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The Epigenome and Cancer Stem Cell Fate: Connected by a Linker Histone Variant

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The molecular features underlying tumor heterogeneity and the role of chromatin components in regulating cell fate within tumors are not well understood. Recently in *Science*, Torres et al. (2016) showed that the linker histone variant H1.0 functions as a chromatin switch that determines self-renewal versus differentiation decisions in cancer stem cells.

Tumors are composed of several cell populations and this heterogeneity poses a challenge to our understanding of cancer development, diagnosis, and treatment choices. In particular, it is thought that self-renewing cells termed cancer stem cells (CSCs) determine expansion and long-term proliferative potential of tumor cells. Recently in *Science*, Torres and colleagues document how intratumor heterogeneity is driven via differential expression of the linker histone H1 variant H1.0 through its role in cancer cell differentiation (Torres et al., 2016) (Figure 1). H1.0 is one of four out of the ten H1 variants whose expression occurs throughout the cell cycle, compared to the six variants whose expression is replication dependent. While some level of functional redundancy is suggested by the viability of knockout mice for several H1 proteins, there is evidence of variant-specific effects on gene regulation and chromatin organization (Alami et al., 2003). Expression levels of the H1.0 variant have long been known to be correlated with terminal differentiation and inversely correlated with neoplastic potential (Lea, 1987); however, its relevance in tumor formation had not been directly assessed.

Torres et al. exploit a previously characterized model system based on experimental transformation of epidermal fibroblasts (Scaffidi and Misteli, 2011). Following transformation, tumor tissue acquires cellular heterogeneity as attested by differential expression of the stem cell surface marker Stage-Specific Embryonic Antigen 1 (SSEA1). By comparing gene expression in SSEA1-positive CSCs and SSEA1-negative cells, they identify the H1.0 gene as a marker of the differentiated cell population. They also analyze a series of human tumors and find that heterogeneity of H1.0 levels is observed in many human cancers and that poor patient survival in multiple cancer types is associated with low H1.0 levels. They conclude that H1.0 level is of prognostic value, uncovering its relevance as a biomarker and potential therapeutic target.

This study also emphasizes a direct role for H1.0 as a driver of stem cell differentiation within tumors. The authors show that H1.0 silencing in CSCs is required to maintain self-renewal ability and tumorigenic potential. Conversely, ectopic expression of H1.0 is sufficient to limit proliferation and promote cellular differentiation. The authors propose that H1.0 expression can lead to silencing of proliferation and oncogenic genes and the expression of differentiation factors. Interestingly, this finding suggests the lack of functional redundancy between H1.0 and other H1 variants, with a unique role for H1.0 in driving CSC differentiation. In contrast, H1.0 knockout mice are viable and fertile and display normal cell proliferation (Sirotkin et al., 1995). Further work will likely follow the lead provided by Torres et al. to investigate the role of H1.0 in endogenous tumor environments and better understand natural heterogeneity from the perspective of chromatin composition.

The next critical question was to understand how H1.0 expression is regulated in stem cells in the context of both development and cancer. Importantly, the authors found that transcriptional silencing is achieved through methylation of a particular CpG-rich DNA region within the H1.0 gene. While the exact mechanism of this methylation switch remains to be uncovered, previous work in other model systems provides interesting clues. In *Xenopus* egg extracts, H1.0 activation can be triggered by increased histone acetylation with TSA treatment (Almouzni et al., 1994), while in differentiating dendritic cells, NF-kappaB can drive H1.0 expression (Gabrilovich et al., 2002). In addition, the H1.0 promoter is poised for activation in human embryonic stem cell lines (marked by the histone post-translational modifications H3K27Me3 and H3K4Me3, characteristic of so called "bivalent promoters"), suggesting that Polycomb-mediated repression may keep the H1.0 gene silent in stem cells (Terme et al., 2011). In contrast, more differentiated cell lines display marks of active transcription at the *H1.0* promoter. This indicates that transcription factors associated with differentiation may durably activate H1.0 transcription, and this may exploit epigenetic switches involving histone variants, DNA modifications, or both. Given the seemingly critical role of H1.0 as driver of differentiation, it will be crucial to dissect these mechanisms during the initial steps of lineage commitment.

Once expressed, how does H1.0 drive differentiation? While H1.0 is mostly recruited to CG-rich regions, the authors showed that H1.0 largely affected AT-rich segment chromatin. In the absence of H1.0, FAIRE-seq experiments indicated that a set of AT-rich regions

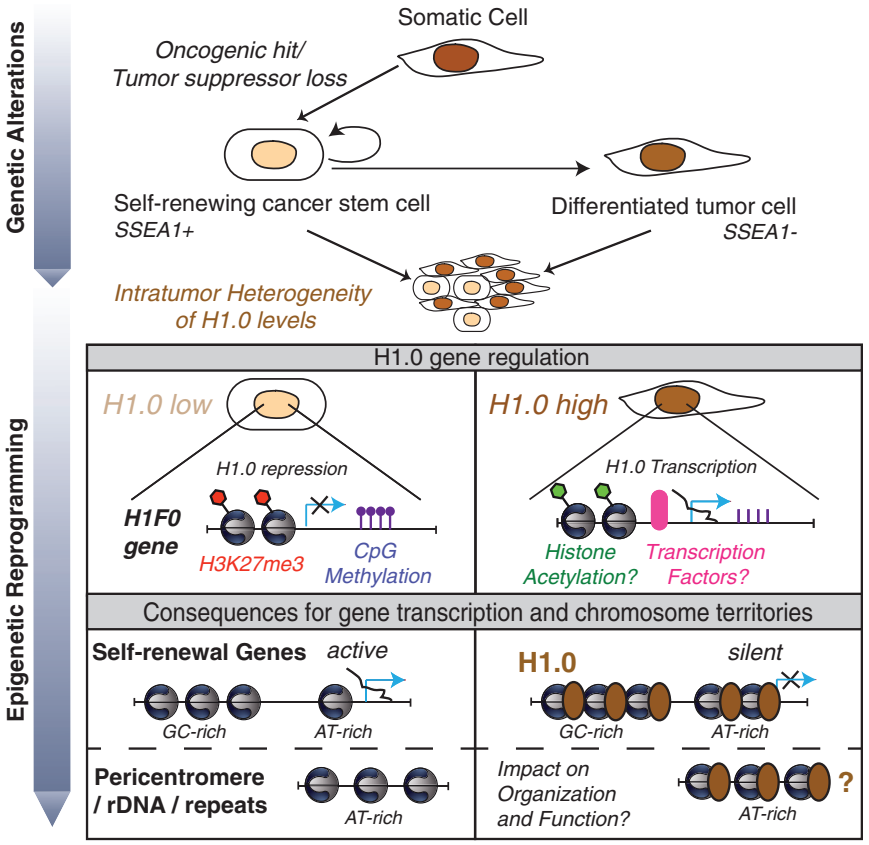

Figure 1. H1.0 Hetergeneity in Tumors Determines Cell Fate Decisions
Following genetic changes that allow tumorigenic growth, cell populations emerge with cancer stem cells (SSEA1+), characterized by low H1.0 levels, and differentiated tumor cells (SSEA1−), with high H1.0 levels. The H1.0 gene itself is regulated by methylation of a CpG shore region within the H1.0 gene body and potential additional epigenetic switches. H1.0 silences self-renewal genes and may impact AT-rich heterochromatin, rDNA, and other repeat regions.

became over-exposed, typically reflecting nucleosome depletion. At these sites associated with genes crucial to proliferation and tumor progression, the authors suggest that the linker histone could play a stabilizing role for nucleosomes. Importantly, beyond genic regions, other chromosome territories may host H1.0 as well and be sensitive to its presence. H1.0 enrichment at AT-rich centromeric and pericentromeric heterochromatin in both mouse and human cells, as well as at a number of repeat regions including ribosomal DNAs, Alu elements, and telomeric satellite sequences, may also be important to consider (Mayor et al., 2015). The integrity of these chromosome landmarks impacts cell cycle progression and could influence stem cell fate decisions. These regions are also marked by the presence of particular histone variants for nucleosomal histones, notably histone H3 variants CenH3 (in centromeric chromatin) and H3.3 (at repeat regions and telomeres). H1.0 in these contexts could help stabilize nucleosomes containing these variants. By regulating their structure and dynamics this could ultimately functionally impact these regions. Further work to dissect the dynamics and roles of H1.0 in the context of heterochromatin will shed light onto these issues.

Finally, if H1.0 is a barrier to stemness, can H1.0 depletion favor induced pluripotency? In light of work highlighting the role of the nucleosome assembly factor CAF-1 in impeding reprogramming (Cheloufi et al., 2015), and the suggested role for H1.0 in stabilizing nucleosomes, it is tempting to speculate that H1.0 may play a similar role as a barrier to reprogramming. Whether this is only relevant in a tumoral context or more generally would be highly interesting. This may be particularly important at regulatory elements that depend on pluripotency factors, where H1.0 could stabilize a nucleosome to block transcription factor access to DNA. Understanding mechanisms at work to control nucleosome stability in the two-way relationship between stem cells and their differentiated counterparts will be an exciting challenge. Whether manipulating these cell fate choices in this way could open therapeutic avenues will be of major interest in the context of strategies in cancer treatment and regenerative medicine.

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