial cells through acquisition of stem cell characteristics.

Key proteins involved in stem cell and cancer cell biology can undergo reversible redox reactions where the cysteine thiol group has been proven to be the primary target (82, 105). For instance, Guo et al. (27) showed that, in vitro, the DNA binding activity of Oct4 is sensitive to abrogation by oxidizing cysteines in the POU domain, which can be reversed by thioredoxin, indicating that Oct4 is a redox sensor. Further study is required to identify the cysteine oxidative modification site(s) in the context of TICs, and to determine how these modifications cross talk with other posttranslational modifications (PTMs) via various signaling pathways. In fact, besides transcriptional and posttranscriptional regulations, a variety of PTMs including phosphorylation, ubiquitination, sumoylation, and glycosylation have been identified as important regulatory mechanisms for Oct4 present in ESCs and ECCs (8). These PTMs play essential roles in regulating structure, activity, and localization of the Oct4 protein and its interactions with other cellular components. The reversible PTMs are well placed to sense, relay, and integrate a variety of extracellular and intracellular signals in pluripotent stem cells (92). The studies on histones have exemplified how combinations of PTMs can function in concert to allow for the storage and transduction of highly specific signals to control epigenetics-based gene transcription. Such combinatorial histone PTMs serve much like a bar code, providing great potential for signal diversity (34). To systematically map out the physiologically relevant PTM sites in Oct4 proteins, Dan et al. (20) developed a cell-free system-based in vitro PTM approach and identified a number of Oct4 phosphorylation sites that were commonly present in all the cellular contexts or specifically present in a particular cellular context, indicating that Oct4 in different cellular environments is controlled by both common and distinct PTM regulatory pathways. Such a system may facilitate fully deciphering the Oct4 PTM bar codes in various cellular contexts including in TICs.

Identification of Oct4 Target Genes in TICs

Genome-wide DNA microarray, chromatin immunoprecipitation (ChIP)-based analyses combined with bioinformatics analyses have identified hundreds of target genes that are transcriptionally controlled by Oct4 in pluripotent stem cells (4, 6, 11, 38, 77, 88). Based on the data from multiple sources, Jung et al. (38) defined six distinct Oct4-binding modules for all the filtered Oct4 target genes: Oct4-Sox2 motif, Oct4 monomer motif, Sox2 monomer motif, no Oct4-no Sox2 motif, Oct4 PORE motif and Oct4 MORE motif. Overall, Oct4, Nanog, and Sox2 form a self-reinforcing and intricately connected network to preserve the characteristics of ESCs by activating the self-renewal genes while suppressing the differentiation genes (71). In comparison, Oct4 target genes in cancer cells are much less identified.

Linn et al. (58) found that Oct4 expression was upregulated in drug-resistant prostate cancer lines concomitant with upregulation of a set of known Oct4 target genes such as MII1, MYB, ILIRN, RPS27, and CUGBP2. To understand the function of Oct4 in cancer, Fang et al. (23) used ChIP sequencing to determine the genome-binding sites of Oct4 in glioblastoma cells. A total of 3,798 potential Oct4 target genes were identified, which fell into major categories such as gene expression, translation, apoptosis, and intracellular transport. Recently, Tang et al. (94) conducted genome-wide profiling of Oct4 target genes in lung cancer cells using ChIP sequencing. Thousands of potential Oct4-binding regions were identified in the lung cancer cell genome. De novo motif and sequence similarity analyses showed that some novel Oct4-binding motifs were present in genes encoding transcription factors such as Sp1, Klf4, ZNF219, and Stat3. Pathway analyses showed that the Oct4 target genes may play key roles in tumorigenesis and important signaling pathways, such as phosphatase and tensin homolog (PTEN) signaling. Oct4 suppressed PTEN expression in an Sp1-dependent manner by recruitment of histone deacetylase (HDAC) 1 and 2, leading to activation of Akt signaling and drug resistance. Meanwhile, Oct4 activated Tenascin-C (TNC) which resulted in cancer metastasis. Xin et al. (100) examined TICs derived from lung adenocarcinoma (LAC) and found that they expressed high levels of Oct4 and matrix metalloproteinase-2 (MMP2) that were associated with highly invasive and migratory capabilities. Further analyses revealed that Oct4 can bind to the promoter of MMP2 and positively regulate its transcription. Taken together, some information about Oct4 target genes has been obtained with the bulk tumor cells, but more systematic and in-depth analyses with enriched TICs are required in future studies to gain deeper insights into the roles of Oct4 in TICs.
Possible Roles of Oct4 in TICs: TIC Self-Renewal and Survival

Gu et al. (26) showed that a fraction of parental or clonally derived human prostate epithelial/hTERT (HPET) cells have self-renewal potential. They expressed ESC markers (Oct4, Nanog, and Sox2) as well as progenitor cell markers (CD44 and Nestin). Clonally derived HPET cells reconstituted the original human tumor in vivo and differentiated into the three prostate epithelial cell lineages, indicating that they arise from common stem/progenitor cells. To determine whether Oct4 expression is associated with the maintenance and expansion of TICs, Kim et al. (42) introduced an Oct4 reporting vector into 4T1 mouse breast cancer cells and sorted Oct4-high and Oct4-low cell populations. They found that Oct4-high cells had a higher tendency to form tumorsphere and a higher expression level of TIC markers such as CD133, CD34, and ALDH1, over Oct4-low cells. In addition, Oct4-high cells exhibited greater tumorigenic potential in vivo. In a subsequent study, Li et al. (53) found that Oct4A was mainly expressed in tumorsphere or cervical TICs, while Oct4B was mainly expressed in differentiated spheres or non-TICs, suggesting that Oct4A but not Oct4B may serve as a putative marker of TICs. In addition, Oct4A overexpression in the SiHa cervical cancer cell line produced typical nonadherent spherical clusters in serum-free medium, with morphologic phenotypes similar to primary tumorspheres. These findings suggest that Oct4A is likely responsible for maintaining self-renewal of TICs, and contributes to tumor initiation.

Although it is well established that in pluripotent stem cells such as ESCs and ECCs, the core pluripotency factors Oct4, Sox2, and Nanog maintain the self-renewal capabilities and pluripotency through regulating their own or each other’s transcription via combinatorial interactions (6, 71), the mechanisms of action of these factors in TICs are much less understood.

Oct4 is reciprocally connected to Akt to maintain self-renewal and survival of the ESCs via the Akt-Oct4 regulatory circuit (92). For instance, Oct4 can directly bind to the promoter region of the TCLI gene and activate its transcription (66). Tcl1 can interact with and fully activate Akt, thereby promoting the survival of ESCs (78). However, an overly activated Oct4/Tcl1/Akt pathway may underlie the resistance of liver TICs to apoptosis and chemotherapeutic drugs (97). In human LAC-derived TICs, IGF-IR activation enhanced Oct4 expression in a dose- and time-dependent manner (57). The phosphorylated Oct4 induces both the EMT/invasion phenotype and the upregulation of Oct4 transcription. This finding renders further support for the aberrant Akt-Oct4 regulatory circuit in TICs and opens a new avenue to target TICs in lung cancers.

EMT and Metastasis

EMT, a critical process for cancer invasion and metastasis, is found to be associated with the stemness property of cancer cells. Mortality of lung cancer patients is more frequently caused by metastasis than their primary tumors. DNA microarray and quantitative RT-PCR analyses revealed an elevated expression of Oct4 and Nanog in LAC (15). Overexpressing Oct4 and Nanog in LAC cells increased the percentage of the CD133-enriched subpopulation and sphere formation and enhanced the tumor-initiating capability of LAC. Meanwhile, such overexpression activated Slug and promoted EMT. Dual knockdown of Oct4 and Nanog suppressed Slug expression, reversed the EMT process, blocked the tumorigenic and metastatic ability, and greatly prolonged the mean survival time of tumor cell-transplanted nude mice. Immunohistochemical analyses confirmed the expression of Oct4, Nanog, and Slug in high-grade LAC, and triple positivity of Oct4/Nanog/Slug was associated with a worse prognosis in LAC patients. These results suggested that Oct4/Nanog may regulate the tumor-initiating capability and promote metastasis of LAC. In a recent study, Chen et al. (13) showed that Oct4 can promote invasion and adhesion of lung cancer cells, which was associated with an increase in mesenchymal markers (vimentin and N-cadherin) and a decrease in epithelial markers such as cytokeratin. Furthermore, Oct4 induced the EMT of lung cancer cells by promoting β-catenin/E-cadherin complex degradation and regulating nuclear localization of β-catenin.

Coexpression of Oct4 and Nanog was also found to endow the MHCC97-L hepatocellular carcinoma cells with TIC properties including self-renewal, extensive proliferation, and high tumorigenic capacity. They promoted the EMT change contributing to tumor migration, invasion, and metastasis in vitro and in vivo. This was achieved mainly through Stat3-dependent Snail activation because silencing Stat3 abrogated Oct4/Nanog-mediated EMT and invasion/metastasis in HCC (104). This finding implicated the Stat3/Snail pathway as a novel therapeutic target for the treatment of progression and metastasis of HCC.

Liu et al. (60) found that follicle-stimulating hormone (FSH) induced both the EMT/invasion phenotype and the upregulation of Oct4 expression in a dose- and time-dependent manner.
in epithelial ovarian cancer cells. In addition, FSH also increased expression of the FSH receptor (FSHR), and knocking down FSHR inhibited FSH-stimulated Oct4 expression, implicating FSHR as a potential regulator for Oct4 transcription. Oct4-specific RNAi blocked the upregulation of Snail and N-cadherin and suppressed the expression of E-cadherin and the morphological changes stimulated by FSH. Thus, this work indicates that Oct4 is an essential mediator in FSH-induced EMT and invasion in epithelial ovarian cancer.

In contrast to the above reports where Oct4 dysfunction inhibited EMT, Hu et al. (31) showed that silencing Oct4 in MCF-7 breast cancer cells improved their migration and invasion capabilities. The EMT-promoting agent TGF-β1 inhibited Oct4 expression in a dose- and time-dependent manner. Knocking down Oct4 also upregulated the expression of two major components of store-operated Ca$^{2+}$ entry channels (SOCs), STIM1 and Orai1, and enhanced SOC-mediated Ca$^{2+}$ influx. Silencing STIM1 blocked the Ca$^{2+}$ influx and rescued the EMT initiated by Oct4 reduction. Although the discrepancy between this report and the above ones is difficult to explain, the dose-dependent effect of Oct4 could be one of the reasons that there was a significantly higher Oct4 level in MCF-7 cells than in other breast cancer cells such as MDA-MB-231 cells (31). Studies in both embryonic development and somatic reprogramming indicated that an intermediate level of Oct4 is associated with maximum stemness or pluripotency, and both very high and very low levels of Oct4 can lead to differentiation (40, 73). Computational modeling of the important interconnections between EMT and stemness suggested that partial EMT is closely associated with stemness (37). To fully understand the roles of Oct4 in tumor metastasis, it is critical for future studies to identify the entire set of EMT-associated genes that are directly or indirectly governed by Oct4 in cancer cells and TICs.

**Drug Resistance**

Multidrug resistance, one of the major causes leading to the ineffectivity of chemotherapeutics, is characterized by the high expression of members of the ATP-binding cassette (ABC) proteins that actively extrude various anticancer drugs and cytotoxic agents from cancer cells. A subpopulation of the prostate cancer cell line 22RV1 had high surface expression of both CD117 and ABCG2 and was therefore termed CD117(+)/ABCG2(+) cells (61). These cells also expressed stem cell markers such as Oct4, Nanog, Sox2, Nestin, and CD133 at relatively high levels. They were highly tumorigenic and resistant to a variety of chemotherapeutics such as cisplatin, paclitaxel, adriamycin, and methotrexate. In those cells, the CpG islands at the ABCG2 promoter were remarkably hypomethylated where histone 3 acetylation and H3K4 trimethylation were highly enriched, providing mechanistic insight into the epigenetic regulation of the ABCG2 gene. It remains to be seen whether Oct4 is required for recruiting the key enzymes for those epigenetic regulations.

Concurrently high expression of Oct4 and ABCG2 was also seen with CXCR4$^+$/CD133$^+$ OVCAR-5 ovarian cancer cells that were resistant to cisplatin (16). For another ovarian cancer cell line, OVCA 433, cisplatin treatment was associated with increased expression of Oct4/Nanog and correlated with enhanced activation of extracellular signal-regulated kinases (ERK1/2) (48). Furthermore, in those ovarian TIC-like cells, the mRNA levels of Snail, Slug, Twist, and MMP2 were significantly upregulated in response to cisplatin and correlated with reduced E-cadherin, increased N-cadherin/vimentin expression, and increased migration, indicating a connection between chemoresistance and EMT. ERK2 inhibitor U0126 partially suppressed cisplatin-induced EMT and TIC markers, suggesting that ERK2 signaling is crucial for cisplatin-induced EMT and TIC phenotypes, and that targeting ERK2 in the presence of cisplatin may reduce the recurrence in ovarian cancer patients (48). Since ERK1/2 can phosphorylate Oct4 in vitro (7), it might be of interest to find out whether ERK1/2-mediated phosphorylation of Oct4 plays a role in chemoresistance.

An intrinsic connection between drug resistance and EMT was also implicated in pancreatic cancer cells. Du et al. (22) showed that chemoradiation-resistant pancreatic cancer cells expressing high levels of Oct4, ABCG2, antiapoptotic protein Bcl-2, and apoptosis-inhibitory protein survivin were more tumorigenic in vitro and in vivo and exhibited more invasive and migratory properties indicative of EMT. Izumiya et al. (33) showed that another anticancer drug, 5-fluorouracil, was also able to induce the expression of Oct4 in Panc-1 pancreatic cancer cells that responded by EMT stimulation. Furthermore, temozolomide (TMZ), the most commonly used antiangioma chemotherapy drug, induced a phenotypic switch of the differentiated glioma cells toward TIC-like glioma stem cells that was accompanied with an increase in Oct4, Sox2, CD133, and ABCG2 expression.

![Fig. 2. Summary of the regulations and roles of Oct4 in TICs. A variety of extracellular and intracellular stimuli can positively or negatively regulate the expression and functionality of Oct4, mainly at the transcriptional and posttranslational levels. By regulating its target genes most of which are to be identified, Oct4 promotes the self-renewal, survival, metastasis, and drug resistance of tumor-initiating cells (TICs).](http://ajpcell.physiology.org/)
Nestin, and a more efficient grafting and invasive phenotype (3). However, future studies are required to establish the exact relationships among TIC formation, drug resistance, and EMT-based tumor metastasis and invasion, and whether Oct4 is a causal factor in these processes.

Conclusions

Mounting evidence has indicated the regulatory mechanisms and functional importance of Oct4 in cancer cells and particularly in TICs (Fig. 2). The evidence for low levels of Oct4 mRNA in those cells is reasonably solid while definitive evidence at the protein level is still required. The participation of Oct4 in various TIC activities such as self-renewal and survival, EMT and metastasis, and drug resistance is mainly implicated from the Oct4 knockdown and overexpression studies. Genome editing techniques such as TALEN or CRISPR/Cas9 would be a more stringent approach to verify such findings. A main challenge for future studies is to unravel the molecular mechanisms of action of Oct4 in TICs, particularly to address the question on how such low levels of Oct4 may function. Combining techniques such as lineage tracing and live cell imaging would allow for studying Oct4 in the in vivo setting, and acquiring cell preparations with high enough quality and purity as well as further optimizing single cell-based analyses are key to unveil the roles of Oct4 in TICs with high reliability. Given the multiple oligomerization and configuration modes of Oct4 and its rather diversified interactions with the target genes and binding partners, Oct4 in TICs is likely to act via mechanisms distinct from those in pluripotent stem cells, and elucidating its full details holds great promise for disabling or eradicating all cancer cells.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Y.-J.W. drafted manuscript; Y.-J.W. and M.H. edited and revised manuscript; Y.-J.W. and M.H. approved final version of manuscript.

REFERENCES


Oct4 IN TUMOR-INITIATING CELLS


62. Mueller T, Luetzkendorf J, Nerger K, Schmoll HJ, Mueller LP. Analysis of OCT4 expression in an extended panel of human tumor cell...


109. Santos-Ramos P, Lakhatcheva M, Garcia-Zepeda EA, Castaneda-Patlan MC, Robles-Flores M. Hypoxia-inducible factors modulate the stemness and malignancy of colon cancer cells by playing opposite roles in the temporal distinct subpopulation of slow-cycling melanoma cells is temporarily distinct subpopulation of slow-cycling melanoma cells is temporarily different subpopulation of slow-cycling melanoma cells.


