Mertk is a therapeutic target in combination with radiation to promote adaptive immune tumor responses

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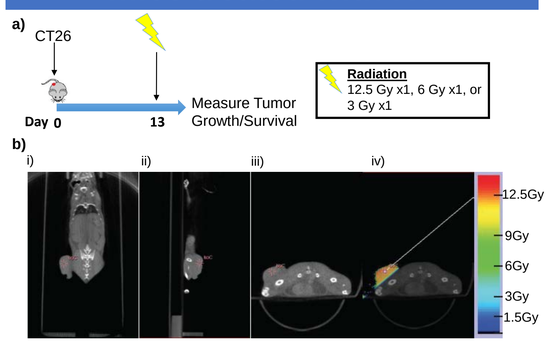
Abstract

Background: Mertk is a member of the Tyro3-Axl-Mertk (TAM) family of receptors and regulates phagocytosis of dying cells by macrophages. Cancer cells killed by radiation therapy direct repolarization of macrophages into immune suppressive phenotypes. Mertk$^{-/-}$ mice grafted with immunogenic tumors have enhanced tumor control following ionizing radiation compared to wild type mice. Gas6 is the endogenous ligand for Mertk and its ability to signal through Mertk requires a post-translational vitamin k-dependent modification that is inhibited by warfarin.

Methods: Mertk$^{-/-}$ and WT mice were injected subcutaneously with 5E4 CT26 cells (BALB/c) or 5E6 Panc02-SIY cells (C57BL/6) and allowed to grow to 5 mm before treatment with 250 µg anti-CD8α antibodies, warfarin (1.25 mg/L drinking water) and subjected to a single dose of ionizing radiation (12.5 Gy) followed by 250 µg of anti-OX40 or PBS I.P. 1-day post-RT. Peripheral blood was collected 6 days after RT and evaluated by Flow Cytometry for SIY-pentamer^{+}CD8^{+} T cells.

Results: Mertk$^{-/-}$ mice have increased survival following RT in CT26 tumor models. CT26 tumor cure in Mertk$^{-/-}$ BALB/c mice was abrogated by depletion of CD8 T cells indicating that ligation of Mertk in tumor macrophages suppresses endogenous anti-tumor immunity following radiation therapy. Similarly, warfarin-treated mice had higher rates of tumor cure following radiation that was also abrogated by CD8 depletion. In C57BL/6 mice, Mertk$^{-/-}$ alone does not affect responses to radiation therapy in the Panc02-SIY tumor model, but the combination of radiation therapy with anti-OX40 co-stimulation of T cell responses resulted in an increase in peripheral blood SIY^{+} CD8 T cells 5 days after treatment, and significantly improved survival compared to radiation alone.

Improved survival in Mertk$^{-/-}$ mice treated with RT



c)

Median Survival (days)

Mouse Strain	Radiation Dose (Gy)			
	0	3	6	12.5
Wild-type	20	20	21	28
Mertk$^{-/-}$	22	62	66.5	not met

Figure 1. a) Mertk wild-type and knock-out mice on a BALB/c background were inoculated with 5*10^4 CT26 cells in the right flank. b)Tumors were allowed to grow to 5 mm before randomizing a single fraction of stereotactic radiation (0, 3,6 or 12.5 Gy). c)Mice were followed for survival.

Tumor cures after RT in Mertk$^{-/-}$ mice are CD8-dependent

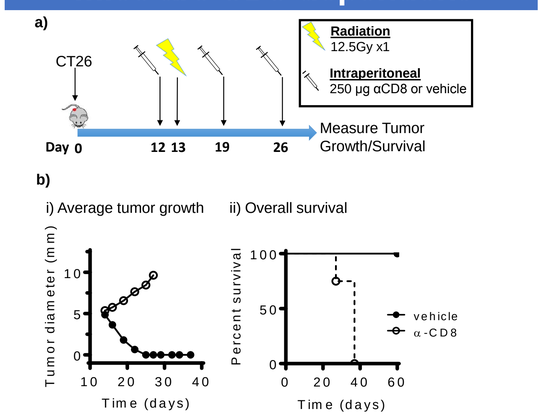


Figure 2. a) Mertk$^{-/-}$ mice on a BALB/c background were inoculated with 5*10^4 CT26 cells in the right flank. Tumors were allowed to grow to 5 mm before randomizing mice to intraperitoneal injections with vehicle (125 µL PBS) or α-CD8 (250 µg). Tumors were then given a single fraction of stereotactic radiation to 12.5 Gy with repeat injections given weekly for a total of 3 doses. Mice were followed for tumor growth and survival. b) vehicle treated mice (●) achieved tumor cure while αCD8-treated mice (O) did not.

Warfarin enhances RT control in a CD8-dependent manner

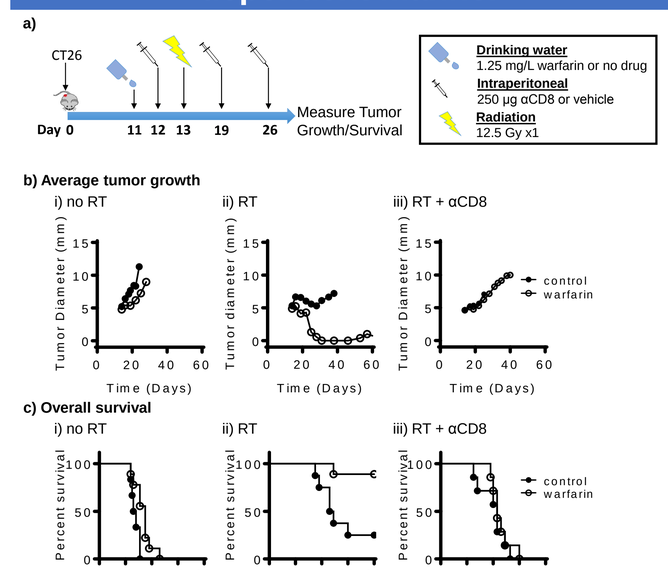
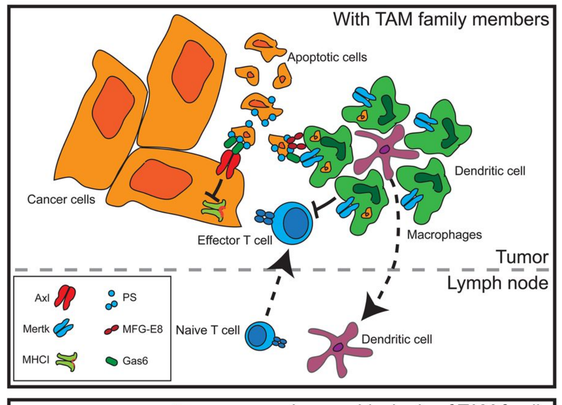
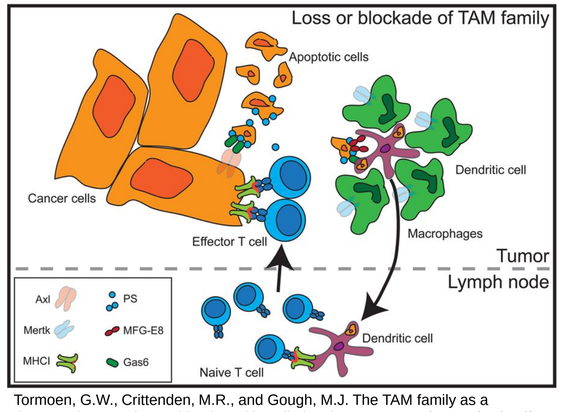


Figure 4. a) BALB/c mice were inoculated with 50,000 CT26 cells in the right flank. Tumors were allowed to grow to 5 mm before randomizing mice to warfarin (1.25 mg/L drinking water) or no drug, then injections with vehicle (125 µL PBS) or αCD8 (250 µg). Tumors were then given a single fraction of stereotactic radiation to 12.5 Gy and repeat injections given weekly for a total of 3 doses. Mice were followed for tumor growth and survival. b) warfarin treated mice (●) exhibited similar tumor growth compared to control mice (O) in the absence of radiation (i), but exhibited increased control of tumor growth compared to control animals when treated with RT (ii), that was abrogated with a CD8-depleting antibody (iii).

Model





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Antigen-specific responses in Mertk$^{-/-}$ mice given RT and OX40

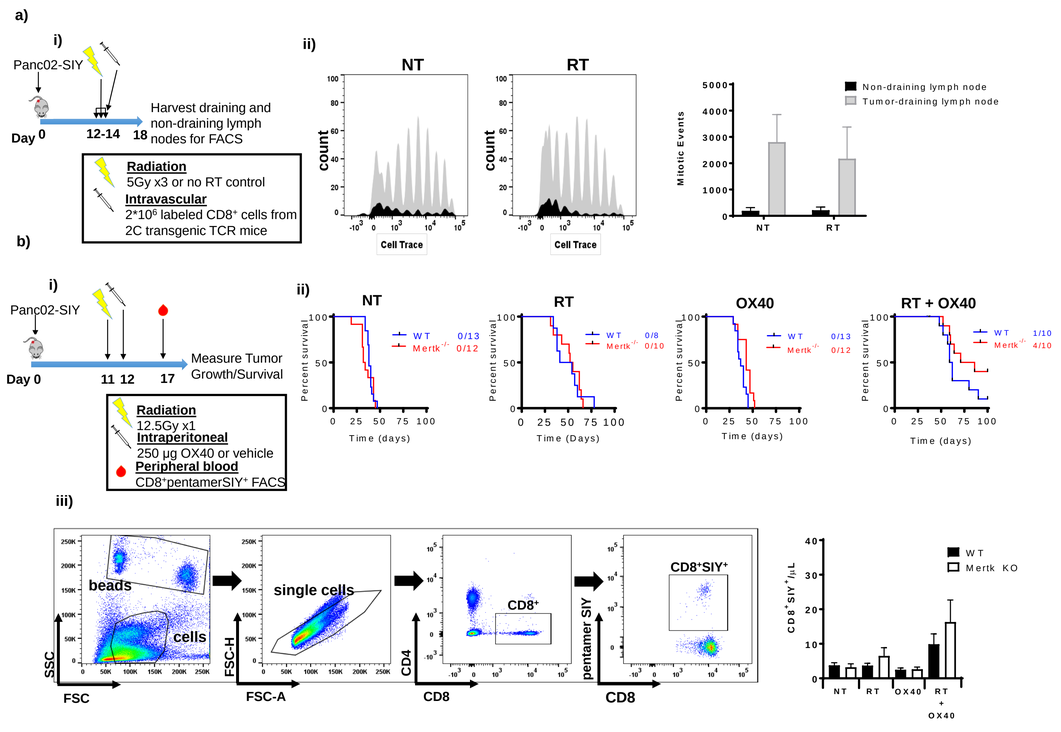

Figure 3. a) C57BL/6 mice were inoculated with 5*10^6 Panc02-SIY cells in the right flank. Tumors were allowed to grow for 12 days before treating with 5Gy stereotactic radiation on 3 consecutive days or no radiation and then injected intravascularly with CellTrace^{TM}-labelled 2*10^6 CD8 T cells from 2C transgenic TCR mice that recognize SIY on MHCI. 4 days later, the tumor draining and contralateral lymph nodes were harvested, processed to single cell suspension and evaluated for CD8 T cell proliferation by flow cytometry. b) Mertk$^{-/-}$ mice on BL/6 background or C57Bl/6 wild-type mice were inoculated with 5*10^6 Panc02-SIY cells in the right flank. Tumors were allowed to grow to 5mm diameter before treating with a single dose of stereotactic radiation to 12.5 Gy or no radiation. One day after RT, mice were given intraperitoneal injections with αCD8 (250 µg) or vehicle (125 µL) and followed for tumor growth and survival (ii). 5 days after treatment, peripheral blood was drawn and evaluated by flow cytometry for CD8+SIY+ T cells (iii).

Conclusions

- Mertk$^{-/-}$ mice show increased responsiveness to RT in immunogenic CT26 tumors
- Mertk$^{-/-}$ mice experience CT26 tumor cure after RT in a CD8-dependent manner
- Poorly immunogenic pancreatic tumors do not have increased responses to RT in Mertk$^{-/-}$ mice
- In poorly immunogenic pancreatic tumors, the combination of RT and anti-OX40 significantly increased overall survival compared to RT alone, and resulted in increased numbers of long-term cures in Mertk$^{-/-}$ mice
- Warfarin alone at 1.25 mg/L in drinking water did not affect growth of CT26 tumor grafts, but enhanced RT control of tumor growth in a CD8-dependent manner similar to Mertk$^{-/-}$ mice
- Mertk expression in tumor macrophages prevents tumor cure by tumor antigen-specific T cells following radiation therapy. In poorly immunogenic tumors, combining RT with additional immune therapy is needed to increase T cell responses, but tumor cure is still limited by expression of Mertk.

Acknowledgements

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