Medicine Personalized JOURNAL

Medical Journal/9 Year/No.32/500000 Rials/2024 Winter/ISSN 2717-3860

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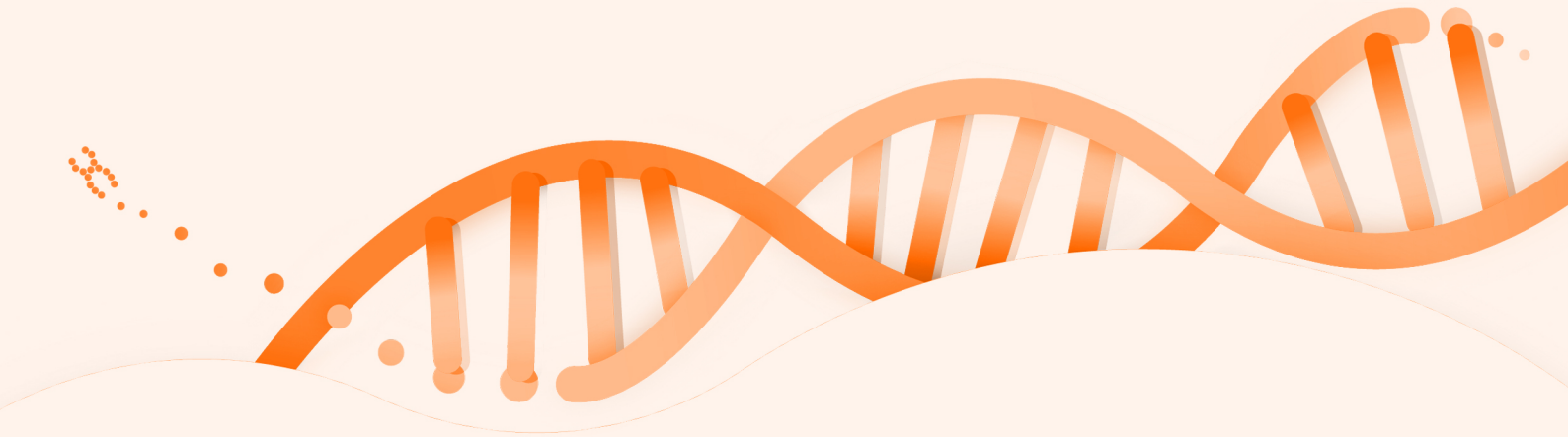

Journal Information

Name: Personalized Medicine Journal
Abbreviated Name: PMJ
Date of First Issue Published: February 2019
Concessionaire: AmitisGen TECH Dev Group
Release Period: Quarterly

Editorial Board Information

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Personalized Medicine Journal
Winter 2024,Volume9,Issue 32

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http://pmjournal.ir Review Article

Winter 2024, Volume 9, Issue 32 (1-7)

New Developments in Cancer Treatment Using CAR T cell Therapy, a Kind of Gene Therapy

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DOI: 10.22034/pmj.2024.2024374.1034

Submitted: 2023-12-06
Accepted: 2024-02-20

Keywords:
Chimeric antigen receptor
Solid tumors
Immunotherapy
Cancer

How to Cite this Article:

A. Sohrabi, M. Saremi. "New Developments in Cancer Treatment Using CAR T cell Therapy, a Kind of Gene Therap " Personalized Medicine Journal, Vol. 9, no. 32, pp. 1- 7.

Abstract:

Recent research has pinpointed cancer as the primary cause of death on a global scale. Various traditional medications and cytotoxic immunotherapies have been established and are now accessible on the market. Given the intricate nature of tumor activity and the multitude of genetic and cellular elements implicated in the development and spread of cancers, it is imperative to create a highly effective immunotherapy that can specifically target tumors at both the cellular and genetic levels. In the clinical context, cancer immunotherapy is growing more and more significant, particularly for tumors that are resistant to traditional chemotherapy and targeted treatments. Chimeric antigen receptor (CAR) T cell therapy is a new method of modifying T cells taken from a patient's blood in a laboratory setting. These modified T cells are created to express artificial receptors that specifically target a particular tumor antigen. These specifically recognize the tumor antigen without the participation of the major histocompatibility complex. The use of CAR therapy has the promise of providing a prompt and more secure treatment regimen for both non-solid and solid malignancies. This study provides a comprehensive analysis of the benefits and progress made in CAR immunotherapy.

INTRODUCTION

Immunological checkpoints are natural mechanisms that impede the immune system's ability to recognize and eliminate normal cells inside the organism. Cancerous cells use this defense route to avoid the detrimental effects of the immune system's activities. If tumor cells cannot use these immunological barriers for defense, the immune system can target and remove them (1). Immune checkpoint blockers are a specific kind of medication created specifically to treat solid tumor malignancies. These medications hinder the function of immunological checkpoints, hence enhancing immune reactions to cancerous growths, namely T-cell reactions (2). Multiple trials have shown the efficacy of immune checkpoint blockade treatment in eliminating tumours. Nevertheless, this treatment has limits since it has been seen to not produce a sufficient amount of tumor-reactive T cells. Furthermore, the responses to tumors that are often activated have limited efficacy, and the generation of memory T cells is inadequate(3).

Adoptive cellular therapy (ACT) is a kind of cancer immunotherapy where cells are taken from an individual or someone else, altered in a lab, and then given back to the recipient (4). ACT has shown greater effectiveness when compared to immune checkpoint blockade therapy, namely in terms of increasing the number of T-cells that fight against tumours and triggering specific immune responses. The progress of modern CAR-T treatment relies on the knowledge and understanding acquired from first ACT methods (5). Research indicates that the ability of T cells to fight tumors is greatly reduced by the suppressive microenvironment of tumors. Subsequently, the concept of isolating, cultivating, and transferring these infiltrating cells was suggested as a potential medicinal therapy. This method has shown to be successful in eliminating several forms of cancer in both experimental and clinical trials (6). CD8+ T cells possess the potential to produce proinflammatory cytokines when they are activated. Proinflammatory cytokines has the capacity to inflict enduring damage on malignant cells. CD4+ T lymphocytes are essential for stimulating B cells to produce antibodies, particularly when they differentiate into plasma cells that generate antibodies. Furthermore, these cells have an essential

part in initiating CD8+ T cell immune responses (7, 8).

Furthermore, it has been shown that the application of interleukin (IL)-2, whether administered systematically or used to grow TILs outside the body, might augment the effectiveness of ex vivo-expanded TILs in fighting against tumors (9). Subsequently, it was shown that the combination of lymphodepletion chemotherapy with ACT may augment the in vivo proliferation and long-term presence of T-cells in individuals with solid tumors or blood cancers (10, 11). CAR-T cells are T cells that have been manipulated genetically to express chimeric antigen receptors (CARs). CARs are synthetic receptors made up of an antigen-binding region derived from the single-chain variable fragment (scFv) of a monoclonal antibody (mAb), linked to an intracellular T-cell activation signaling domain and one or two co-stimulatory domains. Chimeric antigen receptors (CARs) may guide genetically modified T lymphocytes to cancerous cells expressing a specific specific epitope (12, 13). Hence, this article focuses on the introduction of CAR-T cell-based treatment and examines the most recent strategies to overcome the significant challenges that hinder the effectiveness of CAR-T therapy in different tumours.

Overview of CAR-T treatment

Immunotherapy with CAR has been shown to be an effective treatment for several types of malignancy. This therapeutic method is a novel kind of gene therapy that directs T lymphocytes to eradicate cancer cells (14). The initial part of this therapy comprises leukapheresis, which is the procedure of separating an individual's peripheral blood. Apheresis is a frequently used method for removing blood from humans and separating it into its component components, which are then genetically changed before being returned into the patient's system. Blood banks have used apheresis to collect platelets and other blood components for the treatment of many conditions, including hematologic and renal diseases. Therefore, it is seen as a wise strategy for both those in excellent physical condition and those who are unwell (14). The CAR is composed of four elements: a single-chain variable fragment (scFv) serving as the extracellular domain for binding to the target, a spacer domain, a intermembrane domain, and an intracellular area responsible for signalling and activation. When comparing CAR T cells to T cell receptor (TCR) modified cells, CAR T cells have the capability to recognize tumour antigens located on the cell surface independently of HLA molecules. This leads to the stimulation, proliferation, and production of cytokines by antigen-specific T cells, enabling them to combat tumours. CARs has the capacity to recognize different antigens without relying on major histocompatibility complex (MHC) molecules. This characteristic expands their potential use in therapeutic contexts (14).

CAR Architecture

CARs are artificial receptors with a modular structure including four primary elements: (1) an extracellular domain that binds to target antigens, (2) a flexible area that allows movement, (3) a domain that spans the cell membrane, and (4) several domains responsible for intracellular signaling. In this discussion, we will examine the existing ideas that form the foundation of CAR architecture (15).

Antigen binding domain

The antigen binding domain is the specific region of the CAR that provides specificity for the target antigen of interest. Conventionally, the antigen-binding portions are derived from the variable heavy (VH) and light (VL) chains of monoclonal antibodies. Subsequently, these areas are interconnected using a pliable linker to form a unified single-chain variable fragment (scFv) (16). Typically, the single-chain variable fragments (scFvs) present in CARs are engineered to specifically identify cancer antigens located on the external membrane of cells. This recognition triggers the activation of T lymphocytes, bypassing the need for major histocompatibility complex (MHC) molecules. Nevertheless, there have been documented instances of CARs that possess the ability to identify tumor-associated antigens inside cells, using a method like to that of TCRs and necessitating MHC molecules. Several characteristics of the single-chain variable fragment (scFv) have a significant impact on the function of CARs beyond their basic ability to recognize and bind the target epitope. The affinity of CARs plays a vital role in defining their activity since it directly impacts the ability of the antigen-binding domain to bind. For effective detection of antigens on tumor cells, activation of CAR signaling, and T cell activation, CARs must possess an elevated specificity for binding to antigens. Nevertheless, it is important to note that an excessively strong affinity should be avoided, as it might result in the demise of CAR-expressing T cells owing to activation-induced processes and give rise to toxicity (17, 18).

Hinge region

The hinged or spacers area is the extracellular architectural region that links the bound domains to the transmembrane domain. The hinge functions give flexible for overcoming steric hindrance and aid in extending the antigen-binding domain to reach the specific epitope. The chosen hinge significantly affects the operation of CARs due to variations in the size and composition of the hinge area (19). These alterations may impact adaptability, CARs formation, signaling, epitope recognition, activation intensity,

and epitope recognition. Additionally, it has been suggested that the length of the gap is crucial for maintaining a sufficient distance between cells, which is necessary for the development of an immunological connection. The ideal separator dimensions depends on the precise location of the targeted epitope and the level of obstruction on the target cell. Increased spacer length provides enhanced adaptability and improved reachability for epitopes in proximity to the cellular membrane or exhibiting intricate glycosylation patterns. Conversely, shorter spacers demonstrate greater efficacy in binding epitopes that are located at a greater distance from the cell membrane (20).

Transmembrane domain

The transmembrane domain is the most enigmatic component among all the sections of CARs. The fundamental function of the membrane domain is to attach the CAR to the outer surface of the T cell. Nevertheless, there is evidence suggesting that this transmembrane domain may have a role in the functioning of CAR-T cells. Research suggests that the transmembrane domains of CAR have a direct influence on the level and durability of CAR expression. Moreover, they may also participate in signalling or synapse formation and possess the ability to form dimers with endogenous signalling molecules. Most membrane domains are derived from naturally occurring proteins like CD3ζ, CD4, CD8α, or CD28. The study on the influence of different transmembrane domains on CAR function is still inadequate, since the transmembrane domain is often modified to accommodate the requirements of the extracellular spacer region or the intracellular signalling areas. The transmembrane domain of CD3ζ has a crucial function in promoting the activation of T cells via CAR signalling. This is because it plays a crucial role in the process of CAR dimerization and its incorporation into the natural TCRs (21, 22).

Intracellular signaling domain

The first version of CARs, which emerged in the final years of the 1990s, had a signalling domain consisting of either CD3ζ or FcRγ. The stimulation of CAR-T cells in the majority of CARs primarily relies on immunoreceptor tyrosine-based activation motifs generated from CD3ζ. However, relying just on these patterns for signalling is inadequate to stimulate effective T cell responses. The lifetime and durability of these first generation CARs are restricted in vitro (23). The clinical research have substantiated these findings, revealing a low or negligible level of efficacy. The importance of co-stimulation in preserving the durability of CD-19-targeted CAR-T cells was shown via the use of first in in vivo studies of B-cell carcinoma. Introducing a co-stimulatory domain improved the production and multiplication of IL-2 when the antigen was encountered many times (24). Due to the acknowledgment of the importance of co-stimulation in providing durable CAR-T cell therapy, a new version of CARs, referred to as second generation CARs, were created. These CARs are composed of a single co-stimulatory domain linked in a sequential manner with the CD3ζ intracellular signalling domain. Both CD28 and 4-1BB (CD137), which are the two most often approved co-stimulatory motifs by the FDA, have a significant association with heightened levels of patient response. The co-stimulatory domains have unique structural and metabolic features (25, 26).

Antigens used in clinical trials for CAR T-cell therapy targeting solid tumours

EGFRvIII

The rise of CARs has attracted much attention and examination as a result of the favourable results seen from using CD19 CARs in tumour therapy. An in-depth examination is conducted on many antigens linked to tumors to improve the chances of achieving maximum effectiveness. Invasive glioblastoma (GB) is caused by the excessive synthesis of epidermal growth factor receptor (EGFR) and EGFR variant III (EGFRvIII) in different kinds of tumors (27, 28). EGFRvIII presence in a cell often results in cell survival, aggression, formation of new blood vessels, and resistance to both radiation and chemotherapy. EGFRvIII-specific CAR T-cells, which have demonstrated substantial anti-tumor efficacy in preclinical investigations, are now being assessed for inclusion in clinical trials (29). EGFR can maintain a binding site for cetuximab after modifications, however it may lose its domains I and II, as well as its cytoplasmic tail. Consequently, cetuximab has the ability to detect the shortened version of EGFR (huEGFRt), enabling the detection, monitoring, and removal of CAR T-cells that exhibit the truncated EGFR after cetuximab treatment. EGFRvIII is detected in around between 25 and 30% of newly identified GB tumours and is now being examined in clinical trials as a possible therapeutic target for the treatment of GB tumours. Despite the potential for manufacturing cells for therapy and administering them intravenously without complications, two clinical experiments conducted on patients with GB who received treatment with EGFRvIII-targeting CAR T-cells, either co-stimulated solely with 4-1BB or in combination with CD28, did not demonstrate any beneficial effects in radiographic imaging (30, 31).

IL13Rα2

Decreased longevity in people has been connected with a glioma-specific protein called Interleukin 13 receptor α2 (IL13Rα2). A study shown that the application of CAR T-cell treatment led to tumour

decrease. IL13Rα2-specific CARs have the capacity to also identify IL13Rα1 (32). The IL13Rα1-specific scFv is classified as an antigen binding domain, resulting in improved specificity. PET imaging studies show that IL13Rα2-specific CAR T-cells may efficiently migrate to the brain parenchyma, particularly in areas impacted by tumors. Studies enhanced these findings by using an updated design with 4-1-BB co-stimulation (33, 34). The findings demonstrated favourable outcomes for the treatment of GB and a satisfactory degree of tolerance. A different study discovered that although brain and spinal tumors showed a significant decrease in size by therapeutic and PET scans, this improvement was short-lived, lasting around seven months, before the tumors reappeared in other areas (35).

HER2

Studies have shown that human epidermal growth factor receptor 2 (HER2) is excessively produced in different tumors. This overexpression is associated with the development of cancer, indicating that HER2 might be used as a useful indicator for predicting outcomes and as an option for therapy in cancer patients (36). Despite the substantial research conducted in clinical studies on CAR T-cells targeting HER2-expressing tumors, safety concerns have arisen due to the unfortunate death of a colorectal cancer patient who was administered 1×10^{10} third-generation HER2-CAR T-cells (37). Conversely, the phase 1 trial involved administering increasing doses of second-generation HER2-CAR cytomegalovirus (CMV) pp65-bispecific cytotoxic T lymphocytes (CTLs) to 17 patients who had late-stage HER2-positive GB. The trial found that the patients tolerated one or more infusions of 1×10^{8} CTLs well, without experiencing severe toxicities associated to the therapy. Out of the 16 patients who were able to be evaluated, one had a partial response for a duration of over nine months, while seven patients exhibited stable illness that lasted between eight weeks and 29 months. Unfortunately, the status of eight patients worsened after receiving medication. However, the quantities of CAR T-cells in the blood gradually decreased over time, and after 12 months, only two patients still tested positive (38). However, after 18 months, neither patient showed any signs of CAR T-cells, indicating that the HER2-CAR T-cells did not multiply after being administered but managed to survive for almost a year (38, 39).

Difficulties presented by CAR-T cell therapy for solid tumors

Multiple obstacles hinder the use of CAR-T cell therapy in treating solid tumors. Initially, there is a restricted variety of antigens that may be precisely aimed at. Moreover, solid tumors often show varied expression of these antigens. Furthermore, the configuration of the CAR itself presents difficulties. Additionally, there are difficulties linked to the manufacturing of CAR-T cells (40). Furthermore, the capacity of CAR-T cells to efficiently detect and penetrate tumour tissue is inadequate. Furthermore, the presence of an immunosuppressive milieu inside the tumour creates an obstacle. Therefore, there is a pressing need for innovative therapeutic approaches that use lower dosages of CAR-T cells, including highly effective CARs and combination therapies. This section outlines the difficulties linked to CAR-T cell treatment for solid tumors, mostly caused by internal and external variables affecting T cells (40).

Native T-Cell factors

T cell exhaustion

Prolonged activation of effector T cells by receptor-mediated stimulation may induce their differentiation into an exhausted state, leading to decreased longevity of T cells and heightened expression of inhibitory receptors (41). Modifying the CD28 co-stimulatory domain to reduce its activity has been shown to improve the effectiveness of CAR-T cells in organisms. Evidence indicates that CD28 signaling boosts the glycolytic process and promotes the effector memory characteristics, whereas 41BB signaling may improve the oxidative phosphorylation process and sustain the primary memory state (42). The injection of the third-generation CAR, which combines CD28 and 41BB, resulted in a more vigorous proliferation compared to the second-generation CAR that simply utilizes CD28. The density of chimeric antigen receptors on T cells, or on a particular subset of T cells, might influence both the efficacy and possible adverse consequences. Studies indicate that the immunological contact between CAR-T cells and target cells is crucial for the functionality of these cells. Remarkably, the strong attraction of CAR-T cells to certain antigens allows them to identify very little amounts of these antigens. However, this characteristic also raises the possibility of harmful effects on healthy cells around the tumor (43, 44).

CAR-T cell toxicity

The intricacies of CAR-T cell treatment may be categorized under the following groups: Cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) affect the targeted cells and unintended organs due to CAR-T cell therapy, leading to cardiotoxicity and hypersensitivity responses (45). Severe CRS is caused by multiple organ failure, coagulation problems, and respiratory system impairment, presenting a severe danger. ICANS, or immune effector cell-associated neurotoxicity condition, may occur in individuals undergoing CAR-T cell treatment, with a prevalence

between 0% and 50% (46). The symptoms of ICANS include hallucinations, encephalopathy, seizures, and cerebral edema. To reduce these negative occurrences, monoclonal antibodies aimed at the IL-6 receptor, IL-1 receptor antagonist, or granulocyte-macrophage colony-stimulating factor (GM-CSF) might be used. Tocilizumab is licensed for treating CRS, although anakinra and lenzilumab have demonstrated effectiveness in treating both CRS and neurotoxicity. Evaluating and forecasting possible hazards before treatment is still difficult to determine (47).

CAR T cell therapy in conjunction with vaccinations

Therapeutic cancer vaccines are a significant breakthrough in cancer therapy since they specifically activate T cells to identify and eliminate cancerous growths. Several studies have shown that dual-specific T cells, which possess tumor-specific chimeric antigen receptors (CARs) as well as their own T cell receptors (TCRs), display strong expansion and dramatic anti-cancer effects when stimulated with a vaccination containing highly effective immunogens, such as influenza viruses [48]. Preliminary experiments have shown tremendous potential in using virus-specific cytotoxic T lymphocytes (CTLs) for the development of CAR T cells. Notably, in patients with neuroblastoma, cytotoxic T lymphocytes (CTLs) that exclusively target Epstein-Barr virus (EBV) and express GD2 CARs showed a longer period of being active compared to non-virus-specific T cells that were activated (48).

Vaccination offers an alternate approach to enhance the proliferation, activation, and effectiveness of CAR T cells in targeting particular cells inside the body. A commonly used kind of immunization is a viral-based vaccine, such a cytomegalovirus (CMV)-based vaccine, which, when combined with T cell infusion, significantly augments tumour eradication. The administration of a viral vaccine containing gp100 led to enhanced proliferation of T cells and decreased tumour growth in several mouse models (49). A recent study conducted on individuals with B cell acute lymphoblastic leukemia (B-ALL) has shown that viral vaccinations may be used to stimulate natural TCRs, even in the absence of lymphodepletion. This activation enables the efficient proliferation and sustained preservation of CD19-modified virus-specific T lymphocytes. However, the reactivation of viruses and the presence of viruses in the circulation pose possible risks, which necessitates a more comprehensive evaluation of the safety of viral vaccines (50).

Studies conducted in both preclinical and clinical settings have shown that cancer vaccines containing soluble tumor-associated antigens (TAAs) and dendritic cell (DC) adjuvants may successfully activate TAA-specific effector cells and stimulate the production of antibodies. Dendritic cells (DCs) are highly specialized immune cells that have a vital function in both innate and adaptive immunity. They are crucial for the efficacy of immunotherapy. A study showcased the enhanced stimulation, reproduction, and anti-tumor effect of ACT by the implementation of a DC vaccine in live organisms (51). Participants in a melanoma clinical trial received injections of dendritic cells with tumour antigens, followed by the infusion of T lymphocytes that had infiltrated the tumour. Consequently, one man had complete remission while two others maintained a stable state of sickness (52).

THE WAY FORWARD AND CONCLUSION

The realm of synthetic biology and cell engineering offers boundless potential, serving as a basis for the development of groundbreaking pharmaceuticals. Ongoing attempts are being made to create creative strategies to enhance the effectiveness of CAR T-cell treatment. Potential engineering strategies may boost CAR T-cell biological sciences, expanding the method's use in cancer therapy. Enhancing the intricacy of CAR architectures and altering the genes on T-cells might heighten the possible hazards linked to CAR T-cell treatment. Both gene editing and viral transmission technologies include the danger of unintentionally modifying genes that are not the intended target (53).

A targeted insertion of the CAR gene occurred at the TET2 locus, leading to the proliferation of T-cells in a clonal manner. Following that, the clonal population of T-cells saw a natural decrease, emphasizing the possible hazards linked to the use of genetically-modified cells for patient therapy (54, 55). A pilot clinical study has started to evaluate a genetically modified T-cell product that has a transgenic TCR engineered to target NY-ESO-1. This product has been genetically modified utilizing the CRISPR-Cas9 technology. Ongoing monitoring of obstacles linked to gene-editing in trials of CAR T-cell products will aid in detecting potential long-term dangers connected with the emerging field of gene-editing in medicine and assist in devising viable solutions for these issues. The high manufacturing cost of CAR T-cells may rise further because of the use of innovative technological methods. Techniques include using non-viral vectors may reduce manufacturing costs and perhaps enhance affordability. Due to the shorter duration of clinical trials for CAR T-cells in comparison to other medications, it is likely that many CARs may get approval for different illnesses over the next 5-10 years.

REFERENCES

1.Safarzadeh Kozani, P., Safarzadeh Kozani, P., Ahmadi Najafabadi, M., Yousefi, F., Mirarefin, S. M. J., & Rahbarizadeh, F. (2022). Recent advances in solid tumor CAR-T cell therapy: driving tumor cells from hero to zero?. *Frontiers in Immunology*, *13*, 795164.

2.Pardoll, D. M. (2012). The blockade of immune checkpoints

in cancer immunotherapy. *Nature reviews cancer*, *12*(4), 252-264.
3.Jenkins, R. W., Barbie, D. A., & Flaherty, K. T. (2018). Mechanisms of resistance to immune checkpoint inhibitors. *British journal of cancer*, *118*(1), 9-16.
4.Rohaan, M. W., Wilgenhof, S., & Haanen, J. B. (2019). Adoptive cellular therapies: the current landscape. *Virchows Archiv*, *474*, 449-461.
5.Rosenberg, S. A., Spiess, P., & Lafreniere, R. (1986). A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. *Science*, *233*(4770), 1318-1321.
6.Borst, J., Ahrends, T., Bąbała, N., Melief, C. J., & Kastenmüller, W. (2018). CD4+ T cell help in cancer immunology and immunotherapy. *Nature Reviews Immunology*, *18*(10), 635-647.
7.Whiteside, T. L. (2008). The tumor microenvironment and its role in promoting tumor growth. *Oncogene*, *27*(45), 5904-5912.
8.Baxevanis, C. N., Voutsas, I. F., Tsitsilonis, O. E., Gritzapis, A. D., Sotiriadou, R., & Papamichail, M. (2000). Tumor-specific CD4+ T lymphocytes from cancer patients are required for optimal induction of cytotoxic T cells against the autologous tumor. *The Journal of Immunology*, *164*(7), 3902-3912.
9.Umeshappa, C. S., Nanjundappa, R. H., Xie, Y., Freywald, A., Xu, Q., & Xiang, J. (2013). Differential requirements of CD 4+ T-cell signals for effector cytotoxic T-lymphocyte (CTL) priming and functional memory CTL development at higher CD 8+ T-cell precursor frequency. *Immunology*, *138*(4), 298-306.
10.Eberlein, T. J., Rosenstein, M., & Rosenberg, S. A. (1982). Regression of a disseminated syngeneic solid tumor by systemic transfer of lymphoid cells expanded in interleukin 2. *The Journal of experimental medicine*, *156*(2), 385-397.
11.Rosenberg, S. A., Packard, B. S., Aebersold, P. M., Solomon, D., Topalian, S. L., Toy, S. T., ... & White, D. E. (1988). Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. *New England Journal of Medicine*, *319*(25), 1676-1680.
12.June, C. H., O'Connor, R. S., Kawalekar, O. U., Ghassemi, S., & Milone, M. C. (2018). CAR T cell immunotherapy for human cancer. Science, 359(6382), 1361-1365.
13.Park, J. H., Rivière, I., Gonen, M., Wang, X., Sénéchal, B., Curran, K. J., ... & Sadelain, M. (2018). Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. *New England Journal of Medicine*, *378*(5), 449-459.
14.Mohanty, R., Chowdhury, C. R., Arega, S., Sen, P., Ganguly, P., & Ganguly, N. (2019). CAR T cell therapy: A new era for cancer treatment. *Oncology reports*, *42*(6), 2183-2195.
15.Sterner, R. C., & Sterner, R. M. (2021). CAR-T cell therapy: current limitations and potential strategies. *Blood cancer journal*, *11*(4), 69.
16.Chailyan, A., Marcatili, P., & Tramontano, A. (2011). The association of heavy and light chain variable domains in antibodies: implications for antigen specificity. *The FEBS journal*, *278*(16), 2858-2866.
17.Liu, X., Jiang, S., Fang, C., Yang, S., Olalere, D., Pequignot, E. C., ... & Zhao, Y. (2015). Affinity-tuned ErbB2 or EGFR chimeric antigen receptor T cells exhibit an increased therapeutic index against tumors in mice. *Cancer research*, *75*(17), 3596-3607.
18.Huang, R., Li, X., He, Y., Zhu, W., Gao, L., Liu, Y., ... & Zhang, X. (2020). Recent advances in CAR-T cell engineering. *Journal of Hematology & Oncology*, *13*(1), 1-19.
19.Hirobe, S., Imaeda, K., Tachibana, M., & Okada, N. (2022). The effects of chimeric antigen receptor (CAR) hinge domain post-translational modifications on CAR-T cell activity. *International Journal of Molecular Sciences*, *23*(7), 4056.
20.Xu, H., Hamburger, A. E., Mock, J. Y., Wang, X., Martin, A. D., Tokatlian, T., ... & Kamb, A. (2020). Structure-function relationships of chimeric antigen receptors in acute T cell responses to antigen. *Molecular Immunology*, *126*, 56-64.
21.Bridgeman, J. S., Hawkins, R. E., Bagley, S., Blaylock, M., Holland, M., & Gilham, D. E. (2010). The optimal antigen response of chimeric antigen receptors harboring the CD3ζ transmembrane domain is dependent upon incorporation of the receptor into the endogenous TCR/CD3 complex. *The Journal of Immunology*, *184*(12), 6938-6949.
22.Guedan, S., Posey Jr, A. D., Shaw, C., Wing, A., Da, T., Patel, P. R., ... & June, C. H. (2018). Enhancing CAR T cell persistence through ICOS and 4-1BB costimulation. *JCI insight*, *3*(1).
23.Wu, L., Wei, Q., Brzostek, J., & Gascoigne, N. R. (2020). Signaling from T cell receptors (TCRs) and chimeric antigen receptors (CARs) on T cells. *Cellular & Molecular Immunology*, *17*(6), 600-612.
24.Wu, W., Zhou, Q., Masubuchi, T., Shi, X., Li, H., Xu, X., ... & Xu, C. (2020). Multiple signaling roles of CD3ε and its application in CAR-T cell therapy. *Cell*, *182*(4), 855-871.
25.Maher, J., Brentjens, R. J., Gunset, G., Rivière, I., & Sadelain, M. (2002). Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRζ/CD28 receptor. *Nature biotechnology*, *20*(1), 70-75.
26.Kawalekar, O. U., O'Connor, R. S., Fraietta, J. A., Guo, L., McGettigan, S. E., Posey, A. D., ... & June, C. H. (2016). Distinct signaling of coreceptors regulates specific metabolism pathways and impacts memory development in CAR T cells. *Immunity*, *44*(2), 380-390.
27.Daei Sorkhabi, A., Mohamed Khosroshahi, L., Sarkesh, A., Mardi, A., Aghebati-Maleki, A., Aghebati-Maleki, L., & Baradaran, B. (2023). The current landscape of CAR T-cell therapy for solid tumors: Mechanisms, research progress, challenges, and counterstrategies. *Frontiers in immunology*, *14*, 1113882.
28.Tang, C. K., Gong, X. Q., Moscatello, D. K., Wong, A. J., & Lippman, M. E. (2000). Epidermal growth factor receptor vIII enhances tumorigenicity in human breast cancer. *Cancer research*, *60*(11), 3081-3087.
29.Johnson, L. A., Scholler, J., Ohkuri, T., Kosaka, A., Patel, P. R., McGettigan, S. E., ... & Maus, M. V. (2015). Rational development and characterization of humanized anti–EGFR variant III chimeric antigen receptor T cells for glioblastoma. *Science translational medicine*, *7*(275), 275ra22-275ra22.
30.O'Rourke, D. M., Nasrallah, M. P., Desai, A., Melenhorst, J. J., Mansfield, K., Morrissette, J. J., ... & Maus, M. V. (2017). A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. *Science*

translational medicine, *9*(399), eaaa0984.
31.Goff, S. L., Morgan, R. A., Yang, J. C., Sherry, R. M., Robbins, P. F., Restifo, N. P., ... & Rosenberg, S. A. (2019). Pilot trial of adoptive transfer of chimeric antigen receptor transduced T cells targeting EGFRvIII in patients with glioblastoma. *Journal of immunotherapy (Hagerstown, Md.: 1997)*, *42*(4), 126.
32.Brown, C. E., Alizadeh, D., Starr, R., Weng, L., Wagner, J. R., Naranjo, A., ... & Badie, B. (2016). Regression of glioblastoma after chimeric antigen receptor T-cell therapy. *New England Journal of Medicine*, *375*(26), 2561-2569.
33.Krenciute, G., Krebs, S., Torres, D., Wu, M. F., Liu, H., Dotti, G., ... & Gottschalk, S. (2016). Characterization and functional analysis of scFv-based chimeric antigen receptors to redirect T cells to IL13Rα2-positive glioma. *Molecular Therapy*, *24*(2), 354-363.
34.Keu, K. V., Witney, T. H., Yaghoubi, S., Rosenberg, J., Kurien, A., Magnusson, R., ... & Gambhir, S. S. (2017). Reporter gene imaging of targeted T cell immunotherapy in recurrent glioma. *Science translational medicine*, *9*(373), eaag2196.
35.Brown, C. E., Badie, B., Barish, M. E., Weng, L., Ostberg, J. R., Chang, W. C., ... & Jensen, M. C. (2015). Bioactivity and safety of IL13Rα2-redirected chimeric antigen receptor CD8+ T cells in patients with recurrent glioblastoma. *Clinical cancer research*, *21*(18), 4062-4072.
36.Vranić, S., Bešlija, S., & Gatalica, Z. (2021). Targeting HER2 expression in cancer: New drugs and new indications. *Bosnian journal of basic medical sciences*, *21*(1), 1.
37.Morgan, R. A., Yang, J. C., Kitano, M., Dudley, M. E., Laurencot, C. M., & Rosenberg, S. A. (2010). Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Molecular therapy*, *18*(4), 843-851.
38.Daei Sorkhabi, A., Sarkesh, A., Saeedi, H., Marofi, F., Ghaebi, M., Silvestris, N., ... & Brunetti, O. (2022). The basis and advances in clinical application of cytomegalovirus-specific cytotoxic T cell immunotherapy for glioblastoma multiforme. *Frontiers in Oncology*, *12*, 818447.
39.Vitanza, N. A., Johnson, A. J., Wilson, A. L., Brown, C., Yokoyama, J. K., Künkele, A., ... & Park, J. R. (2021). Locoregional infusion of HER2-specific CAR T cells in children and young adults with recurrent or refractory CNS tumors: an interim analysis. *Nature medicine*, *27*(9), 1544-1552.
40.Chung, H., Jung, H., & Noh, J. Y. (2021). Emerging approaches for solid tumor treatment using CAR-T cell therapy. *International journal of molecular sciences*, *22*(22), 12126.
41.Ghajari, G., Heydari, A., & Ghorbani, M. (2023). Mesenchymal stem cell-based therapy and female infertility: limitations and advances. *Current Stem Cell Research & Therapy*, *18*(3), 322-338.
42.Piri-Gharaghie, T., Ghajari, G., Hassanpoor, M., Jegargoshe-Shirin, N., Soosanirad, M., Khayati, S., ... & Mirzaei, A. (2023). Investigation of antibacterial and anticancer effects of novel niosomal formulated Persian Gulf Sea cucumber extracts. *Heliyon*, *9*(3).
43.Morgan, R. A., Yang, J. C., Kitano, M., Dudley, M. E., Laurencot, C. M., & Rosenberg, S. A. (2010). Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Molecular therapy*, *18*(4), 843-851.
44.Ramos, C. A., Rouce, R., Robertson, C. S., Reyna, A., Narala, N., Vyas, G., ... & Dotti, G. (2018). In vivo fate and activity of second-versus third-generation CD19-specific CAR-T cells in B cell non-Hodgkin's lymphomas. *Molecular Therapy*, *26*(12), 2727-2737.
45.Sun, S., Hao, H., Yang, G., Zhang, Y., & Fu, Y. (2018). Immunotherapy with CAR-modified T cells: toxicities and overcoming strategies. *Journal of immunology research*, *2018*.
46.Norelli, M., Camisa, B., Barbiera, G., Falcone, L., Purevdorj, A., Genua, M., ... & Bondanza, A. (2018). Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells. *Nature medicine*, *24*(6), 739-748.
47.Sterner, R. M., Sakemura, R., Cox, M. J., Yang, N., Khadka, R. H., Forsman, C. L., ... & Kenderian, S. S. (2019). GM-CSF inhibition reduces cytokine release syndrome and neuroinflammation but enhances CAR-T cell function in xenografts. *Blood, The Journal of the American Society of Hematology*, *133*(7), 697-709.
48.Huang, M., Deng, J., Gao, L., & Zhou, J. (2020). Innovative strategies to advance CAR T cell therapy for solid tumors. *American journal of cancer research*, *10*(7), 1979–1992.
49.Grenier, J. M., Yeung, S. T., Qiu, Z., Jellison, E. R., & Khanna, K. M. (2018). Combining adoptive cell therapy with cytomegalovirus-based vaccine is protective against solid skin tumors. *Frontiers in Immunology*, *8*, 1993.
50.Lapteva, N., Gilbert, M., Diaconu, I., Rollins, L. A., Al-Sabbagh, M., Naik, S., ... & Rooney, C. M. (2019). T-cell receptor stimulation enhances the expansion and function of CD19 chimeric antigen receptor–expressing T cells. *Clinical Cancer Research*, *25*(24), 7340-7350.
51.Carreno, B. M., Magrini, V., Becker-Hapak, M., Kaabinejadian, S., Hundal, J., Petti, A. A., ... & Linette, G. P. (2015). A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. *Science*, *348*(6236), 803-808.
52.Poschke, I., Lövgren, T., Adamson, L., Nyström, M., Andersson, E., Hansson, J., ... & Kiessling, R. (2014). A phase I clinical trial combining dendritic cell vaccination with adoptive T cell transfer in patients with stage IV melanoma. *Cancer immunology, immunotherapy*, *63*, 1061-1071.
53.Poirot, L., Philip, B., Schiffer-Mannioui, C., Le Clerre, D., Chion-Sotinel, I., Derniame, S., ... & Smith, J. (2015). Multiplex genome-edited T-cell manufacturing platform for "off-the-shelf" adoptive T-cell immunotherapies. *Cancer research*, *75*(18), 3853-3864.
54.Rafiq, S., Hackett, C. S., & Brentjens, R. J. (2020). Engineering strategies to overcome the current roadblocks in CAR T cell therapy. *Nature reviews Clinical oncology*, *17*(3), 147-167.
55.Dimitri, A., Herbst, F., & Fraietta, J. A. (2022). Engineering the next-generation of CAR T-cells with CRISPR-Cas9 gene editing. Molecular Cancer, 21(1), 78.

http://pmjournal.ir Original Article

Winter 2024, Volume 9, Issue 32 (8- 15)

Targeted Screening of Membrane Proteins of Haemophilus Ducreyi with the Aim of Drug Targets Identification

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DOI: 10.22034/pmj.2024.2024174.1032

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Submitted: 2023-11-30
Accepted: 2024-02-22

Keywords:
Sexually transmitted infections
Chancroid
H. ducreyi
Reverse vaccinology

How to Cite this Article:
S. Khorsand-Dehkordi, F. Honarmand, Z. Ahmadzadeh Chaleshtori. "Targeted Screening of Membrane Proteins of Haemophilus Ducreyi with the Aim of Drug Targets Identification " Personalized Medicine Journal, Vol. 9, no. 32, pp. 8- 15.

Abstract:
Chancroid is an STI characterized by the Gram-negative bacteria *Haemophilus ducreyi*. Controlling chancroid is challenging, and the primary treatment accessible is antimicrobial therapy. However, drug resistance has been seen in places where the disease is common. Due to recent global outbreaks of sexually transmitted infections (STIs), it is crucial to continue researchi ng innovative treatment options and prevention measures. We used reverse vaccinology and subtraction genomic methods to determine potential vaccination and therapeutic targets against *H. ducreyi* in silico. We found 56 Secreted proteins, with 159 membrane molecules and 515 cytoplasmic proteins. We assessed their need, operation, and ability to cause disease. We identified 6 potential vaccination targets and three pharmacological targets inside pathogenicity islands. The discovered targets may be utilized in future initiatives to manage chancroid globally.

INTRODUCTION

The Haemophilus ducreyi bacteria bring on a sexually transmitted illness (STI) known as chancroid (1). Chancroid is prevalent in impoverished nations throughout Asia, Africa, and Latin America, suggesting a strong correlation between socioeconomic status and the occurrence of this disease among a specific community (1). In the 1990s, the World Health Organization predicted the global incidence of the condition to be approximately 7 million (2). Assessing the present distribution of chancroid is challenging due to the syndromic care of genital ulcer illnesses and the absence of monitoring and diagnostic methods (3).

Haemophilus ducreyi is a Gram-negative coccobacillus that is fastidious and non-motile (4). It harms catalase, ferments D-glucose, has fine pili and does not create endospores. It has no recognized animal or ecological reservoir and primarily infects the human mucosal epithelium (5). However, it can infect the keratinized stratified squamous epithelium, leading to ulcers, local discomfort, and inguinal lymphadenopathy (5, 6). The bacteria stay outside cells containing white blood cells, collagen, and fibrin. It can avoid being engulfed by white blood cells, which seems to be its primary method of evading the body's immune system and is crucial for its ability to cause disease (5, 7). Ulcerative lesions are linked to mononuclear cell infiltrates in the dermis, mainly CD4þ lymphocytes, which might aid in co-infection with human immunodeficiency virus (HIV) (8).

Haemophilus ducreyi does not lead to general infection, even in individuals with HIV (9). Extragenital lesions may arise due to auto-inoculation. Recent research has revealed H. ducreyi as the cause of persistent epidermal limb ulcerations in both kids and adults that are not sexually transmitted (10). Genital bacteria are categorized into two classes (I and II) based on many phenotypic and genetic variants, particularly the membrane protein, despite the unclear association with non-genital isolates (11). Furthermore, H. ducreyi expresses many virulence factors like lipooligosaccharides, iron-regulated

proteins, outer membrane proteins, toxins, and other secreted products during disease (12). Most oligosaccharide compounds from H. ducreyi include a lactosamine terminal that interacts with sialic acid (11-13). Lipooligosaccharides aid in attaching H. ducreyi to keratinocytes in a laboratory setting. H. ducreyi expresses hemolysin that can break down keratinocytes, macrophages, and T and B cells (14).

Controlling chancroid is challenging because of the lack of a protective immunological response towards further H. ducreyi infections, while a delayed hypersensitivity reaction to H. ducreyi may develop (15). The lesions may last weeks or months and not fully heal without antimicrobial medication. This inadequate response could be because cell-mediated immunity successfully removes intracellular bacteria, but most of H. ducreyi stays extracellular (16). Although the specific reaction that could protect the organism from H. ducreyi infection is unknown, the reality that H. ducreyi is an extracellular bacterium indicates the possibility of a humoral immune response (17). Innate and acquired immune cells, including macrophages, dendritic cells, NK cells, polymorphonuclear leukocytes, memory CD4þ, and effector CD8þ T cells, are attracted to the lesions (18, 19). It remains uncertain whether Th1, Th2, Th17, Th9, Th22, and Treg immune system reaction profiles are effectively regulated in individuals with various disease characteristics. Additional in vitro and in vivo research is required to accurately determine the function of cellular and humoral immune defenses and establish the tolerance or susceptibility of people afflicted by the illness (18, 19).

Due to the population's failure to embrace preventive measures and the absence of a reliable vaccination, the only current treatment options are azithromycin or ceftriaxone (for pregnant individuals) (20). Resistance to antibiotics was reported in endemic regions (21). Furthermore, research on non-genital ulcers indicates the presence of azithromycin-resistant non-genital strains, suggesting the possibility of an external reservoir or tolerance to the typical dosage of azithromycin (22). One goal of investigating H. ducreyi pathogenesis in this setting is to explore pathogenicity variables that might be considered for vaccine development. Due to the advancement of bioinformatics, the development of a vaccine for preventing H. ducreyi epidemics is nearing feasibility, as well as for other sexually transmitted infections (STIs) (23).

The horizontal gene transfer (HGT) process and bacterial evolution have led to the acquisition of novel antibiotic-resistance genes, necessitating the deployment of alternate infection control measures (24). Pathogenic and host genotypes increase the appeal of bioinformatics methods (25). Reverse vaccinology utilizes computational methods to find virulence factors that are surface-exposed or secreted immunogenic proteins capable of binding MHC class I and II molecules for antigen presentation in the host's immune system (26). Successful vaccine potential molecules have established properties such as sub-cellular localization, including signal peptides, transmembrane domains, and antigenic epitopes. More research is needed on the genetic analysis of H. ducreyi. Most do not use reverse vaccinology methods for predicting vaccines or therapeutic targets. This presents new opportunities in the field of comparative genomics.

MATERIALS AND METHODS

Identification of the information

The genomic sequences of 10 H. ducreyi isolates were obtained from the GenBank dataset at the National Center for Biotechnology Information (NCBI). We used the Rapid Annotation utilizing Subsystem Technology (RAST) program to reannotate each of the 10 genotypes. This program standardizes the genome annotations to prevent unexpected outcomes and inaccurate gene interpretation, an important preparatory step for genome analysis.

Finding conserved non-host proteins that are homologous within a species

After applying RAST, we aligned all imported Coding Sequences (CDS) using global alignment techniques. We used the orthoMCL program for orthology definition, employing all-versus-all blast studies with an E-value threshold of 1×10^{-10} and the Markov Cluster (MCL) method. The core genome refers to the coding sequences (CDS) common to all 10 strains. In order to prevent autoimmunity, the medication and vaccination targets should not be similar to human proteins. To identify non-human homologous targets, we utilized orthoMCL (E-value 1×10^{-10}) to contrast the primary genome with the human genome.

Determination of islands with pathogenicity

Genome plasticity refers to the genome's dynamic nature, including DNA acquisition, loss, or reorganization. During this stage, the genomic plasticity studies concentrated on identifying genomic islands, portions of the genome that may have been acquired by horizontal gene transfer (HGT). Genomic islands are predicted based on variations in genome signature such as GC content and codon utilization, the existence of transposases and high levels of virulence, resistance, metabolic, and symbiotic factors, the existence of sequence insertions or flanking tRNA genes, dimensions ranging from 6 to 200 KB, and lack of them in non-pathogenic related

organisms. The Genomic Island Prediction Software (GIPSy) program was used for that.

Using reverse vaccination to identify potential targets for the Haemophilus ducreyi vaccine

The subtractive genome technique was used to determine vaccination targets. We first used the core genome, which comprises the critical genes of the virus, and then conducted BLASTp analysis to forecast the non-host homologous domains. Using the SutfGÍ software, which categorizes proteins into cytoplasmic, secreted, putative surface exposed (PSE), and membrane proteins based on the existence or absence of signal peptides, retention signals, and transmembrane helices, we predicted the subcellular localization of all the proteins from this non-host homologous conserved proteome. We analyzed the secreted proteins for adhesion probability (above 0.51) and MHC I and MHC II binding characteristics using the Vaxijn tool after identification. We identified cleavage locations and transmembrane helices utilizing SignalP and TMHMM, respectively, and determined functional domains using InterProScan. We analyzed the potential targets for their existence in pathogenicity islands (PAIs).

RESULTS

The process in Figure 1 summarizes the essential phases for target identification, the approaches used, and the total number of proteins reported in each step.

Discovering conserved non-host homologous proteins within a species and pathogenicity islands

Ten H. ducreyi strains' sequences were compared (table 1), with H. ducreyi 35000HP serving as the standard strain for the orthoMCL study. The core genome, or 1257 CDSs, consists of coding DNA fragments common to all strains. Taking the human genome as the host genetic code, we discovered that 847 CDSs are non-host homologous molecules.

We also conducted genomic islands identification. H. ducreyi's closest relatives are either harmful to humans or animals or lack a fully sequenced genome, making them unsuitable for predicting genomic islands. To prevent inaccurate adverse outcomes, we utilized two closely similar non-pathogenic organisms, Haemophilus somnus129Pt strain and Manheimia haemolytica USMARC_2286 strain, which are not harmful to humans. We used the GIPSy program for this method. MEGA analysis indicates that H. ducreyi is more closely linked to species within the Pasteurallaceae family rather than other species in the Haemophilus genus, which comprises pathogenic microbes. Additional research conducted phylogenomic research on H. commas 129Pt and M. haemolytica USMARC_2286, which are commensal organisms in animals belonging to the family Pasteurellaceae, revealing a close connection to H. ducreyi. We used GIPSy research to predict three pathogenicity islands and then visualized the findings using the BRIG program. Pathogenicity island prediction (PAI) is crucial to comprehending bacterial evolution, the virulence elements encoded, their mobility and construction, and the relationships between the pathogen and eukaryotic host cell populations (28). As a result, these PAI host infectiousness factors that may be attractive candidates for vaccines that elicit a reaction from the immune system.

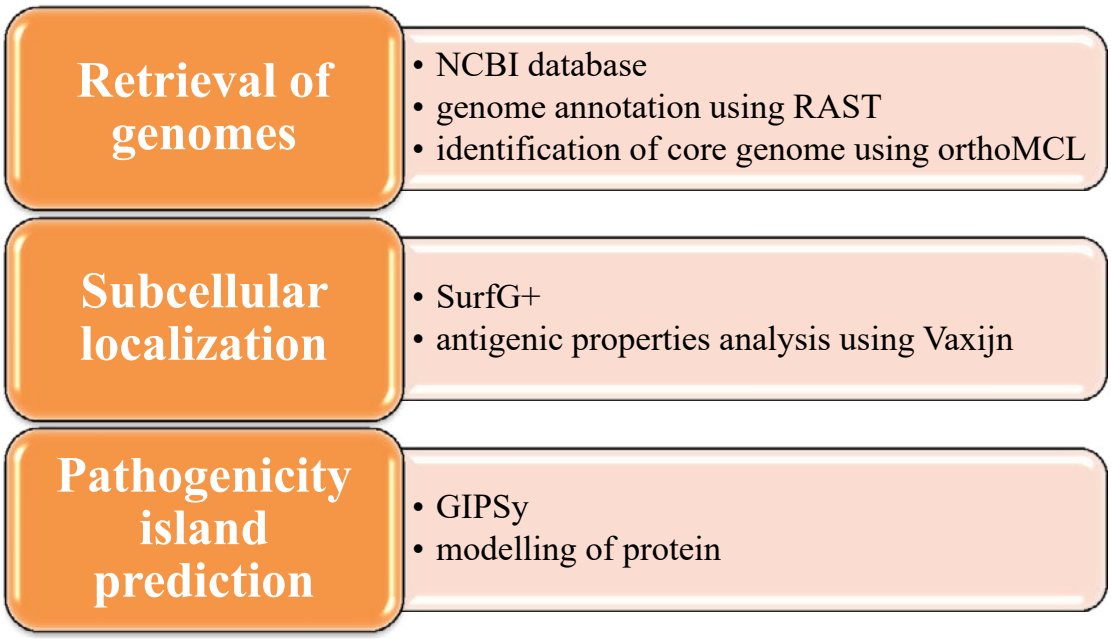

Fig 1. Created a process detailing the approaches used and the total proteins detected at each stage.

Table 1. Details regarding the 10 H. ducreyi strains that were employed in this study.

Strain	GenBank number	Size (MB)	Gene number	GC (%)	Protein number
Hd_GU1	CP011228	1.62	1566	38.10	1407
Hd_GU2	CP011229	1.55	1504	38.00	1340
Hd_GU3	CP011230	1.59	1539	38.10	1385
Hd_GU4	CP011231	1.57	1522	38.10	1363
Hd_GHA1	CP015429	1.62	1551	37.90	1389
Hd_AUSPNG1	CM004377	1.72	1695	38.01	1541
Hd_CLU1	CP011220	1.65	1611	38.20	1450
Hd_CLU2	CP011227	1.59	1557	38.40	1382
Hd_VAN1	CP015424	1.66	1629	38.10	1468
Hd_35000HP	AE017143	1.69	1668	38.20	1509

The lack of certain pathogenicity islands in particular strains may be linked to the variability in virulence genes, various phenotypes, and genetic diversity that categorize H. ducreyi strains into three categories: non-genital, genital class I, and genital class II. The absence of a portion of PAI 2 in genital class II strains (Hd_GU1, Hd_GU2, Hd_GU3, and Hd_GU4) indicates this. PAIs have precarious locations that may be gained or lost over time. Their absence in certain situations may be linked to the inclusion of draft genotypes in the dataset utilized for the research. PAIs are significant because they are a category of GEIs with virulence genes. High densities of two groups of genes are likely to be found inside PAIs: shared genes present in two or more, but not all, strains and singletons (strain-specific genes).

Estimating the potential vaccination target for Haemophilus ducreyi

We analyzed all genetic sequences from H. ducreyi using the reverse vaccinology technique. We examined the genes conserved across many genomes and found they are crucial for pathogenic and non-host homologous organisms. We anticipated the subcellular distribution of protein molecules that are likely to be antigenic by focusing on secreted proteins, surface-exposed proteins, and membrane proteins. We analyzed their MHC I and MHC II binding properties and adhesion probability greater than 0.51, ensuring they did not resemble mammalian proteins. We examined the correlation of virulence factors with pathogenicity islands (PAIs) since they are considered more effective targets due to their encoded nature. Molecules encoded by shared PAIs are suitable candidates, but this selection does not exclude targets from the previous phase. PAIs presence suggests that the proteins might be crucial virulence agents and should be given priority.

The protein's subcellular distribution was forecasted utilizing the SurfGþ program. 847 gene products were discovered, with 332 categorized as probable surface-exposed molecules, secreted proteins, or membrane proteins (Fig 2). We next analyzed 332 proteins in Vaxijn to identify proteins with adhesion probability above 0.51. We discovered 31 proteins from them. We analyzed 31 proteins versus DEG and identified 6 as strictly necessary to the pathogen based on an E-value cut-off of 1×10^{-4}.

The biological data for these 6 proteins was obtained by analyzing cleavage locations and transmembrane helices utilizing SignalP and TMHMM and subsequently forecasting functional domains utilizing InterProScan. Proteins with a molecular weight of 110 kDa or less are preferred candidates for vaccine development due to their ease of purification. The molecular weights of specific proteins were determined utilizing UniProt. All projected proteins fall within this range and may serve as viable vaccine targets (Table 2).

DISCUSSION

Many identified vaccine targets consist of surface-exposed or secreted proteins more likely to be pathogenic or pathogenic, making them good candidates for vaccines (27). We emphasize anticipated proteins in PAIs, but other PSE and secreted proteins, such as the lipoprotein NlpD amino acids and the secreted arginine ABC transporter, are also possible vaccine targets. A01_0584 is a member of the LysM domain, a standard protein module that binds peptidoglycan in bacterium and chitin in eukaryotes (28). The domain was first discovered in enzymes that break down bacterial cell walls and serve as a signal for precise plant-bacteria identification in bacterial pathogenesis (29). Protein A01_0636 is a member of the solute-

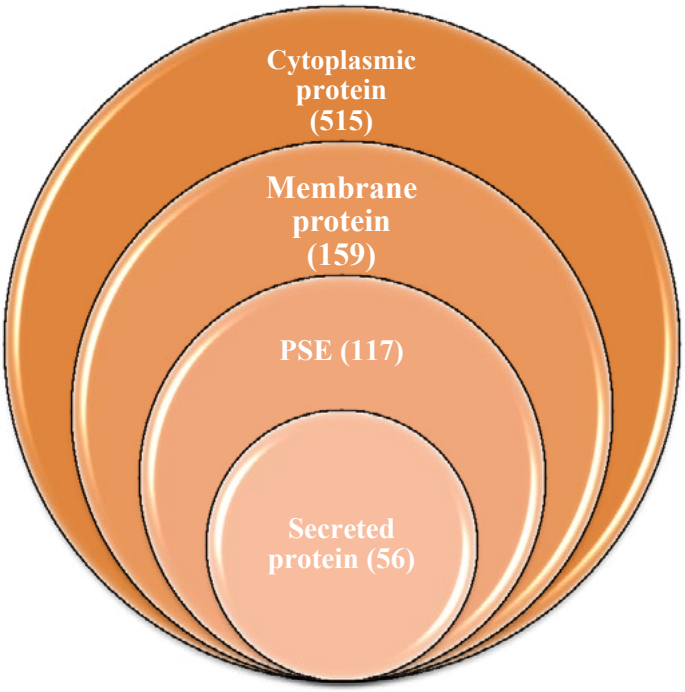

Fig2. Subcellular localization of conserved non-host homologous proteins from H. ducreyi. PSE stands for presumed surface exposed.

binding polypeptide family 3/N-terminal domain of MltF, which plays a role in actively transporting solutes across the cytoplasm membrane (30). In addition, it is crucial to note the discovery of another secreted amino acid, a DNA uptake protein, and a related DNA-binding protein derived from the competence protein molecule ComEA (31). This protein contains a helix-hairpin-helix domain, which enables a cell to absorb external DNA, leading to a process known as transformation. This activity is controlled in reaction to cell-to-cell communication and nutritional circumstances and is common among bacteria, likely serving as a significant route for gene transfer (32).

Most of the outer layer of the protein's composition is situated inside the membrane. It may exhibit sequence diversity in the protecting epitopes found in the exterior loops, enabling evasion of immunity. Research (33) shows that the first stage in most illnesses involves pathogenic bacteria attaching to eukaryotic cells or host proteins in the extracellular matrix. Preventing this connection effectively stops the illness. To avoid issues, we added the recognition of proteins related to adhesion, namely released outer membrane proteins. The first two amino acids are members of the BamA protein family responsible for assembling and integrating beta-barrel molecules into the outer membrane. The third protein is associated with the bacterial surface protein D15 domain. A protein is necessary for maintaining the integrity of the pilus and for its activities, such as adhering to human cells. DsrA was not anticipated to form part of the core genome due to its absence in some strains or the large number of draft genomes included in the analysis. Nevertheless, we must be rigorous in identifying effective targets against all strains (33).

Previous studies, both in laboratory settings and in living organisms, have found H. ducreyi transcripts that are active during human infection, and several of these amino acids were detailed in our findings (33, 34). A study utilized selective capture of transcribed motifs using RNA obtained from pustules of three volunteers infected with H. ducreyi and RNA obtained from bacteria cultured in broth. They discovered many genes in the bacterium that have been recognized as possible factors contributing to virulence, such as the anticipated outer membrane protein (34). A different transcriptomic investigation (35) analyzed the H. ducreyi transcriptome in biopsy samples from human lesions. It contrasted it to the transcriptome of bacteria cultured in mid-log, transition, and stationary phases. H. ducreyi harvested from pustules exhibited upregulated genes related to nutrient transport, anaerobiosis, and fermentation compared to the inoculum in the mid-log phase. These included the anticipated formate efflux transporter focA and the virulence determinant hgbA, which is responsible for haemoglobin uptake. The research only analyzed the transcriptome of the whole lesion at a single point in time. Other virulence indicators may be expressed in varying ways throughout time or by bacteria in various microenvironments within the lesions.

CONCLOSION

Throughout the last century, multiple traditional

Table 2. VaxiJn found a potential candidate vaccination target for Haemophilus ducreyi. TMHMM is a server that predicts transmembrane helices. PSE refers to probable surface-exposed regions, SEC indicates secreted proteins, and MEM stands for membrane proteins.

protein ID	gene	localization	signal-P	TMHMM	InterProScan	**molecular** weight (Da)	**adhesin** probability	**length** (AA)
WP_010944348.1	—	PSE	no	1	etratricopeptide-like helicaldomain	20238	0.550	181
WP_010944572.1	lptC	PSE	no	1	LptC related	21798	0.673	193
WP_010944666.1	—	PSE	no	1	thioredoxin-like fold/ thioredoxin domain/ alkyl hydroperoxide reductase subunit C /thiolspecific antioxidant	19907	0.563	172
WP_010944716.1	—	PSE	yes (between 20 and 21)	0	LysM domain/peptidase M23/ duplicated hybrid motif	40279	0.541	372
WP_010944817.1	—	PSE	no	2	Rossmann-like alpha/beta/ alphas and wich fold/ domain of unknown function DUF218	27983	0.533	248
WP_010945328.1	nlpc	PSE	yes (between 31 and 32)	0	endopeptidase, NLPC/P60 domain	19295	0.519	71

methods have been effectively used in vaccine creation, including the culture of the pathogen and the utilization of biochemical, immunological, and microbiological techniques [74]. This approach is time-consuming, only detects numerous antigens which may not provide protection, and often proves unsuccessful when the infectious agent cannot be grown in a laboratory setting. The genetic revolution has enabled computational vaccinology to achieve optimal outcomes in vaccine formulation by predicting all antigens using in silico methods. Creating a worldwide vaccination for Neisseria meningitidis strains marked the first use of immune informatics in vaccinology. Subsequently, other effective vaccines were developed using reverse vaccinology, including vaccines targeting Listeria monocytogenes, Malarian protozoans, Streptococcus pneumoniae, Porphyromonas gingivalis, Chlamydia pneumonia, and Staphylococcus aureus. Comparative genomics, subtractive genome research, and reverse vaccinology have been effectively used in vaccine development. Due to the failure to prevent global sexually transmitted illnesses and the development of antimicrobial resistance, some infections like syphilis have resurfaced, necessitating the implementation of new techniques for STI management. Preventive strategies such as developing vaccines and medicines against H. ducreyi are necessary to guard against potential chancroid epidemics. The work used in silico reverse vaccinology and subtraction genomic methods to identify potential vaccine and therapeutic targets from the genome of H. ducreyi strains.

Acknowledgments

I would like to thank biotechnology research center

members, who helped with this research.

Conflicts of Interest

The authors declare no conflict of interest.

REFERENCES

1.de Sarom A, Kumar Jaiswal A, Tiwari S, de Castro Oliveira L, Barh D, Azevedo V, Jose Oliveira C, de Castro Soares S. Putative vaccine candidates and drug targets identified by reverse vaccinology and subtractive genomics approaches to control Haemophilus ducreyi, the causative agent of chancroid. Journal of The Royal Society Interface. 2018 May 31;15(142):20180032.

2.Lopez AD, Mathers CD. Measuring the global burden of disease and epidemiological transitions: 2002–2030. Annals of Tropical Medicine & Parasitology. 2006 Aug 1;100(5-6):481-99.

3.Loh AJ, Ting EL, Wi TE, Mayaud P, Chow EP, Santesso N, Falconer J, Ofori-Asenso R, Ong JJ. The diagnostic accuracy of syndromic management for genital ulcer disease: a systematic review and meta-analysis. Frontiers in Medicine. 2022 Jan 3;8:806605.

4.Parija SC. Haemophilus and Bordetella. InTextbook of Microbiology and Immunology 2023 Mar 17 (pp. 579-596). Singapore: Springer Nature Singapore.

5.Martens EC, Neumann M, Desai MS. Interactions of commensal and pathogenic microorganisms with the intestinal mucosal barrier. Nature Reviews Microbiology. 2018 Aug;16(8):457-70.

6.Piri Gharaghie T, Doosti A, Mirzaei SA. Prevalence and antibiotic resistance pattern of Acinetobacter spp. infections in Shahrekord medical centers. Developmental Biology. 2021 Nov 22;13(4):35-46.

7.Piri-Gharaghie T. Polycystic ovary syndrome and genetic factors influencing its development: A review article. Personalized Medicine Journal. 2021 Dec 1;6(23):25-9.

8.Ramdial PK. Dermatopathological challenges in the human immunodeficiency virus and acquired immunodeficiency syndrome era. Histopathology. 2010 Jan;56(1):39-56.

9.González-Beiras C, Marks M, Chen CY, Roberts S, Mitjà O. Epidemiology of Haemophilus ducreyi infections. Emerging infectious diseases. 2016 Jan;22(1):1.

10.Trager JD. Sexually transmitted diseases causing genital lesions in adolescents. Adolescent Medicine Clinics. 2004 Jun 1;15(2):323.

11.Leonard CA, Borel N. Chronic chlamydial diseases: from atherosclerosis to urogenital infections. Current Clinical Microbiology Reports. 2014 Dec;1:61-72.

12.Ghajari G, Nabiuni M, Amini E. The association between testicular toxicity induced by Li2Co3 and protective effect of Ganoderma lucidum: Alteration of Bax & c-Kit genes expression. Tissue and Cell. 2021 Oct 1;72:101552.

13.Yadollahi A, Ghajari G. Transgenic induction in Sesamum indicum with recombinant pBi121 expression construct containing CYP81Q1 and aroA genes using Agrobacterium tomfacensis.

14.Wood GE, Dutro SM, Totten PA. Target cell range of Haemophilus ducreyi hemolysin and its involvement in invasion of human epithelial cells. Infection and immunity. 1999 Aug 1;67(8):3740-9.

15.Brothwell JA, Griesenauer B, Chen L, Spinola SM. Interactions of the skin pathogen Haemophilus ducreyi with the human host. Frontiers in Immunology. 2021 Feb 3;11:615402.

16.Cole LE, Toffer KL, Fulcher RA, San Mateo LR, Orndorff PE, Kawula TH. A humoral immune response confers protection against Haemophilus ducreyi infection. Infection and immunity. 2003 Dec;71(12):6971-7.

17.Cole LE, Toffer KL, Fulcher RA, San Mateo LR, Orndorff PE, Kawula TH. A humoral immune response confers protection against Haemophilus ducreyi infection. Infection and immunity. 2003 Dec;71(12):6971-7.

18.Chávez-Sánchez L, Espinosa-Luna JE, Chávez-Rueda K, Legorreta-Haquet MV, Montoya-Díaz E, Blanco-Favela F. Innate immune system cells in atherosclerosis. Archives of medical research. 2014 Jan 1;45(1):1-4.

19.Ghourchian H, Pecho RD, Karimi-Dehkordi M, Mazandarani A, Ghajari G, Piri-Gharaghie T. Novel Niosome-Encapsulated 2, 5-Diketopiperazine (BHPPD): Synthesis, Formulation, and Anti-breast Cancer Activity. Applied Biochemistry and Biotechnology. 2023 Aug 25:1-22.

20.Rac H, Gould AP, Eiland LS, Griffin B, McLaughlin M, Stover KR, Bland CM, Bookstaver PB. Common bacterial and viral infections: review of management in the pregnant patient. Annals of Pharmacotherapy. 2019 Jun;53(6):639-51.

21.Piri-Gharaghie T, Ghajari G, Tolou-Shikhzadeh-Yazdi S, Aghassizadeh-Sherbaf M, Khorsand-Dehkordi S. Helicobacter pylori strains isolated from raw poultry meat: frequency and molecular characteristics. Scientific Reports. 2023 Jul 10;13(1):11116.

22.Marks M, Mitjà O, Vestergaard LS, Pillay A, Knauf S, Chen CY, Bassat Q, Martin DL, Fegan D, Taleo F, Kool J. Challenges and key research questions for yaws eradication. The Lancet infectious diseases. 2015 Oct 1;15(10):1220-5.

23.Lewis DA. Epidemiology, clinical features, diagnosis and treatment of Haemophilus ducreyi–a disappearing pathogen?. Expert review of anti-infective therapy. 2014 Jun 1;12(6):687-96.

24.Avershina E, Shapovalova V, Shipulin G. Fighting antibiotic resistance in hospital-acquired infections: current state and emerging technologies in disease prevention, diagnostics and therapy. Frontiers in microbiology. 2021 Jul 21;12:707330.

25.Saeed Mohammed A, Ghajari G. Epithelial-Mesenchymal Rransition and its Role in Breast Cancer Metastasis. Personalized Medicine Journal. 2022 Mar 20;7(24):8-13.

26.Piri-Gharaghie T, Doosti A, Mirzaei SA. Identification of antigenic properties of Acinetobacter baumannii proteins as novel putative vaccine candidates using reverse vaccinology approach. Applied Biochemistry and Biotechnology. 2022 Oct;194(10):4892-914.

27.Rawal K, Sinha R, Abbasi BA, Chaudhary A, Nath SK, Kumari P, Preeti P, Saraf D, Singh S, Mishra K, Gupta P. Identification of vaccine targets in pathogens and design of a vaccine using computational approaches. Scientific reports. 2021 Sep 2;11(1):17626.
28.Mesnage S, Dellarole M, Baxter NJ, Rouget JB, Dimitrov JD, Wang N, Fujimoto Y, Hounslow AM, Lacroix-Desmazes S, Fukase K, Foster SJ. Molecular basis for bacterial peptidoglycan recognition by LysM domains. Nature communications. 2014 Jun 30;5(1):4269.
29.Dik DA, Marous DR, Fisher JF, Mobashery S. Lytic transglycosylases: concinnity in concision of the bacterial cell wall. Critical Reviews in Biochemistry and Molecular Biology. 2017 Sep 3;52(5):503-42.
30.Chen YC, Kalawong R, Toyofuku M, Eberl L. The role of peptidoglycan hydrolases in the formation and toxicity of Pseudomonas aeruginosa membrane vesicles. MicroLife. 2022;3:uqac009.
31.Dubnau D, Blokesch M. Mechanisms of DNA uptake by naturally competent bacteria. Annual review of genetics. 2019 Dec 3;53:217-37.
32.Gobbetti M, De Angelis M, Di Cagno R, Minervini F, Limitone A. Cell–cell communication in food related bacteria. International journal of food microbiology. 2007 Nov 30;120(1-2):34-45.
33.Fusco WG, Choudhary NR, Stewart SM, Alam SM, Sempowski GD, Elkins C, Leduc I. Defining potential vaccine targets of Haemophilus ducreyi trimeric autotransporter adhesin DsrA. Monoclonal antibodies in immunodiagnosis and immunotherapy. 2015 Apr 1;34(2):73-82.
34.Bauer ME, Fortney KR, Harrison A, Janowicz DM, Munson RS, Spinola SM. Identification of Haemophilus ducreyi genes expressed during human infection. Microbiology. 2008 Apr 1;154(4):1152-60.
35.Gangaiah D, Zhang X, Baker B, Fortney KR, Gao H, Holley CL, Munson Jr RS, Liu Y, Spinola SM. Haemophilus ducreyi seeks alternative carbon sources and adapts to nutrient stress and anaerobiosis during experimental infection of human volunteers. Infection and Immunity. 2016 May;84(5):1514-25.

http://pmjournal.ir Original Article

Winter 2024, Volume 9, Issue 32 (16- 22)

SIRT1 rs7895833 and SOD50-1bp ins/del Gene Variations in Age-Related Cataract Patients: A Case-Control Study

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DOI: 10.22034/pmj.2024.2024055.1029

Submitted: 2023-12-10
Accepted: 2024-02-18

Keywords:
Oxidative stress
SOD1
SIRT1
Polymorphism
Cataract

How to Cite this Article:

L. Kohan, S. Sharghi, A. Karimi. "SIRT1 rs7895833 and SOD1-50bp ins/del Gene Variations in Age-Related Cataract Patients: A Case-Control Study " Personalized Medicine Journal, Vol. 9, no. 32, pp. 16- 22.

Abstract:

Aim: Oxidative stress is one of the main factors has been implicated in pathophysiology of cataracts. Superoxide dismutase (SOD) can prepare the first line of defense versus detrimental reactive oxygen species (ROS) and Sirtuin (SIRT) confers protection against oxidative stress and retinal degeneration. Correlation of SOD1-50bp ins/del and SIRT1-rs7895833 polymorphisms with risk of cataracts is not studied currently. Therefore, we aimed to explore possible relationship between SOD1 (50bp ins/del) and SIRT1 (rs7895833) polymorphisms with the risk of cataracts in Iranian population.

Methods: Our study design consisted of 200 patients with age-related cataracts and 200 healthy individuals as a control group. After DNA extraction, the identification of polymorphisms was conducted using PCR-based methods and data analysis was done by SPSS software.

Results: A significant difference in *SOD1* DD genotype distribution was observed between studied groups (OR: 3.42, P:0.037), the D allele was more frequent in patients in comparison with controls (OR: 1.68, P:0.009). Also, in the dominant genetic model for the D allele (comparison between ID+DD vs. II), ID+DD genotypes increased the risk of cataracts (OR: 1.62, P: 034). The association between *SIRT1*-rs7895833 polymorphism and cataract was significant in the AG genotype (OR: 2.37, P<0.001) and G allele (OR: 1.97, P<0.001). The *SIRT1*-1 polymorphism increased the risk of cataracts in the dominant tested inheritance model (OR: 2.34, P<0.001). In the combined analysis of two polymorphisms, there is an additive effect of the high-risk putative alleles about the risk of cataracts. Risk estimation according to the number of high-risk alleles showed that $\chi 2$ for linear trend for 0, 1, 2, 3 and 4 putative high-risk alleles is equal to 20.10 (P<0.001).

Conclusion: The results showed that for the first time, *SIRT1* rs7895833 and *SOD1*-50bp ins/del gene variations had additive effects on the risk of cataracts.

INTRODUCTION

Cataracts have been recognized as the opacification of the eye lens with the breakdown of the lens protein microarchitecture that can harmfully influence the light transmission on the retina (1). The WHO estimates that over 94 million people suffer visual impairment due to cataracts worldwide (2). Oxidative stress is currently known as an initiating parameter in the pathogenesis of cataracts. An imbalance between oxidative stress processes, antioxidant protection and repair, promotes cataracts (3). Superoxide dismutase (SOD) is the main enzymatic antioxidant in the lens. SOD prepares the first line of defence versus detrimental reactive oxygen species (ROS) (4). In the lens, SOD1 was determined as the main isoform for about 90% of the whole SOD activity. It is determined that specific SOD1 activity can be reduced as a function of age in the cataract patient's blood (3, 5) as well as in human lenses (6). Previous investigations determined that the decreased activities of the SOD1 isoform in cataractous lenses are commonly associated with protein expression and reduced levels of mRNA transcripts (7). The overexpression of SOD1 is demonstrated to prevent ROS-induced oxidative loss for lenses together with regulating cataract formation (8). In cataractous lenses, impaired activity of SOD1 can be caused by genetic polymorphisms in coding regions along with noncoding regions of the SOD1 gene (9). Several

studies showed an association of SOD1 gene variants with diseases like cancer (10), type 2 diabetes (11), and cardiovascular disease (12).

Sirtuin 1 (SIRT1) is another protein which can protect cells against oxidative stress, regulates glucose or lipid metabolism, and enhances the stability of DNA by binding and deacetylating many substrates (13). The protein of SIRT1 has been reported as a member of the Silent Information Regulator 2 (Sir2) protein family (i.e., a group of nicotinamide adenine dinucleotide (NAD+)-dependent histone deacetylases) (14). This protein can enhance cell survival using the inhabitation of cellular senescence or may apoptosis due to stress, consisting of DNA damage as well as oxidative stress (15). It is richly divided into different tissues together with organs and is determined in the cytoplasm and nucleus of cells from whole the ocular structures consisting of the retina, iris, lens, cornea and ciliary body (16). SIRT1 polymorphisms are associated with many diseases consisting diabetes (17), cancers (18), cardiovascular (19) and neurodegenerative (20) diseases.

Genetic polymorphisms are introduced as rather affecting the genetic risk elements for cataracts and enhancing efforts were focused on detecting the associations among cataract susceptibility and genetic polymorphisms (21). Based on the previous reports, we hypothesized that polymorphisms in the genes of SOD1 and SIRT1 can be associated with susceptibility to cataracts. A number of polymorphisms are specified in SOD1 and SIRT1 genes, which affect gene regulatory regions consisting of promoter. Therefore, in this study for the first time, we selected two common promoter polymorphisms, SOD1 50bp ins/del and SIRT1 rs7895833, to assess their association and genotype combination with age-related cataract in an Iranian population and calculate the additive effect of these two polymorphisms about the susceptibility to age-related cataract.

MATERIAL AND METHODS

Sample collection

This study was conducted following approval from the Ethics Committee of Arsanjan Islamic Azad University (approval ID number: IR.IAU.SHIRAZ.REC.1402.274). In this research, 200 age-related cataract patients and 200 age and sex-matched-healthy individuals are recruited. The control group consisted of subjects without any ocular disorders. Informed written consents are achieved from all participants. Blood samples are collected from cataract patients at Mirhosseini Hospital of Shiraz city (Fars province-Iran). Cataract status was confirmed through a slit lamp examination by a specialist. With the inclusion criteria of: (I) patients aged ≥60 years old; (II) patients diagnosed with age-related cataracts; (III) clinical data of patients was complete and true. Subjects were excluded from the study if they suffered from any metabolic disorder or secondary cataracts due to diabetes or trauma. Also, patients with corneal, fundus, or other diseases that affect vision, i.e., other than cataracts, were excluded.

DNA extraction and Genotyping

DNA was isolated from whole blood by utilizing the salting out method as described in the previous study (22). Analysis of SIRT1 polymorphism was carried out by the Tetra-ARMS PCR method. The polymerase chain reaction is done in a total reaction volume equal to 12.5 µl including 6.25 µl PCR master mix, DNA template of 1 µl (400 ng/ml), 1 µl of each forward and reverse internal primers (10 pm/µl), 0.25 µl of each forward and reverse outer primers (2.5 pm/µl), and 2.75 µl H_2O. For detection of SOD1 polymorphism, polymerase chain reaction was performed in a total reaction of 12.5 µl volume consisting of 6.25 µl PCR master mix, 1 µl DNA template (400 ng/ml), 0.5 µl of each forward and reverse primers (5 pm/µl), and 3.25 µl H_2O. The details of primer sequences are shown in Table 1.

STATISTICAL ANALYSIS

SPSS software version 18.0 was utilized to analyse data. Demographic characteristics and Hardy-Weinberg equilibrium were assessed using the Pearson χ^2 test. Odds ratios with a 95% confidence interval were calculated to examine the relationship between gene polymorphisms and cataract risk. P values less than 0.05 were attended as significant.

RESULTS

The comparison of general data between cataract patients and controls is shown in Table 2. The average age of patients and controls was 64.18±8.83 years. and 63.98±9.03 yrs, respectively. There was no significant difference in mean age between case and control groups (P=0.82).

The distribution of SOD1 50bp ins/del and SIRT1-rs7895833 polymorphisms in cataract patients and healthy individuals is shown in Table 3 and Table 4, respectively. No significant deviations from Hardy-Weinberg equilibrium were found for SOD1 50bp ins/del (χ^2= 0.33, df=1, P=0.56) and SIRT1 rs7895833 (χ^2= 0.15, df=1, P=0.70) in control group.

Our results showed that there was a significant association between SOD1-DD genotype and cataract susceptibility (OR: 3.42, 95%CI: 1.1-10.8, P: 0.037). Also, in the dominant genetic effect of the D allele (comparison between ID+DD vs. II), ID+DD genotypes increased the risk of cataracts and the D allele, as a putative high-risk allele, was associated with increased risk of cataract (OR: 1.68, 95%CI: 1.14-2.48, P: 0.009).

Table1. Details of primers using for genotyping SIRT1 and SOD1 polymorphisms.

Polymorphism	Primers	Sequence (5' to 3')	Annealing Temp (°C)
SIRT-1	FI	GTGGTAAAAGGCCTACAGGCAA	
rs7895833	RI	CTTGCTTCCTAATCTCCATTACGTTTAC	57
	FO	CCTAGCTGGTCTATCTCCCTTACCTC	
	RO	GCACATCTGTGTATCCCCTAGAAAG	
SOD1	F	AATTCCTTACCCCTGTTCTA	
50bp ins/del	R	GGCAGATTTCAGTTCATTGT	60

F: forward; R: Reverse; I: Inner; O: Outer; Temp: Temperature

Table2. Comparison of basic data between patient and control groups.

Variables	Controls (%) N=200	Cases (%) N=200	P-value
Gender			
Female	118 (59)	118 (59)	
Male	82 (41)	82 (41)	NS
Family History			
No	177 (88.5)	149 (74.5)	
Yes	23 (11.5)	51 (25.5)	<0.001
Smoking			
No	164 (82)	168 (84)	
Yes	36 (18)	32 (16)	0.59

NS: not significant

According to SIRT1-rs7895833 genotype distribution, the AG genotype was associated with cataract susceptibility (OR: 2.37, 95%CI: 1.52-3.69, P<0.001). Moreover, the frequency of the rs7895833G allele was significantly higher in cases compared to controls and this high putative allele was associated with an enhanced risk of cataracts (OR: 1.97, 95%CI: 1.36-2.86, P < 0.0001).

To investigate whether the high-risk alleles of SOD1 and SIRT1 had an additive effect on the risk of age-related cataracts, we considered the association between the combination of alleles and the risk of cataracts. The reference group consisted of individuals with the double low-risk alleles of SOD1 and SIRT1. Data analysis showed that there were significant associations between combined alleles and the risk of

Table 3. SOD1-50bp ins/del polymorphism in cataract patients and Controls.

Polymorphism	Cases (%)	Controls (%)	OR (95% CI)	P-Value
Genotypes				
II	136 (68)	155 (77.5)	1	
ID	52 (26)	41 (20.5)	1.44 (0.9-2.31)	0.124
DD	12 (6)	4(2)	3.42 (1.1-10.8)	**0.037**
Alleles				
I	324 (81)	351 (88)	1	
D	76 (19)	49 (12)	1.68 (1.14-2.48)	**0.009**
Dominant model				
II	136 (68)	155 (77.5)	1	
ID+DD	64 (32)	45 (22.5)	1.62 (1.04-2.53)	**0.034**
Recessive model				
II+ID	188 (94)	196 (98)	1	
DD	12 (6)	4 (2)	3.13 (0.99-9.86)	**0.052**

cataracts (Table 5). Also, there was a linear trend in risk associated with 0, 1, 2, 3 & 4, alleles (χ^2: 20.10, P<0.001).

DISCUSSION

The genetic variations are one of the important intrinsic factors can potentially affect many aspects of disease management and its medical treatment. Initially, our research focused on investigating the correlation between genetic variations in the promoter regions of SOD1 and SIRT1 genes and the susceptibility to cataracts. Our findings suggest that these genetic alterations may act as risk factors influencing the development of age-related cataracts in the Iranian population.We found that the G allele of SIRT1-rs7895833 and the D allele of SOD1 50bp ins/del, as high-risk alleles, were associated with an increased risk of cataracts. However, a combination study showed that there were significant associations between combined high-risk alleles and the risk of cataracts; it means that the high-risk alleles of these two polymorphisms had additive effects about the risk of cataracts. According to these results, we reported that individuals with the high risk alleles in these studied genetic variations and specially who had combination of these high risk alleles, are marked for personalized medicine; and these polymorphisms may help in decision to the prevention, diagnosis, and treatment of cataracts in future.

There have been several reports studying the association between various genes as well as several SNPs with cataracts (23, 24). Previous evidence demonstrated that the generation of ROS leads to cross-linking, and aggregation of lens proteins as well as abnormal degradation and was consisted in cataractogenesis (25). The oxidative damage during cataractogenesis can be reduced by cellular defence mechanisms in the eye; SOD1 and SIRT1 proteins play important roles in this situation. As yet there are no studies on the effect of SOD1 50bp ins/del and SIRT1 rs7895833 polymorphisms and the additive effect of these polymorphisms on cataract susceptibility. It has been shown that polymorphisms in promoter regions could affect gene expression and enzyme activity (26,

Table 4. SIRT1-rs7895833 polymorphism in cataract patients and Controls

Polymorphism	Cases (%)	Controls (%)	OR (95% CI)	P-Value
Genotypes				
AA	115 (57.5)	152 (76)	1	
AG	79 (39.5)	44 (22)	2.37 (1.52-3.69)	**<0.001**
GG	6 (3)	4 (2)	1.98 (0.55-7.20)	0.3
Alleles				
A	309 (77)	348 (87)	1	
G	91 (23)	52 (13)	1.97 (1.36-2.86)	**<0.001**
Dominant model				
AA	115 (57.5)	152 (76)	1	
AG+GG	79 (39.5)	44 (22)	2.34 (1.52-3.59)	**<0.001**
Recessive model				
AA+AG	309 (77)	348 (87)	1	
GG	91 (23)	52 (13)	1.51 (0.42-5.45)	0.52

Table 5. Risk estimation according to number of high risk allele

N of high risk allele	Case (%)	Control (%)	OR (95% CI)	P
0	72 (36)	120 (60)	Reference	-
1	94 (47)	63 (31.5)	2.48 (1.61-3.83)	<0.001
2	29 (14.5)	14 (7)	3.45 (1.71-6.96)	0.001
3 & 4	5 (2.5)	3 (1.5)	2.78 (0.64-11.97)	0.17

X^2 for linear trend: 20.10, P<0.001

27). A 50bp ins/del polymorphism (rs36232792) has been recognized in the SOD1 promoter region (1684 bp upstream of the start codon of ATG). Its role is demonstrated with reduced promoter activity together with low mRNA levels in cells can be caused due to the loss of two Sp1 binding sites (28). Attending that the Del allele causes to reduction of the promoter activity of the SOD1. It may change the enzymes' antioxidant capacity which leads to synergistic effects with cataracts induced by oxidative damage subsequently. The results of the present case-control study demonstrate that the risk of cataracts was associated with the Ins/Del polymorphism of SOD1. Although there is no published study regarding the effects of the SOD1 50bp ins/del variant on cataract susceptibility, the role of another genetic variant in the SOD1 gene has already

been studied in the pathogenesis of cataracts. Zhang et al. suggested that the SOD1–251A/G polymorphism may be associated with an increased risk of cataracts (9), also, Celojevic et al. found no correlation between SOD1 intron variants (rs17881180, rs2234694) and age-related cataract (29). Recently, Mahmood et al., reported that there was a significant association between SOD1 rs2070424 polymorphism and the development of cataracts in patients of Karachi, Pakistan (30).

It has been reported that up-regulation of SIRT1 in retinal cells protects cells from apoptotic death induced by anti-retinal antibodies, while down-regulation of SIRT1 causes retinal damage through multiple mechanisms and proposed that SIRT1 may play a role in the retina and optic nerve protections versus degeneration (31). In previous reports, Kilic et al. observed that the oxidative stress index and SIRT1 protein level enhanced in older people [19]. Also, it has been shown that SIRT1 plays crucial roles in regulating longevity, ageing, or in the pathogenesis of age-corresponded metabolic diseases (32). In addition, the explanation of SIRT1 is observed in the lens epithelium of patients along with age-related cataracts, adult retinas, and corneal epithelium (33). Therefore, SIRT1 can protect the retinal cells from apoptotic retinal death and oxidative stress-related retinal damage as well as anti-inflammation (16).

The outcomes of the current investigation demonstrated that the G allele of SIRT1-rs7895833 was associated with an enhanced risk of cataracts. Chen et al. previously reported that SIRT1-rs12778366 could be implicated in the pathophysiology of age-related macular degeneration (34). Furthermore, a recent study by Kaikaryte et al. revealed that the SIRT1 polymorphisms rs7895833 and rs3818292 and also, rs3818292-rs3758391-rs7895833 haplotype G-T-G could be associated with the development of exudative age-related macular degeneration (35). The correlation of SIRT1-rs7895833 polymorphism with diabetes (36), and neurodegenerative diseases, such as primary open-angle glaucoma (37), metabolic syndrome (38), and cardiovascular disease (39) have already been reported.

The results of the present research were influenced by the limited number of controls and cases, which represents a fundamental constraint in our study. Therefore, further efforts, including expanding the sample size and conducting functional experiments, are necessary to elucidate the precise impact of SOD1 and SIRT1 polymorphisms on cataract development.

CONCLUSION

This study represents the initial evidence proposing a potential link between the SIRT1-rs7895833 and SOD1-50bp ins/del polymorphisms with the cataract risk. To validate our findings, additional studies involving larger sample sizes across diverse ethnic groups are warranted.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

Acknowledgements

This paper was extracted from the MSc thesis of Sahar Sharghi. We thank all Control and patient subjects who participated in this study.

Funding

This research didn't receive any specific grant from funding agencies in the public, commercial and not-for-profit sectors.

Author contributions

LK Supervision, Methodology, Reviewing and Editing, SSh and AK Investigations, Statistical analysis, Original draft preparation and Data collection

REFERENCES

1.Sun W, Su L, Sheng Y, Shen Y, Chen G. Is there association between Glutathione S Transferases polymorphisms and cataract risk: a meta-analysis? BMC. Ophthalmology. 2015;15,84.

2.Mackenbrock LHB, Labuz G, Baur ID, Yildirim TM, Auffarth GU, Khoramnia R. Cataract Classification Systems: A Review. Klin. Monbl. Augenheilkd.2024; 241(1),75-83.

3.Lee B, Afshari NA, Shaw PX. Oxidative stress and antioxidants in cataract development. Curr. Opin. Ophthalmol 2023.

4.Younus H. Therapeutic potentials of superoxide dismutase. Int. J. Health. Sci.2018; 12(3),88.

5.Elmazar HM, Elmadbouh I, Mandour SS, Al Ariny GM, Ibrahim AM. Association between cataract progression and ischemia-modified albumin in relation to oxidant–antioxidant profiles in the serum, aqueous humor, and lens. J. Cataract. Refract. Surg. 2018; 44,134–139 (2018).

6.Rong X, Qiu X, Jiang Y, Li D, Xu J, Zhang Y, et al. Effects of histone acetylation on superoxide dismutase 1 gene expression in the pathogenesis of senile cataract. Sci. Rep. 2016; 6,34704.

7.Rajkumar S, Vasavada AR, Praveen MR, Ananthan R, Reddy GB, Tripathi H, et al. Exploration of molecular factors impairing superoxide dismutase isoforms activity in human senile cataractous lenses. Invest. Ophthalmol. Vis. Sci. 2013; 54(9),6224-33.

8.Lin D, Barnett M, Grauer L, Robben J, Jewell A, Takemoto L, et al. Expression of superoxide dismutase in whole lens prevents cataract formation. Mol. Vis. 2005; 11(11),853-8.

9.Zhang Y, Zhang L, Sun D, Li Z, Wang L, Liu P. Genetic polymorphisms of superoxide dismutases, catalase, and glutathione peroxidase in age-related cataract. Mol. Vis.2011; 17,2325.

10.Kopp TI, Vogel U, Dragsted LO, Tjonneland A, Ravn-Haren G. Association between single nucleotide polymorphisms in the antioxidant genes CAT, GR and SOD1, erythrocyte enzyme activities, dietary and life style factors and breast cancer risk in a Danish, prospective cohort study. Oncotarget. 2017; 8(38),62984.

11.Neves AL, Mohammedi K, Emery N, Roussel R, Fumeron F, Marre M, et al. Allelic variations in superoxide dismutase-1 (SOD1) gene and renal and cardiovascular morbidity and mortality in type 2 diabetic subjects. Mol. Genet. Metab. 2012;106(3),359-65.
12.Eskandari-Nasab E, Kharazi-Nejad E, Nakhaee A, Afzali M, Tabatabaei SP, Tirgar-Fakheri K, et al. 50-bp Ins/Del polymorphism of SOD1 is associated with increased risk of cardiovascular disease. Acta. Med. Iran.2014; 52(8),591-5.
13.Cheng Y, Takeuchi H, Sonobe Y, Jin S, Wang Y, Horiuchi H, et al. Sirtuin 1 attenuates oxidative stress via upregulation of superoxide dismutase 2 and catalase in astrocytes. J. Neuroimmunol. 2014; 269(1-2),38-43.
14.Elibol B, Kilic U. High levels of SIRT1 expression as a protective mechanism against disease-related conditions. Front. Endocrinol.2018; 9,614.
15.He J, Zhang A, Song Z, Guo S, Chen Y, Liu Z, Zhang J, Xu X, Liu J, Chu L. The resistant effect of SIRT1 in oxidative stress-induced senescence of rat nucleus pulposus cell is regulated by Akt-FoxO1 pathway. Biosci. Rep.2019; 39(5),BSR20190112.
16.Mimura T, Kaji Y, Noma H, Funatsu H, Okamoto S. The role of SIRT1 in ocular aging. Exp. Eye. Res.2013; 116,17-26.
17.Zhao Y, Wei J, Hou X, Liu H, Guo F, Zhou Y, Zhang Y, Qu Y, Gu J, Zhou Y, Jia X, Qin G, Feng L. SIRT1 rs10823108 and FOXO1 rs17446614 responsible for genetic susceptibility to diabetic nephropathy. Sci. Rep.2017; 7(1),10285.
18.Lv Y, Lin S, Peng F. SIRT1 gene polymorphisms and risk of lung cancer. Cancer. Manag. Res.2017; 9,381-386 9.
19.Kilic U, Gok O, Bacaksiz A, Izmirli M, Elibol-Can B, Uysal O. SIRT1 gene polymorphisms affect the protein expression in cardiovascular diseases. PLoS One.2014; 9(2),e90428.
20.Zhang A, Wang H, Qin X, Pang S, Yan B. Genetic analysis of SIRT1 gene promoter in sporadic Parkinson's disease. Biochem. Biophys. Res. Commun.2012; 422(4),693-6.
21.Wu X, Lai W, Lin H, Liu Y. Association of OGG1 and MTHFR polymorphisms with age-related cataract: A systematic review and meta-analysis. PloS one. 2017; 12(3),e0172092.
22.Kohan L, Mirhosseini M, Mortazavizadeh SM. The risk of relapse in breast cancer patients is associated with MMP-9 gene polymorphism: a prospective study in a sample of the Iranian population. Nucleosides Nucleotides Nucleic Acids. 2022; 41(10),1012-1023.
23.Kafeel S, Bizenjo N, Shivji SS, Keran A, Hashim Z, Nawab SN. DNA Repair Enzyme XRCC4 30 bp Indel Intron 3 Locus Significant Association with Predisposition of Cataract in Senility. Appl Biochem Biotechnol.2024; 196(1),99-112.
24.Zafar S, Khurram H, Kamran M, Fatima M, Parvaiz A, Shaikh RS. Potential of GJA8 gene variants in predicting age-related cataract: A comparison of supervised machine learning methods. PLoS One. 2023; 18(8),e0286243.
25.Berthoud VM, Beyer EC. Oxidative stress, lens gap junctions, and cataracts. Antioxid. Redox. Signal.2009; 11(2),339-53.
26.Harati-Sadegh M, Kohan L, Teimoori B, Mehrabani M, Salimi S. The effects of placental long noncoding RNA H19 polymorphisms and promoter methylation on H19 expression in association with preeclampsia susceptibility. Iubmb Life. 2020; 72(3),413-25.
27.Harati-Sadegh M, Kohan L, Teimoori B, Mehrabani M, Salimi S. Analysis of polymorphisms, promoter methylation, and mRNA expression profile of maternal and placental P53 and P21 genes in preeclamptic and normotensive pregnant women. J. Biomed. Sci. 2019; 26(1),92.
28.Saify K, Saadat M. Influence of a 50bp Ins/Del polymorphism at promoter of the superoxide dismutase-1 on gene expression and risk of heroin dependency. Environ. Health. Prev. Med.2017; 22(1),4.
29.Celojevic D, Nilsson S, Behndig A, Tasa G, Juronen E, Karlsson JO, Zetterberg H, Petersen A, Zetterberg M. Superoxide dismutase gene polymorphisms in patients with age-related cataract. Ophthalmic Genet. 2013; 34(3),140-5.
30.Mahmood A, Aziz Q, Nangrejo R, Ahmed I, Soomro S, Ara I. Superoxide Dismutase (SOD1) Gene Polymorphism in Cataract Patients of Karachi, Pakistan. Journal of the Dow University of Health Sciences (JDUHS). 2022; 16(1),22-6.
31.Kubota S, Kurihara T, Ebinuma M, Kubota M, Yuki K, Sasaki M, Noda K, Ozawa Y, Oike Y, Ishida S, Tsubota K. Resveratrol prevents light-induced retinal degeneration via suppressing activator protein-1 activation. Am. J. Pathol. 2010; 177(4),1725-31.
32.Maeda S, Koya D, Araki SI, Babazono T, Umezono T, Toyoda M, et al. Association between single nucleotide polymorphisms within genes encoding sirtuin families and diabetic nephropathy in Japanese subjects with type 2 diabetes. Clin. Exp. Nephrol. 2011; 15(3),381-390.
33.Zheng T, Lu Y. Changes in SIRT1 expression and its downstream pathways in age-related cataract in humans. Curr. Eye. Res.2011; 36(5),449-55.
34.Chen Z, Zhai Y, Zhang W, Teng Y, Yao K. Single Nucleotide Polymorphisms of the Sirtuin 1 (SIRT1) Gene are Associated With age-Related Macular Degeneration in Chinese Han Individuals: A Case-Control Pilot Study. Medicine (Baltimore). 2015; 94(49):e2238.
35.Kaikaryte K, Gedvilaite G, Vilkeviciute A, Kriauciuniene L, Mockute R, Cebatoriene D, Zemaitiene R, Balciuniene VJ, Liutkeviciene R. SIRT1: Genetic Variants and Serum Levels in Age-Related Macular Degeneration. Life.2022; 12(5),753.
36.Botden IP, Zillikens MC, De Rooij SR, Langendonk JG, Danser AJ, Sijbrands EJ, et al. Variants in the SIRT1 gene may affect diabetes risk in interaction with prenatal exposure to famine. Diabetes Care. 2012; 35(2),424-6.
37.Siwak M, Maślankiewicz M, Nowak-Zduńczyk A, Filipek B, Wojtczak R, Radek M, Kucharska E, Szaflik J, Szaflik JP, Majsterek IJ. Analysis of rs7895833 polymorphism of SIRT1 gene and its influence on the risk occurrence and progression of neurodegenerative disease, such as primary open-angle glaucoma in a Polish population. Journal of Pre-Clinical and Clinical Research. 2022.
38.Tao TT, Lin XH, Tang SJ, Gui WW, Zhu WF, Li H. Association of genetic variants in the Sirt1 and Nrf2 genes with the risk of metabolic syndrome in a Chinese Han population. BMC. Endocrine Disorders. 2022; 22(1),1-8.
39.Yamac AH, Uysal O, Ismailoglu Z, Ertürk M, Celikten M, Bacaksiz A, Kilic U. Premature Myocardial Infarction: Genetic Variations in SIRT1 Affect Disease Susceptibility. Cardiol. Res. Pract. 2019; 8921806.

http://pmjournal.ir Review Article

Winter 2024, Volume 9, Issue 32 (23- 28)

Innovative Functions of Metabolomics in Individualized Health Care: A Review Study in the Field of Metabolomics VitminD Treatment Change MTH1 and MYH Genes Expression in HUVEK Cell

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DOI: 10.22034/pmj.2024.2024193.1033

Submitted: 2023-12-05
Accepted: 2024-02-26

Keywords:
Personalized medicine
Metabolomics
Health Care
Drug Sensitivity

How to Cite this Article:
S. Saremi-Nouri, M. Emami, H. Kabiri, N. Rajaei. "Innovative Functions of Metabolomics in Individualized Health Care: A Review Study in the Field of Metabolomics VitminD Treatment Change MTH1 and MYH Genes Expression in HUVEK Cell " Personalized Medicine Journal, Vol. 9, no. 32, pp. 23- 28.

Abstract:

Variability in medication reactions and illness susceptibility among individuals is often seen in clinical settings. Personalized medicine is now highly esteemed for its focus on prescribing the appropriate medication to each patient. *Metabolomics* is a developing field that thoroughly assesses all metabolite and low-molecular-weight compounds in a biological sample. Metabolic profiling offers a quick overview of a cell›s physiology, making the technique a direct indicator of an organism›s physiological condition. Quantifiable correlations exist between the metabolome and other cellular components such as the genome, transcriptome, proteome, and lipidome. These correlations can be utilized to forecast metabolite levels in biological samples based on mRNA levels. One of the key problems in systems biology is to incorporate metabolomics with other -omics data to enhance comprehension of cellular biology. Metabolomics is used to assess the effectiveness of clinical substances by analyzing the metabolic characteristics of patients before treatment to predict their responses (pharmacometabolomic) and to identify individuals at risk of developing diseases (patient stratification). The rapid progress in metabolomics technique highlights its significant potential for use in customized treatment. We reviewed the unique benefits of metabolomics, including instances in assessing medication treatment and individual stratification, and emphasized metabolomics› promise in personalized medicine.

INTRODUCTION

Interindividual differences in treatment success or illness susceptibility are often seen in clinical settings due to the complex interplay between hereditary and environmental variables (1). The notion of customized medicine, also known as precision medicine, is now of tremendous interest due to revolutionary advancements in biomedical research and high-throughput analytical technologies (2). Personalized medicine aims to optimize treatment by tailoring medication to individual patients for optimal effectiveness and the fewest side effects or to forecast disease vulnerability in groups (3).

Efforts have been made to link medication reactions with host genetic variations, known as pharmacogenomics (4). Despite notable advancements in pharmacogenomics over the past decades, it does not account for the influence of environmental factors and the co-metabolism of host and intestinal microbiota, which are crucial in drug metabolism and disease development (5).

Metabolomics/metabonomics emerged at the end

of the 20th century to study changes in endogenous metabolites due to biological system modifications like cells, tissues, and body fluids, following genomics, transcriptomics, and proteomics (6). Metabolomics combines genetic and environmental influences to analyze biomarkers and investigate illness mechanisms, medication effects, toxicity, and metabolism (5, 6).

Furthermore, pharmacometabonomics, also known as pharmaco-metabolomics, is described as predicting the effects of a medicine or foreign substance on a person using a mathematical model of their metabolite patterns before the intervention (7). Pharmacometabonomics is used to discover metabolic biomarkers that may predict various reactions to medicinal medications by detecting distinct metabolites at the start and linking their changes to treatment results (8). Metabolomics has been used to assess individual vulnerability to illnesses in communities by combining baseline metabotypes with the likelihood of disease incidence, leading to patient stratification (9).

The following mini-review provides a concise overview of commonly used analytical equipment in metabolomics, including nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS). We mainly discussed the unique uses of metabolomics in predicting medication responses and patient classification, highlighting the potential of metabolomics in personalized healthcare soon.

METABOLOMICS TECHNOLOGIES

Metabolomics is a newer field than more established disciplines like genomes, transcriptomics, and proteomics. The rapid progress of metabolomics is attributed to significant advancements in analytical instruments and associated data mining methods during the last decade (10). Three primary metabolomic systems are NMR, GC-MS, and LC-MS. Due to their varied analytical capabilities, these three platforms have been utilized in targeted/untargeted metabolomic research either alone or in conjunction to identify metabolites such as amino acids, organic compounds, lipids, and sugars (11). It is well acknowledged that every technology has strengths and weaknesses in metabolomic research. NMR is often more effective for identifying and quantifying metabolites and for automation, but it has poorer sensitivity and more significant initial costs when compared with MS-based metabolomic methods. GC-MS is a sturdy analytical technique with high sensitivity and allows for easy identification of metabolites utilizing commercial databases and software. However, sample preparation may be time-consuming, and identifying new compounds can be challenging. LC-MS offers greater sensitivity, a broader metabolite detection range, and varied methods, but NMR or GC-MS is more robust and more challenging for chemical identification. Some evaluations have thoroughly compared the merits and downsides of different platforms (10, 11).

Metabolomics for Drug Sensitivity Prediction

A)Simvastatin

These medications are traditional antagonists of HMG-CoA reductase frequently employed to lower LDL-cholesterol levels in the blood and decrease the likelihood of cardiovascular disease (12). Additional biological functions of statins have been documented, including anti-inflammatory actions and immune system regulation. These activities may result in therapeutic advantages or adverse consequences involving type 2 diabetes mellitus and myopathy. Furthermore, individuals clearly differ in how effective simvastatin is as a therapy (13).

Researchers analyzed the lipid profiles before and after treatment using focused on the lipidomics method in favorable and poor participants (24 subjects in each group) based on the percentage alteration in LDL-c decrease or secondary result of C-reactive protein (CRP) in the Cholesterol and Pharmacogenetics (CAP) research (14). Approximately 40 metabolites exhibited substantial changes in excellent participants but not poor rescuers. In excellent responders, 13 saturated or monounsaturated fatty acids were up, while those of 15 polyunsaturated fatty acids (PUFAs) went down. Upon further examination, it was shown that the initial levels of n-6 and n-3 were favorably associated with the decrease in LDL-c, although CE and DG SFA showed a negative correlation. The variables DG-n6 and FA-n3 showed a favorable correlation with the treatment result. Baseline concentrations of PE plasmalogens showed a positive correlation, whereas PC plasmalogens showed a negative correlation with CRP changes post-therapy. The findings suggest that the initial lipid profiles are biomarkers for predicting various responses to simvastatin therapy (15).

They found lower starting point plasma concentrations of both primary and secondary bile acids were closely linked to decreased LDL-c during simvastatin treatment in randomly chosen subjects. This included greater initial LCA, TLCA, GLCA, and coprostanol (COPR) in individuals who responded well to the treatment. The study suggests that the gut microbiota influences the effectiveness of simvastatin via the production of secondary bile acids. Recent research found that the effectiveness of simvastatin as a treatment was reduced by altering the gut microbiota using antibiotics, which suppressed the production of bile acids from cholesterol (15, 16).

B)Aspirin

Aspirin is a commonly used medicine for its strong pain-relieving, fever-reducing, anti-inflammatory, and

blood-thinning properties (17). Approximately 25% of high-risk individuals exhibit resistance to aspirin to platelet activation and atherothrombotic incidents (18). Researchers studied the metabolic processes related to aspirin resistance by analyzing the metabolic patterns before taking aspirin and comparing them to individual responses after aspirin treatment in the Heredity and Phenotype Intervention (HAPI) heart investigation. Seventy-six healthy adults were chosen for their response to collagen-induced ex vivo platelet aggregation. They were divided into 40 good responders and 36 poor responders (19). All participants completed a two-week aspirin therapy. GC/MS-based untargeted metabolomics was used to analyze blood samples taken before and after the dosage. Initially, 18 distinct metabolites were detected in post-dose specimens from all 76 participants, and it was shown that the purine metabolic pathway was notably influenced by aspirin. A comparison was made between excellent and poor responders' purine metabolism pathway metabolites before and after taking an aspirin dosage. Aspirin increased inosine levels in both good- and poor-responders, with more significant levels seen in poor-responders after taking the medication. Subsequently, the medication response-related metabolites were confirmed in a separate cohort of participants (19 good responders and 18 poor responders) within the same research (15, 19).

After conducting a metabolomic study, the authors investigated the genetic connection of purine metabolism-related genes using a "pharmacometabolomics-informed pharmacogenomics" technique. Association studies were conducted between single-nucleotide polymorphisms (SNPs) in nine purine metabolism-related genes and ex vivo platelet aggregation. Researchers identified 51 single nucleotide polymorphisms (SNPs) in the adenosine kinase (ADK) gene related to purine metabolism, significantly correlated with platelet aggregation alterations after aspirin use. The most influential SNP was the intronic variation rs16931294. The G allele was linked to increased platelet aggregation following aspirin exposure compared to the more prevalent A allele. The G allele of rs16931294 was substantially linked to elevated levels of AMP, xanthine, and hypoxanthine before taking aspirin, as well as inosine and guanosine after aspirin treatment, compared to the A allele (19). It was determined that changes in metabolites within the purine metabolism pathway contribute to differences in aspirin effectiveness among individuals. Combining pharmacometabolomics with pharmacogenomics can enhance comprehension of how genetic and metabolic factors influence variations in drug responses.

C)Acamprosate

Acamprosate is an amino acid derivative authorized for treating Alcohol Use Disorders (AUDs). Only a subset of people with Alcohol Use Disorders are responsive to acamprosate treatment. Therefore, it is crucial to identify biomarkers that might forecast the treatment results of acamprosate in individuals with alcohol use disorders in clinical settings (20).

Researchers examined the initial and post-treatment metabolic profiles in a cohort of 120 individuals with alcohol dependence. The sample consisted of 71 individuals who responded well to therapy and 49 who did not react throughout 12 weeks of acamprosate treatment using a pharmacometabolomic method. The scientists first analyzed 36 metabolites in blood samples before and after acamprosate therapy in an identified cohort (51 responses and 39 non-responders) utilizing UPLC-MS/MS. Fourteen different metabolites were found between the starting point and post-treatment of acamprosate. Of the 14 divergent metabolites in the replication cohort, 4 exhibited comparable changes to those in the discovery cohort, with glutamate showing significant alterations. Responders had greater baseline levels of glutamate compared to non-responders. Glutamate concentrations decreased significantly in responders but not in non-responders after acamprosate administration. The baseline ammonia concentrations were more significant in those who responded to acamprosate therapy and decreased following the treatment (15,21).

Furthermore, multivariable logistic regression showed that elevated baseline glutamate or ammonia levels were strong indicators of positive responses to acamprosate therapy. The authors of the animal research found that acamprosate increased glutamine production from glutamate and ammonia via activating glutamine synthetase, leading to a decrease in glutamate levels after acamprosate therapy. Thus, the initial levels of glutamate may serve as a possible indicator for forecasting the treatment results of acamprosate in a clinical setting (21).

Metabolomics in the Identification of Disease Susceptibility Biomarkers

Individuals with the same ailment may respond differently to similar medication treatment due to varied genetic or metabolic backgrounds, leading to significant differences in disease susceptibility across populations. Identifying biomarkers that predict illness risk among individuals is crucial for personalized medicine (22).

Variability in branched-chain amino acids determine the risk of diabetes.

Metabolic illnesses result from the disruption of energy balance control, with obesity being the primary risk factor. Metabolic changes often occur years before metabolic illness develops, and susceptibility

Table1. Details of drugs using for genotyping SIRT1 and SOD1 polymorphisms.

Drugs	Metabolomics	Key Metabolite	Drug Efficacy
Simvastatin	GC/MS-based untargeted metabolomics	LCA, TLCA, GLCA, COPR	reducing plasma LDL-cholesterol
	GC/TOFMS-based untargeted metabolomics	fructose	
Acamprosate	UPLC-MS/MS-based untargeted metabolomics	glutamate	treating Alcohol Use Disorders (AUDs)
Aspirin	GC-MS-based untargeted metabolomics	inosine	antiplatelet aggregation
	MS-based targeted metabolomics	serotonin	

to the condition varies significantly, even among fat individuals. Approximately one-third of obese individuals do not have any metabolic abnormalities, as shown by a recent meta-analysis (23). Discovering metabolic biomarkers that can categorize individuals into distinct risk groups for disease progression is crucial (24, 25). Researchers conducted a nested case-control metabolomic research in the Framingham Offspring Project (26). They assessed the baseline metabolic profiles of 189 individuals who developed diabetes over a 12-year follow-up period and compared them to matched controls. Through paired analysis, researchers found five metabolites at the beginning of the study that showed significant differences between the two groups: three branch-chained amino acids (leucine, isoleucine, and valine) and two aromatic amino acids (phenylalanine and tyrosine). Additional examination revealed that individuals in the highest quartile of plasma amino acids had a minimum of double the risk of developing diabetes over the next 12 years, contrasting those with the lowest concentrations of plasma amino acids. This association was significantly stronger when only three specific branch-chained amino acids were considered. The predictive ability of the discovered amino acids for the development of diabetes was validated in separate replication samples and a random subset of the Framingham Offspring cohort. This work emphasized the significance of amino acids in the development of diabetes and indicated the possibility of identifying metabolic biomarkers for metabolic illnesses by metabolomic analysis (26, 27).

CONCLUSION

Metabolomics is a recent method in comparison to other “omics” disciplines. Metabolomics has been widely tested in clinical and experimental investigations due to the fast advancement of analytical technologies and growing interest in precision medicine. An individual’s metabotype at the beginning of therapy may help predict responses to medication treatment and susceptibility to illnesses throughout the follow-up period. The contrasting metabolic profiles before and after therapy might uncover new pharmacological mechanisms by linking the changed metabolites with related metabolic pathways. To understand how genetic and metabolic factors affect treatment results, it is essential to integrate metabolomics and genomics to uncover the processes behind various medication reactions, as the suggested GWAS-metabolomics approach [39]. Pharmacometabolomics remains in its early stages since most research primarily aims to identify the relationship between initial metabotypes and how individuals respond to drugs or are susceptible to diseases. Baseline metabotypes are often affected by diet, age, medication usage, and gut flora. These factors must be considered when planning pharmacometabolomic research to reduce metabolic biases. Furthermore, the interaction between gut microbiota variations and host metabotype might impact host metabolism and responses to pharmacological treatment, making the combination of metabolomic and gut microbiome studies crucial for a comprehensive knowledge of the functions of gut microbiota. There is limited knowledge about how these “biomarkers” influence drug reactions or disease susceptibility. However, it is crucial to explore the new functions of individual metabolites or their combination in specific conditions through interdisciplinary methods. Although there are large hurdles, confirming the predictive capacity of possible “biomarkers” and defining their functions will be gratifying to hasten the reality of personalized

treatment in clinics. We anticipate that metabolomics and pharmacometabolomics will see growing use and advancement in the future due to technology innovation and their integration with other omics methodologies, driven by a strong interest in customized medicine.

Acknowledgments

I would like to thank biotechnology research center members, who helped with this research.

Conflicts of Interest

The authors declare no conflict of interest.

REFERENCES

1.Oyesola OO, Souza CO. The Influence of Genetic and Environmental Factors and Their Interactions on Immune Response to Helminth Infections. Frontiers in Immunology. 2022 Apr 29;13:869163.
2.Lightbody G, Haberland V, Browne F, Taggart L, Zheng H, Parkes E, Blayney JK. Review of applications of high-throughput sequencing in personalized medicine: barriers and facilitators of future progress in research and clinical application. Briefings in bioinformatics. 2019 Sep;20(5):1795-811.
3.Piri-Gharaghie T. Polycystic ovary syndrome and genetic factors influencing its development: A review article. Personalized Medicine Journal. 2021 Dec 1;6(23):25-9.
4.Pirmohamed M. Personalized pharmacogenomics: predicting efficacy and adverse drug reactions. Annual review of genomics and human genetics. 2014 Aug 31;15:349-70.
5.Dhurjad P, Dhavaliker C, Gupta K, Sonti R. Exploring drug metabolism by the gut microbiota: modes of metabolism and experimental approaches. Drug Metabolism and Disposition. 2022 Mar 1;50(3):224-34.
6.Klassen A, Faccio AT, Canuto GA, da Cruz PL, Ribeiro HC, Tavares MF, Sussulini A. Metabolomics: definitions and significance in systems biology. Metabolomics: from fundamentals to clinical applications. 2017:3-17.
7.Everett JR. Pharmacometabonomics: The prediction of drug effects using metabolic profiling. Concepts and Principles of Pharmacology: 100 Years of the Handbook of Experimental Pharmacology. 2019:263-99.
8.Beger RD, Schmidt MA, Kaddurah-Daouk R. Current concepts in pharmacometabolomics, biomarker discovery, and precision medicine. Metabolites. 2020 Mar 27;10(4):129.
9.Beger RD, Dunn W, Schmidt MA, Gross SS, Kirwan JA, Cascante M, Brennan L, Wishart DS, Oresic M, Hankemeier T, Broadhurst DI. Metabolomics enables precision medicine:"a white paper, community perspective". Metabolomics. 2016 Sep;12:1-5.
10.Pinu FR, Beale DJ, Paten AM, Kouremenos K, Swarup S, Schirra HJ, Wishart D. Systems biology and multi-omics integration: viewpoints from the metabolomics research community. Metabolites. 2019 Apr 18;9(4):76.
11.Wishart DS. Emerging applications of metabolomics in drug discovery and precision medicine. Nature reviews Drug discovery. 2016 Jul;15(7):473-84.
12.Zodda D, Giammona R, Schifilliti S. Treatment strategy for dyslipidemia in cardiovascular disease prevention: focus on old and new drugs. Pharmacy. 2018 Jan 21;6(1):10.
13.Ridker PM. The JUPITER trial: results, controversies, and implications for prevention. Circulation: Cardiovascular Quality and Outcomes. 2009 May;2(3):279-85.
14.Kaddurah-Daouk R, Baillie RA, Zhu H, Zeng ZB, Wiest MM, Nguyen UT, Watkins SM, Krauss RM. Lipidomic analysis of variation in response to simvastatin in the Cholesterol and Pharmacogenetics Study. Metabolomics. 2010 Jun;6:191-201.
15.Li B, He X, Jia W, Li H. Novel applications of metabolomics in personalized medicine: a mini-review. Molecules. 2017 Jul 13;22(7):1173.
16.He X, Zheng N, He J, Liu C, Feng J, Jia W, Li H. Gut microbiota modulation attenuated the hypolipidemic effect of simvastatin in high-fat/cholesterol-diet fed mice. Journal of proteome research. 2017 May 5;16(5):1900-10.
17.Al-Hamadiny SQ, Salman RI, Najman SK, Dilfy SH, Qurabiy HE. Clinical Evaluation of Aspirin Effects on Gastrointestinal Tract and Circulatory System. NVEO-NATURAL VOLATILES & ESSENTIAL OILS Journal| NVEO. 2021 Nov 15:5328-43.
18.Mason PJ, Jacobs AK, Freedman JE. Aspirin resistance and atherothrombotic disease. Journal of the American College of Cardiology. 2005 Sep 20;46(6):986-93.
19.Yerges-Armstrong LM, Ellero-Simatos S, Georgiades A, Zhu H, Lewis JP, Horenstein RB, Beitelshees AL, Dane A, Reijmers T, Hankemeier T, Fiehn O. Purine pathway implicated in mechanism of resistance to aspirin therapy: pharmacometabolomics-informed pharmacogenomics. Clinical pharmacology & Therapeutics. 2013 Oct;94(4):525-32.
20.Hinton DJ, Vázquez MS, Geske JR, Hitschfeld MJ, Ho AM, Karpyak VM, Biernacka JM, Choi DS. Metabolomics biomarkers to predict acamprosate treatment response in alcohol-dependent subjects. Scientific reports. 2017 May 31;7(1):2496.
21.Nam HW, Karpyak VM, Hinton DJ, Geske JR, Ho AM, Prieto ML, Biernacka JM, Frye MA, Weinshilboum RM, Choi DS. Elevated baseline serum glutamate as a pharmacometabolomic biomarker for acamprosate treatment outcome in alcohol-dependent subjects. Translational psychiatry. 2015 Aug;5(8):e621-.
22.Asadipour E, Asgari M, Mousavi P, Piri-Gharaghie T, Ghajari G, Mirzaie A. Nano-Biotechnology and Challenges of Drug Delivery System in Cancer Treatment Pathway. Chemistry & Biodiversity. 2023 Mar 1:e202201072.
23.Bell JA, Kivimaki M, Hamer M. Metabolically healthy obesity and risk of incident type 2 diabetes: a meta-analysis of prospective cohort studies. Obesity reviews. 2014 Jun;15(6):504-15.
24.Doosti A, Amini-Bavil-Olyaee S, Tajbakhsh E, Adeli A, Mahboudi F. Prevalence of viral hepatitis and molecular analysis of HBV among voluntary blood donors in west Iran. The new microbiologica. 2009 Apr 1;32(2):193.
25.Piri Gharaghie T, Doosti A, Mirzaei SA. Detection of T6SS secretory system and membrane purine involved in antibiotic resistance in multidrug-resistant Acinetobacter baumannii isolates. Journal of Microbial World. 2021 May 22;14(1):47-58.
26.Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C,

O'donnell CJ. Metabolite profiles and the risk of developing diabetes. Nature medicine. 2011 Apr;17(4):448-53.
27.Hashemzehi R, Doosti A, Kargar M, Jaafarinia M. Cloning and expression of nlpA gene as DNA vaccine candidate against Acinetobacter baumannii. Molecular biology reports. 2018 Aug;45:395-401.

http://pmjournal.ir Review Article

Winter 2024, Volume 9, Issue 32 (29- 35)

Introducing PROTAC Therapy—a Novel Tailored Approach to Lung Cancer Treatment

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DOI: 10.22034/pmj.2024.712171

Submitted: 2023-12-02
Accepted: 2024-02-15

Keywords:
Cancer
Drug resistance
Targeted therapy
PROTAC therapy

How to Cite this Article:

S. Abareshi, Y. Yousefi, N. Zeynalniya Toosi. "Introducing PROTAC Therapy—a Novel Tailored Approach to Lung Cancer Treatment " Personalized Medicine Journal, Vol. 9, no. 32, pp. 29- 35.

Abstract:

Drug resistance in cancer is a major challenge to properly treating malignancy. Therapies aimed at proteins involved in cancer development may become less effective due to acquired resistance to medications, often resulting from mutations as well as heightened expression of the targeted proteins. Posttranslational modifications (PTMs) like as phosphorylation, methylation, ubiquitination, and acetylation are crucial for regulating protein expression levels. PROTACs are engineered to selectively degrade a specific protein of interest (POI) by ubiquitination, resulting in a regulated decrease in the POI's expression. PROTACs show great potential in targeting hitherto untargetable proteins, such as various transcription factors. PROTACs enhance antitumor immune therapy by specifically modifying certain proteins. Although molecular therapies have advanced, lung cancer remains a major contributor to cancer-related mortality. The management of those with lung cancer is now limited by a lack of targeted therapy choices and the development of acquired drug resistance. Using the intracellular ubiquitin-proteasome system for directed protein breakdown might enhance individualized treatment for lung cancer patients. This study explores the rationale for using PROTAC therapy as an innovative specific therapy and the current advancements in PROTAC development for lung tumors.

INTRODUCTION

Cancer incidence and death rates are on the rise globally, with lung tumors being the most often detected kind, representing 11.6% of all cases. The malignancy of the lung is the primary cause of cancer-related deaths worldwide, accounting for 18.4% of all cancer fatalities and resulting in substantial societal and economic impacts. The five-year survival rate for lung cancers is below 20%. Early-stage lung cancer individuals who had micro-invasive carcinoma and carcinoma had a 5-year survival rate above 100%, whereas advanced-stage lung cancer patients had a survival rate of around 2% (3). Patients with advanced-stage lung cancer should be given a thorough diagnosis and be prioritized for chemotherapy or radiation. Timely detection of lung cancer and appropriate therapy may significantly enhance patients' survival rate by 20%. Smoking is responsible for about eighty percent of fatalities related to lung cancer (4). Factors contributing to the chance of developing lung cancer include exposure to radon, asbestos, long-term and repeated contact to air pollutants, including emissions of polycyclic aromatic hydrocarbons (PAH), and a previous history of cancer of the lungs. The World Health Organization (WHO) categorizes lung cancers into two primary groups: non-small cell lung cancer (NSCLC) representing 80–85% of cases, and small cell lung cancer (SCLC) accounting for the remaining 15%. NSCLC may be classified as adenocarcinoma (LUAD), squamous cell carcinoma (LUSC), and large cell carcinoma (LCC) (5). Every subclass may be subdivided into many groups based on the molecular targetable genetic profile. The 5-year rate of survival for metastatic lung cancer, including both NSCLC and SCLC types, are around 4% (5). Attempts have been made to classify histological subtypes of lung cancer. Each subtype, including adeno, squamous, and small cell carcinoma, has diverse genetic characteristics as identified by the Cancer Genome Atlas via molecular analysis (6). This variety complicates the interpretation

of comprehensive therapy studies that combine clinical outcomes and can miss important therapeutic options designed for specific mutational backgrounds (6).

Both TNM staging and thorough genes are essential for choosing the treatment for patients having lung tumors. Patients undergo biopsy, staging, and genome sequencing to determine suitable therapies for lung tumors, such as surgical removal, radiation, systemic radiation therapy, specific treatment, and immunotherapy. Many individuals with advanced cancer are likely to have tumor development over time as a result of the clonal selection of treatment-resistant tumor cells. Developing innovative strategies to address medication resistance is essential for improving patient results (7).

Although advancements have been made in identifying driver mutations, the outlook for patients with advanced or metastatic NSCLC remains unfavorable. The primary obstacle associated with targeted treatment is the development of acquired resistance (8). Typical resistance mechanisms involve of changes in driving oncogenes, variations in parallel signaling pathways, histologic transformations, and drug tolerance (9). The requirement for innovative treatments that address both the primary mutation and probable resistance pathways is underscored by the resistance and subsequent advancement seen in several individuals. One innovative treatment approach involves the application of target protein degraders (TPD) such proteolysis targeting chimeras (PROTACs) or lysosomal-targeting chimeras (LYTACs). LYTACs and PROTACs use intrinsic cellular mechanisms to specifically eliminate cancer-causing proteins (10). The PROTAC approach is currently employed in laboratory experiments, animal studies, and early-stage clinical trials to evaluate its efficacy against key alterations in various cancer types (11). This study presents the PROTAC innovation, explores advancements in PROTAC innovation for lung cancer, and assesses the potential and obstacles in using PROTACs for clinical applications to improve lung tumor therapy.

Introduction proteolysis targeting chimera

PROTACs are molecules with two different functions that use the natural ubiquitin-proteasome system to target and eliminate certain proteins associated with illnesses like cancer (12). PROTACs are composed of two protein binding molecules that are covalently bonded. One molecule attaches to the protein targeted for degradation, while the other engages the E3 ubiquitin ligase, facilitating ubiquitination and subsequent destruction (13). Proteins are ubiquitinated and then degraded by the proteasome via a process including activation, conjugation, and ligation steps. Ubiquitin is transferred in a step-by-step manner from E1 to E2 and ultimately to the target protein for degradation by the E3 enzyme. Proteasomes break down the protein (14). The PROTAC molecule exploits this mechanism by attaching to the ubiquitin-E2-E3 complex and the desired protein, promoting ubiquitination and eventual degradation of the protein (14). The PROTAC addresses several resistance-related difficulties by fully degrading the protein. Additionally, the PROTAC does not need contact with the active region of the molecule. PROTAC compounds may expedite the breakdown of several target molecules and may need lower dosages compared to direct inhibitors (15). This might result in less systemic adverse effects as compared to direct inhibitors. This approach is rapidly progressing and is being studied in several kinds of malignancies including lung, breast, prostate, and hematologic cancers. The modular structure of PROTAC design provides significant potential, flexibility, and effectiveness in creating new PROTACs to target and break down different intracellular protein substrates (16). Small chemicals that bind to specific regions of target proteins are used in PROTAC development as ligands for the protein-of-interest (POI), rather than conventional small molecule inhibitors (SMIs) that need high affinities to block protein function. Various PROTACs have been created and improved by using a range of small molecule substances, including authorized by the Food and targeted inhibitors, failed clinical trial medicines, and tool compounds (17). For instance, small molecule epidermal growth factor receptor (EGFR) inhibitors and ALK inhibitors were used to create PROTACs that specifically target and destroy EGFR and ALK (18).

Many reported PROTACs use commonly expressed CRBN and VHL E3 ligases, potentially resulting in on-target toxicity (19). PROTACs targeting the same protein exhibit different degradation rates when using either a CRBN or a VHL E3 ligase. Identifying E3 ligases that are unique to tumors and developing specific ligands for them might greatly enhance the effectiveness of tumor-targeted therapies. PROTACs have numerous advantages as compared to conventional small molecule inhibitors (SMIs) (20). PROTACs function by degrading protein targets rather than by limiting their activity. This is expected to provide better suppression in a scenario where the cancer-causing behavior of an objective may happen regardless of its enzymatic function. PROTACs may modify the scaffolding role of certain proteins to enhance the effectiveness of the payloads and overcome tolerance (21). It may target and dismantle multicomponent complexes of proteins that are often considered “undruggable” since blocking one subunit may not deactivate the complex’s function. Furthermore, PROTACs might combat resistance by degrading overexpressed proteins of interest induced by small molecule inhibitors or proteins of interest arising from mutations in the targets (22). PROTACs

have unique event-driven pharmacology, allowing them to trigger many cycles of degradation, unlike SMIs that depend on occupancy-driven pharmacology. PROTAC molecules provide a unique mechanism that enables them to degrade a range of target proteins using minimum drug dosages to achieve the desired medical result. The modular design of PROTACs allows for convenient and adaptable development and enhancement (23).

Advancements in PROTAC technology

PROTACs have become innovative treatments for lung cancer and effective approaches to combat medication resistance in recent times. Several PROTAC medicines have been created to target established goals for therapy in NSCLC, including EGFR, KRAS, ALK, BRAF, and BCL-XL (23). These medications have shown anti-cancer effectiveness in cultured cells and experimental tumor models. Additional improvement of these PROTAC medicines is necessary, coupled with thorough preclinical assessment before advancing to clinical trials.

PROTACs that target EGFR

The main EGFR mutations are EGFREx19Del and EGFRL858R. First and second-generation EGFR-TKIs, including as gefitinib, erlotinib, afatinib, and dacomitinib, were created for targeting these genetic variants directly (24). Osimertinib addressed resistance problems by specifically targeting the EGFRT790M mutation that developed as a result of previous EGFR-TKIs (25). Many patients who first benefit from osimertinib will eventually develop resistance owing to additional EGFR alterations, structural alterations, and heightened MET amplification. Approximately 40% to 50% of patients had novel EGFR mutations, namely in the C797, G796, and L718 sites, leading to cancer recurrence due to resistance to treatment. The majority of advanced-stage patients with lung cancer have reduced effectiveness of EGFR-TKIs due to resistance, emphasizing the requirement for innovative treatment approaches to combat acquired resistance mechanisms such the emergence of new EGFR C797S and T790M mutations (26). PROTAC approach efficiently targets EGFR mutations that are resistant by inducing the degradation of specific EGFR mutants (27). Molecule 4, a PROTAC compound created from afatinib, caused the breakdown of the gefitinib-resistant L858R/T790M mutant EGFR in the H1975 cell line. This work showed that PROTACs may effectively degrade mutant EGFR proteins located on the cell membrane to address drug-resistant EGFR mutations. Building upon this finding, many research teams have created innovative EGFR PROTACs, with some showing effectiveness in inhibiting tumor growth in animal models. The Zhang group has developed accessible EGFR PROTAC, HJM-561, to address treatment resistance in NSCLC resulting from EGFR triple mutations. This drug effectively targets mutant EGFR proteins, showing potent antitumor effects in cell line-derived xenograft (CDX) and patient-derived xenograft (PDX) mice with EGFR Del19/T790M/C797S mutations that did not respond to osimertinib therapy (28). The Zhu group developed potent covalent inhibitors using dacomitinib to target and destroy EGFR (29). The Li group discovered two powerful and selective compounds, 13a and 13b, that target a specific protein and efficiently inhibited the development of malignancies in a laboratory setting (30). CFT8919 has been shown to cause tumor shrinkage in preclinical tumor models that are resistant to first-, second-, and third-generation EGFR-TKIs. It also has the ability to target CNS metastases in the preclinical model (31). These EGFR PROTACs show great promise as candidates for future development and evaluation in clinical research as new treatments to address EGFR-TKI-induced resistance.

PROTACs that target KRAS

Mutations in the KRAS gene have a critical role in the pathogenesis of multiple malignancies. The most common mutation is the KRASG12C (32). Investigators have been working to develop KRAS inhibitors for a long time. KRAS mutations result in a molecule that remains in a very persistent state of activity because of its robust interaction with GTP (33). Protacs designed to target KRAS aim to overcome resistance in KRAS12C and other KRAS mutations. In 2020, the Bond group revealed the initial KRAS PROTAC designed for the KRASG12C (34, 35). The PROTACs utilized ARS-1620 to attach to KRASG12C and thalidomide derivatives, aiming to eliminate the KRAS G12C mutant via CRBN E3-ligase (36). The Crews group developed VHL-based PROTACs by using MRTX849 as the covalent KRASG12C warhead. Their main chemical LC-2 induced the degradation of KRAS and impeded the MAPK signaling pathway, resulting in reduced p-ERK levels across multiple human lung cancer cell lines with the KRASG12C mutation. Nevertheless, this PROTAC did not demonstrate superior antiproliferative activity compared to MRTX849, perhaps because of its covalently permanent character that disrupts the usual catalytic process seen in PROTACs (36).

Focusing on ALK with PROTACs

Anaplastic lymphoma kinase (ALK), a receptor tyrosine kinase belonging to the insulin receptor kinase subfamily, was first discovered via a chromosomal translocation associated with anaplastic large cell lymphoma (ALCL), a form of T-cell non-Hodgkin's lymphoma (37). The discovery of ALK gene

rearrangement represents a significant advancement in treating NSCLC, which makes up approximately eighty percent of those with lung cancer. ALK is essential for brain development since it has a substantial influence on certain neurons in the nervous system. Crizotinib (Xalkori) is the first ALK inhibitor authorized by the FDA for treating patients with metastatic nonsmall cell lung cancer that is ROS1+ or ALK+ (39). Drug resistance and the initiation of a relapse phase during crizotinib treatment have been attributed to mutations located in the ALK kinase domain. There is a scarcity of inhibitors that possess the capability to impede the extensive array of ALK mutants. Cittatinib, the second ALK blocker approved by the FDA, blocks several mutations that cause resistance to crizotinib (40). The subsequent approvals included brigatinib, alectinib, and others (39). In rare instances, inflammatory myofibroblastic tumors manifest in internal organs and soft tissues, such as the brain, pancreas, mouth, epidermis, breast, nerve, gastrointestinal and genitourinary tracts, bone, stomach, kidney, urinal bladder, and ovary. Lorlatinib, a third-generation ALK inhibitor, successfully blocks frequent resistance variants including ALKG1269A and ALKL1196M. However, its effectiveness is compromised by dual alterations like ALKL1196M/D1203N, ALKF1174L/G1202R, and ALKC1156Y/G1269A (41, 42). Ongoing research is focused on developing PROTACs that selectively target mutant ALK due to the many permanent changes seen in ALK-positive NSCLC. Mammalian cells typically have a limited spread of ALK mRNA and protein, which remain at a modest level in adult individuals. Pharmacologically degrading ALK with SNIPER or PROTAC should not cause intolerance in humans based on ALK's physiological role in mammals (43). Hence, it is anticipated that ALK degraders/disruptors, small molecule agents with dual functionalities to breakdown or interrupt ALK, would have minimal negative impacts on clinical health.

Targeting of FAK targeting

A cytoplasmic tyrosine kinase, focal adhesion kinase (FAK) regulates cellular proliferation and signal transductions mediated by integrins. FAK activation is seen in NSCLC with KRAS mutations (44). In vitro, D-PROTAC lowered the FAK protein levels in A427 cells, a KRAS mutant NSCLC cell line, in a dose-dependent manner, resulting in over 90% degradation. For the purpose of treating A427 cells, either defactinib or D-PROTAC was utilized (45). Significantly larger potency characterized D-PROTAC than defactinib. Cell viability was observed to diminish by 70% upon exposure to D-PROTAC. Conversely, treatment with defactinib led to a viability reduction of 24% (46, 47). Furthermore, cell migration and invasion were substantially inhibited by D-PROTAC in comparison to defactinib. Analyzed in vivo investigations used mice with xenograft A427 tumors that were treated with intratumor injections of D-PROTAC or 10 mg/kg defactinib. Following a 21-day period, the tumor volume in the D-PROTAC group escalated by 340 mm3 from its initial value of 100 mm3. In contrast, the defactinib group witnessed a rise of 1500 mm3. In the D-PROTAC group, there was an 89% decline in FAK levels, whereas the reduction observed in the defactinib group was only marginal (47). In the absence of significant damage to neighboring healthy tissue, D-PROTAC appears to be biosafe.

DISCUSSION AND FUTURE PROSPECTS

Targeting tumor therapy has significantly transformed the treatment of various kinds of cancer over the last twenty years. However, the effectiveness of these therapies is often restricted by the emergence of drug resistance. Comprehending the most recent resistance pathways might result in creating advanced medication generations to improve therapeutic effectiveness in people (48). Yet, the increasing expenses and technological challenges associated with combating drug resistance might render this approach unfeasible in the long run. Therefore, there is an urgent requirement to create new therapeutic protocols and treatment approaches. PROTAC technique has revolutionized drug development by providing several benefits compared to traditional protein inhibitors based on occupancy (49). PROTACs are being created for targeting several clinically significant targets, with over a dozen of them progressing to clinical trials, showcasing the significant potential of this novel therapeutic approach. As seen in the instances provided, PROTACs provide an efficient method to combat different types of developing drug resistance to SMIs (50). Due to their event-driven pharmacology, PROTACs are effective in removing the target protein completely and inducing its degradation through target binding rather than disrupting its function. This makes them suitable for treating various mechanisms of resistance generated by target therapy in clinical settings (51). This involves processes such as binding of drugs hindered by small genetic alterations leading to continuous concentrate stimulation, mutations that modify the structure of the binding domain, acquisition of scaffolding activity due to target complex reorganization, overexpression of target proteins, increased competition from natural ligands, and splicing mutations (52).

Overall, PROTACs show promise in addressing the many obstacles encountered in targeted treatments due to drug resistance. PROTAC technology has significant drawbacks, such as the risk of cancer cells developing resistance to PROTACs over time, which has garnered interest from both academics and business (53). The

mechanisms of resistance seen in small molecule inhibitors (SMIs) might also appear in proteolysis-targeting chimeras (PROTACs) due to changes in both the Protein of Interest (POI) or the E3 ligase, which could hinder the formation of a ternary complex by the PROTAC. The medicine's resistance was not generated by mutations disrupting the binding to the Protein of Interest (POI), but rather by genetic modifications that hinder the fundamental components of the Ubiquitin-Proteasome System (UPS) (54). The mutants have mutations impacting the ubiquitination process, leading to varying resistance depending on the specific E3 ligase targeted by the PROTAC (55, 56). Despite the potential development of resistance, PROTACs show increasing promise in disease treatment because to their advantages over standard SMIs, especially in their capacity to quickly provoke resistance in tumors (57). It is premature to determine whether these commitments will result in tangible advantages in medical therapies. We expect to see evidence of concept results from these research shortly due to the current clinical trials and the release of new drugs for testing. Enhancing our capacity to forecast potential drug resistance mechanisms in advance allows us to develop tactics to avoid, impede, or overcome such resistance. PROTACs may be advantageous in reducing resistance since opposition to a VHL-recruiting PROTAC does not always mean resistance to a CRBN-recruiting PROTAC, and vice versa (59). Over 600 E3 ligases in the human genome will be identified, resulting in the discovery of new E3 ligases and ligands suitable for PROTAC development. Using a variety of E3 recruiters may assist preserve therapeutic efficacy and lower the chances of resistance, resulting in the creation of stronger and more efficient treatments for various cancers (60).

CONCLUSION

PROTACs, as a group, have less than ideal physical and chemical properties that hinder their development in the pharmaceutical field. It is essential to evaluate where the PROTACs are located in the lung tissue to treat lung cancer effectively. Additionally, PROTACs have pharmacodynamic effects that go beyond their pharmacokinetics, requiring the development of suitable pharmacodynamic biomarkers to determine dosing regimens. Additional study is needed to ascertain the optimal method of integrating PROTAC technology with existing anti-cancer therapies to achieve the highest clinical efficacy. Novel diagnostic tests are required to evaluate the efficacy of PROTAC in lung tumor xenograft models and immunocompetent animals at preclinical stages, both in laboratory settings and living organisms. Over the last two decades, molecular oncology medicines have heavily concentrated on lung cancer. Ongoing experimental and early clinical trials of a growing range of PROTAC candidates aim to promote customized and targeted treatment for enhancing the prognosis for individuals with advanced lung cancer.

REFERENCES

1.Bade, B. C., & Cruz, C. S. D. (2020). Lung cancer 2020: epidemiology, etiology, and prevention. Clinics in chest medicine, 41(1), 1-24.

2.Thandra, K. C., Barsouk, A., Saginala, K., Aluru, J. S., & Barsouk, A. (2021). Epidemiology of lung cancer. Contemporary Oncology/Współczesna Onkologia, 25(1), 45-52.

3.Ganti, A. K., Klein, A. B., Cotarla, I., Seal, B., & Chou, E. (2021). Update of incidence, prevalence, survival, and initial treatment in patients with non–small cell lung cancer in the US. JAMA oncology, 7(12), 1824-1832.

4.Leiter, A., Veluswamy, R. R., & Wisnivesky, J. P. (2023). The global burden of lung cancer: current status and future trends. Nature Reviews Clinical Oncology, 20(9), 624-639.

5.Zhang, Y., Vaccarella, S., Morgan, E., Li, M., Etxeberria, J., Chokunonga, E., ... & Bray, F. (2023). Global variations in lung cancer incidence by histological subtype in 2020: a population-based study. The Lancet Oncology, 24(11), 1206-1218.

6.Gockel, L. M., Pfeifer, V., Baltes, F., Bachmaier, R. D., Wagner, K. G., Bendas, G., ... & Steinebach, C. (2022). Design, synthesis, and characterization of PROTACs targeting the androgen receptor in prostate and lung cancer models. Archiv der Pharmazie, 355(5), 2100467.

7.Petty, W. J., & Paz-Ares, L. (2023). Emerging strategies for the treatment of small cell lung cancer: a review. JAMA oncology, 9(3), 419-429.

8.Liu, W. J., Du, Y., Wen, R., Yang, M., & Xu, J. (2020). Drug resistance to targeted therapeutic strategies in non-small cell lung cancer. Pharmacology & therapeutics, 206, 107438.

9.Passaro, A., Brahmer, J., Antonia, S., Mok, T., & Peters, S. (2022). Managing resistance to immune checkpoint inhibitors in lung cancer: treatment and novel strategies. Journal of Clinical Oncology.

10.Wang, K., & Zhou, H. (2021). Proteolysis targeting chimera (PROTAC) for epidermal growth factor receptor enhances anti-tumor immunity in non-small cell lung cancer. Drug Development Research, 82(3), 422-429.

11.He, S., Ma, J., Fang, Y., Liu, Y., Wu, S., Dong, G., ... & Sheng, C. (2021). Homo-PROTAC mediated suicide of MDM2 to treat non-small cell lung cancer. Acta Pharmaceutica Sinica B, 11(6), 1617-1628.

12.Li, J. W., Zheng, G., Kaye, F. J., & Wu, L. (2023). PROTAC therapy as a new targeted therapy for lung cancer. Molecular Therapy, 31(3), 647-656.

13.Qi, S. M., Dong, J., Xu, Z. Y., Cheng, X. D., Zhang, W. D., & Qin, J. J. (2021). PROTAC: an effective targeted protein degradation strategy for cancer therapy. Frontiers in Pharmacology, 12, 692574.

14.Qi, S. M., Dong, J., Xu, Z. Y., Cheng, X. D., Zhang,

W. D., & Qin, J. J. (2021). PROTAC: an effective targeted protein degradation strategy for cancer therapy. Frontiers in Pharmacology, 12, 692574.
15.Liu, J., Ma, J., Liu, Y., Xia, J., Li, Y., Wang, Z. P., & Wei, W. (2020, December). PROTACs: A novel strategy for cancer therapy. In Seminars in Cancer Biology (Vol. 67, pp. 171-179). Academic Press.
16. Zhou, X., Dong, R., Zhang, J. Y., Zheng, X., & Sun, L. P. (2020). PROTAC: A promising technology for cancer treatment. European journal of medicinal chemistry, 203, 112539.
17.Kelm, J. M., Pandey, D. S., Malin, E., Kansou, H., Arora, S., Kumar, R., & Gavande, N. S. (2023). PROTAC'ing oncoproteins: targeted protein degradation for cancer therapy. Molecular Cancer, 22(1), 62.
18.Kelm, J. M., Pandey, D. S., Malin, E., Kansou, H., Arora, S., Kumar, R., & Gavande, N. S. (2023). PROTAC'ing oncoproteins: targeted protein degradation for cancer therapy. Molecular Cancer, 22(1), 62.
19.Han, X., & Sun, Y. (2022). Strategies for the discovery of oral PROTAC degraders aimed at cancer therapy. Cell Reports Physical Science.
20.Farnaby, W., Koegl, M., McConnell, D. B., & Ciulli, A. (2021). Transforming targeted cancer therapy with PROTACs: A forward-looking perspective. Current opinion in pharmacology, 57, 175-183.
21.Békés, M., Langley, D. R., & Crews, C. M. (2022). PROTAC targeted protein degraders: the past is prologue. Nature Reviews Drug Discovery, 21(3), 181-200.
22. Burke, M. R., Smith, A. R., & Zheng, G. (2022). Overcoming cancer drug resistance utilizing PROTAC technology. Frontiers in Cell and Developmental Biology, 10, 872729.
23.Zhang, H. T., Peng, R., Chen, S., Shen, A., Zhao, L., Tang, W., ... & Zhang, L. (2022). Versatile Nano-PROTAC-Induced Epigenetic Reader Degradation for Efficient Lung Cancer Therapy. Advanced Science, 9(29), 2202039.
24.Zhang, H., Zhao, H. Y., Xi, X. X., Liu, Y. J., Xin, M., Mao, S., ... & Zhang, S. Q. (2020). Discovery of potent epidermal growth factor receptor (EGFR) degraders by proteolysis targeting chimera (PROTAC). European Journal of Medicinal Chemistry, 189, 112061.
25.Zhao, H. Y., Wang, H. P., Mao, Y. Z., Zhang, H., Xin, M., Xi, X. X., ... & Zhang, S. Q. (2022). Discovery of potent PROTACs targeting EGFR mutants through the optimization of covalent EGFR ligands. Journal of medicinal chemistry, 65(6), 4709-4726.
26.Zhao, H. Y., Yang, X. Y., Lei, H., Xi, X. X., Lu, S. M., Zhang, J. J., ... & Zhang, S. Q. (2020). Discovery of potent small molecule PROTACs targeting mutant EGFR. European Journal of Medicinal Chemistry, 208, 112781.
27.Zhao, H. Y., Wang, H. P., Mao, Y. Z., Zhang, H., Xin, M., Xi, X. X., ... & Zhang, S. Q. (2022). Discovery of potent PROTACs targeting EGFR mutants through the optimization of covalent EGFR ligands. Journal of medicinal chemistry, 65(6), 4709-4726.
28.Du, Y., Chen, Y., Wang, Y., Chen, J., Lu, X., Zhang, L., ... & Zhang, G. (2022). HJM-561, a potent, selective, and orally bioavailable EGFR PROTAC that overcomes osimertinib-resistant EGFR triple mutations. Molecular Cancer Therapeutics, 21(7), 1060-1066.
29.Shi, S., Du, Y., Zou, Y., Niu, J., Cai, Z., Wang, X., ... & Zhu, Q. (2022). Rational design for nitroreductase (NTR)-responsive proteolysis targeting chimeras (PROTACs) selectively targeting tumor tissues. Journal of Medicinal Chemistry, 65(6), 5057-5071.
30.Zhang, W., Li, P., Sun, S., Jia, C., Yang, N., Zhuang, X., ... & Li, S. (2022). Discovery of highly potent and selective CRBN-recruiting EGFRL858R/T790M degraders in vivo. European Journal of Medicinal Chemistry, 238, 114509.
31.Li, K., & Crews, C. M. (2022). PROTACs: past, present and future. Chemical Society Reviews, 51(12), 5214-5236.
32.Liu, J., Xue, L., Xu, X., Luo, J., & Zhang, S. (2021). FAK-targeting PROTAC demonstrates enhanced antitumor activity against KRAS mutant non-small cell lung cancer. Experimental Cell Research, 408(2), 112868.
33.Hyun, S., & Shin, D. (2021). Small-molecule inhibitors and degraders targeting KRAS-driven cancers. International Journal of Molecular Sciences, 22(22), 12142.
34.Yang, F., Wen, Y., Wang, C., Zhou, Y., Zhou, Y., Zhang, Z. M., ... & Lu, X. (2022). Efficient targeted oncogenic KRASG12C degradation via first reversible-covalent PROTAC. European Journal of Medicinal Chemistry, 230, 114088.
35.Bond, M. J., Chu, L., Nalawansha, D. A., Li, K., & Crews, C. M. (2020). Targeted Degradation of Oncogenic $KRAS^{G12C}$ by VHL-Recruiting PROTACs. ACS central science, 6(8), 1367–1375.
36.Jiang, Z., Li, Y., Zhou, X., Wen, J., Zheng, P., & Zhu, W. (2024). Research Progress on Small Molecule Inhibitors Targeting KRAS G12C with Acrylamide Structure and the Strategies for Solving KRAS Inhibitor Resistance. Bioorganic & Medicinal Chemistry, 117627.
37.Yan, G., Zhong, X., Yue, L., Pu, C., Shan, H., Lan, S., ... & Li, R. (2021). Discovery of a PROTAC targeting ALK with in vivo activity. European Journal of Medicinal Chemistry, 212, 113150.
38.Liu, J., Xue, L., Xu, X., Luo, J., & Zhang, S. (2021). FAK-targeting PROTAC demonstrates enhanced antitumor activity against KRAS mutant non-small cell lung cancer. Experimental Cell Research, 408(2), 112868.
39.Gao, H., Zhang, J. Y., Zhao, L. J., & Guo, Y. Y. (2023). Synthesis and clinical application of small-molecule inhibitors and PROTACs of anaplastic lymphoma kinase. Bioorganic Chemistry, 106807.
40.Xie S, Sun Y, Liu Y, Li X, Li X, Zhong W, Zhan F, Zhu J, Yao H, Yang DH, Chen ZS, Xu J, Xu S. Development of Alectinib-Based PROTACs as Novel Potent Degraders of Anaplastic Lymphoma Kinase (ALK). J Med Chem. 2021 Jul 8;64(13):9120-9140.
41.Zhang, C., Han, X. R., Yang, X., Jiang, B., Liu, J., Xiong, Y.,

& Jin, J. (2018). Proteolysis targeting chimeras (PROTACs) of anaplastic lymphoma kinase (ALK). European journal of medicinal chemistry, 151, 304-314.
42.Recondo, G., Mezquita, L., Facchinetti, F., Planchard, D., Gazzah, A., Bigot, L., ... & Friboulet, L. (2020). Diverse resistance mechanisms to the third-generation ALK inhibitor lorlatinib in ALK-rearranged lung cancer. Clinical Cancer Research, 26(1), 242-255.
43.Song, X., Zhong, H., Qu, X., Yang, L., & Jiang, B. (2021). Two novel strategies to overcome the resistance to ALK tyrosine kinase inhibitor drugs: Macrocyclic inhibitors and proteolysis-targeting chimeras. MedComm, 2(3), 341-350.
44.Gao, H., Zheng, C., Du, J., Wu, Y., Sun, Y., Han, C., ... & Rao, Y. (2020). FAK-targeting PROTAC as a chemical tool for the investigation of non-enzymatic FAK function in mice. Protein & Cell, 11(7), 534-539.
45.Liu, J., Xue, L., Xu, X., Luo, J., & Zhang, S. (2021). FAK-targeting PROTAC demonstrates enhanced antitumor activity against KRAS mutant non-small cell lung cancer. Experimental Cell Research, 408(2), 112868.
46.Cromm, P. M., Samarasinghe, K. T., Hines, J., & Crews, C. M. (2018). Addressing kinase-independent functions of Fak via PROTAC-mediated degradation. Journal of the American Chemical Society, 140(49), 17019-17026.
47.Kurim, S. (2018). Towards Novel Effective Combination Therapy for KRAS Mutant Non-Small Cell Lung Cancer (Doctoral dissertation, Université d'Ottawa/ University of Ottawa).
48.Farnaby, W., Koegl, M., McConnell, D. B., & Ciulli, A. (2021). Transforming targeted cancer therapy with PROTACs: A forward-looking perspective. Current opinion in pharmacology, 57, 175-183.
49.Webb, T., Craigon, C., & Ciulli, A. (2022). Targeting epigenetic modulators using PROTAC degraders: Current status and future perspective. Bioorganic & Medicinal Chemistry Letters, 63, 128653.
50.Liu, J., Ma, J., Liu, Y., Xia, J., Li, Y., Wang, Z. P., & Wei, W. (2020, December). PROTACs: A novel strategy for cancer therapy. In Seminars in Cancer Biology (Vol. 67, pp. 171-179). Academic Press.
51.Gao, H., Sun, X., & Rao, Y. (2020). PROTAC technology: opportunities and challenges. ACS medicinal chemistry letters, 11(3), 237-240.
52.Li, X., & Song, Y. (2020). Proteolysis-targeting chimera (PROTAC) for targeted protein degradation and cancer therapy. Journal of hematology & oncology, 13(1), 1-14.
53.Chen, X., Shen, H., Shao, Y., Ma, Q., Niu, Y., & Shang, Z. (2021). A narrative review of proteolytic targeting chimeras (PROTACs): future perspective for prostate cancer therapy. Translational Andrology and Urology, 10(2), 954.
54.Burke, M. R., Smith, A. R., & Zheng, G. (2022). Overcoming cancer drug resistance utilizing PROTAC technology. Frontiers in Cell and Developmental Biology, 10, 872729.
55.Zhou, X., Dong, R., Zhang, J. Y., Zheng, X., & Sun, L. P. (2020). PROTAC: A promising technology for cancer treatment. European journal of medicinal chemistry, 203, 112539.
56.Wang, C., Zhang, Y., Xing, D., & Zhang, R. (2021). PROTACs technology for targeting non-oncoproteins: advances and perspectives. Bioorganic Chemistry, 114, 105109.
57.Chen, C., Yang, Y., Wang, Z., Li, H., Dong, C., & Zhang, X. (2023). Recent Advances in Pro-PROTAC Development to Address On-Target Off-Tumor Toxicity. Journal of Medicinal Chemistry.
58.Martín-Acosta, P., & Xiao, X. (2021). PROTACs to address the challenges facing small molecule inhibitors. European journal of medicinal chemistry, 210, 112993.
59.Qi, S. M., Dong, J., Xu, Z. Y., Cheng, X. D., Zhang, W. D., & Qin, J. J. (2021). PROTAC: an effective targeted protein degradation strategy for cancer therapy. Frontiers in Pharmacology, 12, 692574.
60.Li, Y., Song, J., Zhou, P., Zhou, J., & Xie, S. (2022). Targeting undruggable transcription factors with PROTACs: advances and perspectives. Journal of Medicinal Chemistry, 65(15), 10183-10194.

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Winter 2024, Volume 9, Issue 32 (36-41)

Investigation of Antioxidant and Cytotoxic Effects of Cerium Oxide Nanoparticles Synthesized Using Aqueous Extract of Hyssopus Officinalis Plant on MDA-MB231 Breast Cancer Cell Line

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DOI: 10.22034/pmj.2024.2024143.1031

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Submitted: 2023-11-27
Accepted: 2024-02-30

Keywords:
Cerium Oxide Nanoparticle
Breast cancer
Antioxidant
Hyssopus officinalis extract

How to Cite this Article:
M. Tourani, K. Eghbalpour, N. Eghbalpour, A. Neamati. "Investigation of Antioxidant and Cytotoxic Effects of Cerium Oxide Nanoparticles Synthesized Using Aqueous Extract of Hyssopus Officinalis Plant on MDA-MB231 Breast Cancer Cell Line" Personalized Medicine Journal, Vol. 9, no. 32, pp. 36-41.

Abstract:
Free radicals are naturally produced in the body and are inhibited by the body's antioxidants. The excessive production of free radicals and the inability of the body to remove them lead to oxidative stress in the body, which can lead to many diseases, including cancer. Nanoparticles are compounds that have been given much attention to cancer prevention and treatment, due to their specific biological characteristics and their small size. This study aimed to evaluate the cytotoxic and antioxidant potential of serum oxide nanoparticles synthesized using aqueous extract of Hyssopus officinalis. To perform the MTT assay, first, the MDA-MB231 cancerous cells were cultured, seeded and then treated for 24, 48 and 72 hours. Subsequently, MTT was performed and finally, absorption at 517 nm was recorded. The antioxidant potential of the CeO-NPs was evaluated by estimating the amount of ABTS and DPPH free radicals inhibiting in different concentrations of nanoparticles.
The results showed that the CeO-NPs were able to inhibit the ABTS and DPPH free radicals with a mean concentration (IC50) of about 62 and 31.2 µg / ml. Also, the CeO-NPs inhibited cancer cells with IC50 of about 400 µg/ml, 48 hours after exposure. According to the antioxidant results obtained from this paper, it is suggested that by performing further experiments, this nanoparticle can be used as an antioxidant supplement.

INTRODUCTION

The uncontrollable proliferation and growth of cells in some cases leads to the formation of a mass of cells known as neoplasm or tumor. Some tumors have the ability to spread to other parts of the body, which is called metastasis. Cancer involves many challenges due to the involvement of different cells, tissues and organs of the body, and for this reason its treatments are often non-specific (1). Cancer occurs as a result of disturbing the balance between proliferation and cell death, after which angiogenesis and feeding to cancerous tissue can cause its spread (2). Free radicals are chemically active molecules that are able to react with other molecules and become stable by receiving electrons. These radicals are produced naturally and as a result of natural metabolism in the body, and the body inhibits these radicals through antioxidant defense. But their excessive production causes oxidative stress and damage to vital body molecules, including lipids, proteins, enzymes, DNA and RNA (3). Oxidative stress can lead to damage, followed by diseases such as cancer, heart disease, neurological diseases (such as multiple sclerosis, Parkinson's disease), autoimmune disease, stroke, diabetes, etc (4).

Antioxidant compounds play an important role in preventing oxidative stress in the body. Vitamin E is one of the most important antioxidants in the body. Enzymes such as superoxide dismutase (SOD), glutathione peroxide (GPx) and catalase (5) are part of the body's enzymatic antioxidant defense systems (3). The science of nanotechnology and the use of nanoparticles with different size, shape and surface chemical properties can have wide applications in the field of medicine and treatment (6). The unique properties of nanoparticles have led to their use in rare cancer diagnosis and treatment. Studies show that anticancer drugs on nanoparticles play an effective role in enhancing the drug's performance and destroying

cancer cells. The results of some studies show that nanoparticles contribute to the performance of anticancer drugs in killing cancer cells by producing reactive oxygen species or other unknown mechanisms (7).

Chemical regeneration (8) laser ablation (9) and microwave waves (10) are among the methods used in making nanoparticles, but all these methods are toxic and have potential risks. for the environment (5). Today, the green synthesis method has replaced the previous methods due to its lack of chemicals, cheap price and environmental friendliness. Since this method occurs without the production of toxic substances and by enzymatic and sometimes non-enzymatic processes, it is also called green technology (11).

Cerium oxide nanoparticles are actually the oxidized form of the rare element cerium, which, due to their special characteristics, are able to mimic the activity of superoxide dismutase and catalase and are able to act as scavengers of reactive oxygen species (10) in many biological fields. act Such feature has caused the potential applications of this nanoparticle in biomedicine (12) Medicinal plants have been considered for the treatment of many diseases since ancient times. Hyssop medicinal plant (Hyssopus officinalis L) is a perennial plant of the mint family. The essential oil of this plant is used in the treatment of colds and coughs. It is also widely used as a flavoring agent and in the cosmetic industry. Pinocamphene (50%), alpha-beta-pinene, camphene and sesqui terpene alcohols are among the most important components of the essential oil of this plant. Also, this plant contains flavonoids, tannins and other substances such as diosmin, hyssopin and mucilaginous compounds.

In this research, cerium oxide nanoparticle was synthesized using the aqueous extract of hyssop plant (green method) and then its biological effects (cytotoxicity and antioxidant effect) were evaluated.

MATERIALS AND METHODS

Green synthesis of cerium oxide nanoparticles from aqueous extract of hyssop plant

First, the aqueous extract of hyssop plant was prepared by dissolving 10 grams of hyssop plant powder in 100 ml of distilled water, the resulting mixture was placed on a hot plate stirrer at a temperature of 100 degrees Celsius for 30 minutes, and then it was filtered using filter paper.

To make nanoparticles, 10 ml of prepared extract was combined with 100 ml of cerium nitrate solution and placed on a stirrer at 35°C for one hour. Next, the resulting sediment was separated by a centrifuge, then it was dried, and after confirming the formation of cerium oxide nanoparticles, its biological characteristics were investigated.

Examining the average size of nanoparticles

For this purpose, the powder prepared from nanoparticles in solution was used to check the size of the particles.

Examining the cytotoxic effect of cerium oxide nanoparticles:

MTT test was used to evaluate the toxicity of nanoparticles on cells. For this purpose, the cells were cultured and after reaching the logarithmic phase, they were transferred to a 96-well plate with a density of 5000 cells per well, after 24 hours, the cells were treated with different concentrations of nanoparticles, and then after a period of time Treatment and draining of the treatment medium, MTT solution was added to the cells. The cells were incubated for 4 hours and then the MTT solution was replaced with 100 µl of DMSO. Finally, the absorbance of each well was measured by ELISA reader at a wavelength of 570 nm and the viability of the cells was calculated from the following formula.

Cell viability (%) = (Mean optical absorbance of each well concentration / (mean optical absorbance of control wells)

Investigating the antioxidant capacity of cerium oxide nanoparticles

DPPH test

This test was performed based on the method of Brand-Williams and his colleagues(13). In this method, DPPH free radicals were first produced, and for this purpose, DPPH powder was dissolved in 96% ethanol to obtain a DPPH solution with a concentration of 0.1 mM. Next, the resulting solution was mixed with different concentrations of nanoparticles in an equal ratio, and after incubation for 30 minutes at 37 degrees Celsius, the absorbance of each solution was measured at a wavelength of 517 nm. In this method, BHA was used as a standard antioxidant.

ABTS test

This test was performed based on Miller et al.'s method (Miller & Rice-Evans, 1997). At first, ABTS stock solution was prepared, for this ABTS powder and potassium persulfate were dissolved in deionized distilled water and incubated for 16 hours in the dark at room temperature. After that, to prepare working ABTS, the resulting solution was diluted with distilled water until reaching the absorbance of 0.756 at the wavelength of 734 nm. Finally, the ABTS solution was mixed in a volume equal to different concentrations of nanoparticles, and after one hour of incubation at room temperature, its absorbance was measured at a wavelength of 734 nm.

RESULTS

Evaluating the size of nanoparticles

The results showed that the size of the synthesized

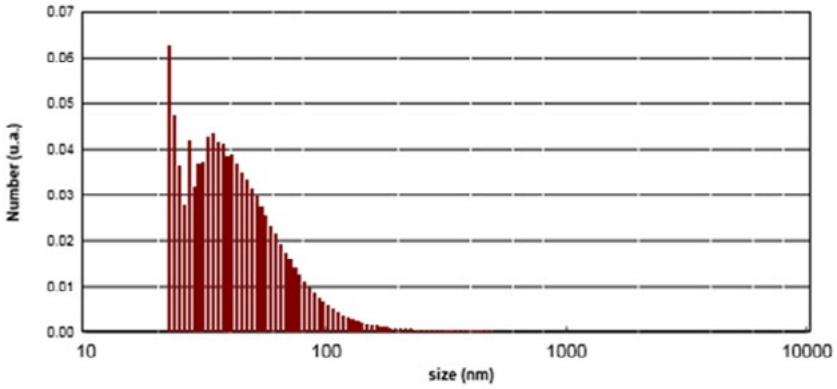

Fig1. Particle dispersion

particles is about 40 nm, which is the best size for biological and biomedical applications of nanoparticles.

Results from TEM and FESEM

Based on the results of TEM and FESEM electron microscopy, the nanoparticles synthesized using the aqueous extract of the hyssop plant have a spherical appearance.

Results from FTIR

FTIR spectrum was recorded in order to determine the effect of hyssop plant on cerium oxide ion. Figure 4-4 shows the FTIR spectrum of the nanoparticle obtained from the aqueous extract of the hyssop plant, in which the 2921/123 cm band is related to the alkyl chain. Also, the 3441/16 cm-1 band in the FTIR spectrum of the nanoparticle is related to the symmetric and asymmetric stretching vibrations of the C-H bond in the four and five positions of the imidazolium ring.

Investigating the toxicity effect of cerium oxide nanoparticles on breast cancer cells (MDA-MB231)

Figure 2 shows the effect of nanoparticle toxicity on breast cancer cells at different nanoparticle concentrations and different treatment times. As can be seen, with the increase in nanoparticle concentration, their toxicity on cells increases. The diagram shows that the nanoparticle at the initial concentration of 62.5 µg/ml is capable of significantly inhibiting cancer cells, and the inhibition rate increases with increasing concentration. In 24 and 48 hours, the IC50 is close to 500 µg/ml, but after 72 hours, the IC50 decreases to about 250 µg/ml, which indicates the effect of concentration- and time-dependent toxicity.

DPPH test

As seen in Figure 3, cerium oxide nanoparticles synthesized using hyssop plant are able to remove DPPH free radicals. As the nanoparticle concentration increases, the inhibition activity of the nanoparticle on free radicals also increases.

Examining the inhibition rate shows that the nanoparticle at a concentration of 62.5 µg/ml is able to inhibit about 50% of free radicals, which indicates the high antioxidant effects of the synthesized nanoparticle.

ABTS test

Figure 4 shows the inhibition of ABTS free radicals

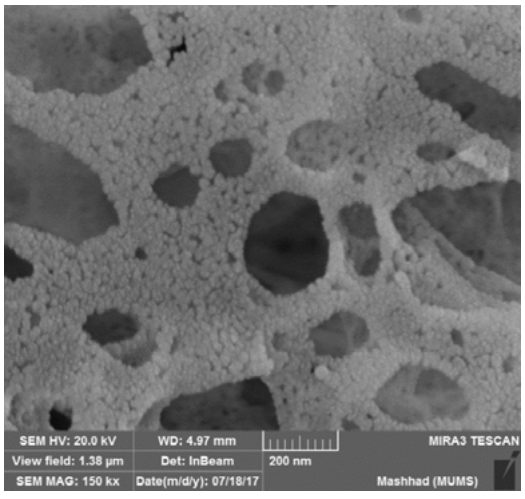

Fig2. Image of cerium oxide nanoparticle obtained from FESEM investigation

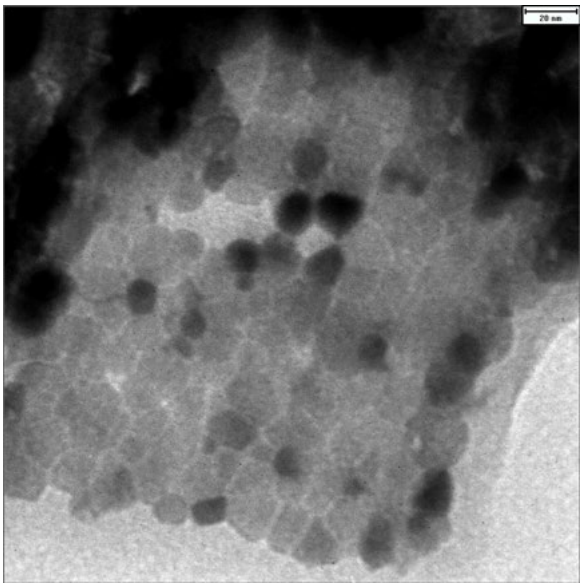

Fig 3. Image of cerium oxide nanoparticle obtained from TEM examination

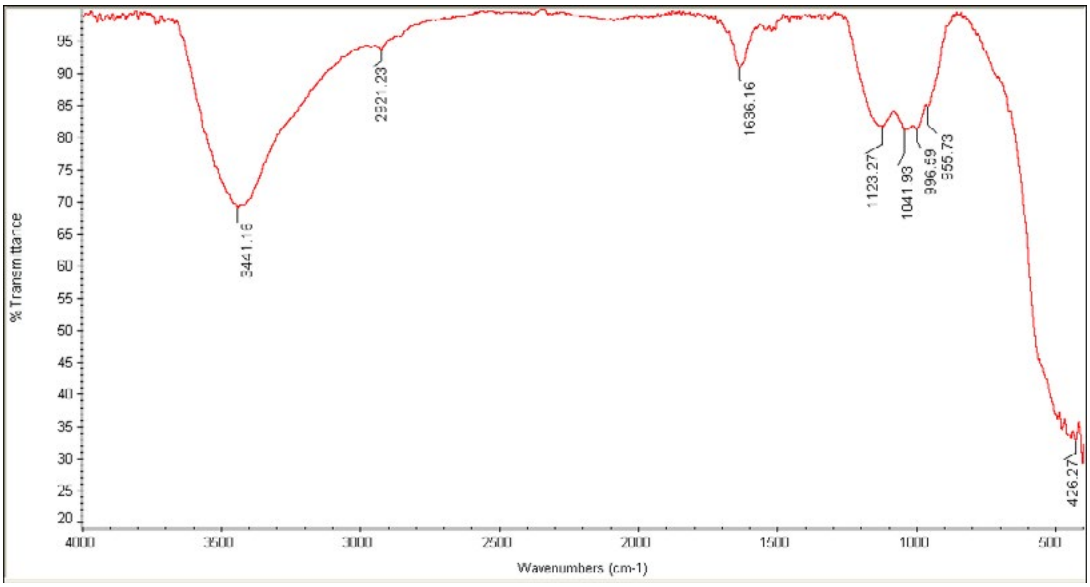

Fig4. FTIR spectrum of cerium oxide nanoparticles

by cerium oxide nanoparticles synthesized from hyssop plant. As can be seen in the figure, with the increase in the concentration of nanoparticles, the inhibition rate of free radicals also increases, so that at a concentration of 500 micrograms/ml of nanoparticles, the inhibition rate is around 95%. The IC50 calculated in this test is about 31.2 µg/ml, which indicates that about 50% of free radicals are inhibited in this concentration.

DISCUSSION

In this study, cerium oxide nanoparticles were first prepared from hyssop plant and then the size of the particles was investigated. After confirming the synthesis of nanoparticles, the effects of cytotoxicity and antioxidant power were evaluated. The use of nanotechnology in the synthesis and optimization of nanoparticles has attracted a lot of attention today. Since nanoparticles synthesized by different methods or from different sources have different biological effects, therefore, investigating the effects of new nanoparticles can open the way for pharmaceutical and therapeutic problems.

The use of nanoparticles with dimensions less than 100 nm in order to deliver and target diagnostic and medicinal agents in medical projects related to cancer has been greatly expanded. Recently, many nanoparticles have been used in targeted drug delivery to malignant tumor cells or reducing the systemic toxicity of anticancer drugs (14) . Examining the effect of toxicity of nanoparticles in inhibiting cancer cells has been evaluated in many studies, for example, Park et al. during a study synthesized cerium oxide

nanoparticles of different sizes (15, 25, 30 and 45 nm) and then the effect of this cytotoxicity Nanoparticles were tested on lung epithelial cells (BEAS-2B) with concentrations of 5, 10, 20 and 40 µg/ml. The results showed that nanoparticles inhibited the growth of cancer cells (15).

In a similar study, Lin et al synthesized cerium oxide nanoparticles with a size of 20 nm and found cytotoxicity on human lung cancer cells with concentrations of 3.5, 10.5 and 23.3 µg/ml at three times 24, 48 and checked for 72 hours. The results showed that the aforementioned nanoparticles induced cell death in a dose- and time-dependent manner similar to the result of cerium oxide nanoparticles synthesized from the hyssop plant (16). Recent studies show that silver nanoparticles also have cytotoxic effects on different cell lines, including Hela and MCF7 cancer cell lines (17). In 2014, Venkatesan et al investigated the anticancer effect of silver nanoparticles produced by green method using water extract of rose petals against human lung adenocarcinoma (A549) by MTT test. The calculated IC50 was 80 µg/ml (18).

In another study silver nanoparticles produced by the green method from cauliflower have antioxidant properties. Antioxidant activity of silver nanoparticles was investigated using DPPH radical scavenging ability and nanoparticles had potential ability to scavenge DPPH radical (19). Furthermore in a similar another study in 2014, the activity of ABTS radical scavenging by silver nanoparticles synthesized from Inonotus obliqqus mushroom was investigated. In this experiment, BHT was used as a standard. The results showed maximum inhibition at 1 mM concentration of about 76% and minimum inhibition at 0.125 mM concentration of about 60%. In general, in this study, similar to the current study, it was shown that increasing the concentration of nanoparticles increases the inhibition of free radicals (20).

In 2004, Giridharan et al used Dodonaea viscosa and Capparis decidua plants to prepare silver nanoparticles and then investigated the antioxidant effects of the synthesized nanoparticles. The results of DPPH and hydroxyl radical removal studies showed that silver nanoparticles obtained from both plants have significant antioxidant activity compared to standard antioxidants. Gold nanoparticles can be mentioned among other synthesized nanoparticles. During another investigation, Inonotus obliqqus plant was used for the synthesis of gold nanoparticles, and then the effect of the synthesized nanoparticles on the inhibition of ABTS free radicals was investigated. The results showed that with the increase in nanoparticle concentration, the amount of free radical inhibition also increases. Investigating the amount of inhibition of gold nanoparticles in different concentrations of 1, 0.5, 0.25, 0.125 mM showed the maximum inhibition of ABTS radical at 1 mM and the minimum inhibition at 0.125 mM). Similarly, Dipankar et al. studied the antioxidant effects of nanoparticles produced from the leaf extract of the blood leaf plant and the results showed that the nanoparticles potentially have concentration-dependent antioxidant activity (21). In another study in 2015, the effect of inhibition of DPPH free radicals by zinc oxide nanoparticles synthesized from Cassiafistula plant extract was calculated in different concentrations of 2, 4, 6, 8 mg in a size of 5-15 nm and the results showed that with increasing concentration from 2000 to 8000 micrograms, the inhibition percentage of DPPH free radicals increases. The IC50 in this study was calculated to be 2853 µg/ml, which is very weak in inhibiting DPPH free radicals compared to the IC50 of the present study (22).

In 2013, Ramamurthy et al. synthesized gold and silver nanoparticles using an aqueous extract of a type of eggplant and further investigated their antioxidant effects with various laboratory methods. The results showed that gold and silver nanoparticles have significant antioxidant power against hydroxyl, superoxide, nitric oxide and DPPH radicals (23).

In another study conducted by Lee et al. in 2013, zinc oxide nanoparticles were first synthesized from the medicinal plant Fagopyrum esculentum and its antioxidant effects were evaluated. Investigating the effects of biomass and the activity of oxidizing enzymes showed an increase in the activity of catalase enzyme and antioxidant glutathione in a concentration-dependent manner (24).

CONCLUSION

The results showed that in the synthesis method, particles with a size of about 40 nm were prepared (Figure 1), which are the appropriate size for further biological investigations. Further investigation of the toxicity effect on breast cancer cells showed that this nanoparticle is able to destroy cancer cells in a time and concentration-dependent manner (Figure 2). The results of the antioxidant power investigation showed that the nanoparticle is capable of inhibiting ABTS free radicals (IC50:31.2) more powerfully than DPPH (IC50:62.5) and overall, this nanoparticle showed high antioxidant effects.

Examining the results of the above studies shows the strong antioxidant properties of different metal nanoparticles. The results obtained from the present study also showed a very high antioxidant property of cerium oxide nanoparticles synthesized using the hyssop plant method, which is consistent with the results of previous studies.

Acknowledgments

This article is a part of the results of the master's thesis, titled as the investigation of the antioxidant properties

and toxicity of cerium oxide nanoparticle synthesized by green method by Hyssopus officinalis plant. The researchers express their thanks and appreciation to all the experts of the research laboratories of the Mashhad Branch, Islamic Azad University who have cooperated in this research.

Funding

This research has performed by personal fund.

Statements and Declarations

There is no statement and declaration.

Conflict of interest statement

There is no conflict of interest.

REFERENCES

1.Fisher R, Pusztai L, Swanton C. Cancer heterogeneity: implications for targeted therapeutics. British journal of cancer. 2013;108(3):479-85.

2.Fiaschi T, Chiarugi P. Oxidative stress, tumor microenvironment, and metabolic reprogramming: a diabolic liaison. International journal of cell biology. 2012;2012:762825.

3.Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaie A. Pesticides and oxidative stress: a review. Medical science monitor : international medical journal of experimental and clinical research. 2004;10(6):Ra141-7.

4.Roome T, Dar A, Ali S, Naqvi S, Choudhary MIJJoe. A study on antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective actions of Aegiceras corniculatum (stem) extracts. 2008;118 3:514-21.

5.Senapati KK, Roy S, Borgohain C, Phukan PJJoMCA-c. Palladium nanoparticle supported on cobalt ferrite: An efficient magnetically separable catalyst for ligand free Suzuki coupling. 2012;352:128-34.

6.Narayanan KB, Sakthivel N. Biological synthesis of metal nanoparticles by microbes. Advances in Colloid and Interface Science. 2010;156(1):1-13.

7.Borovski T, De Sousa EMF, Vermeulen L, Medema JP. Cancer stem cell niche: the place to be. Cancer research. 2011;71(3):634-9.

8.Faure C, Derré A, Neri W. Spontaneous Formation of Silver Nanoparticles in Multilamellar Vesicles. The Journal of Physical Chemistry B. 2003;107(20):4738-46.

9.Zhang ZG, Zhang L, Croll SD, Chopp M. Angiopoietin-1 reduces cerebral blood vessel leakage and ischemic lesion volume after focal cerebral embolic ischemia in mice. Neuroscience. 2002;113(3):683-7.

10.Wijnhoven SWP, Peijnenburg WJGM, Herberts CA, Hagens WI, Oomen AG, Heugens EHW, et al. Nano-silver – a review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicology. 2009;3(2):109-38.

11.Sadeghi B, Rostami A, Momeni SS. Facile green synthesis of silver nanoparticles using seed aqueous extract of Pistacia atlantica and its antibacterial activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2015;134:326-32.

12.Celardo I, Pedersen JZ, Traversa E, Ghibelli L. Pharmacological potential of cerium oxide nanoparticles. Nanoscale. 2011;3(4):1411-20.

13.Bondet V, Brand-Williams W, Berset CJL-FS, Technology. Kinetics and Mechanisms of Antioxidant Activity using the DPPH.Free Radical Method. 1997;30:609-15.

14.Narayanan KB, Sakthivel N. Green synthesis of biogenic metal nanoparticles by terrestrial and aquatic phototrophic and heterotrophic eukaryotes and biocompatible agents. Adv Colloid Interface Sci. 2011;169(2):59-79.

15.Park E-J, Choi J, Park YK, Park KJT. Oxidative stress induced by cerium oxide nanoparticles in cultured BEAS-2B cells. 2008;245 1-2:90-100.

16.Lin W, Huang YW, Zhou XD, Ma Y. Toxicity of cerium oxide nanoparticles in human lung cancer cells. International journal of toxicology. 2006;25(6):451-7.

17.Sukirtha R, Priyanka KM, Antony JJ, Kamalakkannan S, Thangam R, Gunasekaran P, et al. Cytotoxic effect of Green synthesized silver nanoparticles using Melia azedarach against in vitro HeLa cell lines and lymphoma mice model. 2012;47:273-9.

18.Venkatesan B, Subramanian V, Tumala A, Vellaichamy E. Rapid synthesis of biocompatible silver nanoparticles using aqueous extract of Rosa damascena petals and evaluation of their anticancer activity. Asian Pacific Journal of Tropical Medicine. 2014;7:S294-S300.

19.Ranjitham AM, Suja RM, Tiwari SK, editors. INVITRO EVALUATION OF ANTIOXIDANT, ANTIMICROBIAL, ANTICANCER ACTIVITIES AND CHARACTERISATION OF BRASSICA OLERACEA. VAR. BORTRYTIS. L SYNTHESIZED SILVER NANOPARTICLES2013.

20.Nagajyothi PC, Sreekanth TV, Lee JI, Lee KD. Mycosynthesis: antibacterial, antioxidant and antiproliferative activities of silver nanoparticles synthesized from Inonotus obliquus (Chaga mushroom) extract. Journal of photochemistry and photobiology B, Biology. 2014;130:299-304.

21.Devanathadesikan Seshadri V, Vijayaraghavan P, Kim YO, Kim HJ, Ahmed Al-Ghamdi A, Elshikh MS, et al. In vitro antioxidant and cytotoxic activities of polyherbal extracts from Vetiveria zizanioides, Trichosanthes cucumerina, and Mollugo cerviana on HeLa and MCF-7 cell lines. Saudi Journal of Biological Sciences. 2020;27(6):1475-81.

22.Suresh D, Shobharani RM, Nethravathi PC, Pavan Kumar MA, Nagabhushana H, Sharma SC. Artocarpus gomezianus aided green synthesis of ZnO nanoparticles: luminescence, photocatalytic and antioxidant properties. Spectrochimica acta Part A, Molecular and biomolecular spectroscopy. 2015;141:128-34.

23.Hyung S-J, DeToma AS, Brender JR, Lee S, Vivekanandan S, Kochi A, et al. Insights into antiamyloidogenic properties of the green tea extract (-)-epigallocatechin-3-gallate toward metal-associated amyloid-β species. Proc Natl Acad Sci U S A. 2013;110(10):3743-8.

24.Kim TI, McCall JG, Jung YH, Huang X, Siuda ER, Li Y, et al. Injectable, cellular-scale optoelectronics with applications for wireless optogenetics. Science (New York, NY). 2013;340(6129):211-6.

نشریه پزشکی شخصی

فصلنامه پزشکی/ سال نهم/ شماره ۳۲/ قیمت: ۵۰۰۰۰۰ ریال/ زمستان ۱۴۰۲/ شماره شاپا ۳۸۶۰-۲۷۱۷

آینده علم پزشکی، شخصی محور است