Do leukemic cells support the osteoblastic niche?

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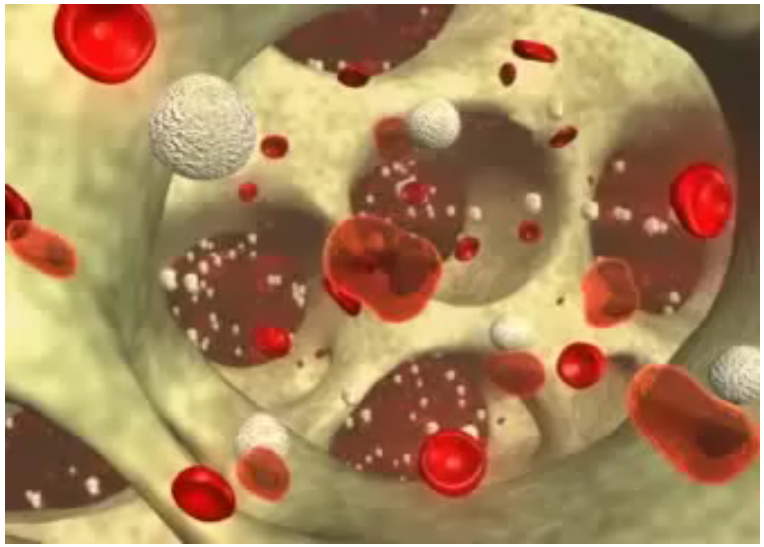
In addition to blood vessels
since many years the **trabecular bone** (endosteum) itself, is also known as **niche** for the persistence of stem cells*.

However, there is still a lack of knowledge regarding the mechanisms of interaction between leukemia and cancer (stem) cells and the bone...

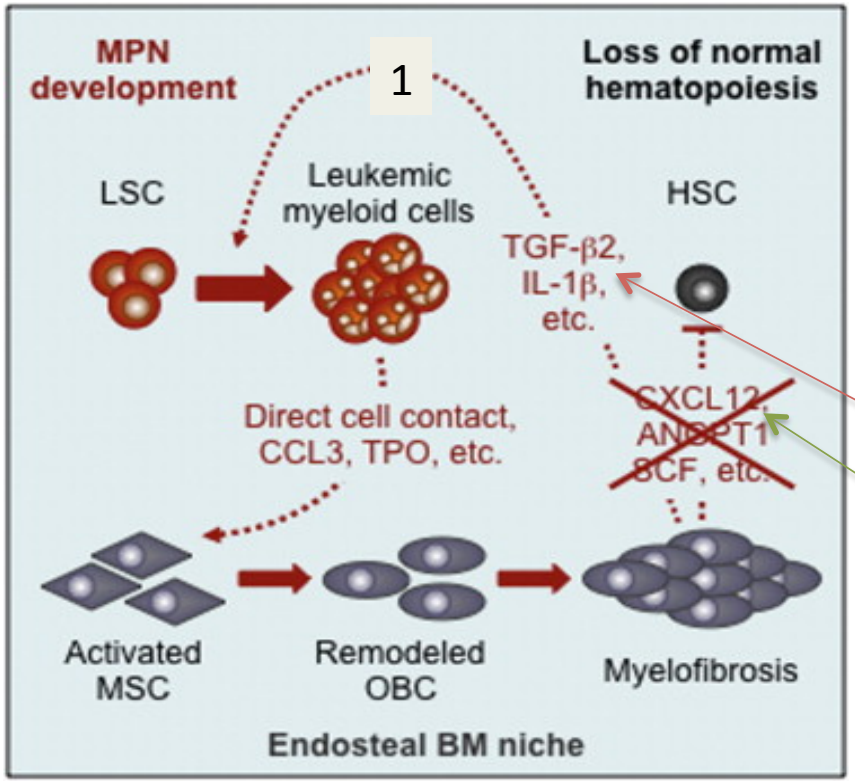


Co-culture involving neoplastic cells and mouse (pre-)osteoblasts has been suggested as a suitable model.
e.g. Schepers K et al, Cell Stem Cell. 2013;13(3):285f

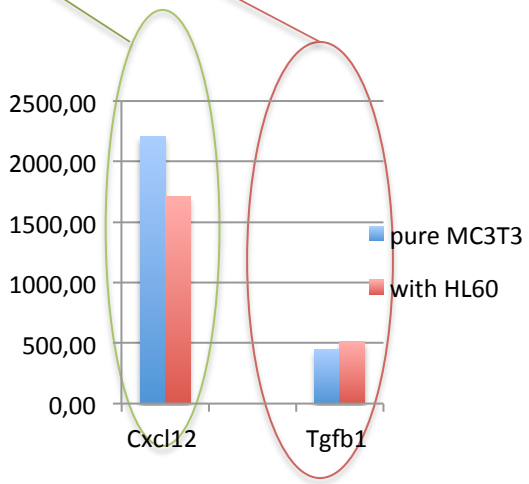
*Wang CQ, Jacob B, Nah GS, Osato M. (2010) Blood Cells Mol Dis;44(4):275ff.
Lymperi S, Ferraro F, Scadden DT. (2010) Ann N Y Acad Sci;1192(1):12ff.

Model system:
The effect of a myeloproliferative neoplasia (MPN) on osteoblasts can be simulated in a co-culture model with mouse - stroma cells and human leukemia cells:

Source: interactive-biology.com



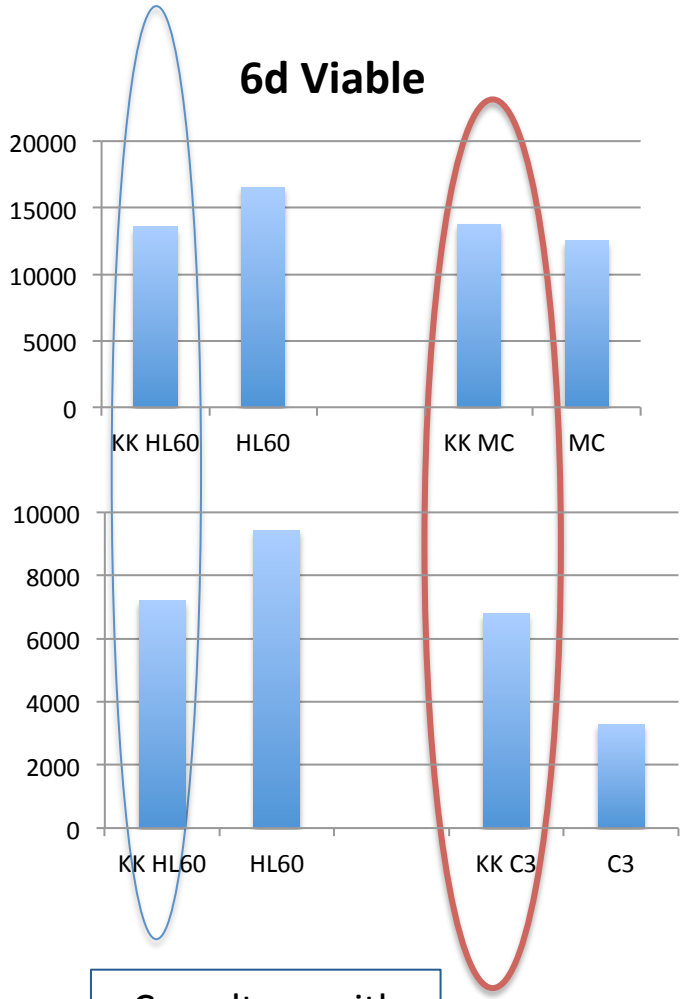
In (pre)malignant diseases osteoblast cells cannot communicate with HCSs via CXCL12 (=SDF-1)
Instead of this:
1. Cytokines are released, which stimulate the differentiation of LSC (leukemia-stemcells) to myeloic leukemia cells &
2. Transform activated mesenchymal stemcells (MSC) into remodeled osteoblasts.
3. **This trends were confirmed in our study of co-culture from HL-60 cells with MC3T3-E1 cells**.



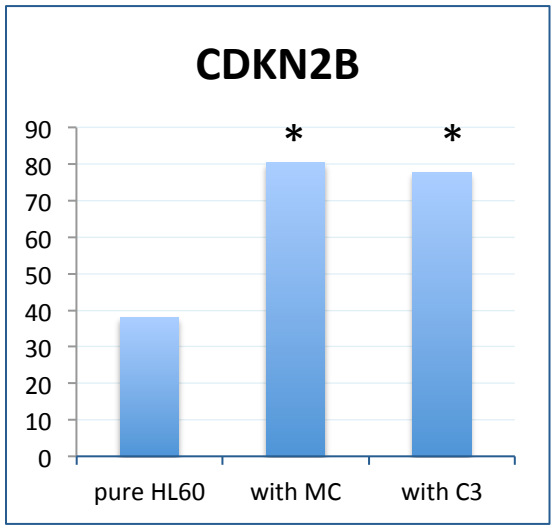
TPO = thrombopoietin (gene name THPO)
TGF-ß = transforming growth factor beta, TGFB

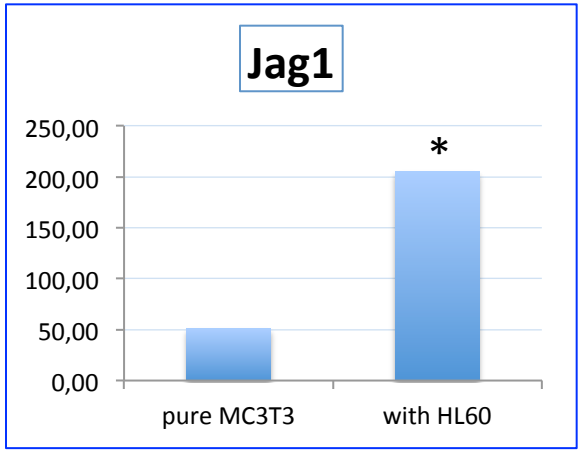
Source: **Schepers K et al, Cell Stem Cell. 2013;13(3):285ff**

MC3T3-E1 cells attenuate proliferation and cells promote quiescence in HL-60 leukemia cells

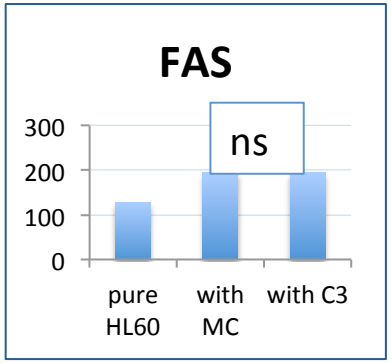


Co-culture with osteoblasts lowers proliferation of HL-60





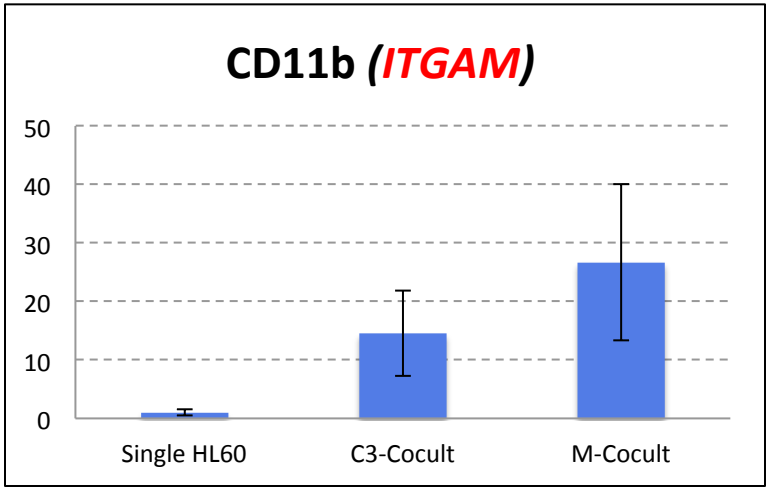
Attenuated proliferation of HL-60 cells coincides with stimulation of osteoblasts the cell cycle inhibitor CDKN2B in HL60 as well as the quiescence inducer Jagged1 (Jag1) in MC3T3-E1, whereas downregulation of pro-apoptotic FAS was not significant in HL-60



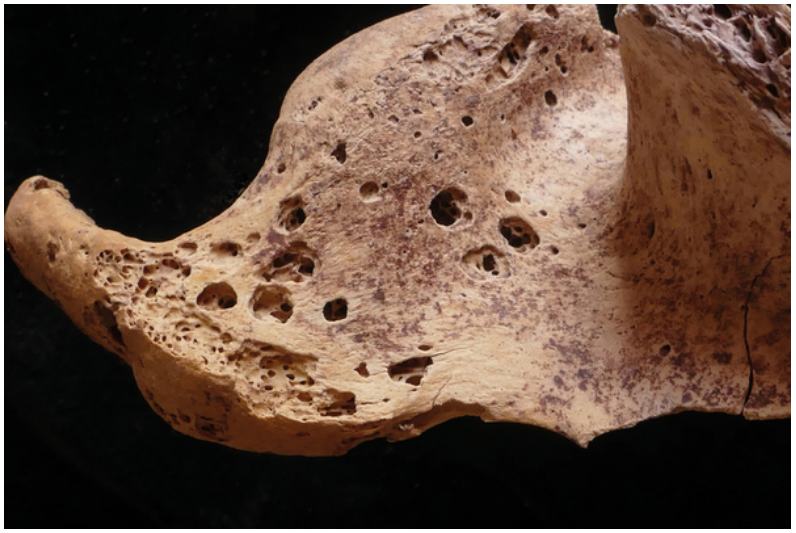
HL-60 cells stimulate proliferation of (pre)osteoblasts (MC3T3-E1 or C3H10T1/2)

Do (mouse) osteoblasts promote differentiation in leukemic cells?

- Data from RT-Q PCR after 6 days co-culture indicated an upregulation of the monocyte-macrophage marker CD11b which is also known as
- **integrin alpha M**
- (data from QPCR were confirmed by gene-chip) in HL-60



- But how does such an integration affect bone ???



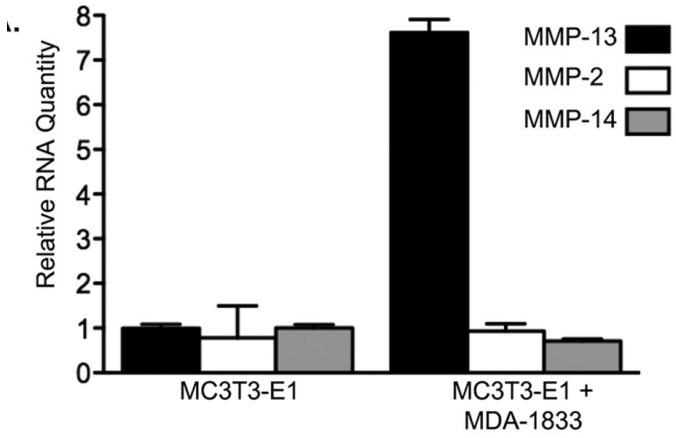
From Egyptian necropolis Qubbet el-Hawa near Aswan (Nature 527, S102–S103; 2015)

- **The deleterious effect of breast cancer on bone was already described in ancient Egypt (2500 BC)**

- **But do leukemia cells also disturb bone cells?**

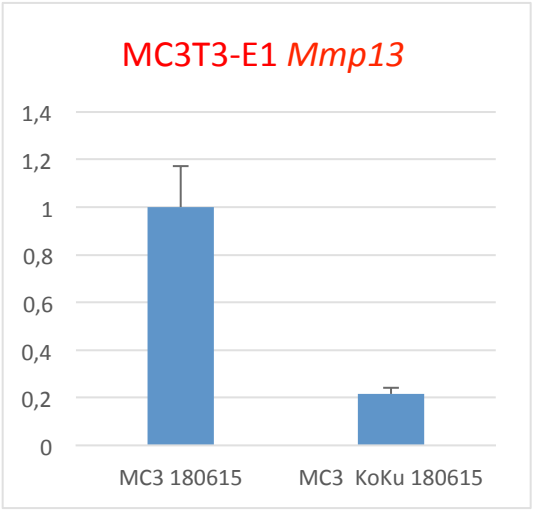
Solid tumor cells (MDA breast cancer) disturb the integrity of MC3T3-E1 osteoblasts.

but HL-60 reduce matrix metalloproteinase (MMP) expression in MC3T3-E1 osteoblasts.



MDA data from Charlotte Morrison et al. J. Biol. Chem. 2011;286:34271-34285

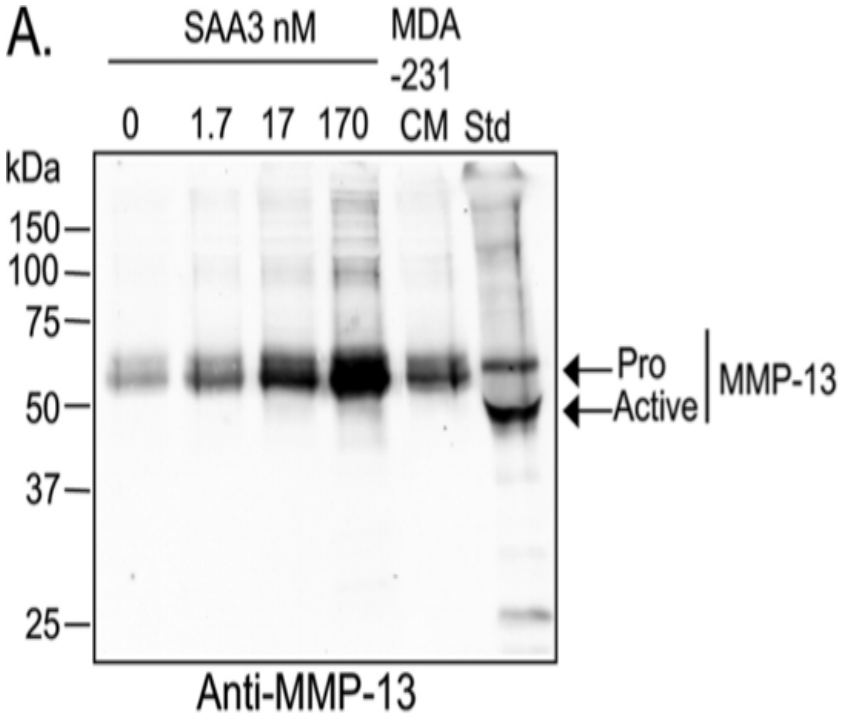
Own data: Downregulation of *Mmp13*, *Mmp2* and *Mmp14* in coculture with HL60



Source: interactive-biology.com

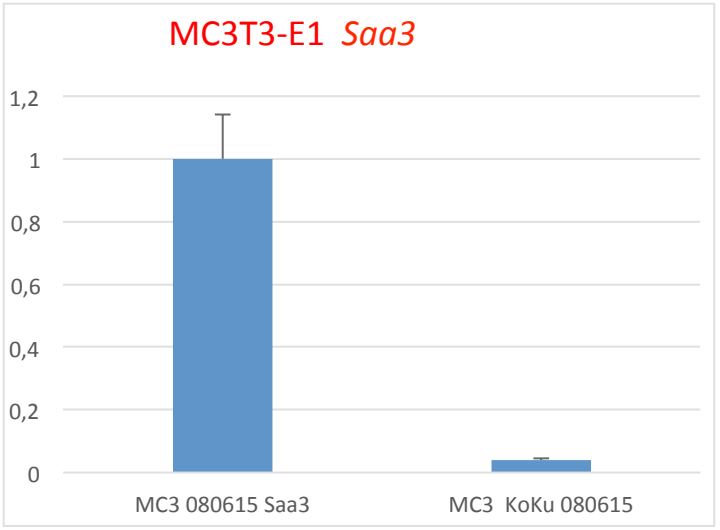
>>>Different mechanisms in breast cancer (MDA) and leukemia cells<<<

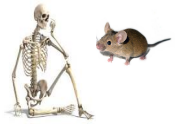
Induction of MMP-13 expression by Pro-inflammatory genes such as SAA3 in MC3T3-E1 cells and validation of SAA3 as a novel MMP-13 substrate.



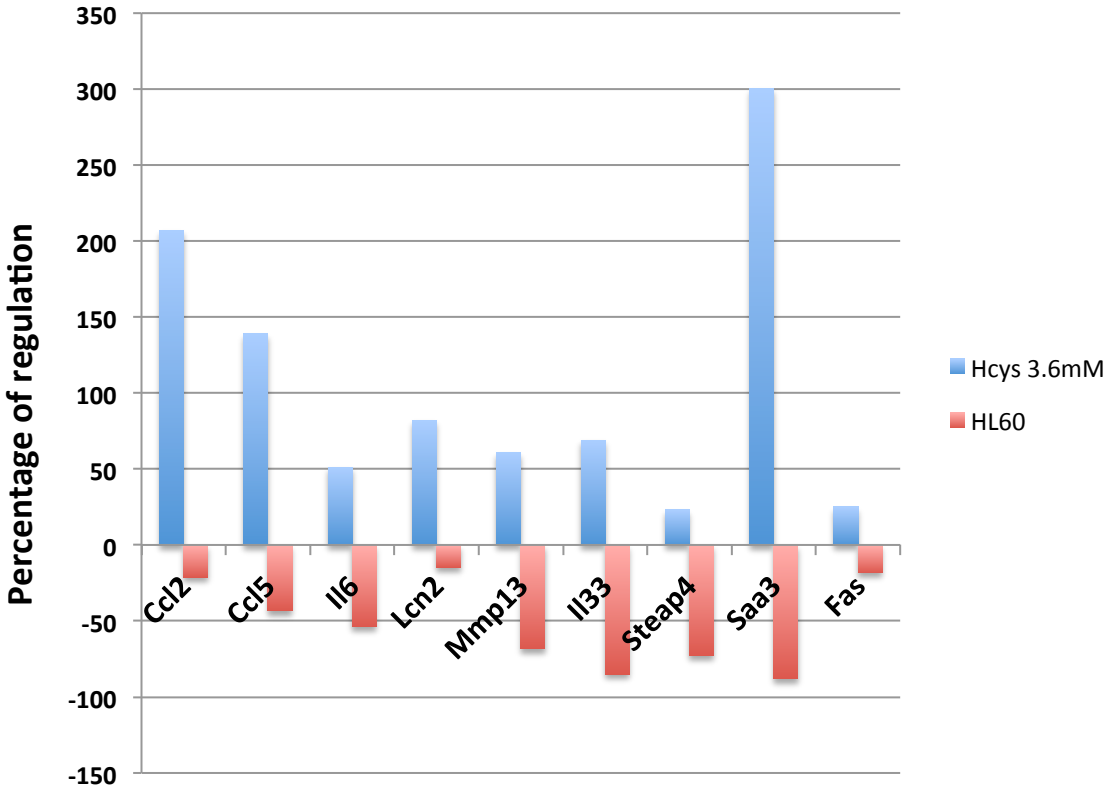
Charlotte Morrison et al. J. Biol. Chem. 2011;286:34271-34285

By contrast, HL60 induces a significant downregulation of *Saa3* in MC3T3-E1 cells:





Pro-inflammatory genes involved in chronic degenerative diseases which are treated by homocysteine (blue)*
in MC3T3-E1 cells are downregulated upon co-culture with HL-60 cells (red bars)



Ccl2 /Ccl5 = chemokine (C-C motif) ligand 2 or 5

Il6 / Il33=Interleukin 6 or 33

Lcn2= lipocalin 2

Mmp13 = matrix metallopeptidase 13

Steap4 = belongs to the STEAP (six transmembrane epithelial antigen of prostate) family

Saa3 = serum amyloid A 3

Fas = Fas cell surface death receptor (belongs to TNF receptor superfamily)

*Data from Thaler et al, The FASEB Journal Vol. 27, 446ff. 2013

This would mean:

- The deleterious effect of breast cancer cells results from a pro-inflammatory phenotype in osteoblasts, which is comparable to the action of homocysteine but
- **HL-60 leukemia cells do the opposite**!

- At least some types of leukemia cells protect the bone cells and undergo quiescence.

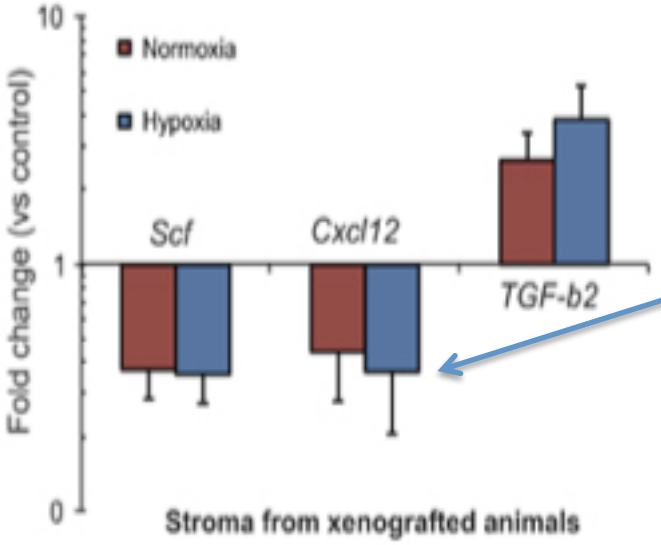
- **Could it be, that human leukemia cells communicate with mouse osteoblasts via microRNAs as packaged in exosomes and vice versa?**

Possible explanation for communication between human leukemia cells and murine osteoblasts:

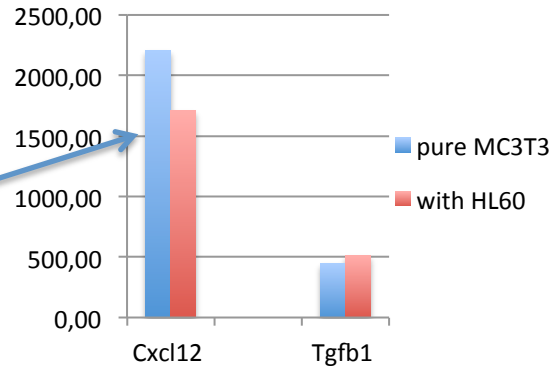
- ***Coordinate regulation of residual bone marrow function***
- ***by paracrine trafficking of AML exosomes***
- *J Huan et al.*
- Leukemia. 2015 Jun 25

AML cells increase exosome production attenuating stromal cell expression of HSC maintenance factors:
...data from human leukemia-exosome xenografts in mice

J Huan et al.
Leukemia. 2015



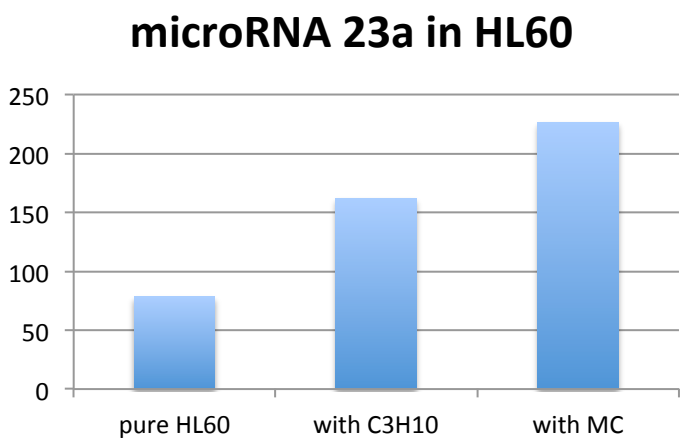
Downregulation of *Cxcl12* in coculture with HL-60 confirms our data

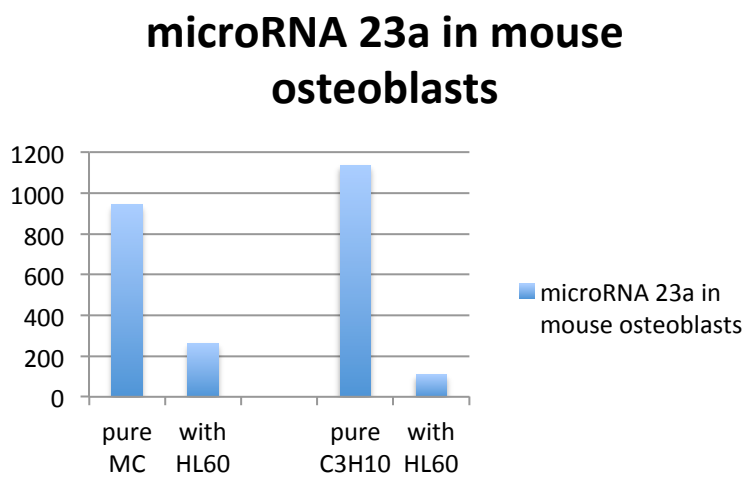


MicroRNA-23a enhances migration and invasion through PTEN in osteosarcoma. (Tian K et al, Cancer Gene Ther. 2015)

- **Upregulation in HL60**

Downregulation in osteoblasts





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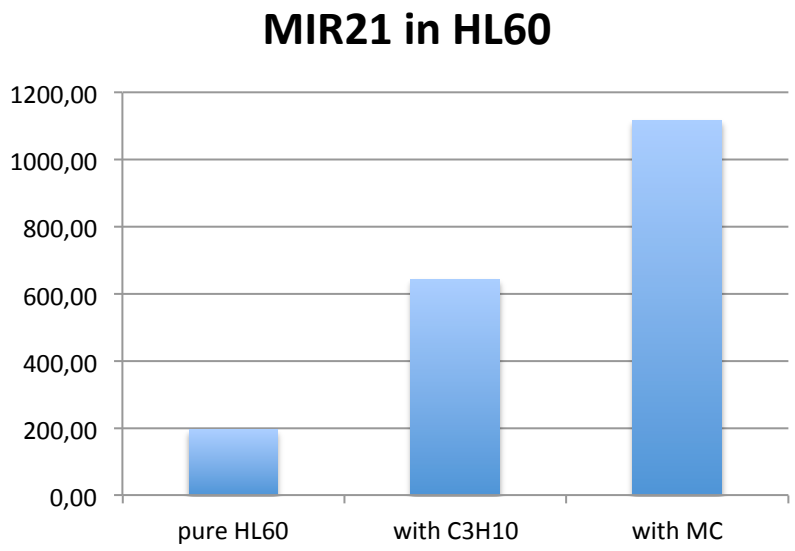
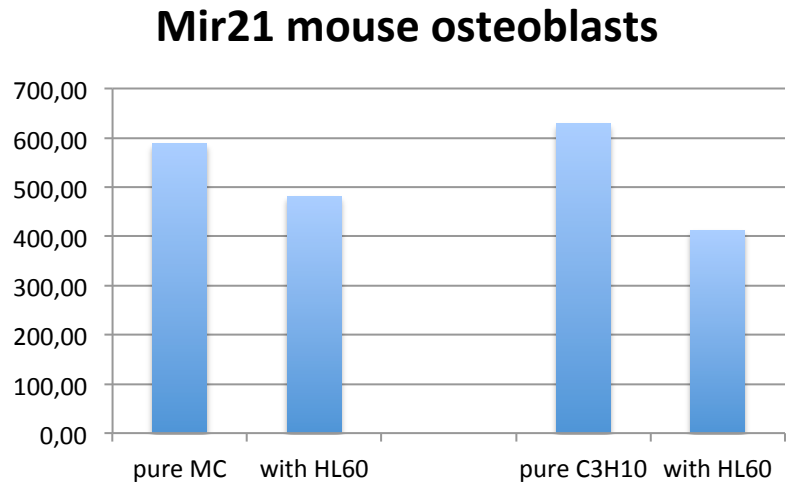
MicroRNA 21 promotes leukemogenesis through increasing the leukemic stem/progenitor cell population
(Pan Y et al, Curr Pharm Des. 20: 5260ff; 2014)





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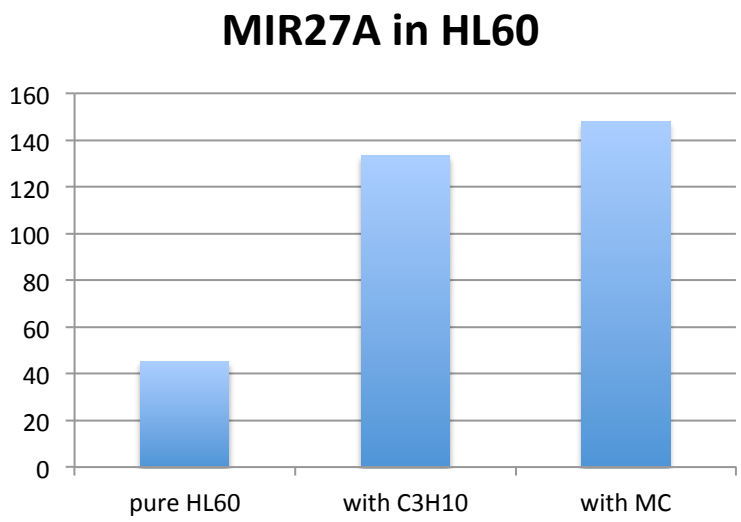
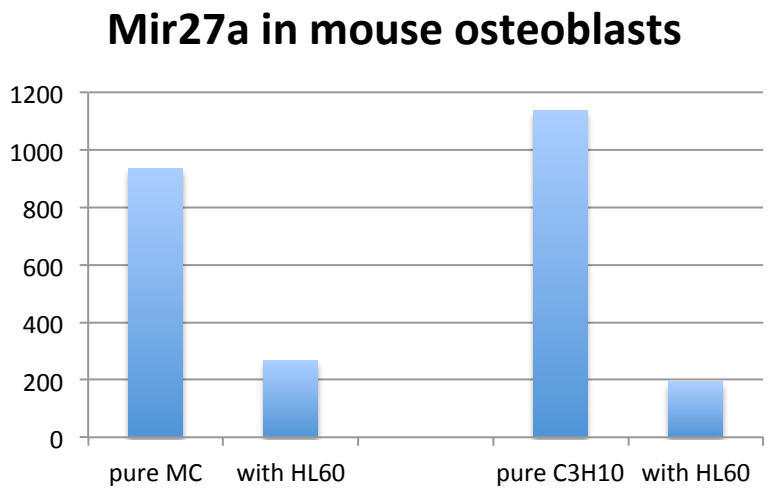
MicroRNA 27a is known for its role as a **tumor suppressor in acute leukemia (**Scheibner KA et al , PLoS One.7:e50895; 2012).
Furthermore, **MIR27 also promotes osteoblast differentiation** (Wang T and Xu Z, Biochem Biophys Res Commun. 2010 Nov 12;402:186ff; 2010).

A significant downregulation of Mir27a in mouse osteoblasts could indicate de-differentiation in co-cultured osteoblasts...





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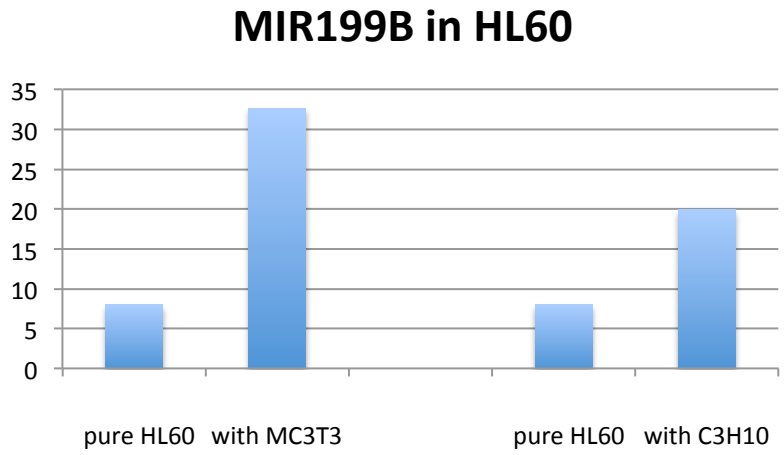
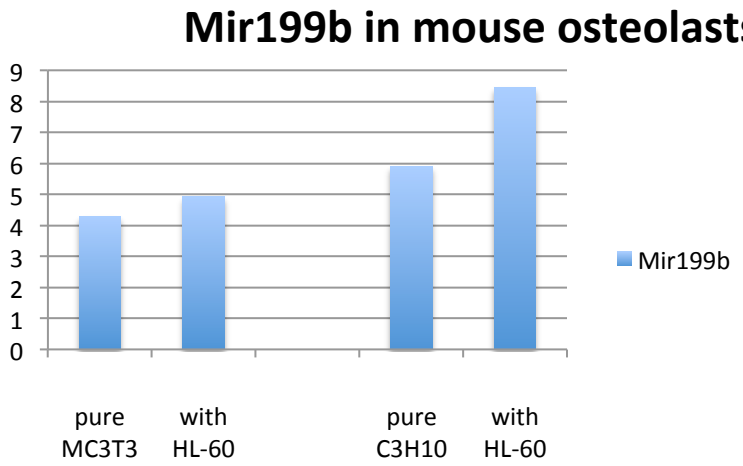
- **MicroRNA 199b** is known for its role as a **tumor suppressor in acute leukemia (**Favreau et al. Exp Hematol Oncol (2016) 5:4).
- However, **MicroRNA 199b also promotes invasion and migration abilities in osteosarcoma** (Zeng H et al, Path Oncol Research, Epub ahead of print] .





Conclusion

- Osteoblasts may attenuate proliferation and induce quiescence of leukemic cells, but
- Leukemia cells support the osteoblastic niche by promoting the expression of anti-inflammatory genes.
- The communication between leukemic cells and appears to be mediated by respective microRNAs.

Thank you ...

Donation of the Strasser Family

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