Elucidating the roles of histone methyltransferase Setd8 and histone H4 lysine 20 mono-methylation (H4K20me1) in heterochromatin formation during erythropoiesis

Keywords: Heterochromatin; histone code; H4K20me1; Setd8/KMT5A/PR-Set7; erythropoiesis

Motivation: Terminally differentiating erythroblasts must rapidly generate heterochromatin and condense their nucleus prior to enucleation. This process coincides with drastic changes in gene expression as globin-related genes must be upregulated while most others become silenced (1). The formation of repressive heterochromatin during differentiation and selective gene silencing is a fundamental biological process important for determining cell identity. The rapid chromatin and nuclear condensation seen during erythropoiesis provides a good model for studying heterochromatin formation. Understanding the mechanisms of heterochromatin formation has broad implications for the *in vitro* production of red blood cells and differentiation of stem cells into selected cell types.

Background: Regions of transcriptionally silent heterochromatin are marked by histone tri-methylation of H3K9, H3K27, and H4K20. The roles of H3K9me3 and H3K27me3 in heterochromatin are established as many of the readers of these marks are known. However, the role of H4K20 methylation in the formation and maintenance of heterochromatin is poorly understood.

Present models of heterochromatin formation in erythroid cells point to deacetylation of H3K9ac, H4K5ac, H4K12ac and H4K8ac, followed by progressive di-methylation of H3K9 (2, 3). In constitutive heterochromatin formation, H3K9me2/3 is recognized by HP1 via a chromodomain and can associate with other nearby HP1 molecules to form highly condensed structures. HP1 can also recruit Suv4-20h1/h2, the di- and tri-methyltransferases for H4K20. Interestingly, double knockout of Suv4-20h1/h2 does not affect heterochromatin formation suggesting H4K20me2/3 is nonessential (4). Prior mono-methylation of H4K20 is necessary for Suv4-20h1/2 activity, catalyzed by Setd8—the sole mono-methyltransferase for this position. Understanding the role of H4K20me1 in heterochromatin formation has been difficult to elucidate as Setd8 deletion is embryonic lethal by the eight-cell stage (5).

Our laboratory has generated erythroid specific Setd8 knockout mice to understand the role of Setd8 in erythropoiesis. Setd8-null erythroblasts had substantially less heterochromatin compared to littermate controls, the majority of which was localized to the nuclear periphery (Fig 1, in submission). Importantly, the overall features of Setd8-null cells are inconsistent with apoptosis or necrosis (6). The severe loss of heterochromatin suggests that Setd8 and/or H4K20me1 are essential for heterochromatin formation.

Aim 1: Delineate the impact of Setd8 deletion on heterochromatin. *We hypothesize that Setd8 and H4K20me1 are essential for heterochromatin formation in erythroid cells.* Extensively self-renewing erythroblasts (ESREs) derived from early fetal liver have proved to be a useful untransformed cell culture model and were first described by the neighboring laboratory of our collaborator, Dr. James Palis (7). ESREs divide daily, and upon induction are capable of terminal differentiation with roughly 90% of the culture forming enucleated reticulocytes (Fig 2). We

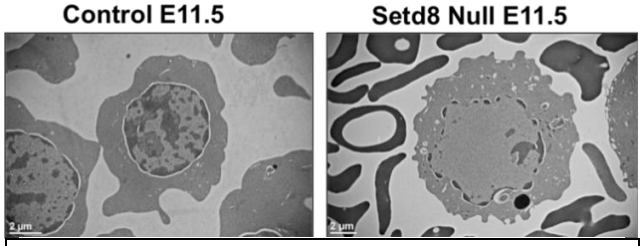

Figure 1: Representative transmission electron micrographs of control and Setd8-null erythroblast at embryonic day 11.5. Knockout was achieved by floxing a critical Setd8 exon and Cre expression was driven by the endogenous erythropoietin promoter. Heterochromatin can be seen as darker regions within the nucleus whereas uncondensed euchromatin remains lighter.

have produced an ESRE line with a tamoxifen inducible Cre and floxed Setd8 alleles allowing for a controlled Setd8 deletion.

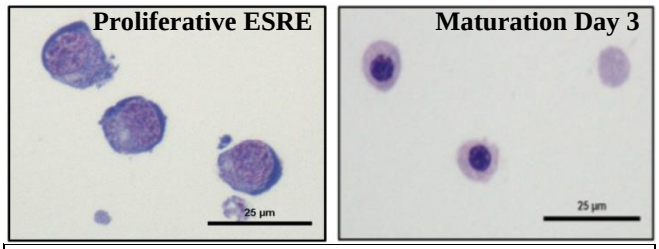

Figure 2: Wright-Giemsa staining of an extensively self-renewing erythroblast culture (left), and following three days of maturation (right). ESREs recapitulate erythroid maturation as nuclei become condensed and capable of ejection, cell size shrinks, and cytosol becomes hemoglobinized with indicative acidophilic staining.

The induction of Cre recombinase and differentiation will be timed so that little to no Setd8 protein is present as the cells undergo terminal maturation. Defects in heterochromatin will then be monitored by Wright-Giemsa staining and loss of important heterochromatin histone marks—H3K9me1/2/3; H3K27me1/2/3; H4K20me1/2/3—will be assessed by Western blot. Setd8 has been shown to recruit Riz1, one of many H3K9 mono-methyltransferases, to the nucleosome (8). Similarly, Suz12—a tri-methyltransferase of H3K27—was detected by mass spectrometry in a Setd8 co-immunoprecipitation experiment with HeLa cell extracts (9). Therefore, we expect a reduction in both H3K9 and H3K27 methylation following Setd8 deletion. Chromatin immunoprecipitation sequencing (ChIP-seq) has been successfully employed by our lab (10) and will be used in conjunction with differential marks identified by Western blot to better understand how Setd8 deletion impacts local chromatin landscape.

Aim 2: Delineate the individual roles of the Setd8 protein and H4K20 mono-methyl mark in heterochromatin formation. Various Setd8 mutations will be transduced into the Setd8-null ESRE line via lentivirus, including a R336G mutation and Δ1-193 truncation. The R336G mutation has been shown to render the protein catalytically dead, but still able to bind the H4 tail (11). The Δ1-193 truncation will remove the N-terminal domain where many protein-protein interactions have been shown to occur, but preserve the catalytic SET domain (8). Defects in heterochromatin can again be monitored by Wright-Giemsa staining, Western blot, and imaging flow cytometry (12). These mutagenic studies will help differentiate the catalytic role of Setd8 from other possible roles in mediating the recruitment of other chromatin remodelers.

Intellectual Merit: Many of the differences in gene expression between differentiated cell types is controlled at the epigenetic level (13). Defining the mechanisms of heterochromatin formation and selective gene silencing is essential to understand the driving mechanisms of cell type specification and modulating stem cell differentiation.

Broader Impacts: With this project, I plan to train undergraduate students interested in biomedical research via the Strong Children's Research Center (SCRC) summer program as our lab has done the last four summers. I plan to share this research at my alma mater, Winthrop University, to encourage students of diverse backgrounds to pursue external research opportunities such as SCRC. I will also share my findings at the multidisciplinary Experimental Biology conference, and in ASBMB Today, a magazine with a broad scientific audience.

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