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**Review Article**

# Anti-cancer Potential of Hydatid Cyst-Derived Antigens: *In Vivo* Insights

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**ABSTRACT**

The global healthcare challenge of cancer remains challenging, requiring innovative approaches to identify potential anticancer agents. The intriguing anti-tumor properties of hydatid cysts produced in their larval stage by *Echinococcus granulosus* (*E. granulosus*) have attracted the attention of many scientists in recent years. This review aimed to delve deeper into the *in vivo* anticancer effects of hydatid cyst-derived antigens and shed light on their mechanisms of action and therapeutic implications for various cancer types. Several bioactive molecules in *E. granulosus* antigens have shown significant anti-cancer activity *in vivo*. Several studies have shown that administering these antigens reduced tumor size while increasing overall survival in breast cancer models. The immune response against tumor cells in lung cancer murine models has also been enhanced by *E. granulosus* antigens, such as antigen B, leading to the regression of tumors and enhanced immunity. Colon cancer cells are sensitized to these antigens as indicated by *in vivo* studies, rendering standard chemotherapy more effective at inhibiting tumor growth. *E. granulosus* antigens also reduce tumor metastasis when applied to *in vivo* melanoma models. *E. granulosus* antigens have demonstrated *in vivo* efficacy as a potential anticancer agent, underscoring their potential as valuable therapeutic agents. There is still much to be discovered about the exact mechanisms of these antigens and their clinical applicability. However, the impressive results observed across a wide range of cancer types underscore the significance of further research into the antigens to overcome cancer *in vivo*. In conclusion, animal model studies reveal the promising potential of *E. granulosus* antigens, particularly hydatid cyst fluid, in inhibiting tumor growth in colon, breast, melanoma, and lung cancers through immune-mediated mechanisms and apoptosis induction. These findings open up new avenues for cancer therapy and immunotherapy research, emphasizing the role of parasite antigens in combatting various cancer types.

## 1. Introduction

Cancer is the first leading cause of death globally, and approximately 9.6 million people were killed by cancer in 2018[1]. In recent years, cancer has become more prevalent. The prevalence of cancer is estimated to increase by 58% by 2040, with 21.6 million new cancer cases diagnosed yearly, and deaths are predicted to increase from 13 million in 2018 to 16.3 million in 2030[2]. Cancer is a disease with heavy economic and treatment costs. Approximately $1.16 trillion was spent annually on cancer by 2020[3]. New treatments and a better understanding of cancer are crucial to reduce mortality and save money. In recent years, evidence has accumulated that some infectious

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agents are capable of causing anti-tumor effects against different types of cancers, such as *Toxoplasma gondii*, *Trypanosoma cruzi*, *Trichinella spiralis*, and *Echinococcus granulosus (E. granulosus)*[4-7]. A retrospective study in Turkey reported that patients with hydatid disease have a significantly lower cancer incidence[8]. Most evidence indicates that *E. granulosus* reduces cancer growth of cancer[9]. The parasite can have two forms, including the infective form and a fluid called hydatid fluid with proteins, lipids, and carbohydrates[10]. In this context, hydatid cysts have garnered attention due to their intriguing interactions with the emerging evidence and host immune system, suggesting that specific components derived from these cysts may possess anticancer properties[11]. These properties could open up new possibilities for developing cancer therapies that are not currently available. An early *in vivo* study found antigenic similarity between hydatid cyst fluid (HCF) and pulmonary carcinoma[12]. There is some evidence that antigens present in *E. granulosus* and certain tumor types modulate host immune responses, resulting in anti-cancer activity[13]. A common characteristic of cancers and parasites is that both express mucin-type O-glycans, which are not typically found on the surface of healthy cells[14]. In cancer cells, o-glycans play critical roles in adhesion, metastasis, and invasion[15]. O-glycosylated TN antigens associated with cancer have been detected in larval and adult extracts of *E. granulosus*, with excretory-secretory protein (ESP) products showing the most significant antigenic activity[16]. Antigens, such as mucin-type O-glycans, have recently been detected in HCF, germinal layers, laminated, and ESP of hydatid cyst protoscoleces[17]. It has been found that Tn antigens induced antibody-mediated immune responses against cancer growth in patients with echinococcosis[18]. It has been reported that mucin-like peptides from *E. granulosus* induce the activation of natural killer (NK) cells in the spleens of immunized mice through factors derived from soluble dendritic cells *in vivo*[19]. Although *E. granulosus*-specific antibodies rarely recognize tumor-derived antigens, the anticancer effects of *E. granulosus* may be attributed to its ability to activate the NK cells and elicit a Th1 response[9]. The serum from patients with hydatid disease had a cytotoxic effect on human lung small cell carcinoma cells, in contrast to control sera from individuals without a history of echinococcosis[20]. Moreover, 40% of mice vaccinated with HCF showed tumor regression in a colon cancer model and adaptive immunological responses in response to tumor rechallenge *in vivo*[13]. According to recent study, neutrophils directly kill *E. granulosus* oncospheres, indicating an antibody-dependent response[21]. In immunotherapy, the adaptive immune response has a long-term memory. Many *in vivo* studies indicate that *E. granulosus* antigens are helpful in the treatment of cancer[9,22]. Researchers need to identify specific molecules of this parasite that reduce cancer risk and can act as future treatments. Therefore, the present study was carried out with the aim of helping to understand the interaction between host immune responses, parasites and cancer for new forms of treatment.

## 2. Life cycle of *Echinococcus granulosus*

The intermediate hosts of *E. granulosus* are domesticated mammals such as sheep, pigs, cattle, goats, and camels[23-26]. A sheep, which has the most fertile hydatid cysts, is a significant intermediate host and is one of the sources of infection for dogs through the consumption of contaminated offal[27]. Dogs, jackals, wolves, coyotes, dingoes, and foxes are the final hosts of *E. granulosus*. Cats, wild cats, and leopards have been reported as parasite hosts, but their effectiveness is low[28]. Even after months outside the body, they are capable of developing and are resistant to external conditions. After being consumed by an intermediate host, they hatch in the small intestine, and the resulting oncospheres invade the blood vessels[29]. During hatching, keratin-like blocks are disaggregated with pepsin pancreatic enzymes, followed by the oncospheres' activation. A muscular oncosphere that has been released attaches itself to the microvilli of the jejunal region of the small intestine and enters the lymphatic or mesenteric venules to lodge in various organs[24]. The distribution of metacestodes in the liver and lungs will likely be affected[30]. It reaches the liver within twelve hours after ingestion and develops into a hydatid cyst if not destroyed by phagocytic cells. In the liver, they form cysts, the origin of *E. granulosus*[31]. It is also possible for *E. granulosus* larvae to grow in the spleen, kidneys, eyes, brain, and long bones[32]. It takes approximately 10-14 days for an embryo to develop into a fully formed metacestode, during which cellular proliferation occurs, hooks on the oncosphere degenerate, and the laminated and germinal layers are formed[33]. These hydatid cysts are ingested or inhaled by a final host, where the protoscoleces project their heads and adhere to the villi of the small intestines to mature into adults[34]. *Echinococcus granulosus* eggs are released every two weeks after detaching a gravid segment after the worm stays in a host for five to 29 months[35]. Furthermore, repeated infections or many cysts may cause the small intestine to become full of adults (Figure 1).

## 3. Hydatid cyst fluid

Hydatid cyst fluid is a complex biological substance primarily composed of water (65-70%), along with proteins (15-20%), lipids (5-10%), carbohydrates (2-3%), enzymes, salts, hormones, immune modulators, growth factors, and other bioactive molecules[36]. Several protein components have been identified within the HCF, including antigen five family members (Ag5), antigen B (AgB), and heat shock proteins (HSPs)[37]. In HCF, Ag5 is also detected, although these proteins' precise role in inhibiting cancer progression has not been determined[38]. Heat shock proteins facilitate protein folding, and cells are protected from stress. It is believed that these components and other proteins and bioactive molecules can exhibit anticancer effects. Additionally, there have been reports that some fraction of HCF lipids show cytotoxic effects

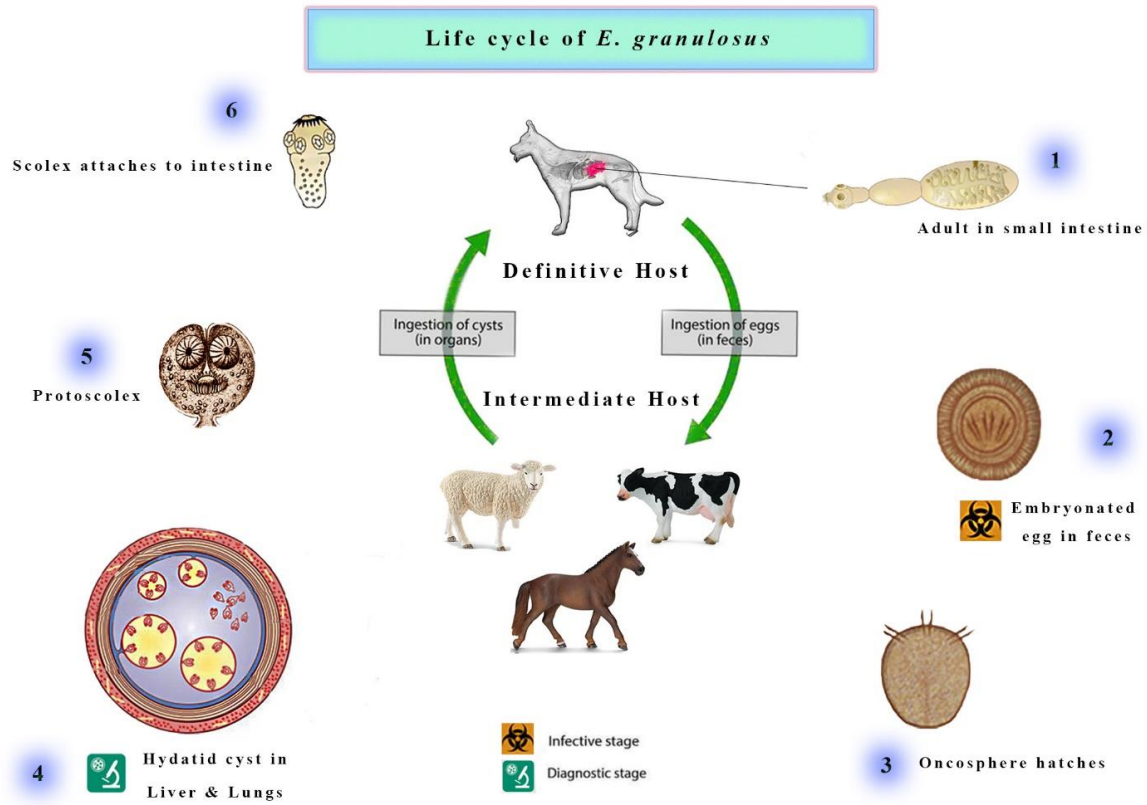


**Figure 1.** The mature *Echinococcus granulosus* (in a broad sense), measuring 2 to 7 millimeters in length, resides within the small intestine of its definitive host. Gravid proglottids expel eggs in the host's feces, which immediately become infectious. When a suitable intermediate host ingests these eggs, they hatch in the small intestine, releasing six-hooked oncospheres that breach the intestinal wall and travel through the circulatory system to various organs, notably the liver and lungs. Within these organs, the oncospheres develop into thick-walled hydatid cysts, gradually enlarging and producing protoscolices and daughter cysts that fill the cyst's interior. The definitive host becomes infected by consuming the cyst-containing organs of the infected intermediate host. Following ingestion, the protoscolices emerge, adhere to the intestinal lining, and mature into adult stages within a span of 32 to 80 days.

against cancer cells[39]. There have been several studies that claim that *E. granulosus* can kill cancer cells or inhibit tumor growth[9,40]. It has been noted above that protoscolex induces cell death in WEHI-164 fibrosarcoma cells and inhibits the growth of melanoma tumors *in vivo*[41,42]. There has been some evidence that different hydatid molecules, especially protoscolex ES, can increase or decrease the number of dead cells in HeLa cell culture[43]. In another *in vivo* study, injection of hydatid fluid (peritoneal or tumor margin) decreased melanoma tumor size in a C57 mouse model[44]. After treatment with AgB fraction, less apoptosis was observed than other molecules. Therefore, AgB possible role deserves further investigation. According to available evidence, hydatid cyst molecules, particularly the ES molecules, may be responsible for the parasite's anti-cancer effects.

## 4. Antigens in hydatid cyst fluid and mechanisms of action

Antigens are molecules that can stimulate an immune response in the body. Antigen B is HCF's most extensively and abundant studied antigen[45]. Several studies have shown that AgB has immunomodulatory properties and is essential in interacting with parasites[46,47]. It is not entirely understood how HCF and its antigens inhibit cancer progression[48]. These components may affect various signaling pathways associated with cell proliferation, angiogenesis, survival, and metastasis[49]. For example, AgB modulates immune responses by causing regulatory T cells (Tregs) to become activated, skewing the cytokine profile towards an anti-inflammatory state potentially creating a non-favorable environment for tumor development[50]. In addition, it has been suggested that some of the lipid fractions present in HCF could interfere with the lipid metabolism pathways or cell membranes that are important for the development of tumors[51]. Although HCF and its antigens have been found to have promising anti-cancer properties, there are many unanswered questions [52]. A deeper understanding of their action mechanisms and therapeutic potential requires further research. Safety concerns are associated with using these substances since they originate from parasites that can cause severe health problems. It is an exciting study area to investigate the potential anti-cancer properties of HCF and its antigens.

## 5. Indirect anti-cancer effect through activation of host immune system

Cancer and *E. granulosus* share common antigens,

which can induce immunological cross-reactions. Evidence shows that high antigen levels in hydatid cysts react with sera from breast cancer patients[53].

In contrast, high levels of antigen in germinal layers and laminated of the cyst react with ES products from cancer cells[54]. Evidence indicates that a 40-kDa antigen found in hydatid fluid can react with sera from patients with breast cancer, as well as monoclonal antibodies raised against this 40-kDa antigen[55]. Therefore, suggesting that *E. granulosus* might induce adaptive immunity through common antigens to exhibit anti-cancer properties is plausible. There is, however, an unclear relationship between the types of immunity in *E. granulosus* and its anti-cancer effects[56]. Th1-polarized immune responses kill cancer cells, whereas Th2-polarized immune responses promote metastasis and tumor progression[57]. The *E. granulosus* induces different types of immunity depending on the type of infection[58]. The Th1-polarized reaction dominates during the invasion of the oncosphere. During the formation and growth of cysts, a th2-polarized response gradually takes over. Upon rupture or death of the cyst, the Th2-polarized answer will quickly be replaced by the Th1-polarized response. Consequently, it is suggested that the anti-cancer effects are due to Th1-polarized responses induced at certain stages of infection[59]. A peptide from *E. granulosus* that resembles the Tn antigen reacted with the mammary adenocarcinoma cells TA3/Ha and pancreatic cancer cells Panc02 (both of which express the antigen strongly), even though the peptide-induced high antibody production[60].

A new study discovered that passive transfer of antisera targeting hydatid fluid, protoscolex antigens, or cyst wall antigens did not counteract the growth of melanoma tumors in mice that had already been exposed to melanoma[9]. Alternatively, spleen cells passively transferred between mice immunized with hydatid fluid, hydatid cyst, or protoscolex would significantly reduce melanoma tumor size and growth rate[61]. Therefore, *E. granulosus* has an anti-cancer effect primarily through a Th1-polarized response[62]. According to the study mentioned above, innate immunity plays an essential role in the anti-cancer effect and adaptive immunity. Accordingly, *E. granulosus* can induce anti-cancer effects directly by secreting proteins with cancer-killing potential and indirectly by activating the host's immune response (especially the T1-polarized response)[9]. However, more research is needed to uncover the mechanisms behind this extraordinary phenomenon.

## 6. Animal models studies

A recent *in vivo* study examined the potential inhibitory effects of *E. granulosus* on exocrine pancreatic cancer using an azaserine-induced rat model[63]. Results showed a significant reduction in Atypical Acinar Cell Foci (AACF) burden in rats receiving both azaserine and protoscolex, suggesting that *E. granulosus* may inhibit precursor neoplastic changes in the pancreas. This study contributes important insights into the potential role of *E. granulosus* in suppressing pancreatic cancer, encouraging further research and therapeutic exploration. A previous study explored the potential of *E. granulosus*, specifically its larval stage, in inhibiting melanoma cancer cell growth[64]. Researchers injected different quantities of viable protoscoleces from the hydatid cyst into mice, followed by melanoma cell injections. The study revealed that mice receiving 100 or 500 protoscoleces displayed significantly smaller tumors compared to the control group, while those receiving 1000 protoscoleces did not exhibit a significant difference. The findings suggest that antigenic similarities between protoscoleces and cancer cells might lead to non-specific antibody-mediated effects on tumor growth. In another *in vivo* study, the therapeutic potential of hydatid cyst antigens in inhibiting melanoma tumor growth in C57/black mice was investigated[65]. Results showed significant tumor growth inhibition in mice treated with these antigens, along with notable differences in cytokine levels, compared to control groups. This suggests that hydatid cyst antigens may possess anti-melanoma properties, potentially linked to immune responses to parasite antigens. These findings hold promise for developing novel approaches in cancer therapy, harnessing parasitic antigens to combat tumors. In a recent study, the therapeutic potential of hydatid cyst antigens on melanoma tumor growth in C57/black mice was investigated[66]. Given the significance of cancer as a leading cause of death in developed countries and previous indications of parasitic infections inducing anti-tumor activity, this research explored the utility of hydatid cyst antigens. The study indicated that treatment with these antigens effectively inhibited melanoma tumor growth in mice. Significant differences in tumor size and cytokine levels between case and control groups suggested an immune response to parasite antigens as a likely mechanism for these anti-melanoma effects. This highlights the potential of hydatid cyst antigens as a novel approach to cancer therapy. The researchers also explored the use of HCF to induce an anti-tumor immune response in a colon carcinoma animal model, and the results revealed that HCF vaccination generated protective immunity, preventing tumor formation in 40% of mice and leading to tumor regression in another 40%[67]. Memory immune responses are also protected against tumor rechallenge. The study identified specific antigens in CT26 colon cancer cells recognized by anti-HCF antibodies, indicating potential immune cross-reactivity. These findings offer promise for developing highly immunogenic anticancer vaccines and present a novel strategy in cancer immunotherapy. A recent study explored the potential anti-tumor effects of HCF and its fractions in a mouse model of colon cancer[68]. The study validated the anti-tumor properties of HCF and a specific fraction, as evidenced by significantly smaller tumor sizes in treated mice compared to control groups. These findings strongly suggest an immune-mediated

mechanism for tumor inhibition by these antigens. This research has implications for developing novel cancer therapies and underscores the promising role of HCF in cancer treatment. In a recent study, the potential of HCF was evaluated for its anti-tumor immune response in a murine model of LL/2 lung cancer[69]. Drawing from previous findings that HCF inhibited colon cancer growth, the study demonstrated that HCF vaccination offered significant protection against lung cancer, both prophylactically and therapeutically. The research highlighted the role of oxidized terminal carbohydrates in HCF, suggesting their involvement in the immune response. It also identified an integrated anti-tumor immune response involving innate and adaptive components and emphasized the crucial role of NK1.1+ cells in mediating this protection. These insights offer a new approach to cancer immunotherapy research. In a recent study on cystic echinococcosis caused by *E. granulosus*, researchers investigated the potential role of arginase, an immune evasion strategy employed by the parasite[70]. They found elevated arginase expression in the peritoneal cells of infected mice, which correlated with reduced CD3 expression in T cells, indicating immunosuppression. This suppression could be reversed by arginase inhibition and L-arginine supplementation. The study also revealed alterations in serum metabolites related to the arginine pathway. These findings provide insights into the immune evasion mechanisms used by *E. granulosus* and their impact on host immune responses. In a recent study, the anti-tumor effects of HCF and AgB were explored in a 4T1 breast tumor model in BALB/c mice[71]. Previous research had hinted at the anti-tumor properties of HCF, and this study provided further insights. The results demonstrated a significant reduction in tumor size in mice injected with HCF and AgB, along with noteworthy changes in cytokine levels. The anti-tumor activity was attributed to immune responses elicited by these antigens, which offers potential implications for breast cancer therapy and cancer immunotherapy studies. In a recent study, the anticancer effects of HCF on a mouse breast cancer cell line were investigated[72]. The research was targetted toward elucidate the mechanisms responsible for these effects. The study confirmed the anticancer potential of HCF and revealed that apoptosis induction played a pivotal role. Specific HCF components, particularly the 78 KDa and glycoprotein fractions, induced over 40% apoptosis, while the AgB fraction was less effective. Understanding these mechanisms could hold promise for developing novel cancer therapies and inform treatment strategies (Figure 2).

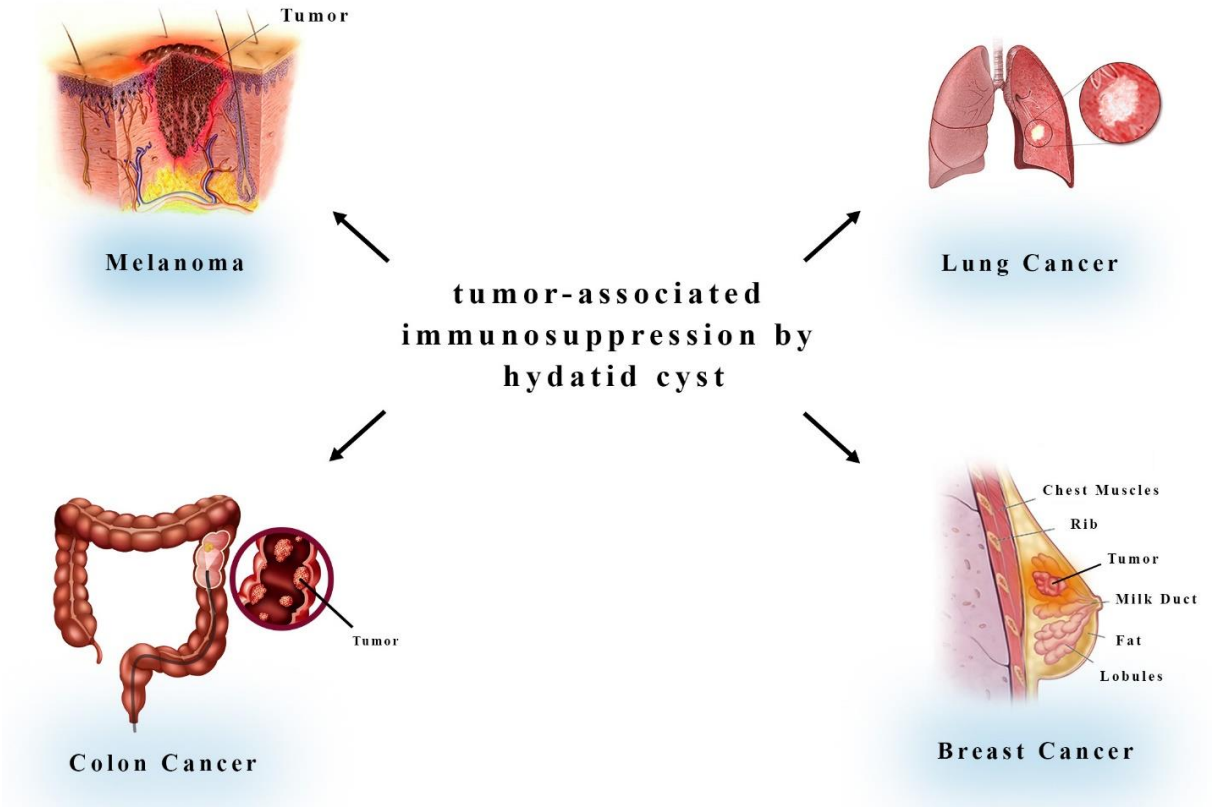


**Figure 2.** Hydatid cyst-associated immunosuppression in cancer. This figure illustrates the impact of hydatid cyst-derived antigens on various cancer types, including colon, melanoma, breast, and lung cancers.

## 5. Conclusion

In conclusion, the extensive body of research presented in these studies underscores the potential anti-tumor effects of *Echinococcus granulosus* antigens, particularly HCF, in the context of various cancer types, including colon, breast, melanoma, and lung cancers. These *in vivo* insights reveal promising avenues for cancer therapy and immunotherapy. The research demonstrates that *E. granulosus* antigens, including protoscoleces and hydatid cyst antigens, can inhibit tumor growth and modulate immune responses, offering significant potential for novel approaches to combat cancer.

These findings suggest that the immune cross-reactivity between parasite antigens and cancer cells may play a pivotal role in suppressing tumor growth, while also highlighting the importance of specific HCF components, apoptosis induction, and integrated anti-tumor immune responses. Additionally, the studies shed light on the potential mechanisms of action and immune evasion strategies employed by *E. granulosus*, providing valuable insights into host immune responses.

Overall, these studies encourage further research and therapeutic exploration in the field of cancer immunotherapy, offering hope for the development of highly immunogenic anticancer vaccines and novel cancer therapies. The potential of HCF and its fractions in inhibiting tumor growth holds promise for improving cancer treatment strategies and underscores the role of parasitic antigens in the fight against cancer.

## Declarations

### *Competing interests*

The authors declare no conflict of interest.

### *Authors' contributions*

Mahsa Mohammadian initiated and guided the conceptualization of the study, defining its core concepts and objectives. The original draft of the manuscript was expertly prepared by Zeinab Hosseini, Mohaddeseh Jamali, and Nikoo Sadat Hasheminezhad, who dedicated their efforts to its creation. Ensuring the document's quality and precision, Razieh Razmi, Rezvan Abbasi, and Negar Jahani were instrumental in the review and editing process. The entire project was overseen and supervised by Mahsa Mohammadian, providing crucial guidance throughout. All authors checked and approved the final version of the manuscript for publication in the present journal.

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### *Ethical considerations*

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

### *Availability of data and materials*

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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