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Immunization using male germ cells and gametes as rich sources of cancer/testis antigens for inhibition of 4T1 breast tumors' growth and metastasis in BALB/c mice

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ABSTRACT

Recently cancer/testis antigens (CTA) have gained lots of attention as targets of immune therapy. However, the therapeutic efficacy of the CTAs single-antigen vaccines is not satisfying due to tumor heterogenicity. Therefore, many studies have focused on the enhancement of their efficacy by utilizing rich sources of tumor-associated antigens for anti-cancer vaccination. In the present study, the testicular germ cells and sperm cells as well-known sources of cancer/testis antigens were investigated for anti-4T1 breast cancer vaccination in BALB/c mice. The testicular germ cells (TGCs) and sperm cells were isolated from male BALB/c mice. The definite number of cells were homogenized and mixed with Bacillus Calmette–Guerin (BCG) for vaccination of female BALB/c mice. The treatment groups underwent 3 times of immunizations with one-week intervals and one week after the last injection, all groups were injected with 4T1 cancer cells. The TGCs + BCG ($259.7 \pm 39\,mm^3$) and Sperm + BCG ($426 \pm 52\,mm^3$) groups exhibited a significant decrease in the tumors' volume in comparison with BCG ($641.3 \pm 102\,mm^3$) and no-treatment ($788.1 \pm 117\,mm^3$) groups. Therefore, the TGCs + BCG immunized mice had the smallest tumors in comparison with all groups ($P < 0.05$). Also, the vital organs of TGCs + BCG (lungs: 6.8 ± 2, liver: 10.1 ± 2) immunized mice exhibited lowest metastatic burden in comparison with the Sperm + BCG (lungs: 13.5 ± 3, liver: 21.1 ± 4), BCG (lungs: 24.3 ± 4, liver: 33 ± 4), and no-treatment (lungs: 26.5 ± 6, liver: 37.3 ± 3) groups. These observations were inconsistent with the tumor-bearing mice survival evaluations as the TGCs + BCG group had longer mean survival time (79.6 ± 12 days) in comparison with other groups (no-treatment: 49.8 ± 8, BCG: 50.5 ± 10, BCG + Sperm: 64.6 ± 7 days). Therefore, TGCs can be a potential source of antigens for the anti-breast cancer immunization and more investigations are necessary.

1. Introduction

The genetic and epigenetic changes cause normal cells transformation to malignant cells and exhibition of new properties including uncontrolled growth, non-stoppable proliferation, apoptosis resistance, angiogenesis, and metastasis [1–4]. The antigens' pattern which is associated with or even responsible for these characterizations, can be appropriate targets for anti-tumor immune response. However, spontaneous immune responses can't adequately inhibit tumors' growth and metastasis [5]. Anti-cancer vaccines can solve this problem by introducing potential antigens to the immune system. Also, they can boost the immune responses against tumor [6]. The targeted antigen for anti-cancer vaccination should exhibit no or highly restricted expression in the normal tissues to prevent autoimmunity. Also, homogeneous and stable expression at the malignant tissue and high immunogenicity are the other important parameters for the selection of appropriate

Abbreviations: CTA, cancer/testis antigen; BCG, Bacillus Calmette-Guerin; PBS, phosphate buffer solution; TGCs, testicular germ cells; s.c, subcutaneously; H&E, hematoxylin and eosin

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target antigens [7]. Therefore, the selection of appropriate antigens for anti-cancer vaccination is a crucial step and can extremely affect the treatment efficacy.

Cancer/testis antigens' (CTA) family is one of the most well-known subtypes of the cancer-associated antigens. CTAs exhibit a restricted pattern of expression at the germ cells and trophoblast tissue. Also, their high expression has been detected in various human cancers. Their stable and specific expression on the cancer cells, high immunogenicity, and lack of expression on the normal cells make them an attractive target for cancer immunotherapy [5,8,9]. Despite these advantages, one major obstacle has limited their success in the clinical trials which is their heterogeneous expression, even within individual patients [10]. The tumor consists of different subclones and each subclone has its own antigen expression pattern [11]. Therefore, activating the immune system against a specific CTA can eradicate its related subclone. However, this treatment can cause selection of other subclones which are negative for this CTA, to proliferate and dominate the tumor [12]. Also, the tumors' antigen editing and poor antigen presentation are the other obstacles against single antigen-based cancer vaccines [13,14]. These pitfalls have made the single-antigen cancer vaccines not to meet the expectations. Therefore, targeting of multiple antigens can have attracted lots of attention for the enhancement of cancer vaccines efficacy [14–16].

The testicular germ cells (TGCs) are a rich source of different CTAs. CTAs are divided into two groups including X-CTAs which are encoded by the X chromosome and non-X CTA are distributed throughout the genome. X-CTAs are predominantly expressed in proliferating germ cells (spermatogonia). The genes for the non-X CTAs are usually expressed during later stages of germ cells' differentiation (spermatocytes). Therefore, cells at the different stages of spermatogenesis process exhibit different patterns of CTAs expression [10,17,18]. In addition, the final product of the spermatogenesis process, male gametes, express a vast variety of CTAs [19–22]. Therefore, male germ cells and gametes can be potential sources of antigens for anti-cancer immunization.

In the present study, the homogenized male germ cells and gametes were utilized as rich sources of CTAs for anti-breast cancer immunization. According to the best of our knowledge, this is the first time to use male germ cells and gametes for activating of the anti-tumor immune response. In this study, female BALB/c mice were immunized by the BALB/c male germ cells and gametes. The immunization was done in multiple times and the Bacillus Calmette-Guerin (BCG) was used as the adjuvant in both treatment groups. No-treatment and BCG injected mice were used as control groups. After completion of the immunization course, the mice were injected with the 4T1 breast cancer cells. The treatments' efficacy at the TGCs + BCG and Sperm + BCG immunized groups was investigated according to the tumors' growth, metastasis, and tumor-bearing mice survival time in comparison with control groups.

2. Materials and methods

2.1. Animal care and ethics

This study was approved by the institutional review committee of Arak University of Medical Sciences and all procedures were reviewed and approved by Institutional Animal Care and Ethics Committee of Arak University of Medical Sciences according to their guidelines for care and use of the laboratory animal. The mice were obtained at standard conditions: 24 ± 2 °C temperature, 50 ± 10% relative humidity, and 12 h light/12 h dark. All mice were fed sterilized standard chow and water *ad libitum*. The mice were sacrificed through injection of the ketamine and xylazine mixture.

2.2. Sperm isolation

20 male BALB/c mice (23 ± 2 g, 8-week-old) were purchased from the Pasteur Institute of Tehran, Iran. Animals were euthanized following anesthesia with the intraperitoneal injection of a mixture of ketamine (45 mg/kg) and xylazine (35 mg/kg). A vertical midline lower abdominal incision was made. Then, testes and epididymides were carefully dissected out. The adhering connective tissues were dissociated under a 20× magnification provided by a stereo zoom microscope (Olympus, Japan). Epididymal sperms were collected by chopping of the caudal epididymis in 1 mL of human tubular fluid (HTF) solution by 18-gauge needles. Then, the parts were incubated for 10 min at 37 °C in the cell culture incubator to allow sperm to swim out of the epididymal tubules. The sperm counts were obtained by the standard hemocytometry method as described previously [29]. Briefly, after dilution of epididymal sperm to 1:20 in HTF medium, approximately 10 μL of this diluted specimen was transferred to each of the counting chambers of the hemocytometer, which was allowed to stand for 5 min in a humid chamber to prevent drying. The cells sedimented during this time and were counted with a light microscope at 400×. The sperm count was expressed as the number of sperms per milliliter.

2.3. Testicular germ cells isolation

According to previous studies [23,24], the harvested testes were decapsulated by two pairs of iris forceps and mechanically digested by multiple aspirations through pipette tips (eight aspirations through 2-mm diameter tips followed by 10 aspirations through 1-mm diameter tips) into a 50-mL syringe following the addition of 20 mL PBS. Mechanical digestion was continued until the tubules were completely dissociated. Thereafter, the tubules were allowed to settle by gravity and washed three times with PBS. Supernatants containing the interstitial cells were discarded. The tubules were transferred to a 50 mL culture flask and subjected to digestion in saline containing collagenase type V (1 mg/mL) and DNAse (1 mg/mL) for 25 min in a shaking water bath (120 cycles per min at 37 °C). The suspension was then aspirated three times with a pipette and incubated for an additional 5 min in a 37 °C shaking water bath. This isolation procedure resulted in a single cell suspension consisting of Sertoli cells and germ cells as well as an eligible number of peritubular cells. Finally, the cell suspension was purified, by panning which exploits the fact that Sertoli cells will adhere to the surface of the plastic culture dish while germ cells remain in suspension.

2.4. Mice immunization

60 female BALB/c mice were purchased from the Pasteur Institute of Tehran, Iran. After one-week acclimatization, the mice were randomly divided into five groups (n = 15). All the mice were five to six weeks old (19 ± 2). Mice of group 1 (no-treatment) were subcutaneously (s.c) injected with 100 μL PBS. Mice of group 2 (BCG) were s.c injected with 2×10^6 BCG cells alone. Both TGCs and sperm cells were mechanically macerated in sterile PBS by a homogenizer. Mice of group 3 (Testicular germ cells (TGCs) + BCG) were s.c immunized with 2×10^6 TGCs + 2×10^6 BCG. Mice of group 4 were s.c immunized by a mix of 2×10^6 sperms + 2×10^6 BCG cells. Each mouse at each group underwent three times of injections with one-week intervals. The immunization schedule is illustrated in Table 1.

2.5. Breast cancer cells culture

Murine mammary carcinoma cell line (4T1) was purchased from Pasteur Institute of Tehran, Iran. The cells were cultured in RPMI 1640 medium (Sigma-Aldrich, Germany) containing 10% fetal bovine serum (FBS) (Sigma-Aldrich, Germany) and 1% antibiotics mixture containing penicillin (Sigma-Aldrich, Germany) and streptomycin (Sigma-Aldrich,

Table 1
Immunization schedule of the groups.

Groups	1st injection	2nd injection	3rd injection
1 (n = 15)	PBS	PBS	PBS
2 (n = 15)	BCG	BCG	BCG
3 (n = 15)	TGCs + BCG	TGCs + BCG	TGCs + BCG
4 (n = 15)	Sperm + BCG	Sperm + BCG	Sperm + BCG

PBS: phosphate buffer solution, BCG: Bacillus Calmette-Guerin, n: number of mice per group, testicular germ cells: TGC.

Germany) was added to the final solution. The cells were incubated in a humidified incubator at 37 °C in a 5% CO_2 atmosphere.

2.6. Breast tumor implantation

One week after the last injection of the immunization process, 4T1 cancer cells were harvested from culture flasks by trypsin (Sigma, USA) and washed three times with PBS. 10^6 cells which were suspended in 50 μL serum-free DMEM-F12 (Sigma, USA) were s.c injected at the fourth mammary fat pad of each mouse. The mice were monitored daily for the growth of implanted cancer cells and tumor formation. When the tumors became palpable, in order to determine tumors' volume, the greatest longitudinal diameter (length) and the greatest transverse diameter (width) of the tumors were determined every 4 days. Tumors' volume based on caliper measurements were calculated by the following Eq. (1):

$$\text{Tumor volume} = \text{Length} \times \text{Width}^2 \times 0.52 \quad (1)$$

2.7. Metastasis analysis

40 days after the cancer cells implantation, seven mice from each group were sacrificed and their lungs and liver were harvested. Then, lungs and livers were fixed in 10% formalin neutral buffer solution and embedded in paraffin. In the next step, dehydration was done and tissues were blocked. Thin sections about 5 μm were prepared and stained by hematoxylin and eosin (H&E). Histological photographs were obtained using a digital light microscope (Olympus, Japan) to evaluate the metastatic colonies.

2.8. Statistical analysis

Statistical analyses and graphics were performed using JMP 11.0. All data, including tumors volume, and metastatic colonies were compared between the different groups by One-way ANOVA by Tukey's *post hoc* tests. Survival times were analyzed by Kaplan-Meyer using log-rank test. Statistical significance was set at $P < 0.05$ (*: $P < 0.05$, ns: not significant).

3. Results

3.1. Effect of testicular germ cells and sperm cells vaccination on the 4T1 breast tumors' growth

The testicular germ cells and sperms were isolated from male BALB/c mice and homogenized to be used as the source of CTAs for anti-tumor immunization. One group (n = 8) of female BALB/c mice were immunized with 2×10^6 TGCs + 2×10^6 BCG cells. The other treatment group (n = 8) was vaccinated by 2×10^6 sperms + 2×10^6 BCG cells. The BCG was used as the immunoadjuvant for attracting the immune cells to the site of injection and activating the immune system. Also, to identify the effect of BCG on the tumors' growth, one group of mice (n = 8) were injected with BCG alone. Another group of mice didn't undergo any treatment and were injected just with PBS to be used as the no-treatment group. The immunization process contained three times of injection with one-week intervals (Table 1). One week after the last injection of the immunization process, the 4T1 breast cancer cells were s.c implanted. As Fig. 1 illustrated, the BCG injection *per se* didn't cause significant inhibition in the tumors' growth in comparison with the no-treatment group ($P > 0.05$). However, the TGCs + BCG and Sperm + BCG immunized mice exhibited significant ($P < 0.05$) inhibition of the tumors' growth in comparison with both the BCG and no-treatment groups. Also, significant ($P < 0.05$) difference was observed between the TGCs + BCG ($259.7 \pm 39\,mm^3$) and Sperm + BCG ($426 \pm 52\,mm^3$) immunized mice tumors' volume. Therefore, the TGCs + BCG immunized mice had the smallest tumors in comparison with all groups.

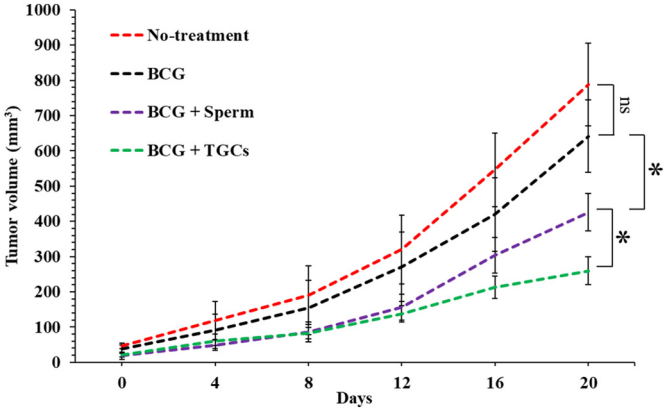

Fig. 1. The tumors' growth progression at different groups (n = 8). The tumors' volume was calculated every 4 days (*: $P < 0.05$, ns: not significant).

3.2. Effect of testicular germ cells and sperm cells vaccination on the formation of metastatic colonies at the vital organs

Metastasis is the main cause of cancer patients' death [25]. Therefore, metastasis evaluation was one of the main axes of our study. The metastatic burden at the vital organs of mice was investigated 40 days after the cancer cells implantation (Fig. 2). Histopathological evaluations demonstrated that the Sperm + BCG and TGCs + BCG groups exhibited a significantly lower number of metastatic colonies at lungs (Fig. 2A) and livers (Fig. 2B) of the tumor-bearing mice. The Sperm + BCG and TGCs + BCG vaccinations could significantly decrease the metastatic colonies at the tumor-bearing mice liver in comparison with BCG and no-treatment groups (Fig. 2C–F). Therefore, activation of the immune response against testicular germ cells and sperm cells' antigens can significantly inhibit metastasis of the 4T1 breast tumor.

3.3. Effect of testicular germ cells and sperms cells vaccination on the 4T1 breast tumor-bearing mice survival time

The mice survival curves are illustrated in Fig. 3. The mean survival time for no-treatment and BCG groups (n = 8) was 49.8 ± 8 and 50.5 ± 10 days, respectively. However, significantly higher survival time was observed for the BCG + Sperm (64.6 ± 7 days) and BCG + TGCs (79.6 ± 12 days) immunized mice. Therefore, utilizing from the sperms and TGCs as the sources of antigens for anti-tumor immunization can significantly increase 4T1 breast tumor-bearing mice survival time.

4. Discussion

The main goal of anti-cancer vaccination is to introduce the tumor-associated antigens to the immune system for activating an effective anti-tumor immune response [26–28]. After the antigens' inoculation, the dendritic cells (DC) internalize the antigens by phagocytosis. Then

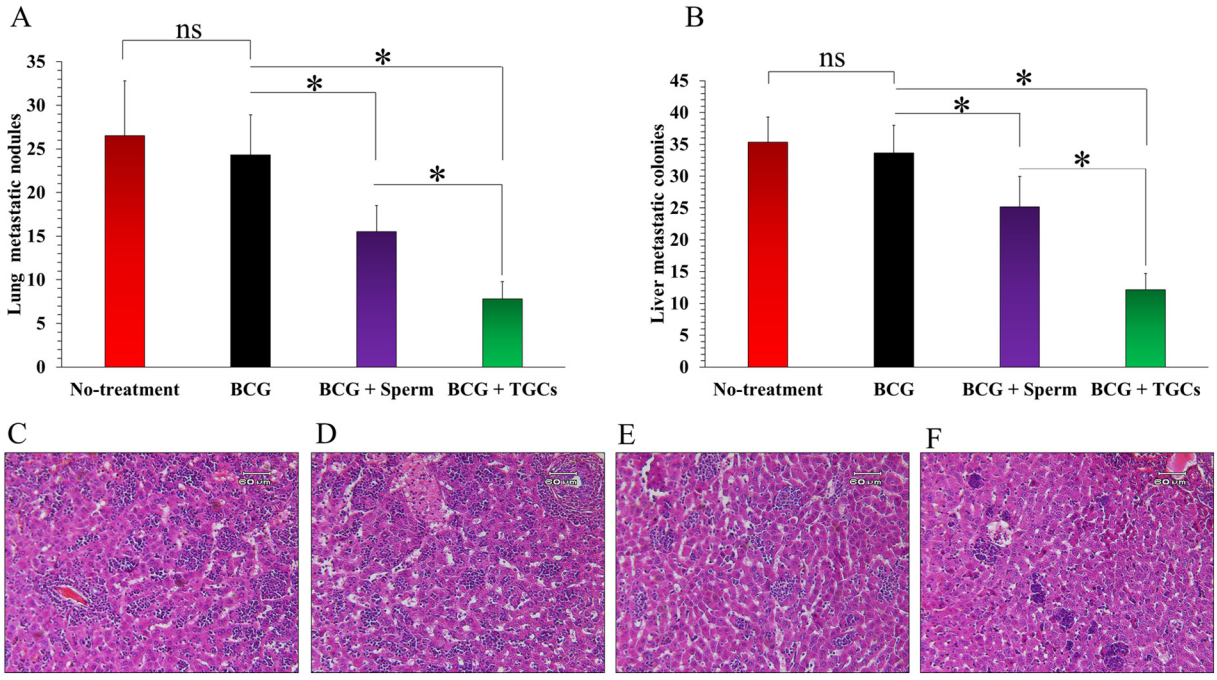

Fig. 2. Evaluation of the metastasis formation at the vital organs (liver and lungs) of the tumor-bearing mice at different groups (n = 7), 40 days after the cancer cells implantation (*: $P < 0.05$, ns: not significant). Metastatic colonies formation at (A) the lungs and (B) livers of the tumor-bearing mice at different groups. The photograph of the H&E stained liver sections of (C) the no-treatment, (D) BCG, (E) BCG + Sperm, (F) BCG + TGCs groups.

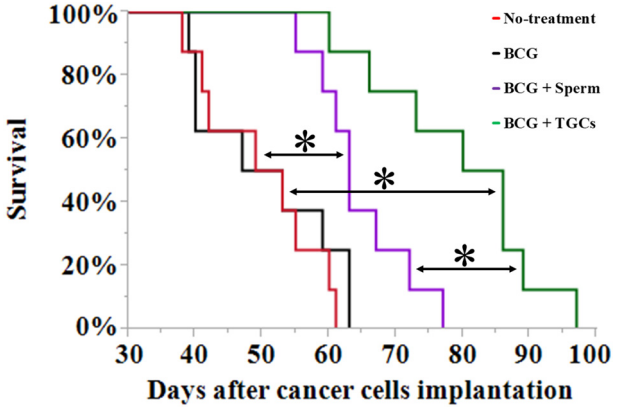

Fig. 3. The survival curves of tumor-bearing mice at different groups (n = 8) (*: $P < 0.05$, ns: not significant).

after, they maturate and differentiate for presenting the cancer antigens to naive CD4+ T helper cells and naive CD8+ cytotoxic T cells in lymph nodes. These cells will proliferate and attack the cancer cells at the primary and metastatic sites [29]. Also, the DCs can activate the NK cells. The activated NK cells will recognize and eradicate the target cancer cells [30].

Presentation of appropriate antigens to the immune system can deeply affect the anti-tumor vaccination efficacy. However, tumor heterogeneity is a considerable challenge in designing effective cancer vaccines. Tumor heterogeneity means that the tumor consists of different subclones with different properties which are associated with the expression of different patterns of antigens [11,31]. Vaccination against a single antigen only can target a limited number of the tumor's subclones. Also, the targeted antigen can be hidden by the cancer cells' due to antigen editing or poor antigen presentation of the cancer cells [32,33]. Therefore, targeting multiple cancer-associated antigens enhance vaccination efficacy [34].

Therefore, different sources of antigens have been introduced for anti-cancer immunization. Whole tumor cell vaccination is a promising method to present a vast variety of tumor's antigens to the immune system [35]. However, the low immunogenicity of tumor cell materials is the main pitfall of this method. Also, the reproducing of these vaccines is costly and time-consuming. Moreover, the availability and quality of the tumor cells' materials are the other big challenges against this vaccination approach [36]. Therefore, finding rich sources of antigens with high immunogenicity is one of the main current challenges for anti-cancer vaccination.

Testicular germ cells express many antigens which can be detected at the cancer cells. This restricted expression at the testicular germ cells and malignant cells caused to name them as cancer/testis antigens [37]. Among the different types of tumor antigens, CTAs represent highly promising therapeutic targets due to high immunogenicity and restricted expression at the cancer cells. In healthy adults, most of the CTAs just can be detected at the testicular germ cells and sperms [5]. However, CTA expression is not completely restricted to the cancer cells or testis and some of them can be detected in normal tissues including the pancreas, liver, spleen, normal lymphocytes, and somatic ciliated epithelia [38–40]. For example, SP17 antigen as a CTA was detected in human somatic ciliated epithelia which caused raise of some concerns for anti-SP17 immune therapy [39,41,42]. On this basis, it has been hypothesized that SP-targeted vaccines could cause off-target effects, such as malabsorption and/or respiratory infections. So far, the results of a phase I clinical trial with SP17-pulsed dendritic cells exhibited significant anti-tumor immune responses and no major side effects. The anti-SP17 humoral response was not associated with evident side effects. This observation is the same as what happens in the vasectomized men, that although SP17 auto-antibodies can be detected in their blood, it do not produce any toxicity [43,44].

The testis is an immune-privileged site which causes the decrease or absence of immune tolerance to CTAs. Therefore, the immune system will recognize CTAs as neoantigens when expressed in cancer cells and can activate a strictly tumor-specific immune response [45]. Immune responses against CTAs are frequently observed in cancer patients, and there is an association between CTAs expression and cytolytic activity of tumor immune infiltrates [46,47]. Thus, CTAs represent the promise of highly specific immune targeting of a wide range of human cancers.

The other advantage of CTAs is their determinate role in the cancer cells' properties. Multiple CTAs have been shown to support cancer cells' growth, proliferation, and metastasis. In addition, inhibiting of these CTAs activity can significantly affect the cancer cells' capacity to proliferate [37]. CTAs are more frequently express at metastatic colonies. An important feature of cells with metastatic capability is increased motility and invasive potential. Interestingly, several CT antigens, including MAGE-C2, GAGE, XAGE1, CAGE, and CT45A1, have been demonstrated to enhance both phenotypes [48–50].

Selection of appropriate animal models is critical in cancer vaccine research. The 4T1 is a transplantable tumor cell line that has several characteristics that make it a suitable experimental animal model for breast cancer. Unlike most *in vivo* tumor models, the 4T1 cancer cells are implanted at the anatomical correct site of breast tumor. Also, it can highly invasive and can spontaneously metastasize to multiple distant organs and its metastatic distribution exhibits high similarity with human mammary cancer [51]. In addition, 4T1 cells express different CTAs including MAGE-B [52], and BORIS [52] and many other CTAs [53].

In this study, TGCs and sperm cells were utilized as rich sources of antigens for anti-breast cancer vaccination against 4T1 tumors. BCG was used as the immunoadjuvant agent. At the last day of measuring tumors' diameters, it was apparent that the TGCs + BCG ($259.7 \pm 39\,mm^3$) and Sperm + BCG ($426 \pm 52\,mm^3$) vaccinations caused about 67% and 45% decrease in the 4T1 breast tumors' volume in comparison with no-treatment group ($788.1 \pm 117\,mm^3$). Also, TGCs + BCG and Sperm + BCG vaccinations caused significant ($P < 0.05$) decrease in the formation of lungs and liver's metastatic colonies in comparison with the control groups. In addition, TGCs + BCG and Sperm + BCG immunizations caused about 29 and 14 days increase of the 4T1 breast tumor-bearing mice survival time. Also, the mean life span of the TGCs + BCG immunized mice were 15 days more than the Sperm + BCG group. Therefore, both testicular germ cells and sperms can be used as the appropriate sources of antigens for anti-breast cancer vaccination. Although TGCs + BCG vaccination exhibited better therapeutic effect, their isolation is extremely harder than sperms. Therefore, one of the main advantages of utilizing sperm is its easier isolation and better availability in comparison to TGCs. However, TGCs exhibit a significantly higher number of CTAs with higher intensity in compassion with sperm. Several CTAs are expressed in TGCs during spermatogenesis process. For example, spermatogonia express MAGE-A, GAGE, NY-ESO-1, SSX, SAGE1, NXF2, TDRT1.TES15. Also, spermatocytes exhibit different CTAs including SCP1, ADAM2, SPO11, TSP50, BORIS, TPTE, LDHC, TPX1 during meiosis. The spermatozoa which are more differentiated than spermatogonia and spermatocytes, express SPANX, ADAM2, SP17, ACRBP, CAGE, AKAP4, ZNF645, and TPTE which are well-known CTAs [5,17,54]. In addition, previous studies have hypothesized that sperm fibrous sheath proteins may be appropriate targets for developing successful cancer vaccines due to the expression of two sperm fibrous sheath proteins including sperm protein 17 (SP17) and calcium-binding tyrosine-phosphorylation (CBYR) regulated protein, in the tumors of unrelated histological origin and their capability to induce T cell-based immune responses [20,55–62]. Also, some of these antigens or their related antibodies can be used as diagnostic and prognostic cancer biomarkers. Gupta et al. have shown that circulating levels of anti-SP17 antibodies were significantly elevated in esophageal squamous cell carcinomas patients when compared with normal subjects [63–65].

These CTAs function in the cancer cells have been demonstrated. The MAGE-A, MAGE-B, MAGE-C, GAGE, PAGE4, and CAGE expression cause apoptosis resistance. Also, MAGE-C2, GAGE, XAGE1, CAGE, CT45 A1 can acceleration metastases. More therapeutic efficacy of the TGCs + BCG vaccination can be attributed to the expression of higher numbers and intensity of CTAs in comparison to sperm [5,17,54].

Many more *in vitro* and *in vivo* experiments in different cancer models are needed for evaluation of this anti-cancer immunization approach in different aspects. It is obvious that testicular germ cells isolation, purification, and expansion is considerably more time and cost consuming than sperm isolation and purification. Also, germ cells donation needs more invasive procedures in comparison with sperm donation and patients may exhibit better compliance for sperm donation than germ cells donation. Therefore, although testicular germ cells immunization exhibited better therapeutic effect in comparison with sperm immunization according to this study, utilizing from sperms immunization may receive more attention for clinical trials. It's better to utilize from close family sperms donation to avoid significant protein polymorphism. In our idea, this anti-cancer immunization approach can be more effective if be used as a therapeutic vaccination approach after surgical resection of the primary tumor mass, the same as OncoVax vaccination approach. But sperm or germ cells will be used instead of autologous tumor cells [66]. After resection of the primary tumor, this vaccination approach may inhibit tumor recurrence and metastasis formation.

5. Conclusions

CTAs have gained lots of attention as targets of anti-tumor immune therapy. However, the therapeutic efficacy of the CTAs single-antigen vaccines is not satisfying due to the tumor heterogenicity. Therefore, many studies have focused on the enhancement of their efficacy by utilizing rich sources of tumor-associated antigens for anti-cancer vaccination. In the present study, the testicular germ cells and sperm cells as well-known sources of cancer/testis antigens were investigated for anti-4T1 breast cancer vaccination in BALB/c mice. Significant inhibition of 4T1 breast tumors' growth and metastasis was observed by both TGCs + BCG and Sperm + BCG vaccinations in comparison with control groups. Also, the TGCs + BCG vaccination exhibited a better therapeutic effect in comparison with Sperm + BCG group, which can be attributed to the higher number of CTAs expression by germ cells in comparison with sperm. However, sperm cells are more available than TGCs and their isolation is more time and cost consuming especially in the human. Therefore, both TGCs and sperms can be appropriate sources of antigens for anti-cancer vaccination. However, more investigations are needed.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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