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Metabolic programming of adult hematopoietic stem cell function by prenatal folate status

Anna Beaudin, University of Utah

妊娠期 Folate deficient nutrients ：HSC↓、altered glycolysis, impaired TCA cytle, Fatty acid
妊娠期 folate supplemented: HSC↑
Folate supplemented HSC: down of protein synthesis, nucleotide biosynthesis
Metabolism 変化が epigenetic changes を誘導し、それによって metabolism の変化が生じるのが面白い。

ROS and TLR signaling balance eat me and don't eat signals to determine stem cell clonal selection by macrophages

Len Zon, Boston Children's Hospital

Calreticulin is an eat me signal on HSPCs that mediates macrophage interaction.
TLR3 as a surface CALR inducer in a ROS-induced context.
B2m is a don't eat me signal (* MHC Class 1 とは independent).
Human MYC overexpression induce erythroleukemia in fish.
Leukemia cell secrete apelin, an angiogenic peptide necessary for HSC function.
Apelin induces clonal expansion of leukemia associated endothelial cells.
造血幹細胞において、TLR3 リガンドが b2M の発現を誘導し、b2M が don't eat me シグナルとして働いて、macrophage による貪食を防ぐ。

HSCs engage a cytoprotective nucleolar stress response that provides resilience during aging

Emmanuelle Passegué, Columbia University Irving Medical Center

Nucleolar rH2AX is associated with impaired protein translation in old HSCs
mTOR is not associated
ISR (統合ストレス) is not associated
Nucleolar stress → p53 activation in old HSCs
ActD induced nucleolar stress induction makes young HSCs to old HSCs
Mdm2 C305F mutant mice: no binding to Rpl11, no activation of nuclear stress-induced p53
Nucleolar stress response inactivation impairs young HSC function
Aged mutant mice: compromised HSCs.
Nucleolar stress response is critical to maintain HSCs.

一部の old HSC では nuclear stress response が亢進しており、そのような細胞は quiescent で造血再構築能が高いとのこと。

A branch chain amino acid rheostat controls hematopoietic stem cell replicative lifespan
Marie-Dominque Filippi, Cincinnati Children's Hospital

Functional analysis of LT-HSCs bosed on division history (GFP) and mitochondrial membrane potential (TMRE)

GFP-high (low division history), TMRE low (low mitochondria): best HSC
Division history generates a low potent myeloid biased HSC
GFP high HSC: BCAA catabolism, mTOR suppression
Bcat2 inhibition accelerates HSC cell cycle and myeloid skewing
Alpha ketoisocaproic acid (ロイシンの中間代謝物、サプリメントして使われている) restores the functions of HSCs with division history
サプリメントで、造血幹細胞が若返り？

Elevated mitochondrial membrane potential is a therapeutic vulnerability in Dnmt3aR878H-driven Clonal Hematopoiesis
Jennifer Trowbridge, The Jackson Laboratory

Dnmt3a hematopoiesis has a competitive advantage in IGF1 KO mice
(* IGF1 は有名な長寿遺伝子、IGF1 が BM microenvironment で減少すると造血幹細胞の老化が進むという情報あり)
Dnmat3a mutant HSPCs maintain oxidative phosphorylation in IGF1 KO mice
Dnmt3a-mutant HSCs have elevated mitochondrial membrane potential
Dnmt3a-mutant HSCs are sensitive to mitochondrially targeted compounds
MitoQ reduced Dnmt3a-mutant competitive advantage in vivo

Native Clonal Hematopoiesis in the mouse
Margaret Goodell, Baylor College of Medicine

Mouse 24-36 months old で clonal hematopoiesis を評価
3 month vs 30 month HSC (CD150+) nad MPP(CD150-)
Somatic mutation: 60 mutations at 3 mo, 150 mutations at 30 mo
Phylogenetic tree: No collapse of polyclonality in mice (ヒトと違う)

HSCs and MPPs are independent (most MPPs did not have a clear HSC)
Murine clone outgrowth during aging, but clone size is small
いくつか共通点はあるが、マウスではヒト CH ほどはっきりとした clone 増殖は認められない。寿命が短いためか、SPF で飼育されているためか？

Role of ubiquitin E3 ligase UBR5 in Myelodysplastic Syndromes with Trisomy 8
Shannon Buckley, University Utah of Salt Lake

Ubiquitin E3 ligase UBR5 is highly expressed in MDS Trisomy 8
UBR5 overexpression leads to bone marrow failure
UBR5 OE only perturb adult hematopoisis
No effect on fetal liver hematopoiesis
Depletes HSC pool and function
Increases DNA damage

Trisomy 8 induces chromatin conformation changes and inter-chromosomal interaction that compromise function of hematopoietic stem cells
Goro Sashida, Medical Sciences Kumamoto University

Generation of Trisomy 8 mice (ヒト 8 番染色体を丸ごとマウス ES 細胞に導入)
Reduced repopulation capacity of stem and progenitor cells
Increased expression of inflammatory genes and stem cell, myeloid genes
Inter-chromosomal interaction with Y chromosome inducing active compartment
UTY bound to promoter and enhancer regions enriched with RUNX binding motif
Runx1 deletion mitigated the impaired hematopoiesis in a part of trisomy 8 mice

上記 2 つ Trisomy 8 関連の研究。Buckley: 8 番染色体上の UBR5 の発現が増えることが造血幹細胞機能阻害、Sashida: Y 染色体との aberrant interaction が造血障害を引き起こすとの結論。Trisomy 8 では 8 番染色体上の遺伝子の発現量が増加することが疾患発症の原因と一般に考えられているが、後者のメカニズムが興味深い。

HDAC7 is necessary for hematopoietic stem and progenitor cell function
Sara Meyer, Thomas Jefferson University
Hdac7 knockout mice depleted of B cells, increase myeloid cells
Reduced BFU-E.
Decreased HSCs

EGR1 down in Hdac7-/- mice
HDAC7 KO マウスの解析。HSC は減るようだが、それほど激しくはない。Erythroid にも phenotype が出ている。Enzymatic activity が重要かは現時点では不明とのこと。

Genotype-phenotype mapping via single-cell multi-omics identifies therapeutic vulnerabilities in VEXAS Syndrome
Dan Landau, Weill Cornell Medicine

Single cell analysis of WT and UBA1 mutated cells (臨床サンプルを用いた同一検体での比較は、変異細胞の性質解明に極めて強力)
UBA1 mutated NK cells show high degree of cytotoxic signatures
UBA1 mutated HSCs: myeloid bias, inflammation and UPR
UBA1 mutated HSCs: UPR activates IRE1 and PERK-ATF4 modules
UBA1 mutated HSCs: ATF4 accessibility increased
UBA1 inhibition in CD34+ cord blood cells ex vivo shows increased ATF4
PERK inhibition reverses myeloid bias

UBA1 変異細胞は inflammation や UPR が亢進しており、in vitro で培養した場合の増殖は大幅に低下しているが、なぜか in vivo では増加する。**その鍵を握るのが、PERK-ATF4 とのこと**。

UBA6 inhibition selectively targets mutant-UBA1 cells in VEXAS syndrome
Dan Starczynowski, Cincinnati Children's Hospital

AAV-medisated knockin of UBA-M41V (VEXAS syndrome の mutation) in THP1 cells
UBA1-M41V: UPR and TP53 signaling
Pan Ubiquitinaton: no difference probably because the compensation of UBA6
UBA-M41V cells are sensitive to UBA6 inhibition

Characterization and relevance of immunophenotypic changes following Menin inhibition in AML
Sanam Loghavi, MD Anderson Cancer Center

Monoblastic cells: CD64+ CD117-
Menin inhibitor 治療後は、immunophenotype(myeloid vs monocytic)の変化が頻繁に起こるとのこと。

Trib1 regulates neutrophil differentiation, lifespan, and function
Warren Pear, University of Pennsylvania

Trib1 loss → neutrophil increase
Peripheral lifespan of neutrophil increased
Trib1delta Mrp8-Cre mice: neutrophils display an aged-like phenotype

Chromothripsis orchestrates leukemic transformation in blast phase MPN through targetable amplification of DYRK1A
John Crispino, St. Jude Children's Research Hospital

BP(blast phase)-MPN: chr21amp (amplification of a region of chromosome 21) frequently detected
Chr21amp is a late clonal event triggering leukemic evolution
24 genes lie in the minimally amplified region of chr21amp
DYRK1A is the most likely mediator of the adverse chr211amp phenotype
DYRK1A is highly expressed in chr21amp cells
DYRK1A: Dually specificity tyrosine (Y) Regulated Kinase 1A
Loss of DYRK1A suppresses proliferation and survival of HEL cells and chr21amp BP-MPN
DYRK1A promotes activity of the DEAM complex through phosphorylation on LIN52
DYRK1A is a candidate driver of genomic instability in Down Syndrome
DYRK1A interferes with DREAM complex mediated DNA repair
DYRK1A loss leads to enhanced JAK/STAT activation
DYRK1A increases BCL2 expression

The RBM15-MKL1 fusion protein mediated m6A RNA modification promotes leukemia by regulating expression of Fzd genes
Stephanie Halene + Diane Krause, Yale University School of Medicine

Non-Down Syndrome-AMKL fusion protein RBM15-MKL1
RBM15: RNA binding motif protein 15
Key regulator of m6A writer
Recruits METTL3 to RNA
RBM15-MKL1-transduced HEL cells:
Up of TGFB receptor activity, Wnt singalling, Down megakaryocytosis

RNA binding and m6A localization: similar to wild-type RBM15
METLL3 inhibitor was effective on RBM15-MKL1 driven AML, prolonged survival in AMKL engrafted mice
FDZs were upregulated by RBM15-MKL1 and downregulated by METTl3i

IGF2BP1 is essential for ETO2/GLIS2 leukemogenesis via stabilization of primitive hematopoietic transcriptional programs
Lynn Lee, Cincinnati Children's Hospital

In vivo CRISPR Screen in AMKL using Top 100 upregulated genes: IGF2BP1 depleted
IGF2BP1: M6A reader, stabilizes MYC
IGF2BP1 knockdown inhibits AMKL proliferation, inducing cell cycle arrest, apoptosis and differentiation
IGF2BP1 expression declines with hematopoietic maturation
IGF2BP1-bound transcripts highly enriched for hematopietic gene
IGFBP1 loss downregulates E2F-mediated gene expression

DNMT3A:R882H is not required for disease maintenance in AML, but is associated with increased leukemia stem cell frequency
Ravindra Majeti, Stanford University

Patient samples: DNMT3A-R882 correction does not eliminate leukemogenenicity
LIC is same in primary recipients
LIC decreased for corrected cells
iPS derived AML in vivo correction: no difference
global methylation no change
2 次移植でようやく変化あり：R882 で engraftment increased

Primary U2AF1S34F mutated hematopoietic cells are sensitive to nonsense-mediated RNA decay disruption in vivo
Matthew J. Walter, Washington University School of Medicine

MLL-AF9 U2AF1-S34F dox system
(MLL-AF9 細胞に U2AF1-S34F mutation を発現させるシステム)
U2AF-S34F + MLL-AF9 cells are sensitive to NMD inhibition
K562 U2AF1S34F-Luc sensitive to NMD inhibition

Unresolved R loops can lead to DNA damage by NMD inhibition and S34F mutation

SETDB1 mediated gene regulation contributes to methionine demand in leukemia
Andrew Muntean, University of Michigan Medical School

SETDB1 overexpression inhibit myeloid leukemogenesis
SLC44A1 is negatively correlated with SETDB1
SLC44A1 is essential for leukemia survival
SLC44A1: major cell surface choline transporter
Choline Kinase (ChoK) is important for leukemogenesis
Leukemic cells display increased methionine metabolism
Choline は、ミトコンドリアで CHDH と BHMT によって methionine に変換される
Methionine restriction accelerated MLL-ENL leukemia in immune competent mice
(過去に Xenograft model を使った実験で、Methionine restriction で白血病発症が遅延した、という論文があるが、免疫正常のマウスの実験系では白血病発症が促進された。Methionine 制限で T-cell がダメになる一方、白血病は SL44A1 up→Choline up→Mehionine up しているので、影響を受けにくいため、と推測される)

Lineage plasticity in response to therapy is a targetable mechanism of resistance in AML
Andrew Volk, Cincinnati Children's Hospital
Ven/Aza treatment changes AML lineage identity
Ven/Aza/G-CSF very effective

IRF5 defines a new high-risk inflammatory T-lineage acute lymphoblastic leukemia subtype
Anastasia N. Tikhonova, University of Toronto Princess Margaret Cancer Center

Subset of T-ALLs : IRF5 high, resembles ETP inflammatory T-lineage
IRF5-high T-ALL: BCL2 high and sensitive to venetoclax
＊IRF5-high と low T-ALL は、surface marker では区別がつかない。マーカー上は同じようにみえても、細胞内での転写制御状態は異なっており、RNA-seq 等で遺伝子発現をみてはじめてわかるとのこと。

Targeting polyamine metabolism in acute myeloid leukemia stem cells
Courtney Jones, Cincinnati Children's Hospital

The polyamine spermidine levels are elevated in LSCs (metabolic analysis of LSCs and HSCs)

DENSpm: a polyamine analog causing polyamine depletion
Selective depletion of colony formation of Primary AML in vitro and in vivo
RNA-seq: Polyamine depletion promotes LSC differentiation
Decreases Protein synthesis only in LSCs
Reduced eIF5A
Decrease the abundance of select proteins: HBO1
Polyamine depletion targets LSCs in part by impairing HBO1 protein expression
Forced expression of HBO1 partly rescued the effect of polyamine depletion

Identification of leukemia stem cell subsets with distinct transcriptional, epigenetic and functional properties
John Dick, University of Toronto Princess Margaret Cancer Center

OCI-AML22: a novel system to model primary AML hierarchy
Chr9 LSC* chromatin variant as essential for tumor initiation
LSC transposable element 121 score (LSCTE121) is associated with poor outcome in AML
CRISPRi decreases chromatin access: reduced engraftment of AML cells
CD112 prospectively segregates distinct LSC types
CD112 low: immature and slow
CD112 high: differentiated and rapid

Targeting mitochondrial calcium uptake to eradicate Venetoclax-resistant Acute Myeloid Leukemia Stem Cells
Craig Jordan, University of Colorado, Denver
ROS-low : LSCs
BCL2 has non-canonical role in regulating intracellular calcium transport
Increased Serca
Venetoclax resistant cells: increased calcium
MCU knockdown impairs metabolism of venetoclax resistant AML
Mitoxantrone phenocopies MCU knockdown in venetoclax resistant primary specimens
(Mitoxantrone は抗がん剤だが、100 nM では nonDNA damage detectable なので、MCU inhibitor として働いていると思う、とのこと)

Separating and uniting genetic and non-genetic hierarchies in human AML
Eirini Papapetrou, Mount Sinai

SAR-iPSCs model of synthetic leukemogenesis

SRSF2, ASXL1, NRAS

RAS mutations are always present as late events in AML

NRAS G12D is an obligatory late mutation

RAS mutation transform GMPs harboring preexisting mutations

R+SA vs SA+R: gene expression different

RAS-MT RAS-WT comparison: RAS-MT more monocytic

N/KRAS mutation, but not monocytic stage, is assocaiated with poor outcomes in AML patients to venetoclax

RAS mutations confer clinical resistance to VEN not because they produce monocytic cells,
but because they change the expression of BCL2 family genes: high MCL1, low BCL2