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Exploring the Potential of Echinococcus Granulosus Antigens in Immunotherapy for Cancer

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ABSTRACT

Background: Echinococcus granulosus, intrigues researchers due to its unique biology and immunogenicity. Recent studies show a negative relationship between echinococcosis incidence and cancer progression, emphasizing significant similarities between E. granulosus and cancer antigens. This article will review the anti-cancer effects of E. granulosus antigens and their application in cancer immunotherapy.

Main Body: It begins with an introduction, highlighting the significance of E. granulosus antigens in contemporary cancer treatment. Subsequently, an overview of hydatid cysts, the source of these antigens, elucidating their structure and immunogenic properties is provided. Our review extends to the interactions between echinococcosis and both the innate and acquired immune systems, shedding light on the mechanisms involved. We delve into the intriguing connections between echinococcosis and cancer, exploring the direct and indirect anti-cancer effects of these antigens and their potential in cancer immunotherapy. To offer a balanced perspective, we also weigh the pros and cons of using E. granulosus antigens in cancer therapy.

Conclusion: In conclusion, the promising attributes of E. granulosus antigens, as highlighted throughout this review, suggest a bright future for their utilization in cancer therapy. Their ability to trigger potent immune responses and target cancer cells holds great potential for the development of effective and tailored therapies, offering new hope for improved cancer treatment outcomes. Further research and clinical trials are needed to fully realize the potential of E. granulosus antigens in the field of cancer immunotherapy.

1. Introduction

Cancer is still one of the most important health challenges in the world and its impact on public health is increasing day by day. According to GLOBOCAN global statistics, cancer is one of the main causes of death and disability worldwide, which includes approximately 10 million deaths in 2020 (47). Therefore, continuous pursuit of innovative and effective treatment approaches is necessary to fight this terrible disease. In recent years, immunotherapy has emerged as a promising strategy that enhances the power of the immune system to target and fight cancer cells (48). In this context, the potential of antigens derived from different sources has been investigated in cancer treatment, and among them, hydatid cyst antigens are known as an interesting candidate.(42 ,13)

Hydatid cysts, resulting from the larval stage of the worm Echinococcus granulosus, are common in certain regions of the world. According to the World Health Organization report, more than one million new cases of echinococcosis are reported annually, and this disease has significant health and economic consequences in endemic areas (35). The protoscolex, which is the infective stage of the parasite, contains antigenic components that stimulate a strong immune response in the host. It has been observed that the immune system has a strong defence mechanism against hydatid cysts, which leads researchers to investigate the potential of echinococcal antigens in other fields, especially in cancer immunotherapy (11, 32). The immunogenicity of these antigens, together with their ability to induce a specific immune response, has made them attractive targets for the development of innovative therapeutic strategies against cancer.

However, despite these promising findings, the challenges and limitations of fully exploiting the potential of immunotherapy based on hydatid cyst antigens should be considered. The heterogeneity of tumour antigens in different types of cancer poses an important obstacle that requires further research to identify antigens that can target a wider range of tumours. In addition, optimization of antigen formulation, delivery methods, and cost-effective manufacturing are areas that require careful investigation for the successful translation of protoscolex antigen-based therapies into clinical practice. The purpose of this article is to review the potential of echinococcal antigens as a promising candidate for cancer therapy, emphasizing its unique immunogenic properties and discussing the current preclinical evidence supporting its use.

Hydatid cysts: a brief review

Hydatid cysts are pathological structures formed by the larval stage of the Echinococcus granulosus worm. This parasitic infection, also known as Cystic echinococcosis (CE), affects both humans and various animals, especially in areas where this parasite is endemic (18). The life cycle of Echinococcus includes two hosts: definitive hosts, usually canids, and intermediate hosts, including humans and various herbivores. In humans, infection occurs through the consumption of eggs, which can be the cause of contamination of food, water, or other environmental sources. Larvae can migrate through the bloodstream to different organs, which generally affect the liver and lungs, but can also spread to other organs and lead to the formation of hydatid cysts (Figure 1).(22)

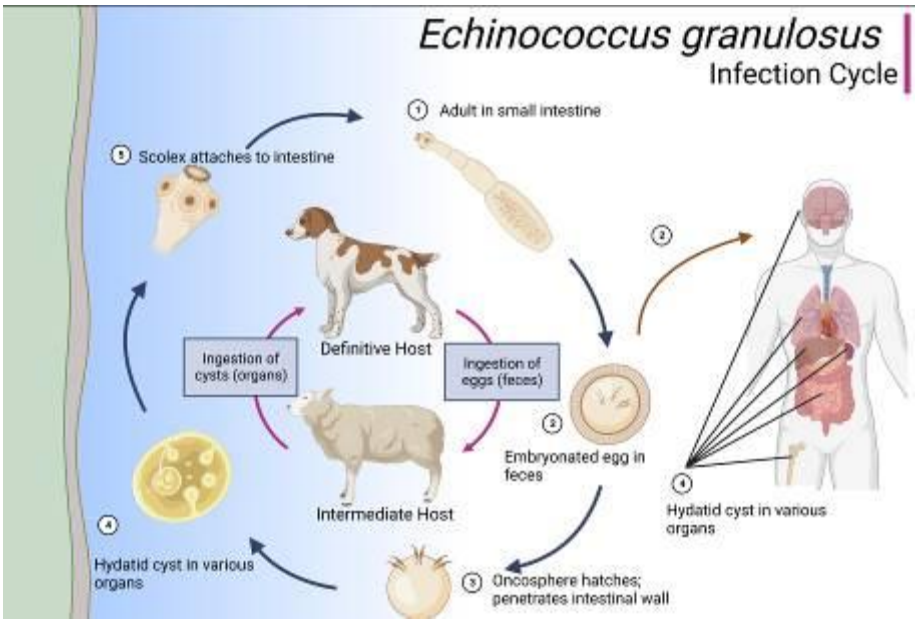

Figure 1. Life cycle of Echinococcus granulosus. Definitive hosts of the parasite are canines and intermediate hosts will be infected through consumption of meat contaminated with hydatid cysts. Then the parasite will go through its maturity stages in the definitive host and will leave the host's body in the form of eggs through faeces (13). Figure provided by www.biorender.com.

Hydatid cysts are characterized by a fluid-filled sac called a cysticercus surrounded by a thick, fibrous wall called an odonticia or pericyst. This cyst grows slowly over several years and gradually enlarges, causing pressure on the surrounding tissues (19). The growth rate can vary depending on the host's immune response and the location of the cyst. In some cases, hydatid cysts can reach very large sizes with a diameter of more than 15 cm (20). The clinical appearance of hydatid cysts varies depending on the location, size and number of cysts present. Often, the infection remains asymptomatic for a long time, and these cysts may be discovered incidentally during routine medical examinations or imaging studies. However, when symptoms do occur, they can be diverse and vary from vague abdominal discomfort to more specific manifestations related to the affected organs (24).

In the liver, the most common site of infection, hydatid cysts can cause liver enlargement (hepatomegaly) and pain in the right upper quadrant of the abdomen. Grown cysts can exert pressure on adjacent structures and lead to biliary obstruction, jaundice, or portal hypertension (26, 30). The presence of a cyst in the lung causes cough, chest pain, fever and breathing problems. Rupture of the cysts can lead to severe complications such as anaphylactic shock due to the release of the contents of the cysts into the bloodstream. Infectious cysts may lead to the spread of daughter cysts to other parts of the body, resulting in secondary infections and additional clinical manifestations.(39)

Research efforts aimed at understanding the molecular and immunological aspects of hydatid cysts have increased our knowledge of host-parasite interactions and the underlying immune evasion mechanisms employed by the parasite. Investigating host-specific immune responses to Echinococcus can provide valuable insights into the development of novel immunotherapeutic strategies against other diseases, including cancer. In addition, similarities between hydatid cysts and tumours, especially in terms of immune evasion and immunosuppressive microenvironments, have led researchers to explore the potential of hydatid cyst antigens in cancer immunotherapy (2, 4). The unique immunogenic properties of hydatid cyst antigens make them attractive for the development of immunotherapies that can stimulate strong and selective immune responses against cancer cells while sparing healthy tissues.

Hydatid cyst antigens

1. Structure and features

The structure and characteristics of hydatid cysts play an important role in their pathogenesis and clinical manifestations. Hydatid cysts are complex structures that consist of two separate layers: an outer pericyst and an inner endocyst (Figure 2). The pericyst, also known as the endodontic layer, is a fibrous layer that surrounds and protects the cyst and prevents the spread of infection to the surrounding tissues (19). This layer is mainly composed of collagen fibres and connective tissue components that contribute to the thick and durable nature of the cyst wall. The germinal layer, or endocyst, is the innermost layer responsible for the production of protoscolexes and daughter cysts during cyst growth and development.(28)

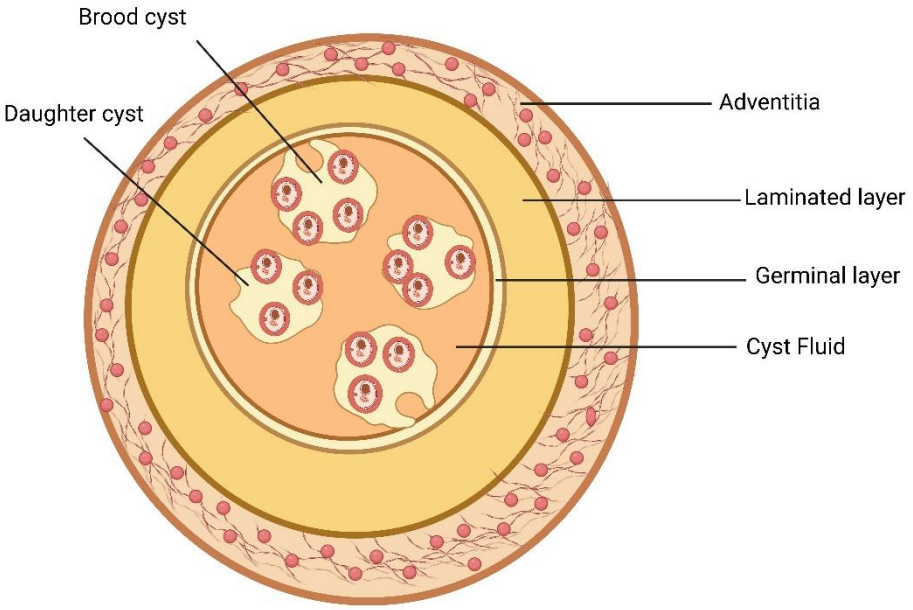

Figure 2. Structure of hydatid cyst. Figure provided by www.biorender.com

Protoscolexes are infectious structures that grow from the germinal layer into the cyst. These small, bud-like structures are equipped with hooks and suckers that enable them to firmly attach to host tissues. Protoscolexes have a distinctive morphology consisting of a two-lobed shape and a central rostrum equipped with a hook. These hooks play an important role in the stabilization of protoscolexes within the cyst and facilitate their survival and growth during the life cycle of E. granulosus (29).

The cyst fluid that fills the hydatid cyst cavity is a complex mixture containing various components derived from the parasite and the host. Cyst fluid consists of hydatid fluid, a clear, viscous substance that serves as a nutrient-rich medium for parasite growth. For example, a relationship between the level of newly produced protein and the creation of suckers on protoscolexes has been mentioned. This indicates a potential increase in protein production during the development of protoscolexes in the special region from which the suckers originate (17). In addition to hydatid fluid, cystic fluid may contain daughter cysts, isolated protoscolexes, and cellular debris. Hydatid fluid contains immunogenic components including antigens that can stimulate an immune response in the host (10). The composition of cystic fluid can vary depending on factors such as the stage of cyst expansion, the

location of the cyst inside the host, and specific factors of the host.(25)

Understanding the complex structure and characteristics of the hydatid cyst is crucial for understanding the immunological properties of echinococcal antigens and their potential applications in cancer therapy. The unique characteristics of hydatid cysts, such as the presence of protoscolexes and immunogenic cyst fluid, make them suitable candidates for investigating new immunotherapy strategies against cancer. Further research on immunological interactions between host and parasite can provide valuable insights into the development of targeted and effective cancer therapies.

2 .3. Immunogenicity and antigenic characteristics

The successful survival of parasites is due to their ability to use different escape strategies that ensure their continuous presence in the host's body. For example, E. granulosus uses two different mechanisms to evade the host's immune response. The first is a passive escape mechanism in which the parasite avoids the destructive effects of an immune system attack by developing into a hydatid cyst. The second mechanism, immune modulation, is that the parasite actively interacts with the host's immune system and reduces the effects of the host's response. These complex tactics enable survival and continuation of the parasite's life cycle within the host (46).

However, despite these escape mechanisms, the immune system still can recognize the parasite due to the expression of distinct antigens during different stages of development. The main source of antigenic stimulation is the contents of hydatid cysts, including protoscolex; However, the germinal layer of the cyst acts as a barrier against the host's immune cells. Therefore, for antigenic stimulation, grooves or tears must occur in the germinal layer. Such an event also occurs following manipulation of the cyst, for example, during surgery or perforation of the cyst (41).

The response of the host's immune system against hydatid cysts is classically divided into innate and acquired immunity. Protoscolex antigens are multifaceted and different components stimulate distinct immune responses. It should be noted that specific proteins and glycoproteins present in Protoscolex antigens have been identified as key immune targets. These antigens can induce antibody production, especially IgG subtypes that play a critical role in modulating immune responses against the parasite. In addition, Protoscolex antigens can activate cellular immune responses, including the generation of cytotoxic T lymphocytes (CTLs), which can directly target and kill infected cells.(51)

1 .2 .3. Echinococcosis And Innate Immunity

Innate immunity is the first line of defence against all kinds of parasites, which can detect pathogen-associated molecular patterns (PAMPs) through membrane pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) and domain-like oligomerization receptors. to detect nucleotide binding (NLRs: Nucleotide-Binding Oligomerization Domain (NOD) Like Receptors). These receptors are expressed by host innate immune cells, including macrophages, neutrophils, endothelial cells, dendritic cells (DCs), and lymphocytes, which regulate immune responses through various host defence mechanisms (6).

Hydatid fluid is a complex mixture of distinct antigens of host and parasite origin. Antigen B (AgB), one of the most abundant antigens of E. granulosus, is a heat-stable lipoprotein of 160 kDa with three subunits at 8, 12, 16, and 20 or 24 kDa, accounting for about 10% of the total hydatid fluid content. Immunolocalization studies indicate the presence of this antigen, which is a type of protease inhibitor with the ability to inhibit neutrophil recruitment, in the germinal layer of the metacercaria and the membrane of the protovasculature (54). According to Shepherd et al.'s studies, the 12 kDa AgB subunit plays an important role in the parasite's escape mechanism from the host's innate immunity (44). Changes in the activity level of macrophages and suppressing the production of effective cytokines are among the mechanisms through which AgB plays a role in modulating the host's immune response. It has also been found that the presence of parasite metabolites can affect the differentiation, maturation, and function of dendritic cells and natural killer cells (NKs), thereby contributing to

disease progression.(33)

There is still no detailed information about the mechanisms related to host susceptibility/resistance to hydatid cysts. However, studies indicate the presence of some molecules associated with the host's innate immune cells (such as NKs, macrophages, neutrophils, mast cells, basophils, eosinophils, and dendritic cells) in patients with cystic echinococcosis (6).

Normally, the primary immune response to E. granulosus infection is the maturation of dendritic cells and altered differentiation of monocytes to evade the immune system and establish chronic infection. Research conducted on BALB/c mice infected with E. granulosus showed an increase in the number of macrophages, dendritic cells, Tregs, and myeloid-derived suppressor cells (MDSCs), and the expression of arginase-1 (ARG-1). It is in many myeloid cells that inhibit T lymphocyte response to E. granulosus antigens (55). It was already known that the secretory-discharge products of E. granulosus can cause immune tolerance in dendritic cells to disrupt the host's immune response; However, the presence of some components of these secretion-excretion products, including protovascular antigens similar to cancer antigens, can stimulate the differentiation and maturation of dendritic cells towards the activation of the specific immune system. The delivery of such stimulating antigens to dendritic cells occurs through extracellular vesicles from protovasculatures and metastodes of E. granulosus.(12)

The presence of some inflammatory cells, mainly neutrophils and macrophages, is a sign of cellular immune response in the acute echinococcosis stage (32). Active neutrophils at this stage can secrete a type of protease called neutrophil elastase (NE), which is capable of digesting the parasite's body and attracting other neutrophils through chemotaxis. In response, E. granulosus oncospheres express a protein called EgKI-1, which is capable of inhibiting NE and helps the parasite escape from the host's immune system (51).

Among other mechanisms that occur in the direction of the escape of the parasite from the host's immune system, it is observed in the stage of chronic echinococcosis and the case of hydatid cyst rupture. The leakage of hydatid fluid in this situation attracts neutrophils. The influx of neutrophils to kill protoscolex can be a reason for the production of antigen B by hydatid cyst fluid (44). Antigen B, as a protease inhibitor, prevents chemotaxis of neutrophils and gives the parasite a chance to cause secondary echinococcosis by producing larger cysts (38). Therefore, it can be said that preventing the influx of neutrophils by inhibiting NE secretion and chemotaxis is one of the important mechanisms of parasite escape from the host's immune system in acute and chronic stages.

The high activity of neutrophils in killing E. granulosus oncospheres indicates the presence of an antibody-dependent cellular response. On the other hand, we know that an anti-tumor immune response is needed to overcome acute malignancies (44). Considering that we need the memory of immune response for successful immunotherapy, the existence of immune memory can be seen as a reason for the resistance of echinococcosis patients against some cancers (38). Of course, there is also evidence of a decrease in the Th1 immune response and an increase in the level of breast cancer metastasis to the liver following the injection of 4T1 mouse breast cancer cells into mice with secondary echinococcosis.(50)

In addition to the fact that neutrophils play an important role in host defence, they can also have pro-tumour and anti-tumour effects in cancer patients. Many people with advanced cancers show high levels of neutrophils in their blood, although the exact mechanisms behind this presence are unknown. Recent evidence has shown that neutrophils in the tumour microenvironment are actively involved in the initiation of tumour growth, progression, metastasis and angiogenesis. Consequently, potent inhibitors of neutrophil elastase have been tested as anticancer drugs.(52)

3. 2. 2. Echinococcosis and acquired immunity

It has been found that both Th1 and Th2 cell types are present in the host's acquired immune response

to E. granulosus. Therefore, by identifying modulating antigens affecting Th1 or Th2 polarity, it can be determined that the body's immune system is sensitive/resistant to the parasite. In the immune response to infections, cytokines produced by Th lymphocytes are involved in the regulation of antibody isotype production, and in particular, Th2 cytokines regulate the synthesis of immunoglobulin E (IgE) and IgG4. Several pieces of evidence show that the sera of patients with cystic echinococcosis contain all isotypes of specific AgB antibodies. The predominant binding of IgG4 to AgB suggests a link between AgB and Th2 activation (36).
EgTeg, a new molecule isolated by examining the cDNA library obtained from E. granulosus, is located in the skin of the protoscolex and the germinal layer of the cyst wall, and is a type of coat protein that is probably related to the survival of the parasite. This study showed that EgTeg is an immune regulatory molecule: it inhibits chemotaxis and primarily induces IL-4-positive T lymphocytes and non-complement repair antibodies. As a result, it can be said that this antigen activates a type of Th2 immune response associated with chronic infection. The anti-inflammatory response induced by helminths enables parasite survival in the host by limiting potentially destructive inflammatory responses.(3)

For the first time in a preliminary study in 1979, antigenic similarity between lung carcinoma and hydatid cyst fluid was reported (53). Of course, it should be noted that some of these common antigens induce the suppression of the anticancer immune response. Mucin O-glycans are among the most important common antigens between parasites and cancer cells. In cancers, mucin O-glycans are involved in metastasis, invasion and cell adhesion. In E. granulosus parasite, Tn O-glycosylated antigens are present in larval and adult extracts (14). Recently, antigens, which may be mucin O-glycans, have also been identified in hydatid cyst fluid, lamina and germinal layers, and secretory/excretory products of hydatid cyst protoscolexes. The immunological cross-reaction of hydatid cyst fluid antigens with the serum of cancer patients was higher than with the serum of healthy individuals. Therefore, antibodies produced against these parasitic O-glycosylated antigens can be useful against cancer. Mucin-like peptides obtained from E. granulosus have been found to induce the increase of active natural killer cells in the spleen of immunized mice in a process mediated by factors derived from soluble dendritic cells.(45)

Also, the treatment of spleen cells with Egmuc peptides in immunized mice could increase the immune response and kill pancreatic tumour cells in vitro. Of course, more detailed studies showed that these anticancer effects were mostly due to the increased production of IL-12p40p70 and IL-6 and as a result, increased NK cell activity, which seems to be more of a Th1-like response (38). It has also been found that the cytotoxic effects of serum on human small-cell lung carcinoma cells were higher among patients with echinosis than among healthy people (23). Furthermore, 40% of mice vaccinated with hydatid cyst fluid induced tumour recurrence in a colon cancer model as well as an acquired immune response against tumour rechallenge (11).
Therefore, it can be said that the results obtained from different studies support the use of specific antigens of E. granulosus in preventing/treating some types of cancer. These studies show that the high structural similarity of E. granulosus antigens with cancer antigens will create a long-term memory in the immune system and prepare for possible confrontation with cancer cells. However, more studies are still needed to finally confirm these antigens as treatment/prevention options.

E. granulosus and cancer: a complex relationship

It has been proven that various worms including liver fluke Clonorchis sinesis and Opisthorchis viverini (causing cholangiocarcinoma) and blood fluke Schistosoma japonicum (known risk factor for liver carcinoma and colon cancer), Schistosoma mansoni (probable cause of colon cancer)) and Schistosoma haematobium (which leads to bladder cancer) are among the carcinogens in humans. On the other hand, there is also evidence that certain helminth infections can induce anticancer activities, such as the pig worm Trichinella spiralis, which can protect infected mice against tumor growth and metastasis (21).
The simultaneous presence of E. granulosus infection and cancer such as liver cancer, lung

carcinosarcoma, liver mucosal adenoma cyst, kidney sarcoma, kidney adenocarcinoma, or ovarian epithelial tumor and lymphoepithelioma-like gastric carcinoma have been widely reported (1, 4).

However, the exact relationship between E. granulosus and cancer remained unknown until the last decade, until a study in Turkey (with a prevalence rate of 1-2% for E. granulosus) found that the rate of solid tumour formation among infected patients It is inferior to cystic echinococcosis. This study, which was conducted on 2086 people undergoing solid tumour surgery between 1990 and 2001, showed that only two patients had both diseases at the same time (38). This accidental discovery prompted researchers to investigate the anticancer effects of E. granulosus antigens. After that, the results of studies conducted by different research groups have confirmed this hypothesis. For example, it has been proven that protoscolexes in hydatid cysts (larval stage of E. granulosus) can induce cell death in WEHI-164 fibrosarcoma cells in vitro (16). In addition, vaccination with hydatid cyst fluid in a mouse model with experimental colon cancer CT26 caused tumour recurrence (10). Also, the simultaneous injection of live protoscolex and melanoma cells can lead to a significant decrease in tumour growth compared to the control group in a mouse model (5). Overall, this evidence shows that E. granulosus can show protective effects against certain types of cancers in vitro and in vivo.

Mechanism of action of anticancer effects of E. granulosus

Considering that the investigation of the relationship between the E. granulosus parasite and pelvic cancer is relatively new, detailed information about the mechanism of action of the anticancer effects of this parasite has not yet been obtained. However, the anticancer effects of E. granulosus can be classified into two general categories: direct cell killing effects and indirect effects through the host's immune system (Figures 3 and 4**).**

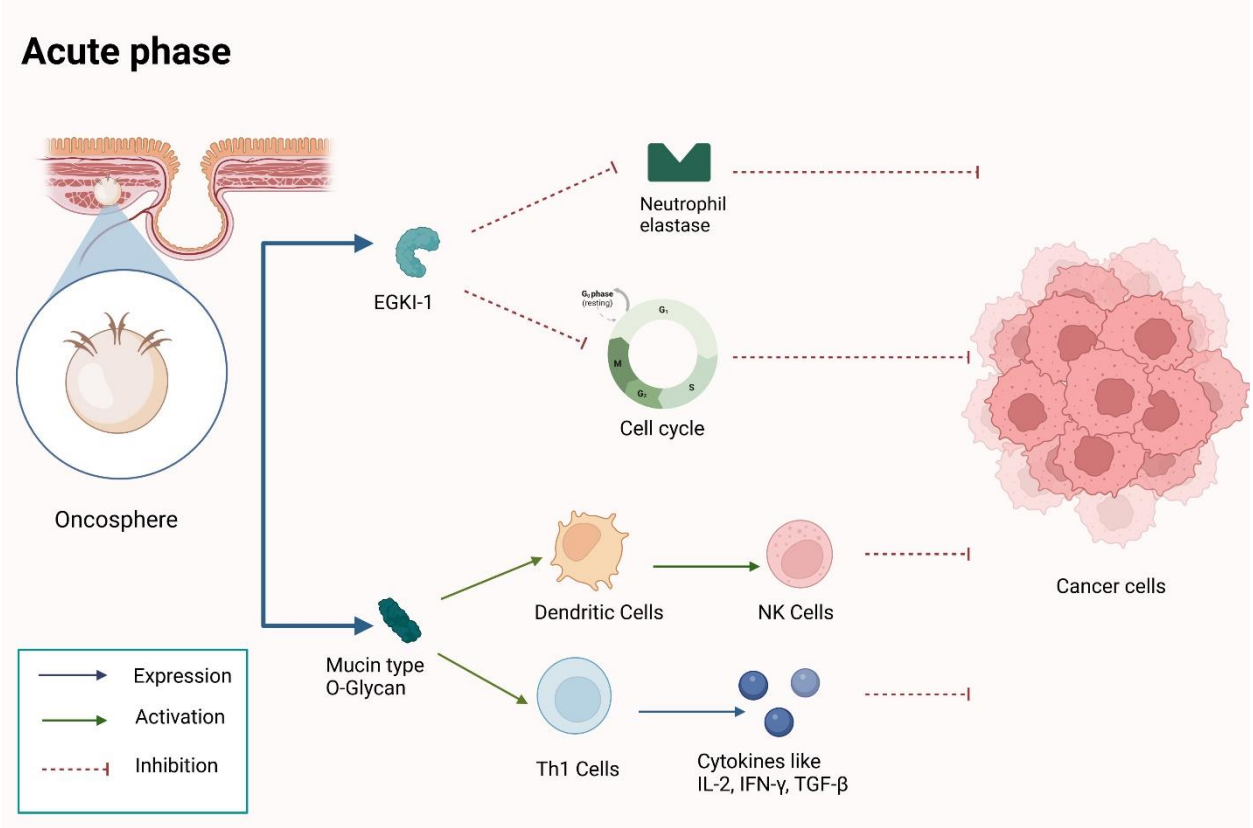

Figure 3. Possible mechanisms responsible for the anticancer effects induced in the acute phase of Echinococcus granulosus infection. In the acute phase, the release of EgKI-1 by the oncosphere strongly inhibits neutrophil elastase and disrupts the cell cycle, thus exerting anticancer effects on various types of cancer cells. At the same time, mucin O-glycans will be recognized by the host

immune system and activate innate and Th1-polarized immune responses that have protective effects against cancer. Image courtesy of www.biorender.com.

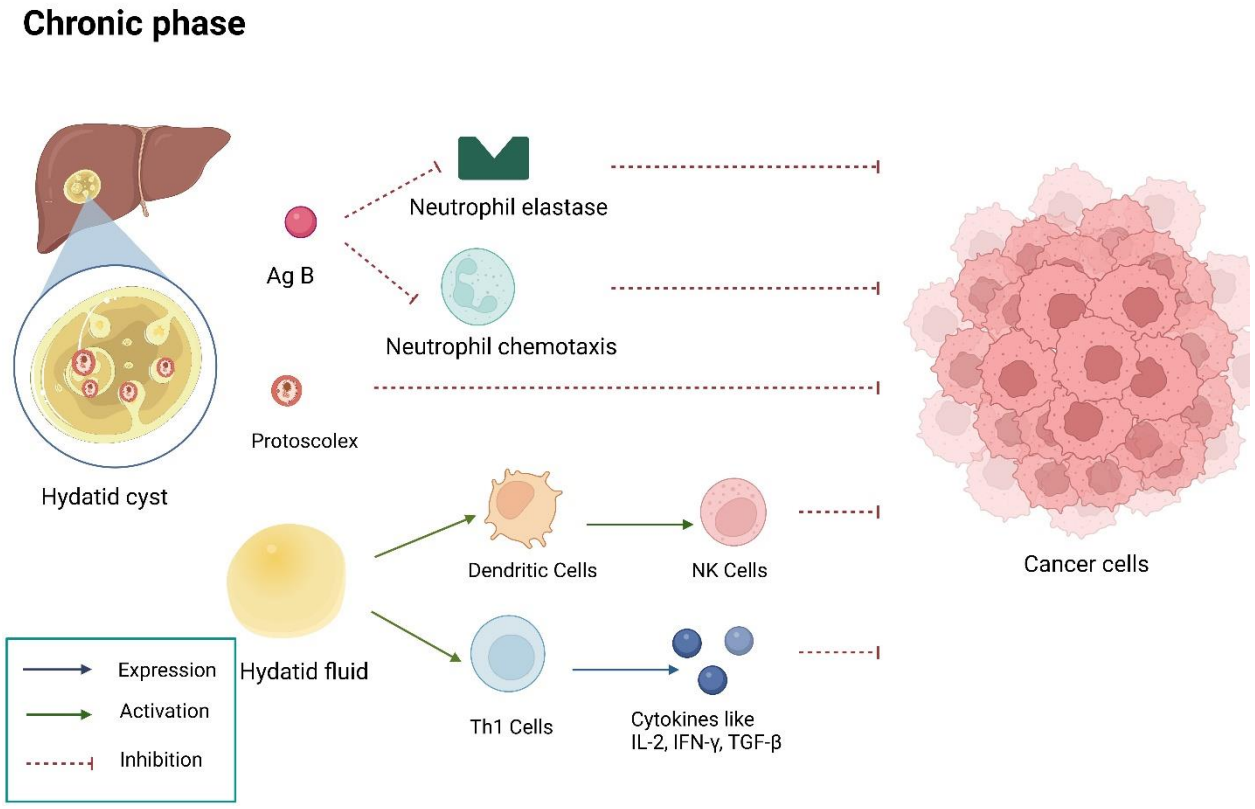

Figure 4. Possible mechanisms responsible for the anticancer effect induced in the chronic phase of Echinococcus granulosus infection. At this stage, the contents of the hydatid cyst will be released to the infection sites due to the rupture of the cyst. The highly immunogenic contents of hydatid cysts rapidly activate the host's innate immunity and cause a shift from Th2 to Th1 response, which has host protective effects against cancer. Antigen B (AgB), another potent inhibitor of neutrophil elastase, is highly expressed in hydatid cysts and may exert its anticancer effects through inhibition of neutrophil elastase and neutrophil chemotaxis. At the same time, protovasculature can also play a direct role in anticancer effects. Image courtesy of www.biorender.com.

5. 1. Direct effects of echinococcosis on cancer through the property of cell killing

There is evidence that echinococcosis parasites can directly kill cancer cells in addition to inducing antibody-mediated immunity. It has been found that hydatid cyst protoscolexes can induce cell death in WEHI-164 fibrosarcoma cells in vitro and inhibit melanoma tumour growth in vivo (16). However, the specific molecules involved in this process remain largely unclear. In an in vivo study on C57BL/6 mice, treatment with hydatid cyst fluid at the same time as injection of melanoma cells resulted in decreased tumour growth. However, in this study, it is not clear whether the control group received heme alum as an auxiliary control or whether it was injected intraperitoneally into the tumour margin (14). The worrying point in this research is the possible effect of alum as an adjuvant in stimulating the Th2 immune response in mice.

Interestingly, one study showed that treatment of HeLa cells with various hydatid cyst molecules, specifically Protoscolex excretory/secretory molecules, could increase the number of dead cells or decrease the number of viable cells in the culture medium; Also, the treatment of Vero cells with the same molecules did not increase the number of dead cells or decrease the number of living cells in the culture medium (21). As mentioned in the previous sections, probably the presence of high amounts

of antigen B (AgB) as a NE in hydatid cysts is one of the reasons for these strong effects. The effects of AgB on the breast cancer cell line were also investigated and it was found that the rate of cell death decreased significantly after treatment with this antigen (15).
Another protein that needs further investigation is the EgKI-1 molecule in the E. granulosus oncosphere, which was mentioned in the previous sections. A study that investigated the anticancer effects of this protein in both in vitro and in vivo conditions indicated a significant reduction in the rate of apoptosis after treatment with this protein in both conditions (37). Therefore, in general, it can be said that the liquid contents of the hydatid cyst play an important role in the occurrence of the parasite's cytotoxic effects

. 5. 2. Indirect anticancer effect through the activation of the host's immune system

As mentioned in the above section, the anticancer effect has been seen in the contamination of different parasites. Like parasitic infections, cancer cells can also stimulate innate and acquired immunity in the growth process (38). Therefore, it can be expected that some parasitic antigens similar to cancer antigens during chronic infection can create memory in the immune system and thus prepare this system for a possible encounter with cancer cells.
In this regard, there is evidence of similarity between parasite antigens and certain types of cancer, especially mucin O-glycans (51). For example, antigens such as Tn antigen (CD175) and TF antigen (or CD176) are among the most popular antigens in cancer treatment research, because in many types of cancer (including bladder, cervix, colon, ovary, stomach, lung and prostate) are observed (9). The first report of antigen similarity between E. granulosus and cancer was in the 1970s, which showed a clear sedimentary band from the immunoreactivity of hydatid fluid and serum of lung carcinoma patients (53). After that, the presence of both CD175 and CD176 antigens was reported in E. granulosus larval and adult worm extracts. Antigens such as Tk and TF have also been reported in adult worms (14). Of course, common antigens other than mucin O-glycans have also been identified. For example, a non-glycosylated 27 kDa molecule is shared between human breast cancer and E. granulosus (43). In addition, in a study on the antitumor immune response induced by hydatid cyst fluid in an animal model of colon cancer, heat shock protein 70 (hsp 70) of E. granulosus has shown high homology with CT26 colon cancer cell mortalin (about 60%) (11). In addition, it has been proven that common antigens between E. granulosus and cancers can show immunological cross-reactivity. In general, it can be assumed that the induction of the host's immune response against certain types of cancer after echinococcosis infection can be caused by the presence of these common antigens.
Previous studies have shown that both cellular immunity and humoral immunity induced by the protozoan parasite Trypanosoma cruzi protect against various types of cancer in a mouse model (40). But in the case of E. granulossus, such information is not available. In the onco-immunology paradigm, it has been found that the polarized Th1 response plays a central role in killing cancer cells and preventing tumor growth, while the polarized Th2 response increases tumor progression and metastasis (7). As with E. granulossus, the immune response induced by the parasite varies during different stages of infection: 1) during the oncosphere invasion, a polarized Th1 response will predominate; 2) in the process of cyst formation and growth, the polar Th2 response will gradually take over the responsibility; 3) At the time of cyst rupture or parasite death, the polarized Th2 response will rapidly change to the polarized Th1 response (51). Therefore, it can be said that the activation of Th1 response is the reason for the anticancer effects of the parasite (49). Furthermore, this could explain the conflicting results of some epidemiological and laboratory studies, which show that Echinococcosis cysti can promote the development and progression of cancer (50.(

Echinococcal antigens and cancer immunotherapy

In the long-term fight against cancer, there are four general approaches: surgery, chemotherapy, radiotherapy, and recently immunotherapy, which has become a suitable treatment method for cancer (87). Inspired by the success of Immune Checkpoint Inhibitors (ICIs) over the past decade, oncologists and immunologists have made every effort to ensure further success. However, the success achieved with ICIs is still limited, thus requiring further avenues to be explored. The accidental discovery of

the anticancer effect induced by E. granulosus infection has attracted much attention. Understanding the underlying mechanisms can provide new insights into the regulation of immunotherapy.

After nearly 40 years of extensive research with limited success, it is now believed that a cancer vaccine may have the potential to convert immunodeficient tumors to ICI therapy through T cell immune activation (21). In this context, the ideal cancer vaccine should first be able to stimulate a strong Th1-specific response (as a Th2 response may facilitate cancer progression and metastasis) and then a tumor-specific response (inducing acquired immunity specifically against the cells cancerous and preserving normal cells). Many tumor-specific antigens have been identified and exploited. However, most of them fail to induce an appropriate and effective immune response, including mucin O-glycans, sTn, and tumor-associated T antigen, which are found almost exclusively on cancer cells and are widely used in the diagnosis and prediction of cancer progression. have been used (56). It should be noted that despite the production of specific immunoglobulin G and the conversion of serum immunoglobulin M to immunoglobulin G in a multicenter phase III study, the sTn-based vaccine was not beneficial for women with metastatic breast cancer (31). A Th2-dominant response could be an explanation for the failure of this vaccine, thereby highlighting the importance of a Th1-dominant response.

As previously mentioned, mucin O-glycans have now been found in both adult and larval stages of E. granulosus and in patients with cystic echinococcosis (21). On the other hand, accumulating evidence has shown that hydatid cyst antigens can induce anticancer effects against several types of cancer in vitro and mouse models (15, 23, 45). Furthermore, immunization of mice with hydatid cyst antigens resulted in high expression of interferon-gamma (34). The existence of these evidences indicates the role of mucin O-glycans in stimulating the Th1 immune response and preparing the immune system to fight against cancer. Therefore, it can be said that mucin O-glycans of parasitic origin are one of the successful options for cancer vaccine design. Since there are many differences between the immune system of humans and mice, further studies are needed to confirm this hypothesis.

Advantages and challenges of using echinococcal antigens for cancer treatment

Immunotherapy based on echinococcal antigen has several advantages that make it a promising candidate for cancer treatment.

First of all, echinococcal antigens have unique immunogenic properties (4, 14, 16). These antigens have the ability to activate specific immune responses against cancer cells, which leads to the recognition and elimination of tumor cells by the immune system. Second, immunotherapy based on echinococcosis antigens has the potential to induce long-term immune memory (11, 13). By stimulating the production of memory T cells, the immune system can retain information about tumor-specific antigens. This memory response enables a rapid and robust immune response upon re-encounter with cancer cells expressing the same antigens (27). This immunological memory provides stable and durable protection against tumor recurrence and can significantly improve long-term outcomes for cancer patients.

Another advantage of immunotherapy based on echinococcal antigens is their potential for combination therapy. For example, the combination of protoscolex antigen with other immunomodulatory agents such as immune checkpoint inhibitors or adjuvants can increase the effect of immunotherapy (42). Synergistic effects can be achieved by targeting multiple pathways involved in immune response modulation, leading to enhanced antitumor immune responses and improved therapeutic outcomes.

In addition, immunotherapy based on echinococcal antigens has shown the potential for minimal side effects. By selectively targeting cancer cells that express specific antigens, this treatment can spare healthy cells and tissues from the harmful side effects often associated with conventional treatments such as chemotherapy and radiotherapy. This targeted approach can help minimize toxicity and improve the overall quality of life of cancer patients undergoing treatment.

However, although immunotherapy based on echinococcal antigen is promising, it also faces several challenges and limitations that must be considered for its successful implementation in cancer treatment. One of the main challenges is the heterogeneity of tumor antigens. Different types of cancer

exhibit diverse antigenic profiles, which makes it challenging to identify echinococcal antigens that can effectively target a wide range of tumor cells.(8)

Another limitation is the optimization of echinococcal antigen formulation and delivery. The immunogenicity of echinococcal antigens can be affected by factors such as the choice of adjuvants, dose and route of administration. Finding the optimal formulation that enhances antigen immunogenicity while ensuring patient safety and tolerance is an important aspect of successful immunotherapy. Furthermore, the cost and scalability of echinococcal antigen-based immunotherapy present significant challenges. Large-scale production and purification of echinococcal antigens can be complex and expensive. Standardization of production processes and optimization of production methods are necessary to make available and affordable this treatment method. Overcoming these challenges will be critical to ensure the widespread availability and cost-effectiveness of echinococcal antigen-based immunotherapy.

Future prospects and potential applications

potential applications and avenues for exploration. First, more research is needed to expand the understanding of echinococcal antigen immunotherapy in different types of cancer. Currently, most studies are focused on preclinical investigations in a limited number of cancer types. Conducting robust clinical trials involving large populations of patients with different types of cancer will help evaluate the efficacy, safety, and feasibility of using echinococcal antigen-based therapies in a broader setting.

In addition, the development of combination therapies containing antigen and other immunomodulatory agents is promising. Combining antigen with immune checkpoint inhibitors, cancer vaccines, or other immunotherapeutic agents can potentially enhance the antitumor immune response by targeting multiple immune pathways simultaneously. In addition, identifying new antigens or engineering antigenic constructs with high immunogenicity can open new avenues for cancer treatment. Advances in antigen discovery techniques, such as proteomics and genomics, may help identify other antigens that are highly expressed on cancer cells and are capable of eliciting strong immune responses.

Finally, the potential application of echinococcal antigen-based immunotherapy could extend beyond primary tumor treatment. This method can be used as an adjuvant treatment method to prevent cancer recurrence or as a neoadjuvant treatment method to stimulate the immune system before removing the tumor through surgery. Furthermore, investigating the potential of echinococcal antigen immunotherapy in combination with other therapies, such as chemotherapy or radiation therapy, may increase overall treatment responses and improve disease outcomes.

The future of immunotherapy based on echinococcal antigens is promising, with several

Conclusion

Echinococcal antigen-based immunotherapy is a promising candidate for cancer treatment. The unique immunogenic properties of echinococcal antigens, together with their ability to activate specific immune responses against cancer cells, have made them attractive targets for immune system inhibition in the fight against cancer. Preclinical studies have shown the ability of these antigens to inhibit tumor growth, increase immune responses, and synergize with other immunomodulatory agents. However, several challenges and limitations must be considered to fully realize the potential of cancer treatment with this method. Heterogeneity of tumor antigens, optimization of antigen formulation and delivery, and cost-effective production are key areas that require further research. Furthermore, large clinical trials are needed to confirm the efficacy and safety of echinococcal antigen-based therapies in different cancer types and patient populations. With continued progress and collaboration in this field, the use of echinococcal antigens can revolutionize cancer treatment and provide new avenues for targeted and personalized approaches that can improve treatment outcomes

and overall cancer management.

References:

1 -Ahmadi, A., F. Barekati, K. Amini, A. Rasooli and M. Mazrouei. 2022. The Role of Zoonotic Helminths in Causing Cancer. Zoonosis 2: 9-24.
2 -Ahmadi, A., M. Moazeni and M. Shaddel. 2020. Subcutaneous Hydatid Cyst in Laboratory Mice: Is it a Suitable Method for Evaluating Therapeutic Agents against Hydatid Cyst? Archives of Razi Institute 75: 75-81.
3 -Amri, M., D. Mezioug and C. Touil-Boukoffa. 2009. Involvement of IL-10 and IL-4 in evasion strategies of Echinococcus granulosus to host immune response .European cytokine network 20: 63-68.
4 -Asouli, A., S. Sadr, H. Mohebalian and H. Borji. 2023. Anti-Tumor Effect of Protoscolex Hydatid Cyst Somatic Antigen on Inhibition Cell Growth of K562. Acta parasitologica 68: 385-392.
5 -Badri-Chookami, M., S. M .Sharafi, R. Rafeie, M. Bahadoran, N. Pestechian and H. Yousofi-Darani. 2014. Effect of Alive Protoscoleces of Hydatid Cyst on the Growth of Melanoma Cells in Mouse Model. Journal of Isfahan Medical School 32: 486-492.
6-Bakhtiar, N. M., A. Spotin, M. Mahami-Oskouei, E. Ahmadpour and A. Rostami. 2020. Recent advances on innate immune pathways related to host–parasite cross-talk in cystic and alveolar echinococcosis. Parasites & Vectors 13: 232.
7-Basu, A., G. Ramamoorthi, G. Albert, C. Gallen, A. Beyer ,C. Snyder, G. Koski, M. L. Disis, B. J. Czerniecki and K. Kodumudi. 2021. Differentiation and Regulation of TH Cells: A Balancing Act for Cancer Immunotherapy Frontiers in Immunology
8 -Benvenuto, M., C. Focaccetti, V. Izzi, L. Masuelli, A. Modesti and R. Bei. 2021. Tumor antigens heterogeneity and immune response-targeting neoantigens in breast cancer. Seminars in Cancer Biology 72: 65-75.
9-Berois, N., A. Pittini and E. Osinaga. 2022. Targeting Tumor Glycans for Cancer Therapy: Successes, Limitations, and Perspectives. Cancers 14.
10 -Berriel, E., T. Freire, C. Chiale, E. Rodríguez, G. Morón, G. Fernández-Graña, M. Crispo, N. Berois and E. Osinaga. 2021. Human hydatid cyst fluid-induced therapeutic anti-cancer immune responses via NK1.1+ cell activation in mice. Cancer Immunology, Immunotherapy 70: 3617-3627.
11 -Berriel, E., S. Russo, L. Monin, M. F. Festari, N. Berois, G. Fernández, T. Freire and E. Osinaga. 2013. Antitumor activity of human hydatid cyst fluid in a murine model of colon cancer .TheScientificWorldJournal 2013: 230176.
12 -Cao, S., W. Gong, X. Zhang, M. Xu, Y. Wang, Y. Xu, J. Cao, Y. Shen and J. Chen. 2020. Arginase promotes immune evasion of Echinococcus granulosus in mice. Parasites & vectors 13: 49.
13 -Chen, J., W. Liao and H .Peng. 2022. Toxoplasma gondii infection possibly reverses host immunosuppression to restrain tumor growth Frontiers in Cellular and Infection Microbiology
14 -Daneshpour, S., M. Bahadoran, S. H. Hejazi, A. A. Eskandarian, M. Mahmoudzadeh and H. Y. Darani. 2016. Common antigens between hydatid cyst and cancers. Advanced biomedical research 5: 9.
15 -Daneshpour, S., A. H. Kefayat, M. R. Mofid, S. Rostami Rad and H. Yousofi Darani. 2019. Effect of Hydatid Cyst Fluid Antigens on Induction of Apoptosis on Breast Cancer Cells. Advanced biomedical research 8: 27.
16 -Darani, H. Y. and M. Yousefi. 2012. Parasites and cancers: parasite antigens as possible targets for cancer immunotherapy. Future oncology (London, England) 8: 1529-1535.
17 -GALINDO, M., M .J. GONZALEZ and N. GALANTI. 2002. Echinococcus granulosus protoscolex formation in natural infections. Biological Research 35: 365-371.
18-Gessese, A. T. 2020. Review on Epidemiology and Public Health Significance of Hydatidosis. Veterinary medicine international 2020: 8859116.
19 -Golzari, S. E. J. and M. Sokouti. 2014. Pericyst: the outermost layer of hydatid cyst. World journal of gastroenterology. p. 1377-1378.
20 -Goussard, P., E. Eber, L. Mfingwana, P. Nel, P. Schubert, J. Janson, R. Pitcher and C. le Roux. 2022. Paediatric pulmonary echinococcosis: A neglected disease. Paediatric Respiratory Reviews 43: 11-23.
21-Guan, W., X. Zhang, X. Wang, S. Lu, J. Yin and J. Zhang. 2019. Employing Parasite Against Cancer: A Lesson From the Canine Tapeworm Echinococcus Granulocus Frontiers in Pharmacology
22 -Hawash, Y. 2020. Parasitic Liver Diseases. In: Radu-Ionita F., Pyrsopoulos NT., Jinga M., Tintoiu IC., Sun Z., Bontas E, editors. Liver Diseases: A Multidisciplinary Textbook. Cham. Springer International Publishing. p. 161-172.
23 -Karadayi, S., S. Arslan, Z. Sumer, M. Turan, H. Sumer and K. Karadayi. 2013. Does hydatid disease have protective effects against lung cancer? Molecular Biology Reports 40: 4701-4704.
24 -Khalili, N., P. Iranpour, N. Khalili and S. Haseli. 2023. Hydatid Disease: A Pictorial Review of Uncommon Locations. Iranian Journal of Medical Sciences 48: 118-129.
25-Khanfar, N. 2004. Hydatid disease: a review and update. Current Anaesthesia & Critical Care 15: 173-183.
26 -Kosmidis ,C. S., K. Papadopoulos, C. M. Mystakidou, C. Sevva, C. Koulouris, N. Varsamis, S. Mantalovas, V. Lagopoulos, V. Magra, V. Theodorou, S. Ouzouni, N. Iason Katsios, P. Axi, K. Sapalidis and I. Kesisoglou. 2023. Giant Echinococcosis of the Liver with Suppuration: A Case Report and Review of the Literature. Medicina.
27 -Lau, C. M. and J. C. Sun. 2018. The widening spectrum of immunological memory. Current Opinion in Immunology 54: 42-49.
28-Malik, A. A. and S. u. Bari. 2019. Biology of the Echinococcus .Human Abdominal Hydatidosis. Singapore. Springer Singapore. p. 1-13.

29 -Manterola, C., J. Rivadeneira, S. D. Pogue, C. Rojas, C. Manterola, J. Rivadeneira, S. D. Pogue and C. Rojas. 2023. Morphology of Echinococcus granulosus Protoscolex. International Journal of Morphology 41: 646-653.
30-Milani, N., N. Majidi and L. Goshayeshi. 2021. Hydatid cyst of the liver with portal thrombosis: A case report. Authorea Preprints.
31 -Miles, D., H. Roché, M. Martin, T. J. Perren, D. A. Cameron, J. Glaspy, D. Dodwell, J. Parker, J. Mayordomo, A. Tres, J. L. Murray, N. K. Ibrahim and t. T. S. Group. 2011. Phase III Multicenter Clinical Trial of the Sialyl-TN (STn)-Keyhole Limpet Hemocyanin (KLH) Vaccine for Metastatic Breast Cancer. The Oncologist 16: 1092-1100.
32 -Moghaddam, S. M., S. Picot and E. Ahmadpour. 2019. Interactions between hydatid cyst and regulated cell death may provide new therapeutic opportunities. Parasite (Paris, France) 26: 70.
33 -Motran, C. C., L. F. Ambrosio, X. Volpini, D. P. Celias and L .Cervi. 2017. Dendritic cells and parasites: from recognition and activation to immune response instruction. Seminars in immunopathology 39: 199-213.
34 -Noya, V., S. Bay, M. F. Festari, E. P. García, E. Rodriguez, C. Chiale, C. Ganneau, F. Baleux, S. Astrada, M. Bollati-Fogolín, E. Osinaga and T. Freire. 2013. Mucin-like peptides from Echinococcus granulosus induce antitumor activity. International journal of oncology 43: 775-784.
35-Organization, W. H. and others. 2011. Report of the WHO informal working group on cystic and alveolar echninococcosis surveillance, prevention and control, with the participation of the Food and Agriculture Organization of the United Nations and the World Organisation for Animal Health, 22-23.
36-Ortona, E., P. Margutti, F .Delunardo, V. Nobili, E. Profumo, R. Riganò, B. Buttari, G. Carulli, A. Azzarà, A. Teggi, F. Bruschi and A. Siracusano. 2005. Screening of an Echinococcus granulosus cDNA library with IgG4 from patients with cystic echinococcosis identifies a new tegumental protein involved in the immune escape. Clinical and experimental immunology 142: 528-538.
37 -Ranasinghe, S. L., G. M. Boyle, K. Fischer, J. Potriquet, J. P. Mulvenna and D. P. McManus. 2018. Kunitz type protease inhibitor EgKI-1 from the canine tapeworm Echinococcus granulosus as a promising therapeutic against breast cancer. PloS one 13: e0200433.
38 -Ranasinghe, S. L. and D. P. McManus. 2018. Echinococcus granulosus: Cure for Cancer Revisited. Frontiers in medicine 5: 60.
39-Rawat, S., R. Kumar ,J. Raja, R. S. Singh and S. K. S. Thingnam. 2019. Pulmonary hydatid cyst: Review of literature. Journal of family medicine and primary care 8: 2774-2778.
40 -Ribeiro Franco, P. I., J. R. do Carmo Neto, M. P. Miguel, J. R. Machado and M. R. Nunes Celes .2023 .Cancer and Trypanosoma cruzi: Tumor induction or protection? Biochimie 207: 113-121.
41 -Sayek, I., M. B. Tirnaksiz and R. Dogan. 2004. Cystic Hydatid Disease: Current Trends in Diagnosis and Management. Surgery Today 34: 987-996.
42-Shakibapour, M ,.A. Kefayat, M. Reza Mofid, B. Shojaie, F. Mohamadi, S. Maryam Sharafi, M. Mahmoudzadeh and H. Yousofi Darani. 2021. Anti-cancer immunoprotective effects of immunization with hydatid cyst wall antigens in a non-immunogenic and metastatic triple-negative murine mammary carcinoma model. International Immunopharmacology 99: 107955.
43 -Sharafi, S. M., R. Rafiei, R. Rafiei, M. Hadipour, H. Shirzad, H. Khanahmad and H. Y. Darani. 2016. A Nonglycosylated 27 KDa Molecule as Common Antigen between Human Breast Cancer and Echinococcus granulosus Hydatid Cyst Wall. Advances in Breast Cancer Research 05No.02: 6.
44 -Shepherd, J. C., A. Aitken and D. P. McManus. 1991. A protein secreted in vivo by Echinococcus granulosus inhibits elastase activity and neutrophil chemotaxis. Molecular and Biochemical Parasitology 44: 81-90.
45 -Shooraj, M., E. Hajipour and S. A. Mahdavi. 2022. Immunological Examination of Echinococcus granulosus as a Candidate for Cancer Treatment and Vaccine Development: A Review Study TT -. mazu-tbsrj 4: 0.
46 -Siracusano, A., F. Delunardo, A. Teggi and E. Ortona. 2012. Host-parasite relationship in cystic echinococcosis: an evolving story. Clinical & developmental immunology 2012: 639362.
47 -Sung, H., J. Ferlay, R. L. Siegel, M. Laversanne, I .Soerjomataram, A. Jemal and F. Bray. 2021. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians 71: 209-249.
48 -Taefehshokr, N., B. Baradaran, A. Baghbanzadeh and S. Taefehshokr. 2020. Promising approaches in cancer immunotherapy. Immunobiology 225: 151875.
49-Tez, S. and M. Tez. 2015. Echinococcus and cancer: unsolved mystery. Parasite immunology. p. 426.
50 -Turhan, N., G. Esendagli, O. Ozkayar, G .Tunali, C. Sokmensuer and O. Abbasoglu. 2015. Co-existence of Echinococcus granulosus infection and cancer metastasis in the liver correlates with reduced Th1 immune responses. Parasite Immunology 37: 16-22.
51-Veríssimo, C. D. M., C. Graeff-Teixeira, M .K. Jones and A. L. Morassutti. 2019. Glycans in the roles of parasitological diagnosis and host-parasite interplay. Parasitology 146: 1217-1232.
52 -Wu, L., S. Saxena, M. Awaji and R. K. Singh. 2019. Tumor-Associated Neutrophils in Cancer: Going Pro. Cancers.
53 -Yong, W. K., D. D. Heath and T. Savage. 1979. Possible antigenic similarity between pulmonary carcinoma and cysts of Echinococcus granulosus. British medical journal 1: 1463-1464.
54 -Zhang, W. and D. P. McManus. 2006. Recent advances in the immunology and diagnosis of echinococcosis. FEMS Immunology & Medical Microbiology 47: 24-41.

55 -Zhang, X., W. Gong, C. Duan, H. Cai, Y. Shen and J. Cao. 2022. Echinococcus granulosus Protoscoleces-Derived Exosome-like Vesicles and Egr-miR-277a-3p Promote Dendritic Cell Maturation and Differentiation. Cells.
56- Zhang, Y., L. Sun, C. Lei, W. Li, J. Han, J. Zhang and Y. Zhang. 2022. A Sweet Warning: Mucin-Type O-Glycans in Cancer. Cells.