Supplementary Methods

Colony Forming Assay

Methylcellulose colony-forming assay were performed using Methocult GF M3434 (Stem Cell Technologies) in 35-mm dishes. Colony count scoring and re-plating were repeated every 7 days.

Wright Giemsa Staining

Smears of mice peripheral blood or cytospin slides of mice BM cells or colony-forming cells were stained using Camco Stain Pak (Cambridge Diagnostic). Morphology images were obtained using Motic BA310 microscope system (Motic).

Gene Set Enrichment Analysis (GSEA)

Microarray gene expression data (GSE54663) for CD34^{+} human cord blood cells transduced with constitutively active HIF1A or empty vector was used to generate HIF1A induced gene set. Three-fold upregulated 364 genes in the constitutively active HIF1A transduced cells compared to control cells were used. The data were tested for enrichment in human MDS cohort (1) using GSEA (v2.2.3)(2). The following parameters were used; 1,000 gene_set permutations, Signal2Noise ranking metric, and descending order real mode for gene sorting.

Data set (GSE28359) for CD34^{+} human BM cells treated with 1,000 nM SR1 was used to generate AHR target gene set. The top 150 downregulated genes in SR1 treated cells compared to control cells were used as previously described (3). GSEA analysis was run in Log2 RNA-Seq expression data of c-Kit^{+} BM cells from *Vav1*-Cre/Control mice and *Vav1*-Cre/TPM mice (n = 2 per each group). The following parameters were used; 1,000 gene_set permutations, Diff_of_Classes ranking metric, and descending order real mode for gene sorting.

Mitochondrial DNA Assay

Total DNA was extracted from c-Kit$^+$ BM cells using Master Pure DNA purification kit (Epicentre). As previously described (4,5), the mitochondrial DNA (*NADH dehydrogenase subunit 2*, *Nd2*; mitochondrial genome) amount relative to nuclear DNA (*Non-metastatic cells 1*, *Nme1*; nuclear genome) was determined by quantitative RT PCR.

Cell Culture

CD34$^+$ cord blood cells were cultured as previously described (6). Cells were incubated for 24 hours in normoxia in the culture medium containing each of TCA cycle metabolites; dimethyl succinate (DMS), diethyl fumarate (DEF), dimethyl fumarate (DMF), diethyl malate (DEM), dimethyl malate (DMM), and dimethyl-α-ketoglutarate (DMKG). All the TCA cycle metabolites were purchased from Sigma-Aldrich.

Dot Blot Analysis

DNA from the c-Kit$^+$ BM cells was purified using Master Pure DNA purification kit (Epicentre). Purified 2μg DNA was boiled in 0.4M NaOH for 10 min at 95 °C and then neutralized with 1M NH_4COOH for 10 min on ice. Sample was serially diluted four-fold and spotted on a nylon membrane (Amersham Hybond-N+, GE Healthcare). The membrane was then incubated for 2 hours at 80 °C and blocked using Phosphate Buffered Saline (PBS) with Tween 20 containing with 5% skim milk for 1 hour at room temperature. Anti-5-methylcytosine (5-mc) antibody (clone 33D3, Millipore) or anti-5-hydroxymethylcytosine (5-hmC) antibody (Active Motif) were used as first antibodies overnight at 4 °C. Anti- rabbit (NA934V, GE Healthcare) and mouse IgG antibody (NA931V, GE Healthcare) were used as secondary antibodies.

References

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