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Cancer-like epigenetic derangements of human pluripotent stem cells and their impact on applications in regeneration and repair

Jeffrey S Huo[1,2], Stephen B Baylin[3] and Elias T Zambidis[1,2]

A growing body of work has raised concern that many human pluripotent stem cell (hPSC) lines possess tumorigenic potential following differentiation to clinically relevant lineages. In this review, we highlight recent work characterizing the spectrum of cancer-like epigenetic derangements in human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC) that are associated with reprogramming errors or prolonged culture that may contribute to such tumorigenicity. These aberrations include cancer-like promoter DNA hypermethylation and histone marks associated with pluripotency, as well as aberrant X-chromosome regulation. We also feature recent work that suggests optimized high-fidelity reprogramming derivation methods can minimize cancer-associated epigenetic aberrations in hPSC, and thus ultimately improve the ultimate clinical utility of hiPSC in regenerative medicine.

Addresses
[1] Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
[2] Division of Pediatric Oncology, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD 21205, USA
[3] Division of Cancer Biology, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD 21205, USA

Corresponding author: Zambidis, Elias T (ezambid1@jhmi.edu)

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Introduction

Human pluripotent stem cells (hPSC) are stable cell lines that can be indefinitely propagated in culture and have enormous potential for use in regeneration and repair of human disease and injury. The discovery of methods to isolate human embryonic stem cells (hESC) from pre-implantation embryos [1], and the derivation of human induced pluripotent stem cell (hiPSC) lines from human differentiated cells with defined factors [2,3] inaugurated the practical development of that potential. However, from the beginning, concern existed regarding the degree to which these artificially derived hPSC lines truly recapitulated the normally regulated embryonic pluripotent state.

Most hPSC lines share remarkably similar superficial measures of pluripotency (such as cell surface markers and teratoma formation in immunocompromised mice), but possess distinct cell line-dependent variations and lineage skewing in their potency of differentiation. This has been observed among both hESC [4–6] and hiPSC lines [7–12]. In efforts to understand the mechanisms underlying this skewing in differentiation potency, hPSC were found to have significant variation in transcriptomes and epigenomes [13–15]. In particular, the reactivation of self-renewal and de-differentiation inherent in the reprogramming process of hiPSC induces aberrations in patterns of transcription, methylation [16•,17•,18,19] and hydroxymethylation [20,21] that are not observed in hESC derived directly from pre-implantation human embryos.

This review synthesizes research suggesting that the aberrant epigenetic regulation observed in many hPSC lines may potentially confer increased tumorigenic potential in their use for regeneration and repair of diseased tissues. We detail the growing evidence of parallels between aberrant epigenetic regulation in cancer, and epigenetic aberrations which arise during establishment and subsequent propagation of hPSC cell lines that are generated with methods involving ectopic expression of defined pluripotency factors which are also oncogenes. We also highlight emerging evidence of aberrant X-chromosome regulation in many hPSC lines that may further have cancer-related implication. Finally, we feature recent research suggesting the potential of optimizing derivation conditions to minimize or avoid these cancer-associated epigenetic aberrations. Together, these emerging findings strongly indicate the need for further research to more completely understand the mechanisms underlying the development (or avoidance) of hPSC-associated epigenetic aberrations. The development of derivation methods that produce hPSC lines that more faithfully recapitulate the normal, non-cancerous pluripotent state is needed.

Cancer-associated promoter hypermethylation, histone modification, and hPSC tumorigenic safety

Concern regarding reprogramming-associated epigenetic aberrations in hPSC initially focused on risks introduced

by hiPSC derivation with viral constructs. The most commonly employed methods of hiPSC derivation utilized overexpression of reprogramming transcription factors (e.g., *MYC, KLF4, OCT4*) that also have established roles in oncogenesis [22]. Methods of hiPSC generation employing viral vectors to express these defined factors, posed oncogenic risks associated with the viral integration and constitutive expression of proto-oncogenes [23]. Thus, hiPSC generated by such standard methodology might theoretically trigger a latent oncogenic potential, which could manifest as formation of tumors upon differentiation and implantation in clinical contexts.

Recent reports have provided validation of these theoretical concerns. For example, when 21 hiPSC lines derived from five different hiPSC induction methods were differentiated in parallel to cartilage, four of the 21 human iPSC lines generated cartilage *in vitro* that contained abnormal tumor-like glandular histology with expression of CEA and CA19-9 tumor markers, as well as forming glandular epithelial cells following transplantation into SCID mice [24•]. Similarly, foci of malignant-like characteristics are more consistently found in teratomas generated by incompletely reprogrammed and partially-reprogrammed hiPSC, as assessed by blinded histologic comparisons [25••]. These data correlated with previous findings of overexpression of cancer-associated genes in hPSC-derived hepatocytes, endothelial cells, and neural crest cells, versus corresponding primary tissues [26]. Finally, parallel differentiation of 40 hiPSC lines into dopaminergic neurons revealed seven 'differentiation-defective' clones that formed teratomas after transplantation into NOD/SCID mouse brains [27•]. Together, these studies demonstrate that among hiPSC sharing similar superficial measures of pluripotency, at least some hiPSC harbor the potential to form tumors upon differentiation and transplantation *in vivo*. Understanding the molecular basis for this inherent oncogenic potential harbored by hiPSC is critical both to enable screening of iPSC for safety, and for refinement of derivation methods to reduce this oncogenic potential.

Human iPSC derivation methods that achieved only partial reprogramming to a bona fide pluripotent state resulted in hiPSC lines that exhibit malignant histology [25••,28]. Likewise, in mouse models, premature termination of reprogramming leads to malignant tumor formation [29]. Dissection of the underlying epigenetics reveal an emerging model that suggests individual hiPSC lines may exist epigenetically on a continuum between normal embryonic cells (and hESC) on one end, and frankly aberrant embryonic carcinoma or cancer lines on the other (summarized in Figure 1). This model synthesizes together mechanistically related studies of epigenetic changes in cancer and associated reprogramming.

Central to this model has been the discovery that reprogramming can establish epigenetic states resembling those seen in cancer. For example, abnormal DNA hypermethylation at gene promoters established persistent silencing of tumor-suppressor genes and other key regulators leading to tumorigenesis [30]. Systematic comparison of normal stem cells, hiPSC lines, and cancer lines revealed a spectrum of aberrant, cancer-like promoter DNA hypermethylation and gene silencing, from normal hESC lacking cancer-like aberrations, to hiPSC that displayed progressively greater degrees of cancer-like promoter hypermethylation and gene silencing abnormalities [25••,31]. This pattern of promoter hypermethylation in cancer cell lines and some hiPSC, but reduced in other hiPSC and hESC, has been recapitulated in subsequent independent comparisons [27•]. These findings clearly suggest that the process of inducing self-renewal and de-differentiation to convert differentiated cells to hiPSC, may also steer those same cells toward an aberrant cancer-like epigenetic state not seen in hESC.

Further studies have directly linked aberrant, cancer-like DNA promoter hypermethylation with epigenetic states central to pluripotency. Pluripotent stem cells are characterized by a set of key developmental genes bearing 'bivalent' chromatin marking with repressive H3K27me3 and activating H3K4me3 histone marks. This balance of repressive and activating 'bivalent' chromatin marks upon these key promoters, and its associated occupancy by Polycomb-repressive complexes, leaves stem cells 'poised' for rapid reconfiguration upon appropriate developmental signals to establishment of a terminal differentiated state [32]. A succession of papers demonstrated links between cancer-associated promoter hypermethylation and pluripotent stem cell epigenetic marks. For example, Polycomb-mediated H3K27me3 was found to pre-mark genes for *de-novo* DNA methylation in a colon cancer cell line [33], and Polycomb group targets were found to be 12 times more likely to have cancer-specific promoter DNA hypermethylation [34]. Building upon these earlier studies, a more direct, explicit connection between these two fundamental epigenetic mechanisms was recently established through integration of genome-wide epigenetic data between stem cells and cancer cells. Over 75% of genes bearing CpG-island DNA hypermethylation specifically in cancers, were found to be 'bivalently' marked in stem cells. This aberrant hypermethylation among 'bivalent' marked genes was enriched among developmental regulators. The methylation status of the genes within this intersecting cancer/stem cell hypermethylation module, when correlated with whole-genome methylation data from hundreds of patient samples, crisply segregated colon and breast cancer patients with previously established subtypes with better and worse prognoses [35••]. Together, these findings explicitly correlate cancer as an epigenetically deranged state that intersects with an underlying stem cell self-renewal program.