Linker histone H1.0 generates epigenetic and functional intratumor heterogeneity

Cancer originates from a single cell, however most human cancer cells exhibit intratumor heterogeneity and display distinct phenotypes. Functional heterogeneity is responsible for this complex diversity, and is evident at various levels. Phenotypic and functional heterogeneity can arise in the same tumor due to environmental, genetic, and reversible changes [1]. Functional heterogeneity allows researchers to ensure that cancer stem cells are in different states and to provide reactions to regulatory influences at a population level [2]. Phenotypic and functional heterogeneity can be traced to distinct differentiation states, and if epigenetic changes were to occur during tumor growth, it could establish hierarchies that affect the long-term proliferation of cancer cells. However, the mechanism and the contribution to functional heterogeneity of this is unknown. In this study, Torres et al. (2017) identified an epigenetic mechanism that established intratumor heterogeneity through generating hierarchically organized human tumors. They found that H1.0, which is found in dividing and non-dividing cells, inhibits tumor maintenance through reversible silencing which affects the differentiation states and self renewal potential which drive tumor growth [3].

Torres et al. (2017) used their own system to model functional intratumor heterogeneity, which uses the CSC (cancerous) marker SSEA1. SSEA1 has uncontrolled proliferation potential and multipotency [4]. These are also known as self renewing tumor cells. Among differentially expressed genes of SSEA1+ and SSEA1- cells, *H1F0* which encodes H1.0, displayed consistent downregulation of SSEA1+ cells in multiple tumors. Further examination of protein levels via quantitative immunofluorescence microscopy of tumor sections showed lower levels of H1.0 in self renewing SSEA1+ cells. This was also confirmed by using flow cytometry, supporting the association between H1.0 levels and proliferation potential. This was also seen in other cancer tissues as well, suggesting that they exhibit heterogeneous expression of H1.0 and that cells with undifferentiated phenotypes with expressed CSC show low levels of H1.0.

Aberrant DNA-methylation is a significant factor in downregulating cancer, such as the *TERT* oncogene, *p15* tumor suppressor and *BRCA1* . DNA methylation may occur by CpG island methylator phenotypes or constitutional DNA methylation [5]. In healthy adult tissues, H1F0 is unmethylated and highly expressed. To see if DNA methylation affects *H1F0* levels in tumors, bisulfite sequencing analysis of *H1F0* locus. They also compared SSEA1+ cells with other highly differentiated cells. SSEA1+ was found to be highly methylated on multiple CpG islands. Further examination using ChIP revealed high levels of H3K27ac in the CGI shore, suggesting that an enhancer is present. Targeted methylation of the CGI shore was shown to control *H1F0* expression by silencing *H1F0* in self-renewing tumor cells.

H1.0 loss was shown to upregulate AT rich sites at self renewal sites, and H1.0 seemed to be enriched around the TSS. Combined with the fact that AT regions may be thermodynamically unstable when wrapped around nucleosomes, perhaps H1.0 represses the AT regions by

stabilizing nucleosomes at promoters. Altered nucleosome occupancy without H1.0 depends on GC content. When a knockout was performed, the AT regions showed more nucleosome alterations, becoming more occupied in the absence of H1.0. This shows that nucleosomes were forced to occupy regions of higher AT content. H3K27ac and H3K27me3 was mapped by ChIP and showed that altered nucleosomes induced by H1.0 loss, and that upregulated self renewal related genes did show significant differences in the ac and me3 content at their promoters following the H1.0 knockdown. This suggests that the changes in gene expression were likely a consequence of altered nucleosome occupancy. Furthermore, in AT rich regions the loss of H1.0 destabilizes nucleosome-DNA interactions, while increasing accessibility to regulatory elements for self-renewal.

The evidence discussed here points to genetic and non-genetic sources of intratumor heterogeneity. Here, Torres et al. (2017) found that epigenetic states controlled by integral parts of chromatin define cell subpopulations that contribute to tumor maintenance. The experimental epigenetic states were seen to alter the self-renewal and differential balance of cancer cells showed the importance of chromatin based pathways. Additionally, cells that silence H1.0 preserve their proliferation ability, however cells that re-express the protein repress oncogene gene networks and express a different differentiated phenotype that has limited proliferation potential. They propose that because of these findings, cells that are insensitive to differentiation cues and are capable of permanently silencing H1.0 can act as self-renewing tumor maintenance. Restoring high levels of H1.0 in cancer cells may be beneficial by enhancing the differentiation process.

This paper provides extremely valuable insight into cancer proliferation mechanisms. The results were well prefaced in the introduction. Because of this class, I was able to understand a significant portion of the results which greatly helped me to draw conclusions about the paper. They thoroughly explained their past experiments and methods, which made the figures easier to understand. I appreciated that they did not only use one type of human cancer cell, and used cells from the breast and brain. However, in the introduction, I was not familiar with the term “phenotypic and functional heterogeneity” which is a recurring point in the study. I would have liked for the introduction to have focused more on the definition and purpose for using it as a way to measure the impact of the results. I would also have liked to see more investigation in breast cancer tumor cells. There have been other studies that show SSEA1 contributing to malignant phenotypes in breast tumors, which also require SSEA1 to be present so tumor cells interact with endothelial tissue, attributing this to proliferation [6]. Additionally, something that was alluded to, but not thoroughly discussed in the paper was the tumor microenvironment. It appears to be a significant source of variation when discussing tumor treatment [7]. How does individual microenvironments impact heterogeneity? How does cell signaling and signal transduction play a role not only in proliferation, but supporting this environment for growth? How can we utilize CTM (tumor interacting proteins), TEC (tumor endothelial cells), CAF (cancer associated fibroblasts), and more with the regulation of H1.0[7]? In the future, I look

forward to more research surrounding the impacts and therapeutics on individual tumor cells using epigenetic regulation.

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