



Features and applications of Ehrlich tumor model in cancer studies: a literature review

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Background and Objective: Breast cancer is the most prevalent cancer worldwide, responsible for a large number of deaths, especially among women. Therapeutic options for breast cancer include surgery, radiotherapy, chemotherapy, hormone therapy, and immunotherapy, but further studies of the pathogenesis of this disease and new treatments are still needed. *In vitro* and *in vivo* cancer models are important research tools. Murine Ehrlich tumors are one of these models, especially for hormone-positive breast cancer. The present narrative review discusses characteristics of the Ehrlich tumor model, laboratory manipulations of Ehrlich cells (ECs), and applications in pharmacological, pathological, and translational studies.

Methods: This review was based on scientific articles, books, and theses on Ehrlich tumors. We searched the PubMed, SciELO, Google Scholar, Google, and Clarivate databases.

Key Content and Findings: Hormone-positive ECs produce solid Ehrlich carcinoma (SEC) and ascitic Ehrlich carcinoma (AEC), with different features and applications. The presence of SEC or AEC induces systemic and immunological alterations that are similar to cancer in humans, what makes this model applicable to different studies in the cancer field.

Conclusions: Ehrlich tumors are a relevant tool for improving our understanding of the pathogenesis of breast cancer and investigating the tumor microenvironment, side effects of therapies, and new treatment options. Despite some limitations, such as the absence of an invasive phenotype to produce metastasis, both SEC and AEC are relevant in preclinical and translational studies of breast cancer.

Keywords: Tumor animal model; breast cancer; preclinical studies; translational studies; cell manipulation

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Introduction

In 2020, breast cancer became the most common cancer worldwide, surpassing lung cancer. Among women, breast cancer is the leading cause of cancer deaths. In the same year, more than 2.26 million new cases of breast cancer and nearly 685,000 deaths from breast cancer occurred worldwide (1). With these high numbers, breast cancer has enormous social and familial impacts. One notable impact is the creation of orphans that is attributed to maternal cancer mortality. There were 1,047,000 new orphans in 2020, and

25% (258,000) of these were attributable to maternal deaths from breast cancer. These children experience health and education disadvantages throughout their lives (2).

Breast cancer also affects health systems in many countries by having high treatment costs, especially in advanced stages of the disease (3,4). Breast cancer is among the top five cancers with the highest economic costs, together with tracheal, bronchus, and lung cancer; colon and rectum cancer; liver cancer; and leukemia (1). Costs that are associated with breast cancer in the United States

Table 1 Search strategy of this literature review

Items	Specification
Date of search	February to June 2023
Databases and sources searched	PubMed, SciELO, Google Scholar, Google, Clarivate, including thesis and books
Search terms	“Ehrlich tumor”; “Ehrlich tumor” and “breast cancer”, “metabolism”, “treatment”, “translational”, “features”, “immunological”, “cancer”, “oxidative stress”, “protein expression”, “immune response”, “cachexia”
Timeframe	1947–2023
Inclusion and exclusion criteria	Inclusion: articles, books, and thesis with recognizable authors and citations. Exclusion: articles with only abstract available; sources published in languages others than English, Portuguese, or Spanish
Selection process	All authors selected and shared with each other the published literature sources

increased from \$26.8 to \$29.8 billion from 2015 to 2020 (5). A substantial proportion of these costs are associated with breast cancer treatment, which is based on the tumor subtype and stage of progression, with or without metastasis.

According to specific subtypes, breast cancer is classified based on the expression of estrogen receptors (ERs), progesterone receptors (PRs), and human epidermal growth factor 2 (HER2): luminal A (HER2⁻), luminal B (HER2^{+/-}), HER2 overexpression (ER⁻/PR⁻/HER2⁺), and triple-negative (ER⁻/PR⁻/HER2⁻), the latter of which is the most severe (6). The importance of ER α in the origin of breast cancer was recently suggested based on the induction of dicentric chromosome bridge formation and breakage that cause the amplification of key oncogenes, including *ERBB2/HER2* and cyclin D1 (*CCND1*), in breast cancer cells (7).

Therapies for breast cancer are based on the cancer subtype and include surgery, chemotherapy, radiotherapy, and immunotherapy. Numerous anticancer drugs are currently available, but these treatments have low efficacy and high toxicity, leading to cancer progression, cancer recurrence, and low survival (8). New safe and effective therapies with low toxicity and a lower cost are still needed. *In vitro* and *in vivo* models are important tools for pharmacological investigations. Ehrlich tumors in mice are one of these models, used for more than a century. Ehrlich cells (ECs) have characteristics that are very close to human breast cancer cells, representing a hormone-positive tumor model. The present review discusses the cellular, biochemical, and genetic characteristics of the Ehrlich tumor model, its applications in pharmacological, pathological, and translational studies, and laboratory research manipulations of Ehrlich tumor cells. We present this article in accordance with the Narrative Review

reporting checklist (available at <https://tbc.amegroups.com/article/view/10.21037/tbcr-23-32/rc>).

Methods

This narrative review was based on scientific articles on Ehrlich tumors that have been published by scientists from several countries. The literature search was performed in the PubMed, SciELO, Google Scholar, Google, and Clarivate (Web of Science) databases. *Table 1* presents the search strategy.

Historical approach of Ehrlich tumors

Ehrlich tumors are a model that is largely used in cancer research, both solid and ascitic forms (9). This tumor is considered a carcinoma. By definition, this means a cancer that arises in epithelial tissue of the skin or lining of internal organs. The Ehrlich tumor originated in breast tissue. It is a spontaneous mammary adenocarcinoma in female mice that was first transplanted by its namesake Paul Ehrlich. Ehrlich and Apolant [1905] used an Ehrlich tumor in an experiment by transplanting tumor tissues subcutaneously from mouse to mouse. The first experiments with Ehrlich tumors were performed with solid Ehrlich carcinoma (SEC). Later, in 1932, the tumor cells began to be manipulated as ascitic Ehrlich carcinoma (AEC). After 1940, studies began to standardize both types of Ehrlich tumors, adapting them for qualitative and quantitative research (10). Since then, AEC cells have spread rapidly among research institutes globally (11).

Since the establishment of Ehrlich tumors, they have been applied to studies of the experimental oncology,

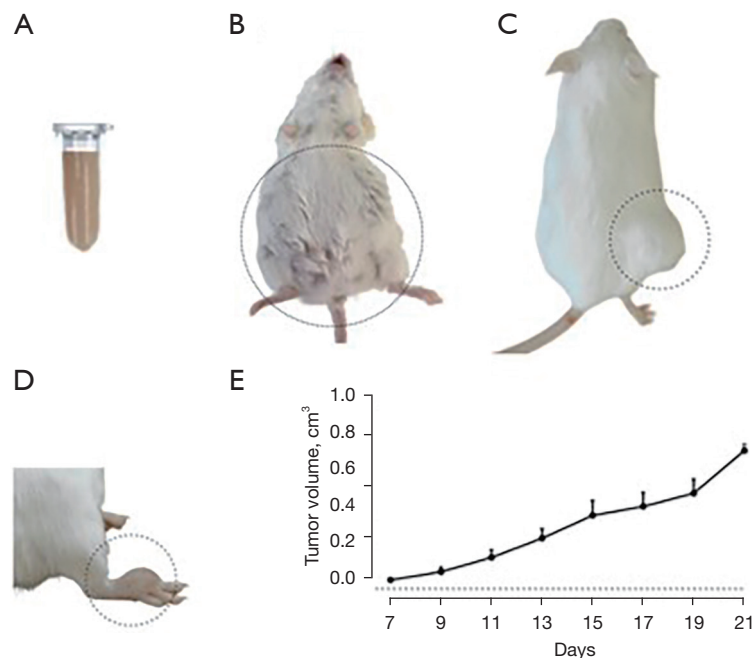


Figure 1 Macroscopic characteristics of ascitic and solid forms of Ehrlich tumors. (A) Ascitic liquid collected from peritoneum of Swiss mouse. (B) Ascitic Ehrlich tumor 10 days after intraperitoneal cell inoculation in a female Swiss mouse. (C) Solid Ehrlich tumor 21 days after subcutaneous cell inoculation. (D) Solid Ehrlich tumor 5 days after cell inoculation in the paw. (E) Time-course (days) of SEC growth (tumor volume) after subcutaneous inoculation of 2×10^6 cells. Sources: A-D, images kindly provided by Dr. Claudia Rita Corso (Federal University of Paraná); E, unpublished data from Dr. Débora Rasec Radulski (Federal University of Paraná). SEC, solid Ehrlich carcinoma.

biochemistry, pharmacology, and carcinogenesis of breast cancer. Since 1948, approximately 14,500 articles that mentioned this tumor have been published. According to PubMed [2023], the highest scientific literature production with Ehrlich tumors was reached in the 1960s, with a decrease after the 1990s. From 2010 until now, published articles that mention Ehrlich tumors have again increased. This tumor has been used to investigate systemic alterations that are induced by tumors, the sensitivity of tumor cells to chemotherapies, and the antitumor potential of synthetic and natural products.

Ehrlich tumor types and characteristics

ECs can produce ascitic or solid tumors, depending on the site of inoculation. AEC originates from intraperitoneal inoculation, where tumor cells may lead to an increase in peritoneal vascular permeability, generating an aggressivity tumor form (10,11). Macroscopically, ascitic fluid (Figure 1A,1B) is viscous and colored light gray or beige. Over time, it may develop a reddish color as a result of intraperitoneal hemorrhage. The death of the animal with AEC occurs

between 10 and 14 days after cell inoculation, depending on different situations, such as (I) the concentration of inoculated cells, (II) the amount of fluid in the peritoneal cavity, which causes abdominal pressure and compression of the organs, and (III) the number of passages, in which repeated transplantation increases malignancy and tumor proliferation (10). Intraperitoneal passages gradually increase the cell proliferation rate, resulting in an ascitic tumor that resembles human tumors that are sensitive to chemotherapy (11).

In contrast, SEC is obtained by subcutaneous inoculation in the dorsal region (Figure 1C), paws (Figure 1D), or upper region of the head (12-19), inducing a solid tumor 14 days after inoculation (20,21), which is palpable from day 8 (16,18) and can be maintained by day 42 after inoculation (21). Tumor growth can be observed exponentially (Figure 1E) on a daily basis, in which the tumor mass is fibrous, firm, and well-defined (10). This model has been frequently used in pharmacological studies, considering the longest survival time (compared to other models), high transplantable capability, fast and undifferentiated growth, no regression, and low malignancy (9,11,21).

SEC tumor cells are anaplastic and pleomorphic and have a high nucleus/cytoplasm ratio. The nucleus is composed of nucleoli and loose chromatin, and a high mitotic level can be identified. The stromal constitution comprises delicate capillaries and collagen fibers. It is also possible to identify areas of necrosis and hemorrhage. These cells still express anti-proliferating cell nuclear antigen (PCNA) with nuclear labeling, indicating lower positivity at day 7 than at day 14. AEC exhibits histological features that are similar to SEC but with larger cells. However, anti-PCNA staining was higher in the 7-day group than in the 14-day group, indicating that the difference in PCNA expression is related to a difference in the growth curve between the ascitic and solid forms (22).

Histologically, SEC is composed of neoplastic cells, connective tissue, and polymorphonuclear lymphocytes, which are all part of a well-defined capsule. Cellular composition is heterogeneous in different zones of SEC, such as in central areas relative to the periphery, with inflammatory cells near a necrotic zone. The periphery of the tumor is characterized by neoplastic cells, frequently in mitosis, and large heteroplasmy with polymorphism in both cells and the nucleus. Other areas are composed of cells with different phenotypes in acinus-like structures (9).

Evaluations of SEC have shown that the tumor has a capsule that is detectable by Doppler ultrasound, although this is not present in all cases, and it also exhibits blood flow, which may be reduced to central or distinct foci of necrosis. Tumor development varies according to its vascularization. Greater vascularization is associated with a faster growth rate. Conversely, the absence of blood flow and a homogeneous echotexture are associated with a slower growth rate of tumor cells. Additionally, extratumor vessels have a significantly higher resistivity index (RI) and pulsatility index (PI) than tumor vessels (23). Treatment with polysaccharides from sweet green pepper was shown to reduce vascular endothelial growth factor (*Vegf*) gene expression and vessel diameter but not the number of vessels in SEC in mice that were treated orally for 21 days. This effect was related to a reduction of tumor growth in these animals (18), confirming that vascularization is a key factor for SEC development.

EC preparation and manipulation

ECs can be used both *in vivo* and *in vitro*. Regardless of the way they are eventually used, these cells must undergo an obligatory *in vivo* phase (i.e., serial intraperitoneal passages). The intraperitoneal passage process guarantees a degree of

malignancy that is similar to the parental tumor, which can be explanted from a mouse and re-inoculated in another mouse or cultured *in vitro* (24). Frozen cells are maintained in a culture medium, such as Eagle, supplemented with 10% fetal bovine serum (FBS) (25), or in a solution of FBS and 10% dimethylsulfoxide (DMSO), and kept in a -80°C freezer or liquid nitrogen vials.

The intraperitoneal passage period is approximately 6–10 days (16,26). Afterward, animals with AEC are euthanized, and 1 ml of sterile phosphate-buffered saline (PBS) is injected in the peritoneal cavity to dilute the liquid tumor, followed by a small incision of the peritoneum to allow ascitic fluid collection under aseptic conditions. The cells are then washed, centrifuged at 4°C , and resuspended to measure cell viability (26,27). Cell viability is usually evaluated by trypan blue staining and must be above 95% to be considered satisfactory for application in experiments and SEC formation; otherwise, the process of intraperitoneal passages is continued (26). The washing and resuspension of Ehrlich carcinoma cells are generally performed using isotonic solutions, such as PBS and sodium chloride (NaCl). Schachtschabel & Foley (25) reported that although Ehrlich carcinoma cells are tolerant cells, in a hypertonic environment they abandon their epithelial morphology to become similar to fibroblasts, with an extended appearance, in addition to an increasing cell duplication time.

The subsequent standard cell dose that is used is based on the number of cells that are needed to generate a lethal ascitic tumor in 100% of inoculated mice, which is 10^6 viable cells. Under these conditions, animals develop an ascitic tumor and die within 30 days (24). On day 8 of tumor growth, there are 140–280 million cells/mL of ascitic fluid, in a total volume of 5–8 mL (24). Most often, a higher number of inoculated cells results in a shorter duration of intraperitoneal passage (28). The recommended intraperitoneal inoculation volume is below 0.2 mL, sometimes without specifying the exact number of cells (27,29). *Figure 2* shows the process of EC manipulation for the development of SEC and AEC.

Immune and systemic alterations caused by Ehrlich tumors

Immunoinflammatory regulation

Differences in the inoculation and constitution of AEC and SEC tumors are marked by differences in the immune response, metabolism, and other pathophysiological aspects.

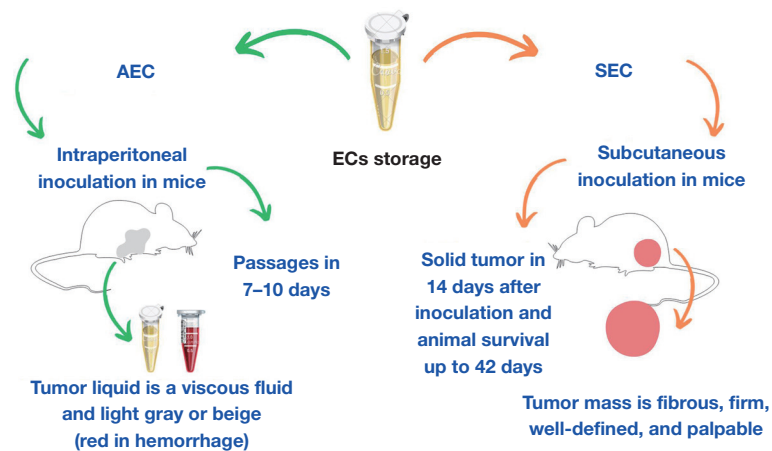


Figure 2 Sequence of EC manipulation for inoculation and AEC and SEC development. EC, Ehrlich cell; AEC, ascitic Ehrlich carcinoma; SEC, solid Ehrlich carcinoma.

Some studies reported relevant immune responses against these cells, but others reported escape strategies of tumors against the immune system. Most studies describe AEC as responsible for promoting higher immune modulations compared with SEC (30).

Tumor cell proliferation is marked by an immune modulation response, in which immune cells interact with tumor cells in the tumor microenvironment. Ozaslan *et al.* reported that AEC has a higher capacity for proliferation and immune evasion because it does not have a specific tumor transplant antigen (11). Instead, in AEC, splenic cells are altered, such as a decrease in CD4 and CD8 T cells, an increase in B-cell number (which peaked 2 days after tumor inoculation), and an increase in the macrophage population (31). Inzhevatkin *et al.* reported the suppression of metabolic processes of macrophages that were associated with tumors during tumor growth. Neutrophils are another important cell population in tumor development (32). AEC plays a paradoxical dual role in these cells, in which neutrophils are responsible for controlling tumor development in susceptible animals (e.g., Swiss mice), but in mice that are resistant to tumor development [e.g., CAF1 (BALB/c A/J) F1 mice], neutrophils improve tumor growth (33).

Additionally, thymic damage occurs at late stages of cancer, specifically at day 21 after tumor inoculation, marked by the depletion of CD4⁺ and CD8⁺ cells in peripheral blood (34). All actions of immune cells in the tumor microenvironment result in cytokine expression, especially proinflammatory cytokines [e.g., interleukin-1 (IL-1), IL-4, IL-6, IL-17, and tumor necrosis factor α], the

enzymes myeloperoxidase and *N*-acetylglucosaminidase, nitric oxide, and the anti-inflammatory cytokine IL-10 (17,18,35). IL-17 is stimulated in CD4⁺ cells by suppressor cells that derive from myeloid-derived suppressor cells and tumor-associated macrophages (35). IL-6 is mainly involved in cachexia and metabolic changes (36). IL-1 β increases on day 10 after inoculation but declines after this period, whereas interferon γ (IFN- γ) progressively increases afterward (37). Therefore, AEC is marked by a systemic inflammatory process, with neutrophilia, an increase in macrophages, thrombocytopenia, splenomegaly, and splenic hematopoiesis (31,38-40).

Peritumor inflammatory infiltrates in SEC are composed of mononuclear and polymorphonuclear leukocytes, with moderate intensity between days 2 and 7 post-inoculation. Da Silva *et al.* also showed a positive correlation between the intensity of inflammatory infiltrate and animal weight and tumor size (41). The main lymphocytes are CD3⁺, CD4⁺, CD8⁺, and Foxp3. Increases in CD3⁺ and Foxp3 and decreases in CD4⁺ and CD8⁺ were linked with tumor development (41). Additionally, SEC cells that were inoculated in the intraplantar area in mice were characterized by local neutrophilic infiltration with an extensive necrotic area, which may be responsible for mechanical allodynia, thermal hyperalgesia, and paw edema (42).

Metabolic changes

SEC can induce metabolic dysregulation, with a systemic proinflammatory state, skeletal muscle wasting, lipolysis, and increases in lactate dehydrogenase, creatine kinase (9),

and aspartate aminotransferase (16-19). Cancer cachexia involves metabolic regulation in skeletal muscle after SEC cell implantation, which is linked with glucose oxidation, and changes in energy homeostasis and in insulin metabolism (43). Insulin secretion and glucose homeostasis are normally affected by tumor cells that consume high amounts of glucose. SEC-bearing animals exhibited decreases in glycemia, hepatic glycogen, body weight, insulinemia, and insulin sensitivity 14 days after tumor cell inoculation, associated with increases in plasma free fatty acids and triglycerides (43). From our experience, SEC can reduce hepatic glycogen without affecting glycemia and reduces the total activity of hepatic cytochrome P (8,44). Another study found that SEC also modifies fatty acid composition 23 days after tumor inoculation (45).

AEC was also associated with lower peroxidation compared with liver tissue, based on the thiobarbituric acid reactive substances method (46). The authors suggested that the resistance to lipid peroxidation is linked to lower levels of lipids and fatty acids and an increase in α -tocopherol per milligram lipid in AEC compared with the liver. However, in our experience with measuring levels of lipoperoxidation in SEC by the ferric-xylenol orange (FOX) method (47), the lipoperoxidation ratio can be higher in Ehrlich tumor tissue (4.9–10.0 nmol hydroperoxides/min/mg of protein) than in liver homogenate (2.6–8.0 nmol hydroperoxides/min/mg of protein; (8,16-19,44). Additionally, mitochondria in ascitic ECs are also resistant to lipid peroxidation mainly through the action of succinate and a glutathione-dependent mechanism (48).

Protein expression

ECs express different proteins with oncogenic characteristics that promote a series of metabolic and morphological transitions in tumor cells. These modifications are linked to structural deterioration and decreases in adenosine triphosphate concentrations and RNA and DNA synthesis (10).

Among the expressed proteins are S100A6 and CacyBP/SIP (calyculin-binding protein and siah-1 interacting protein), which are purified in ECs and play an important role in carcinogenesis. S100A6 belongs to the S100 protein family. It is a low-molecular-weight calcium-binding protein that is also found in epithelial cells, fibroblasts, and tumor cells with high metastatic activity. CacyBP/SIP protein was discovered during analyses of S100A6. It interacts with

seven in absentia homolog 1 (Siah-1) and suppressor of kinetochore protein 1 (Skp1), which are involved in the ubiquitination and degradation of β -catenin (49). In addition to tumor proteins, animals with Ehrlich tumors produce serum factors, specifically serpins, which are essential for tumor growth, tumor invasion, and apoptosis, causing an increase in the cell mitotic index from 15% to 80%. Serpins are also involved in processes of coagulation, fibrinolysis, and inflammation (50,51). Importantly, when this protein is inhibited, Ehrlich tumor-bearing animals exhibit an increase in survival from 14–20 days to 90 days (51).

Importantly, the aggressivity and resistance of AECs are linked with overexpression of the chaperone heat shock protein 70 (HSP70), which is responsible for cell proliferation, invasion, metastasis, and death. This principally occurs as an adaptive modulation of stationary ECs under conditions of chronic oxygen and nutrient deficiency (20,52). Another protein that is modulated in AEC is multidrug resistance-associated protein 1 (Mrp1), originally discovered as a cause of multidrug resistance in tumor cells. In this case, Mrp1 increased when AEC was exposed to fractional irradiation (53). Consequently, the cells can adapt and modulate their expression, increasing cell survival. Inducible nitric oxide synthase (iNOS) and cyclooxygenase 1 (COX1) protein expression in AEC increased on day 2, with peak levels at day 10 post-inoculation (37).

Ehrlich-bearing mouse welfare and physiological changes

Animal welfare and adequate animal handling are indispensable for obtaining reliable research results. Physiological changes can impair the application of tumor models to pharmacological and pathological studies. One example is immunological changes that are observed in the cohabitation of healthy and tumor-bearing mice. Maragno-Correa *et al.* reported a decrease in the frequency of CD4⁺, CD8⁺, and CD19⁺ in spleen cells in ascitic tumor-bearing animals (54). Cohabitation experiments involved pairs of animals, in which one animal was inoculated intraperitoneally with Ehrlich tumor cells, and the other was named the companion of a sick partner (CSP) that received no stimulation. For the control group, one of the animals was treated with 0.9% NaCl intraperitoneally, and the other was named the companion of a healthy partner (CHP) (55-57). These studies demonstrated numerous neurological, endocrine, and immunological changes in CSP animals. In addition to these changes, an increase in tumor growth was observed when CSP animals were inoculated

Table 2 Murine Ehrlich carcinoma applications in research and translational investigations

Tumor type	Applications	References
Solid and liquid tumor	Anticancer potential of probiotics, spices, plant specimens, seaweed, and nanoparticles, alone or in combination with routine anticancer drugs	(8,19,61-66)
Solid tumor	Chemopreventive effects of plant extracts, polysaccharides, and oligosaccharides	(8,16,18)
Solid tumor	Antitumor/therapeutic effects of plant extracts, polysaccharides, oligosaccharides, and chemical compounds	(8,16,18,44,66-69)
Solid and liquid tumor	Anticancer immunization	(60)
Solid tumor	Neoplastic cachexia	(9)
Solid tumor	Cardiac dysfunction caused by neoplastic cachexia	(27)
Solid tumor	Pain model in cancer, indicated by mechanical and thermal hyperalgesia	(70)
Solid tumor	Translational model of immune response	(30)

with tumor cells (56). Differences were found between males and females, demonstrating different strategies in fighting the tumor. Male animals in the study by Machado *et al.* exhibited an increase in catecholamines and decreases in phagocytosis and oxidative burst (56). Morgulis *et al.* found that females exhibited increases in stress-related behavior, serum corticoid levels, and tumor growth (57). Additionally, CSP animals, when immunized with an ovalbumin booster, exhibited a series of immunological changes, such as decreases in bone marrow cell counts and plasma levels of IL-10 and IFN- γ , associated with increases in eosinophil and neutrophil counts, IL-4, IL-5, and plasma levels of norepinephrine (55). The authors indicated that the stress of cohabitation with a sick animal caused physiological and behavioral changes that increased susceptibility to Ehrlich tumor development (56).

Research applications of Ehrlich tumor model

Tumor models in preclinical studies

ECs represent a multifaceted preclinical tumor model that is applied in both pathophysiological and pharmacological screening studies for cancers. This is not limited to classic neoplasms (i.e., liquid or solid) it also extends to systemic involvement, generating such conditions as hepatotoxicity and higher levels of neoplastic markers, such as α -fetoprotein and tumor carcinoembryonic antigen, which are often observed in oncology practice (58,59). In addition to this versatility, the advantages of the model include fast tumor growth and high rates of tumor occurrence after transplantation (60). The antineoplastic potential of a wide

variety of substances has already been tested, including probiotics, polysaccharides, oligosaccharides, spices, plant extracts, isolated compounds, seaweed, off-label drugs alone or in combination with routine anticancer drugs, and countless variations of formulations, including nanoparticles (8,16-19,61-65). *Table 2* shows some applications of solid and liquid models of Ehrlich carcinoma in preclinical studies.

In addition to studies of chemoprevention (71), another important point is the possibility of anticancer immunization, which can be investigated in mice that are challenged with AEC and SEC (60). These effects were more pronounced when the challenge was minimal (1,000 ECs), whereas at a medium challenge (100,000 ECs), the effect was virtually lost. Despite the translational limitation in this case, because it is a transplantable neoplasm (as opposed to what occurs in humans with an intrinsic malignant transformation), these results reinforce the importance of the immune system in fighting cancer and how animal models can broaden our knowledge. Efforts have been made to understand immune responses that are induced by ECs (30). Although coming from a similar origin, there is a distinct profile of the body's response to ascitic and solid tumors (see section Immunoinflammatory regulation).

The Ehrlich tumor model is still important for clarifying controversial scientific issues, serving as links between *in vitro* and *in vivo* findings. One interesting example was provided by Mostafa *et al.*, who found promising results in the application of mesenchymal stem cells in cancer cell culture, an effect that was opposite to observations in SEC under conditions of both local and systemic treatment. Mesenchymal stem cells, although presenting anti-inflammatory and regenerative effects, are affected

by the tumor microenvironment, which directs them to a tumorigenic pathway only in the *in vivo* environment (72).

Furthermore, anticancer studies are important in terms of improving the quality of life of cancer patients. SEC triggers catabolic activities that are similar to patients with neoplastic cachexia (9). This model becomes interesting because it reproduces systemic proinflammatory characteristics, the loss of skeletal muscle, and lipolysis that are seen in human cancer patients (73). The aggressiveness of this condition is usually an experimental limiting factor, but in this model, it was possible to evaluate animals for 28 days without complications. Thus, effects of drugs on tumor growth and improvements in paraneoplastic metabolic syndromes can be studied simultaneously. Another important finding with regard to neoplastic cachexia concerns cardiac dysfunction. In this field, 24-day SEC altered cardiac structure and function, including a reduction of left ventricular wall thickness and ejection fraction, and caused degeneration, death, and muscle atrophy in the hearts of tumor-bearing mice (27). The pathogenesis of these changes is based on the suppression of mammalian/mechanistic target of rapamycin and consequent activation of autophagy. Cardiac dysfunction, in addition to being a consequence of cancer itself, is even more often caused by anticancer treatment (74).

The presence of SEC is also associated with mechanical and thermal hyperalgesia, as seen in human patients with breast cancer in the preoperative period (42). In this pain model, ECs are inoculated into the subcutaneous region of the hind paw in mice (*Figure 1D*), leading to local tumor development and the activation of dorsal root ganglia neurons via transient receptor potential vanilloid 1 (70). This pain-like clinical condition is responsive to the opioid morphine, which does not interfere with the tumor growth rate, so it can be used as an independent *in vivo* model of oncological pain.

Tumor animal models in translational studies

Translational research is complex and conducted over a long period of time. Researchers need to verify results precisely, while shortening the period between the discovery of innovations (75). The complexity of translation between tumor models occurs mainly according to the diversity of these models, which are not directly comparable. However, the models are descriptive, generally emphasizing the initial response to tumor survival of the animal and addressing tumor growth characteristics, drug effects, and in some cases resistance (76,77).

AEC is an allograft transplantation model that originates from a mouse mammary adenocarcinoma. Considering this characteristic, which is associated with rapid growth in suspension and sensitivity to chemotherapies, it can be used in translational research for breast cancer (78). SEC presents immune responses that are analogous to human breast cancers, representing a translational response, mainly to the hormone-positive breast cancer (30). Additionally, the 4T1, EMT6, TM40, and D2A1 cell lines are also considered allograft transplant models, whereas xenographic transplants include such human breast cancer cell lines as BT20, BT474, MCF-7, MDA-MB-231, and MDA-MD-453. Other animal models that are used in translational research include chemical models (e.g., 7,12-dimethylbenz[*a*]anthracene and *N*-methyl-nitrosourea), physical models that utilize radiation, biological models that utilize lentivirus infection, and genetic models that can be transgenic (e.g., oncogene activation) or knockout (e.g., through the inactivation of tumor suppressor pathways) (78).

Thus, *in vitro* and *ex vivo* models are fundamental to understanding tumor biology, identifying specific biomarkers, and searching for promising therapeutic pathways. This allows the generation of clinical results in oncology and clarification of pathways that are observed in clinical practice. However, the adopted models need to mimic the human condition, which is a challenge (79).

Limitations and conclusions

Despite several applications and advantages of the Ehrlich tumor model, such as strong reproducibility, low cost, and easy maintenance, this model has some limitations. Metastases are responsible for 90% of cancer deaths (80). ECs are not able to exhibit the invasive phenotype to produce metastasis, regardless of the route of administration of tumor cells. To analyze mechanisms that are related to metastasis in breast tumors, other models are more useful, such as those that generate lung metastases spontaneously with the inoculation of MCF-7 cells in the intra-iliac artery in mice. More recently, genetically engineered mouse models have been developed that are capable of producing spontaneous metastases in the lungs and lymph nodes (81). Another limitation of the Ehrlich model is the inability to accurately mimic defense responses to the tumor in humans. This is mainly attributable to the loss of histocompatibility complex antigens in its ascitic form (82). This is believed to be the reason for its ability to proliferate in almost all mouse lines, such as Swiss and C57BL/6, despite the fact that

most studies use female Swiss mice.

New *in vitro*, *in vivo*, and *ex vivo* models targeting different systems are necessary, which can complement existing advantages and overcome distinct disadvantages. Among these, tumor-derived organoids and organotypic cultures from fresh tumor tissue are examples of sophisticated systems (79). However, these systems also have limitations. Some of them cannot be propagated, or they cannot be easily preserved. Thus, Ehrlich tumors are a relevant tool for understanding breast cancer pathogenesis, investigating the tumor microenvironment, systemic alterations that are produced by cancer, the side effects of therapies, and discovering new treatments. Both SEC and AEC are important in preclinical and translational studies, mainly involving hormone-positive breast cancer. Indirectly, these models help to understand and minimize the problems faced in therapies and social issues related to the breast cancer, such as the impact of the disease on patients' family.

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are

appropriately investigated and resolved.

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