IL-4 AND IL-13 SYNERGIZE TO INCREASE GROWTH AND CCL4 CHEMOKINE mRNA EXPRESSION BY 4T1 MAMMARY ADENOCARCINOMAL TUMOR CELLS

Antonio Harris[1], Daniel Pina[2], Sharon Njoki[3], Michael Donkor[4], Byron Quinn[1] and Harlan P. Jones[4]
[1]Langston University, Langston, Oklahoma; [2]Texas State University, San Marcos, Texas
[3]University of Texas at Dallas, Dallas, Texas; [4]University of North Texas Health Science Center, Fort Worth, Texas

THE UNIVERSITY of NORTH TEXAS HEALTH SCIENCE CENTER at FORT WORTH

ABSTRACT

Approximately, 1 in 8 women in the US will develop invasive breast cancer and 1 in 33 will die. Breast Cancer is a major health disparity for women all over the globe, having the largest impact on African American women under the age of 45. Despite medical advances, the 5-year survivorship among breast cancer patients remains significantly low largely due to high recurrence rates and propensity for metastasis to distal organs. (e.g., lung, liver, brain). Thus, further knowledge of the mechanisms which promote tumor development and progression is needed to advance cancer treatment. Tumor cells are known to secrete and express immune modulators as a mechanism to invade host antitumor immune responses. Using an experimental murine tumor cell line, our preliminary results demonstrate the expression of interleukin-4 receptor (IL-4R). We hypothesize that IL-4R activity plays a role in mediation of tumor cell function. In this study, 4T1 mammary adenocarcinoma cells were exposed to IL-4R ligands, interleukin-4 (IL-4), interleukin-13 (IL-13) and their ability to influence TNF-α, TGF-β1 and CCL4 expression by 4T1 cells. Our results demonstrated an increase growth and CCL4 mRNA expression in response to IL-4 in combination with IL-13, suggesting a potential target to mitigate tumor progression.

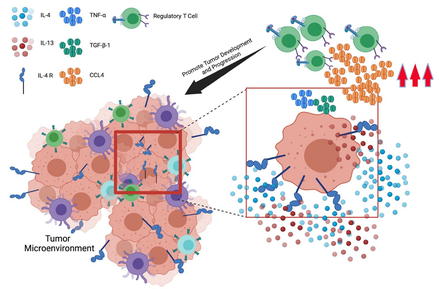
INTRODUCTION

Breast cancer is the second most common cancer among women in the United States. A major obstacle in the treatment of invasive breast cancer is its propensity to metastasize to distal organs, resulting in poor survivorship. Mechanisms which promote tumor progression include the ability of the tumor microenvironment to escape immune defenses (2,4). Specifically, the tumor microenvironment is capable of secreting immunosuppressive cytokines and chemokines which promote growth and spread of tumor cells (2,4,6). Both IL-4 and IL-13 cytokines are anti-inflammatory cytokines that regulate immunomodulatory responses by suppressing pro-inflammatory cellular signals. Previous studies have show that tumor cells can express IL-4 and their cognate IL-4 (IL-4R). Receptor as well as chemotactic factors that can recruit immunosuppressive cells and promoting tumor growth (1,4,5). To gain further insight of the role of IL-4 and IL-13 on tumor cell-mediated immune suppression, studies were conducted to assess the effects of IL-4 and IL-13 on tumor cell growth, cytokine and chemokine expression. Using the murine breast cancer cell line, 4T1 mammary adenocarcinoma, we demonstrate the synergistic effect of IL-4 and IL-13 on tumor growth and CCL4 chemokine mRNA expression. Our findings support our working hypothesis that IL-4R signaling increases cytokine and chemokine factors by tumor cells which promote the downstream recruitment and activation of immunosuppressive cells, thereby promoting tumor development and progression.

HYPOTHETICAL MODEL

The tumor cell microenvironment is highly immunosuppressive. Chemokines and cytokines expressed by the tumor microenvironment can promote immune suppression. Here we propose that increased CCL4 production and ligation to CCR5 expressing T regulatory cells promote the development and progression of the tumor microenvironment (Figure. 1.)

Figure1. Role of CCL4 in promoting immune evasion by tumor cells



METHODS

- **Cell Cultures**: 4T1 cells were seeded in T-75 culture flasks and incubated at 37ºC at 5% CO_2 until reaching 70% confluency.
- **6 Well Cultures**: 4T1 cells were seeded in 6 well culture plates and stimulated with 20 ng/mL of IL-4, IL-13, or a combination of IL-4, IL-13. or culture media as a control.
- **Microscopic Visualization**: Light field images were taken on 2 consecutive days. Each image represents the centermost aspect of the 6 well plate in triplicate at 10X magnification
- **Total RNA Extraction:** Total RNA from 4T1 cells were extracted using RNA easy column separation techniques (Invitrogen)
- **Quantitative RT-PCR :** cDNA was generated using a starting Total RNA concentration of 1 µg per reaction and MLV (Molony murine leukemia virus) reverse transcriptase (Promega Corp., Madison, WI, USA). After cDNA synthesis, real-time PCR was performed using SYBR green-based amplification techniques. PCR was performed in a 20 µl reaction volume using the StepOne system (Applied Biosystems Inc., Foster City, CA, USA).
- **Quantitation of mRNA gene expression:** TNF-α, TGF-β 1 and CCL-4 was quantified using $\Delta\Delta CT = \Delta CT(\text{target gene}) - \Delta CT(\text{GAPDH})$ methods (3).

RESULTS

Figure 2. Synergistic Effect of IL-4 and IL-13 on 4T1 Tumor Cell Expansion

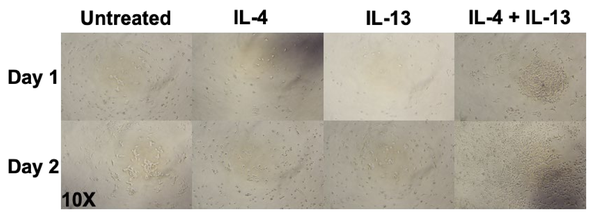


Figure 2. Qualitative growth characteristics of 4T1 Cells in response to IL-4, Il-13 or IL-4 + IL-13. 4T-1 cells (295,000) were seeded in 6 well plates in the presence of absence of IL-4 (5ng/ml), IL-13 (5ng/ml) or in combination. Images represent one of triplicate wells under light microscopic conditions (10X) depicting the center of each well.

Figure 3. Quality Control of Total RNA Extracted From 4T1 Cells

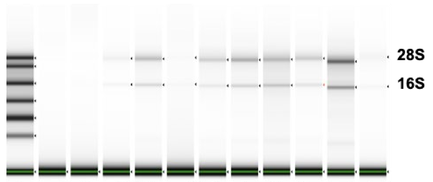


Figure 3. Total RNA gel integrity. Quality control of total RNA. Extracted from 4T1 tumors cells was determined using automated electrophoresis using Agilent 4200 tapestation (Santa Clara, CA). Lanes. 1, 2-control; lanes3,4,5-IL-4; lanes 6,7,8-IL-13; lanes 8,10,11-IL-4+IL-13.

Figure 4. IL-13 Increases CCL4. mRNA Expression by 4T1 Tumor Cells

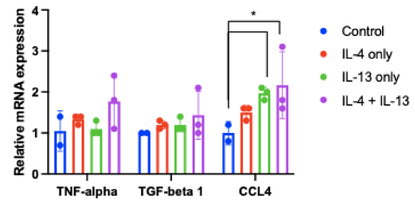


Figure 4. Cytokine and chemokine mRNA gene expression by 4T1 cells in response to IL-4, IL-13 or IL-4+. IL-13. Bars represent mean +/- standard error (n=2) for control and (n=3) for treatment groups.

CONCLUSIONS

- IL-4 plus IL-13 enhances the growth of murine 4T1 adenocarcinoma. Mammary tumor cells
- TNF-α, TGF-β and CCL4 mRNA gene expression. By 4T1 adenocarcinoma. Mammary tumor cells was not significantly altered by IL-4 or IL-13 alone.
- IL-4 plus IL-13 significantly increased CCL4 mRNA gene expression by 4T1 adenocarcinoma. Mammary tumor cells

REFERENCES

1. Venmar, K. T., Carter, K. J., Hwang, D. G., Dozier, E. A., & Fingleton, B. (2014). IL4 receptor IL4α regulates metastatic colonization by mammary tumors through multiple signaling pathways. Cancer research, 74(16), 4329–4340. https://doi.org/10.1158/0008-5472.CAN-14-0093
2. Choi, P., & Reiser, H. (1998). IL-4: role in disease and regulation of production. Clinical and experimental immunology, 113(3), 317–319. https://doi.org/10.1046/j.1365-2249.1998.00690.x
3. Ho-Pun-Cheung, A., Bascoul-Mollevi, C., Assenat, E., Boissière-Michot, F., Bibeau, F., Cellier, D., Ychou, M., & Lopez-Crapez, E. (2009). Reverse transcription-quantitative polymerase chain reaction: description of a RIN-based algorithm for accurate data normalization. BMC molecular biology, 10, 31. https://doi.org/10.1186/1471-2199-10-31
4. Esquivel-Velázquez, M., Ostoa-Saloma, P., Palacios-Arreola, M. I., Nava-Castro, K. E., Castro, J. I., & Morales-Montor, J. (2015). The role of cytokines in breast cancer development and progression. Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research, 35(1), 1–16. https://doi.org/10.1089/jir.2014.0026
5. Nakashima H, Fujisawa T, Husain SR, Puri RK. Interleukin-13 receptor α2 DNA prime boost vaccine induces tumor immunity in murine tumor models. J Transl Med. 2010 Nov 10;8:116. doi: 10.1186/1479-5876-8-116. PMID: 21067607; PMCID: PMC2993653.
6. Hu, G. N., Tzeng, H. E., Chen, P. C., Wang, C. Q., Zhao, Y. M., Wang, Y., Su, C. M., & Tang, C. H. (2018). Correlation between CCL4 gene polymorphisms and clinical aspects of breast cancer. International journal of medical sciences, 15(11), 1179–1186. https://doi.org/10.7150/ijms.26771

ACKNOWLEDGEMENTS

Research reported in this work was supported by the National Cancer Institute (NCI) of the National Institutes of Health (NIH) under Award Number 1 P20 CA233355-01. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. Research reported in this work was supported by the National Lung Heart Blood Institute (NHLBI) of National Institutes of Health (NIH) under Award Number 2 R25HL007786-26. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.