Infectious Agents and Cancer

REVIEW

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The role of helminths and their antigens in cancer therapy: insights from cell line models

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Abstract

Background Recent articles have explored the effect of worms on cancer cells. This review focused on various cell cultures employed to understand which cells are more commonly and less utilized.

Methods The present review analyzed studies published between 2013 and 2023 to obtain information about different cell cultures used in cancer studies involving helminths. Databases such as PubMed, Google Scholar, HINARI, and the Cochrane Library were searched.

Results This search yielded 130 records, but 97 papers were excluded because they were either irrelevant to the research topic (n = 72) or contradicted the research idea (n = 25).The remaining twenty-one articles focused on different types of worms, such as *Echinococcus granulosus, Clonorchis sinensis, Opisthorchis felineus, Opisthorchis viverrini, Trichinella spiralis, Toxocara canis,* and *Heligmosomoides polygyrus*.

Conclusion Due to the presence of numerous antigens, parasites at different growth stages can impact various cells through unknown mechanisms. Given the high diversity of antigens and their effects, artificial intelligence can assist in predicting initial outcomes for future studies.

Keywords Cancer, Antigen, Treatment, Helminths

Introduction

Cancer is a chronic illness resulting from the abnormal growth of cells that can spread to other parts of the body and cause widespread metastases, ultimately leading to death [1]. According to estimates, there were 19.3 million new cases of cancer and almost 10 million cancer-related deaths worldwide in 2020 [2]. Breast cancer, lung cancer, and prostate cancer are the most commonly diagnosed cancers, but lung, liver, and stomach cancers are the most deadly [2, 3]. Despite progress in cancer treatment, new approaches are needed to cure this disease and reduce its high mortality rate [2–4].

Certain parasites have been linked to the development of cancer, such as *Opisthorchis viverrini* and *Clonorchis sinensis*, which may cause cholangiocarcinoma (a type of liver cancer) [5, 6]. However, some parasitic infections or their antigens can protect the host and may possess anticancer properties. For instance, *Echinococcus granulosus*, *Toxocara canis*, *Taenia crassiceps*, and *Trichinella spiralis* are examples of such parasites [7–14]. Recently, there has been a significant focus on helminthic therapy. The potential of helminths (parasitic worms) to have antitumor effects has been studied in vitro, and the therapeutic value of these parasites in cancer treatment has been extensively studied [15–18]. Therefore the effects of parasites and their antigens on various types of cells, including immune cells, epithelial cells, and neurons, as well as

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different organs, such as the liver, lungs, and brain, have been investigated in vitro and in vivo [19–21].

Using nonliving models such as cell cultures or animal tissues to study the effects of parasites may be preferable before conducting final studies on living organisms [22–24]. This review has focused on various cell cultures employed in this field to understand which cells are more commonly used and which are less utilized or not used on which research studies or tests have been employed.

Methods

Registration

This meta-analysis and systematic review were carried out following the PRISMA standards. The whole procedure was prospectively registered in PROSPERO (CRD42023483762).

Literature search strategy and classification

A thorough search for information across various databases was conducted until June 2023. Different keywords and phrases related to other types of worms, such as flukes, tapeworms, trematodes, cestodes, nematodes, and roundworms, along with their antigens, were used to find relevant information These words were combined using "OR" and/or "AND" to refine this search. In addition, specific words such as cancer, tumor, cell line, and in vitro culture were included to further narrow our search results (Table 1). Two researchers were involved in searching for information from books, journals, and other sources [25, 26].

Inclusion and exclusion criteria

Two researchers thoroughly examined each article's title, abstract, and full text to determine the most relevant articles. All irrelevant or duplicate articles were immediately eliminated. To be considered, research and review articles had to meet the following rigorous criteria:

A. The article must explicitly discuss the connection between worms and cancer.
B. The article must have a clear and definite final thought or opinion.
C. The article must have a minimum of four reliable sources for its information.
D. In the event of multiple articles containing the same crucial information, only the newest and most detailed article was chosen.
E. The article must provide compelling evidence of how cancer and worms or worm-related molecules impact each other.

Any articles that didn't meet these criteria were immediately excluded. To be specific:

F. The article must address the relationship between helminths (parasitic worms) and cancer.
G. The article must provide a clear and definitive result regarding the connection between helminths and cancer. Any article with an uncertain or ambiguous result was not considered for inclusion [27].

Assessment of risk of bias and reporting quality of included studies

The quality of the research methods was evaluated in this study based on own writer's established criteria since there is no standardized mechanism for assessing the quality of in vitro experiments. Two separate reviewers evaluated the papers selected for review using these criteria. Table 2 displays the criteria used to determine the likelihood of selection, performance, or detection

Table 1 The table identifies the search keywords for each database along with the corresponding number of articles

Search engine	Search terms	Search results 2023/06/03
PubMed	Trematoda, Cestoda, Nematodes, Neoplasm, Cell line, Antigens	114
HINARI	Flukes, Tapeworms, Trematodes, Cestodes, Nematodes, Roundworms, Antigens, Cancer, Tumor, Cell lines,	99
Scopus	Cestodes, Nematodes, Roundworms, Antigens, Cancer, Tumor, Cell line "	122
Google Scholar	fluke parasites, tapeworm infection, trematode treatment, cestode therapy, nematode research, roundworm diagnosis, antigen detection, antigen research, cancer treatments, cancer cell line, tumor study, cell line culture, in vitro culture methods, parasitic worms, parasite research, fluke disease, tapeworm medicine, trematode infection, cestode study, nematode genetics, roundworm genome, cancer antigens, tumor antigens, cell line technology, in vitro testing, parasitic infections, parasitology, nematode diseases, fluke control, tapeworm control,	113
Total articles after excluding duplicates	–	130

Table 2 The criteria used to determine the likelihood of selection, performance, or detection bias leading to systematic errors

	Questions	Yes	Partly	No	Unknown risk
Reporting quality	Is the cell origin and cell type used reported?	Reported	Not reported	Not reported	–
	Is the duration of exposure reported?	Reported	Not reported	Not reported	–
	Is the frequency of exposure reported?	Reported	Not reported	Not reported	–
Performance bias	Is the temperature controlled?	Yes	Yes	No	Not reported
	Was the exposure blinded?	Yes	–	No	Not reported
	Was the exposure randomized?	Yes	–	No	Not reported
Selection bias	Is the cell vitality scored/measured?	Yes	–	No	Not reported
Detection bias	Were the methods the same for control and exposure treatment?	Yes, independent measurements	Dependent measurements	No	
	Were the data measurements randomized?	Yes		No	Not reported
Other Bias	Was there no industry sponsoring involved?	Yes		No	

bias leading to systematic errors, which were classified as "low," "moderate," or "high" levels of prejudice. When evaluation criteria were missing, risks in papers were classified as "Risk Unknown." It was also assessed reporting quality, which refers to the lack of repeatability caused by inaccurate or missing data. The presence or absence of necessary information regarding the study design and experimental controls were indicated as "Yes/partly" or "No," respectively.

Data analysis

The included articles were meticulously put in an Excel form based on various categories, such as the type of helminths and antigens, cell lines, origins of the cell lines, cell type, advantages of using this media, and the proposed mechanism for preventing cancer. The data was thoroughly analyzed, and basic calculations were performed to understand the information better. The findings were then presented in a well-organized and easy-to-read table [28]. A flow diagram was drawn for finding, classifying, and including articles in the retrieval and identification process [29].

Results

Search results and study selection

It searched several databases, such as PubMed, Google Scholar, HINARI, and the Cochrane Library, and obtained 130 records. These records were saved in Endnote X8. Then, 97 papers were excluded because their titles were irrelevant to research topic (n = 72) or went against the research idea (n = 25). Afterward, a comprehensive analysis of the content of 33 relevant studies was conducted. After investigating and analyzing keywords, this review examined 21 selected articles and their results and methods (as shown in Fig. 1). The exclusion of the final 11 studies from the results section was due to unrelated reports, the absence of a valid and reliable questionnaire, and studies focusing on non-cancer subjects or in-vivo tests.

Characteristics of the studies included in this review

In this systematic review, a total of twenty-one articles were analyzed. Most of these articles were published between 2019 and 2022, as indicated in Table 3. This review examined different types of worm antigens, including those of *Echinococcus granulosus, Clonorchis sinensis, Opisthorchis felineus, Opisthorchis viverrini, Trichinella spiralis, Toxocara canis*, and *Heligmosomoides polygyrus*. A range of cell lines were also analyzed, including MPSIEC, HCC, HepG2, A375, CCA, JAWSII, H69, HuCCT, and others. These cell lines originate from various sources, such as the intestine, liver, skin, bile ducts, bone marrow, lung, breast, and pancreas.

The Becerro-Recio article published in 2022 demonstrated the benefits of using parasite antigens and MPSIEC cell lines. The interaction between FhNEJ (*Fasciola hepatica* parasite antigen) and MPSIEC triggers a rapid change in the expression of FhNEJ protein in response to the host epithelial barrier. This interaction involves cathepsins L3 and L4, as well as several immune regulatory proteins. When MPSIECs are stimulated with FhNEJ, changes in the protein profile related to immunomodulation and cell–cell interactions occur. Additionally, it leads to a significant decrease in the expression of proteins associated with ribosome function. This study also revealed that the cells responded to parasite stimulus by showing changes in the expression of proteins related to immunomodulation and cell–cell interactions, and there was a notable decrease in the function of ribosomes [30].

Hepatocellular carcinoma (HCC) cells have the unique advantage of extensive migration capacity, which allows them to invade the host's normal liver tissue to varying degrees depending on the environment

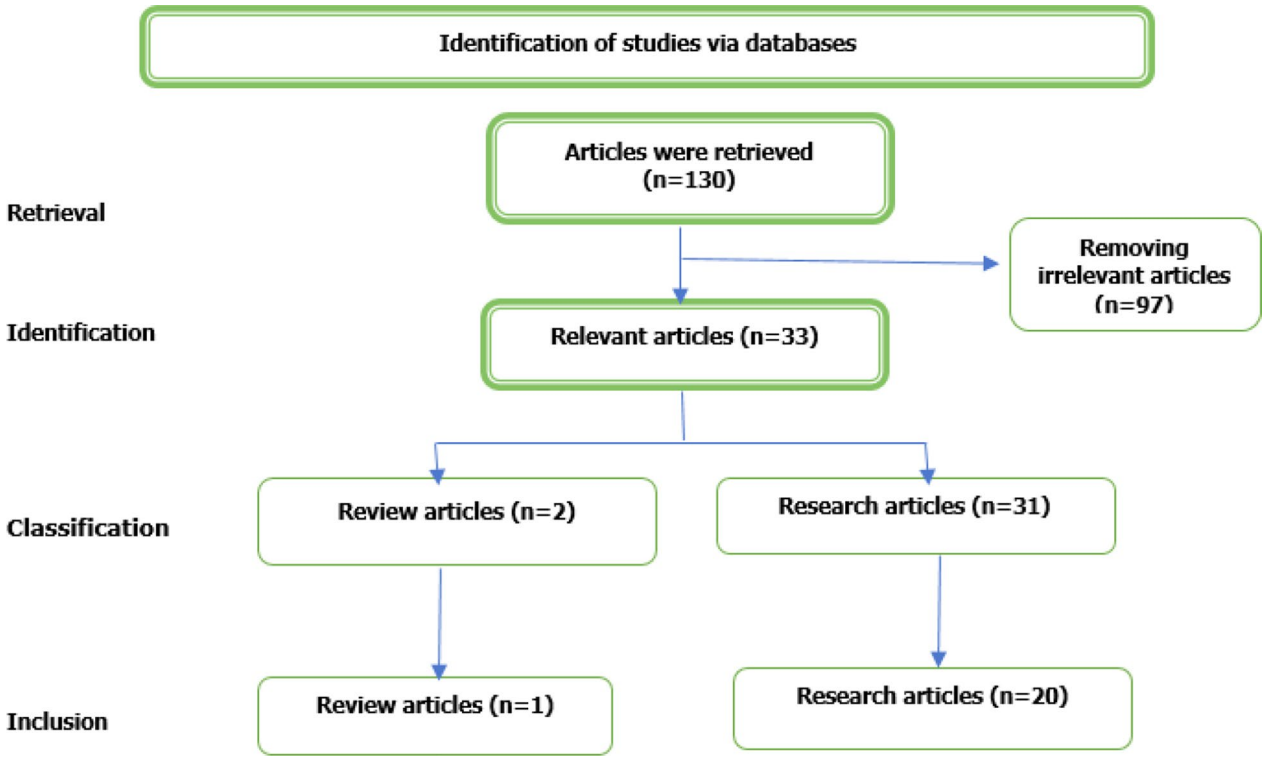

Fig. 1 Flow diagram of article retrieval, identification, classification, and inclusion

[31]. The benefits of using a melanoma cancer cell line are mentioned in another article by Barati. The authors evaluated the effects of AgB, extracted from the hydatid cyst fluid of the *Echinococcus granulosus* larval stage, on the melanoma B16F10 cell line and confirmed that AgB inhibits proliferation and promotes apoptosis. For this study, two cell lines were chosen for investigation: HEK293 as a normal cell line, and B16F10 as a cancer cell line. The study results showed that HEK293 cells, a normal cell line, were less sensitive to AgB than the B16F10 cancer cell line, which was highly sensitive to AgB. The study also revealed that AgB has a unique role in inhibiting the cell cycle, ultimately leading to a decrease in the population of the cells in the S and G2/M phases [32]. In another study by Buathong, after treatment with *Opisthorchis*-like eggs, the number of cells in the S phase of the cell cycle increased in the HeLa and MDA-MB_231 cell lines. These changes in the cell cycle can help identify new molecules specific to cancer that can target mitosis and optimize combination therapies in the future [33]. In another article, researchers used HuCCT1 cell culture derived from the epithelium of the biliary tract. They found that *Clonorchis sinensis* excretory-secretory products increased the migration and invasion of malignant cells [34].

Reporting quality and risk of bias

Significant differences were observed in the quality of the reporting across all of the reviewed studies, as depicted in Fig. 2, however, all the publications provided cell type, length, and frequency information. Our evaluation of the risk of bias indicated significant or unknown hazards. In 80% of the trials, cell viability was measured before or after exposure. The most essential unknown risks were associated with unreported blinding and randomized exposure and assessments. While over 90% of the publications discussed controlling the temperature during exposure, only 50% included the error range, and the measurements were usually taken outside of the culture area, suggesting that this variable may be a significant artifact. Among the twenty publications that dealt with industry sponsorship, two included authors affiliated with or employed by the company in question, raising concerns about the possibility of biased results.

Discussion

It is essential to understand the complex interactions between parasites and their hosts. However, studying living organisms directly can pose ethical, logistical, and practical challenges. Therefore, researchers are increasingly turning to non-living models, such as cell cultures, to

Table 3 Characteristics of studies included in this review

Cell line	origin	Cell type	Helminth	Ags	Year	Country	Reference
HepG2 SK-OV-3 A549	Liver Ascites Lung	Hepatocellular ovarian Epithelial	*Trichinella spiralis*	ESP*	2022	Thailand	[12]
Panc02TA3/Ha	Pancreasbreast	syngeneic pancreatic tumor syngeneic mammary adenocarcinoma	*Echinococcus granulosus*	Mucin-like peptides (MUC6*) Tn antigen	2013	Uruguay	[15]
CCA*	bile duct	Cholangiocarcinoma	*O. viverrini*	Metacercariae	2020	Thailand	[33]
CCA*(HuCCT1)	The epithelium of the biliary tract	Cholangiocarcinoma	*Clonorchis sinensis*	Excretory-secretory products	2017	Korea	[34]
MPSIEC*	Intestinal	Epithelial	*Fasciola hepatica*	Tegument and Somatic FhNEJ*	2022	Uruguay	[35]
Human HCC* HepG2	Liver	Hepatcellular carcinoma	*Echinococcus granulosus*	Echinococcus granulosus PSCs*from the liver hydatid cysts	2022	China	[36]
A375	Skin	Melanoma cell	*Echinococcus granulosus*	1.Fertile and Infertile (Hydatid cyst fluid) 2. Fertile (containing protoscoleces (PSCs*) and infertile (without PSCs*) liver hydatid cyst	2022	Iran	[37]
CCA-OF	bile duct	Cholangiocarcinoma	*Opisthorchis felineus*	*O. felineus* metacercariae	2021	vimentin	[38]
JAWSII	Bone marrow	an immortalized immature DC line	*Opisthorchis felineus*	Extract hemozoin from *Opisthorchis felineus*	2019	China	[39]
CCA*(HuCCT1)H69	bile duct	Cholangiocarcinoma	*Clonorchis sinensis*	Excretory-secretory products (ESPs*)	2019	Korea	[40]
MCF-7T47D MDA-MB-231FaDu HeLa SCC15 CJMNFF	Breast Breast-Breast Pharynx-cervicaltongue skinforeskin	breast adenocarcinomabreast ductal carcinoma	*Echinococcus granulosus*	Oncosphere (EgKI-1*)	2019	Australia	[41]
RBE-GRNPLC-GRN	liver	CholangicarcinomaHepatocellular carcinoma	*C. Sinensis*	The mRNA sequence annotated with granulin (CsGRN*)	2017	China	[42]
HuCCT-1Cho-CK Choi-CK	bile duct	Cholangiocarcinoma	*Clonorchis sinensis*	Excretory–secretory products	2017	Korea	[43]
KKU-M214 H-69	liver lung	Cholangiocarcinoma cholangiocyte	*O. viverrini*	Excretory-secretory products	2014	Thailand	[44]
CCA* (HuCCT1)	bile duct	Cholangiocarcinoma	*Clonorchis sinensis*	Excretory–secretory products let-7a let-7i miR-16 miR-24 miR-31 miR-93 miR-95 miR-124a miR-136 miR-153 miR-181d miR-185 miR-195 miR-199a-3p miR-342-5p miR-373	2014	Korea	[45]
HuCCT1	Epithelial bile duct	Cholangiocarcinoma	*C. sinensis*	Excretory–secretory products (metacercariae)	2014	Korea	[46]
NCI-H209/An1L929	Bone marrowAdipose	Lung small cell carcinomafibroblasts(control)	*Echinococcus granulosus*	sera from patients with hydatid cysts	2013	Turkey	[47]

Table 3 (continued)

Cell line	origin	Cell type	Helminth	Ags	Year	Country	Reference
NIH-3T3 MMNK1	whole mouse embryos Fetal liver	murine fibroblasts Cholangiocyte	*O. viverrini*	Crude ESP*	2012	Thailand	[48]
HCT116	colon	colon cancer cell	*Heligmosomoides polygyrus*	excretory-secretory	2020	UK	[49]
AGS HT-29 Caco 2	Stomach Colon	gastric cancer colon adenocarcinoma colon adenocarcinoma	*Toxocara canis*	excretory-secretory troponin protein	2022	IRAN	[50]
4T1	Mammary gland	Epithelial	*Echinococcus granulosus*	Antigen B	2019	Iran	[51]

* *FhNEJ* Newly excited juvenexcited *F. hepatica*, *PSCs Echinococcus granulosus* protoscoleces, *EgKI-1* Kunitz type protease inhibitor, *CsGRN* the mRNA sequence annotated with granulin, *MUC6* mucin-like peptides, *ESP* the excretory/secretory product, *MPSIEC* mouse intestinal epithelial cells, *HCC* hepatocellular carcinoma and *CCA* cholangiocarcinoma

examine parasite behavior and the effects of their antigens. This review explored the diverse range of cell cultures used in cancer treatment research, with a specific focus on the use of worms and their antigens. By using these nonliving models, scientists can acquire essential knowledge before conducting final studies involving living organisms. In the future, helminth antigen therapy could be a potential treatment option for patients who do not respond well to traditional therapies. These antigens may effectively generate more anticancer activity, causing fewer adverse effects in most cell lines. However, the fact that worm antigens can also be used to treat cancer is often overlooked. This review highlights the use of cell culture in parasitology laboratories. This review has compiled information about different cell cultures, research studies, or tests employed in this field of parasite antigens and their impact on tumors.

Various cell lines including MPSIEC, human HCC HepG2, A375, CCA, JAWSII, CCA (HuCCT1), H69, MCF-7T47D, MDA-MB-231, FaDu HeLa SCC15, CJM, NFF, RBE-GRN, PLC-GRN, Cho-CK, Choi-CK, KKU-M214, H-69, Panc02, TA3/Ha, NCI-H209/An1, L929, NIH-3T3, MMNK1, HCT116, AGS, HT-29, Caco 2, 4T1, SK-OV-3 and A549, have been utilized in these specific studies. These cell lines originate from various cell types, including epithelial cells, hepatocellular carcinoma cells, melanoma cells, cholangiocarcinoma cells, an immortalized immature DC line, breast adenocarcinoma cells, breast ductal carcinoma cells, cholangiocytes, pancreatic tumor cells, syngeneic mammary adenocarcinoma cells, lung small cell carcinoma cells, fibroblasts, murine fibroblasts, colon cancer cells, gastric cancer cells, colon adenocarcinoma cells, and ovarian cells. Various types of cells respond differently to parasite antigens depending on their origin. According to the results, parasites such as *Clonorchis* and *Opisthorchis* and related antigens have been investigated for their effect on liver cells and bile ducts. Still, their effects on other healthy and cancerous cells have not been evaluated. The effect of *Echinococcus* and its antigens, from the category of Cestodes, on more diverse cells has been investigated, for example, the effect of this parasite on liver, lung, skin, pancreas, and bone marrow cells has been studied. Among nematodes, worms such as *Trichinella* and *Toxocara* with liver, lung, and colon cells have been evaluated. It can be suggested that cells from different sources are adjacent to other parasites and their antigens, and the effect of each parasite is investigated separately in each cancer tissue. In addition, examining healthy tissues in addition to cancerous tissues may also be helpful. Alternatively, assessing the animal model of a particular cancerous tissue along with its healthy tissues is advisable.

Different helminths, including *Fasciola hepatica, Echinococcus granulosus, Opisthorchis felineus, O. viverrini, Clonorchis sinensis, Heligmosomoides polygyrus, Toxocara canis* and *Trichinella spiralis* have mentioned in these articles. Other parasites like *Fasciolepsis boski*, *Taenia saginata*, *Taenia solium*, *Necator, Ancylostoma,* and the other important helminth parasites are not listed in Table 3. In a 2023 review article, different parasites such as the larval stage *of Echinococcus granulosus* (hydatid cyst), *Trichinella spiralis, Toxoplasma gondii, Trypanosoma cruzi*, and *Acanthamoeba castellanii* were mentioned because they can potentially be used in cancer immunotherapy [52]. It seems that the effect of most parasites on cancer cells and healthy cells has not been investigated enough, and there is an opportunity to work on these diverse parasites.

Various types of helminths and their different antigens and immature forms have been tested and analyzed in numerous studies. Some of the examples include the testing of tegument and somatic antigens, protoscoleces from liver hydatid cysts, metacercariae, excretory-secretory products (ESPs), oncosphere, the mRNA sequence annotated with granulin, and mucin-like peptides. Different parts of parasites in different stages of development

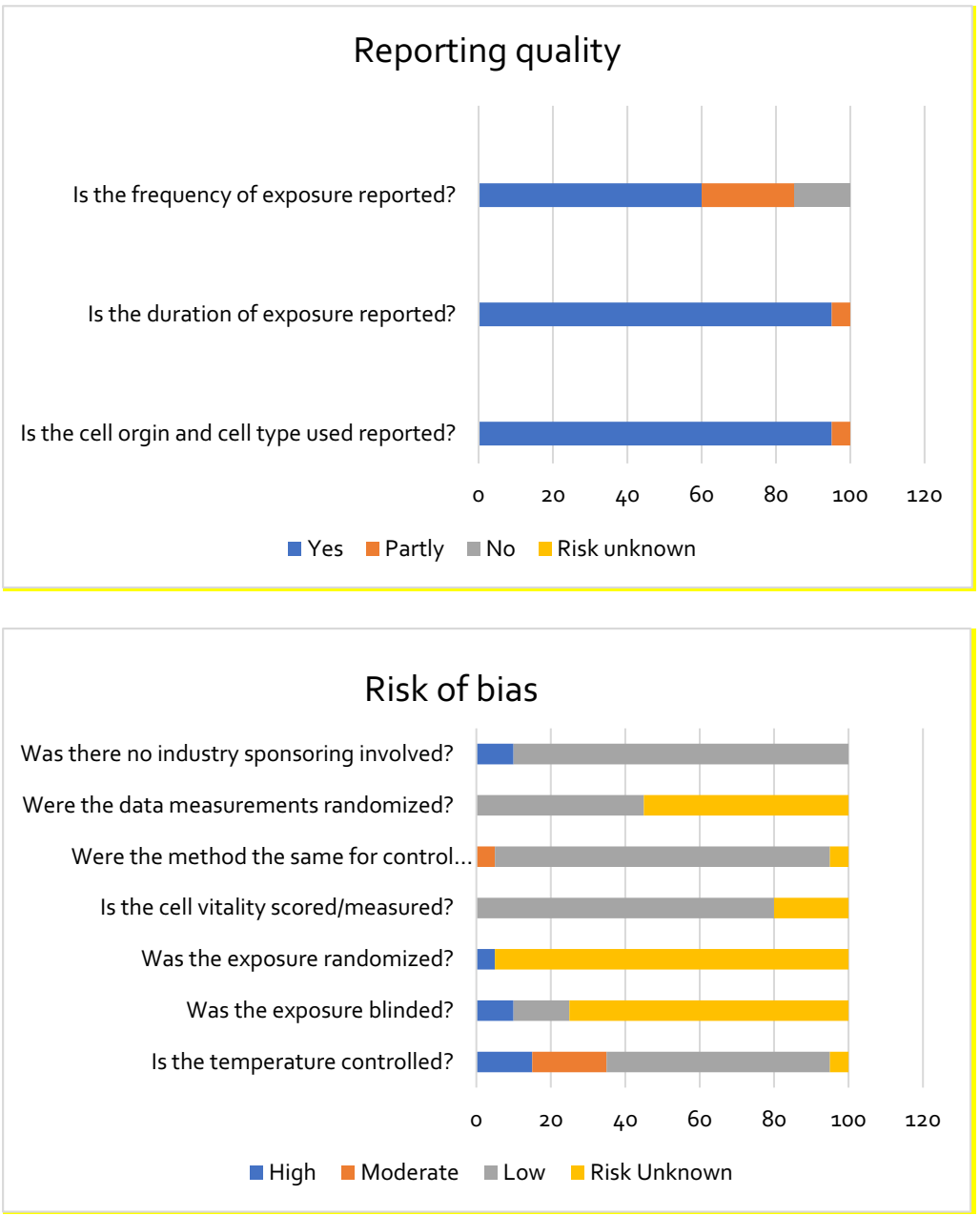

Fig. 2 The evaluation process involved scoring 20 papers based on their reporting quality and the possibility of bias. Each paper was assessed for the inclusion of crucial study facts, and labeled as "Yes," "Partly," or "No" accordingly. Researchers categorized the bias risk of each paper as "Low," "Moderate," or "High." If the bias risk was not disclosed or discussed, the paper was marked as "Risk unknown" and given a corresponding score. The final score was the percentage average of all 20 papers

can produce different antigens, the effect of which can be investigated in various cell cultures. It seems that molecular investigation of these antigens and artificial intelligence can effectively estimate their possible impact on cancerous and healthy tissue. It appears that this subject can accommodate various future research topics.

Although the anticancer effects of parasites have been observed in vitro, the mechanism by which antigens and helminths interact with different cancerous cells to kill them remains unclear. It is believed that inhibition of cell proliferation, induction of apoptosis, and direct toxicity may be involved. Recently, a group of researchers reported that a protease inhibitor, which is highly expressed by the oncosphere of *E. granulosus*, could inhibit the growth of various human cancer cells without affecting normal cell growth [53]. In 2008, researchers

made a ground-breaking discovery of specific molecules present in parasites that induce apoptosis exclusively in cancer cells while leaving normal cells unscathed. One example is the *Trypanosoma cruzi* surface molecule gp82, which can induce apoptosis in melanoma cells while sparing normal melanocytes. These molecules are promising candidates for cancer therapy because they can selectively target cancer cells without damaging healthy cells [54]. Additionally, anti-*T. spiral* antibodies showed a cross-immune response with myeloma cell SP2/0 antigens [55]. Furthermore, antibodies against *T. cruzi* cross-reacted with human colon and breast cancer cell lines. These antibodies selectively react with cancer cells but not normal cells and could serve as candidates for selective cancer therapy in humans [56]. Finally, parasites in different stages of growth can have different effects on different cells due to the presence of large amounts of various antigens, and this effect occurs via different mechanisms. Considering the high variety of antigens and their different effects, artificial intelligence may help predict the initial results.

Conclusion

In conclusion, exploring parasites, particularly helminths, and their antigens, as a novel approach to cancer therapy represents a promising frontier in medical research. The utilization of various cell cultures derived from different cell types has enabled scientists to study the effects of these organisms on cancer cell lines. Although these parasites' in vitro anticancer properties have been documented, the precise mechanisms of action remain elusive. Further research is essential to unravel how these antigens and helminths interact with cancerous cells, which could lead to breakthroughs in treatment strategies and improve outcomes for patients battling this devastating disease.

Author contributions

Raheleh Rafiei-Sefiddashti contributed to the conception and design of the work and revised the draft. Ali Kheirandish contributed to the acquisition, analysis, and interpretation of data, revised the draft, and approved the submitted version. Gita Alizadeh contributed to the acquisition, analysis, and interpretation of data, drafted the work, and approved the submitted version. Maryam Alipour, Mahnaz Jaafari, Mahdis Radfar, and Tina Bybordi contributed to the acquisition and have approved the submitted version. Raheleh Rafiei-Sefiddashti approves the submitted version (and any substantially modified version that involves the author's contribution to the study). This includes: (i) ensuring that original data, figures, materials, and code on which the submission is based are preserved according to best practices in the field to make them retrievable for reanalysis; (ii) confirming that the presentation of data, figures, materials, and code accurately reflects the original; and (iii) anticipating and minimizing obstacles to sharing the data, materials, and code described in the work. All authors have agreed to be personally accountable for their contributions. This is to ensure that any questions regarding the accuracy or integrity of any part of the work, even if they were not personally involved, are properly investigated, resolved, and documented in the literature.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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