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RAPID COMMUNICATION

Profiling of histone H3 trimethylation and distinct epigenetic pattern of chromosome Y in the transformed bronchial epithelial cells induced by consecutive arsenic treatment

To the Editor:

Environmental arsenic is a known human carcinogen that is associated with cancers in lung, liver, skin, breast, bladder, kidney, prostate, etc.[1] However, the underpinnings of malignant transformation of the normal cells in response to environmental arsenic exposure remain poorly understood, in part due to mixed signaling pathways elicited by arsenic, especially the inorganic trivalent arsenic (As^{3+}). In the present report we profiled the trimethylation status of histone H3 through global chromatin immunoprecipitation followed by sequencing (ChIP-seq) in the As^{3+}-induced transformed cells and found an overall gain of active methylation of histone H3, the trimethylation of lysine 4 of histone H3 (H3K4me3), along with a diminished enrichment of the repressive marker H3K27me3 in the genome in the transformed cells relative to the control cells. Many oncogenic genes responsible for cell proliferation and self-renewal of the cancer stem-like cells are enriched with H3K4me3 along with a reduced level of H3K27me3 and/or H3K9me3. In contrast, some genes encoding proteins with tumor suppressor-like activity showed reduced enrichment of the active marker, H3K4me3, but elevated levels of the repressive markers, H3K27me3 and H3K9me3. These data, thus, suggest that the effect of As^{3+} on histone methylations that define the chromatin configuration and gene expression dynamics of the genome and the cell fate decision may contribute significantly to the carcinogenicity of As^{3+}.

We had previously shown that treatment of the non-cancerous human bronchial cell line BEAS-2B cells with 0.25 μM As^{3+} consecutively for 6 months induces transformation and the generation of the cancer stem-like cells.[2] To understand how such a treatment causes malignant transformation, global ChIP-seq was performed to profile the epigenetic pattern, specifically, the trimethylation of the key lysine residues in histone H3 protein, in both the parental BEAS-2B cells and the cells treated with 0.25 μM As^{3+} for 6 months (As^{3+}-transformed cells). It had been well-documented that histone H3 lysine4 trimethylation (H3K4me3) is an active marker for gene transcription, whereas H3K9me3 and H3K27me3 are repressive markers for transcription due to the configuration of heterochromatin that prevents access of transcription factors to their target genes.[3] As exhibited in Figure 1A, the transformed cells showed an overall enhancement of H3K4me3. In contrast, a significant decrease of H3K27me3 was noted in the transformed cells. There is no measurable difference of H3K9me3 on the global genome scale between the parental cells and the As^{3+}-transformed cells. By scanning the entire chromosome regions of the randomly selected chromosomes using UCSC Genome Browser, except Y chromosome, all other chromosomes showed notable increase of H3K4me3 and decrease of H3K27me3, as exampled for chromosome 3 (Fig. 1B). It was striking to note that all of the examined histone H3 trimethylation markers, H3K4me3, H3K9me3 and H3K27me3, are nearly wiped out on chromosome Y in the As^{3+}-transformed cells, except in the centromere regions (red box in Fig. 1B, right panel). This epigenetic pattern is also reflected on the majority of the individual genes in chromosome Y, such as *UTY*, a demethylase of H3K27me3, and *KDM5D* that demethylates H3K4me3 as well as H3K4me2, in the transformed cells.

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Complete loss of H3K4me3 on *KDM5D* can block the expression of KDM5D, which may be attributed to the overall enhancement of H3K4me3 in the transformed cells as indicated in Figure 1A. As comparison, the patterns of H3K4me3, H3K9me3 and H3K27me3 on another allosome, chromosome X, were indistinguishable from all autosomes (data not shown). Similar to other chromosome, the chromosome X in the transformed cells also exhibited a notable enhancement of H3K4me3 along with a reduced level of H3K27me3.

Relative to the parental cells, we found 283 genes showed pronouncedly increased, and 77 genes showed decreased enrichment of H3K4me3 in the As^{3+}-transformed cells. Only 16 genes and 13 genes showed significant increase and decrease, respectively, of H3K9me3 in the transformed cells. For another repressive marker H3K27me3, 234 genes had an enhanced enrichment and 479 genes had a reduced level of H3K27me3, respectively (Fig. S1A). To get insights into which sets of genes are more likely to acquire an overall active epigenetic status in the transformed cells, we next conducted gene ontology pathway assay for the biological process of these genes with an active epigenetic status. As indicated in Figure 1C, the top-ranked pathways of these genes are largely centered on embryonic organ development, skeletal development, epithelium development, nervous system development, stem cell development, and morphogenesis. NCI-Nature pathway analysis suggested that many of these genes are in the pathways associated with cancer cell growth or proliferation, such as signaling of VEGF, ER, ErbB, β-catenin, SMAD2/3, S1P1, BMP, etc (Fig. 1D). The active status of S1P1 pathway in the transformed cells is in agreement with an enhanced metabolism of sphingolipids as demonstrated in a metabolomic analysis of the As^{3+}-transformed cells (Fu, et al, in press).

To further understand how As^{3+} induces transformation, we also analyzed some genes that were not included in above pathway assay but had been linked to human cancers. As shown in Figure S2, most of these indicated genes, including *PDGFRA*, *KIT*, *MYC*, etc., displayed an enhanced enrichment of H3K4me3, with or without concomitant decline of H3K27me3, or H3K9me3. Intriguingly, IKBKB (IKKβ) and NFKB1 (p50), the two key molecules for NF-κB activation and activity, showed an identical epigenetic pattern with an enhanced H3K4me3 and a decrease of H3K9me3 in the transformed cells.

It has been proposed that genes having a reduced level of H3K4me3 with or without an increase of H3K27me3 or H3K9me3 are in a repressive epigenetic status that prevented expression of these genes. ChIP-seq data showed a total of 327 of such genes in the As^{3+}-transformed cells. NCI-Nature pathway analysis of these genes suggested an over-representation of the pathways in LKB1, p38 and p53 (Fig. S3A), all of which are known tumor suppressors. In the

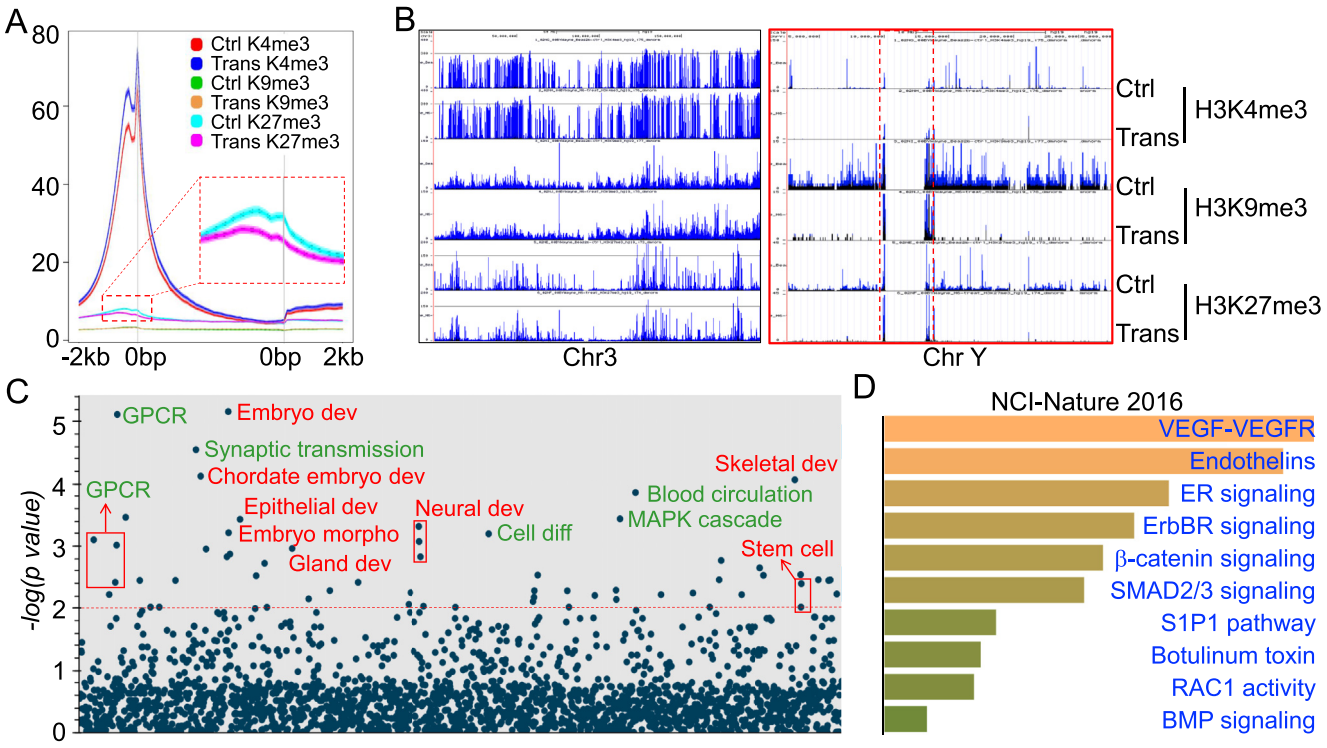

Figure 1 The As^{3+}-transformed cells showed enhanced active chromatin markers. **(A)** Histone methylation profiles of ChIP-seq for H3K4me3, H3K27me3 and H3K9me3 between parental cells and transformed cells. Average plots of the genebodies were shown. Trans: transformed cells. **(B)** Genome Browser screenshots of ChIP-seq for the entire regions of chromosomes 3 and Y. **(C)** Manhattan Plot shows major pathways that are featured with active chromatin status (increased H3K4me3 and decreased H3K9/27me3) in the transformed cells. **(D)** NCI-Nature 2016 pathway assay for these genes with active chromatin status in the transformed cells.

transformed cells, complete loss of H3K4me3 was noted on the tumor suppressors *MARK2*, *TP73* and *IRX2* (Fig. S3B). Both *TP73* and *IRX2* also showed gain of H3K27me3 and/or H3K9me3 (green arrows in Fig. S3B). Other tumor suppressors, including *ATF3*, *ARID3A*, *TNFRSF10C*, *SMARCD3*, *SLURP1*, *LYPD2*, and *LYNX1*, showed either a reduced level of H3K4me3, or increase of H3K27me3 and/or H3K9me3 (Fig. S3C).

In summary, based on the enrichment levels of the trimethylation of three most important lysines of histone H3, our data unraveled an overall active epigenetic status in the transformed cells induced by consecutive As^{3+} treatment, especially for those genes contributing to the proliferation and oncogenesis. In contrast, many tumor suppressor-like genes exhibited a repressive epigenetic status in the transformed cells. This epigenetic pattern provided a new mechanistic explanation on As^{3+} carcinogenesis. It was unexpected that chromosome Y in the transformed cells showed a nearly complete loss of the enrichment signals for H3K4me3, H3K9me3 and H3K27me3. At this moment, we have no clue on the biological or pathological significance of such a finding. There is no conclusive epidemiological evidence showing influences of environmental As^{3+} exposure on male fertility,[4] despite some scattering reports indicating infertility, gonad dysfunction and low sperm quality among individuals who exposed to As^{3+}.[5] Meanwhile, the unique histone methylation pattern of chromosome Y may be not a result of aneuploidy loss of chromosome Y in the transformed cells, because several genes in chromosome Y showed 1- to 4-fold increased expression relative to the non-transformed cells as determined by transcriptomic analysis (data not shown). Taken together, the findings presented here will be fundamental for understanding how As^{3+} causes human diseases and designing appropriate prevention and intervention strategies of cancers resulted from environmental As^{3+} exposure.

Conflict of interests

The authors declare no conflicts.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2021.11.005.

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