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Development of novel dual inhibitor of CXC Chemokine receptor 4 and Mcl-1 against Multiple Myeloma

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Abstract

Multiple myeloma (MM) is a neoplastic plasma-cell disorder. This is characterized by clonal proliferation of malignant plasma cells in the bone-marrow (BM) microenvironment, monoclonal protein in blood or urine, and associated organ dysfunction. The treatment options approved by FDA are immune-modulatory agents, proteasome inhibitors, and autologous stem cell transplantation (ASCT). Unfortunately, MM remains uniformly fatal owing to intrinsic or acquired drug resistance and the median survival time is 3 to 5 years. Thus, there is a great need for novel strategies to combat MM. The intimate relationship of myeloma cells to BM microenvironment is "hallmark of myeloma". The homing of MM cells to the BM, mediated by the chemokine stromal cell-derived factor-1α (SDF-1α) and its receptor CXCR4 has important functional sequelae. The BM microenvironment constituting cells secrete chemokines, cytokines, and growth factors such as interleukin 6 (IL6), vascular endothelial growth factor (VEGF), SDF-1α, and tumor necrosis factor α (TNFα) etc. These growth factors either secreted by MM or BM microenvironment cells (e.g. stromal cells) contribute in activation of several signaling pathways including nuclear factor-κB (NF-κB); phosphatidylinositol 3-kinase (PI3K)-Akt; Ras-Raf-MAPK kinase (MEK)-extracellular signal regulated kinase (ERK); and the Janus kinase 2 (JAK2)-signal transducer and activator of transcription 3 (STAT3). Activation of these pathways has been associated with increased expression of several anti-apoptotic proteins such as Bcl-2, Bcl-xL, Mcl-1, and XIAP. Collectively, these discoveries highlight that interaction of MM cells to BM microenvironment not only promote growth, survival and migration of MM cells, but also confer resistance to conventional chemotherapy. We hypothesized that an agent capable of inhibiting the migration of myeloma cells to bone marrow and suppressing the expression of survival protein Mcl-1 would be a better option for MM treatment. We have synthesized a novel dual inhibitor of CXCR4 and Mcl-1. Our *in vitro* data suggests that this molecule inhibits the expression of CXCR4 and Mcl-1 and survival of MM cells.

Introduction

- MM cells are highly dependent on the bone marrow (BM) microenvironment for growth and survival.[1]
- Migration of cells through the blood to the BM niches requires active navigation, a process termed homing.
- One of the most extensively studied chemokines in homing is stromal cell-derived factor 1α [SDF-1α; also known as CXC chemokine ligand 12 (CXCL12)] and its receptor, CXCR4.[2]
- The BM microenvironment constituting cells secrete chemokines, cytokines, and growth factors such as interleukin 6 (IL6), vascular endothelial growth factor (VEGF), SDF-1α, and tumor necrosis factor α (TNFα) etc. [3]
- These growth factors contribute in activation of several signaling pathways including nuclear factor-κB (NF-κB); phosphatidylinositol 3-kinase (PI3K)-Akt; Ras-Raf-MAPK kinase (MEK)-extracellular signal regulated kinase (ERK); and the Janus kinase 2 (JAK2)-signal transducer and activator of transcription 3 (STAT3). Activation of these pathways has been associated with increased expression of several anti-apoptotic proteins such as Bcl-2, Bcl-xL, Mcl-1, and XIAP. [4]
- Mcl-1 is one of the most highly amplified genes in a variety of human cancers including MM and contributes in drug resistance.[4] Thus, Mcl-1 becomes unique target to overcome resistance in MM.
- Gambogic acid (GA) is a xanthone derived from the resin of Garcinia hanburyi (also called mangosteen). We have previously shown that GA inhibits CXCR4 in MM.[5]
- We modified GA by adding MCl-1 inhibitor moiety in order to develop this molecule as a dual inhibitor of CXCR4 and Mcl-1. We investigated whether the gambogic acid (GA) analog (s) can modulate survival and drug resistance of MM cells.

Hypothesis

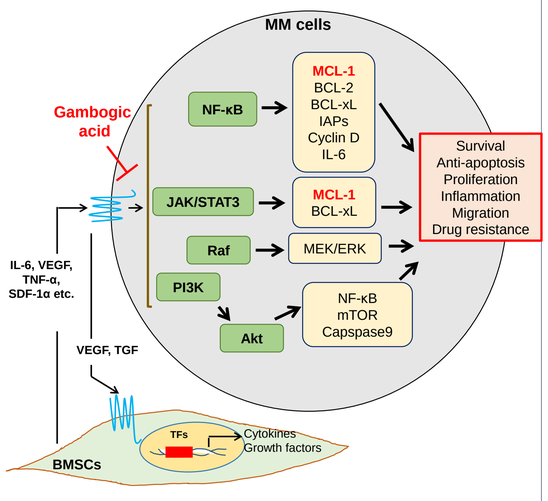
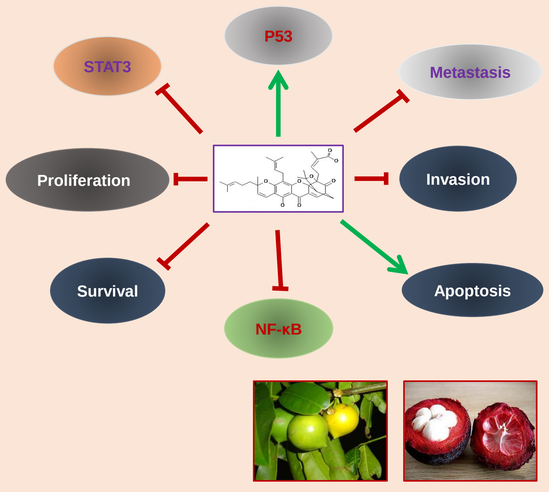


Figure 1. Intimate relationship between MM and BM microenvironment. BMSCs secrete cytokine a growth factors, these growth factors activate several pathways in MM. GA targets these pathways.

Gambogic acid - A hope for MM



Garcinia species (False mangosteen)

Results

GA interacts with CXCR4 and modulates the expression of CXCR4 protein of MM cells

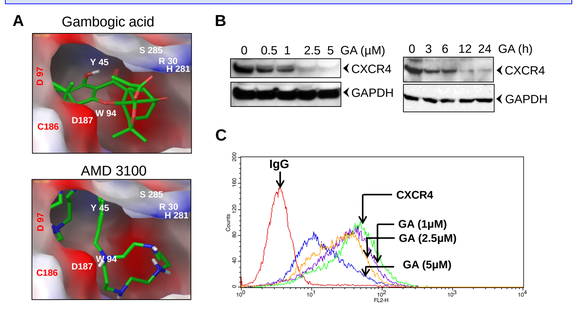


Figure 2. *A*, GA was docked onto CXCR4, and compared with AMD3100. CXCR4 residues within 5 Å distance from the ligands are shown with gray carbon atoms and ligands shown with green carbon atoms. Oxygen atoms are colored red, nitrogen blue, and polar hydrogen white. ***B***, GA suppresses CXCR4 levels in a dose- and time- dependent manner. ***C***, Surface expression of CXCR4 in GA treated MM cells was determined by flow cytometer.

GA analog reduces cell viability and induces apoptosis through inhibition of Mcl-1

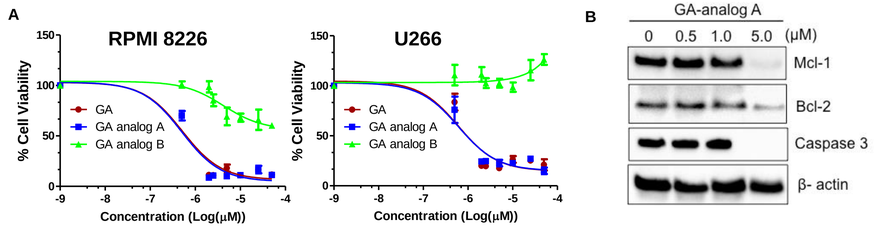


Figure 3. *A,* GA analogs reduce the survival of MM cells. MM cells (RPMI 8226 and U266) were treated with different concentration of GA, and its analogs for 48h. Cell viabilities were assessed using MTT assay. As shown in the figure treatment of GA and its analog effectively kill MM cells. Prism software was used to plot the graph. ***B,*** Effect of GA analog on survival protein was investigated. The human MM RPMI 8226 cells were treated with the indicated amount of GA analog for 24h. Treatment of GA analog modulates expression of anti-apoptotic proteins such as Mcl-1, Bcl-2, and caspase 3.

Conclusion and future directions

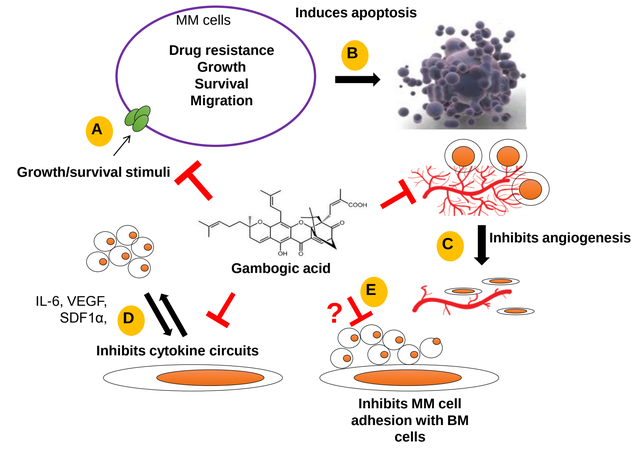


Figure 4: Our data showed that GA and its analog mediate their effects by targeting MM cells and the BM microenvironment. **(A)** GA targets CXCR4 receptor, **(B)** directly inhibits MM cell growth, **(C)** inhibits angiogenesis, **(D)** decrease cytokine production and sequelae such as osteoclastogenesis, and whether inhibits MM cell adhesion to BM accessory cells **(E)** will be explored.

References

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