

A quantitative systems approach to identify paracrine mechanisms that locally suppress immune response to Interleukin-12 in the B16 melanoma model

Yogesh M. Kulkarni¹, Emily Chambers², A. J. Robert McGray³, Jason S. Ware¹, Jonathan L. Bramson³, and David J. Klinker II^{1,2,4}

Short Abstract — Identifying local mechanisms for immunosuppression is a key knowledge gap for improving the efficacy of immunotherapies for cancer. Leveraging concepts from the analysis of physiological systems, an integrated *in vitro-in silico-in vivo* approach was developed to evaluate competing hypotheses regarding tumor-mediated suppression of immune response to Interleukin-12, a key cytokine that helps shape anti-tumor immunity. Collectively, the data suggest that (1) biochemical cues associated with epithelial-to-mesenchymal transition can shape anti-tumor immunity through paracrine action and (2) remnants of the immunoselective pressure associated with evolution in cancer include both sculpting of tumor antigens and expression of proteins that proactively shape anti-tumor immunity.

Keywords — Model-based inference, high content assays, proteomics.

I. SUMMARY

Interleukin-12 (IL12) enhances anti-tumor immunity when delivered to the tumor microenvironment [1]. However, local immunoregulatory elements dampen the efficacy of IL12. The identity of these local mechanisms used by tumors to suppress immunosurveillance represents a key knowledge gap for improving tumor immunotherapy [2].

From a systems perspective, local suppression of anti-tumor immunity is a closed-loop system - where system response is determined by an unknown combination of external inputs and local cellular cross-talk [3]. Here, we recreated this closed-loop system *in vitro* and combined quantitative high content assays, *in silico* model-based inference, and a proteomic workflow to identify the biochemical cues responsible for immunosuppression. Following an induction period, the B16 melanoma cell

model, a transplantable model for spontaneous malignant melanoma, inhibited the response of a T helper cell model to IL12. This paracrine effect was not explained by induction of apoptosis or creation of a cytokine sink, despite both mechanisms present within the co-culture assay. Tumor-derived Wnt-inducible signaling protein-1 (WISP-1) was identified to exert paracrine action on immune cells by inhibiting their response to IL12. Moreover, WISP-1 was expressed *in vivo* following intradermal challenge with B16F10 cells and was inferred to be expressed at the tumor periphery. A similar pattern of WISP-1 is observed by immunohistology in human melanoma. Collectively, the data suggest that (1) biochemical cues associated with epithelial-to-mesenchymal transition can shape anti-tumor immunity through paracrine action and (2) remnants of the immunoselective pressure associated with evolution in cancer include both sculpting of tumor antigens and expression of proteins that proactively shape anti-tumor immunity.

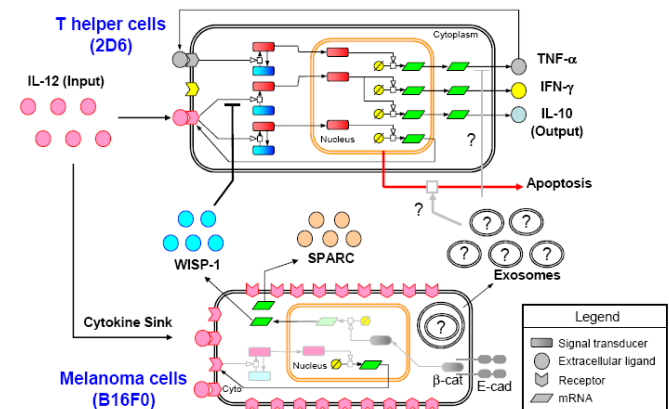


Figure 1: A quantitative systems approach was used to reveal paracrine mechanisms for tumor-mediated suppression of immune response to Interleukin-12.

REFERENCES

- [1] Kerkar SP et al. (2010) *Cancer Res* **70**, 6725-6734.
- [2] Fox BA et al. (2011) *J Transl Med* **9**, 214.
- [3] Klinker DJ (2010) *Mol Cancer* **9**, 242.

Acknowledgements: This work was funded by NSF and NCI/NIH.

¹Department of Chemical Engineering and Mary Babb Randolph Cancer Center, West Virginia University, Morgantown, WV USA.

²Department of Immunology, Microbiology and Cell Biology, West Virginia University, Morgantown, WV USA.

³Dept. Of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, CANADA

⁴Corresponding author. E-mail: david.klinker@mail.wvu.edu