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The role of pigmentation in tumor treatment with virus-like drug conjugate belzupacap sarotalocan (AU-011) in an vitro and vivo model

Introduction

A virus-like drug conjugate belzupacap sarotalocan (bel-sar , AU-011) [1]

- Virus-like particle conjugated to phthalocyanine photosensitizer
- Binds to tumor specific glycosaminoglycans (GAGs) on cell membrane to deliver a therapeutic payload
- For the potential treatment of Indeterminate Lesions and small Uveal Melanoma (UM) in clinic

The mechanism of light activated bel-sar[1,2,3]

- In situ tumor ablation
- Induce immunogenic cell death
- Local acute inflammatory response
- Systemic immune response

Pigmentation is a poor prognostic factor in uveal melanoma [4]

- Associated with loss of chromosome 3
- Correlated with a poor survival

Pigmentation is a barrier for applying laser treatment in UM [5]

- Limited tissue penetration
- Quencher of singlet oxygen
- Verteporfin photodynamic therapy induced less tumor regression in pigmented UM

PURPOSE: To investigate the role of pigmentation in tumor behavior and its impact on anti-tumor efficiency of bel-sar treatment

Methods

Cell lines were used for evaluation in both in vitro and vivo models

- B16F10 wild type (wt)
- B16F10 tyrosinase knockout (TYR ko) cell line

Pigmentation and ultrastructure of melanosome were visualized via

- pellet
- light microscopy
- Electron microscopy

The vitro cytotoxicity and DAMP exposure were assessed by

- Apoptosis marker (Annexin-V, AV), necrosis marker (Propidium Iodide, PI)
- Damage associtaed molecular patterns (DAMPs), such as
  - Calreticulin (CRT)
  - Heat shock proteins 90 (HSP90)

The vivo model was established

- In syngeneic C57BL/6 mice, subcutaneous model
- Tumor micro-environment was analysed by FACS (flow cytometry)

References:

1. Kines, R.C, 2018. *Molecular Cancer therapeutics* 17.2 : 565-574
2. Huis In't Veld, R.V, 2023. *Cancer Immunology Immunotherapy* : 1-18.
3. Kines, R.C, 2021. *Cancer immunology research 9*(6), 693-706.
4. Gelmi, M.C, 2023. *Ophthalmology Science*, 100297.
5. Yordi, S, 2021. *Survery of Ophthalmology* 66.4, 552-559

Figure 1: Loss of visible pigmentation and melanosome ultrastructure in B16F10 ko cells

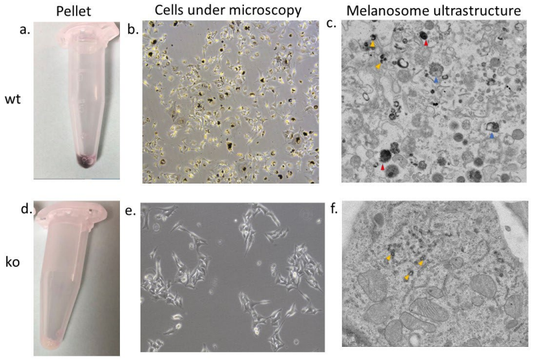


Figure 2: In vitro cytotoxicity and exposure of DAMPs induced by bel-sar treatment.

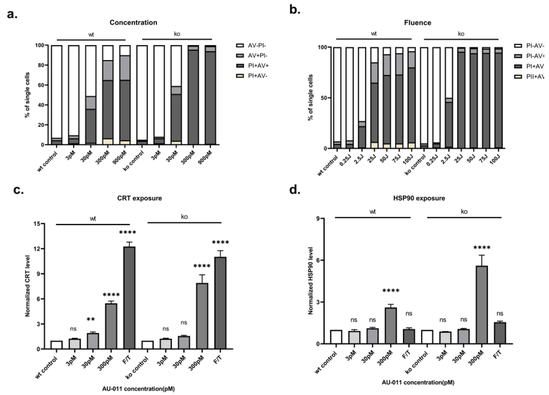
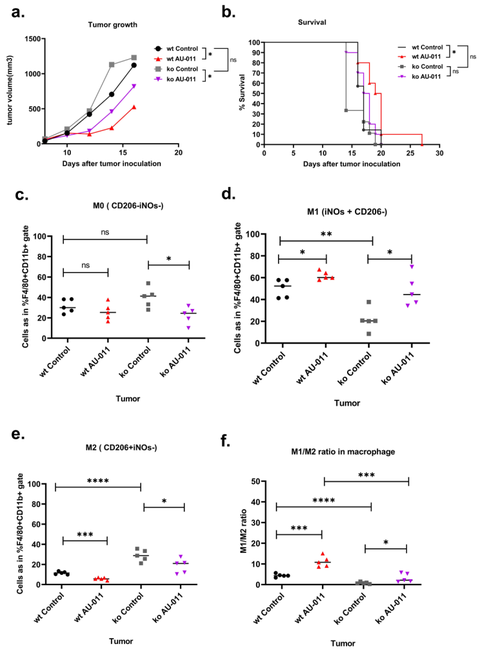


Figure 3: Tumor regression and macrophage infiltration in wt and TYR ko tumors after bel-sar treatment



Results:

KO TYR produced a non-pigmented cell line with underdeveloped melanosomes (Fig. 1)

- Light grey pellet
- Non pigmented cells
- Only early stages of melanosomes

Bel-sar treatment induced immunogenic cell death (Fig. 2)

- Near complete cell death of both cell lines
- Enhanced exposure of DAMPs, CRT and HSP90
- Regardless of pigmentation

Bel-sar treatment induced tumor growth delay and a shift to M1 macrophage (Fig. 3)

- Pigmented tumors contained more M1 and fewer M2 macrophages
- Bel-sar treatment gave a shift to M1 macrophage in both models

Conclusions

- **Pigmentation influenced the type of infiltrating macrophages in tumors, with more M1 macrophages in pigmented tumors than non-pigmented tumors.**
- **Bel-sar induced immunogenic cell death independent of pigmentation**
- **Bel-sar treatment also induced tumor growth delay and stimulated further M1**

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