MYELODISPLASTIC SYNDROMES: CAOS AND ORDER

October 26, 2018
IRST, Meldola

Hematopoietic stem cells and Mesenchymal stem cells in myelodysplasia

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DISCLOSURE

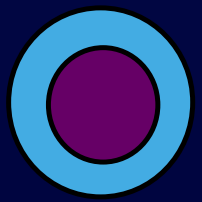
Mauro Krampera

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other

I have no real or apparent conflicts of interest to report influencing this presentation

MDS pathogenesis

HSPC genetic instability



↓

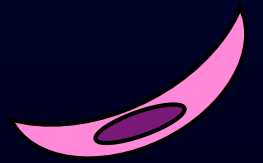
Mutational profile
(somatic genetic abnormalities involved in RNA splicing, i.e. SF3B1, SRSF2)

↓

Phenotypic aberrations

+

Abnormalities in the BM microenvironment, i.e.



- altered hematopoietic–stromal cell interactions
- deregulated production of growth factors and hematopoietic modulators

Role of the BM microenvironment in MDS pathogenesis

OPEN

Leukemia (2015) **29,** 259–268

www.nature.com/leu

REVIEW

Myelodysplasia is in the niche: novel concepts and emerging therapies

E Bulycheva[1], M Rauner[2], H Medyouf[3], I Theurl[4], M Bornhäuser[1,5], LC Hofbauer[2,5] and U Platzbecker[1]

- MDS is not only a disease of the HSCs, but of the entire BM microenvironment and bone metabolism
- Interactions between mesenchymal stem and progenitor cells (MSPC) and hematopoietic stem and progenitor cells (HSPC) contribute to the pathogenesis of MDS and associated disorders

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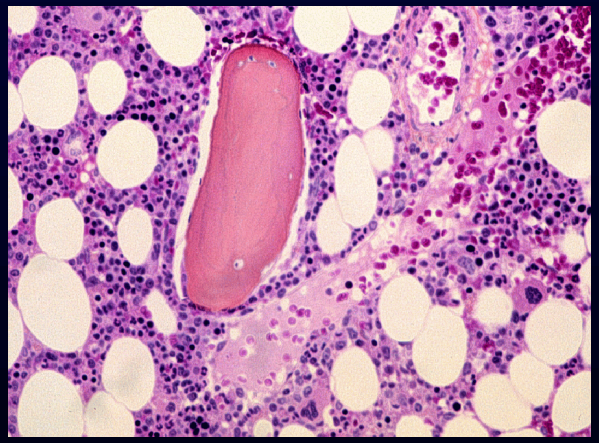
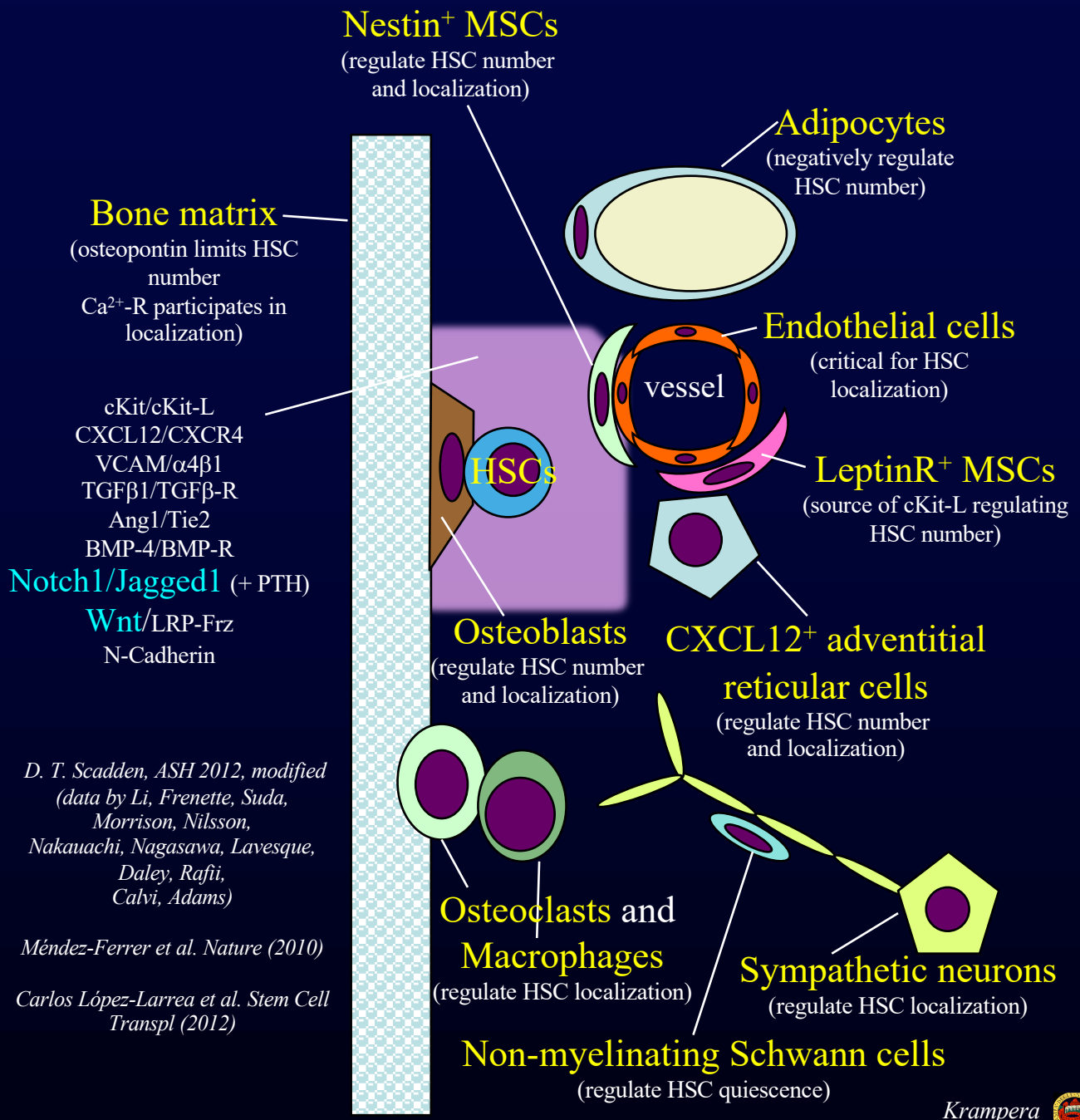
Bone marrow hematopoietic stem cell niche

Discrete and specialized microenvironmental space where interactions occur, through direct contact and soluble factors, amongst:

HSCs
"Stromal cells"
Extracellular bone matrix
(ialuronic acid, glycosaminoglycans, osteopontin, etc.)

leading to a finely tuned regulation of HSC functional properties





D. T. Scadden, ASH 2012, modified (data by Li, Frenette, Suda, Morrison, Nilsson, Nakauachi, Nagasawa, Lavesque, Daley, Rafii, Calvi, Adams)

Méndez-Ferrer et al. Nature (2010)

Carlos López-Larrea et al. Stem Cell Transpl (2012)

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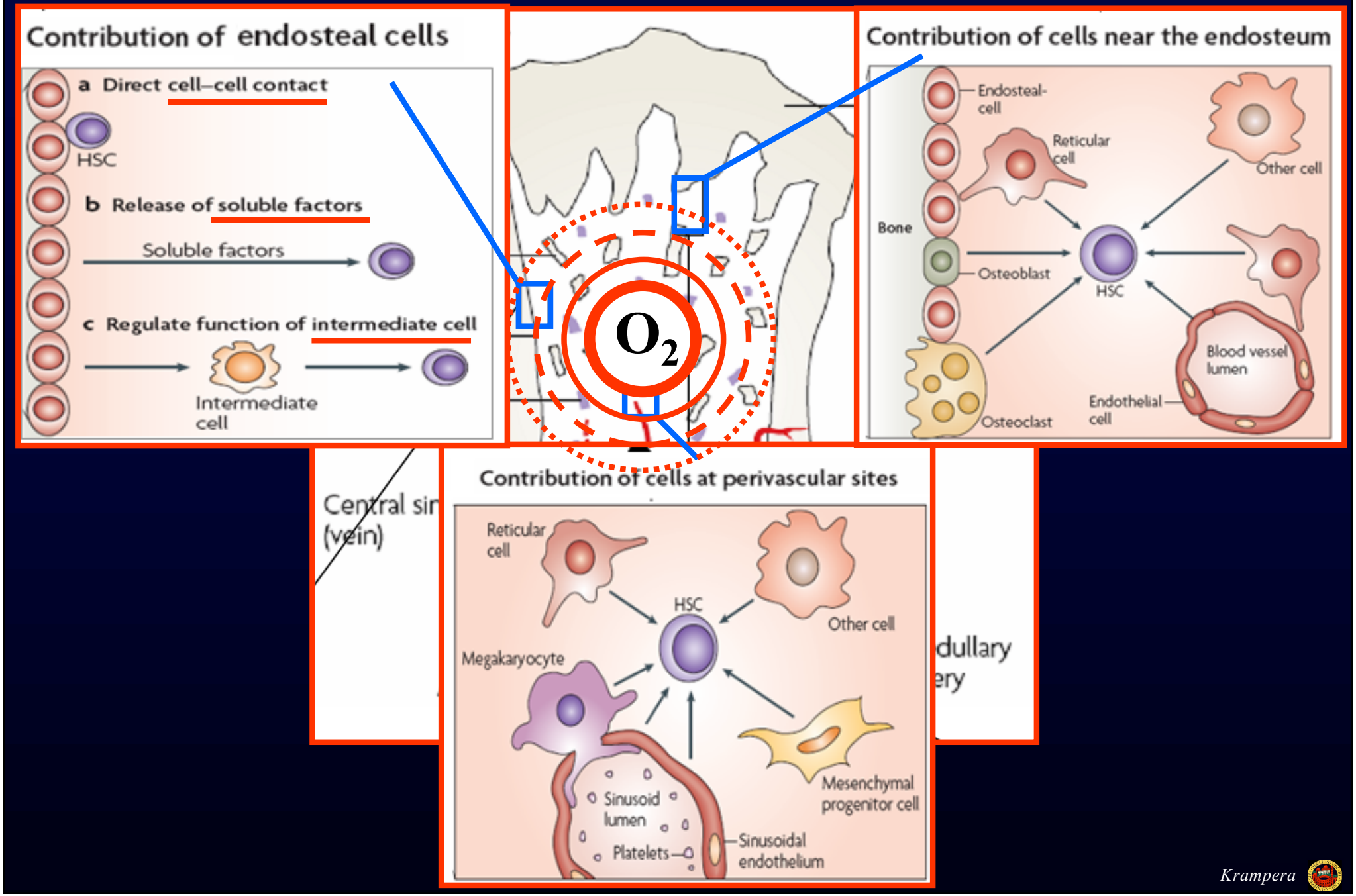
HSC niche



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Bone marrow hematopoietic stem cell niche

Riserva
Automantenimento
Ripopolamento midollare

NICCHIA STROMALE
Cellule staminali quiescenti

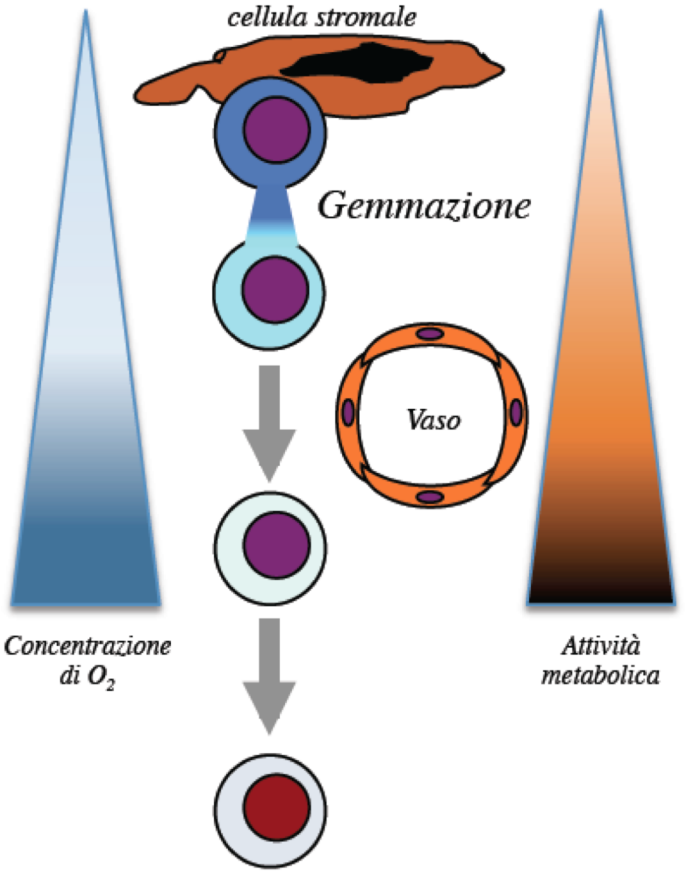
Indirizzo differenziativo
Proliferazione
Differenziazione
Maturazione

Progenitori *"committed"*

NICCHIA VASCOLARE

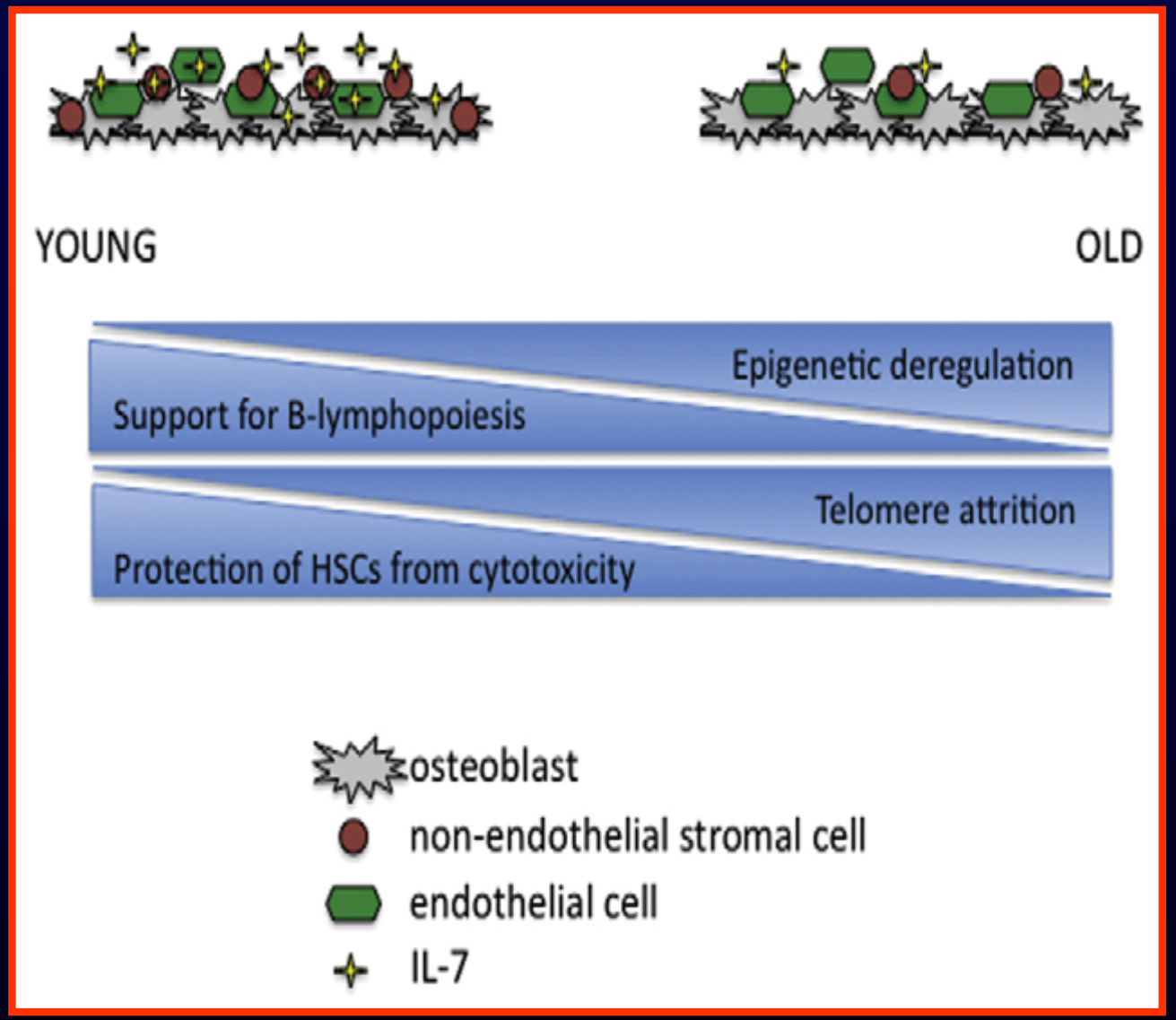
Attività funzionale

Tessuto maturo



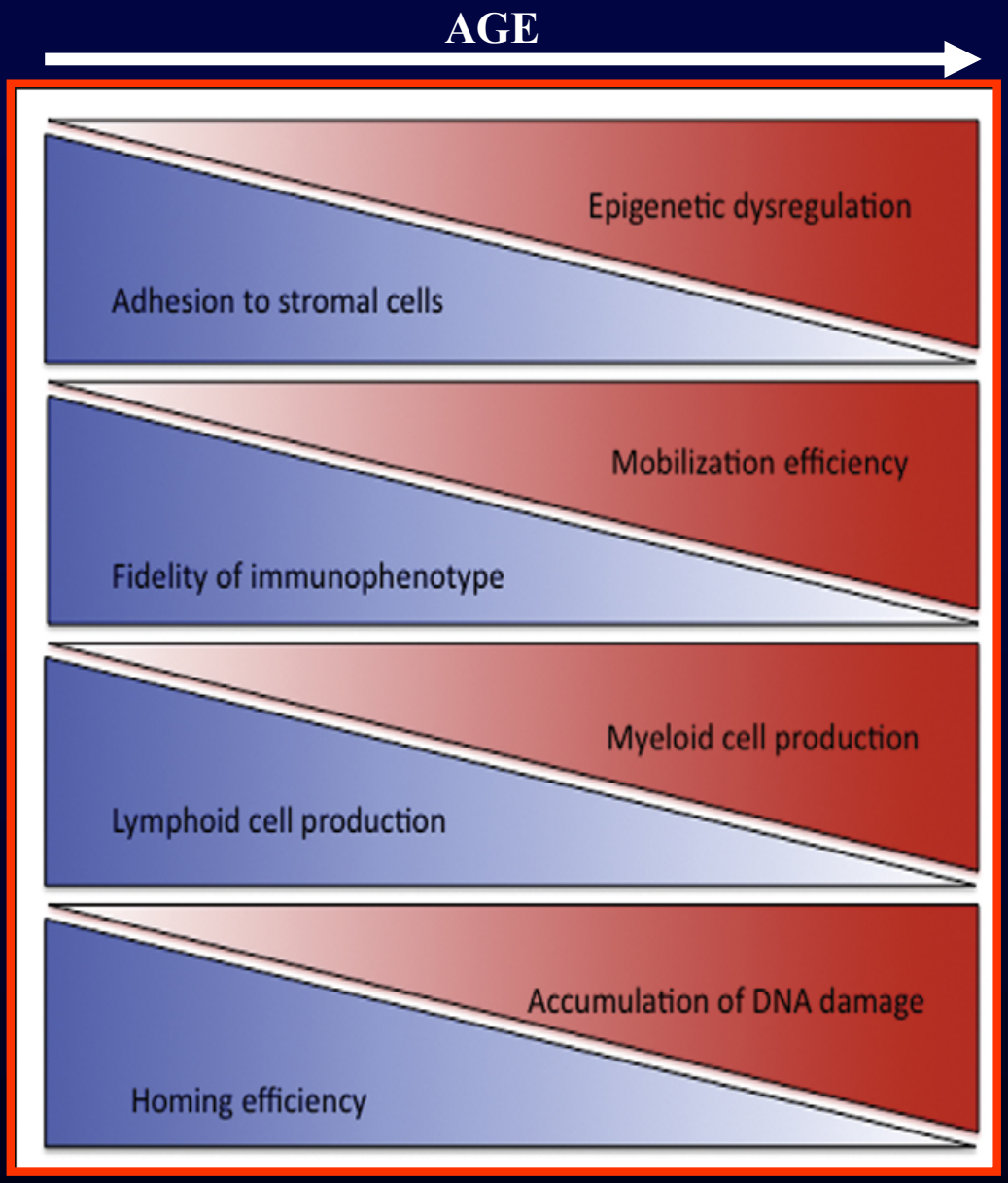
Krampera M. Fisiologia dell'emopoiesi. In Corradini P – Foà R. *Manuale di Ematologia*, revisione 2018.

HSC stromal niche ageing

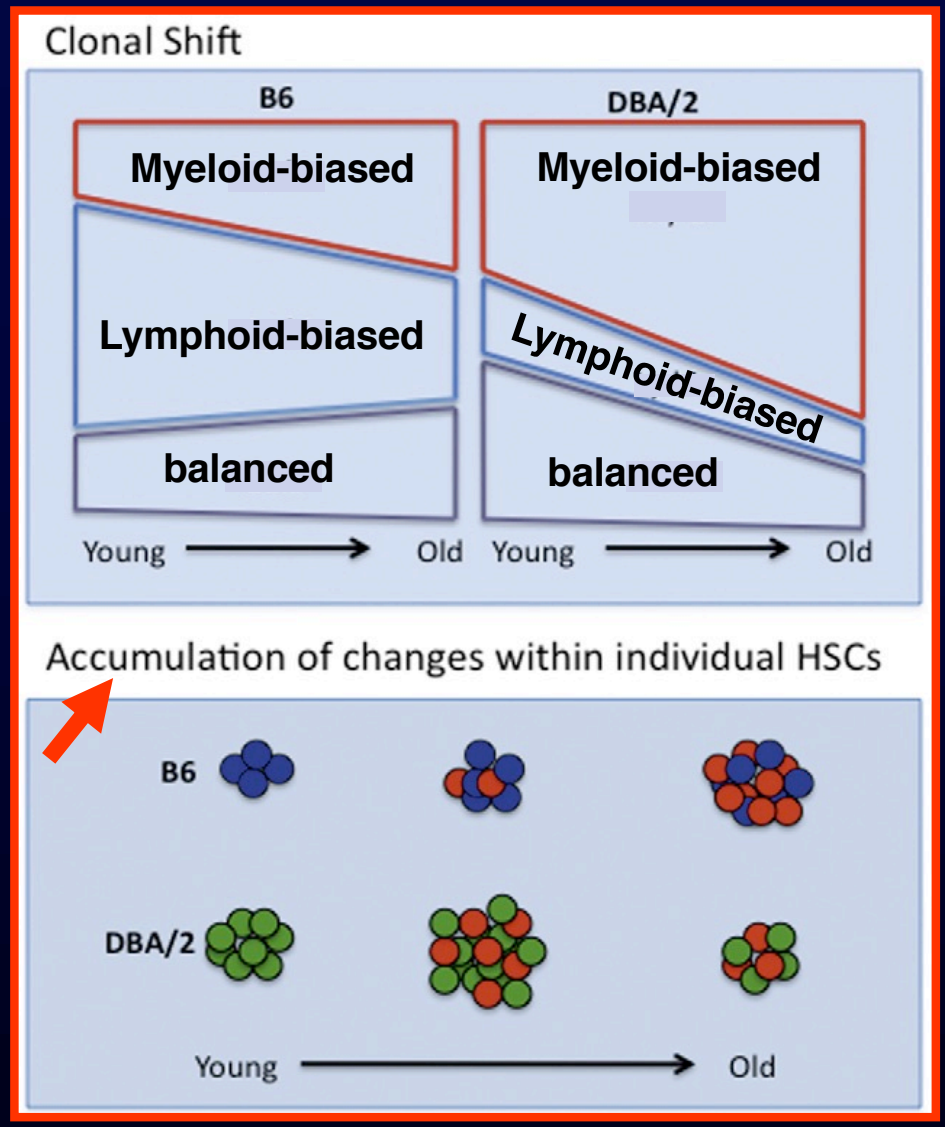


Waterstrat A, et al. Effects of aging on hematopoietic stem and progenitor cells Curr Op Immunol 2009, 21:408–413

HSC ageing



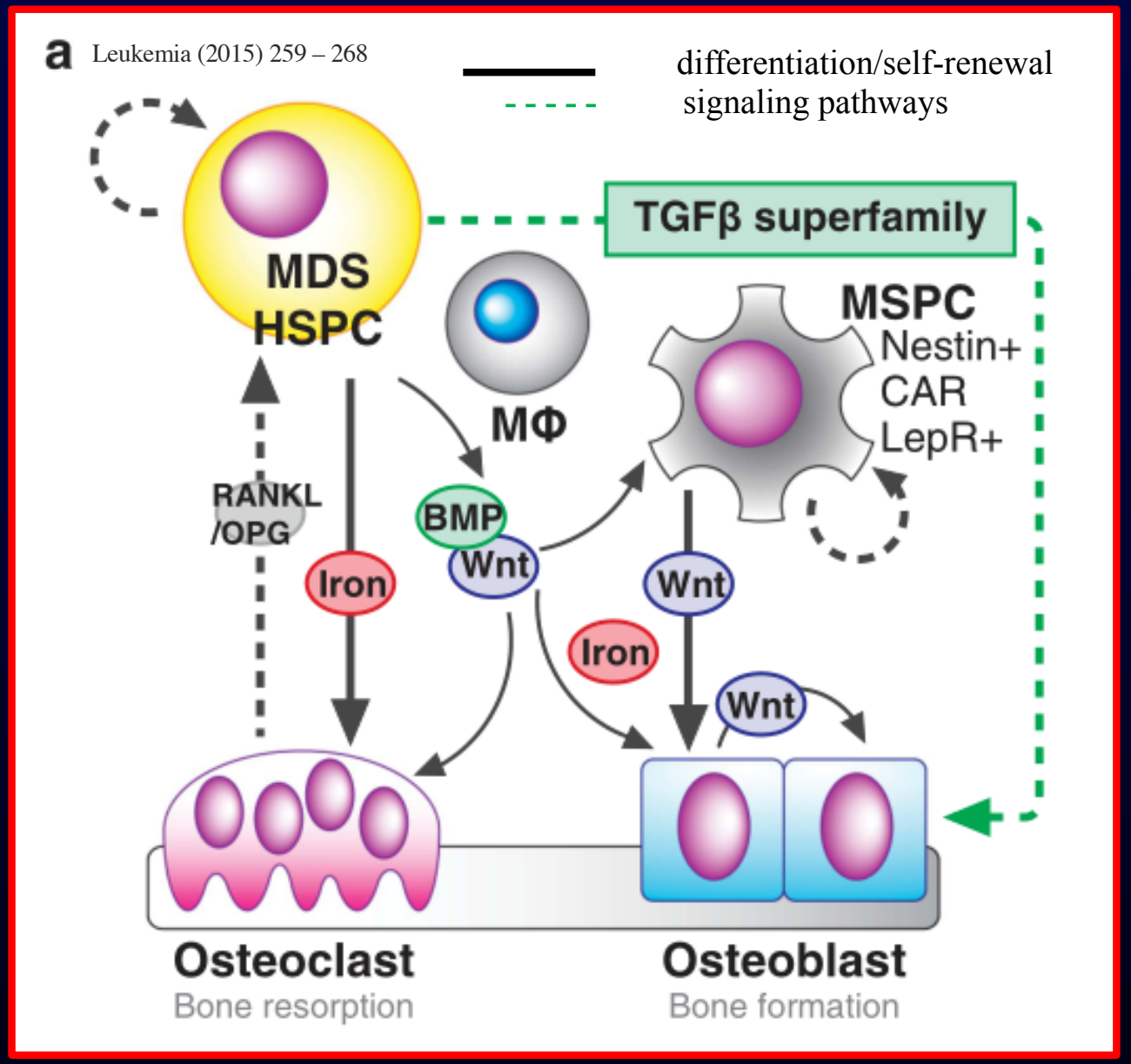
2 models



Waterstrat A, et al. Effects of aging on hematopoietic stem and progenitor cells Curr Op Immunol 2009, 21:408–413

Role of the BM microenvironment in MDS pathogenesis

Cellular and humoral components within the osteo-hematopoietic niche



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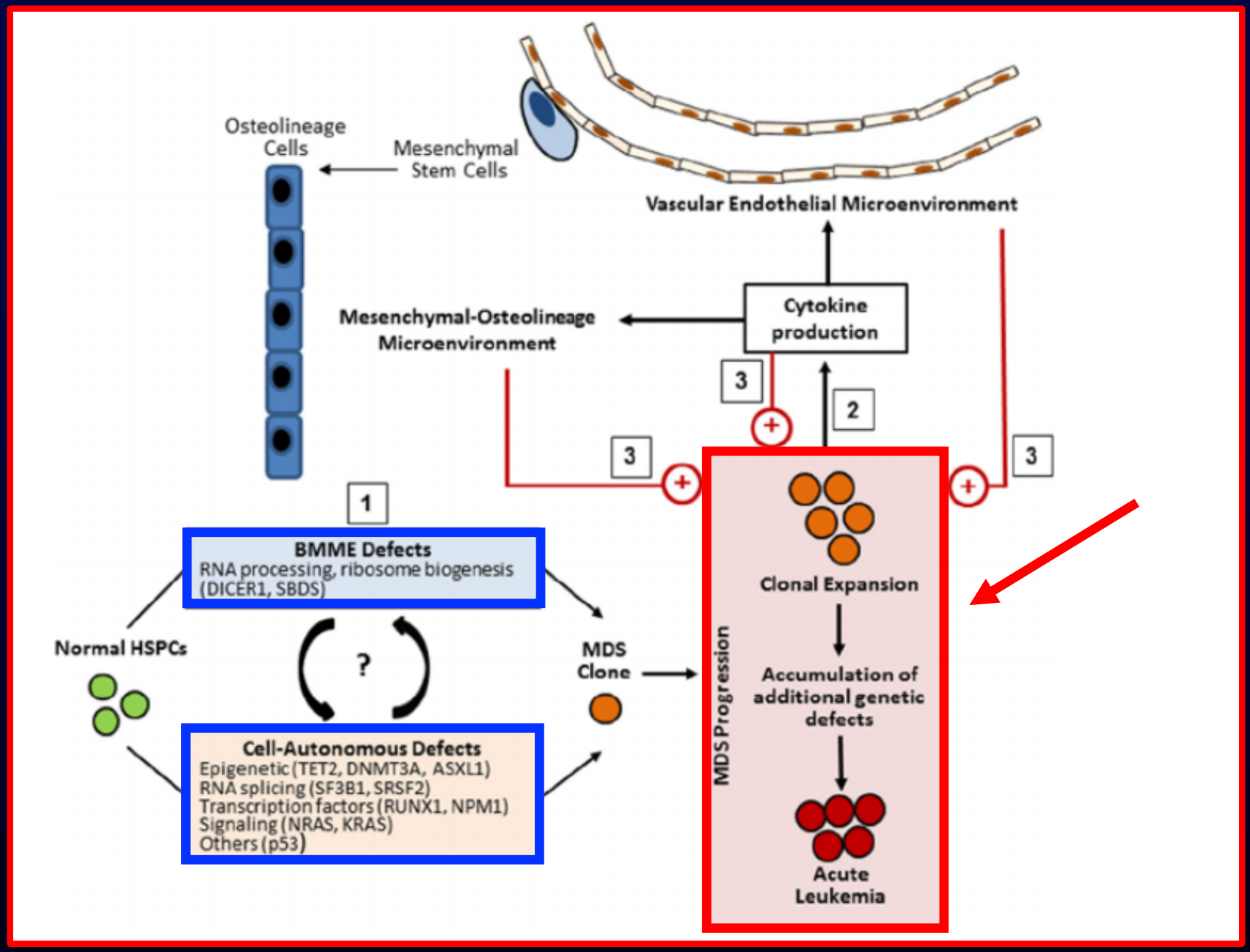
Role of the BM microenvironment in MDS pathogenesis



Li et al. 2017

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Role of the BM microenvironment in MDS pathogenesis

1- Animal models revealing BM microenvironment-induced MDS

2- Alterations of the cellular components of the niche in MDS patients

3- Signalling defects within the osteo-hematopoietic niche

4- Iron overload and dysregulation of iron homeostasis

Role of the BM microenvironment in MDS pathogenesis

1- Animal models revealing BM microenvironment-induced MDS

Published in final edited form as:
Nature. 2010 April 8; 464(7290): 852–857. doi:10.1038/nature08851.

nature
International journal of science

Bone progenitor dysfunction induces myelodysplasia and secondary leukemia

Marc H.G.P. Raaijmakers[1,2,3,*], **Siddhartha Mukherjee**[1,2,3,4,*,#], **Shangqin Guo**[1,2,3], **Siyi Zhang**[1,2,3], **Tatsuya Kobayashi**[5], **Jesse A. Schoonmaker**[1,2,3], **Benjamin L. Ebert**[6], **Fatima Al-Shahrour**[6], **Robert P. Hasserjian**[7], **Edward O. Scadden**[1,2,3], **Zinmar Aung**[1,2,3], **Marc Matza**[1,2,3], **Matthias Merkenschlager**[8], **Charles Lin**[9], **Johanna M. Rommens**[10], and **David. T. Scadden**[1,2,3,4]

- Selective *Dicer1* deletion (miRNA processing endonuclease) in MSC osteoprogenitors induces markedly abnormal hematopoiesis and eventually AML
- Dicer1−/− osteoprogenitors display reduced levels of Sbds, the gene mutated in Shwachman-Bodian-Diamond Syndrome (BM failure and AML predisposition)
- Deletion of Sbds in osteoprogenitors largely mimics Dicer1 deletion
- (MSPCs from MDS patients exhibit a low expression of Dicer1 and DROSHA)

Role of the BM microenvironment in MDS pathogenesis

1- Animal models revealing BM microenvironment-induced MDS

Myelodysplasia in *Dicer*$^{-/-}$ mice (Raaijmakers et al. Nature 2010)

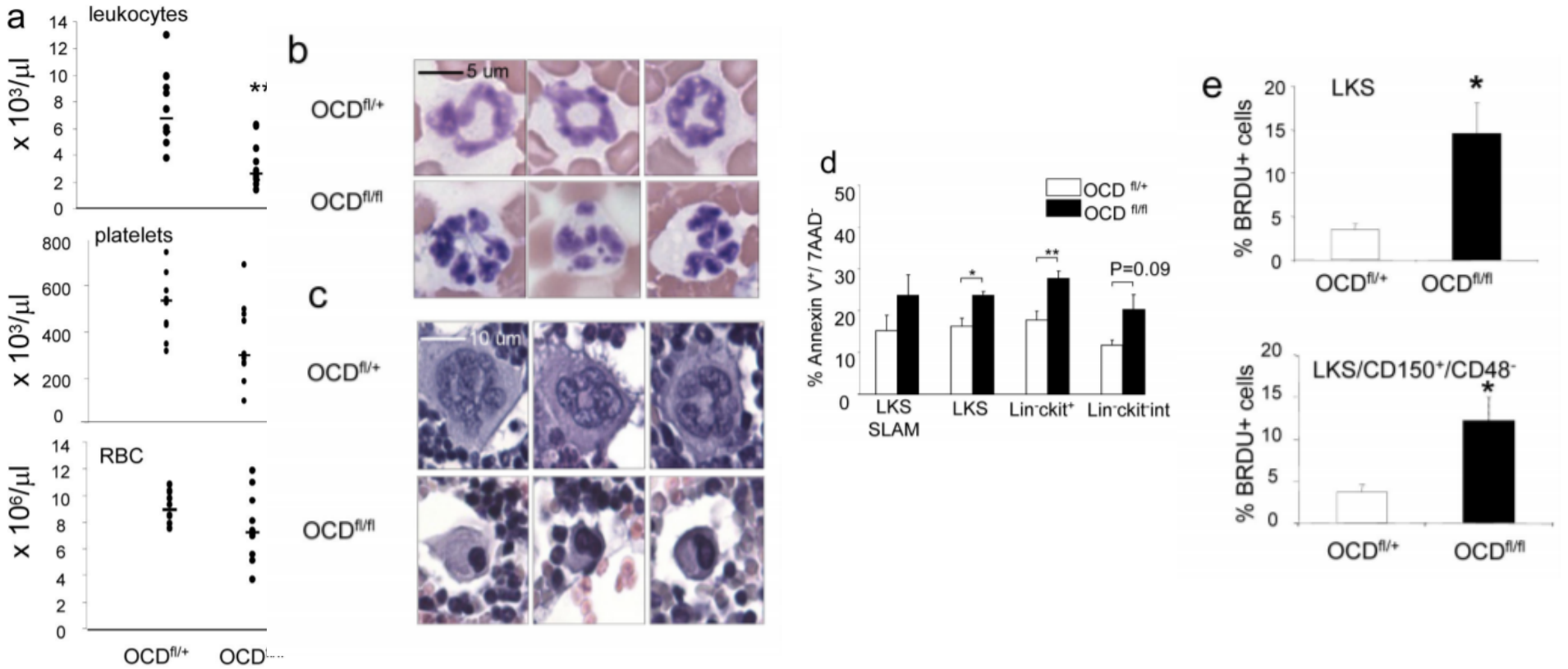


Figure 2. Myelodysplasia in OCD fl/fl mice a, Leukopenia with variable anemia (p=0.16) and thrombocytopenia (p=0.08) in OCD fl/fl mice (n=10). b, blood smears showing dysplastic hyperlobulated nuclei in granulocytes c, bone marrow sections showing micro-megakaryocytes with hyperchromatic nuclei d, increased apoptosis of hematopoietic progenitor cells in OCD fl/fl mice. (n=4) e, increased proliferation of hematopoietic progenitor cells as shown by in vivo BRDU labeling (n=4). Data are mean ± s.e.m. * p≤0.05, **p≤0.01. RBC=red blood cells, LKS= lineage −C-kit+ Sca1+ cells LKS-SLAM= lineage −C-kit+ Sca1+ CD150+ CD48− cells L-K+= lineage−c-kit+ cells L-K-int=lineage−Ckit intermediate, BRDU= bromodeoxyuridine.

AML with soft tissue infiltration in Dicer1-deleted mice

Dicer1

INITIATING EVENT

Abnormal

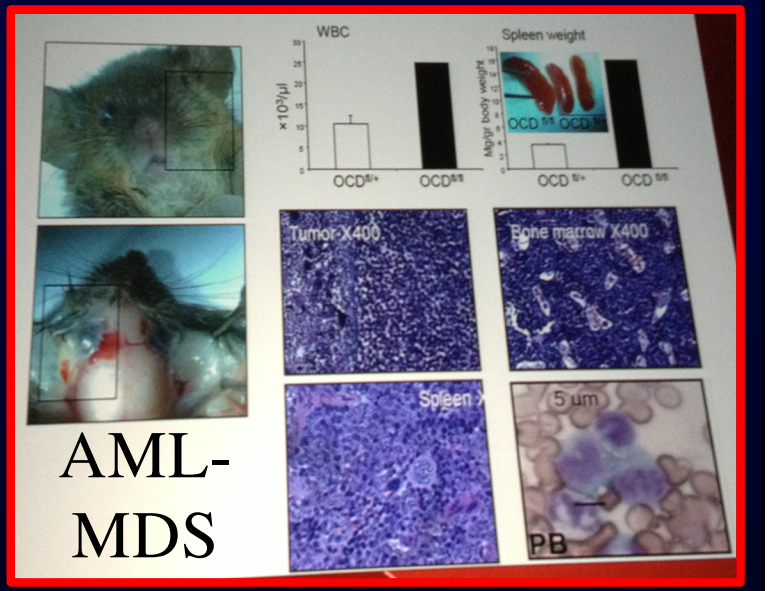
(SECONDARY & TERTIARY EVENT)

Genotype: normal
Phenotype: normal

HSC Transplant

MDS

Genotype: mutated
Phenotype: malignant



AML-MDS

from David T. Scadden, *ASH 2012, modified;*
Raaijmakers et al. *Nature 2010; 464: 852-857*

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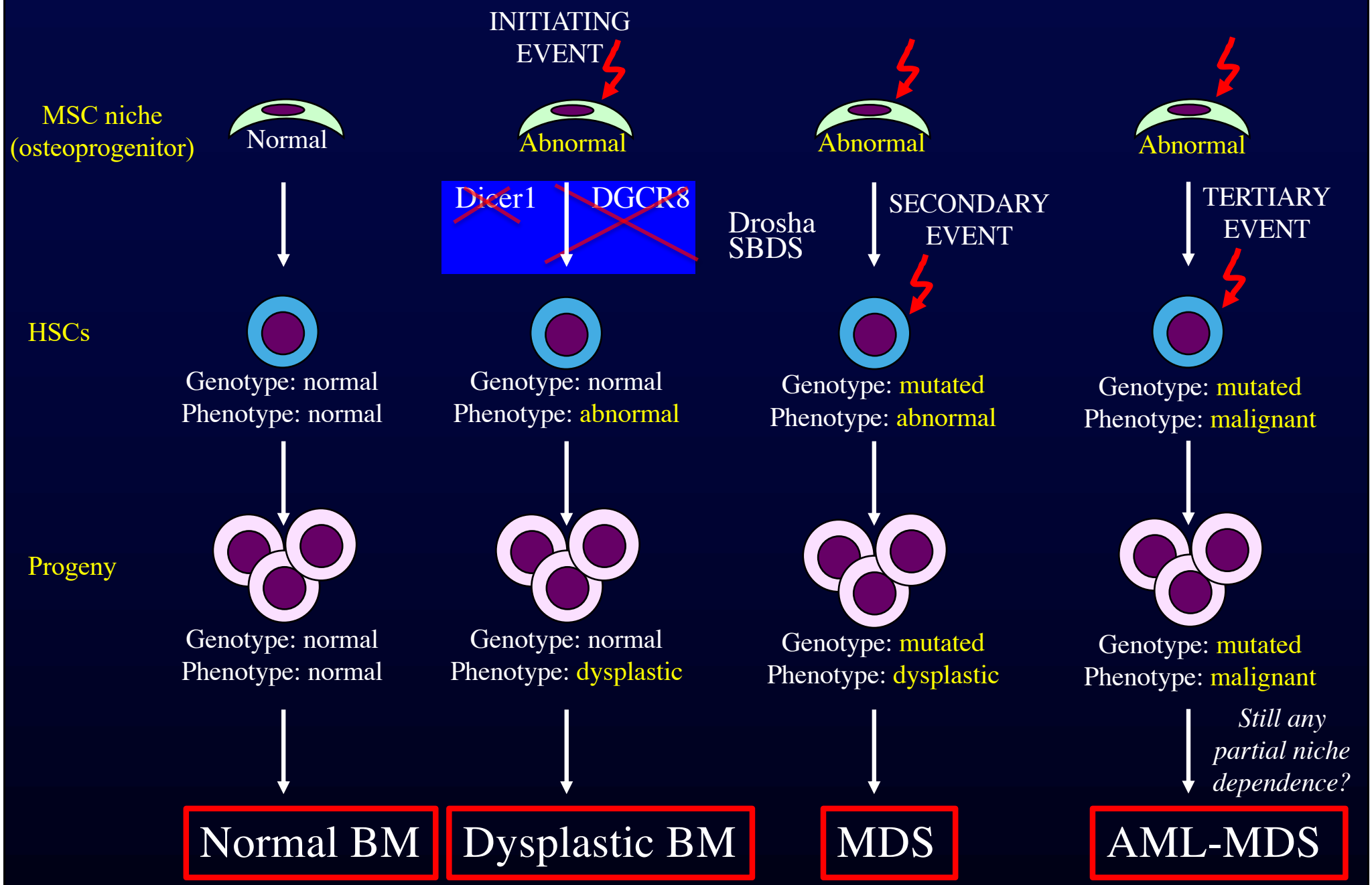
Bone marrow HSC niche: oncogenesis model



from David T. Scadden, *ASH 2012, modified;* Raaijmakers et al. *Nature 2010; 464: 852-857*

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Role of the BM microenvironment in MDS pathogenesis

1- Animal models revealing BM microenvironment-induced MDS

Table 1. Murine models of MDS exhibiting bone marrow microenvironment involvement

	Genetic alteration		Model features		
Model	Gene	Location	MDS	Microenvironment	References
Osx-GFP-Cre^{+} Dicer$^{fl/fl}$ mice	*Dicer1* deletion	Microenvironment (osteoprogenitors targeted by Osterix)	• Cytopenias • Dysplasia • Progression to acute leukemia • Hypercellular marrow • Increased apoptosis	• Osteoblastic dysfunction (impaired osteoblastic differentiation) with downregulation of the *Sbds* gene • Increased vascularity	[55]
Osx-GFP-Cre^{+} Sbds$^{fl/fl}$ mice	*Sbds* deletion	Microenvironment (osteoprogenitors targeted by Osterix)	• Cytopenias • Dysplasia • Increased apoptosis • Genotoxic stress	• Growth retardation with skeletal defects due to impaired osteoblastic differentiation • S100A8 and S100A9 overexpression by *Sbds*-deficient osteoprogenitor cells drive HSPC genotoxic stress • Increased vascularity	[55,87]
NUP98-HOXD13 (NHD13)-transgenic mice	*NUP98-HOXD13* fusion transgene expression	Hematopoietic cells (targeted by Vav regulatory elements)	• Cytopenias • Dysplasia • Progression to acute leukemia • Hypercellular marrow • Impaired differentiation • Increased apoptosis	• Age-dependent alterations of mesenchymal osteolineage cells and skeletal phenotype • Increased vascularity	[88–90]
S100A9-transgenic mice	*S100A9* overexpression	Hematopoietic cells (targeted by H2K promoter)	• Cytopenias • Dysplasia • Increased pyroptosis	• Expansion of CD33^{+} myeloid-derived suppressor cells leading to production of hematopoiesis-suppressive cytokines (IL-10 and TGF-β) • Caspase-1 activation, NLRP3 inflammasome complex formation, and pyroptosis induction in BM cells	[91,99]

Li et al. 2017

Role of the BM microenvironment in MDS pathogenesis

1- Animal models revealing BM microenvironment-induced MDS

Cell Stem Cell 2014;14(6):824-37

Myelodysplastic Cells in Patients Reprogram Mesenchymal Stromal Cells to Establish a Transplantable Stem Cell Niche Disease Unit

Hind Medyouf,[1,11,13,*] Maximilian Mossner,[2] Johann-Christoph Jann,[2] Florian Nolte,[2] Simon Raffel,[3] Carl Herrmann,[4,5] Amelie Lier,[3] Christian Eisen,[3] Verena Nowak,[2] Bettina Zens,[1,3] Katja Müdder,[1,3] Corinna Klein,[1,3] Julia Obländer,[2] Stephanie Fey,[2] Jovita Vogler,[2] Alice Fabarius,[2] Eva Riedl,[6] Henning Roehl,[7] Alexander Kohlmann,[8] Marita Staller,[8] Claudia Haferlach,[8] Nadine Müller,[2] Thilo John,[9] Uwe Platzbecker,[10] Georgia Metzgeroth,[2] Wolf-Karsten Hofmann,[2] Andreas Trumpp,[1,3,11,12,*] and Daniel Nowak[2,12]

- Xenograft model of low-risk MDS: the first proof of concept that patient-derived stromal cells drive propagation of human MDS stem cells *in vivo*
- Intrabone co-injection of low-risk MDS patient-derived CD34+ cells + MSPCs into immunocompromised mice leads to long-term engraftment of *bone fide* MDS cells (strong myeloid bias and clonality tracking). CD34+ cells-only injection is highly ineffective
- Patient-derived MSPCs are more efficient than healthy age-matched MSPCs in supporting MDS stem cells
- a number of processes involved in cellular cross-talk are deregulated in MDS-MSPCs

Role of the BM microenvironment in MDS pathogenesis

2- Alterations of the cellular components of the niche in MDS patients

Table 1. Overview of published data on the potential role of MSPCs and derivates in the pathogenesis of MDS

PROs	*CONs*
■ Cytogenetic aberrations in MDS-MSPCs[62,63,74]	■ Normal cytogenetics in MDS-MSPCs[64,66]
■ Lower expression of *Dicer1*, *DROSHA*,[56,57] *AURKA*, *AURKB*[75] genes in MDS-MSPCs	■ Normal structure, proliferation and differentiation potential of MDS-MSPCs[62,63,64,66]
■ Altered immunophenotype in MDS-MSPCs: decreased CD44 and CD49e,[70] CD90, CD104 and CD105[68] expression, increased CXCL12 expression[71]	■ Normal HSC support by MDS-MSPCs[62,63,64]
■ Impaired proliferation and differentiation capacity of MDS-MSPCs[58,65,70]	
■ Impaired cytokine production, including IL-32, by MDS-MSPCs[58]	
■ Deregulation of Wnt signaling pathway in MDS-MSPCs[82,89]	
■ Impaired HSPC support by MDS-MSPCs[65,66]	

Bulycheva et al. 2015

Abbreviations: HSPC, hematopoietic stem and progenitor cell; IL-32, interleukin-32; MDS, Myelodysplastic syndrome; MSPC, mesenchymal stem and progenitor cell.

- Stromal cells fail to support HSC trafficking into the microenvironmental niche
- Cytogenetic abnormalities in MSPCs (mostly in Chr 1 and 7, different from those detectable in HSPCs) in up to 50% of MDS patients
- Monocytes from MDS patients fail to upregulate matrix MMP-9 gene expression in response to stromal signals. MMP-9 promote the egress of cells from the BM: non-responsive monocytes accumulate over time, whereas inducible levels of MMP-9 decline, thus resulting in hypercellularity in the BM of patients with MDS
- Macrophages interfere with interactions between MSPCs and HSPCs in MDS through increased synthesis of TNF-α

Role of the BM microenvironment in MDS pathogenesis

3- Signalling defects within the osteo-hematopoietic niche

- Controvertial role of secreted cytokines and adhesion molecules in MDS

Leukemia (2010) 24, 748–755

www.nature.com/leu

ORIGINAL ARTICLE

Lenalidomide induces cell death in an MDS-derived cell line with deletion of chromosome 5q by inhibition of cytokinesis

A Matsuoka[1], A Tochigi[1], M Kishimoto[1], T Nakahara[1], T Kondo[1], T Tsujioka[1], T Tasaka[1], Y Tohyama[2] and K Tohyama[1]

Available online at www.sciencedirect.com
ScienceDirect
Leukemia Research 32 (2008) 1407–1416
Leukemia Research
www.elsevier.com/locate/leukres

Functional analysis of myelodysplastic syndromes-derived mesenchymal stem cells

Eugenia Flores-Figueroa[a,*], Juan José Montesinos[a], Patricia Flores-Guzmán[a], Guillermo Gutiérrez-Espíndola[b], Rosa María Arana-Trejo[c], Sebastián Castillo-Medina[a], Adrián Pérez-Cabrera[c], Erika Hernández-Estévez[a], Lourdes Arriaga[d], Hector Mayani[a]

Contents lists available at ScienceDirect
Leukemia Research
journal homepage: www.elsevier.com/locate/leukres

Disease progression mechanism in myelodysplastic syndromes: Insight into the role of the microenvironment

Mariko Ishibashi, Hideto Tamura, Kiyoyuki Ogata*

- Canonical Wnt signaling deregulation in MDS-MSPCs

Impaired Wnt–Pathway Signalling and Reduced Expression of Senescence–Associated Markers in Bone Marrow Mesenchymal Stem Cells of Patients with Myelodysplastic Syndromes

Konstantia I. Pavlaki, Maria-Christina Kastrinaki, Aristea Batsali, Anthi Demetriadou, Maria Velegraki, Irene Mavroudi, Michael Klontzas, Anna Psaraki, Maria Psyllaki, Charalampos Pontikoglou, and Helen A. Papadaki

Blood 2011 118:272;

Erythropoietin inhibits osteoblast function in myelodysplastic syndromes via the canonical Wnt pathway

Ekaterina Balaian,[1]* Manja Wobus,[1]* Heike Weidner,[1] Ulrike Baschant,[2,3] Maik Stiehler,[4] Gerhard Ehninger,[1] Martin Bornhäuser,[1,5] Lorenz C Hofbauer,[2,3,5] Martina Rauner[2,3]** and Uwe Platzbecker[1,3,5]**

Haematologica 2018
Volume 103(1):61-68

Wnt / β-Catenin signaling pathway

Canonical Wnt pathway

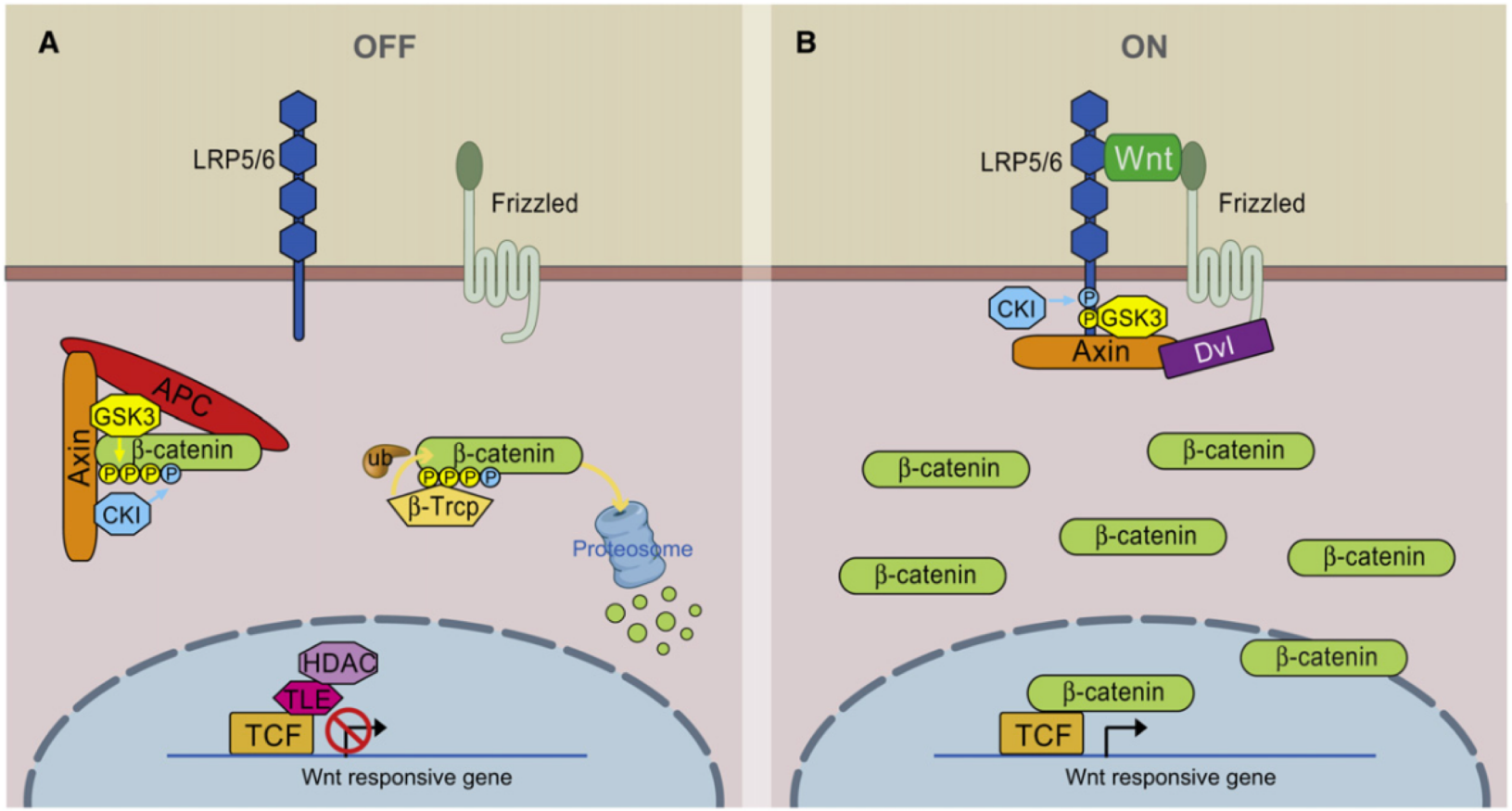


Figure 1. Overview of Wnt/β-Catenin Signaling

(A) In the absence of Wnt, cytoplasmic β-catenin forms a complex with Axin, APC, GSK3, and CK1, and is phosphorylated by CK1 (blue) and subsequently by GSK3 (yellow). Phosphorylated β-catenin is recognized by the E3 ubiquitin ligase β-Trcp, which targets β-catenin for proteosomal degradation. Wnt target genes are repressed by TCF-TLE/Groucho and histone deacetylases (HDAC).

(B) In the presence of Wnt ligand, a receptor complex forms between Fz and LRP5/6. Dvl recruitment by Fz leads to LRP5/6 phosphorylation and Axin recruitment. This disrupts Axin-mediated phosphorylation/degradation of β-catenin, allowing β-catenin to accumulate in the nucleus where it serves as a coactivator for TCF to activate Wnt-responsive genes.

MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. Dev Cell. 2009 Jul;17(1):9-26

Wnt / β-catenin signaling pathway

Canonical Wnt pathway

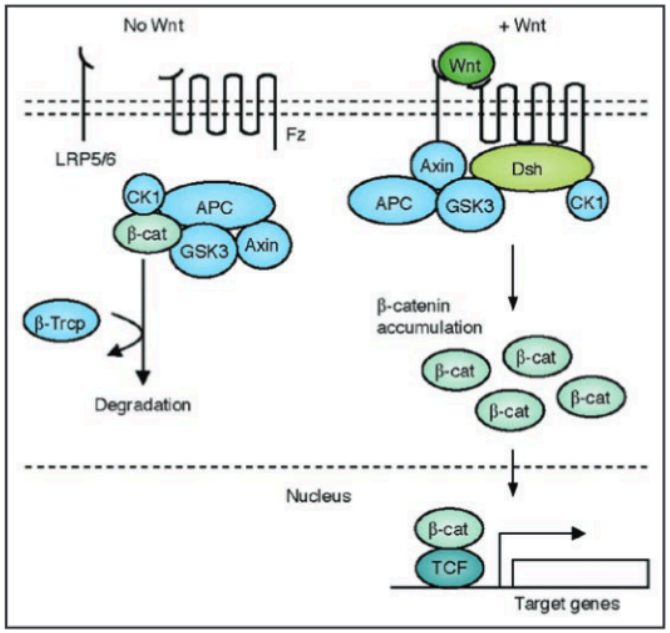


Figure 1. A schematic representation of the canonical Wnt signal transduction cascade. Left, in the absence of Wnt ligand, a complex of Axin, APC, GSK3-β, CK1 and β-catenin is located in the cytosol. β-catenin is dually phosphorylated by CK1 and GSK3-β and targeted degraded by the proteosomal machinery mediated by β-TrCP. Right, with Wnt stimulation, signaling through the Fz receptor and LRP5/6 co-receptor complex induces the dual phosphorylation of LRP6 by CK1 and GSK3-β and this allows for the translocation of a protein complex containing Axin from the cytosol to the plasma membrane. Dsh is also recruited to the membrane and binds to Fz and Axin binds to phosphorylated LRP5/6. This complex formed at the membrane at Fz/LRP5/6 induces the stabilization of β-cat via either sequestration and/or degradation of Axin. β-catenin translocates into the nucleus where it complexes with Lef/Tcf family members to mediate transcriptional induction of target genes.

Non-canonical Wnt pathways

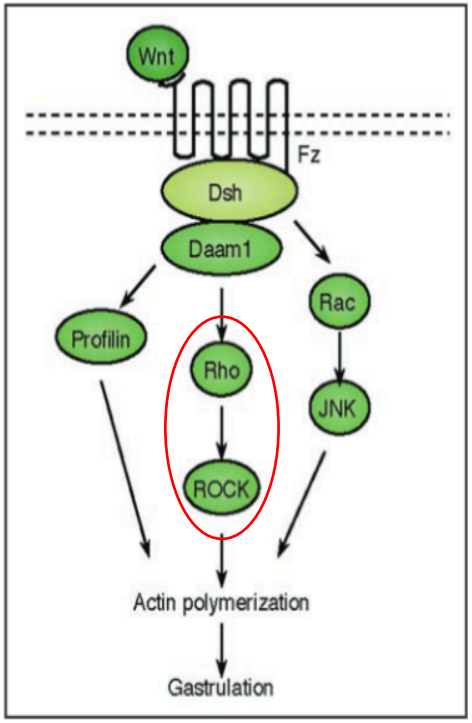


Figure 2. A schematic representation of the Planar Cell Polarity transduction cascade. Wnt signaling is transduced through Fz independent of LRP5/6 leading to the activation of Dsh. Dsh through Daam1 mediates activation of Rho which in turn activates Rho kinase (ROCK). Daam1 also mediates actin polymerization through the actin binding protein Profilin. Dsh also mediates activation of Rac, which in turn activates JNK. The signaling from Rock, JNK and Profilin are integrated for cytoskeletal changes for cell polarization and motility during gastrulation.

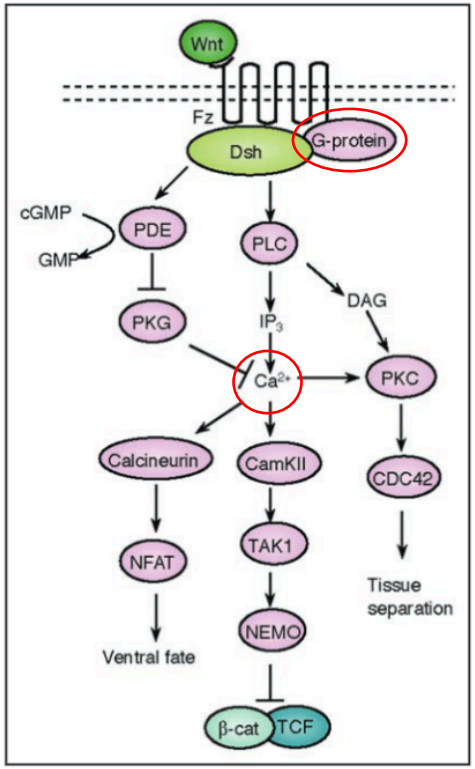
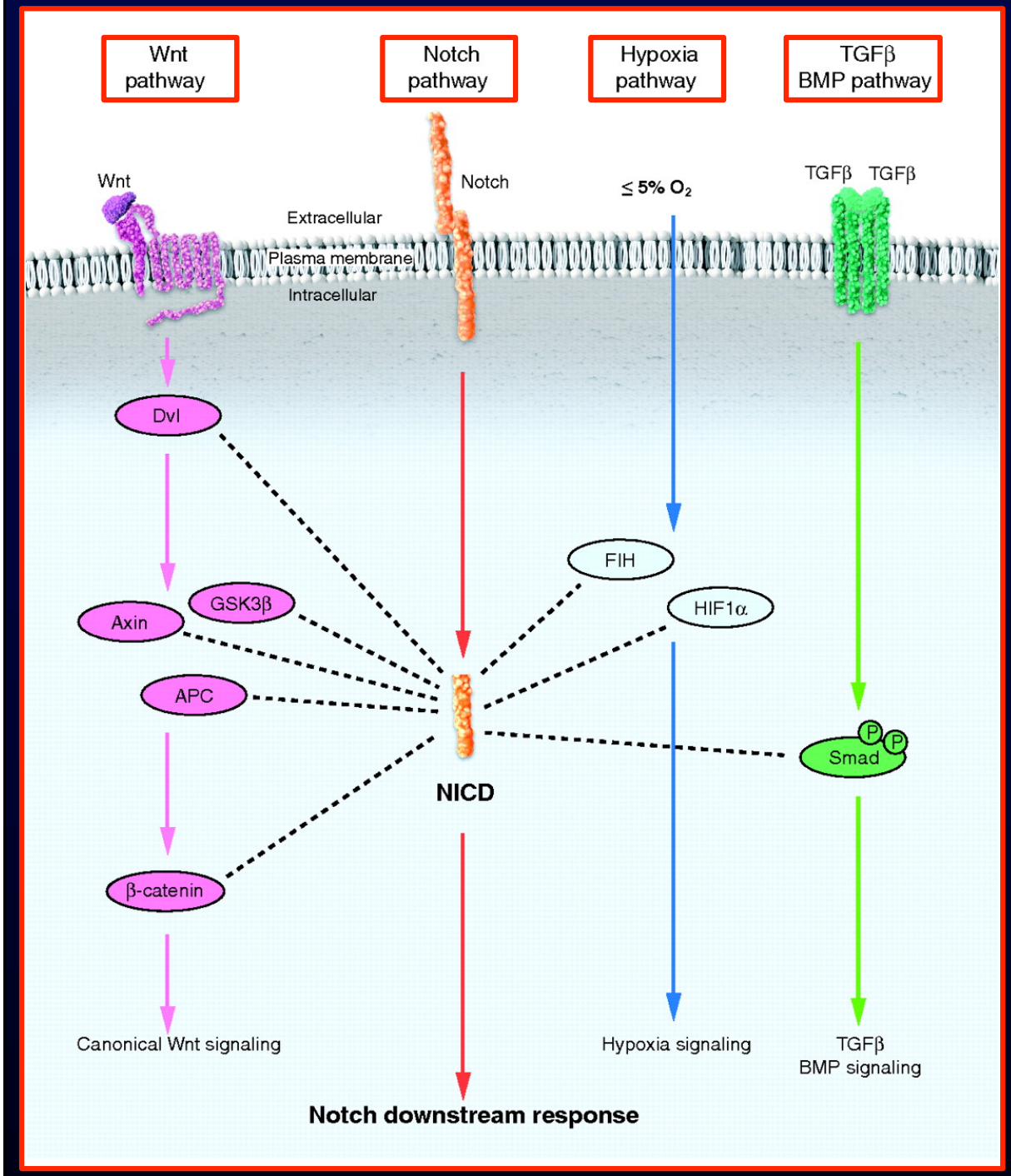
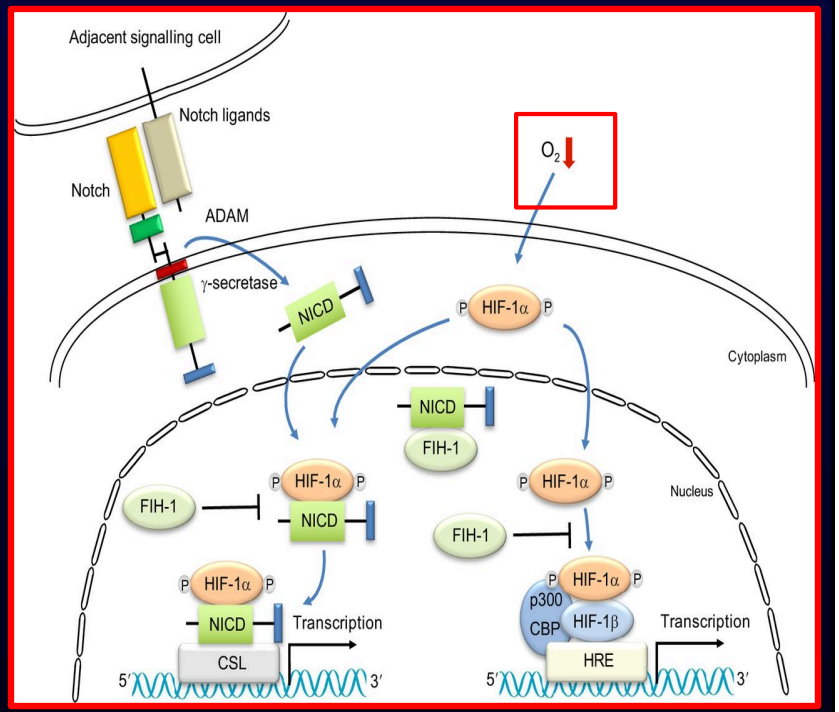


Figure 3. A schematic representation of the Wnt/Ca^{2+} signal transduction cascade. Wnt signaling via Fz mediates activation of Dsh via activation of G-proteins. Dishevelled activates the phosphodiesterase PDE which inhibits PKG and in turn inhibits Ca^{2+} release. Dsh through PLC activates IP3, which leads to release of intracellular Ca^{2+}, which in turn activates CamK11 and calcineurin. Calcineurin activate NF-AT to regulate ventral cell fates. CamK11 activates TAK and NLK, which inhibit β-catenin/TCF function to negatively regulate dorsal axis formation. DAG through PKC activates CDC42 to mediate tissue separation and cell movements during gastrulation.

Komiya Y1, Habas R. Wnt signal transduction pathways. Organogenesis. 2008 Apr;4(2):68-75

Cross-interactions of different signalling pathways in normal hematopoiesis





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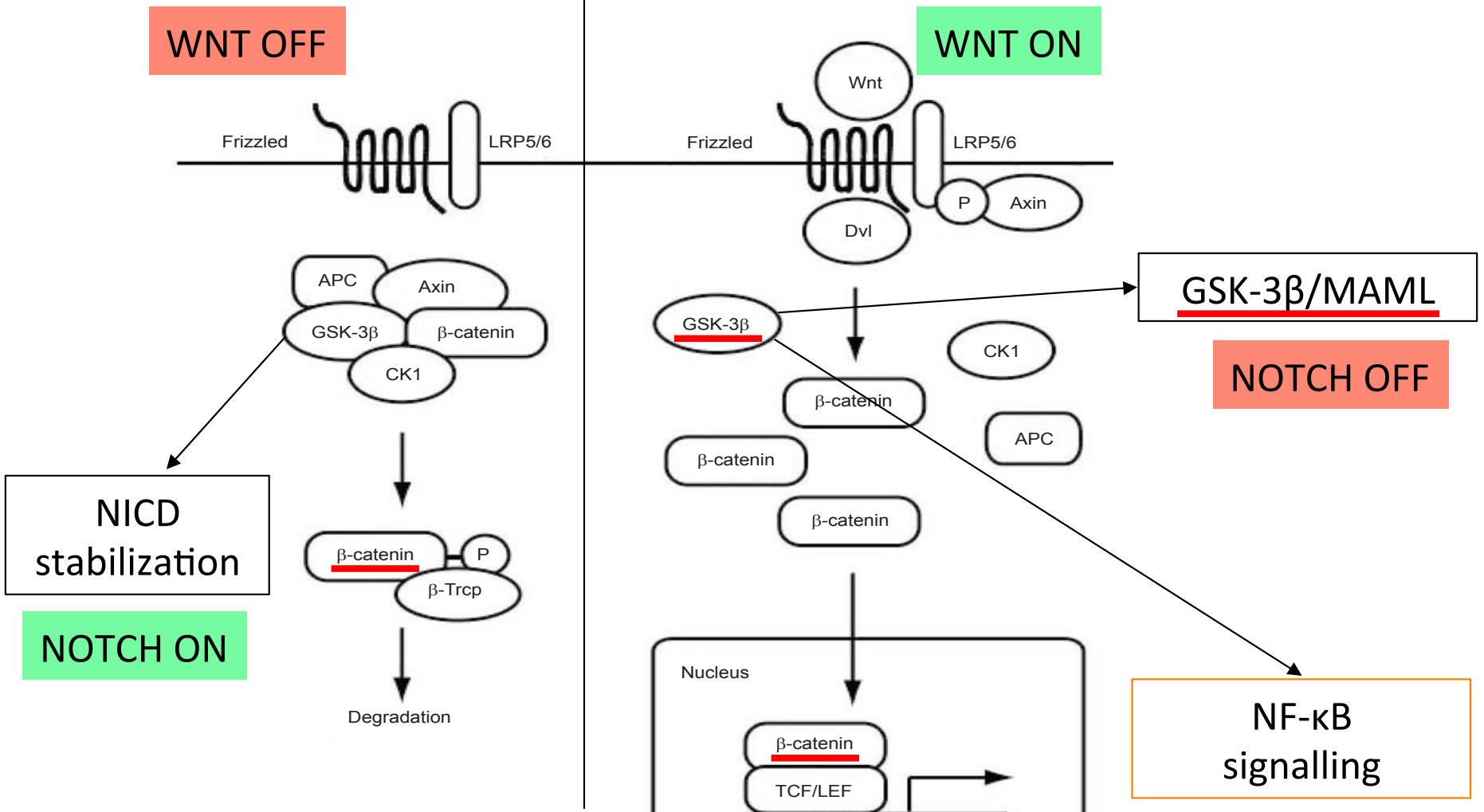
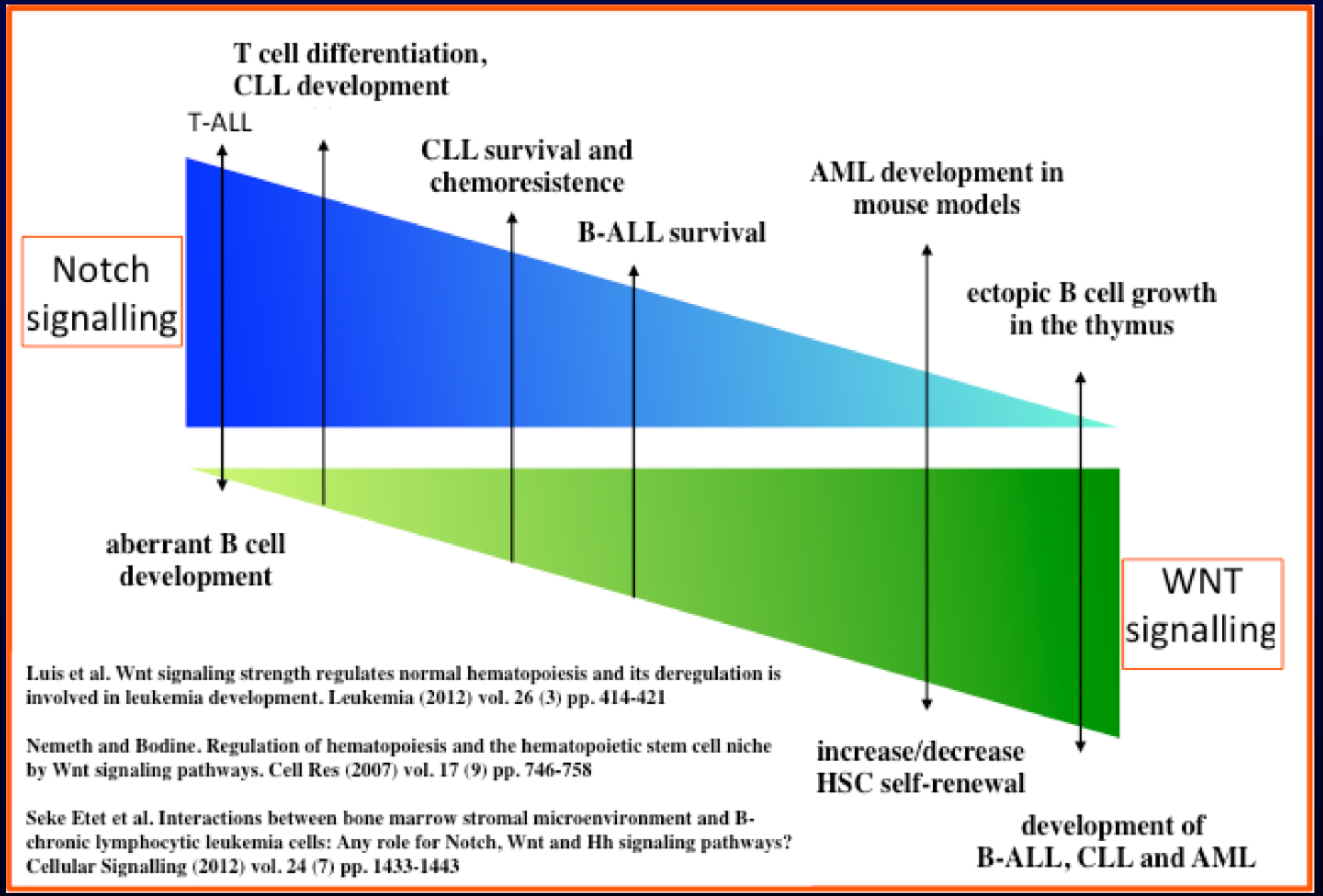
Notch / Wnt balance in normal hematopoiesis



modified from Nemeth and Bodine, Regulation of hematopoiesis and the hematopoietic stem cell niche by Wnt signaling pathways. Cell Res (2007) vol. 17 (9) pp. 746-758

Notch / Wnt balance in neoplastic hematopoiesis



Luis et al. Wnt signaling strength regulates normal hematopoiesis and its deregulation is involved in leukemia development. Leukemia (2012) vol. 26 (3) pp. 414-421

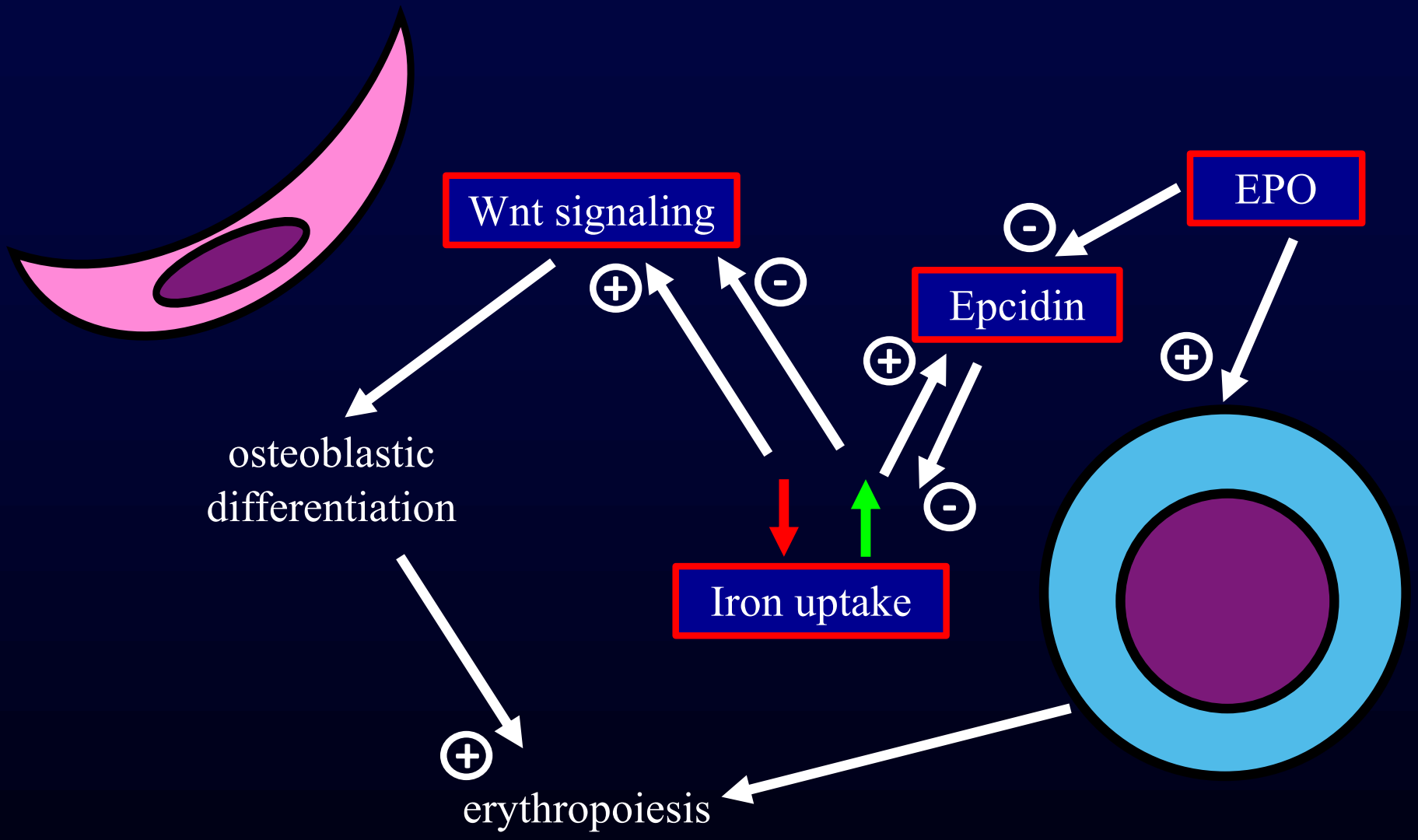
Nemeth and Bodine. Regulation of hematopoiesis and the hematopoietic stem cell niche by Wnt signaling pathways. Cell Res (2007) vol. 17 (9) pp. 746-758

Seke Etet et al. Interactions between bone marrow stromal microenvironment and B-chronic lymphocytic leukemia cells: Any role for Notch, Wnt and Hh signaling pathways? Cellular Signalling (2012) vol. 24 (7) pp. 1433-1443

Role of the BM microenvironment in MDS pathogenesis

3- Signalling defects within the osteo-hematopoietic niche

4- Iron overload and dysregulation of iron homeostasis



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Role of the BM microenvironment in MDS pathogenesis

3- Signalling defects within the osteo-hematopoietic niche

4- Iron overload and dysregulation of iron homeostasis

- Iron depletion can activate Wnt/β-catenin and induce osteoblastic differentiation of MSPCs
- Deregulated Wnt signaling in MDS - MSPCs disrupts iron regulation (→ accumulation) and is an important factor in MDS pathogenesis

Impaired Wnt-Pathway Signalling and Reduced Expression of Senescence-Associated Markers in Bone Marrow Mesenchymal Stem Cells of Patients with Myelodysplastic Syndromes

Konstantia I. Pavlaki, Maria-Christina Kastrinaki, Aristea Batsali, Anthi Demetriadou, Maria Velegraki, Irene Mavroudi, Michael Klontzas, Anna Psaraki, Maria Psyllaki, Charalampos Pontikoglou, and Helen A. Papadaki

Blood 2011 118:272;

Published in final edited form as:
Leukemia. 2012 March ; 26(3): 414–421. doi:10.1038/leu.2011.387.

Wnt signaling strength regulates normal hematopoiesis and its deregulation is involved in leukemia development

Tiago C. Luis[1,2], **Michiko Ichii**[3], **Martijn H. Brugman**[2], **Paul Kincade**[3], and **Frank J.T. Staal**[1,2]

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Role of the BM microenvironment in MDS pathogenesis

3- Signalling defects within the osteo-hematopoietic niche

4- Iron overload and dysregulation of iron homeostasis

- MDS-MSPCs exhibit a lower mineralization in response to Epo due to Wnt dysregulation
- Decrease of sensitivity of erythroid progenitors to Epo in TfR2−/− mouse model (TfR2 is a component of the Epo-R complex) → Epo increase → iron overload

Erythropoietin inhibits osteoblast function in myelodysplastic syndromes via the canonical Wnt pathway

Ekaterina Balaian,[1*] Manja Wobus,[1*] Heike Weidner,[1] Ulrike Baschant,[2,3] Maik Stiehler,[4] Gerhard Ehninger,[1] Martin Bornhäuser,[1,5] Lorenz C Hofbauer,[2,3,5] Martina Rauner[2,3**] and Uwe Platzbecker[1,3,5**]

Haematologica 2018 Volume 103(1):61-68

CLINICAL TRIALS AND OBSERVATIONS

Predictive factors of response and survival in myelodysplastic syndrome treated with erythropoietin and G-CSF: the GFM experience

Sophie Park,[1] Sophie Grabar,[2] Charikleia Kelaidi,[3] Odile Beyne-Rauzy,[4] Françoise Picard,[5] Valérie Bardet,[5] Valérie Coiteux,[6] Geneviève Leroux,[7] Pascale Lepelley,[8] Marie-Thérèse Daniel,[9] Stéphane Cheze,[10] Béatrice Mahé,[11] Augustin Ferrant,[12] Christophe Ravoet,[13] Martine Escoffre-Barbe,[14] Lionel Adès,[3] Norbert Vey,[15] Lina Aljassem,[16] Aspasia Stamatoullas,[17] Lionel Mannone,[18] Hervé Dombret,[19] Keith Bourgeois,[20] Peter Greenberg,[21] Pierre Fenaux,[3] and François Dreyfus,[1] for the GFM group (Groupe Francophone des Myélodysplasies)

p-ERK1/2 is a predictive factor of response to erythropoiesis-stimulating agents in low/int-1 myelodysplastic syndromes

Emilie Frisan,[1,2,3,5] Patrycja Pawlikowska,[1,2,3] Cecile Pierre-Eugène,[1,2,3,4] Vivian Viallon,[5,6] Laure Gibault,[5,7] Sophie Park,[1,2,3,5,8] Patrick Mayeux,[1,2,3] François Dreyfus,[1,2,3,5,8] Françoise Porteu,[1,2,3] Michaëla Fontenay[1,2,3,4,5]

RED CELLS, IRON, AND ERYTHROPOIESIS

Transferrin receptor 2 is a component of the erythropoietin receptor complex and is required for efficient erythropoiesis

Hana Forejtniková,[1,2] *Maud Vieillevoye,[1,2] *Yael Zermati,[1,2] Mireille Lambert,[1,2] Rosa Maria Pellegrino,[3] Soizic Guihard,[1,2] Muriel Gaudry,[1,2] Clara Camaschella,[4] Catherine Lacombe,[1,2,5] Antonella Roetto,[4] †Patrick Mayeux,[1,2] and †Frédérique Verdier[1,2]

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Role of the BM microenvironment in MDS pathogenesis

3- Signalling defects within the osteo-hematopoietic niche

4- Iron overload and dysregulation of iron homeostasis

- Iron overload is very common in MDS (blood transfusions and inefficient erythropoiesis
- Iron overload has adverse effects on bone homeostasis (inhibition of Wnt signalling)
- In the process of iron overload, hyper-production of hepcidin occurs
- In MDS hepcidin levels are very heterogenous depending on the subtype, with the lowest level in RARS and the highest in RAEB and CMML, and EPO levels

Expression of Genes Regulating Iron Metabolism in Hepatocyte Cell–Line HepG2 Induced by Sera from MDS Patients.

Laura Breda, Hussam Ghoti, Stefano Rivella, Gideon Rechavi, Ioav Cabantchik, and Eliezer A. Rachmilewitz

Blood 2007 110:4612;

Articles Myelodysplastic Syndromes

Inappropriately low hepcidin levels in patients with myelodysplastic syndrome carrying a somatic mutation of *SF3B1*

Ilaria Ambaglio,[1] Luca Malcovati,[1,2] Elli Papaemmanuil,[3] Coby M. Laarakkers,[4,5] Matteo G. Della Porta,[1] Anna Gallì,[1,2] Matteo C. Da Vià,[1,2] Elisa Bono,[1,2] Marta Ubezio,[1,2] Erica Travaglino,[1] Riccardo Albertini,[6] Peter J. Campbell,[3] Dorine W. Swinkels,[4,5] and Mario Cazzola[1,2]

Role of the BM microenvironment in MDS pathogenesis

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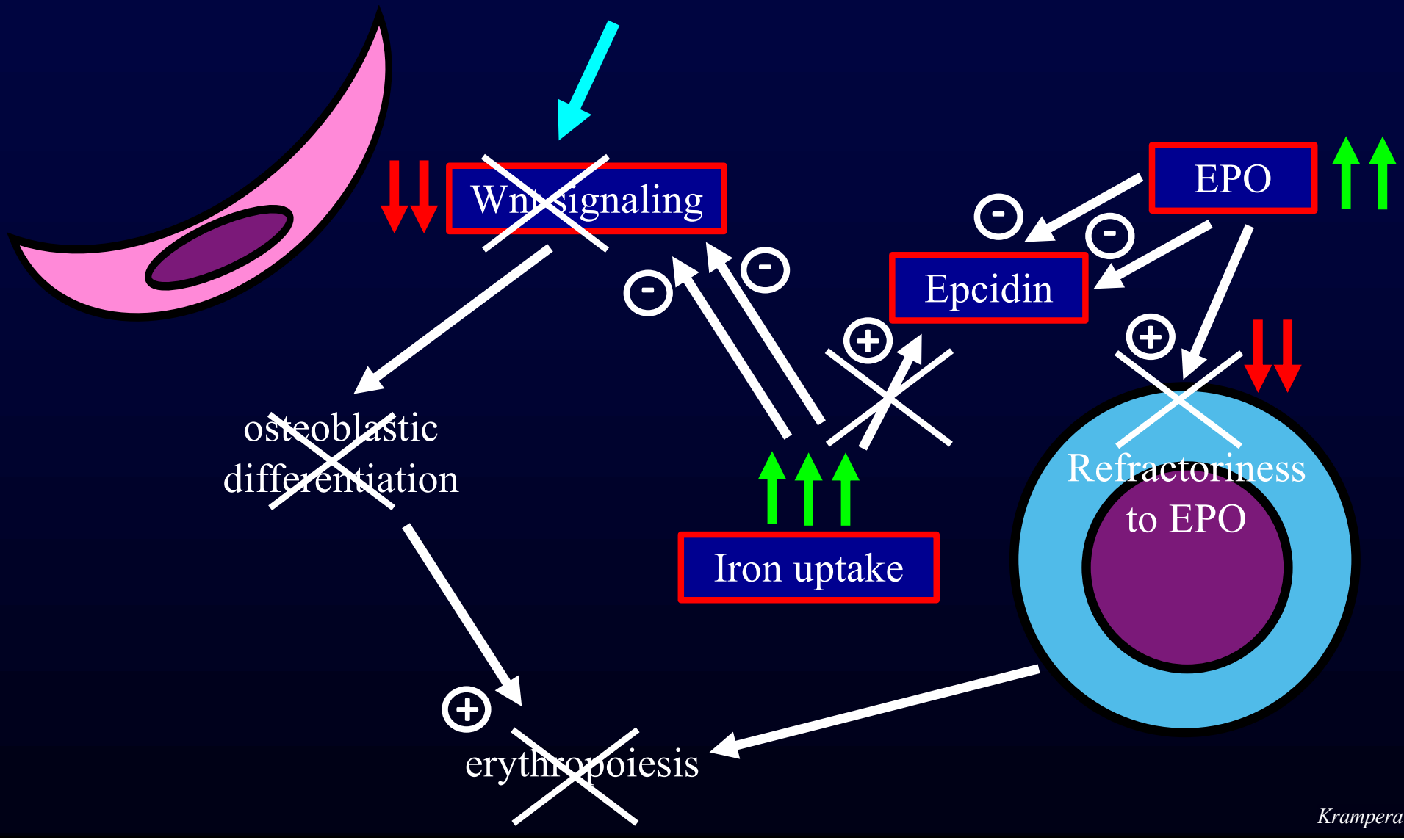
4- Iron overload and dysregulation of iron homeostasis



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Role of the BM microenvironment in MDS pathogenesis

3- Signalling defects within the osteo-hematopoietic niche

Table 2. Aberrant signaling molecules in the MDS microenvironment

Signaling molecule	Involvement in MDS
CCL3	Mesenchymal osteolineage dysfunction [85,89,101–103]
TGF-β	Hematopoietic suppression [146]
	Secreted by myeloid cells downstream of S100A9-CD33 signaling [99]
S100A9 and S100A8	Production by mesenchymal stromal cells in human MDS correlates with leukemic progression [87]
	Induces expansion of $CD33^+$ myeloid-derived suppressor cells and induces secretion of suppressive cytokines TGF-β and IL-10 [99]
	Induces caspase-1-mediated pyroptotic cell death and IL-1β production [91]
	Induces HSPC genotoxic stress [87]
IL-1β	Increased cell death in MDS marrow [144]
	Increased production downstream of S100A9-mediated caspase-1 activation [91]
VEGF	Increases marrow vascularity [112–116] to potentially increase O_2 and nutrient delivery to malignant cells
	Direct stimulation of malignant cell proliferation [129]
	Overexpressed by myeloid precursors in MDS [129], megakaryocytes [113], and mesenchymal stromal cells [76,83]

Li et al. 2017

Hypoxia and MDS

- Hypoxia / low oxygen availability contributes to both normal and malignant hematopoiesis
- HIF-1 and HIF-2 (hypoxia inducible transcription factors) are the key mediators of the cellular response to hypoxia
- In MDS patients, HIF-1 expression correlates with poor patient survival and disease progression
- The strong hypoxic gene expression profile of supportive MDS-MSCs, in comparison to healthy MSCs, suggests that hypoxia and HIF-1 signaling may influence the malignant behavior of MDS-MSCs

Hypoxia-inducible factor-1α expression indicates poor prognosis in myelodysplastic syndromes

Hongyan Tong, Chao Hu, Zhengping Zhuang, Lijun Wang & Jie Jin

CellPress

Myelodysplastic Cells in Patients Reprogram Mesenchymal Stromal Cells to Establish a Transplantable Stem Cell Niche Disease Unit

Hind Medyouf,[1,11,13,*] Maximilian Mossner,[2] Johann-Christoph Jann,[2] Florian Nolte,[2] Simon Raffel,[3] Carl Herrmann,[4,5] Amelie Lier,[3] Christian Eisen,[3] Verena Nowak,[2] Bettina Zens,[1,3] Katja Müdder,[1,3] Corinna Klein,[1,3] Julia Obländer,[2] Stephanie Fey,[2] Jovita Vogler,[2] Alice Fabarius,[2] Eva Riedl,[6] Henning Roehl,[7] Alexander Kohlmann,[8] Marita Staller,[8] Claudia Haferlach,[8] Nadine Müller,[2] Thilo John,[9] Uwe Platzbecker,[10] Georgia Metzgeroth,[2] Wolf-Karsten Hofmann,[2] Andreas Trumpp,[1,3,11,12,*] and Daniel Nowak[2,12]

Published in final edited form as:
Exp Hematol. 2007 January ; 35(1): 21–31. doi:10.1016/j.exphem.2006.08.017.

Enhanced growth of myelodysplastic colonies in hypoxic conditions

James Edwin Thompson, **Joseph Patrick Conlon**, **Xiaowei Yang**, **Patricia Vanessa Sanchez**, and **Martin Carroll**
Division of Hematology and Oncology, University of Pennsylvania, Philadelphia, Pa., USA

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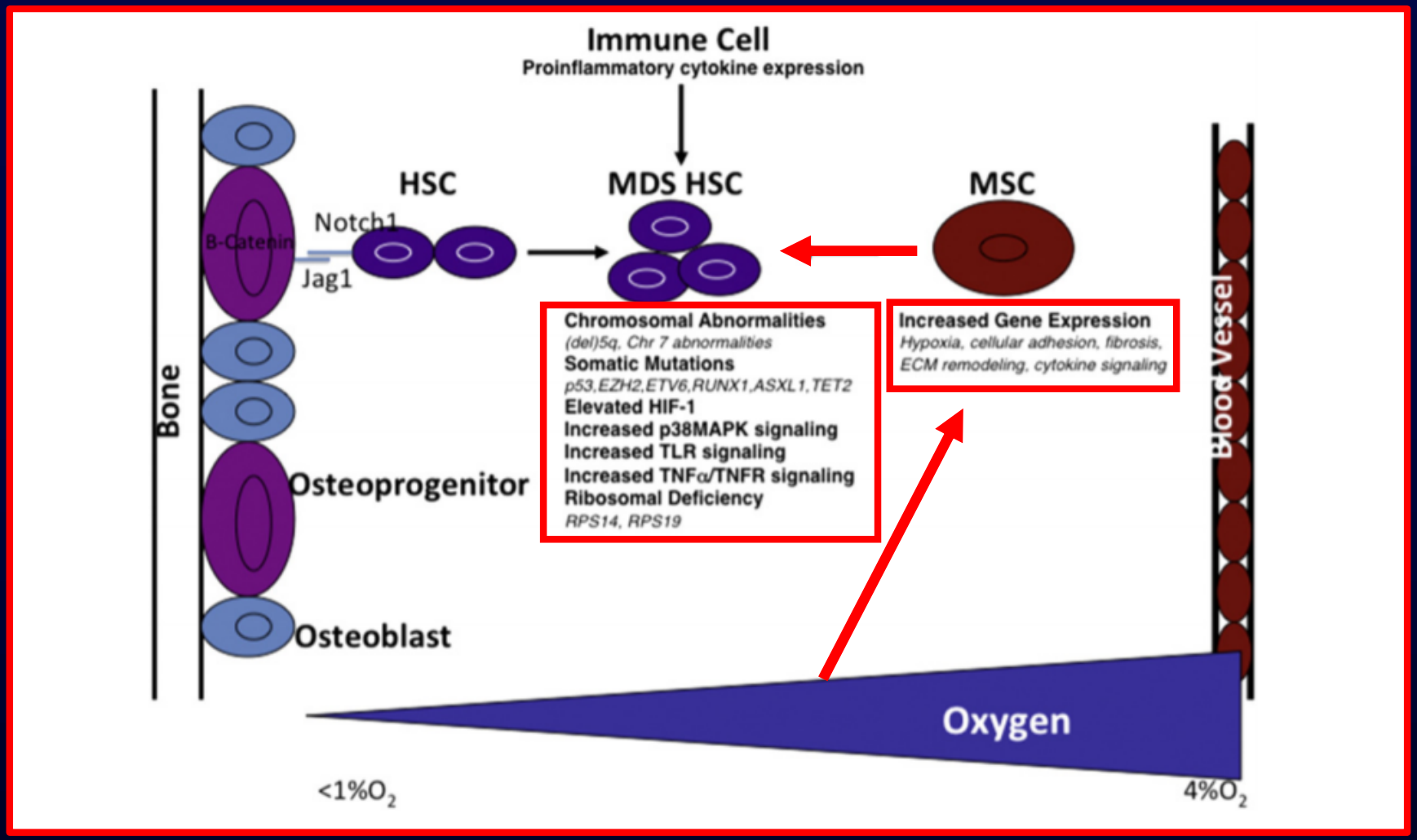
Hypoxia and MDS



Immune cells, osteoblasts, and signaling molecules that influence MDS-HSC signalling and functions. Hypoxia also has the capacity to directly and indirectly influence the behavior of MDS HSCs

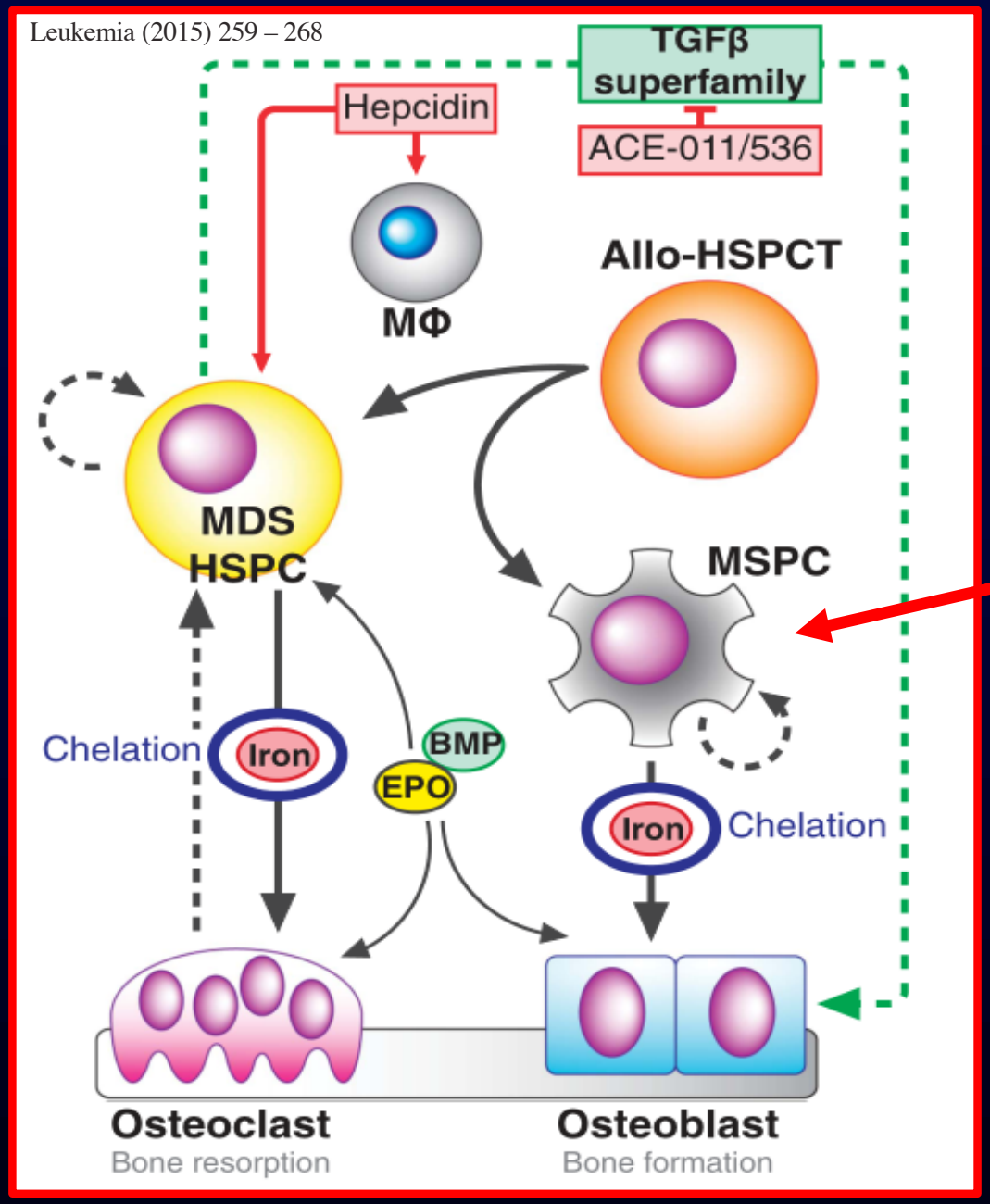
Potential therapeutic targets of the osteo-hematopoetic niche

Table 2. Currently available therapeutic options for patients with MDS and their potential impact on the functional properties of the osteo-hematopoietic niche

Agent	*Mechanism*
HMA (azacitidine/decitabine)	* Demethylation of Wnt-antagonist gene promoters and reduction of the non-phosphorylated β-catenin in HSPCs[105] * Upregulation of BMP-2, -4 and -6 expression in osteoblasts[106]
Lenalidomide	* Inhibiting angiogenesis, suppressing the production of proinflammatory cytokines (for example, TNFα), inhibiting cytokinesis in MDS cell lines[78] * Decrease of CXCL12 secretion by MSPCs, thereby detaching HSPC from their niche; enhancement of CD29 (integrin β1) expression[69] * Increase in expression of adhesion molecules in HSPCs; increase in CXCL12 and ICAM-1 secretion by MSPCs, thus improving their hematopoiesis-supporting capacity[72]
ACE-011/ACE-536	* Trap of important soluble factors (that is, activin, BMP-2, BMP-6) secreted by stromal cells[107,112] * Modulating of the SMAD signaling pathway, which leads to changes in the transcription of SMAD-regulated target genes[113]
Iron chelation	* Activation of Wnt/β-catenin pathway and induction of osteoblastic differentiation of MSPCs[117]

Abbreviations: BMP, bone morphogenetic protein; HMA, hypomethylating agent; HSPC, hematopoietic stem and progenitor cell; ICAM-1, intercellular adhesion molecule-1; MDS, myelodysplastic syndrome; MSPCs, mesenchymal stem and progenitor cells; TNFα, tumor necrosis factor-α.

Potential therapeutic targets of the osteo-hematopoetic niche



Wnt agonists ?

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Extracellular vesicles in MDS

Haematologica. 2016 Apr;101(4):437-47. doi: 10.3324/haematol.2015.134932. Epub 2016 Jan 22.

Extracellular vesicle miR-7977 is involved in hematopoietic dysfunction of mesenchymal stromal cells via poly(rC) binding protein 1 reduction in myeloid neoplasms.

Horiguchi H[1], Kobune M[2], Kikuchi S[1], Yoshida M[1], Murata M[3], Murase K[1], Iyama S[1], Takada K[1], Sato T[1], Ono K[1], Hashimoto A[1], Tatekoshi A[1], Kamihara Y[1], Kawano Y[1], Miyanishi K[1], Sawada N[3], Kato J[1].

Circulating exosomal microRNAs in acquired aplastic anemia and myelodysplastic syndromes

Valentina Giudice,[1] Lauren G. Banaszak,[1] Fernanda Gutierrez-Rodrigues,[1] Sachiko Kajigaya,[1] Reema Panjwani,[1] Maria del Pilar Fernandez Ibanez,[1] Olga Rios,[1] Christopher K. Bleck,[2] Erin S. Stempinski,[2] Diego Quinones Raffo,[1] Danielle M. Townsley[1] and Neal S. Young[1]

Microvesicles from Mesenchymal Stromal Cells Are Involved in HPC-Microenvironment Crosstalk in Myelodysplastic Patients

Sandra Muntión, Teresa L. Ramos, María Diez-Campelo, Beatriz Rosón, Luis Ignacio Sánchez-Abarca, Irena Misiewicz-Krzeminska, Silvia Preciado, María-Eugenia Sarasquete, Javier de las Rivas, Marcos González, Fermín Sánchez-Guijo, María-Consuelo del Cañizo

Extracellular vesicles (EVs)

Exosomes	40-100 nm in diameter Alix, TSG101, Tetraspanin, Heat shock protein
Shedding microvesicles or Ectosomes	50-1000 nm in diameter Phosphatidylserine RNA, Protein
Apoptotic bodies	50-5000 nm in diameter Phosphatidylserine DNA

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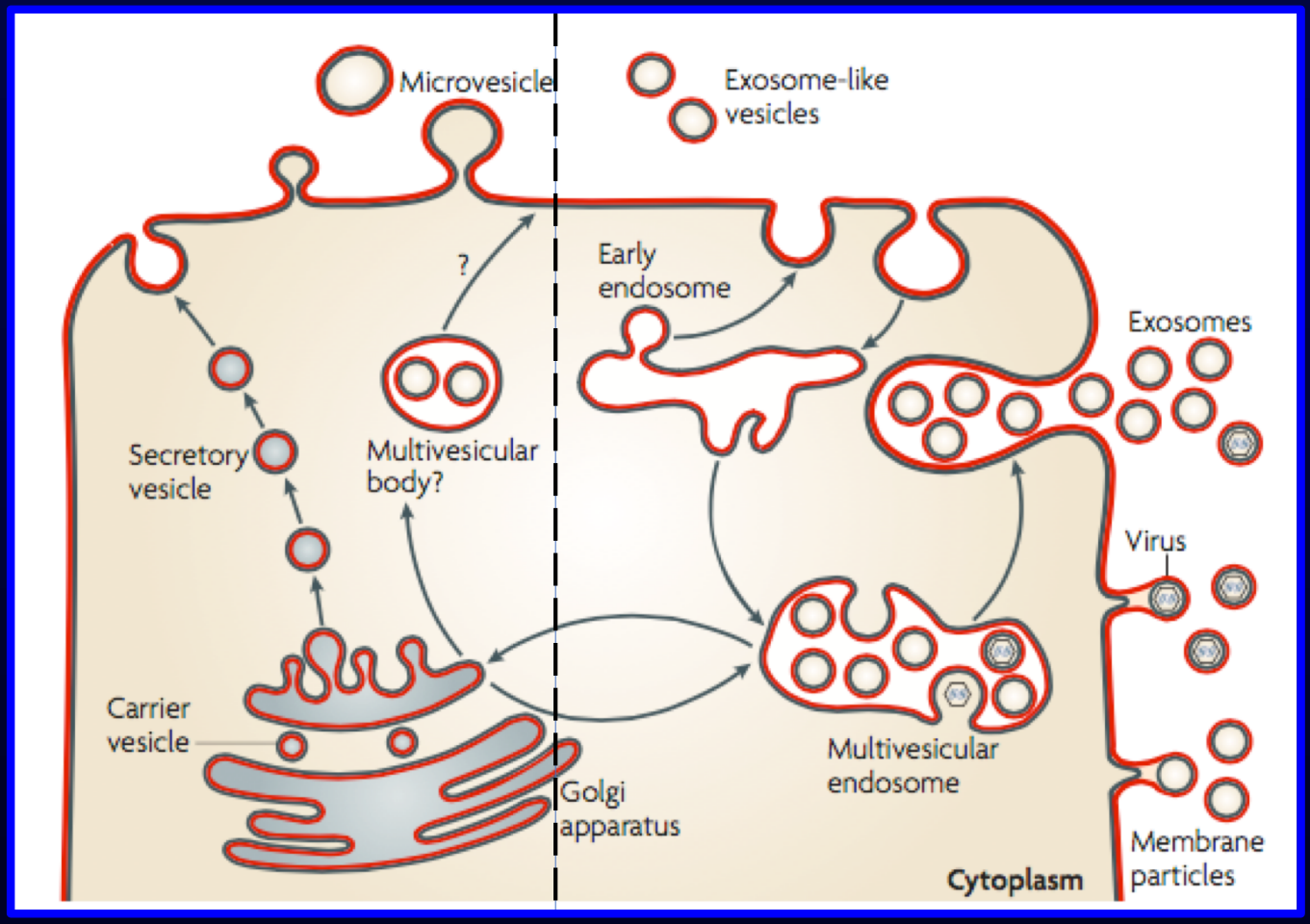
Extracellular vesicle biogenesis



Different types of secreted membrane vesicles: Intracellular trafficking either between subcellular compartments or towards the plasma membrane for secretion of soluble proteins occurs through carrier and secretory vesicles that contain intraluminal components. Secreted vesicles can form inside internal compartments from where they are subsequently secreted by fusion of these compartments with the plasma membrane. Vesicles generated in multivesicular endosomes are called exosomes once secreted.
Nat Rev Immunol. 2009 Aug;9(8):581-93.

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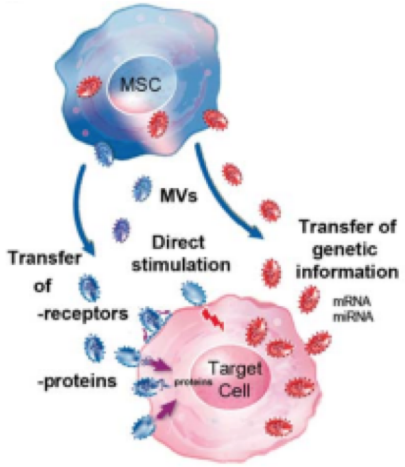
Extracellular vesicles (EVs)

Potential advantages for cells

- different biologically active molecules towards the same cell target
- lipid envelope → protection from degradation + rapid internalization
- surface proteins → binding to specific receptors → different cell targets

SMALL MOLECULE CONCENTRATIONS → MAXIMUM BIOLOGICAL EFFECTS

(even in case of negligible quantities, undetectable with standard techniques)



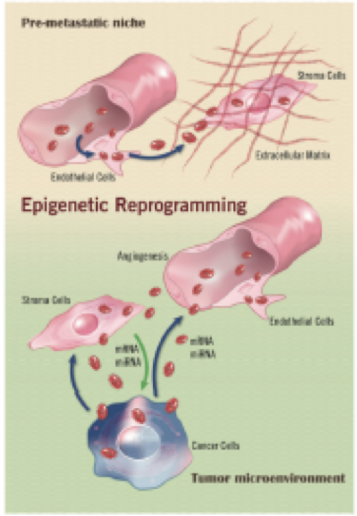
Nephrol Dial Transplant (2012) 27: 3037–3042

Short- and long-range communication

autocrine

paracrine

endocrine



Am J Cancer Res 2011;1(1):98-110

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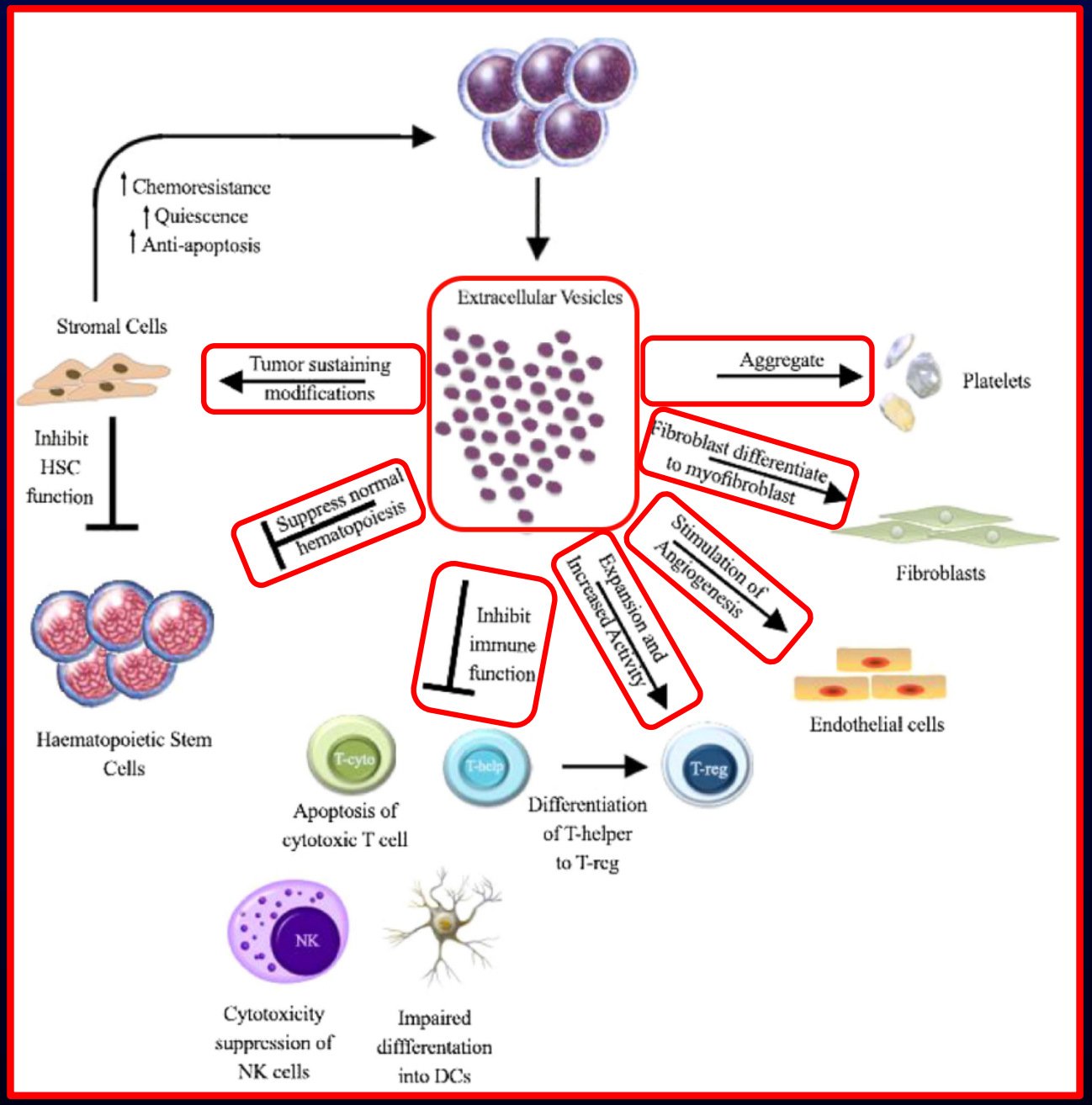
The role of the EVs in hematopoiesis



Pando et al. *Leukemia Research* 2018

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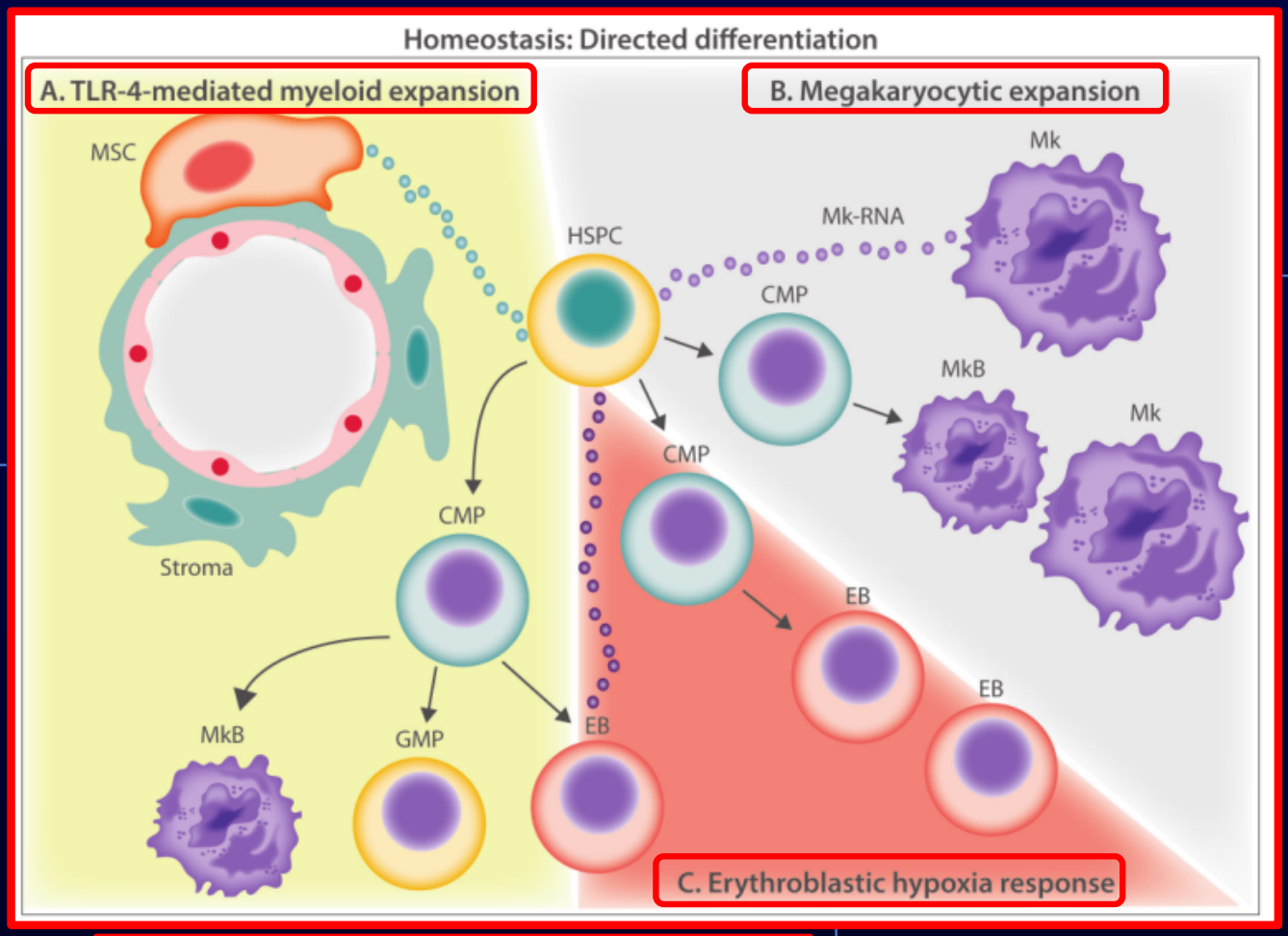
EV crosstalk in BM microenvironmental homeostasis

MSC-derived EVs signal to HSPCs through the TLR-4 pathway, resulting in myeloid biased expansion



CMP: common myeloid progenitor;
EB: erythroblast;
GMP; granulocyte monocyte progenitor;
HSPC: hematopoietic stem and progenitor cell;
Mk: megakaryocytes;
MkB: megakaryoblast

Hypoxia induces erythroid blast cells to release EVs containing miR-486 which increases erythroblastic differentiation by targeting Sirt1 in HSPCs

Megakaryocyte-derived EVs are internalized by HSPCs and increase differentiation of new megakaryocytes through RNA-mediated signaling

Butler et al. *Haematologica* 2018

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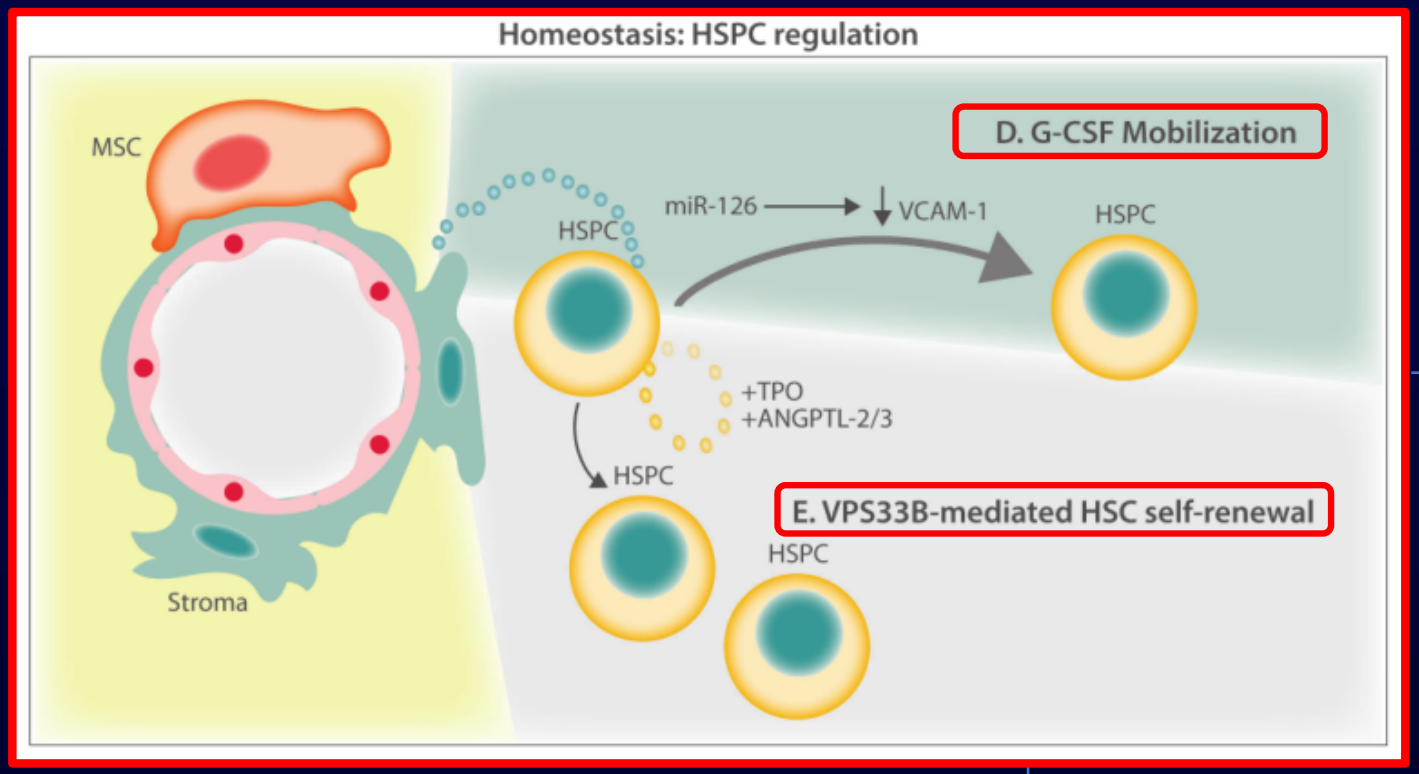
EV crosstalk in BM microenvironmental homeostasis



Butler et al. *Haematologica* 2018

G-CSF infusion stimulates the release of EVs containing miR-126 that act to down-regulate VCAM-1 in HSPCs, resulting in their mobilization out of the BM

HSPCs self-regulate stem cell potential by packaging and releasing critical secretory proteins through the exosomal pathway via VPS33B

ANGPTL-2/3; angiopoietin-like protein 2 and 3; G-CSF: granulocyte colony-stimulating factor; HSPC: hematopoietic stem and progenitor cell; TPO: thrombopoietin; VCAM-1: vascular cell adhesion molecule; VPS33B: vacuolar protein sorting-associated protein 33B.

EV crosstalk in BM microenvironmental homeostasis

Table 2A. Physiological regulation of hematopoiesis by extracellular vesicles.

EV origin	Recipient cell	Cargo	Molecular target and effect	Functional event	Reference
Reticulocytes	Macrophage	Transferrin receptor		Release and recycling of transferrin receptor during enucleation and maturation of erythrocytes	[9]
MSCs	HSPCs		TLR4: binding/activation	TLR4 signaling results in myeloid biased expansion and skewed hematopoietic repopulation potential of HSPCs	[17]
Megakaryocytes	HSPCs	Mk-RNA	ICAM-1: binding/entry CD63: binding/entry CD18: binding/entry CD11b: binding/entry	Selective differentiation of progenitors into functional megakaryocytes	[61]
Erythroleukemia cells	HSPCs	miR-486-5p	Sirt1: downregulation	Promotes erythroid differentiation in response to hypoxia	[63, 64]
G-CSF stimulated BM stroma	Stroma ECs HSPCs	miR-126	VCAM-1: downregulation	Down regulation of VCAM1 leads to mobilization of HSPCs out of the niche and into peripheral blood	[52]
Aged mouse BM cells	Young mouse BM stromal cells	miR-183-5p	HMOX1: downregulation	Reduced proliferative ability of stromal cells and decreased osteogenic differentiation	[65]
Mouse embryonic stem cells	HSPCs	Wnt3, Oct4,	SCL: upregulation, HoxB4: upregulation GATA2: upregulation MAPK p24/44: phosphorylation	Expansion of HSPCs and expression of markers associated with early HSC states	[66]
HSPCs	HSPCs	TPO, ANGPTL2, ANGPTL3	Autocrine signaling loop: maintains stemness	VPS33B mediated release of exosomes is required for maturation of secretory growth factors and maintaining cell stemness	[45]

Butler et al. *Haematologica* 2018

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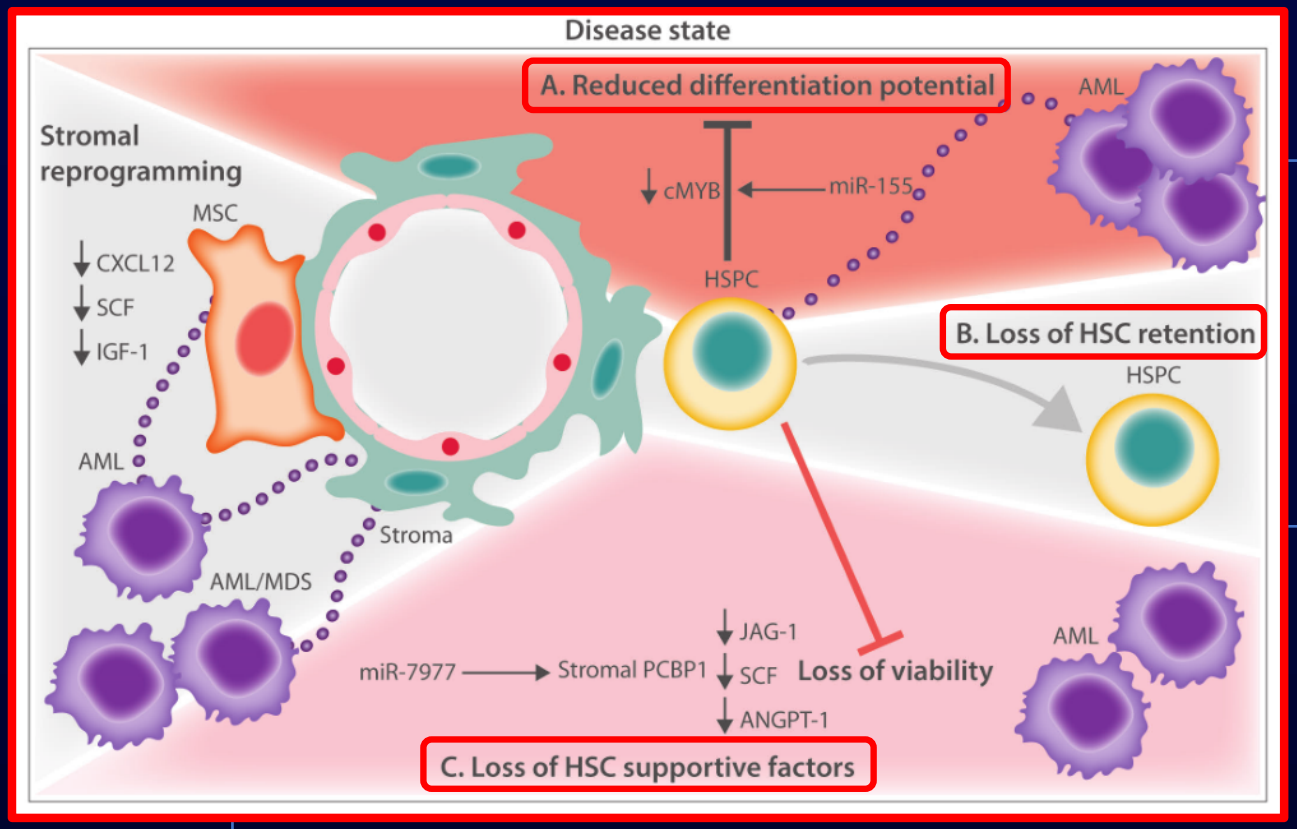
EV crosstalk in the leukemic microenvironment



Butler et al. *Haematologica* 2018

EVs from AML blasts traffic miR-155 to HSPCs and down-regulate critical transcription factors (c-MYB), resulting in reduced differentiation potential

AML EVs reprogram MSCs and stromal cells, and downregulate niche retention factor CXCL12 resulting in mobilization of HSPCs from the BM

AML and MDS EVs promote the loss of HSPC supportive factors (CXCL12, SCF, IGF-1) through the trafficking of miR-7977 to supportive stroma, leading to reduced HSPC viability and hematopoietic potential

AML: acute myelogenous leukemia;
ANGPT-1: angiopoi-etin 1;
CXCL12: C-X-C motif chemokine 12;
HSPCs: hematopoietic stem and progenitor cells;
IGF-1: insulin-like growth factor 1;
MDS: myelodysplastic syndrome;
PCBP1: poly(rc) binding protein 1;
SCF: stem cell factor.

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Extracellular vesicle crosstalk in the pathophysiological regulation of hematopoiesis

Table 2B. Pathophysiological regulation of hematopoiesis by extracellular vesicles.

EV origin	Recipient cell	Cargo	Molecular target and effect	Functional event	Reference
AML blasts	HSCPs	miR-150/155	cMYB; downregulation	Suppression of cMYB in HSPC reduces clonogenicity and leads to down regulation of niche retention factor CXCL12 and mobilization of HSPCs to peripheral blood	[13, 51]
AML blasts	BM Stroma		CXCL12: downregulation SCF: downregulation IGF1: downregulation DKK1: upregulation	Down regulation of HSC-supportive factors and suppression of hematopoiesis and osteolineage development by upregulating Dkk1 expression in BM stroma	[14]
AML and MDS cells	MSCs	miR-7977	PCBP1: downregulation Jagged1: downregulation SCF: downregulation ANGPT1: downregulation	Reduced HSC-supportive growth factors and hematopoiesis-supportive capacity of MSCs	[70]
MDS patient MSCs	$CD34^+$ progenitor cells	miR-10a/15a	P53: transcriptional dysregulation MDM2: transcriptional dysregulation	Alteration of HSCPs viability and clonogenicity	[71]
CML cells	BM stroma	Amphiregulin (EGFR-ligand)	EGFR: activation MMP9: upregulation IL8: upregulation	Alteration of BM microenvironment leading to increase attachment and proliferative advantage of CML cells	[72]
Melanoma cells	BM progenitors		c-MET	Mobilization of BM progenitors and upregulation of proinflammatory molecules at sites of macrophage trafficking leading to promotion of melanoma invasion and metastasis	[73]

Butler et al. *Haematologica* 2018

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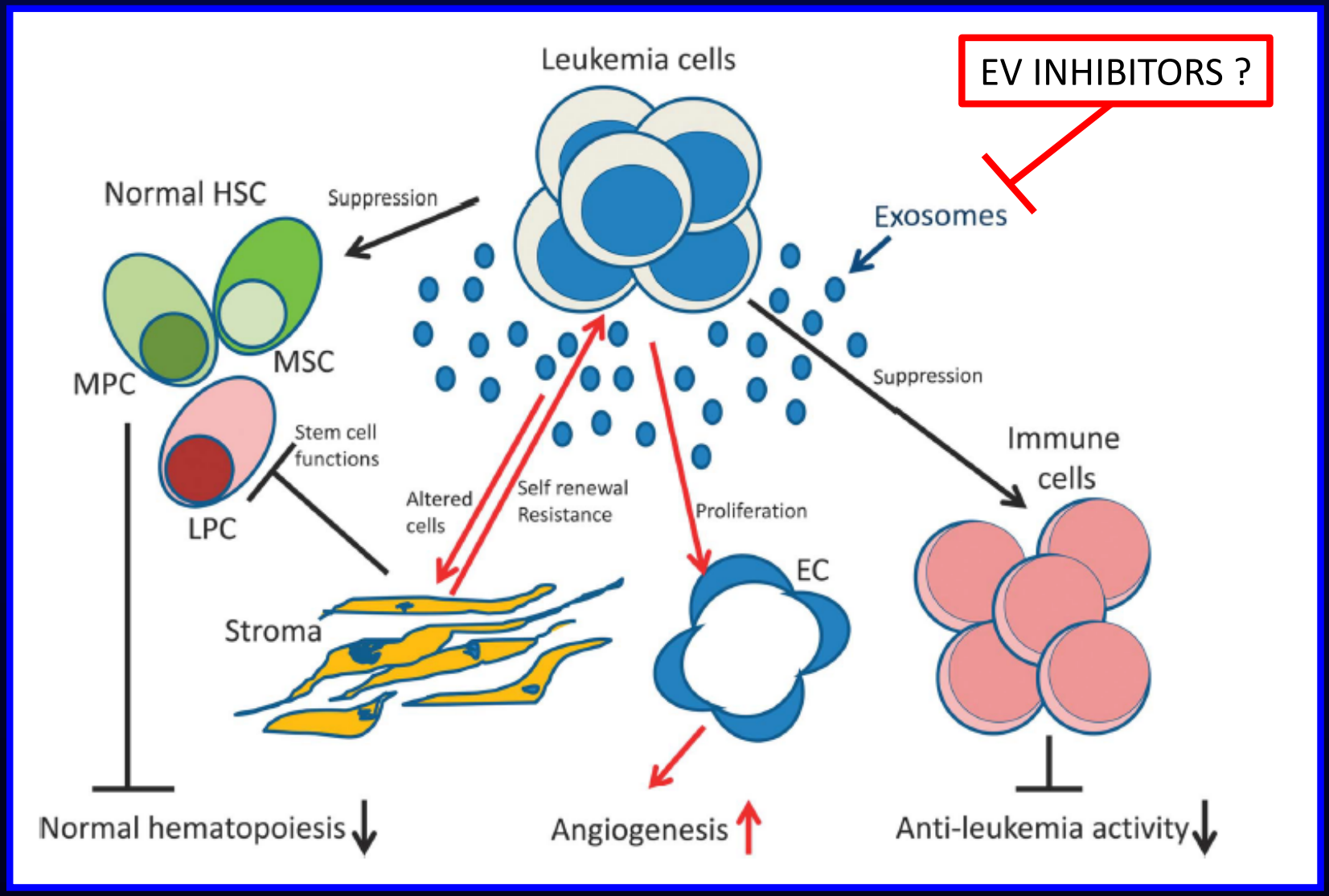
Role for EVs in pathological hemato-/lymphopoiesis

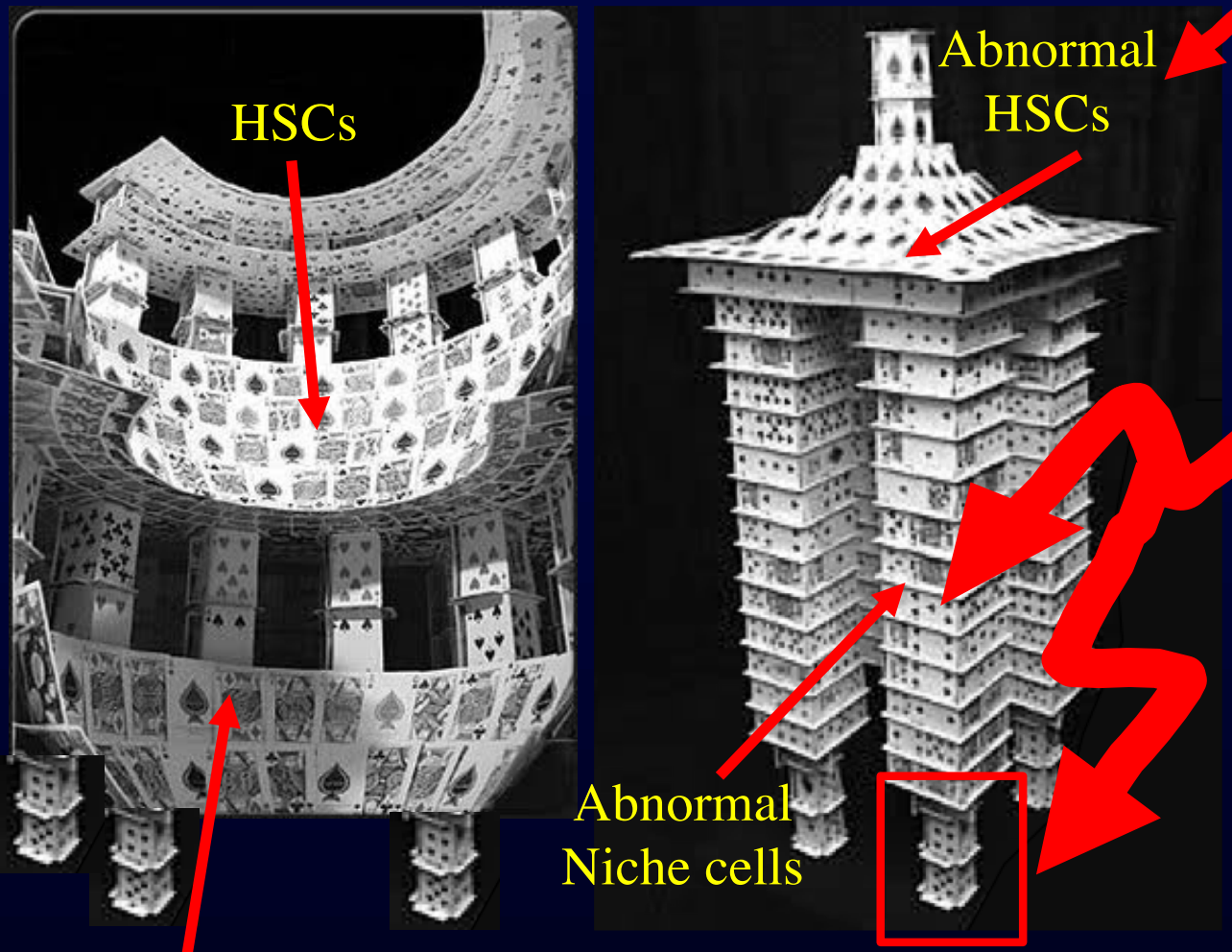


Boyiadzis M, Whiteside TL. The emerging role of tumor exosomes in hematological malignancies. *Leukemia 2017;31:1259-68*

CONCLUSIONS

Normal hematopoiesis

MDS hematopoiesis



Mutation-specific target-therapy

Microenvironment-directed therapy

Niche cells

- Intercellular pathways
- Soluble factors / EVs

- Wnt / β-catenin
- Epo
- Iron metabolism

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Section of Hematology, Department of Medicine
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