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AURKA is a potential target for multiple myeloma therapy

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ABSTRACT

Multiple myeloma (MM) is an incurable disease with the second most frequent hematopoietic malignancy. In this study, we found that the expression of Aurora kinase A (AURKA) was significantly increased in patients with high–risk multiple myeloma, especially in the proliferation subgroups. MLN8237, a small molecule AURKA inhibitor, inhibited MM cell proliferation by inducing cell apoptosis and injury. Thus, we speculate MLN8237 is a potential therapeutic agent for MM and AURKA may be a potential target for MM treatment.

Keywords: multiple myeloma, AURKA, MLN8237

INTRODUCTION

Multiple myeloma (MM) is an incurable B–cell malignancy characterized by excessive proliferation of plasma cells, from which all patients eventually relapse[1,2]. The survival time span of MM patients is long: from several months to more than 15 years, indicating that there is genetic heterogeneity characterized by various degrees of differentiation and diverse molecular genetic characteristics in MM patients. According to different gene expression profile, Zhan *et al.*[3] classified MM patients into eight subgroups, such as CD1 and CD2 subgroup, MAF/MAFB (MF) spike group, MMSET spike group (MS), myeloid–like group (MY) and proliferation group(PR), etc. They concluded that MF, MS and PR subgroups with poor outcomes represented high–risk MM compared to the other five groups, with PR subgroup having the worst outcome in the progress of MM. Over the past decades, many treatment methods have been developed for MM patients, including chemotherapy, autologous stem cell transplantation among others. However, all MM patients eventually relapse and die of MM[4].

To predict drug resistance and establish a novel target of therapy in MM, Zhou *et al.*[5] analyzed a serial of sequential samples by gene expression profiling (GEP). Patients were taken from baseline, during different periods of intensive treatment with tandem autologous transplants and at relapse. The study revealed chromosomal instability (CIN) is highly correlated to drug resistance, rapid disease relapse and progression. Aurora kinase A(AURKA) is one of the most significantly differentiated genes in the CIN signature. As a member of the Aurora kinase family, AURKA plays a key role in cell cycle by regulating centrosome segregation and maturation, and bipolar spindle formation in mitosis[6]. Overexpression of AURKA leads to genomic instability and tumor occurrence[7]. The functions of AURKA have been well explored in solid tumors[8], such as bladder cancer, breast cancer and medullary thyroid cancer[9–11]. MLN8237, known as a kind of highly selective inhibitor of AURKA, can induce cytotoxicity and cell–cycle arrest in MM, which is currently applied to patients with malignant tumors in phase 2 clinical trials[12].

In this study, we evaluated the expression of AURKA in myeloma cells and MM subgroups. Our findings indicate that AURKA expression is associated with MM patient survival. In addition, we

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showed that MLN8237 is a promising therapeutic agent in MM.

MATERIALS AND METHODS

MM cell lines

Human MM cell lines ARP1 and 8226 cells were maintained in complete media–RPMI 1640 medium (GIBCO, Grand Island, NY,USA) containing 10% fetal calf serum (GIBCO,USA) and penicillin/streptomycin (100 μg/mL, Sigma, St. Louis, MO,USA) at 37℃ with 5% CO_2.

Reagents

Antibodies were purchased from Santa Cruz Biotechnology (Dallas, Texas,USA) and Cell Signaling Technology (Danvers, MA,USA). MLN8237 was from Millennium (Cambridge, MA,USA).

Flow cytometry

The Annexin V Apoptosis Detection Kit APC (eBioscience, San Diego, CA,USA)was used to detect apoptosis according to the protocol through a FACS Scan flowcytometer (Becton–Dickinson, San Jose, CA,USA)[13,14].

Cell proliferation

The cells were counted with a hemocytometer and the dead cells were stained by trypan blue.

Gene expression profiling (GEP) and data analysis

GEP adopted Affymetrix U133 Plus 2.0 microarrays was as described in the previous articles[3,15,16]. In this study, GEP data sets of primary myeloma are GSE2658 and GSE19554.

Statistical analysis

Kaplan–Meier curve and log–rank test were performed to plot and analyze the patient survival data. More than two groups were analyzed with one–way ANOVA, and paired groups were analyzed by *t* test. $P < 0.05$ was considered to be significant.

RESULTS

AURKA expression is significantly increased in MM cells

The expression of AURKA was analyzed in normal plasma cells(NP), monoclonal gammopathy of undetermined significance cells(MGUS, a pre–MM disease), and plasma cells of MM patients according to the GEP database***(Fig.1A)***. The results showed that expression of AURKA significantly elevated in MM cells compared to that of NP and MGUS cells. And AURKA expression in PR subgroup was highest in eight subgroups of MM***(Fig.1B)***. Moreover, the level of AURKA increased with extreme progression in the relapsed samples compared to the corresponding baseline sample in 88 paired MM samples***(Fig.1C)***. In addition, AURKA expression in 9 MM patients increased terrifically along with the following four serials at diagnosis, pre–1st, post–2st and post–2nd autologous stem cell transplants (ASCT) ***(Fig. 1D)***. These results revealed that overexpressed AURKA in MM cells is responsible for MM cell pro–

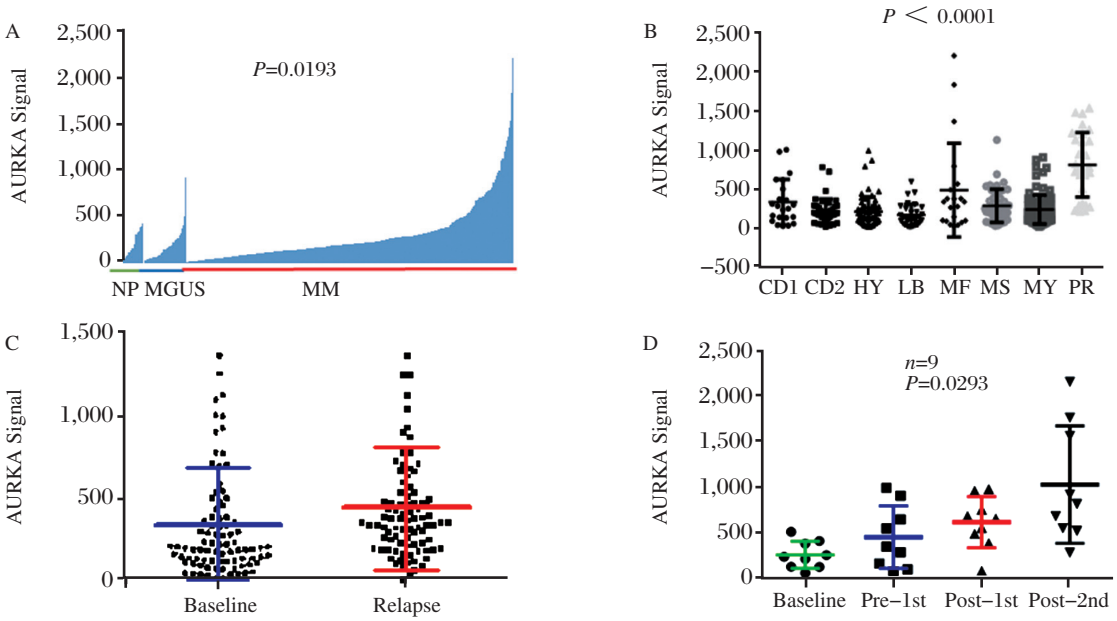

Fig. 1 **AURKA expression increases in MM patients.** (A) AURKA expression in NP, MGUS and MM. (B) AURKA expression in eight subgroups of MM. (C) AURKA expression in 88 pairs MM samples of baseline and relapse phrases. (D) AURKA expression in myeloma patient samples collected at diagnosis, pre–1st, post–1st and post–2nd autologous stem cell transplant (ASCT) was plotted.

liferation and drug resistance.

MLN8237 inhibits MM cell proliferation

We examined the antitumor effect of MLN8237, a selective AURKA inhibitor, on ARP1 and 8226 MM cells. MLN8237 induced the inhibition of cell proliferation and viability in ARP1 and 8226 cells in a dose–dependent manner***(Fig.2A)***. Flow cytometry detection with annexin V, a sensitive index of early cell apoptosis, showed that MLN8237 treatment remarkably induced apoptosis in two MM cells***(Fig.2B)***. The clonogenic soft agar assay was conducted in ARP1 and 8226 MM cells with two doses of MLN8237. The result showed that MLN8237 inhibited colony formation in both ARP1 and 8226 cells***(Fig. 2C)***. These findings

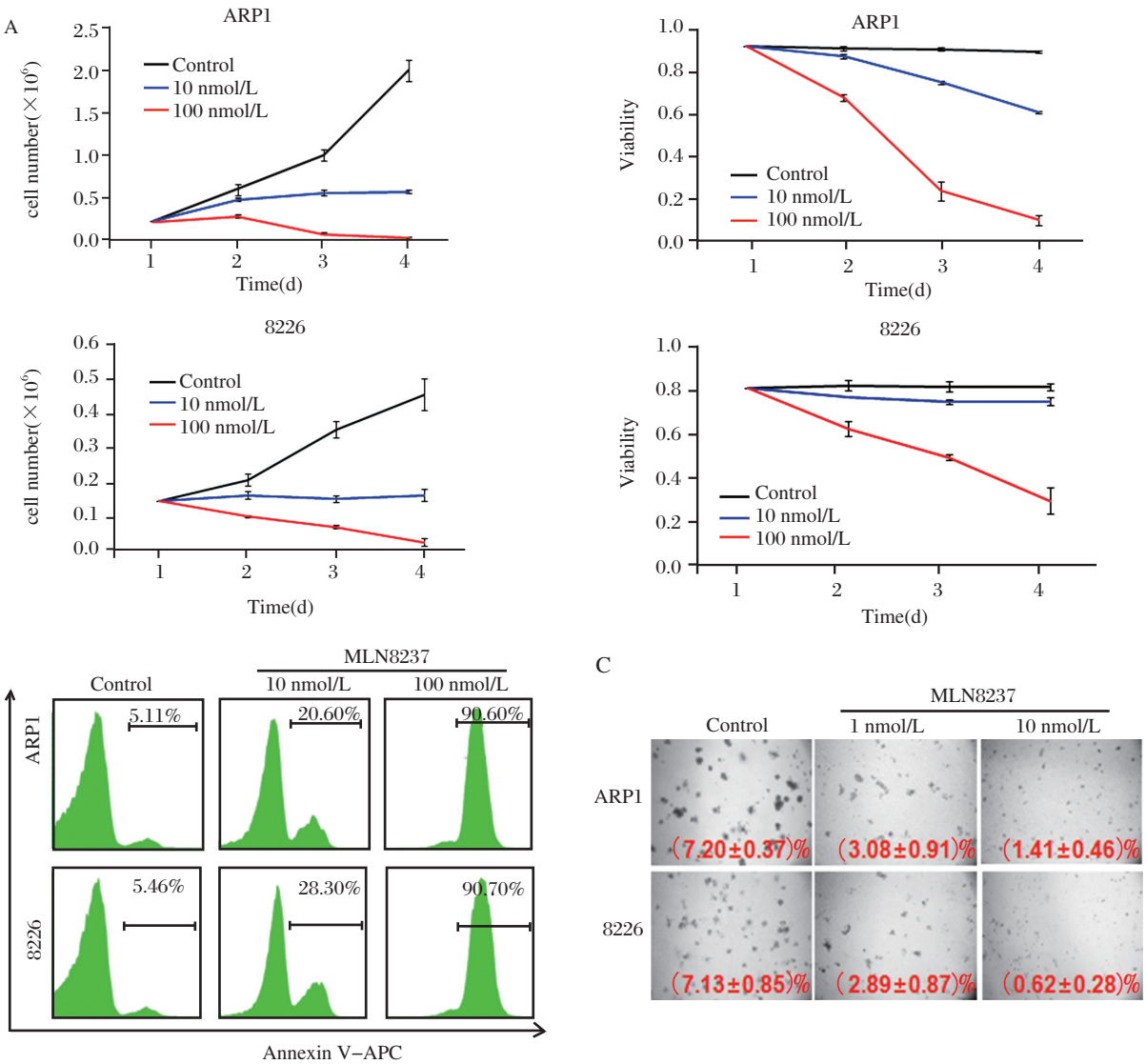

Fig. 2 **MLN8237 inhibits MM cell proliferation.** (A) Cell growth curve of ARP1 and 8226 MM cells with MLN8237 treatment. (B)Apoptosis of ARP1 and 8226 MM cells treated with different dose of MLN8237. (C) The clonogenic soft agar assay was performed in ARP1 and 8226 MM cells with two doses of MLN8237 treatment.

suggest that MLN8237 inhibited proliferation of MM cells by inducing cells apoptosis.

AURKA expression are correlated with MM patient´s survival

To verify the association of AURKA expression to clinical outcome, we searched the database of total therapy cohort (TT2, TT3, TT6). We found that MM patients with high expression of AURKA suffered poor clinical outcomes compared to low–MTDH patients ***(Fig. 3)***. Elevated AURKA expression is linked to significantly shorter time duration of overall survival (OS). This suggests that AURKA acts as an oncogene in MM as well.

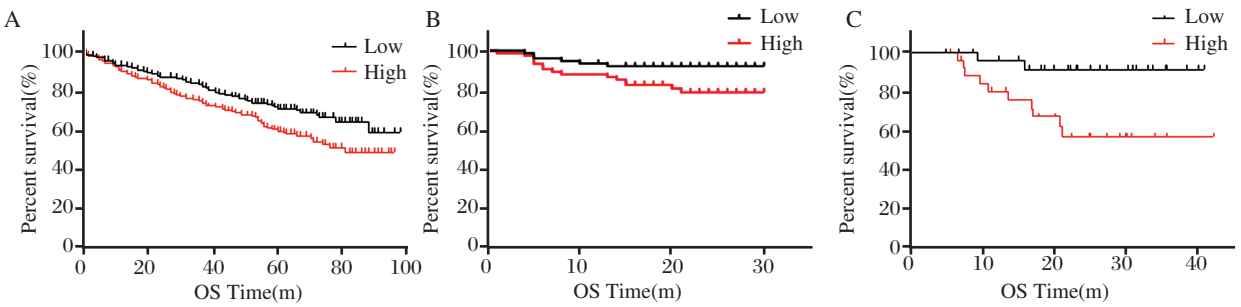

Fig.3 **Kaplan–Meier curve survival analyses are performed in TT2, TT3, TT6 cohorts.** Overall survival curves of TT2 (A),TT3(B),TT6(C) cohort were illustrated.

DISCUSSION

Targeted therapies for MM, such as proteasome inhibitors, can greatly improve the outcomes in patients. However, most patients eventually relapse and die of MM. It is therefore of great importance to find a proper target in MM. As a serine/threonine protein kinase regulating centrosome function, AURKA is definitely involved in the process of numerous cancers including breast cancer, liver cancer, bladder cancer, etc. Our previous study indicated that AURKA might be a poor prognostic marker in MM patients. This work is to confirm the role of AURKA in the development of MM.

Based on the GEP database, AURKA expression significantly increased in high–risk patients compared to NP and MGUS groups. Furthermore, elevated AURKA was found in MM patients who relapsed or received chemotherapy and autologous stem cell transplantation. The results indicated that AURKA expression was associated with MM progression, and the overexpression of AURKA may lead to MM cell proliferation and drug resistance. We also analyzed the effect of selective AURKA inhibitor MLN8237 *in vitro*. MLN8237 treatment arrested the growth rate of ARP1 and 8226 MM cell lines by inducing cell apoptosis. The result suggests that AURKA can promote the proliferation of MM cells. The clinical TT serial cohort analysis was consistent with above results. The overall survival time of MM patients with high AURKA is remarkably shorter than low AURKA expression patients.

In summary, AURKA overexpression can promote MM progression. AURKA inhibitor, MLN8237, can suppress MM cell growth and induce apoptosis. Our findings provide insight into the role of AURKA for further study in MM, and we believe AURKA may act as a promising therapeutic target in MM in the future.

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References

[1] Fairfield H, Falank C, Avery L, et al. Multiple myeloma in the marrow: pathogenesis and treatments. *Ann N Y Acad Sci,* 2016,1364: 32–51.

[2] Libby EN, Becker PS, Burwick N, et al. Panobinostat: a review of trial results and future prospects in multiple myeloma. *Exp Rev Hematol*, 2015, 8(1):9–18.

[3] Zhan F, Huang Y, Colla S, et al. The molecular classification of multiple myeloma. *Blood,* 2006, 108(6):2020–8.

[4] Shank BR, Brown VT, Schwartz RN. Multiple myeloma maintenance therapy: a review of the pharmacologic treatment. *J Oncol Pharm Pract*, 2015, 21(1):36–51.

[5] Zhou W, Yang Y, Xia J, et al. NEK2 induces drug resistance mainly through activation of efflux drug pumps and is associated with poor prognosis in myeloma and other cancers. *Cancer Cell*, 2013, 23(1): 48–62.

[6] Ke YW, Dou Z, Zhang J, et al. Function and regulation of Aurora_Ipl1p kinase family in cell division. *Cell Res,* 2003, 13(2):69–81.

[7] Chiang CM. p53–Aurora A mitotic feedback loop regulates cell cycle progression and genomic stability. *Cell Cycle,* 2012, 11(20):3719–20.

[8] Baba Y, Nosho K, Shima K, et al. Aurora–A expression is independently associated with chromosomal instability in colorectal cancer. *Neoplasia,* 2009, 11(5):418–25.

[9] Necchi A, Pintarelli G, Raggi D, et al. Association of an aurora kinase a (AURKA) gene polymorphism with progression–free survival in patients with advanced urothelial carcinoma treated with the selective aurora kinase a inhibitor alisertib. *Invest New Drugs,* 2017, 35(4): 524–8

[10] Kozyreva VK, Kiseleva AA, Ice RJ, et al. Combination of Eribulin and Aurora A inhibitor MLN8237 prevents metastatic colonization and induces cytotoxic autophagy in breast cancer. *Mol Cancer Ther,* 2016, 15(8):1809–22.

[11] Tuccilli C, Baldini E, Prinzi N, et al. Preclinical testing of selective Aurora kinase inhibitors on a medullary thyroid carcinoma–derived cell line. *Endocrine,* 2016, 52(2):287–95.

[12] Gorgun G, Calabrese E, Hideshima T, et al. A novel Aurora–A kinase inhibitor MLN8237 induces cytotoxicity and cell–cycle arrest in multiple myeloma. *Blood,* 2010,

115(25): 5202–13.

[13] Yang Y, Gu C, Luo C, et al. BUB1B promotes multiple myeloma cell proliferation through CDC20_CCNB axis. *Med Oncol,* 2015, 32(3):81.

[14] Li Y, He N, Zhai C. Peperotetraphin inhibits the proliferation of human prostate cancer cells via induction of cell cycle arrest and apoptosis. *Med Oncol,* 2015, 32(2):1–6.

[15] Zhou W, Yang Y, Gu Z, et al. ALDH1 activity identifies tumor–initiating cells and links to chromosomal instability signatures in multiple myeloma. *Leukemia,* 2014, 28(5):1155–8.

[16] Yang Y, Zhou W, Xia J, et al. NEK2 mediates ALDH1A1–dependent drug resistance in multiple. *Oncotarget,* 2014, 5(23):11986–97.

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