Stress-induced signals promote T cell-tumor cell interactions following anti-CTLA4 therapy

Maria Grazia Ruocco
NYU School of Medicine
4T1 mouse breast cancer model

Anti-CTLA4 mAb 9H10 in combination with local RT but NOT alone induced anti-tumor CD8 T cell responses that inhibited the primary tumor and its metastases

CD8 T cells infiltrating the regressing tumors were CXCR6+

Demaria et al., Clin. Cancer Res. 11:728-34, 2005
Matsumura et al., J Immunol 181:3099-3107 2008
Hypothesis

Ionizing radiation alters the expression of cell surface molecules on tumor cells, resulting in changes in the interactions between tumor cells and T cells.

Aim

A better understanding of tumor cell-T cell interactions will contribute to improve clinical approaches.
Experimental model

injection of 4T1 tumor cells

**short term**

1. Day 1
2. Day 2
3. Day 3
4. Day 5
5. Day 6
6. Day 16 imaging

**long term**

1. Day 13
2. Day 14
3. Day 15
4. Day 18
5. Day 22 imaging

RT RT
9H10
9H10
Blood vessels
T cells
2nd harmonic
T cells infiltrate the tumor microenvironment
Control

Blood vessels
T cells
4T1 tumor cells
9H10

Blood vessels
T cells
4T1 tumor cells
RT+9H10 leads to tumor eradication
Blood vessels
T cells
4T1 tumor cells
RT+9H10

Blood vessels
T cells
4T1 tumor cells
RT and 9H10 combined treatment increases the duration of T cells-tumor cell interactions
**In vitro T cell migration assay**

Diagram showing a cell suspension adhering to a glass surface, with antibodies for ICAM-1, anti-CD3, and anti-CTLA-4.
Anti-CTLA4 induces high T cell motility and prevents stable T cell-tumor cell interactions
In vitro T cell migration assay
Soluble anti-CTLA4 fails to override a bound anti-CD3 stop signal
ICAM-1 and Ra1 expression is increased in 4T1 tumor cells following *in vivo* RT.
Rae1-β

- A stress-activated molecule, expressed on transformed cells.
- Binds to NKG2D, expressed on effector cells including activated cytolytic CD8 T cells.
- Interaction of Rae1 with NKG2D expressed on anti-tumor CD8+ T cells is important for immune-mediated tumor inhibition in the 4T1 tumor model (Nam JS at al. Cancer Res., 2008).
Rae1-β converts the anti-CTLA4 ‘go’ signal into a ‘stop’ signal

αCD3+9H10  αCD3+9H10+αNKG2D

[Diagram showing experimental results]
*In vivo* anti-NKG2D antibody treatment: reverses the effects of RT+9H10
Conclusions

• RT+9H10 increases the duration of T cell-tumor cell interactions in vivo.

• 9H10 treatment alone increases T cell migration decreasing the duration of T cell- tumor cell interactions.

• RT upregulates ICAM1 and Rae1 on tumor cells

• Blocking the interactions between Rae1 and NKG2D prevents stable interactions suggesting that these molecules are important for formation of stable IS and T cell activation.
Acknowledgments

Noriko Kawashima
Julie Huang
Mengling Liu
Silvia Formenti

Michael Dustin and Sandra Demaria
p<0.0001