Editorial

Macrohistone H2A1 takes the center stage in cancer research as a regulator of stemness

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Cancer stem cells (CSCs) are cancer cells that possess characteristics associated with normal stem cells, specifically the ability to give rise to all cell types found in a particular cancer sample. CSCs are therefore tumorigenic, and may generate tumors through the stem cell processes of self-renewal and differentiation into multiple cell types. Such cells are hypothesized to persist in tumors as a distinct population and cause relapse and metastasis by giving rise to new tumors. In fact, as CSCs form a small proportion of the tumor, this may not necessarily select for drugs that act specifically on the stem cells. The CSC theory suggests that conventional chemotherapies kill differentiated or differentiating cells, which form the bulk of the tumor but do not generate new cells (1). A population of CSCs, which gave rise to it, could remain untouched and cause relapse. Therefore, development of specific therapies targeted at CSCs holds hope for improvement of survival and quality of life of cancer patients, especially for patients with metastatic disease. To this aim, we need to understand a great deal about the genetic and epigenetic mechanisms controlling CSC stemness and tumorigenic potential. Epigenetic mechanisms of nuclear chromatin remodeling are increasingly recognized as crucial factors in the pathophysiology of cancer. In fact, tumorigenic alterations within cells are triggered at the cellular level by changes in gene transcriptional patterns dependent on the degree of nuclear chromatin compaction. The latter is regulated at several levels, allowing transcriptional plasticity. A recently emerged alternative mechanism of transcriptional plasticity is the replacement of canonical histones, around which DNA is wrapped (H2A, H2B, H3 and H4), with the incorporation of histone variants, mostly of histones H2A or H3 (2-4). Variant histones have evolved crucial roles in chromosome segregation, transcriptional regulation, DNA repair, sperm packaging and other processes such as cell proliferation. Histone variants emerged early in eukaryotic evolution and were later displaced for packaging roles by the canonical histones, the synthesis of which is coupled to DNA replication. Differences among histone variants in their stability, DNA wrapping, specialized domains that regulate access to DNA, and post-translational modifications, underlie the diverse functions that histone variants have (4). Among core histones, the H2A family exhibits highest sequence divergence, resulting in the largest number of variants known. Strikingly, H2A variants differ mostly in their C-terminus, including the docking domain, strategically placed at the DNA entry/exit site and implicated in interactions within the nucleosome. Moreover, the acidic patch, important for internucleosomal contacts and higher-order chromatin structure, is altered between different H2A variants. Consequently, H2A variant incorporation has the potential to strongly regulate DNA organization on several levels resulting in meaningful biological output. H2A histone variants include H2AX, H2A.Z, H2A.Bbd and mascrohistone H2A (MacroH2A) (4).

MacroH2A (comprising of two different genes encoding similar proteins, macroH2A1 and macroH2A2) is the largest among H2A histone variants and among all histones, and it is believed to act as a strong transcriptional modulator that can either repress transcription, or activate it in response

to as yet undefined nutrients or growth signals (5-11). The highest degree of diversification among histone H2A variants is to be found in their C-termini, regarding both length and amino acid sequence. Accordingly, macroH2A is composed of a domain 66% homolog to histone H2A, and it stands out because of its unique structure, whereby a C-terminal linker connects the histone fold domain to a macro domain. This domain protrudes from the compact structure of the nucleosome, likely affecting the function and organization of the surrounding chromatin, and is conserved in multiple functionally unrelated proteins throughout the animal kingdom. Initially thought to be present only in vertebrates, there is now evidence of macroH2A presence also in invertebrates (12).

The impact of macroH2A, and in particular of macroH2A1, on transcriptional processes has now come to take a center stage in the plasticity of stem cell differentiation and in the pathogenesis of a growing number of cancer types (13-16). MacroH2A1 levels negatively correlate with the self-renewal capacity of the pluripotent stem cells and regulate the delicate balance between self-renewal and differentiation of embryonic and adult stem cells (13). Moreover, macroH2A1 can act as a powerful oncogene or tumor suppressor in a context-dependent and isoform-specific manner (14). In the cancer of the bladder, arising from the the urothelium lining the urinary bladder and being the 9th leading cause of cancer, the alternative splicing of macroH2A1 pre-mRNA can regulate aggressiveness and progression (17). Despite its increasingly appreciated role in cancer progression and stemness in non transformed cells, until now the role of macroH2A1 in CSC in any cancer type was unknown.

The recent Oncogene report from Park *et al*. titled "MacroH2A1 downregulation enhances the stem-like properties of bladder cancer cells by transactivation of Lin28B" fills this gap, and it demonstrates that the macroH2A1 has a pivotal role in the bladder tumor progression and the regulation of stem-like characteristics of bladder cancer cells, through regulation of the Lin28B/let-7 pathway (18). How to define CSC in a unequival manner *in vitro* or *ex vivo* remains a matter of debate to the community and it depends of the type of cancer. Indipendently of the type of cancer studied, it is now widely accepte that CSC are characterized by an enhanced ability to migrate and metastasize, an enhanced epithelial-mesenchymal transition (EMT) (the process by which epithelial-like cells lose their cell polarity and cell-cell adhesion, and gain migratory and invasive properties to become mesenchymal stem cells), a larger size of tumors compared to cancer cells when inoculated into nude or immunocompromised mice. All these CSC properties are met when bladder cancer cells are silenced for macroH2A1 expression in the study of Park *et al*. (18), where it was observed enhanced proliferation and expression of EMT markers, and spectacularly increased tumor size in xenograft orthotopic mice models. Importantly the re-introduction of macroH2A1 restored the formation of smaller tumors. These authors accompanied the analysis of CSC aggressive properties with a detailed molecular and cell biology analysis. First bladder CSC depleted in macroH2A formed clearly spheres in 3D cultures; second, they commenced to express high levels of stemness markers OCT-4, c-MYC and KLF4; third, they displayed improved DNA damage repair upon irradiation; fourth they have an increased side population evaluated by the efflux of DNA binding dye Hoechst 33342 and, fifth, these cells displayed lower reactive oxygen species (ROS) levels (18).

A mentioned above, the concept that macroH2A1 is a key regulators of stem-like properties is not new, although this is the first time that it is extended to CSC, e.g., bladder cancer cells. In this study, Park *et al*. provide key molecular insights in the mechanism involved in the macroH2A1-dependent bladder cancer stemness. By using a commercially available PCR array of the 84 most important genes involved in CSC potential, they identify as a top hit LIN28B. LIN28 encodes an RNA-binding protein that binds to the let-7 pre-microRNA and blocks production of the mature let-7 microRNA in mouse embryonic stem cells, in pluripotent embryonal carcinoma cells and other stem cell types including CSC, to regulate their self-renewal (19). In this study, Park *et al*. show a nearly perfect anti-correlation in immuno-staining scoring between macroH2A1 and LIN28B in normal human bladder versus bladder cancer (18). MacroH2A1-dependent LIN28B activation led to let-7 microRNA family (a-b-c-d-e-f-g-i) suppression. Over-expression of LIN28B in bladder cancer cells recapitulated the pro-proliferative, pro-invasiveness and pro-metastatic effects induced by macroH2A1 depletion (18). Moreover LIN28B overexpression triggered the appearance of the very same CSC-like properties observed in bladder cancer cells upon deletion of macroH2A1 (18). Park *et al*. finally attempt to provide an epigenetic mechanism for macroH2A1-dependent LIN28B, analyzing promoter occupancy and competition with established chromatin remodeling factors p300, EZH2 and SUV39H1. Their analysis showed that the depletion of

mH2A1 significantly increased the occupancy of p300 on Lin28B promoter regions subsequent acetylation of lysine 27 on histone H3 (H3K27ac). Conversely, the localization of EZH2 on Lin28B promoter regions was restricted by mH2A1 depletion thereby decreasing the level of tri-methyl H3K27 (H3K27me3) at the promoter regions of the Lin28B locus (18), finally suggesting that the reciprocal bindings of coactivators and corepressors mediated by mH2A1 on the Lin28B promoter regulate the Lin28B expression and its downstream pathway, thereby possibly governing CSC potential.

The study from Park *et al*. recognizes for the first a macroH2A1/LIN28B epigenetic signaling pathway that could be targeted to eradicate bladder CSC, a primary driver for bladder cancer relapsing. MacroH2A1 is a histonic protein that, as such, does not present with enzymatic pockets or binding domain that could be easily druggable. The exciting finding that human macroH2A1 binds the SirT1-metabolite O-acetyl-ADP-ribose (OAADPR) through its macro domain (20) did not seem to open new therapeutic avenues so far, although providing for the first time a direct molecular link between cellular metabolism and the epigenetic machinery. Other approaches using histone mimics to disrupt chromatin complexes that have been shown to be effective to block inflammatory pathways in macrophages might be more promising (21). Even if LIN28B overexpression induces a strong stemness phenotype in bladder cancer cells, recapitulating the one induced by macroH2A1 depletion, the study from Park *et al*. did not analyze CSC transcriptome with deep sequencing; leaving out the possibility that macroH2A1 would act on the miRNome, LINE-1 elements or on lncRNAs. Moreover, the genomic action of macroH2A1 can not be reduced to the promoter of one gene (LIN28B) because (I) genome occupancy of macroH2A1 is typically abundant in introns and intergenic regions, not only in promoters; (II) it is unclear how displacement of macroH2A1 genome occupancy can occur during increase in carcinogenesis without artificial manipulation and how this correlates with significant changes in gene expression at the global genomic/transcriptomic level (8,9). Previous deep seequencing studies suggested indirect oncogenic transcriptional programs independent of changes in macroH2A1 genomic occupancy: variations in macroH2A1 transcriptional activities without changes in genome occupancy have been reported in breast cancer cells and in hepatocellular carcinoma cells (8,9). If this is the case for bladder cancer cells remains unsolved. Moreover, macroH2A1 exists as two alternatively exon-spliced isoforms, macroH2A1.1 and macroH2A1.2, which differ just for few amino acids. In most cancers studied to date, with the exception of hepatocellular carcinoma, macroH2A1.1 acts as a tumor suppressor cancer types, whereas the role of macroH2A1.2 is oncogenic (14). MacroH2A1.1 but not macroH2A1.2 has been found decreased in bladder cancer (17). The role of macroH2A1 splicing variants and the molecular determinants for their functional differences in bladder cancer and bladder CSC would need further investigation. As standard oncologic treatments, such as chemotherapy, radiotherapy and surgical resection, can only shrink the bulk tumor, with the tumor tending to relapse, therapeutic strategies focusing on targeting CSCs and their microenvironmental niche will address the ineffectiveness of traditional cancer therapies to eradicate the CSCs that otherwise result in therapy resistance. The combined use of traditional therapies with targeted CSC-specific agents may target the whole cancer and offer a promising strategy for lasting treatment and even cure. Enhanced knowledge on the epigenetic circuitry implicated in CSC maintenance and potential will drive towards this direction.

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Footnote

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