P989 DYNAMIC CHANGES IN THE HISTONE H3 LYSINE 27 TRIMETHYLATION EPIGENETIC LANDSCAPE FOLLOWING RUXOLITINIB ADMINISTRATION

Topic: 15. Myeloproliferative neoplasms - Biology & Translational Research

Graeme Greenfield¹, Mary Frances McMullin², Ken Mills³

¹ PGJCCR, Queen’s University Belfast, Belfast, United Kingdom; ² CME, Queen’s University Belfast, Belfast, United Kingdom; ³ PGJCCR, Queen’s University Belfast, Belfast, United Kingdom

Background: Dysregulation of normal epigenetic processes is increasingly recognised as a feature of myeloid malignancy generally and implicated in the pathogenesis of MPN. Epigenetic changes may directly result from the mutation of key epigenetic regulators including TET2, ASXL1 and EZH2 or indirectly from the activation of intracellular signalling cascades. We have previously observed that ruxolitinib, a JAK1/2 inhibitor, may alter the epigenetic landscape of histone modifications in MPN. CUT&RUN is a novel experimental approach providing high resolution profiling of epigenetic modifications and transcription factor binding.

Aims: To comprehensively profile the H3K27me3 epigenetic landscape of JAK2 V617F positive cells resulting from JAK inhibition and determine the dynamic changes occurring between short term and persistent ruxolitinib therapy to identify potential genetic contributors to pathogenesis and options for future therapeutics.

Methods: JAK2 V617F positive UKE1 cells were cultured in duplicate in the presence of 1000nM ruxolitinib or DMSO 0.1% vehicle control continuously for approximately five weeks. Samples were harvested at 37 days for CUT&RUN analysis using H3K27me3, EZH2 and IgG (negative control) antibodies. CUT&RUN performed using EpicypHER CUTANA Kit according to manufacturer instructions. Libraries prepared using NEBNext® Ultra™ II DNA Library Prep kit with 50 base pair, paired end sequencing undertaken on Illumina NovaSeq 6000. Resulting FASTQ files were trimmed using Trim Galore! and aligned to reference genome hg38 using Bowtie2. Duplicate reads were removed using SAMtools rmdup and peaks called with MACS2 callpeak on the usegalaxy.eu platform. Differential peak analysis undertaken with Diffbind package in R. Samples were normalised to library size prior to differential analysis with DESeq2 methodology. Bigwig files generated using deepTools bamcoverage for visualisation.

Results: UKE1 cells remain sensitive to ruxolitinib after a period of treatment with high dose ruxolitinib lasting over 5 weeks. CUT&RUN libraries were sequenced to a minimum depth of 3.6 million unique reads per sample. Initial comparisons between Day 37 high dose treated cells and vehicle control revealed 1004 differentially enriched H3K27me3 peaks. Of these, 545 (54.2%) peaks were increased in the ruxolitinib treated cells and 459 (45.7%) peaks were increased in the control cells. Analysis of the difference between day 37 and day 1 high dose ruxolitinib treated cells revealed 290 peaks differentially enriched. Of these peaks, 279 (96.2%) were increased and 11 (3.8%) were decreased at day 37. Analysis of the overlap between these results identified 33 peaks increased at D37 in comparison to DMSO control and day 1 treatment samples.

Analysis of the differential binding patterns and direct visualisation of the results in a genome browser revealed particular genetic locations of interest including the promoter region of SPRY1, a potential regulator of erythropoiesis and JAK signalling, UGCG and BTBD11. Figure 1 shows an example. Differential H3K27me3 was also noted at regions associated with PLXNA1 and NRP2 genes which act as co-receptors for class III semaphorin signalling.

Image:
Summary/Conclusion: The dynamic changes in the H3K27me3 epigenetic landscape of JAK2 V617F positive cells following persistent high dose ruxolitinib administration has potential therapeutic implications and may sensitise cells to epigenetic therapies. Specific gene loci identified demonstrating differential H3K27me3 may offer some insight into JAK2 V617F mediated pathology and require further investigation.