Analysis of drug resistance in multiple myeloma and the interaction with mesenchymal stem cells by scRNA-seq

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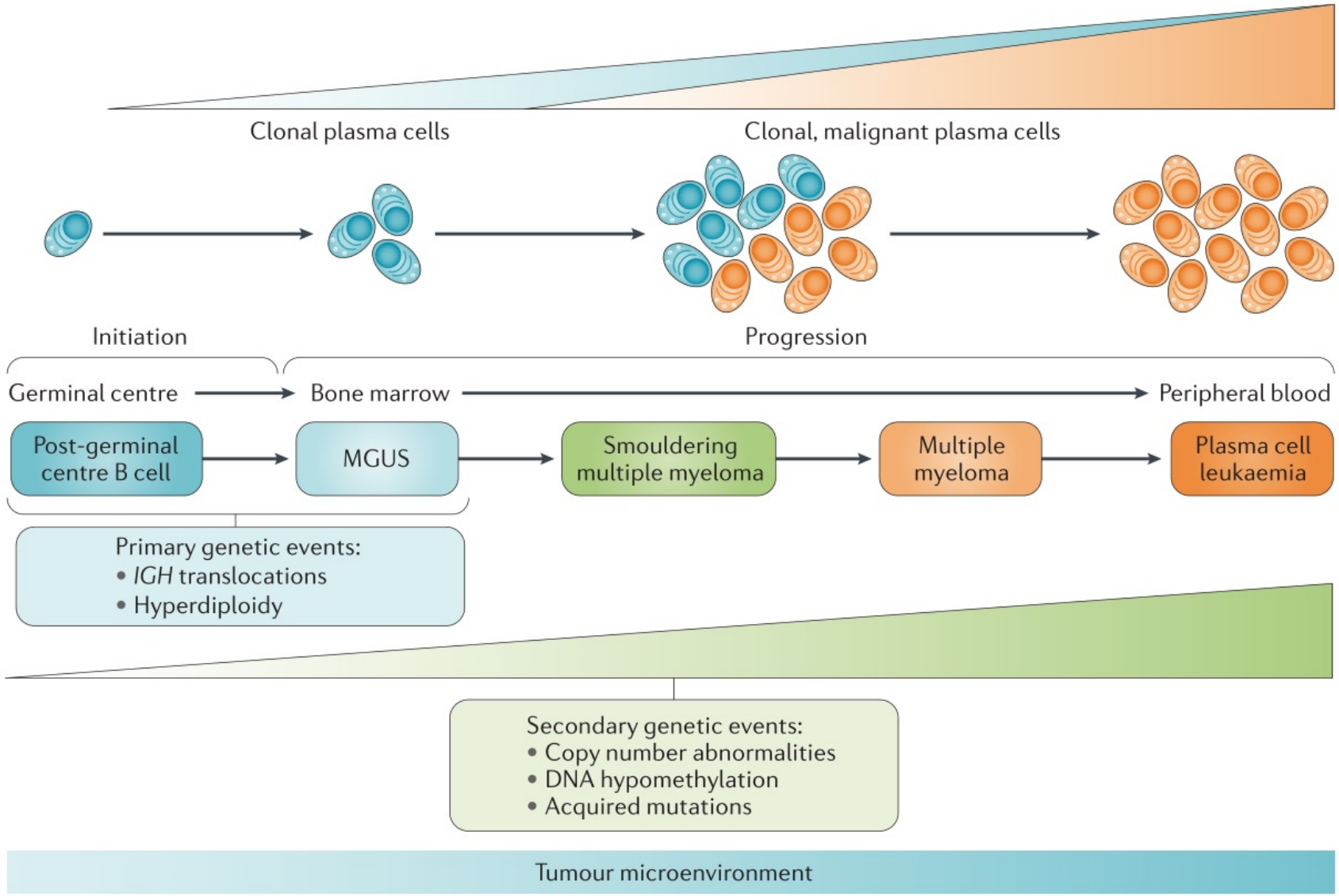
November 11 (CST), 2020

Multiple myeloma (MM) is a cancer of plasma cells



Shaji K. Kumar et al., Nature Reviews Disease Primers (2017)

Symptoms of multiple myeloma

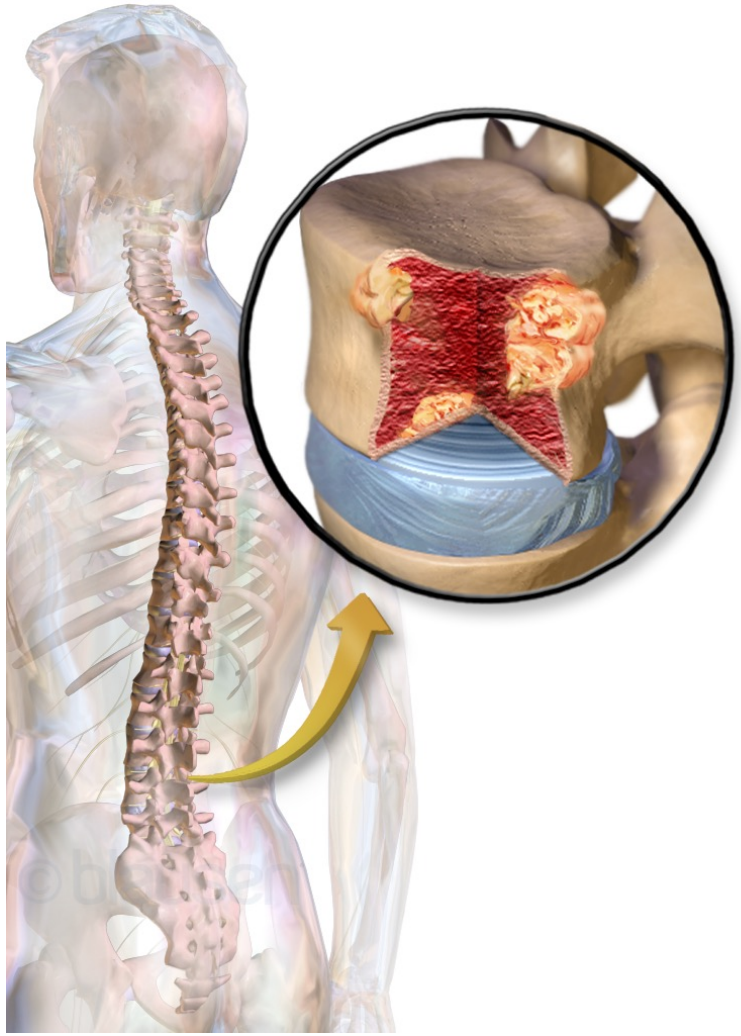
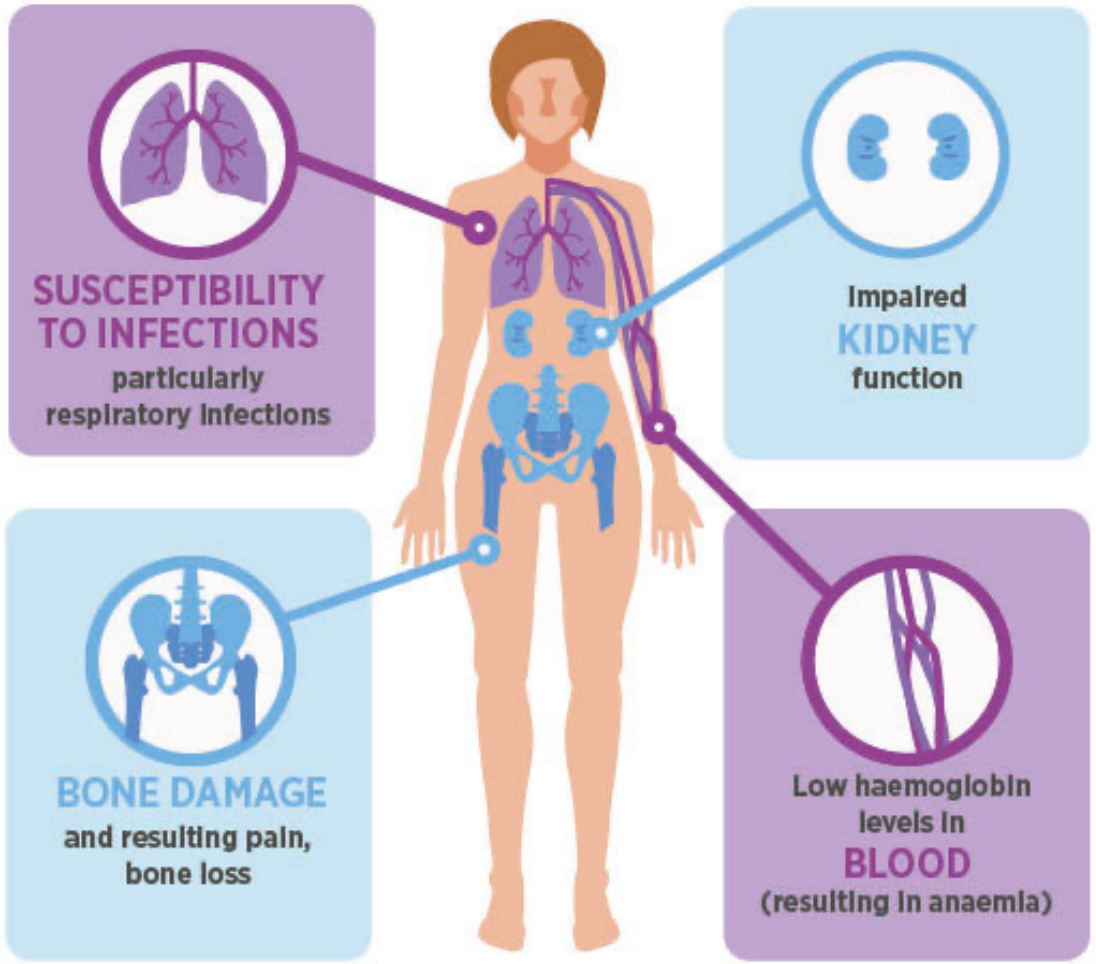


Illustration showing the most common site of bone lesions in vertebrae



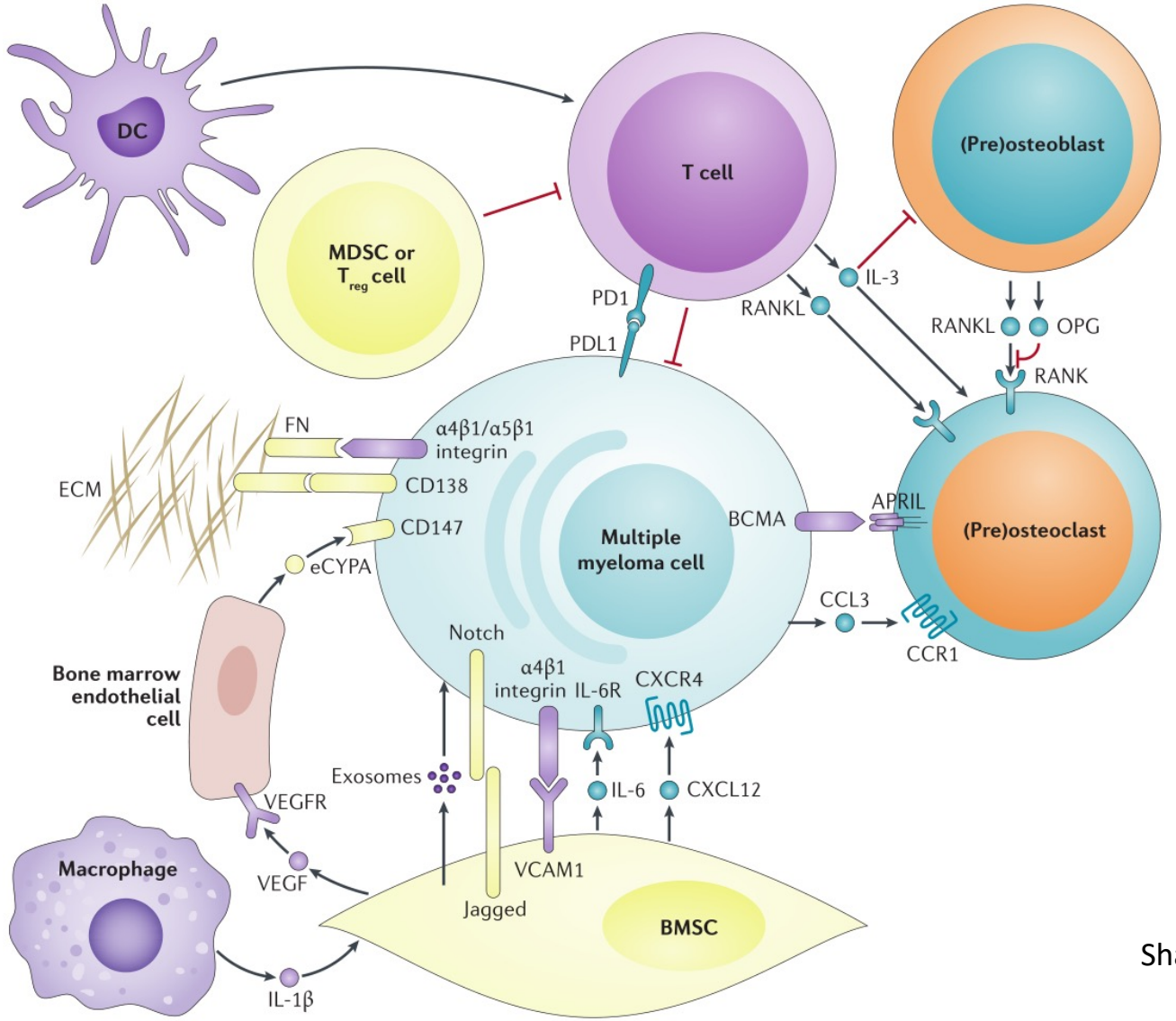
https://en.wikipedia.org/wiki/Multiple_myeloma
https://minajamyelooma.fi/en/multiple-myeloma/symptoms/

Mechanisms of drugs resistance in multiple myeloma

Agents	Mechanism of action	Mechanisms of resistance
Proteasome inhibitors (**bortezomib**, carfilzomib and ixazomib)	Inhibition of activity of the 26S proteasome; Inhibition of NF-κB activity; induction of apoptosis by activation caspase-8 and caspase-9; downregulates the expression of adhesion molecules on PCM cells	Upregulation of the proteasomal system; point mutations of the PSMB5 gene and overexpression of the proteasome β5 subunit; increased expression of the MARCKS protein
Corticosteroids (prednisone, dexamethasone methylprednisolone)	Induction of apoptosis of PCM cells; reduction in mitochondrial transmembrane potential	Functional defect of the glucocorticoid receptor; overexpression of the oncogenes FGFR3 and MYC
Chemotherapeutc agents (alkylating drugs – melphalan, cyclophosphamide), anthracyclines (doxorubicines)	DNA damage; immunostimulatory activity by inhibiting interleukin-6	Up-regulation of P-gp; increased ABCG2 expression; RECQ1 over-expression; overexpression of Bcl-xL
Immunomodulatory drugs (thalidomide, lenalidomide, pomalidomide)	Targeting PCM cells in the BM microenvironment; triggering caspase-8-mediated apoptosis	Downregulation of CRBN expression; deregulation of IRF4 expression
Monoclonal antibodies, (daratumumab, elotuzumab)	Antibody-dependent cellular cytotoxicity, macrophage-mediated phagocytosis	Downregulation of CD38 expression; upregulation of CD55 and CD59 on the PCM cells

Pawel Robak et al., Cancer Treatment Reviews (2018)

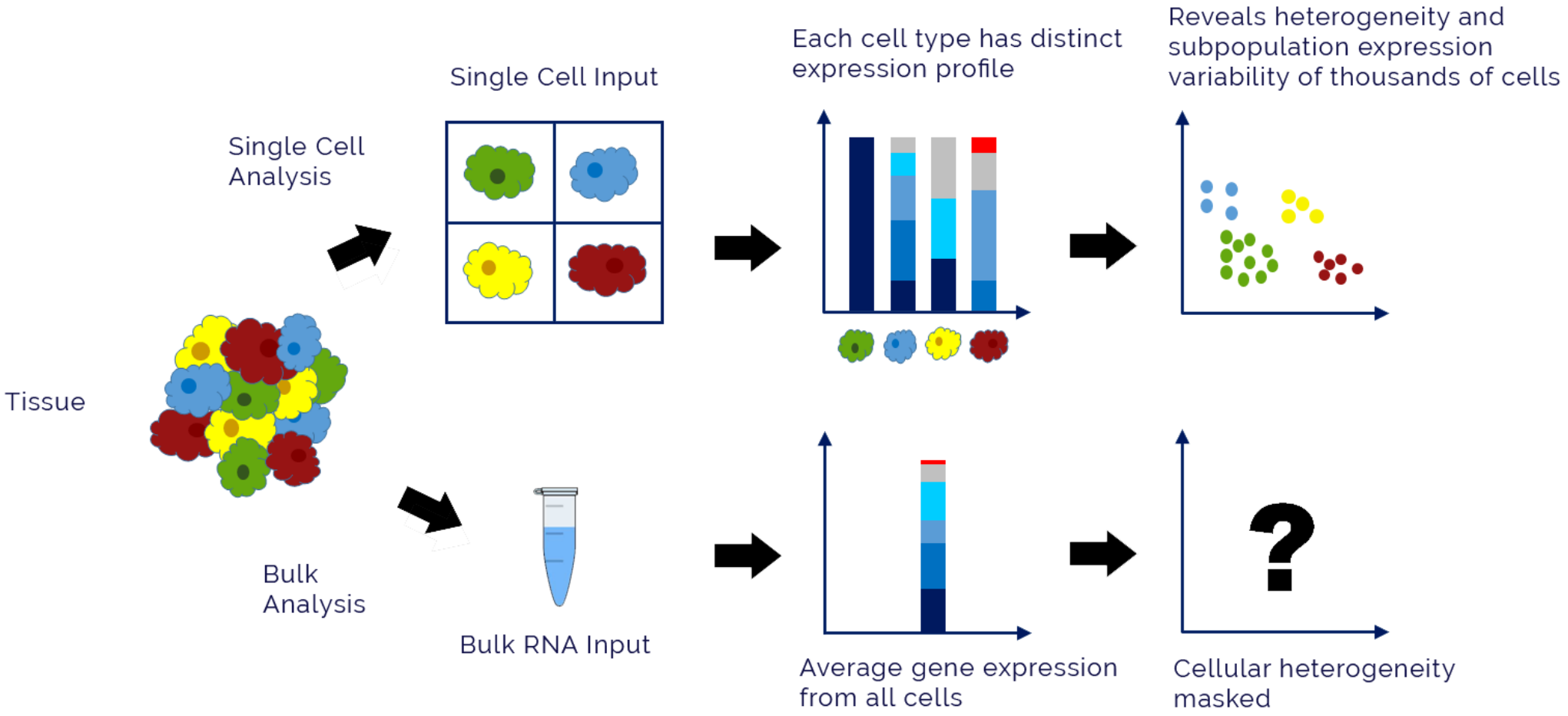
Interactions between MM and bone marrow microenvironment



1. **Effects on MSC differentiation program?**
2. **Effects on MM proliferation, migration and drug resistance?**

Shaji K. Kumar et al., Nature Reviews Disease Primers (2017)
Song Xu et al., Leukemia (2018)

Single-cell RNA-seq can reveal hererogeneity

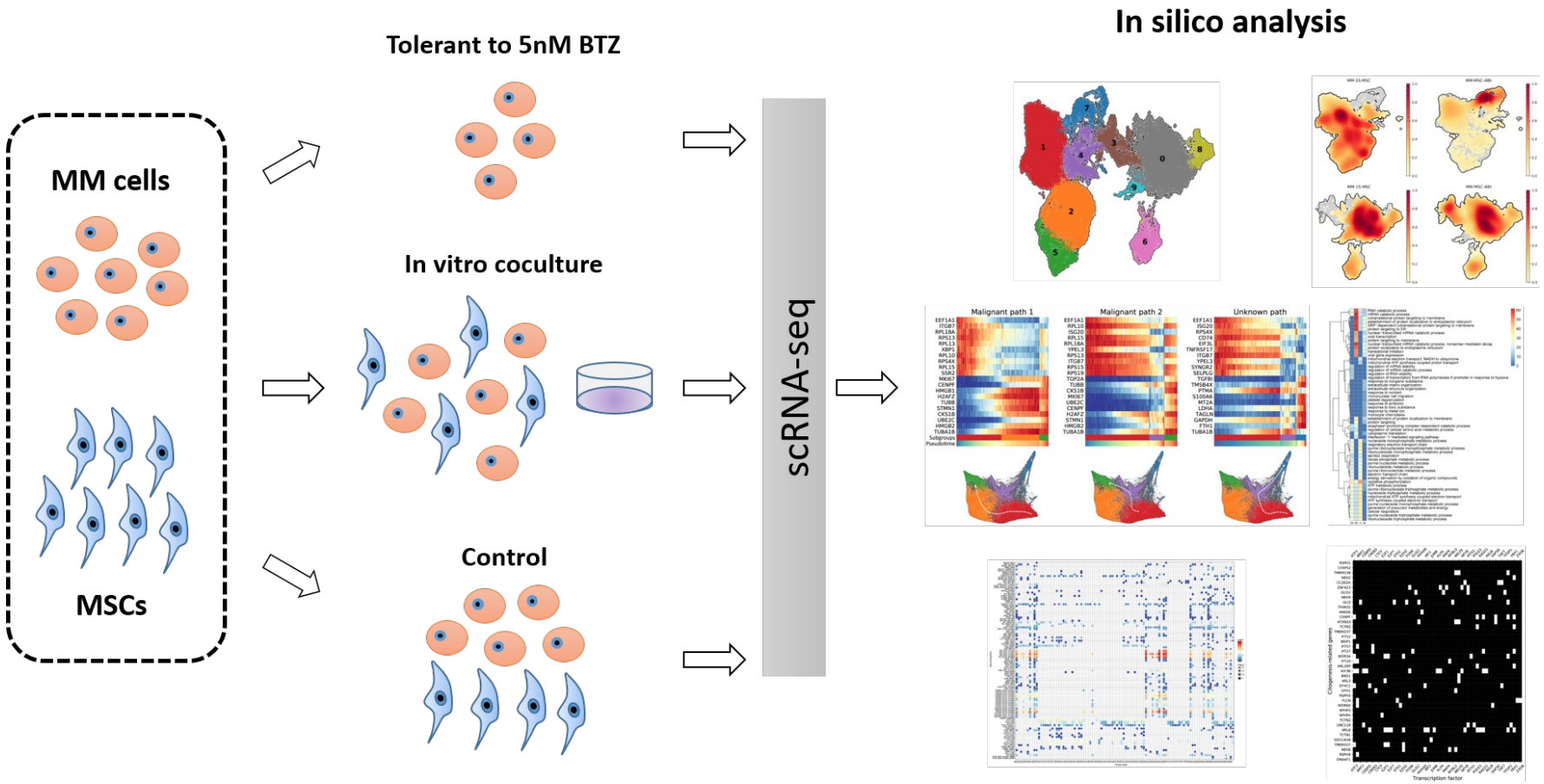


MM heterogeneity and MSC multiple lineage potentials

→ single-cell profiling

https://www.lcsciences.com/discovery/applications/transcriptomics/single-cell-rna-seq-sequencing-service/

Graphical abstract



Collaborator: Prof. Zhiqiang Liu lab,
Tianjin Medical University, Tianjin, China

Transcriptome quantification and quality control pipeline

Raw data

CellRanger count

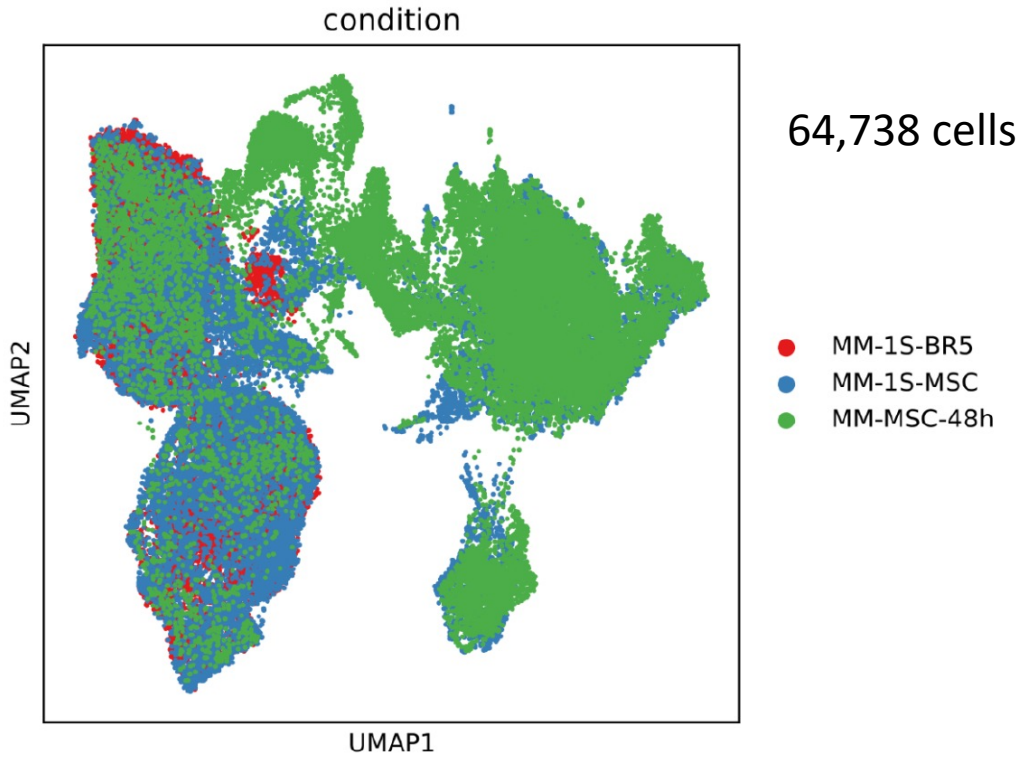
Feature-barcode matrices

CellRanger aggr

Aggregated feature-barcode matrix (69714 barcodes x 33538 features)

Cell QC
Gene QC

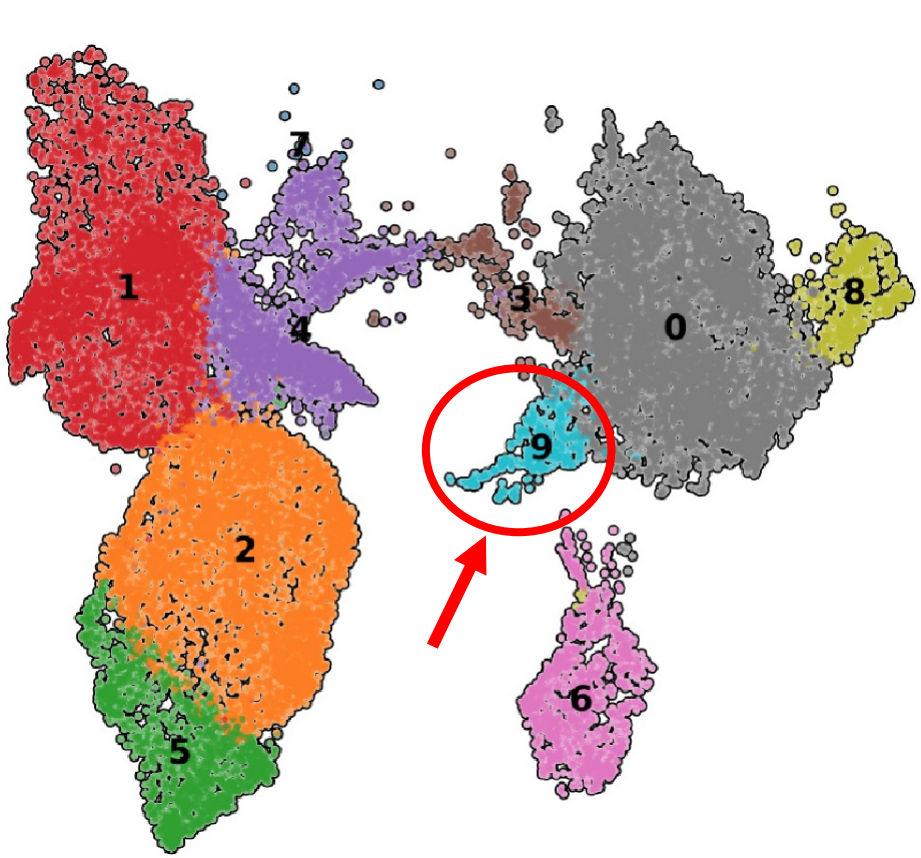
Cleaned feature-barcode matrix (64738 x 17884)



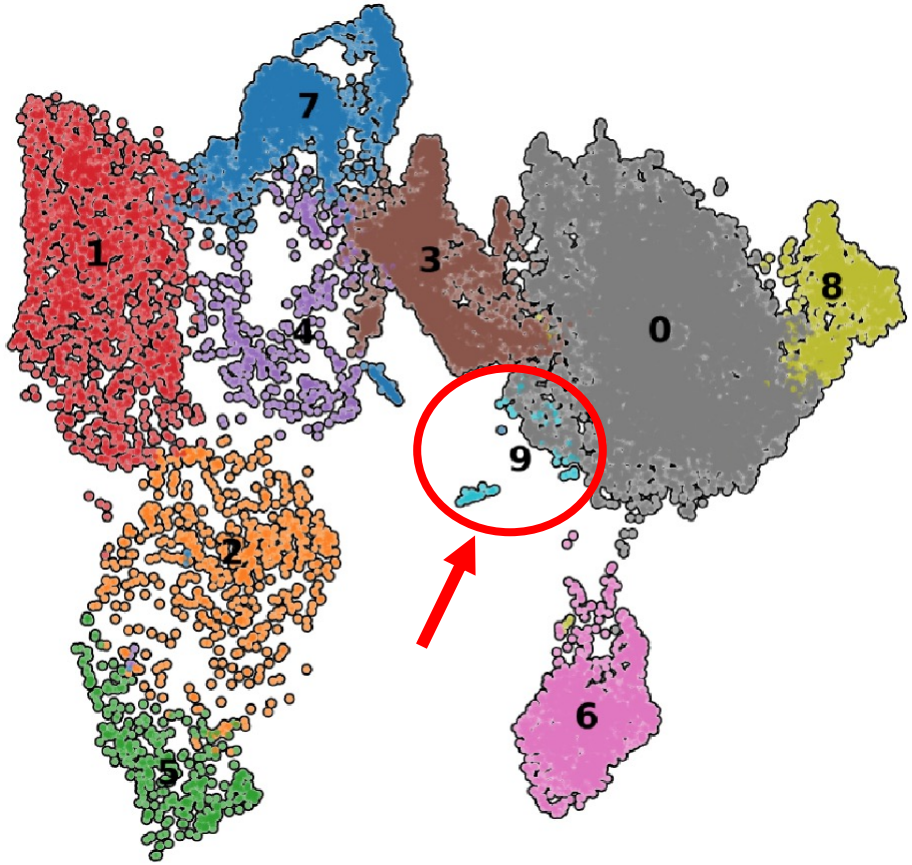
64,738 cells

Co-culture effects on MSC differentiation program?

Subgroup 9 shrinks during MM-MSC interaction



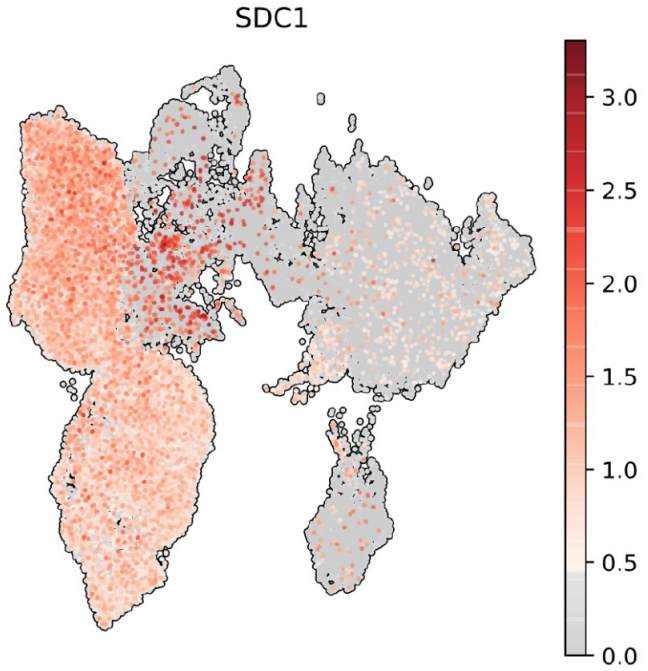
Control



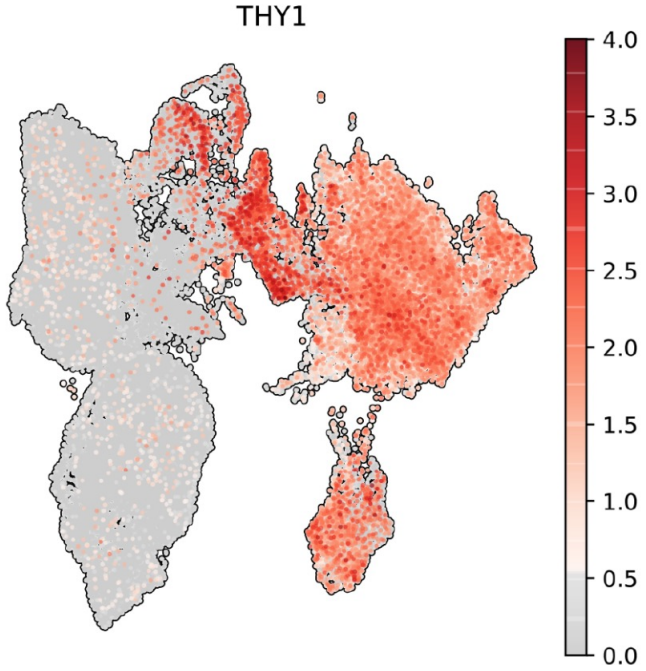
Co-culture

Subgroup 9 represents osteogenic lineage MSC

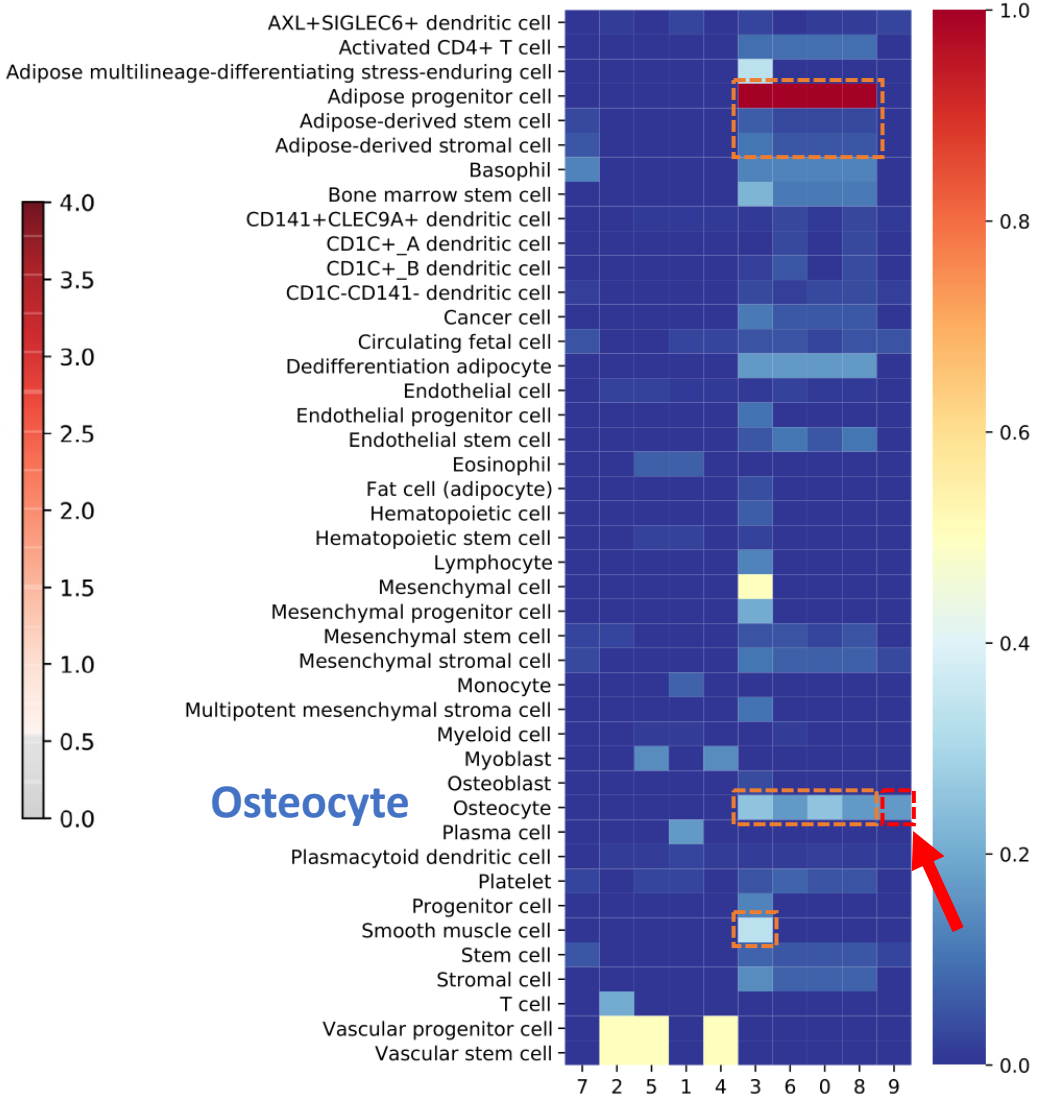
MM marker



MSC marker



Overlap score (normalized by num. of ref. marker)

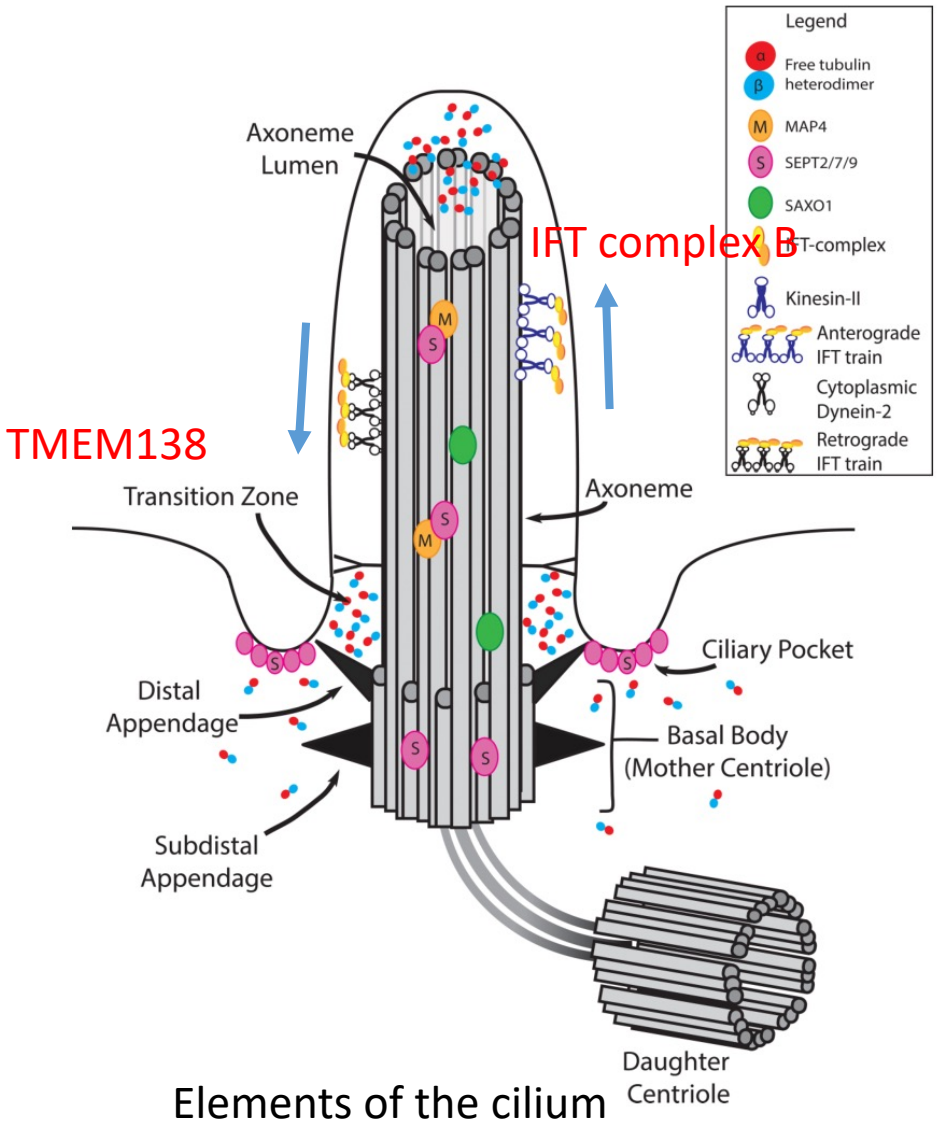


Reduction of subgroup 9 is consistent with bone lesion symptom in MM

MM-mediated inhibition of MSC ciliogenesis causes MSC osteoblast differentiation repression

- Our collaborator's previous experiments show that MM cells can inhibit MSC differentiation to osteoblast by repressing MSC ciliogenesis.
- The mechanisms that primary cilium regulates osteoblast differentiation is known (by receiving extracellular fluid signal and Ca^{2+} signal).
- How MM represses MSC ciliogenesis is unclear.

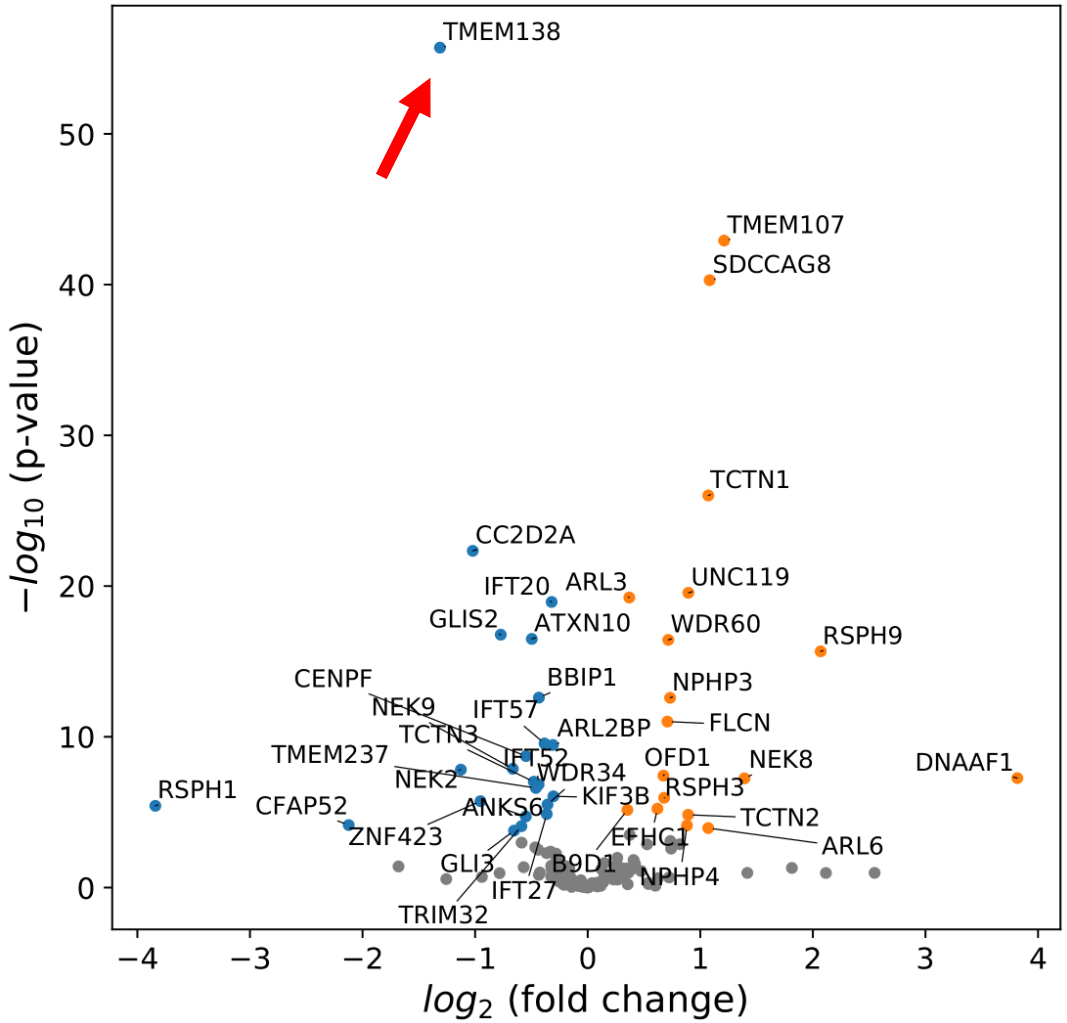
Identification of differentially expressed cilium-associated genes



Elements of the cilium

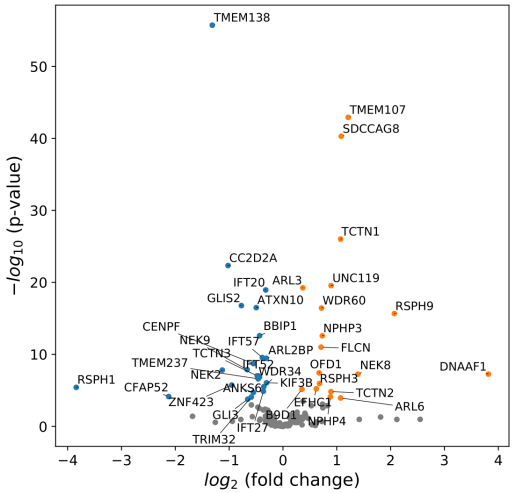
--- Mary Mirvis et al., Biochemical Journal (2018)

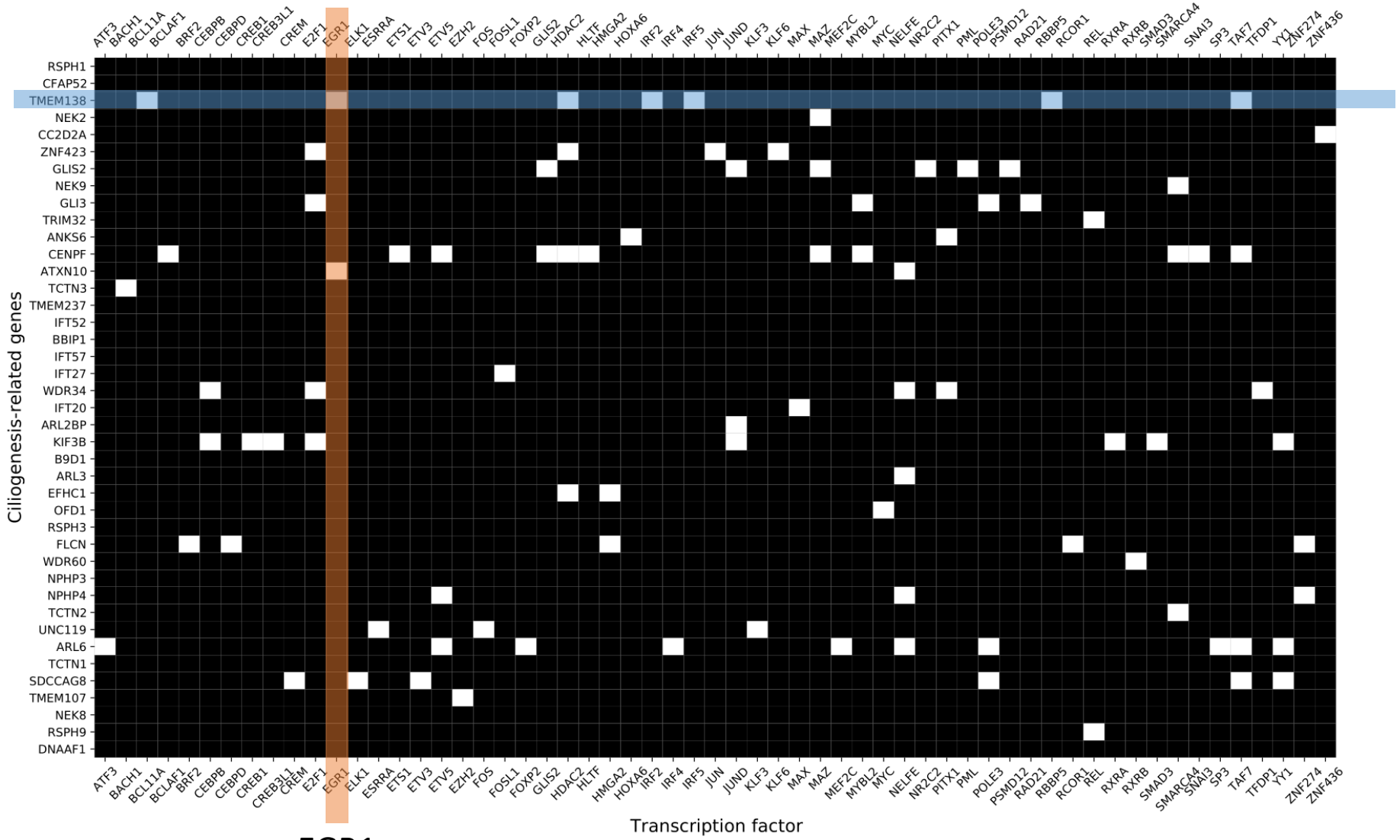
Components of IFT complex B and transition zone are down-regulated significantly



Prediction of transcription factors of ciliogenesis genes

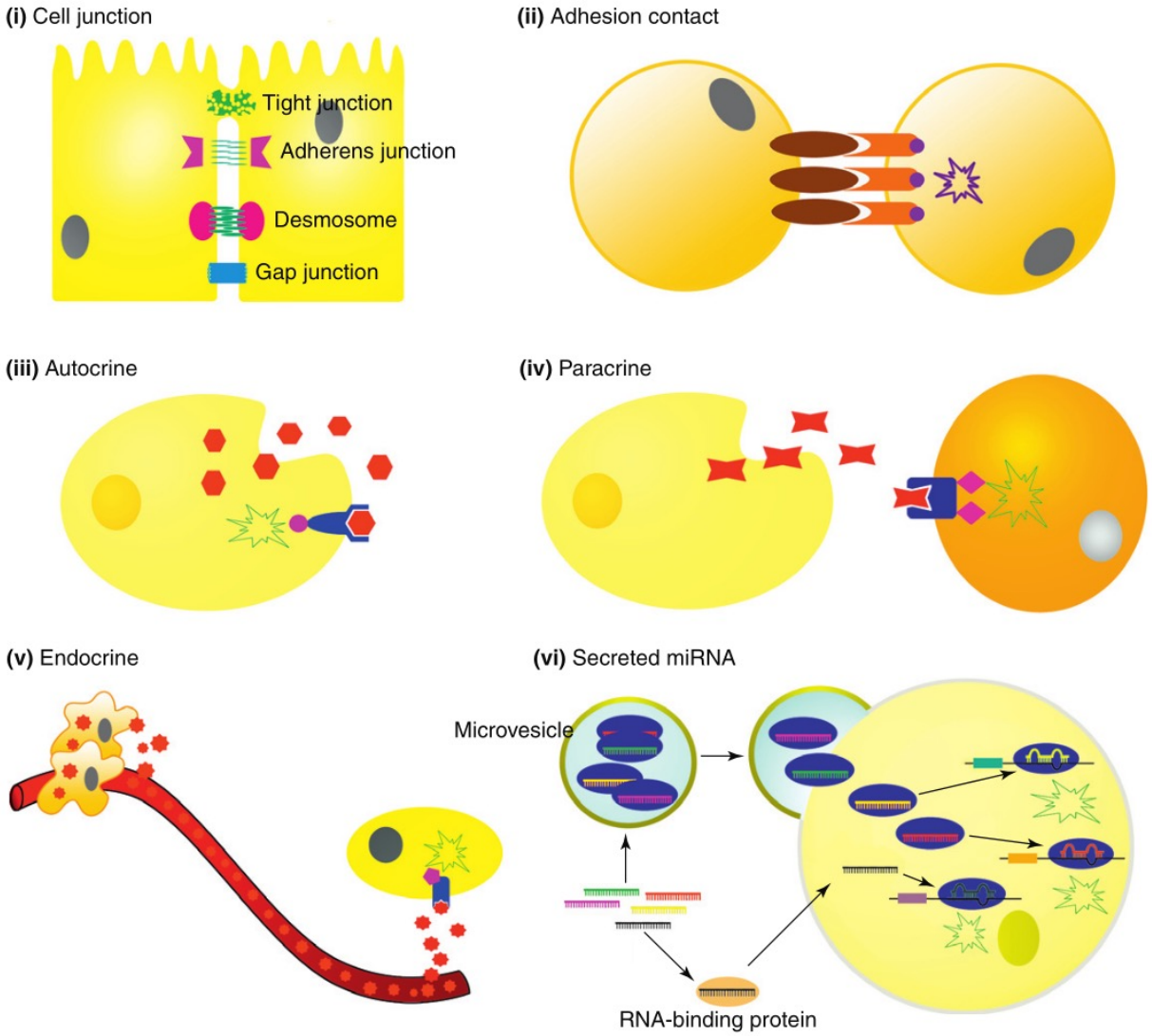
TMEM138





EGR1 ← highly downregulated after co-culture

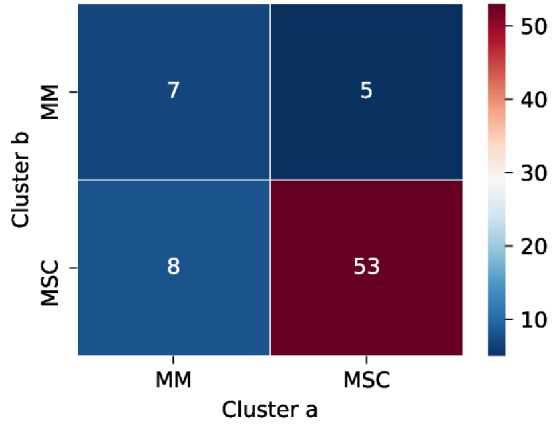
How do MM cells send signals to repress MSC ciliogenesis?

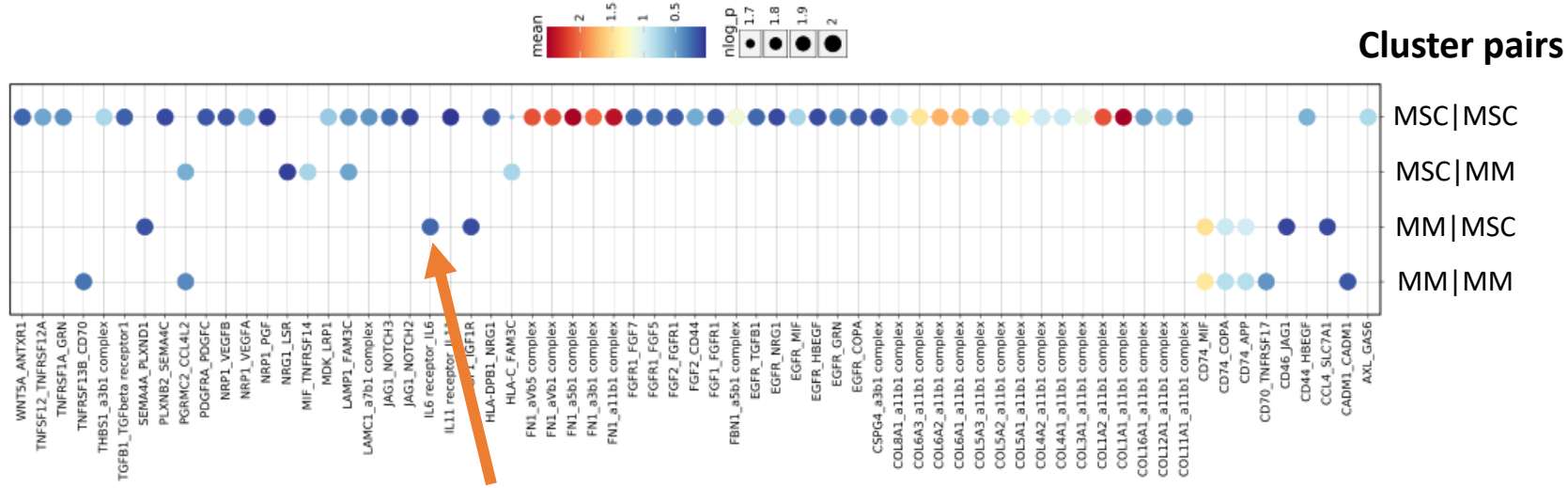


Single-cell transcriptomes contain ligand-receptor (LRP) expression information which can be used to infer specifically MM-to-MSC LRPs.

Xi Chen el al., Trends in Cell Biology (2012)

Identification of intercellular ligand-receptor pairs (LRPs)





IL6 receptor_IL6
MM ← MSC

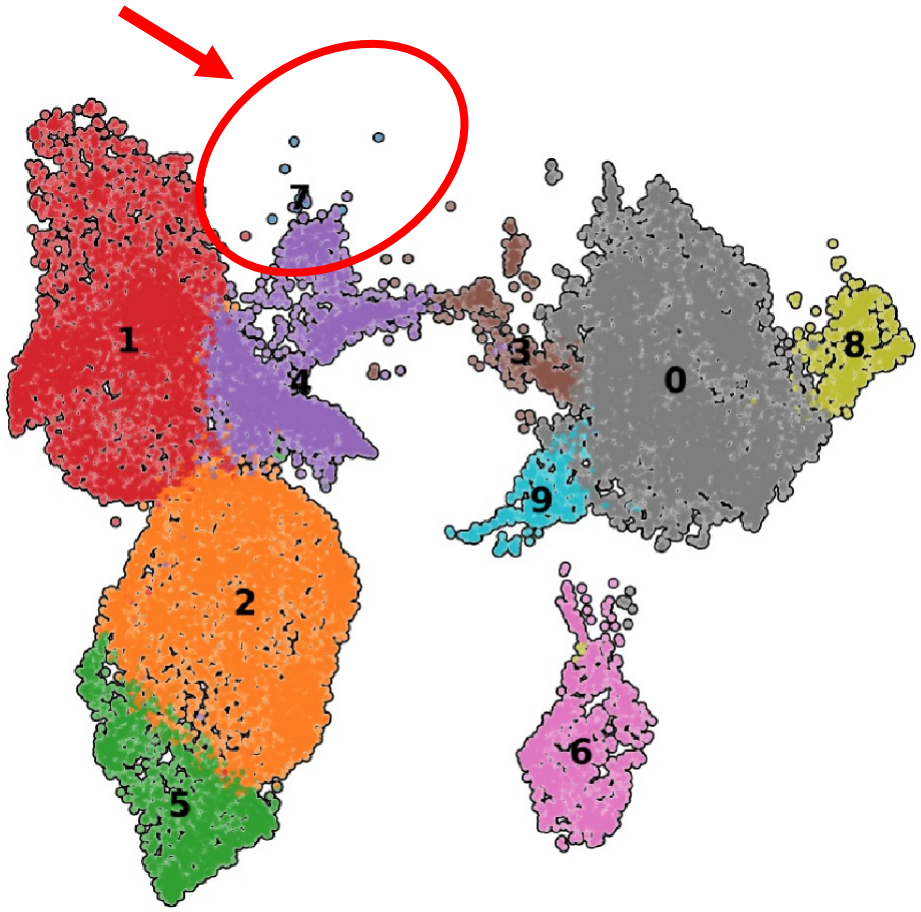
A wellknown LRP promoting MM proliferation validates the LRP prediction to some extent

interacting_pair	secreted	receptor_a	receptor_b	cell_pair
CCL4_SLC7A1	TRUE	FALSE	TRUE	MM|MSC
IGF1_IGF1R	TRUE	FALSE	TRUE	MM|MSC
SEMA4A_PLXND1	FALSE	FALSE	TRUE	MM|MSC
HLA-C_FAM3C	TRUE	TRUE	FALSE	MSC|MM
LAMP1_FAM3C	TRUE	TRUE	FALSE	MSC|MM
PGRMC2_CCL4L2	TRUE	TRUE	FALSE	MSC|MM
NRG1_LSR	TRUE	TRUE	TRUE	MSC|MM

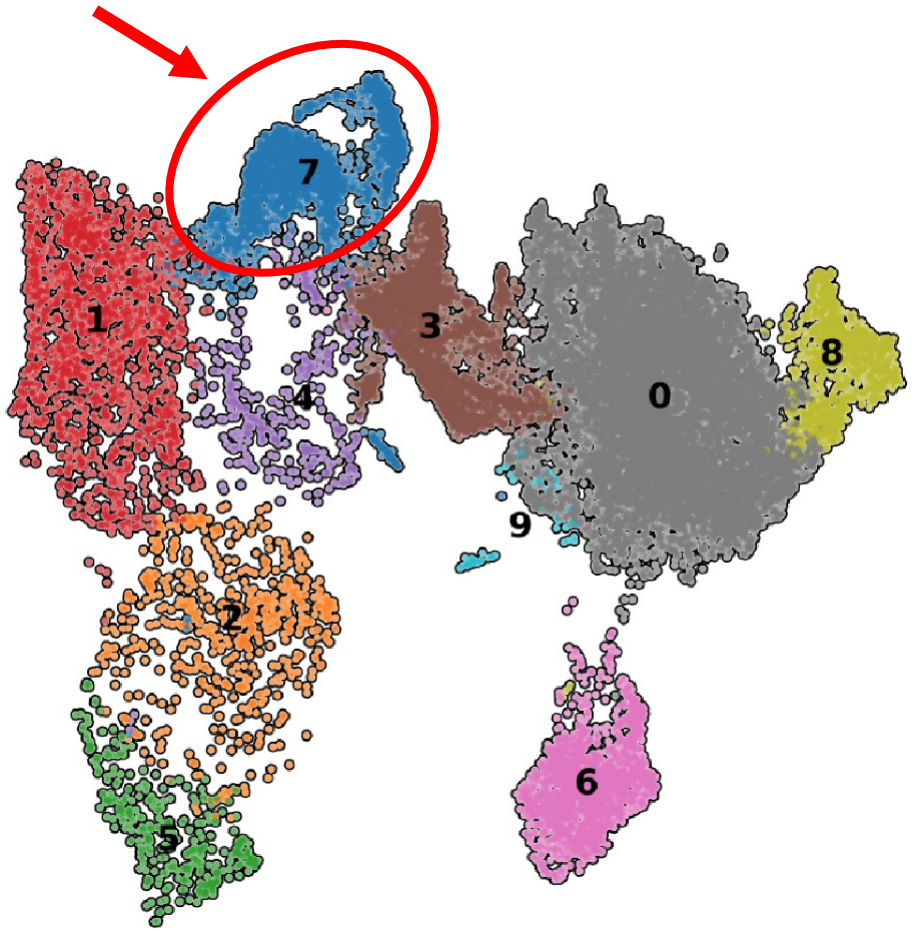
MM → MSC direction LRPs

Co-culture effects on MM proliferation, migration and drug resistance?

Subgroup 7 shows up during MM-MSC interaction



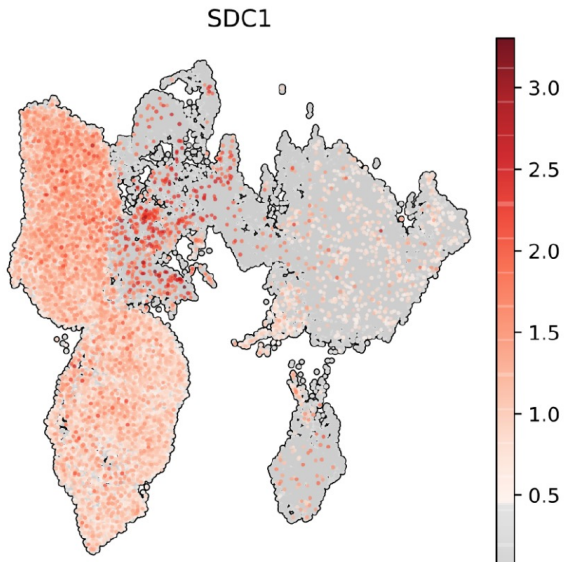
Control



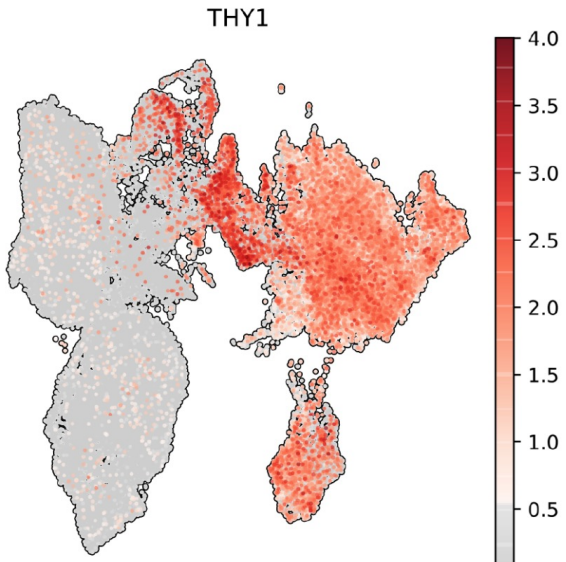
Co-culture

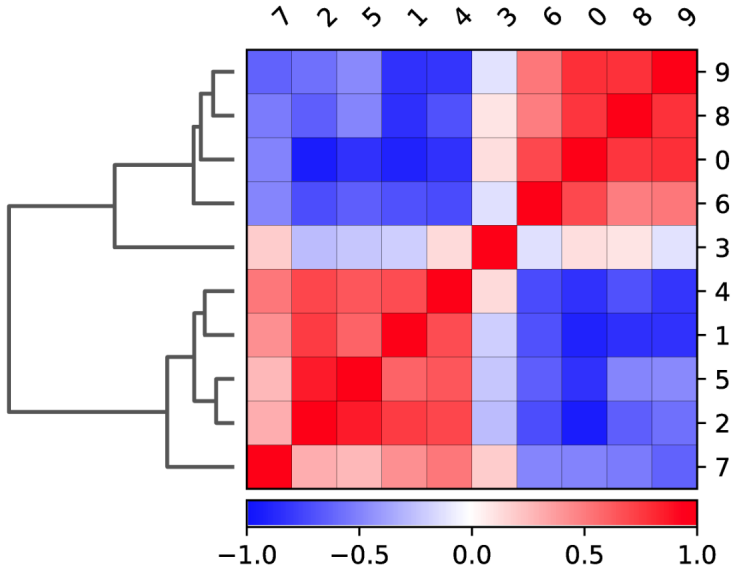
Subgroup 7 generated during MM-MSC interaction probably represents cancer stem cell (CSC)

MM marker

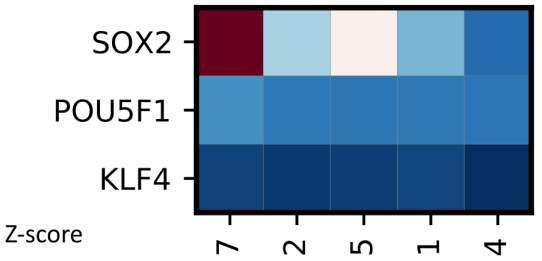


MSC marker

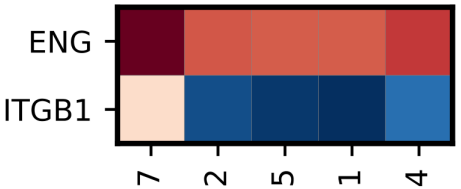




Canonical stem cell markers

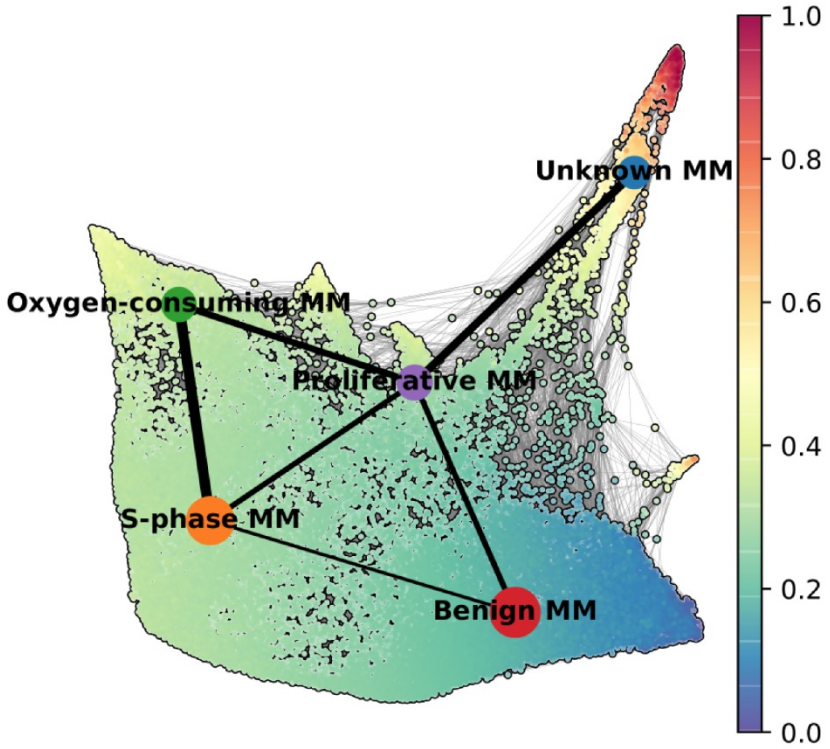


MMCSC markers

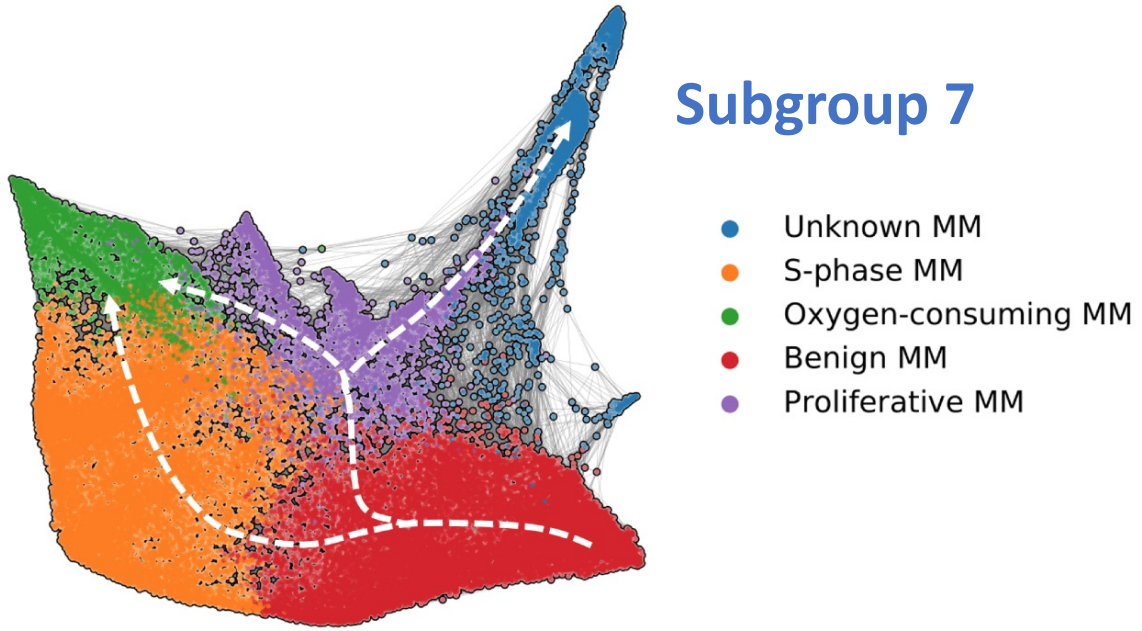


Mariana Bleker de Oliveira et al., Blood (2016)

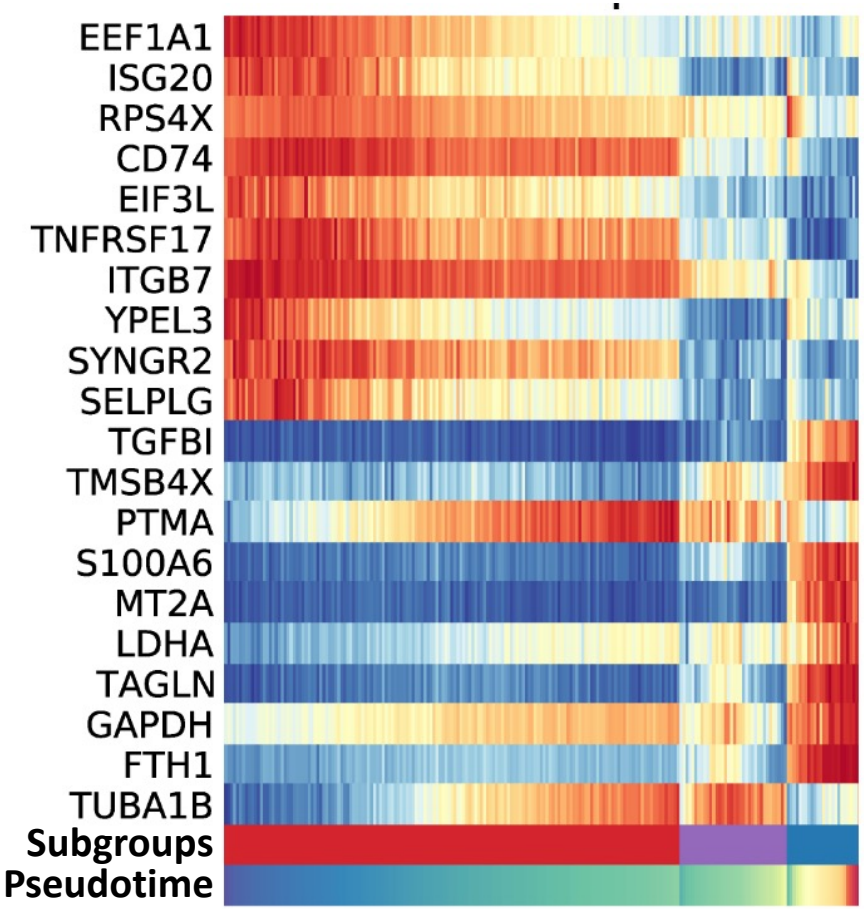
Transition trajectory reconstruction of MM cells indicates potential reprogramming path of subgroup 7

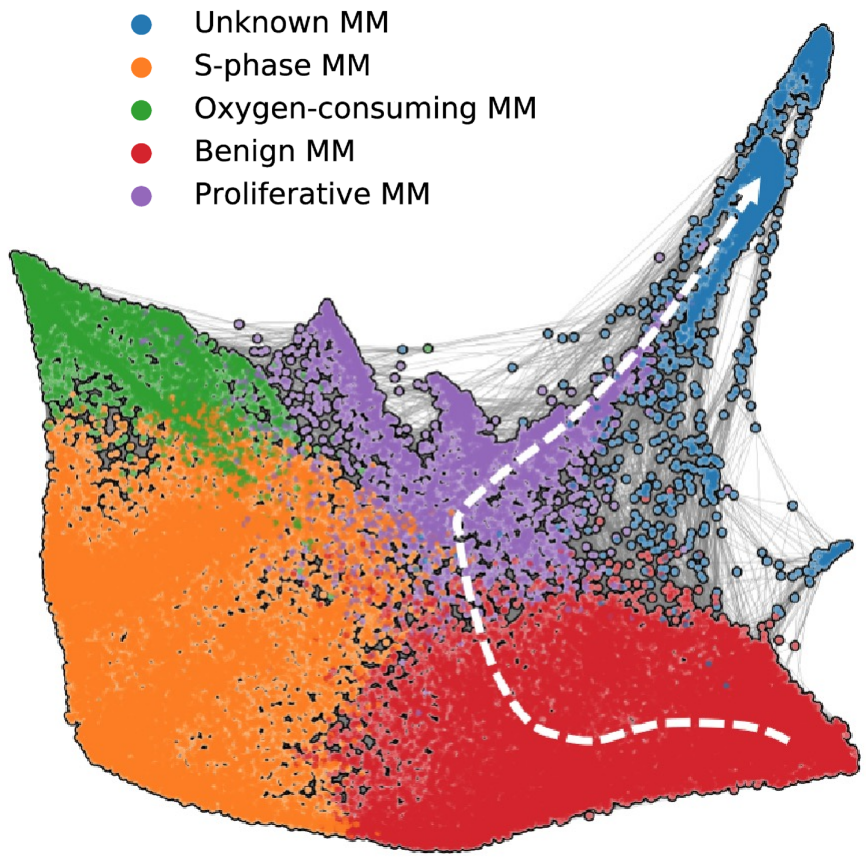


Benign MM as start point



Identification of dynamically expressed genes along MM transition path to subgroup 7

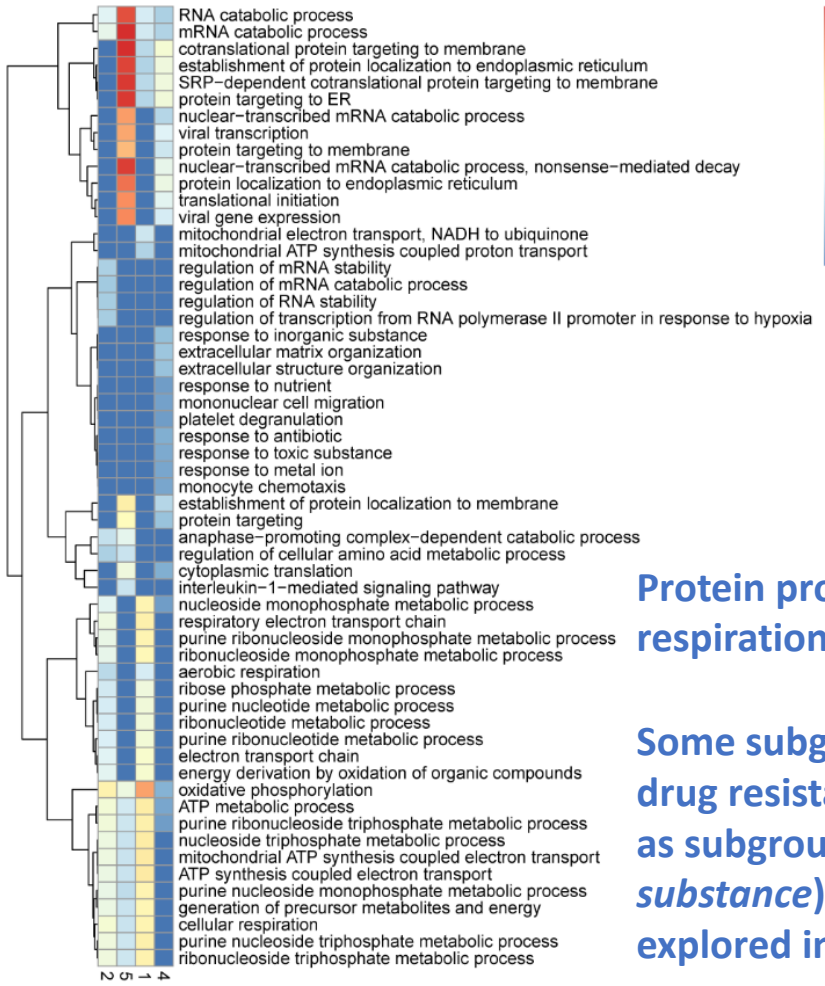




Which genes are responsible for the transition process?

MM-MSC interaction shifts MM transcriptome towards a drug resistant direction

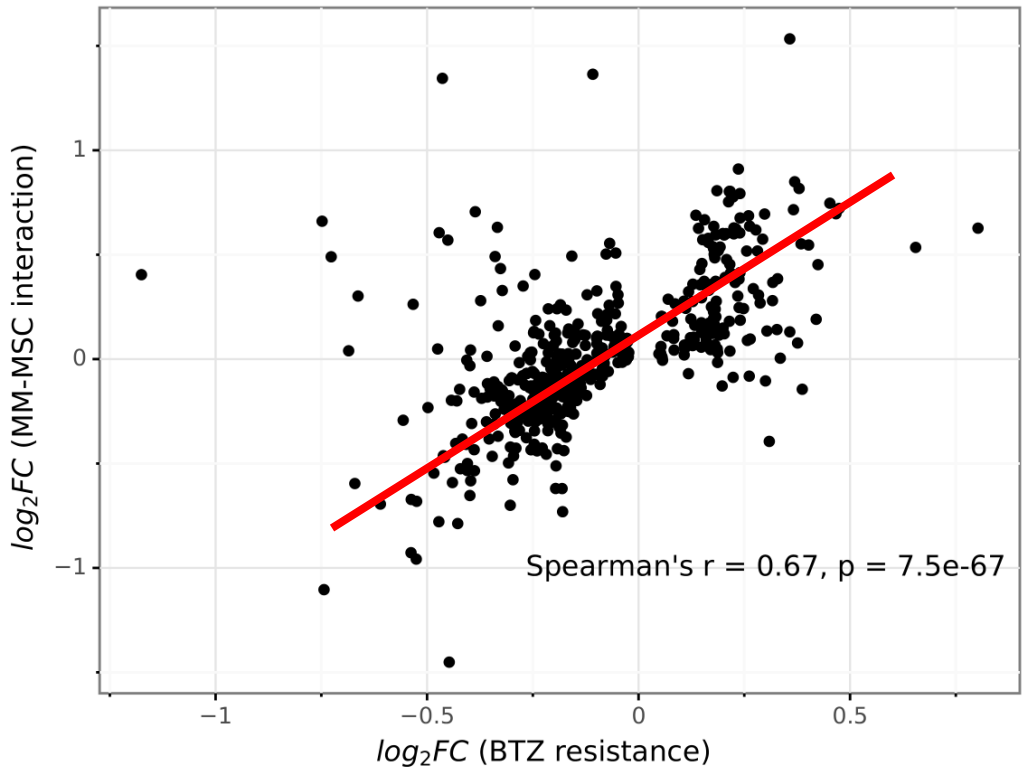
Drug resistance experiment



Protein processing, cellular respiration, etc.

Some subgroups show particular drug resistance pathways (such as subgroup 4's *response to toxic substance*) which may be explored in our future analysis

Dots represent top 500 DE genes in MM drug resistance experiment



Summary

1. Osteogenic lineage MSC group shrinks during MM-MSC interaction and potential mechanisms (cilia-related) regulating this process is inferred.
2. A MM subtype (subgroup 7) with the characteristics of stem cells nearly only show up after MM-MSC co-culture. The potential transition path from benign MM to the stemness MM and potentially responsible genes are identified.
3. MM subtypes show different drug resistance pathways and MSC can promote MM drug resistance.
4. These data characterize interactions between MM and MSC, providing clues next experiments can follow.

Acknowledgement

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- Prof. Xiujie Wang
- Members in the lab

Tianjin Medical University

- Prof. Zhiqiang Liu
- Members in Liu lab

Thanks for your attention!