32P

ENZYCHEM LIFESCIENCES

KRIBB Korea Research Institute of Bioscience & Biotechnology

Tumor growth inhibition effect of PLAG by regulation of neutrophil infiltration in ICI insensitivity B16F10 melanoma

Guen Tae Kim[1], Eun Young Kim[1], Su-Hyun Shin[1], Hyowon Lee[1], Se Hee Lee[1], Jae Sam Lee[1], Ji Won Choi[1], Ki-Young Sohn[1] And Jae Wha Kim[2]

[1]Biology R&D, Enzychem Lifesciences, 10F aT Center 27 Gangnam-daero, Seoul, South Korea; [2]Immunetherapy Convergence Research, Korea Research Institute of Bioscience and Biotechnology, 125 Kwahak-ro, Daejeon, South Korea

ABSTRACT

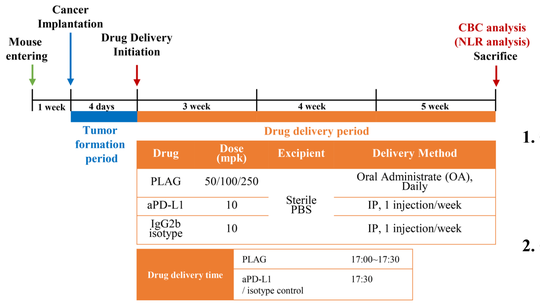
Background : Malignant melanoma (MM) is a representative tumor with a low survival rate due to its early diagnosis difficulty and poor treatment efficacy. Melanoma usually promotes its growth via tumor-infiltrating neutrophils (TINs), which inhibit the activation of immune cells that prevent cancer cells such as cytotoxic T lymphocytes. Therefore, the best option for MM treatment is to prevent excessive neutrophil infiltration into cancer lessons.

Methods ; A syngeneic mouse model was used (n=6) to investigate the enhanced anti-tumor effect of anti-programmed cell death-ligand 1 (aPD-L1) with various dosages of 1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol (PLAG). A B16F10 melanoma cell was s.c. inoculated into the C57BL/6 mice and maintained for 4-days. After that, PLAG was daily administered orally with different dosages (50/100/250 mpk) for 3 weeks with or without 10 mpk aPD-L1 (10F.9G2). A PD-L1 antibody was delivered via IP injection once a week. Tumor growth was calculated in 3-day intervals.

Results : In this current study, we investigated the effects of PLAG and aPD-L1 in a B16F10 MM mouse model. We observed significant tumor growth inhibition in the mice treated with both PLAG and PLAG/aPD-L1, but not in aPD-L1 treated one (p<0.05). Abnormal levels of lymphocyte, neutrophil and neutrophil-to-lymphocyte ratio (NLR) shown in tumor-bearing mice have been restored to normal status in PLAG and PLAG/aPD-L1-treatment. In addition, the infiltration of active neutrophils (Ly6G and Myeloidperoxide positive) was significantly reduced (p<0.05). Since PLAG significantly inhibits the release of neutrophil maturation (G-CSF) and infiltration (Mip-2), it seems that active neutrophils couldn't easily penetrate tumors. Furthermore, PLAG effectively reduced the adenosine release in the tumor microenvironment (TME) and serum, thereby controlling unnecessary massive neutrophil migration (p<0.05).

Conclusions : Collectively, our finding shows that PLAG effectively inhibits MM progression and may represent a novel therapeutic strategy for MM with high TINs, which is very difficult to use aPD-L1 therapy.

EXPERIMENTAL DESIGN



Drug	Dose (mpk)	Excipient	Delivery Method
PLAG	50/100/250	Sterile PBS	Oral Administrate (OA), Daily
aPD-L1	10		IP, 1 injection/week
IgG2b isotype	10		IP, 1 injection/week

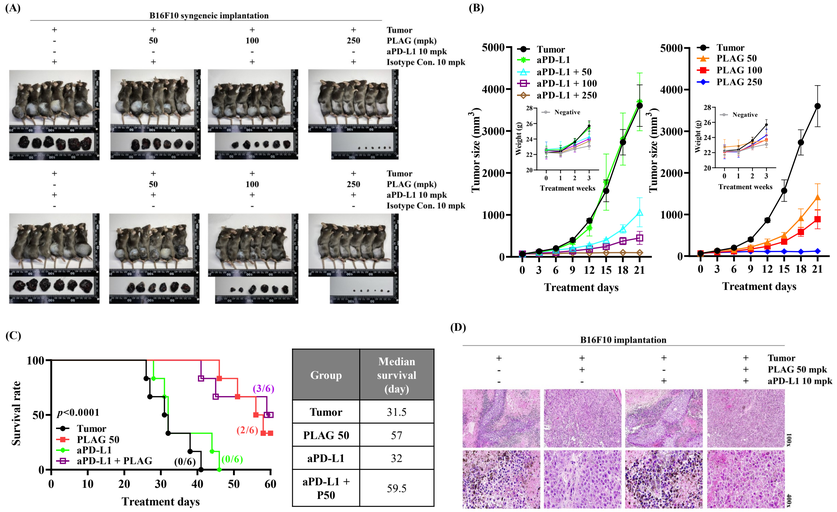
Drug delivery time		
	PLAG	17:00~17:30
	aPD-L1 / isotype control	17:30

1. Compound concentration
- PLAG : 50, 100, 250 mpk
- aPD-L1 : 10 mpk
- Isotype control : 10 mpk

2. Compound delivery
- O.A : PLAG (QD)
- I.P : aPD-L1, Isotype control (QW)

RESULT

1. PLAG inhibits the overgrowth of melanoma

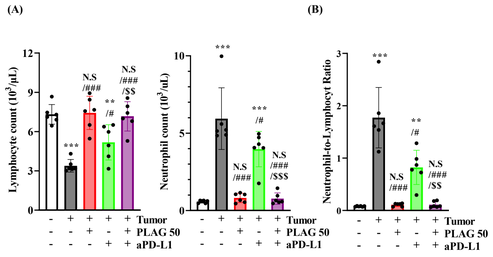


Group	Median survival (day)
Tumor	31.5
PLAG 50	57
aPD-L1	32
aPD-L1 + P50	59.5

PLAG and PLAG/aPD-L1 inhibit melanoma growth in a B16F10 melanoma

(A) Tumor burden and tumor size were measured on the sacrificed day in control and PLAG/aPD-L1. **(B)** Tumor size changes in each treatment group, measured at 3-day interval. **(C)** 60-days survival rate diagram by treatment days. **(D)** Assessment of pigmentation in MM tissue sections at the marginal area.

2. NLRs in blood of B16F10 mice are restored to normal status by PLAG treatments

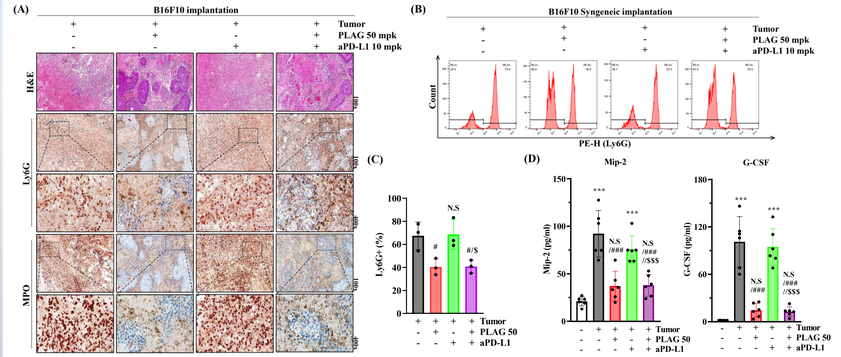


Cotreatment with PLAG and aPD-L1 inhibits melanoma growth in a B16F10 melanoma mice

(A) Numbers of lymphocytes and neutrophils in the blood of B16F10 mice **(B)** NLR calculated in each treatment group was analyzed by CBC.

Compared with the negative control: **P<0.002, ***P<0.001; Compared with the tumor only: ###P<0.001; Compared with the aPD-L1 only: $$$P$<0.002, $$$$P$<0.001 (each experiment n=6). N.S, Not significant. Mean ± SD.

3. Tumor-recruited neutrophils are significantly reduced in PLAG-treatments

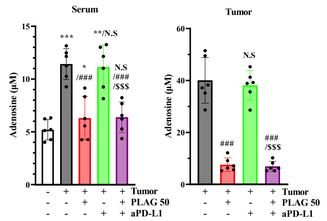


Analysis of tumor-infiltrating neutrophils (TINs) in tumors treated with PLAG/aPD-L1

(A) TINs were measured in B16F10 mice in each treatment group (Control, PLAG alone, aPD-L1 alone, PLAG/aPD-LI) by IHC staining with anti-Ly6G and anti-MPO antibodies. **(B)** Among CD45$^+$ cells (leukocytes), Ly6G$^+$/CD11b$^+$ (neutrophils) populations were measured by FACS. **(C)** Quantification of TINs (Ly6G$^+$) in tumors. **(D)** Levels of G-CSF and Mip-2 were measured by ELISA.

Compared with the negative control: ***P<0.001; Compared with the tumor only: #P<0.033, ###P<0.001; Compared with the aPD-L1 only: $$P$<0.033, $$$$P$<0.001 (each experiment n=6). N.S, Not significant. Mean ± SD.

4. Excessive adenosine release is inhibited by PLAG treatments



Analysis of released adenosine treated with PLAG and aPD-L1

Analysis of extracellular adenosine in the TME and serum of B16F10 innoculated mice.

Compared with the negative control: *P<0.033, **P<0.002, ***P<0.001; Compared with the tumor only: ###P<0.001; Compared with the aPD-L1 only: $$$P$<0.001 (each experiment n=6). N.S, Not significant. Mean ± SD.

CONCLUSION

- **PLAG inhibits the growth of B16F10 malignant melanoma by an average 85.5% and restored OS.**
- **Antitumor effect of PLAG was due to the effective control of excessive neutrophil infiltration into the tumor.**
- **The phenomenon appears to be caused by lowered expression of neutrophil infiltration-related factors (ie, Mip-2, G-CSF) and inhibition of excessive adenosine production.**
- **These results suggest that PLAG therapy may be leveraged to effectively inhibit malignant melanoma in human patients to help promote complete recovery.**

This work was supported by the grants (IGM0382211 and IGM0402111) from Enzychem Lifesciences and KRIBB Research Initiative Program (KGM5252221).