MAF and NF-κB Signaling Pathways Regulate Expression of APOBEC3B in Multiple Myeloma

Valeriy V Lyzogubov, Ruslana G Tytarenko, Cody Ashby, Faith E Davies, Gareth J Morgan, Brian A Walker

Myeloma Institute, University of Arkansas for Medical Sciences, Little Rock, AR, USA

INTRODUCTION

- APOBECs are a source of mutations in ~50% of human cancers.
- High expression of *APOBEC3B* (A3B) is associated with a poor prognosis and drug resistance.
- There is an enrichment for APOBEC signature mutations and a higher mutational load in the MAF (t(14;16)/t(14;20)) subgroup of multiple myeloma (MM).
- It is not clear whether over-expression of A3B is due to direct activation by MAF or indirectly through downstream pathways.

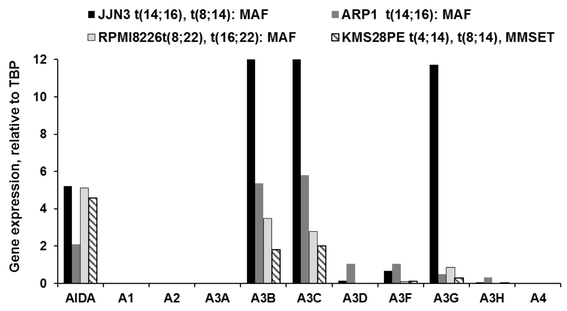
METHODS

- Gene expression profile (GEP).
- qPCR.
- Western blotting.
- DNA cytosine deaminase activity assay.
- Immunofluorescent analysis.
- Laser confocal microscopy.
- Inducible lentiviral shRNA MAF and A3B knockdown.
- Expression of A3B-HA using ViraPower T-Rex lentiviral expression system.
- Effect of PMA on A3B expression.
- Effect of Bortezomib on A3B expression.

RESULTS

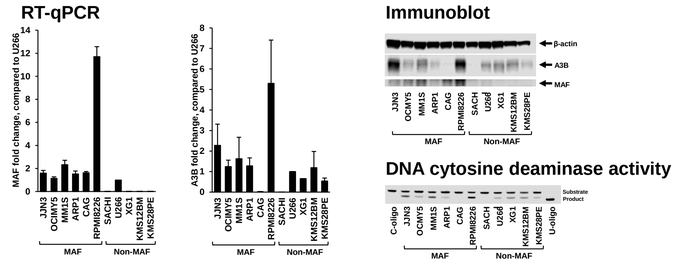
- By microarray, high levels (log_2>10) of A3B mRNA were found in approximately half of multiple myeloma patients.
- The majority of t(14;16) *MAF* expressing patients had high levels of A3B.
- Increased expression of *A3B* was also detected in 72% of MM cell lines.
- In t(14;16) and t(14;20), which over-express *MAF* and *MAFB* respectively, MM cell lines there was a strong positive correlation between *MAF* and *A3B* mRNA expression (r^2= 0.51 and r^2=0.43, respectively).

Expression of AID/APOBECs in MM cell lines



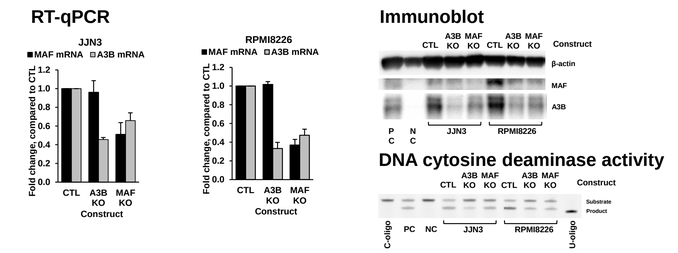
- *AIDA, A3B, A3C, A3D, A3F A3G* and *A3H* mRNA were detected in MM cell lines by RT-qPCR. The panel of primers was designed in Dr. R.S. Harris' lab (Refsland et al., 2010).

APOBEC3B and *MAF* expression in MM cell lines



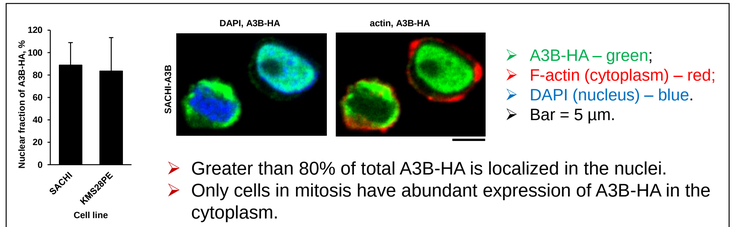
- MAF group cell lines have high expression of *MAF* mRNA and protein.
- Only U266 from the non-MAF group has *MAF* mRNA and very low MAF protein expression.
- There is a strong positive correlation (r^2=0.68) between *MAF* and *A3B* mRNA expression in cell lines from the MAF group and no such correlation in the non-MAF group (r^2=0.09).

Down-regulation of *MAF* results in reduced expression of *APOBEC3B* and loss of enzymatic activity



- Upon down-regulation of *MAF* by shRNA, JJN3 and RPMI8226 showed reduced expression of A3B protein (-33% and -29%, respectively) with accompanying reduction in cytosine deaminase activity (-23% and -26%, respectively).

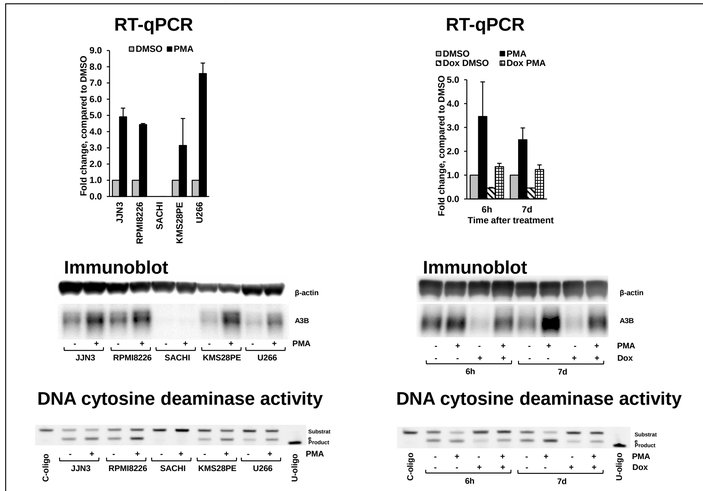
APOBEC3B localizes to the nucleus



- A3B-HA – green;
- F-actin (cytoplasm) – red;
- DAPI (nucleus) – blue.
- Bar = 5 µm.

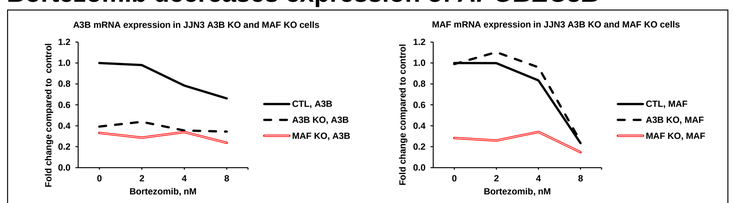
- Greater than 80% of total A3B-HA is localized in the nuclei.
- Only cells in mitosis have abundant expression of A3B-HA in the cytoplasm.

PMA increases expression of *APOBEC3B*



- PMA increased the expression of *A3B* mRNA in MM cell lines 6 hours after treatment. The highest fold change in A3B to PMA was detected in JJN3, RPMI8226 and U266 cell lines.
- Down regulation of A3B by shRNA prevented the stimulatory effect of PMA in JJN3 within 6 hours of treatment and lasted for 7 days after treatment.

Bortezomib decreases expression of *APOBEC3B*



- Bortezomib (8 nM) reduces expression of MAF and A3B in JJN3 cells transduced with TRIPZ control (CTL) construct within 48 h. All tested doses of Bortezomib have no effect on A3B mRNA levels in JJN3 A3B and MAF knock-out (KO) cells.
- Bortezomib (8 nM) decreases expression of MAF mRNA in JJN3 A3B KO cells.

CONCLUSIONS

1. Knock-down of *MAF* results in decreased expression and activity of APOBEC3B, indicating that MAF is a regulator of APOBEC3B expression in multiple myeloma.
2. Expression of APOBEC3B in t(14;16) cases is dependent upon MAF expression but can also be increased through activation of the PKC/NF-κB signaling pathway, resulting in increased DNA damage.

Myeloma Institute
University of Arkansas for Medical Sciences

Contact: VVLyzogubov@uams.edu

There are no conflicts of interest to disclose