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POSTER ABSTRACTS

506.BONE MARROW MICROENVIRONMENT

CYP26 Activity Maintains the Bone Marrow Niche and Stem Cell Homeostasis

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Vitamin A and its active compound, retinoic acid (RA), manifest pleomorphic activities in hematopoiesis. Vitamin A homeostasis is maintained systemically through hepatic CYP26. Emerging data suggests that CYP26 mediated RA metabolism also plays a role in controlling hematopoietic stem and progenitor cells (HSPCs) behavior (Ghiaur G et al. PNAS 2013, Schonberger K et al. Cell Stem Cell 2022).

We generated *Cyp26a1/ Cyp26b1* inducible knockout (CYP26KO) using ROSA26CreERT *Cyp26a1/ Cyp26b1* conditional mice to study the impact on hematopoiesis and the bone marrow microenvironment (BME). Tamoxifen induces complete CYP26KO in these mice and subsequently higher retinoid levels and systemic inflammation (Snyder JM et al. FASEB J 2020). Here, we characterize the hematopoietic, bone and bone marrow (BM) phenotype of these mice. Compared to the CYP26 wild type controls (CTR), we observed leukocytosis and mild anemia as well as signs of extramedullary hematopoiesis in CYP26KO mice (Table).

Spleen histology and flow cytometry showed an increased number of all mature elements, including megakaryocytes as well as increased absolute numbers of all hematopoietic progenitor populations (HPCs): LSK, CMP, GMP and MEP (Table). BM analysis showed decreased cellularity and myeloid biased in CYP26KO mice (Table). There was an increased frequency of colony forming unit cell (CFU-C) in the BM of CYP26KO mice but overall preserved absolute numbers when accounted for cellularity (Table). We observed increased frequency of LSK (Table) but decreased numbers of CMP, GMP and MEP in the BM of CYP26KO mice. Both CFU assay and flow cytometry showed myeloid biased HPCs found in the BM of CYP26KO mice (Table). When analyzing the stem cell compartment, short term stem cells and multipotent progenitor cells number were decreased in CYP26KO BM ($p<0.01$), but the long-term HSCs (LT-HSC) were relatively preserved (0.04 ± 0.01 vs. 0.17 ± 0.04 x10 ^{6}/femur, CTR vs CYP26KO, $p<0.05$). Both flow cytometry and CFU assay confirmed the presence of circulating HPCs in CYP26KO mice (Table). Thus, hematopoiesis of CYP26KO mice migrated away from the BM and into extramedullary tissues. The hematopoietic phenotype was most likely due to the cell extrinsic role of CYP26 since bone marrow transplantation experiments confirmed that a CYP26KO recipient (regardless of the genotype of the hematopoietic cells) and not CYP26 wild type recipient recapitulates the observed hematopoietic findings.

This observation, together with necropsy findings of thin, frail bones in CYP26KO mice, prompted a closer look at the BM matrix/microenvironment of these mice. CYP26KO mice had smaller BM cavities, decreased trabecular bone and an increased fat/bone marrow volume ratio (Table). Consistent with these findings, CYP26KO mesenchymal stroma cells (MSC) had no osteoblastic differentiation potential in vitro but showed preserved adipogenic potential. The number of CFU-fibroblasts was decreased in the BM of CYP26KO mice but were found circulating in the PB and were dramatically increased in the spleen of these mice (Table).

Single cell RNAseq showed quantitative and qualitative alterations in the BME populations with relative reduction of *Cxcl12* abundant MSC2, which explained the qRT-PCR observation of lower levels of *Cxcl12* mRNA in MSC isolated from CYP26KO mice. More so, *Spp1* mRNA was downregulated in various MSC populations of CYP26KO bone marrow, consistent with their osteopenia and with potential implications for the observed HSPC phenotype. Receptor ligand analysis revealed that various components of the BME have impaired interactions with the extracellular matrix. Thus, the leading hypothesis is that absence of CYP26 leads to a dysregulated BME and to migration of a HSCs-niche unit out of the BM and into secondary hematopoietic organs. Interestingly, scRNAseq experiments uncovered upregulation of *S100a8/9* in the CYP26KO neutrophil progenitors, which may be in response to systemic stress. The observed bone alterations (osteopenia and increased BM fat) coupled with noted changes in hematopoiesis (i.e. myeloid bias and inflammatory stress) may represent early signs of aging of the bone and bone marrow and suggest a central role of retinoid homeostasis in this process.

Disclosures No relevant conflicts of interest to declare.

Table. Summary of analyzed parameters

Phenotype	*Parameter*	*CTR*	*CYP26KO*	*Statistical considerations*
Clinical phenotype	WBC (x10^3/µL)	5.97 ± 0.74	21.12 ± 7.58	p<0.01
	HG (g/dL)	16.20 ± 0.57	13.96 ± 1.49	p<0.01
Bone marrow hematopoiesis	Cell number (x10^6/ per femur)	14.52 ± 1.34	8.16 ± 0.92	p<0.01
	CFU-C frequency (per 25,000 BM cells)	44.33 ± 3.72	69.83 ± 8.10	p=0.02
	LSK frequency (percentage of alive cells)	0.38 ± 0.07	0.86 ± 0.13	p<0.01
	LSK absolute number (x10^3/ per femur)	75±19	31±9	p<0.01
Spleen hematopoiesis	Spleen weight (grams)	0.10±0.01	0.17±0.04	p<0.05
	Spleen cellularity (x10^6)	110± 14	202± 55	p<0.05
	CFU-C frequency (per 25,000 cells)	12.5±1.91	18.67±4.04	p<0.01
	LSK frequency (percentage of alive cells)	0.5±0.2	1.6±0.7	p<0.05
	LSK absolute numbers (x10^6/ per spleen)	0.26±0.08	2.18±1.43	p<0.05
Peripheral blood hematopoiesis	CFU-C frequency (per 200µL)	46.5 ± 21.25	179.5 ± 86.12	p<0.05
Bone phenotype	Bone marrow cavity volume (mm^3)	1.89±0.16	0.67±0.18	p<0.01
	Trabecular number (per mm^3)	1.66±0.14	1.01±0.26	p<0.01
	Trabecular thickness (µm)	10.9±0.8	5.8±2.3	p<0.01
Bone marrow fat phenotype	Adipocyte volume (percentage of BM cavity)	6.75±2.02	11.2±1.75	p<0.01
Distribution of colony forming unit fibroblast (CFU-F)	Bone marrow (CFU-F/femur)	224±41	94±40	p<0.05
	Spleen (CFU-F/spleen)	0.8±0.84	28.67±11.01	p<0.05
	Blood (animals with CFU-F/animals tested)	0/14	5/15	p<0.01

Figure 1

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