Introduction

Multiple myeloma (MM) is a plasma cell malignancy that accounts for more than 10% of all blood cancers and may progress to plasma cell leukemia (PCL). Despite treatment, virtually all patients become unresponsive to treatment. RNA editing is a post-transcriptional pre-mRNA processing activity that represents an unexplored potential source of clonal molecular heterogeneity contributing to therapeutic resistance. In particular, adenosine deaminase acting on RNA (ADAR) 1, which exists in two isoforms, one constitutive and one inflammation-responsive, has been associated with disease progression and cancer stem cell (CSC) maintenance. The aim of this study was to investigate whether enhanced ADAR1 expression and activity contributed to therapeutic resistance of MM and PCL.

Procedures

1) ADAR Quantification: Whole gene and isoform-specific qRT-PCR was used to detect ADAR1 expression in PCL and MM primary samples and in human MM cell lines (HMCL).
2) RNA Editing Detection: We developed a RNA editing site-specific qPCR (RESS-qPCR) assay to detect RNA editing in cancer stem-cell associated transcripts.
3) Therapeutic Resistance Assay. A MM cell line was exposed to lenalidomide continuously in vitro to establish a model of therapeutic resistance.
4) Development of a humanized PCL mouse model: We established novel in vivo PCL primagrafts by intrahepatic transplantation of primary total mononuclear cells into neonatal RAG2-/-gc-/- mice.

Results

Approximately, 30% of MM patients in the MM Genomic Initiative dataset harbor copy number amplifications of the ADAR locus on chromosome 1q21, which portends a poor prognosis. We observed significantly increased ADAR1 expression in primary PCL samples and aberrant RNA editing of the stem cell transcription factor GLI1 and the DNA cytidine deaminase APOBEC3D. Notably, high-ADAR1-expressing PCL cells successfully engrafted in RAG2-/-gc-/- mice. As the inflammation-responsive isoform of ADAR1 was upregulated in primary samples, we sought to explore the effects of the anti-MM agent and immunomodulatory drug lenalidomide on ADAR1 expression and activity. Continuous in vitro exposure to lenalidomide led to increased ADAR1 mRNA and protein level and a potent induction of RNA editing activity. Increased RNA editing was detected in several cancer and stem cell-associated transcripts, including GLI1, APOBEC3D, AZIN1 and MDM2. Notably, this aberrant RNA editing activity was associated with increased self-renewal capacity in vitro and a cancer stem cell phenotype.

Conclusions

ADAR1 overexpression and deregulated RNA editing represents a unique source of RNA and proteomic diversity, and may confer a survival and self-renewal advantage to MM cells. This research identifies ADAR1 as a new diagnostic and therapeutic target in MM, and establishes a robust humanized PCL primagraft model for future pre-clinical testing of ADAR1 modulatory agents.