Finding the Achilles' heel of an incurable cancer: the role of the Rap GTPases in multiple myeloma homing and pathogenesis

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1 Introduction

Multiple myeloma (MM) is the **second most prevalent blood cancer** after non-Hodgkin's lymphoma. Despite the recent breakthroughs in MM treatment, MM remains **uniformly fatal** due to intrinsic or acquired **drug resistance** [1]. Thus, there is a need to develop novel MM therapeutics to extend patient survival and possibly cure MM.

In MM, **antibody-secreting B lymphocytes** become malignant, spread through the bloodstream, and migrate into the **bone marrow** (BM) where they cause severe **bone damage**, the primary cause of mortality [2]. **Homing into the BM** and **adhesion to BM stromal cells** is required for the prolonged survival, pathology, and drug resistance of MM cells.

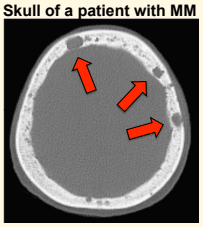
Quick Facts about MM:

- currently regarded as incurable
- 2008 estimates from Health Canada and NIH:

Country	New Cases	Deaths
Canada	2,100	1,350
US	19,920	10,690

- average lifespan from diagnosis is 3 years

Skull of a patient with MM

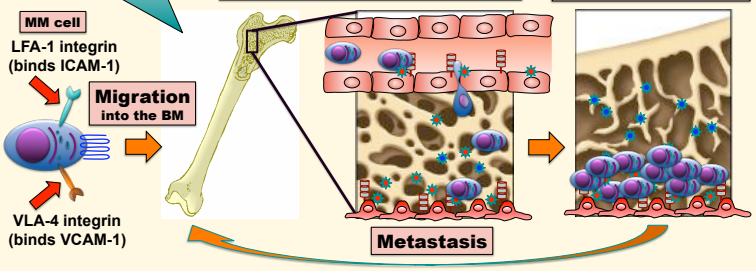


Baur-Melnyk *et al.* (2008) [6]

Adhesion and migration are important in MM

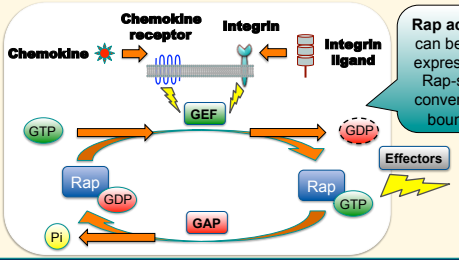
Adhesion to BM endothelial cells → transendothelial **migration** → **adhesion** to BM stromal cells → secretion of RANKL

Adhesion-induced proliferation, survival, drug resistance, and RANKL-mediated bone resorption by osteoclasts



= BM endothelial cell
= BM stromal cell
= Integrin (e.g. LFA-1, VLA-4)
= Integrin ligand (e.g. ICAM-1, VCAM-1)
= Chemokine receptor (e.g. CXCR4)
= Chemokine (e.g. SDF-1α, MIP-1α)
= RANKL (osteoclast differentiation factor)

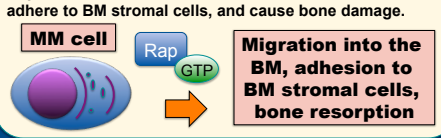
Our lab has shown that activation of the Rap GTPases (**Rap**) is critical for **B-lymphocyte migration and adhesion** [3-5]. Furthermore, interfering with the adhesion of MM cells to BM stromal cells has recently been proposed as a therapeutic strategy [2], but the role of Rap in MM has not been addressed to date. If blocking Rap activation prevents MM dissemination and bone damage, Rap or its effectors could be **novel therapeutic targets** for treating MM.



Rap activation in B cells can be **blocked** by over-expressing **RapGAPII**, a Rap-specific GAP that converts Rap to its GDP-bound (inactive) form

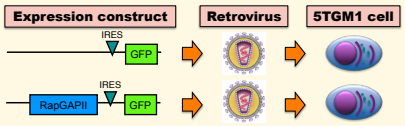
2 Hypothesis

Rap activation is critical for MM cells to home to the BM, adhere to BM stromal cells, and cause bone damage.

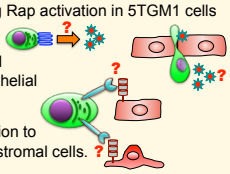


3 Research Plan

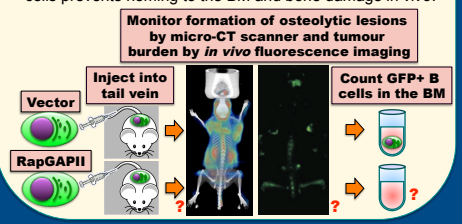
- **Aim 1:** transduce the murine MM cell line 5TGM1 with either an empty vector or the RapGAPII expression constructs.



- **Aim 2:** determine if blocking Rap activation in 5TGM1 cells *in vitro* prevents:
  - chemoattractant-induced transwell and transendothelial migration;
  - integrin-mediated adhesion to BM endothelial and BM stromal cells.

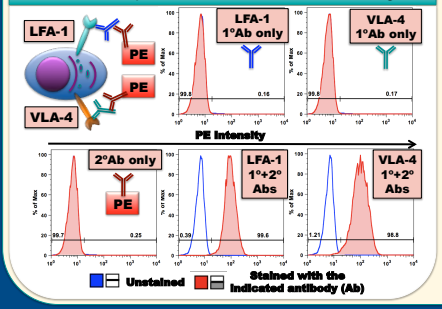


- **Aim 3:** determine whether blocking Rap activation in 5TGM1 cells prevents homing to the BM and bone damage *in vivo*.

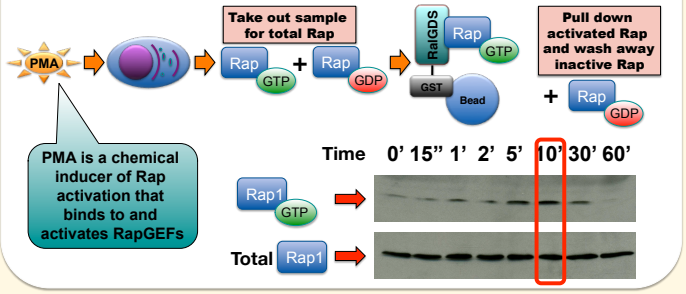


4 Results

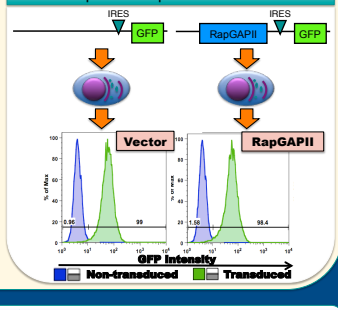
5TGM1 cells express surface LFA-1 and VLA-4 integrins



Rap activation in 5TGM1 cells can be induced with 100 nM PMA and peaks at 10 minutes



5TGM1 cells were transduced with the vector or RapGAPII expression constructs



5 Conclusions

1. 5TGM1 cells express high levels of LFA-1 and VLA-4 integrins on their surface.
2. Stimulation with 100 nM PMA for 10 minutes can be used as a positive control of Rap activation in 5TGM1 cells.
3. Pure populations of GFP⁺ 5TGM1 cells were obtained following transduction with either the vector or RapGAPII expression constructs.
4. Average GFP intensity is similar in both transduced 5TGM1 cell populations, suggesting similar amounts of construct expressed.

6 Immediate Goals

1. Investigate whether incubation of 5TGM1 cells in wells coated with LFA-1 and VLA-4 integrin ligands (e.g. fibronectin, ICAM-1, VCAM-1) can induce Rap activation.
2. Check by flow cytometry if 5TGM1 cells express the BM homing receptor CXCR4 on their surface.
3. Determine if RapGAPII is expressed and if it blocks Rap activation in response to 100 nM PMA in GFP⁺ 5TGM1 cells transduced with the RapGAPII expression construct.

7 Acknowledgements

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8 References

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