The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 20(2): S2: 482-486, 2025 www.thebioscan.com

Targeting Inflammation-Induced Immune Evasion in Colorectal Cancer: Zinc Pyrithione Downregulates PD-L1 Expression to Restore Antitumor Immunity

V BRAHMAIAH[1], G DEEPTHI REDDY[1], B Y KAVITHA[1], Dr G DEEPIKA*[1]

1. Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Begumpet, Hyderabad-16, Telangana, India.

DOI: 10.63001/tbs.2025.v20.i02.S2.pp482-486

KEYWORDS
Zinc Pyrithione, PD-L1, colorectal cancer, immune evasion, immunotherapy, tumor microenvironment, epithelial-mesenchymal transition, IFN-γ, in vitro

Received on:
03-01-2025

Accepted on:
20-03-2025

Published on:
25-04-2025

ABSTRACT

Background: Colorectal cancer is adept at modulating immune responses, particularly in the context of chronic inflammation, with immune evasion being a critical factor in its progression. Inflammation can induce the expression of Programmed Death-Ligand 1, leading to T cell inactivation and tumor immune escape. Zinc Pyrithione has demonstrated anti-inflammatory and anticancer properties; however, its potential to modulate immune checkpoint pathways and restore immune function under inflammatory conditions remains largely unexplored. This study investigates the immunomodulatory role of ZnPT in counteracting inflammation-induced immune escape in colorectal cancer.

Methods: In vitro experiments were conducted using HT-29 and SW480 colorectal cancer cell lines to simulate an inflammation-induced immunosuppressive tumor microenvironment. Cells were stimulated with lipopolysaccharide to induce inflammation, followed by treatment with ZnPT. PD-L1 expression was assessed at both mRNA and protein levels. A co-culture model with peripheral blood mononuclear cells was employed to evaluate the impact of ZnPT on immune cell-tumor interactions, with interferon-gamma secretion quantified via ELISA as a measure of immune activation.

Results: LPS stimulation significantly increased PD-L1 expression in colorectal cancer cells, confirming the induction of an immune escape phenotype. Subsequent treatment with ZnPT effectively restored IFN-γ secretion in PBMC co-cultures, indicating a reversal of inflammation-induced immune suppression. In HT-29 cells, LPS exposure reduced IFN-γ levels, but ZnPT treatment led to a dose-dependent increase in IFN-γ secretion. Similarly, in SW480 cells, IFN-γ levels decreased after LPS treatment, but ZnPT administration elevated IFN-γ production.

Conclusion: ZnPT can modulate PD-L1 expression and enhance immune responsiveness in the tumor microenvironment. These findings suggest the potential of ZnPT as a therapeutic agent for reversing immune suppression and restoring effective anti-tumor immune responses in colorectal cancer.

INTRODUCTION

Colorectal cancer is a significant global health challenge, characterized by its ability to evade immune surveillance, particularly within an inflammatory tumor microenvironment[1]. The capacity of colorectal cancer cells to modulate the immune response is a critical factor in disease progression, necessitating novel therapeutic strategies that can restore effective anti-tumor immunity [2]. One such mechanism involves the upregulation of Programmed Death-Ligand 1, an immune checkpoint protein that suppresses T cell activity and promotes tumor immune escape, making it a prime target for therapeutic intervention [3]. Current immunotherapies, such as PD-1/PD-L1 inhibitors, have shown efficacy in a subset of colorectal cancer patients, particularly those with microsatellite instability-high tumors; however, a significant proportion of patients do not respond to these treatments, highlighting the need for alternative approaches to enhance anti-tumor immunity [4]. Inflammation, a hallmark of colorectal cancer development, plays a complex role in shaping the tumor microenvironment and influencing immune responses. The chronic inflammatory state can induce the expression of PD-L1, further contributing to immune evasion and resistance to immunotherapy [5].

Zinc Pyrithione, a coordination complex of zinc, has been recognized for its antimicrobial, antifungal, and anti-inflammatory properties, and has demonstrated promising anticancer activity in various malignancies[6]. Recent studies suggest that ZnPT can induce oxidative stress-mediated apoptosis and interfere with oncogenic signaling pathways in cancer cells, indicating its potential as a therapeutic agent. Furthermore, ZnPT has been shown to modulate the expression of genes involved in cell cycle regulation and apoptosis, supporting its role in suppressing tumor growth [7], [8]. The tumor microenvironment, comprising immune cells, stromal elements, cytokines, and signaling molecules, plays a crucial role in cancer progression[9], [10]. Tumor-associated macrophages, tumor-associated

neutrophils, and myeloid-derived suppressor cells are pivotal in influencing the nature of the tumor microenvironment and can serve as both positive and negative mediators of tumor growth [11]. The complex interplay between cancer cells and immune cells within the tumor microenvironment determines the balance between immune-mediated tumor suppression and tumor-induced immune tolerance [12], [13]. Understanding the mechanisms by which cancer cells evade immune detection and suppression is critical for developing effective immunotherapeutic strategies. Therefore, approaches that target both cancer cells and the tumor microenvironment hold great promise for improving treatment outcomes in colorectal cancer. This study aims to elucidate the immunomodulatory effects of ZnPT on colorectal cancer cells within an inflammatory microenvironment, with a focus on PD-L1 regulation and restoration of anti-tumor immune responses.

MATERIALS AND METHODS

Cell Culture and Reagents:

The human colorectal cancer cell lines HT-29 and SW480 were obtained from the American Type Culture Collection and cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. These cell lines were chosen as representative models of colorectal cancer with differing genetic backgrounds and responses to therapy, allowing for a comprehensive evaluation of ZnPT's effects. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO2. Zinc Pyrithione was purchased from Sigma-Aldrich and dissolved in dimethyl sulfoxide to create a stock solution. Lipopolysaccharide was used to induce inflammation in colorectal cancer cells.

PBMC Isolation and Co-culture Experiments:

Peripheral blood mononuclear cells were isolated from healthy donor blood samples using density gradient centrifugation with Ficoll-Paque. PBMCs were then co-cultured with colorectal cancer cells to simulate the tumor microenvironment and assess the impact of ZnPT on immune cell function. The co-culture experiments were designed to evaluate the direct effects of ZnPT on immune cell activation and cytokine production in the presence of tumor cells.

PD-L1 Expression Analysis:

The expression levels of PD-L1 in colorectal cancer cells were measured using flow cytometry. Cells were incubated with a fluorescently labeled anti-PD-L1 antibody, and the fluorescence intensity was quantified using a flow cytometer. Quantitative real-time PCR was used to assess PD-L1 mRNA levels in colorectal cancer cells treated with ZnPT, providing insights into the mechanisms underlying the observed changes in PD-L1 protein expression.

Statistical Analysis:

Data from all experiments were analyzed using GraphPad Prism software. Statistical significance was determined using Student's t-test or ANOVA, with a p-value less than 0.05 considered statistically significant. The results were expressed as mean ± standard deviation, with all experiments independently repeated at least three times to ensure reproducibility and reliability The reagents used in the experiments, including cell culture media, supplements, and antibodies, were carefully selected to ensure optimal cell viability and accurate measurement of PD-L1 expression.

Results

To evaluate the immunomodulatory potential of Zinc Pyrithione (ZnPT) in reversing inflammation-induced immune escape in colorectal cancer (CRC), a series of in vitro experiments were conducted using HT-29 and SW480 cell lines. The effects of ZnPT on PD-L1 expression at both the mRNA and protein levels were analyzed following lipopolysaccharide (LPS) stimulation. Additionally, functional immune responses were assessed using a PBMC co-culture model with IFN-γ quantification. The results are described stepwise below:

1. ZnPT Suppresses LPS-Induced PD-L1 mRNA Expression in CRC Cells

LPS stimulation significantly upregulated PD-L1 mRNA expression in both HT-29 and SW480 cell lines (Figure A and B), confirming successful induction of an inflammation-driven immune escape phenotype. In HT-29 cells, PD-L1 mRNA levels increased from **3.63 ± 0.25** in the untreated control to **6.23 ± 0.35** upon LPS exposure ($p < 0.001$). Following ZnPT treatment at increasing concentrations (0.59, 1.19, and 2.39 μM), PD-L1 mRNA expression was markedly reduced to **2.87 ± 0.25**, **1.87 ± 0.15**, and **0.37 ± 0.46**, respectively ($p < 0.01$ to $p < 0.001$ compared to LPS-treated) (Table1).

Similarly, in SW480 cells, LPS elevated PD-L1 mRNA expression from **3.33 ± 0.30** to **6.23 ± 0.30** ($p < 0.001$). ZnPT treatment at 0.44, 0.88, and 1.76 μM concentrations resulted in a significant dose-dependent suppression of PD-L1 mRNA to **1.96 ± 0.21**, **1.50 ± 0.10**, and **0.78 ± 0.15**, respectively. These reductions were statistically significant and demonstrate the potent transcriptional downregulation of PD-L1 by ZnPT (Table1).

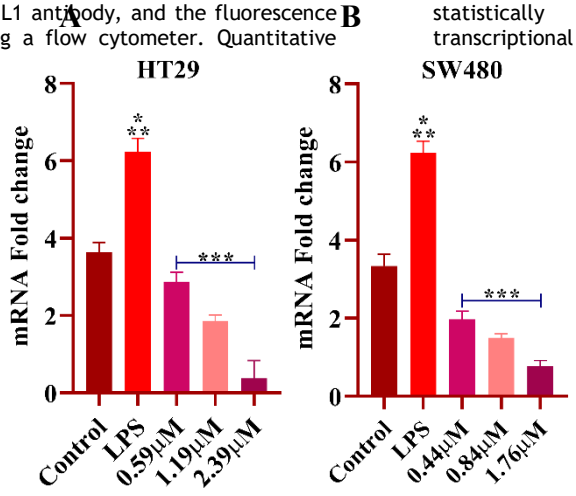

Figure A & B: Zinc Pyrithione suppresses LPS-induced PD-L1 mRNA expression in HT-29 and SW480 cells. Bar graph showing dose-dependent downregulation of PD-L1 mRNA levels in both cell lines following LPS stimulation and ZnPT treatment. Data normalized to control and expressed as mean ± SD. One-way ANOVA used for statistical analysis.

Name of the cell line	(PD-L1) Mean of Fold change Expression ± S.D.				
HT29	Control	LPS	0.59μM	1.19μM	2.39μM
	3.633 ± 0.251	6.233 ± 0.351	2.866 ± 0.251	1.866 ± 0.152	0.373 ± 0.456
SW480	Control	LPS	0.44μM	0.88μM	1.76μM

	3.33 ± 0.305	6.23 ± 0.305	1.96 ± 0.208	1.5 ± 0.1	0.78 ± 0.152

Table1: Quantitative real-time PCR analysis showing relative PD-L1 mRNA levels in HT-29 and SW480 colorectal cancer cells after LPS stimulation and treatment with different concentrations of Zinc Pyrithione. Values are expressed as mean ± SD (n = 3). Statistical analysis performed using one-way ANOVA; p < 0.05 considered significant.

2. ZnPT Inhibits PD-L1 Protein Expression in a Dose-Dependent Manner

Western blot analysis supported the qRT-PCR findings by demonstrating a strong, dose-dependent reduction in PD-L1 protein levels upon ZnPT treatment (Figure C & D). In HT-29 cells, LPS increased PD-L1 protein levels from a normalized control value of **1.00 ± 0.00** to **2.07 ± 0.21** ($p < 0.001$). Treatment with ZnPT at 0.59 µM, 1.19 µM, and 2.39 µM suppressed PD-L1 protein expression to **0.36 ± 0.02**, **0.34 ± 0.05**, and **0.26 ± 0.05**, respectively ($p < 0.01$ to $p < 0.001$ compared to LPS-treated group) (Table2).

In SW480 cells, the LPS-induced increase in PD-L1 protein expression was from **1.00 ± 0.00** (control) to **1.90 ± 0.10** ($p < 0.001$). ZnPT treatment at 0.44 µM, 0.88 µM, and 1.76 µM reduced expression to **0.76 ± 0.05**, **0.45 ± 0.04**, and **0.06 ± 0.03**, respectively (Table2). The strongest suppression was observed at the highest dose, nearly abolishing PD-L1 protein expression, indicative of potent anti-inflammatory and immune-restorative potential. (Figure E & F)

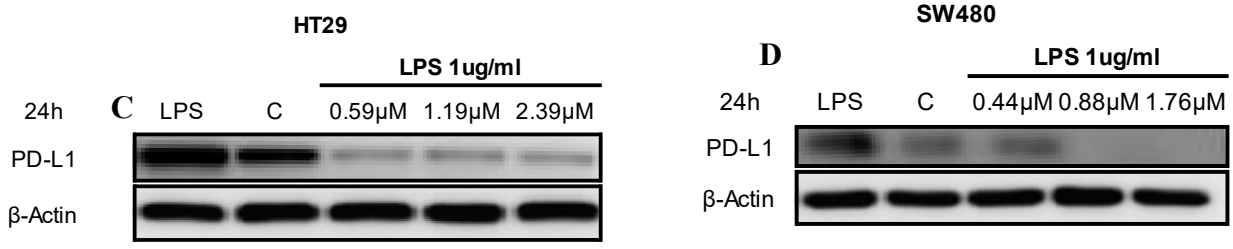

Figure C & D: Western blot image illustrating dose-dependent suppression of PD-L1 protein in SW480 cells post ZnPT treatment. β-Actin confirms equal protein loading across lanes.

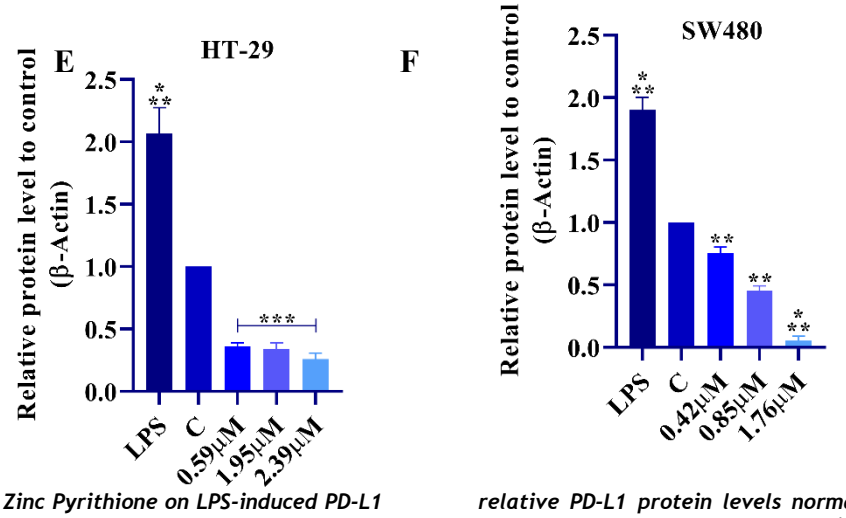

FIGURE E & F: Effect of Zinc Pyrithione on LPS-induced PD-L1 protein levels in HT-29 and SW480 cells. Bar graph representing relative PD-L1 protein expression (normalized to β-Actin) in HT-29 and SW480 cells treated with LPS and various concentrations of ZnPT. Results show a dose-dependent decrease in PD-L1 protein expression.

Table2: Dose-dependent effect of Zinc Pyrithione on LPS-induced PD-L1 protein expression in HT-29 and SW480 cells. Densitometric analysis of Western blot data quantifying relative PD-L1 protein levels normalized to β-Actin. Values represent mean ± SD from three independent experiments. Statistical significance determined by one-way ANOVA

Name of the cell line	(PD-L1) Mean of Relative protein level to control (β-Actin) ± S.D.				
HT29	LPS	Control	0.59µM	1.19µM	2.39µM
	2.066 ± 0.208	1 ± 0	0.364 ± 0.024	0.340 ± 0.049	0.258 ± 0.048
SW480	LPS	Control	0.44µM	0.88µM	1.76µM
	1.9 ± 0.1	1 ± 0	0.756 ± 0.047	0.450 ± 0.042	0.058 ± 0.031

3. ZnPT Restores IFN-γ Secretion in LPS-Suppressed PBMC Co-cultures

To assess the functional restoration of immune activity, IFN-γ secretion was measured in PBMC co-cultures with ZnPT-treated CRC cells. LPS treatment drastically suppressed IFN-γ levels in HT-29 and SW480 co-cultures (Figure G). In HT-29 cells, IFN-γ secretion dropped from **655.7 ± 23.5 pg/mL** (control) to **35 ± 13.2 pg/mL** after LPS exposure ($p < 0.001$). ZnPT treatment reversed this suppression in a dose-dependent manner, increasing IFN-γ secretion to **176.3 ±**

23.5, **366 ± 30.5**, and **845 ± 55 pg/mL** at 0.59, 1.19, and 2.39 µM, respectively ($p < 0.01$ to $p < 0.001$ vs LPS) (Table3).
In the SW480-PBMC co-culture, LPS reduced IFN-γ secretion from **758.7 ± 56.2 pg/mL** to **48 ± 19 pg/mL** ($p < 0.001$). Treatment with ZnPT restored secretion levels to **171 ± 30**, **369 ± 27**, and **1,145 ± 47.7 pg/mL** at 0.44, 0.88, and 1.76 µM concentrations, respectively. The most significant increase was observed at 1.76 µM, exceeding baseline control levels, suggesting ZnPT not only reversed suppression but enhanced immune responsiveness beyond normal levels ($p < 0.001$) (Table3).

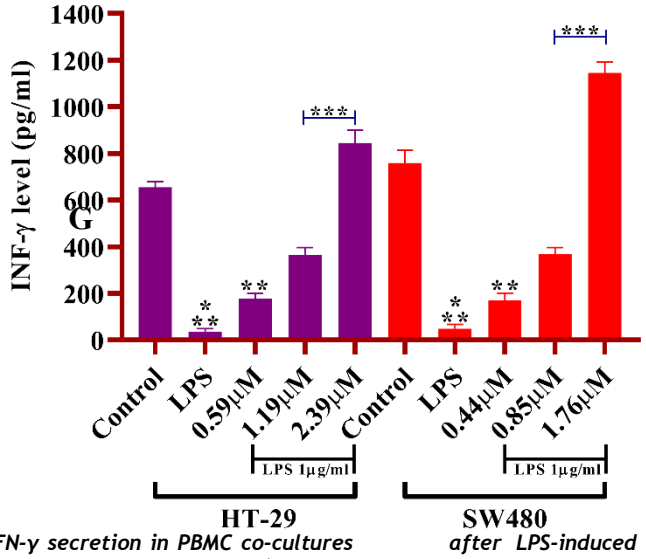

Figure G: Restoration of IFN-γ secretion in PBMC co-cultures following Zinc Pyrithione treatment. Bar graph showing IFN-γ levels measured by ELISA in PBMC co-culture supernatants after LPS-induced suppression and ZnPT treatment. Data demonstrate ZnPT-induced restoration of immune function in a concentration-dependent manner.

Table3: Effect of Zinc Pyrithione on IFN-γ secretion in PBMC co-cultures with LPS-stimulated HT-29 and SW480 cells. ELISA-based quantification of IFN-γ (pg/mL) secreted in PBMC co-cultures with LPS-treated CRC cells and subsequent Zinc Pyrithione treatment. Data expressed as mean ± SD (n = 3). Significant differences observed across treatment groups (p < 0.05, p < 0.01, *p < 0.001).

Name of the cell line	Mean of INF-γ level (pg/ml) ± S.D.				
HT29	**Control**	**LPS**	**0.59µM**	**1.19µM**	**2.39µM**
	655.7 ± 23.5	35 ± 13.2	176.3 ± 23.5	366 ± 30.51	845 ± 55
SW480	**Control**	**LPS**	**0.44µM**	**0.88µM**	**1.76µM**
	758.7 ± 56.2	48 ± 19	171 ± 30	369 ± 27	1145 ± 47.7

DISCUSSION

The tumor microenvironment plays a pivotal role in colorectal cancer progression, with immune evasion being a critical hallmark[14]. PD-L1 overexpression in cancer cells facilitates immune evasion by binding to PD-1 on T cells, leading to T cell inactivation and suppressed anti-tumor immunity [15]. Tumors exhibiting suppressed interferon-gamma signatures have been shown to display reduced responsiveness to immunotherapeutic interventions, thereby highlighting the critical role of IFN-γ in mediating effective anti-tumor immunity; consequently, therapeutic strategies aimed at restoring IFN-γ expression hold promise for enhancing the efficacy of cancer treatment.[15], [16]. These findings support the further investigation of ZnPT as a potential therapeutic agent for colorectal cancer, particularly in combination with other immunomodulatory or chemotherapeutic drugs. Here, the capacity of ZnPT to modulate PD-L1 expression and enhance anti-tumor immune responses provides a rationale for its use as an adjunct therapy in colorectal cancer. Targeted therapies that interfere with the signalosome of PD-L1 with small molecules may provide a more economical alternative than using recombinant antibodies [17]. While the canonical function of PD-L1 involves the suppression of anti-tumor immune responses, emerging evidence suggests a more complex role for this molecule within the tumor cell itself. Our findings demonstrate that Zinc Pyrithione can reverse this immune suppression by downregulating PD-L1 expression and restoring the secretion of IFN-γ. The multifaceted role of zinc in modulating immune responses underscores its significance in cancer prevention and therapy [18]. Zinc may also act on the conformation of the p53 protein of the tumour cells [19]. The cytotoxic/tumor suppressor effects of zinc provide the opportunity for the development of new chemotherapeutic agents for treatment and prevention of specific cancers [20]. Intriguingly, our study demonstrates that ZnPT markedly attenuates epithelial-mesenchymal transition in HT-29 and SW480 colorectal cancer cell lines when exposed to inflammatory conditions and TGF-ß stimulation, suggesting a potential role in preventing tumor progression and metastasis.
The ability of ZnPT to modulate IFN-γ levels further supports its role in enhancing anti-tumor immunity. Our study has several limitations. The mechanism by which zinc affects the interferon signaling pathway is unknown. First, the experiments were conducted in vitro, and further in vivo studies are needed to validate these findings.
Second, the specific signaling pathways involved in ZnPT-mediated downregulation of PD-L1 need to be further elucidated.

CONCLUSION

In conclusion, our study provides compelling evidence that Zinc Pyrithione can effectively downregulate PD-L1 expression in colorectal cancer cells and restore immune effector function. These findings suggest that ZnPT holds great promise as a novel therapeutic agent for enhancing anti-tumor immunity and improving the efficacy of immunotherapy in colorectal cancer.

REFERENCES

- [1] Y. I. Nussbaum et al., "Current and Prospective Methods for Assessing Anti-Tumor Immunity in Colorectal Cancer," International Journal of Molecular Sciences, vol. 22, no. 9. Multidisciplinary Digital Publishing Institute, p. 4802, Apr. 30, 2021. doi: 10.3390/ijms22094802.
- [2] D. Ciardiello et al., "Immunotherapy of colorectal cancer: Challenges for therapeutic efficacy," Cancer Treatment Reviews, vol. 76. Elsevier BV, p. 22, May 04, 2019. doi: 10.1016/j.ctrv.2019.04.003.
- [3] V. Prima, L. N. Kaliberova, S. A. Kaliberov, D. T. Curiel, and S. Kusmartsev, "COX2/mPGES1/PGE 2 pathway regulates PD-L1 expression in tumor-associated macrophages and myeloid-derived suppressor cells," Proceedings of the National Academy of Sciences, vol. 114, no. 5, p. 1117, Jan. 2017, doi: 10.1073/pnas.1612920114.
- [4] D. S. Chen, B. Irving, and F. S. Hodi, "Molecular Pathways: Next-Generation Immunotherapy—Inhibiting Programmed Death-Ligand 1 and Programmed Death-1," Clinical Cancer Research, vol. 18, no. 24, p. 6580, Oct. 2012, doi: 10.1158/1078-0432.ccr-12-1362.
- [5] Y. Jiang, X. Zhao, J. Fu, and H. Wang, "Progress and Challenges in Precise Treatment of Tumors With PD-1/PD-L1 Blockade," Frontiers in Immunology, vol. 11. Frontiers Media, Mar. 12, 2020. doi: 10.3389/fimmu.2020.00339.
- [6] M. Chen et al., "Anti-tumour activity of zinc ionophore pyrithione in human ovarian cancer cells through inhibition of proliferation and migration and promotion of lysosome-mitochondrial apoptosis," Artificial Cells Nanomedicine and Biotechnology, vol. 48, no. 1, p. 824, Jan. 2020, doi: 10.1080/21691401.2020.1770266.
- [7] S. Scavo and V. Oliveri, "Zinc ionophores: chemistry and biological applications," Journal of Inorganic Biochemistry, vol. 228. Elsevier BV, p. 111691, Dec. 09, 2021. doi: 10.1016/j.jinorgbio.2021.111691.
- [8] C. Zhao et al., "Repurposing an antidandruff agent to treating cancer: zinc pyrithione inhibits tumor growth via targeting proteasome- associated deubiquitinases." Jan. 10, 2017.
- [9] P. Pęczek, M. Gajda, K. Rutkowski, M. Fudalej, A. Deptała, and A. M. Badowska-Kozakiewicz, "Cancer-associated inflammation: pathophysiology and clinical significance." 2023.
- [10] K. B. Kennel and F. R. Greten, "Immune cell - produced ROS and their impact on tumor growth and metastasis," Redox Biology, vol. 42. Elsevier BV, p. 101891, Feb. 06, 2021. doi: 10.1016/j.redox.2021.101891.
- [11] C. J. Perry et al., "Myeloid-targeted immunotherapies act in synergy to induce inflammation and antitumor immunity," The Journal of Experimental Medicine, vol. 215, no. 3, p. 877, Feb. 2018, doi: 10.1084/jem.20171435.
- [12] D. O. Croci and M. Salatino, "Tumor Immune Escape Mechanisms that Operate During Metastasis," Current Pharmaceutical Biotechnology, vol. 12, no. 11. Bentham Science Publishers, p. 1923, Nov. 01, 2011. doi: 10.2174/138920111798376987.
- [13] R. Kim, M. Emi, and K. Tanabe, "Cancer cell immune escape and tumor progression by exploitation of anti-inflammatory and pro-inflammatory responses," Cancer Biology & Therapy, vol. 4, no. 9. Taylor & Francis, p. 924, Sep. 01, 2005. doi: 10.4161/cbt.4.9.2101.
- [14] M. Cirone, A. Garufi, L. D. Renzo, M. Granato, A. Faggioni, and G. D'Orazi, "Zinc supplementation is required for the cytotoxic and immunogenic effects of chemotherapy in chemoresistant p53-functionally deficient cells," OncoImmunology, vol. 2, no. 9, Sep. 2013, doi: 10.4161/onci.26198.
- [15] J. Cha, L.-C. Chan, C. Li, J. L. Hsu, and M. Hung, "Mechanisms Controlling PD-L1 Expression in Cancer," Molecular Cell, vol. 76, no. 3. Elsevier BV, p. 359, Oct. 24, 2019. doi: 10.1016/j.molcel.2019.09.030.
- [16] L. F. Mager, M.-H. Wasmer, T. T. Rau, and P. Krebs, "Cytokine-Induced Modulation of Colorectal Cancer," Frontiers in Oncology, vol. 6. Frontiers Media, Apr. 19, 2016. doi: 10.3389/fonc.2016.00096.
- [17] D. Escors et al., "The intracellular signalosome of PD-L1 in cancer cells," Signal Transduction and Targeted Therapy, vol. 3, no. 1. Springer Nature, Sep. 21, 2018. doi: 10.1038/s41392-018-0022-9.
- [18] D. Skrajnowska and B. Bobrowska-Korczak, "Role of Zinc in Immune System and Anti-Cancer Defense Mechanisms," Nutrients, vol. 11, no. 10. Multidisciplinary Digital Publishing Institute, p. 2273, Sep. 22, 2019. doi: 10.3390/nu11102273.
- [19] D. K. Dhawan and V. D. Chadha, "Zinc: a promising agent in dietary chemoprevention of cancer.," PubMed, Dec. 2010, Accessed: Feb. 2025. [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/21245614
- [20] L. C. Costello and R. Franklin, "Cytotoxic/tumor suppressor role of zinc for the treatment of cancer: an enigma and an opportunity," Expert Review of Anticancer Therapy, vol. 12, no. 1. Taylor & Francis, p. 121, Dec. 08, 2011. doi: 10.1586/era.11.190.