

Characterizing the effects of the Polycomb and DNA methylation silencing mechanisms on the MYOD1 promoter.

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Epigenetic mechanisms regulate the genome in normal tissue and are implicated reprogramming the genome in cancer, resulting in aberrant gene expression and cellular transformation. Such events during carcinogenesis involve DNA methylation, covalent modification of core histone proteins, and altered nucleosome positioning at gene promoters. While each of the aforementioned modulations is dependent on specific classes of proteins, a high level of combinatorial control must be maintained to ensure the correct chromatin conformation and cellular stability.

DNA methylation and Polycomb group proteins repress gene expression through different means. The process of DNA methylation results in a methyl group being added to the cytosine ring of DNA. Polycomb group proteins facilitate the addition of three methyl groups on lysine 27 of Histone H3 (H3K27me3). Aberrant DNA methylation of CpG island promoters have been linked to cancer development; while Polycomb group proteins have largely been implicated in the regulation of developmental genes. We have recently reported that prostate cancer cells exhibit a gain in DNA methylation upon loss of H3K27me3 (3), suggesting a mechanistic switch from normal somatic cells to their cancerous counterparts. These results are consistent with previous reports suggesting that in most cases DNA methylation and H3K27me3 are largely mutually exclusive (2, 4, 5). Here we sought to investigate how nucleosome occupancy and the complement of histone modifications are different in cancer cells silenced by DNA methylation or H3K27me3.

To study the differences in chromatin structure present in promoters silenced by methylation or H3K27me3, we utilized the human myogenic differentiation gene 1 (MYOD1). MYOD1 is a tissue specific gene that is usually silenced by H3K27me3 in non-muscle cells. However, previous work has shown that the MYOD1 promoter can be silenced by DNA methylation in some transformed cells (6). Therefore, we were able to study the differences between the promoter structures by using three different cell lines – an expressing muscle cell line (RD), a normal fibroblast cell line (LD419) and a colorectal carcinoma cell line (RKO). We confirmed that H3K27me3 is enriched across the promoter region of MYOD1 in LD419 cells. In contrast, neither expressing RD cells, nor RKO colorectal carcinoma cells exhibited H3K27me3 in the same region. Histone H3 occupancy was higher across the MYOD1

promoter in both silenced cell lines compared to expressing RD cells, which contain a nucleosome free region upstream of the transcriptional start site. To this end, we confirmed that the H3K4me3 mark, an activating histone modification, is enriched across the MYOD1 gene in the RD cells. As expected, H3K4me3 is not present at the MYOD1 promoter in either cell line in which MYOD1 is silenced. We further sought to confirm whether additional repressive histone modifications are associated with MYOD1 when silenced. We investigated the role of the H3K9me3 modification in MYOD1 silencing, which binds to chromatin to repress transcription through inhibition of RNA Polymerase II elongation. H3K9me3 was enriched across the MYOD1 promoter in both the LD419 fibroblast (H3K27me3 silenced) and RKO colorectal (DNA methylated) cell lines compared to the expressing RD cells. Collectively these data reveal that DNA methylation and the H3K27me3 histone modification occur independent of one another at the MYOD1 promoter, however, a more compact and restrictive chromatin structure is generated in either case (Figure 1).

In order to investigate differences in nucleosome positioning at the MYOD1 promoter, we examined the chromatin structure surrounding the transcriptional start site of MYOD1 using a powerful high-resolution, single molecule technique (Methylation Sensitive Promoter Analysis; M-SPA; 1). While a nucleosome free region is located immediately upstream of the transcriptional start site in expressing RD cells, the same site is occupied by a nucleosome in H3K27me3 silenced fibroblasts. We are currently investigating the possibility that there may be differences in nucleosome stability when marked by H3K27me3 compared with DNA methylation (Figure 1). Preliminary results indicate the presence of an unstable nucleosome in LD419 fibroblasts immediately upstream of the transcriptional start site, which may not be present in cells silenced by DNA methylation. By determining the presence of a switching from normal somatic cellular phenotypes to DNA methylation through H3K27me3 silencing, we may be able to potentially target preventative therapeutics at the most relevant stages of carcinogenesis.

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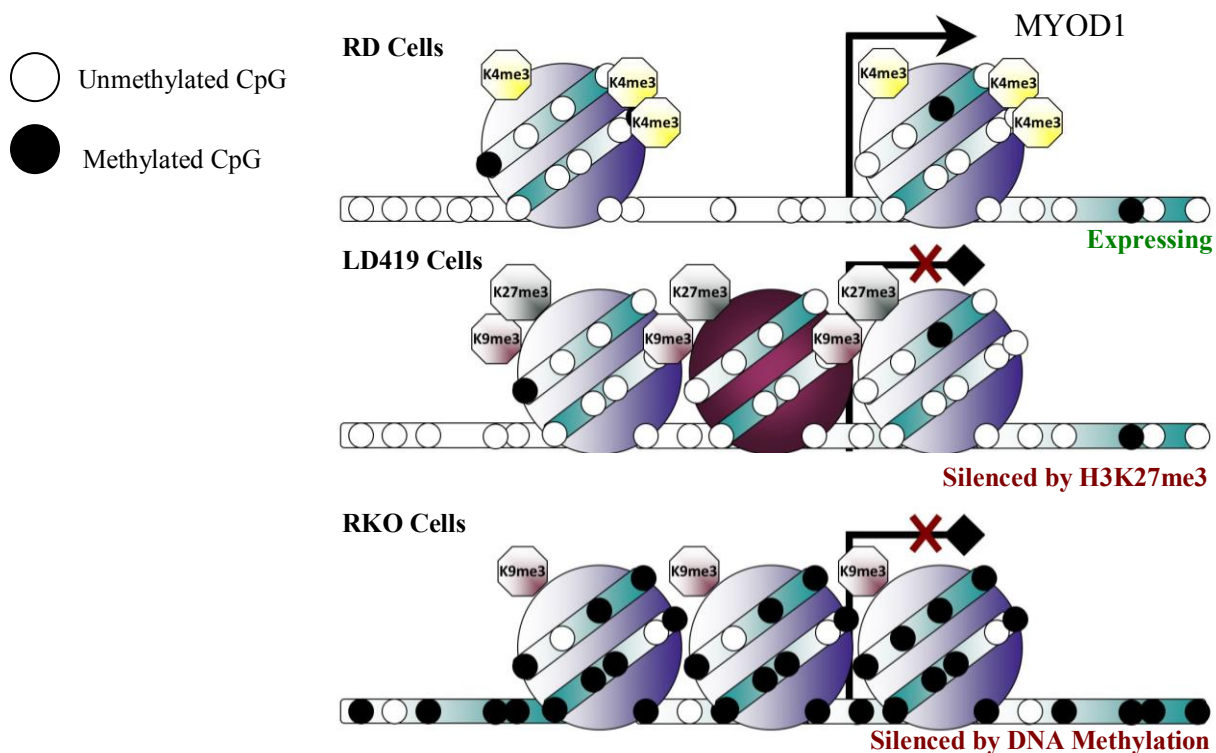


Figure 1: A model for epigenetic silencing of the MYOD1 promoter. A nucleosome free region is located upstream of the start site in active RD Cells, in contrast to silenced LD419 and RKO cells. An unstable nucleosome (red) is found in this region in H3K27me3 silenced cells. Transformed RKO cells lose H3K27me3 and gain DNA methylation at the MYOD1 promoter, which we propose promotes a less dynamic chromatin structure. Silenced promoters are enriched for H3K9me3. We hypothesize the existence of a mechanistic switch during carcinogenesis.