IL-12 Induced Regulation of the JAK-STAT Pathway in the Tumor Microenvironment of Acute Myeloid Leukemia

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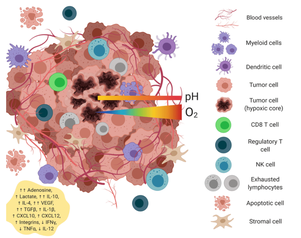
Introduction

- **Acute Myeloid Leukemia** is a significant hematological cancer of the blood
- Accounts for approximately 1/3 of all leukemia diagnoses in the world
- The five-year survival rate of acute myeloid leukemia is only **31.9**%
- Acute myeloid leukemia diagnosis and therapeutic methods have not increased or been enhanced in the past 30 years, since 1990

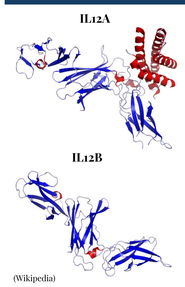
Tumor Microenvironment

- The tumor microenvironment is a known and significant factor in the poor prognosis in addition to continuation and progression of cancer
- Complex environment around a tumor composed of cancerous cells, stromal tissue, and the extracellular matrix
- Frequently, the cancerous cells and the tumor itself **engineer this environment** to inhibit immune and similar suppression cells from progressing towards the tumor
  - ❖ Production of cancerous growth factors
  - ❖ Modifications of the extracellular matrix to promote **tumor development**
  - ❖ Mediated by vascular endothelial growth factor **(VEGF)**
- This self-engineering and immune inhibition is often the factor for the difficulty of treatment and diagnosis



(Wikipedia)

Interleukin-12 | IL-12

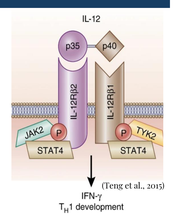


(Wikipedia)

- Interleukins are a collection of **cytokines**, which are proteins and signaling molecules secreted by leukocytes
- Interleukin-12 is a heterodimeric protein consisting of two primary receptors: **IL-12Rβ1 & IL-12Rβ2**
- Secreted by dendritic cells, macrophages, neutrophils, helper T cells, B cells, and similar antigen-presenting cells
- Encoded by two genes: **IL12A (p35)** consisting of a bundle of four alpha helices and **IL12B (p40),** consisting of three beta sheets
- Associated with the stimulation of interferon gamma, or **IFN-γ**, tumor necrosis factor, or **TNF-α**, and similar ideals
- IL-12 has been observed as a plausible anti-cancer drug, though no significant influence on cancer suppression has been proven
- IL-12 binding to its receptor initiates a signal transduction pathway referred to as the **JAK-STAT pathway**

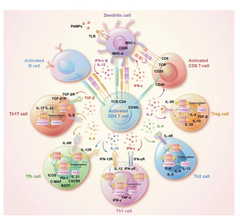
The JAK-STAT Pathway

- Upon IL-12 binding, IL-12Rβ2 experiences **tyrosine phosphorylation**, introducing binding region for non-receptor tyrosine kinase **TYK2** and tyrosine-protein kinase **JAK2**
- This initiates the **JAK-STAT signal transduction cascade** that ultimately expresses transcription factors associated with the upregulation of STAT genes oriented around cell proliferation, apoptosis, and tumorigenesis
- Following ligand binding by IL-12, the receptors dimerize to decrease the proximity of JAK in which the JAKs phosphorylate their corresponding JAKs on tyrosine residues within a regions called **activation loops**, a process referred to as **transphosphorylation**



(Teng et al., 2015)

IFN-γ
T_H1 development



(Xue et al., 2023)

- The activated JAKs then phosphorylate the tyrosine residues present on the IL-12 receptor, introducing binding sites for proteins consisting of **SH2 domains**, allowing STATs to binding to this receptor with their SH2 domain
  - Induces tyrosine phosphorylation of STATs by JAKs, dissociating the STAT from the IL-12 receptor
- These activated STATs then construct heterodimers or homodimers, in which the SH2 domains of each STAT binds the phosphorylated tyrosine of the corresponding STAT, eliciting **dimer translocation** towards the cell nucleus for transcription
- To bypass the nuclear membrane and lamina and enter the nucleus, STAT dimers surpass the nuclear pore complexes through an amino acid sequence, referred to **nuclear localization signals, NLS,** in which it experiences binding of proteins referred to as **importins** and progresses into the nucleus
  - A protein referred to a GTP-binding nuclear protein Ran, or **Ran**, binds to the importins, then releasing them from the STAT dimer and introducing it into the nucleus
- Increase and stimulated JAK-STAT pathway will accelerate the transduction pathway and upregulate the associated STAT genes, a significant of which is **STAT4.**

Experimental Design

Research Question

What is the influence of increasing IL-12 concentrations on tumor suppression within Acute Myeloid Leukemia in addition to its correlation with JAK-STAT signaling expression and upregulation of the STAT4 gene? Though IL-12 has been hypothesized to be an anti-cancer drug,

Engineering & Scientific Objective

The primary scientific objective of this study is to observe a decrease in tumor mass extent by at least 50%. In addition, a similar objective is to observe an increase in STAT4 relative expression, interferon gamma and TNF-α aggregation, and CD4+ T cell concentration, by at least 10%

Predicted Results

As the IL-12 concentration in acute myeloid leukemia cells increases, the tumor mass will progressively decrease though the STAT4 expression, interferon gamma and tumor necrosis factor alpha aggregation, helper T cell concentration, and extent of inflammation will increase. At a specified threshold, however, these increasing values will plateau excluding inflammation.

Susceptible Concerns

Within the in-silico environment, there are no evident harms regarding the research study as it is computationally-centric. This relies on previously established models, however, that may decrease the validity and application of the data, indicating that an in-vivo validation is imperative.

Independent Variable & Dependent Variable

In this study, the independent variable is the concentration of IL-12 acute myeloid leukemia cells. The dependent variable is the corresponding concentrations of VGEF to assess the versatility of the tumor microenvironment, interferon gamma, TNF-alpha, and CD4 expression to assess heightened immune responses, and STAT4 expression to assess the association between IL-12 and the JAK-STAT pathway.

Constants

The tumor location in which it is in the bone marrow, cell type regarding acute myeloid leukemia cells, and tumor type, in which it is a type one solid tumor.

Hypothesis

If IL-12 is implemented in myeloid cancer cell lines, its binding affinity to its receptor on myeloid cells will increase to amplify the expression of the JAK-STAT signaling transduction pathway and upregulate the gene STAT4, increasing interferon gamma responses and tumor suppression in its microenvironment and promoting immune responses as a therapeutic target to myeloid cancers.

Materials & Methodology

Critical Materials

There were numerous critical materials to ensure the validity of this research study, the greatest being a computer to conceptualize and analyze data in addition to the genomic analytical software itself referred to as **UCSC Xena.** UCSC Xena is a functional genomic visualization and analytical software developed by the University of California Santra Cruz and is a critical application for in-silico analyses of gene expression, phenotypic expression, cross-correlation of variables, and similar ideals. Oriented primarily around cancer and generates genomic statistics to assess the true significance of findings. Acute Myeloid Leukemia data is from TCGA.

UNIVERSITY OF CALIFORNIA SANTA CRUZ

University of California Santa Cruz (2024)

High-Level Procedure & Analytical Process

1. ❖ Access the UCSC Xena model at https://xenabrowser.net/
2. ❖ Select the visualization tab
3. ❖ Select the study tab and choose TGCA Acute Myeloid Leukemia
4. ❖ Create a subgroup for each gene, or independent variable, in the study
5. ❖ Complete a differential expression analysis through assessing the means of expression at distribution IL-12 concentrations
6. ❖ Utilize statistical and analytical methods to quantify and visualize the data
7. ❖ Determine the relative extent of IL-12 induce regulation on the dependent variables through viewing charts in box, violin, or scatter plot
8. ❖ Finalize the data and ensure validity through repeating analyses and confirming analysis
9. ❖ Utilize to the User Guide Docs https://ucsc-xena.gitbook.io/project to troubleshoot or advance study

Analysis & Discussion

IL12Rβ1 & VEGFA

- Increased IL12Rβ1 concentration **decreased** the gene expression of VEGFA
- **VEGFA**, or vascular endothelial growth factor A, is significantly associated with the versatility and the adaptability of the tumor microenvironment
- Thus, decreased expression indicates that IL12Rβ1 was able to directly able to modulate the tumor microenvironment through suppressing ECM genes that promote tumor responses

IL12Rβ1 & TNF

- Increased IL12Rβ1 concentration had a **positive correlation to TNF** signaling, though the distribution is random and does not have a defined trajectory
- This indicates that IL-12 is associated with TNF, though is **not a primary factor** due to the lack of statistical significance in its association
- Increased TNF did not increase the day until death but rather **decreased it**, which will be discussed further

IL12Rβ, CRP, & IL6R

- CRP, or **C-reactive protein,** is an indicator of inflammation and similar ideals
- Increased IL12Rβ1 was associated with drastic rises in CRP levels, indicating that IL-12 may induce inflammation, possibly promoting the tumor microenvironment
- **IL6R** is a similar inflammatory indicator in which there was a significant decrease in less concentration of IL12Rβ1

IL12Rβ1 & IFNG

- IL-12 IL12Rβ1was unable to directly promote interferon gamma aggregation
- This indicates that IL-12 must rely on STAT4 to elicit interferon gamma, implying that the decreased interferon gamma levels were due to decreased expression of STAT4

IL12Rβ1 & STAT4

- IL12Rβ1 lacked a specific correlation to STAT4 levels, in which increased IL-12 revealed a **decrease in STAT4**
- This implies that IL-12's activation of the JAK-STAT pathway may not only be limited to the STAT4 gene, but may induce transcription of numerous genes that **nullify its effect**

IL12Rβ1 & CD4

- Increasing IL12Rβ1 was positively correlated to CD4+ levels, implying that IL-12 was correlated to promoting the **differentiation and development of T helper cells**
- Higher CD4+ levels may indicate greater immune responses induced by IL-12; however, high CD4 levels were associated with low STAT4 levels, decreasing the correlation among the two

STAT4 & IFNG

- Validity of the results were defined by the STAT4 & IFNG mapping that shows a true **positive correlation** among the variables as precautionary action towards the accuracy of the data results.

Conclusion

- Analysis the data shows that the **hypothesis was not supported** due to the decreased regulations found by STAT4, indicating that IL-12 experiences complications in regulating the JAK-STAT pathway, a possible reasoning for why IL-12 studies have not shown significant cancer effects
- This lack of significant correlation to STAT4 may have been through the transcription or activation external biological variables that only **nullified the STAT4 genes**
- IL-12 induced **inflammation** may have a been suppressing variable and further promoted tumor adaptability despite the external benefits
- IL-12 itself was unable able to directly mediate IFN gamma, TNF alpha, and similar immune responses
  - This was most likely due to **the lack of STAT4 amplification**
- Though there was no direct correlation between IL-12 and IFN gamma as well as TNF alpha, positive associations were present
  - Increases in TNF alpha decreased the days until death, confirming the **dual responses of TNF alpha**
- There was a distinct negative correlation between the increasing activity of IL-12 and vascular endothelial growth factor alpha (VEGFA)
  - This indicates that IL-12 was able to modulate and **mediate the activity of VEGFA**, signifying its implications in stagnating and suppressing tumors in acute myeloid leukemia
- Increasing IL-12 promoted CD4+ levels, in which it is a protein on the surface of Th1 or helper T cells
  - This implies that IL-12 was able to elicit **and upregulate the immune responses** through humoral and cell-mediated immunity
- Higher IL-12 levels exponentially increased inflammation in the primary site of the tumor, indicating that increased IL-12 may both promote and suppress the tumor