WEP Warwick Evans Publishing

Transactions on Materials, Biotechnology and Life Sciences
ISSN: 2960-1932, eISSN: 2960-2319 | Volume 6
FBSE 2024

Construction of DNA Vaccine of Extracellular Region of Vascular Endothelial Growth Factor Receptor 2 and Its Antitumor Activity in Vivo

Yao Sang [1, *]

School of Life Sciences, Jilin University, Changchun City 130012, China

*Corresponding author: Yao Sang, e-mail: 13309173169@163.com

Abstract: Introduction: it constructed recombinant DNA vaccine of extracellular region of vascular endothelial growth factor receptor 2 (VEGFR2) and analyzed its in vivo activity against H22 transplanted hepatoma. Methods: the gene fragment of VEGFR2 extracellular region 1-3 was amplified, and the pcDNA3.1(+)/exVEGFR2 recombinant plasmid was constructed. The expression of exVEGFR2 was detected by Western blot. Subsequently, liposome (LP) pcDNA3.1(+)/exVEGFR2 complex (LP-pcDNA3.1(+)/exVEGFR2) was prepared and immunized to C57BL/6 mice. The cytotoxic activity mediated by cytotoxic T cells (CTLs) was analyzed by 51Cr release assay. H22 tumor bearing C57BL/6 mouse model was prepared. Quadriceps femoris muscle was injected with normal saline (saline group, AG), LP-pcDNA3.1(+) 100 μL (1 μg/μL, LP-pcDNA3.1(+) group, BG), and LP-pcDNA3.1(+)/exVEGFR2 100 μL (1 μg/μL, LP-pcDNA3.1(+)/exVEGFR2 group, CG). Tumor growth and death were recorded, and microvessel density (MVD) was evaluated by CD31 immunohistochemical staining. Results: a 1252 bp of exVEGFR2 sequence was amplified, and a specific band expressing 44 kDa molecular weight was constructed by transfecting pcDNA3.1(+)/exVEGFR2 into COS-7 cells. After immunizing C57BL/6 mice for 6 weeks, CTLs experiment suggested that LP-pcDNA3.1(+)/exVEGFR2 could effectively mediate the toxic effect of VEGFR2 positive H22 cells. The in vivo antitumor activity experiment found that as against AG and BG, the tumor volume, weight, and MVD were markedly reduced, the tumor latency time was markedly prolonged, and the average survival time was also markedly prolonged in CG ($P < 0.05$). Conclusion: LP-pcDNA3.1(+)/exVEGFR2 can effectively activate C57BL/6 mice to produce specific anti-VEGFR2 immune response, and then produce anti-tumor cell immunotoxicity in vitro and in vivo. Inhibiting angiogenesis can effectively prolong the survival time of H22 tumor bearing mice.

Key words: VEGFR2; extracellular region; DNA vaccines; antitumor; angiogenesis; survival

1. Introduction

Malignant tumors are diseases caused by malignant proliferation of cells, with aggressive and metastatic characteristics [1]. The occurrence of malignant tumors is related to a series of abnormal gene changes triggered by the stimulation of chemical, physical, viral, and other factors and long-term joint action [2]. The growth, invasion, and metastasis of malignant tumors almost depend on neovascularization to provide adequate nutrition [3]. Therefore, inhibiting or destroying tumor angiogenesis (anti-tumor angiogenesis therapy), is one of the hot spots in oncology research.

At present, the known pro angiogenic factors related to tumor angiogenesis include vascular endothelial growth factor (VEGF), transforming growth factor (TGF), platelet derived growth factor (PDGF), etc., and the anti angiogenic factors include angiostatin, endostatin, platelet factor 4, etc. [4-6]. VEGF is the most widely studied angiogenesis related factor. VEGFR2 is the cell surface receptor of VEGF. VEGF can bind with VEGFR2 and form receptor dimer, and participate in angiogenesis, vascular development, vascular permeability, and other functions after activating downstream signaling pathways [7]. Blocking VEGFR2 signaling pathway can effectively inhibit tumor growth [8]. Inducing the immune response against VEGFR2 and inhibiting the expression of VEGFR2 through some way is one of the effective and economical methods in the potential anti-tumor angiogenesis therapy. LP vaccine is a new vaccine drug formulation developed in recent years. LP loaded DNA or mRNA vaccine delivery system has the characteristics of strong targeting, high

encapsulation efficiency, and excellent cell affinity [9]. Majzoub et al. (2014) [10] suggested that the complex constructed by DNA plasmid and cationic LP can make up for the defect of simple DNA vaccine immune activation, and can also activate innate immune activity in the body to play an anti-tumor role. However, there are relatively few studies on the preparation of LP VEGFR2 extracellular region complex DNA vaccine.

In this article, LP VEGFR2 extracellular 1~3 DNA vaccine was prepared, and its immune activation and antitumor activity in vivo and in vitro were tested. It provides reference for the development and application of new therapeutic vaccines for anti-tumor angiogenesis therapy.

2. Materials and methods

2.1. Materials and reagents

C57BL/6 mice (Suzhou Cyagen Biotechnology Co., Ltd., China); Trizol reagent, radio immunoprecipitation assay (RIPA), DNA connection kit (Thermo Fisher Scientific, China); DNA reverse transcription kit (Solarbio, USA); pcDNA3.1(+) vector (Shanghai North Connaught Biotechnology Co., Ltd., China); DH5α competent cells (Shanghai Sigma Aldrich, China); COS-7 cells and H22 cells (ATCC, USA); Bicinchoninic acid (BCA) assay protein concentration detection kit and enhanced chemiluminescence (ECL) kit (Shanghai Beyotime Biotechnology Co., Ltd., China); RPMI-1640 culture medium (Gibco, USA); CD31 polyclonal antibody and exVEGFR2 polyclonal antibody (Santa Cruz Biotechnology, USA).

2.2. Construction and identification of pcDNA3.1(+)/exVEGFR2

Total RNA was extracted from spleens of 14 d C57BL/6 mice under sterile conditions by Trizol method, and cDNA library was synthesized and constructed by DNA reverse transcription kit. The amplification primers of the extracellular region 1-3 of VEGFR2 were designed. The upstream primer was 5'-CGGATAACCTGGCTGACC-3', and the downstream primer was 5'-AGGGATTCGGACTTGACTGC-3'. The amplified product was electrophoresed on 1% agarose gel, and the target fragment was recovered by cutting the gel. The amplified product was connected with pcDNA3.1(+) empty plasmid by using DNA connection kit to obtain pcDNA3.1(+)/exVEGFR2 recombinant plasmid. Subsequently, the pcDNA3.1(+)/exVEGFR2 recombinant plasmid was transformed into DH5α competent cells, and the cells were routinely plated for culture. The positive monoclonal colonies were picked out and propagated in LB medium, and then sequenced for identification.

2.3. Detection of pcDNA3.1(+)/exVEGFR2 expression

According to the LP transfection method, pcDNA3.1(+)/exVEGFR2 recombinant plasmid and pcDNA3.1(+) empty plasmid were transfected into COS-7 cells, and normal COS-7 cells were used as negative control. After transfection, the cells were cultured at 37℃ and 5% CO2 for 72 h. The cells were collected and RIPA lysate was added to extract total protein. The protein concentration was determined by BCA method. 50 μg protein was subjected to gel electrophoresis, transferred to polyvinylidene fluoride membrane by wet transfer method, and blocked with 5% skimmed milk powder overnight. VEGFR2 extracellular region 1-7 (1:200) and GAPDH (1:1000) primary antibodies were added, incubation overnight at 4℃. HRP labeled secondary antibody (1:1000) was applied, incubation at 37℃ for 1 h. After washing the membrane, ECL chemiluminescence staining was performed for development under a gel imaging system, with GAPDH as a control.

2.4. Preparation of LP LP-pcDNA3.1(+)/exVEGFR2

Lecithin, cholesterol, and C18H38 were mixed in a ratio of 5:5:2 to 100 mL chloroform solution, which was rotated and evaporated under reduced pressure at 200 r/min and 30℃. When it appeared as thin films, it was dried under reduced pressure and vacuum for 15 min. The film was dissolved in

ultrapure water at 50℃ for 45 min, and then placed in a water bath at 35℃ for 10 min. After sealing the film, it stood at room temperature overnight. After 15 min of phacoemulsification, 0.22 and 0.10 μm filters were used for filtration three times. LP and pcDNA3.1(+)/exVEGFR2 recombinant plasmids were mixed at 3:1 (w/w) to obtain LP-pcDNA3.1(+)/exVEGFR2, which was dissolved in 5% glucose solution for future use.

2.5. CTLs experiment

Thirty 8-week-old female C57BL/6 mice were randomly divided into AG, BG, and CG, with 10 mice in each. Mice in AG, BG, and CG were immunized with 20 μg/w 0.9% sodium chloride, LP-pcDNA3.1(+), and LP-pcDNA3.1(+)/exVEGFR2 intramuscularly for 6 times. After continuous immunization for 5 weeks, spleen cells of mice were collected as effector cells, mixed with VEGFR2 positive H22 target cells labeled with Na251CrO4, and seeded in a 96-well plate. 0.1 mL target cells + 0.1 mL complete medium were as the control release well, and 0.1 mL target cells + 0.1 mL 2% SDS were as the maximum release well, incubation at 37℃ and 5% CO2 for 4 h. 100 μL supernatant was applied to detect the absorbance of each well, and the specific lysis was calculated according to the following equation.

$$\text{Specific lysis (\%)} = (\text{release well of experimental group} - \text{control release well})/(\text{maximum release well} - \text{control release well}) \times 100\% \quad (1)$$

2.6. In vivo anti-tumor activity detection

(1) Tumor bearing model construction and grouping

The ascites of 8-week-old female C57BL/6 mice was extracted under sterile conditions, and the cells were made into 5 × 107/mL suspension with RPMI-1640 culture medium, and then 0.2 mL H22 cell suspension was subcutaneously injected into the right chest of mice at 2 h to prepare 30 tumor bearing mouse models. The tumor bearing mouse models were randomly divided into AG, BG, and CG, with 10 mice in each. Mice in AG, BG, and CG were injected intramuscularly with 100 μL of 0.9% sodium chloride, 100 μL 1 μg/μL of LP-pcDNA3.1(+), and LP-pcDNA3.1(+)/exVEGFR2 at the muscular fat of quadriceps muscle, respectively, for 10 d/time, and were immunized for 3 times. The size and volume of tumors were detected by vernier caliper, and the survival time of mice was recorded. After death, tumors were separated and weighed.

(2) Tumor MVD detection

The mouse tumor tissues were fixed and paraffin sections were made. After dewaxing, they were incubated with 3% H_2O_2 for 5 min at 25℃, rinsed with distilled water, and soaked in phosphate buffer (PBS) for 5 min before antigen retrieval. 10% goat serum was blocked for 10 min at 25℃, and CD31 (1:1000) primary antibody was added dropwise, incubation overnight at 4℃, and rinsing with PBS for 3 times × 5 min. Biotin labeled secondary antibody was added dropwise, incubation for 30 min at 25℃, rinsing with PBS for 3 times × 5 min. HRP labeled streptavidin was added dropwise, incubation for 30 min at 25℃, rinsing with PBS for 3 times × 5 min. After DAB chromogenic treatment, it was rinsed with tap water, counterstained with hematoxylin, and sealed with neutral gum. The staining was observed under a light microscope, and five fields were randomly selected for MVD counting.

2.7. Statistical treatment

SPSS 23.0 software was employed. Data were expressed by (mean ± sd) or %, and t-test or chi-square test were adopted. When $P < 0.05$, it was considered statistically meaningful.

3. Results

3.1. VEGFR2 extracellular region 1-3 amplification and recombinant plasmid verification

The 1252 bp gene fragment in the extracellular region 1-3 of VEGFR2 was amplified. After agarose gel electrophoresis and detection, a specific product with a length of about 1200 bp was found. The recombinant plasmid pcDNA3.1(+)/exVEGFR2 was constructed and verified by double digestion and electrophoresis. A specific digestion product of about 1200 bp was also found and verified by sequencing (Figure 1).

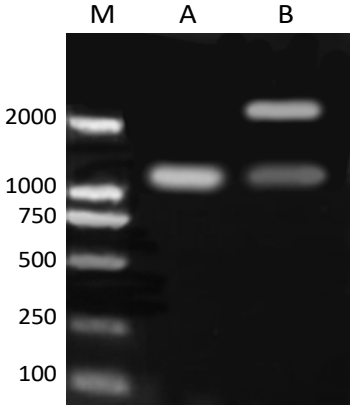

Figure. 1 Verification of agarose gel electrophoresis detection.

Note: M is a DNA marker with a length of 2000 bp; A is the verification of amplification products of VEGFR2 extracellular region 1-3; B is the verification of the double digestion product of pcDNA3.1(+)/exVEGFR2 recombinant plasmid

3.2. Expression verification of LP-pcDNA3.1(+)/exVEGFR2 recombinant plasmid

The expression of VEGFR2 extracellular region 1-3 in COS-7 cells was detected, and it was found that there was no expression of VEGFR2 extracellular region 1-3 in negative control group and BG. However, in the CG, the specific protein expression of VEGFR2 extracellular region 1-3 was about 44 kDa in length (Figure 2).

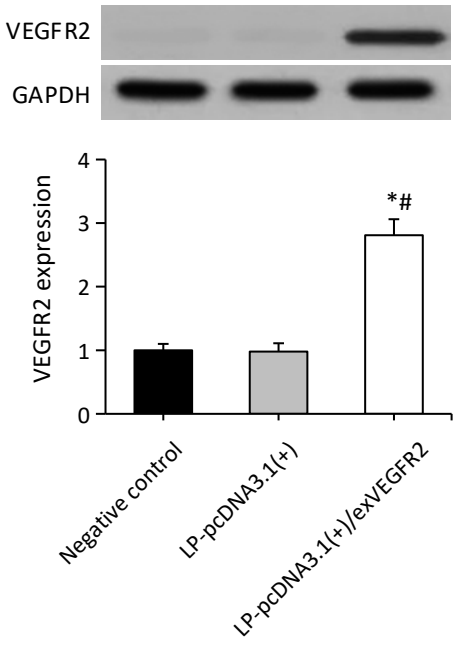

Figure. 2 Verification of VEGFR2 extracellular region 1-3 protein expression.

Note: * as against negative control group, # as against BG, $P < 0.05$

3.3. CTLs validation of LP-pcDNA3.1(+)/exVEGFR2

The release of cytotoxic ^{51}Cr mediated by CTLs from spleen cells of C57BL/6 mice after immunization was detected. T cells in AG and BG induced VEGFR2 positive H22 cytotoxic effect, and ^{51}Cr release did not change markedly in different T cells target effector cell ratios. The ^{51}Cr release in CG increased with the increase of target effector cell concentration (1-64 times), and was markedly greater than that in AG and BG ($P < 0.05$) (Figure 3).

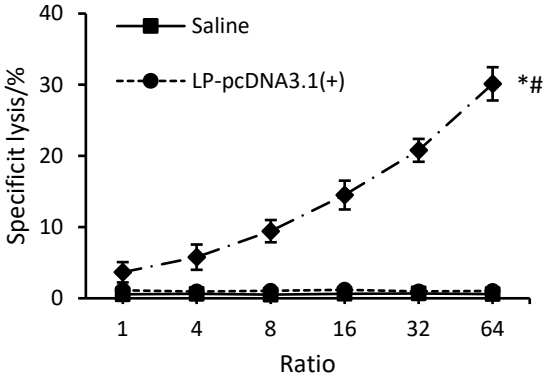

Figure. 3 Experimental results of CTLs in vivo.

Note: * as against AG, # as against BG, $P < 0.05$

3.4. Validation of in vivo antitumor activity of LP-pcDNA3.1(+)/exVEGFR2

The tumor growth of C57BL/6 tumor bearing mice after immunization was compared. With the extension of tumor bearing time, the tumor volume of mice in AG, BG, and CG gradually increased. There was no visible difference in tumor volume between AG and BG, while that of CG was markedly smaller as against AG and BG ($P < 0.05$). Meanwhile, as against AG and BG, the tumor mass was markedly reduced, and the tumor latency was markedly prolonged in CG ($P < 0.05$) (Figure 4).

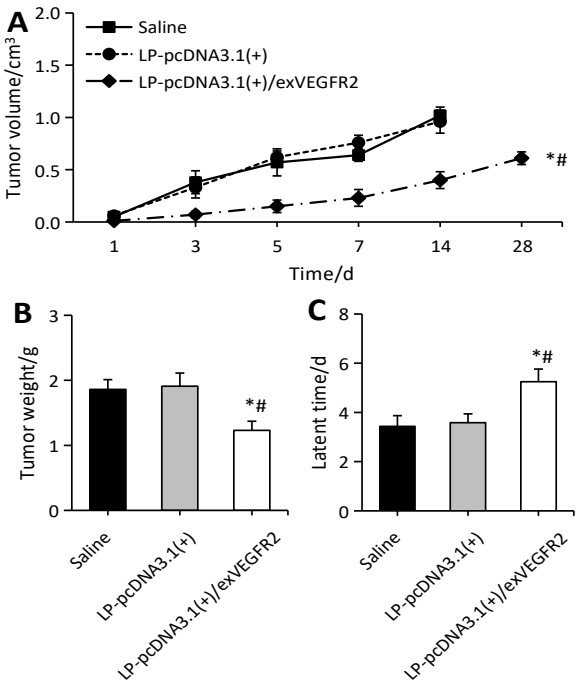

Figure. 4 Comparison of tumor growth in tumor bearing mice.

Note: A: time-tumor volume curve; B: tumor weight; C: tumor latency; * as against AG, # as against BG, $P < 0.05$

The tumor MVD of C57BL/6 tumor bearing mice after immunization was compared. There was not clearly different in tumor MVD between AG and BG, while the tumor MVD of CG was markedly smaller than that of AG and BG ($P < 0.05$) (Figure 5).

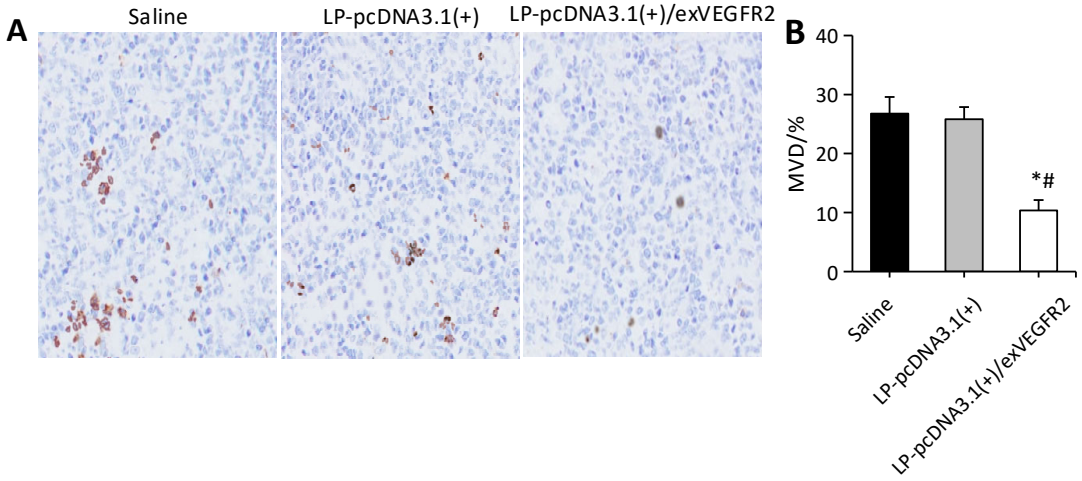

Figure. 5 Tumor MVD in tumor bearing mice.

Note: A: CD31 immunohistochemical staining observation, × 200; * as against AG, # as against BG, $P < 0.05$

The survival of C57BL/6 tumor bearing mice after immunization was compared. The average survival time of mice in AG, BG, and CG were (14.1 ± 1.6) d, (14.5 ± 1.3) d, and (25.1 ± 2.5) d, respectively. There was not obviously different in survival time between AG and BG, while the survival time of CG was markedly longer than that of AG and BG ($P < 0.05$) (Figure 6).

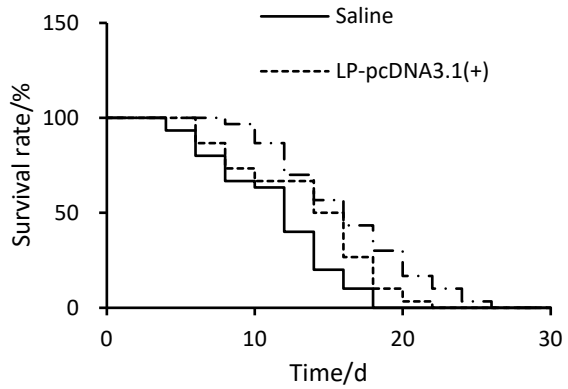

Figure. 6 Time-survival curve of tumor bearing mice.

4. Discussion

Anti-tumor angiogenesis therapy has become a research hotspot in the treatment of malignant tumor diseases. VEGF is a highly specific mitogen of vascular endothelial cells, which can selectively stimulate the proliferation and migration of vascular endothelial cells, and also promote angiogenesis and enhance vascular permeability [11]. Studies have confirmed that the expression levels of VEGF and its receptor VEGFR are abnormally elevated in 70% of metastatic malignant tumors, so it is an ideal tumor antigen target [12,13]. Bevacizumab is a class of synthetic anti VEGF monoclonal antibodies, which can block the VEGFA pathway. Studies have found that bevacizumab used in the treatment of lung cancer, metastatic breast cancer, colon cancer, and other malignant tumors can markedly improve the survival rate of patients and prolong the overall survival [14-16]. However,

bevacizumab and other treatment methods belong to passive immunotherapy, and there will be relatively obvious toxic and side effects in the treatment process, which has the characteristics of dose increasing effect and maintenance treatment, and the treatment cost is high, and there is a risk of drug resistance [17]. Therefore, vaccine active immunotherapy has become a hotspot of tumor treatment strategies. VEGFR2 is composed of extracellular region, transmembrane region, and intracytoplasmic tyrosine kinase region, and the extracellular region is responsible for recognizing and binding target antigens [18]. VEGF binds to the extracellular region of its receptor to form VEGFR2 dimers, activating downstream signaling [19]. In this article, the spleen was isolated from C57BL/6 mice and the extracellular region of VEGFR2 was amplified, and the pcDNA3.1(+)/exVEGFR2 recombinant plasmid was prepared. After transfection into COS-7 cells, the target protein with a molecular weight of 44kDa was successfully expressed.

Under normal circumstances, the body has immune tolerance to self antigens and will not produce immune response. When stimulated by cross antigens and non-specific immune cell stimulants, it can induce autoimmune reactions in the body and act on cells or tissues with target antigens [20]. The immune activity of exogenous DNA plasmid vaccine is low, so it can't effectively induce the body to generate sufficient immune response. LP DNA plasmid vaccine is a new type of vaccine drug dosage form that has attracted more attention at present [21]. Zhao et al. (2021) [22] prepared a novel LP-polymer hybrid nanoparticle pSFV-MEG/LNPs. After immunization, the humoral and cellular immune responses of pSFV-MEG/LNPs were 1.58 times and 1.05 times higher than pSFV-MEG. Luo et al. (2023) [23] prepared CpG-DNA multi targeted LP vaccine, which can fine tune the efficiency of ER targeted antigen delivery, regulate the presentation of exogenous antigen proteins through the histocompatibility complex I or II pathway, and can effectively stimulate humoral and cellular immune responses. Valentin et al. (2022) [24] suggested that LP nano formulations expressing HIV-1 Gag and Gag conserved regions can provide suboptimal priming of T cell responses as heterologous DNA enhanced vaccine regimens, and are preventive vaccines for immunotherapeutic intervention against HIV infection and other chronic diseases. In this article, LP-pcDNA3.1(+)/exVEGFR2 composite gene vaccine was prepared, and CTLs experiments confirmed that the vaccine could activate immune T cell-mediated tumor cell immune killing effect. To further understand the anti-tumor effect of LP-pcDNA3.1(+)/exVEGFR2, the H22 tumor bearing mouse model was prepared in this article. It was found that LP-pcDNA3.1(+)/exVEGFR2 could markedly inhibit tumor growth, reduce MVD, and prolong tumor latency and overall survival of mice after intramuscular injection of LP-pcDNA3.1(+)/exVEGFR2 into quadriceps femoris. In the tumor microenvironment, a variety of cells can secrete pro tumor angiogenic factors, among which VEGF and VEGFR can activate a variety of signaling pathways, including PI3K/AKT, MEK/MAPK, β-catenin/VE-cadherin, which promote endothelial cell proliferation, migration, and invasion, and enhance vascular permeability and promote vasculogenic mimicry [25-28]. The LP-pcDNA3.1(+)/exVEGFR2 vaccine can stimulate the specific immune activation effect in H22 tumor bearing mice and block the downstream signaling, thus inhibiting angiogenesis.

5. Conclusion

The LP-pcDNA3.1(+)/exVEGFR2 compound gene vaccine has specific immune activating effect and anti-tumor activity in vitro and in vivo. However, this article only analyzed the anti-tumor effect of the vaccine on H22 tumor bearing mice. In the future, it is necessary to further prepare various types of tumor bearing mouse models to explore the anti-tumor immune activity of LP-pcDNA3.1(+)/exVEGFR2. In conclusion, the results of this article can provide experimental basis for the development of anti-tumor vaccines.

References

[1] de Visser KE, Joyce JA. The evolving tumor microenvironment: From cancer initiation to metastatic outgrowth. Cancer Cell, 2023; 41(3): 374-403.

[2] Schwartz SM. Epidemiology of Cancer. Clin Chem, 2024; 70(1): 140-149.

[3] Gupta J, Tayyib NA, Jalil AT, Hlail SH, Zabibah RS, Vokhidov UN, Alsaikhan F, Ramaiah P, Chinnasamy L, Kadhim MM. Angiogenesis and prostate cancer: MicroRNAs comes into view. Pathol Res Pract, 2023; 248: 154591.

[4] Ahmad A, Nawaz MI. Molecular mechanism of VEGF and its role in pathological angiogenesis. J Cell Biochem, 2022; 123(12): 1938-1965.

[5] Vimalraj S. A concise review of VEGF, PDGF, FGF, Notch, angiopoietin, and HGF signalling in tumor angiogenesis with a focus on alternative approaches and future directions. Int J Biol Macromol, 2022; 221: 1428-1438.

[6] Li M, Popovic Z, Chu C, Krämer BK, Hocher B. Endostatin in Renal and Cardiovascular Diseases. Kidney Dis (Basel), 2021; 7(6): 468-481.

[7] Ozawa M, Ohtani H, Komatsuda A, Wakui H, Takahashi N. VEGF-VEGFR2 inhibitor-associated hyaline occlusive glomerular microangiopathy: a Japanese single-center experience. Clin Exp Nephrol, 2021; 25(11): 1193-1202.

[8] Ni H, Guo M, Zhang X, Jiang L, Tan S, Yuan J, Cui H, Min Y, Zhang J, Schlisio S, Ma C, Liao W, Nister M, Chen C, Li S, Li N. VEGFR2 inhibition hampers breast cancer cell proliferation via enhanced mitochondrial biogenesis. Cancer Biol Med, 2021; 18(1): 139-154.

[9] Christodoulides M, Humbert MV, Heckels JE. The potential utility of liposomes for Neisseria vaccines. Expert Rev Vaccines, 2021; 20(10): 1235-1256.

[10] Majzoub RN, Chan CL, Ewert KK, Silva BF, Liang KS, Jacovetty EL, Carragher B, Potter CS, Safinya CR. Uptake and transfection efficiency of PEGylated cationic liposome-DNA complexes with and without RGD-tagging. Biomaterials, 2014; 35(18): 4996-5005.

[11] Ahmad A, Nawaz MI. Molecular mechanism of VEGF and its role in pathological angiogenesis. J Cell Biochem, 2022; 123(12): 1938-1965.

[12] Mabeta P, Steenkamp V. The VEGF/VEGFR Axis Revisited: Implications for Cancer Therapy. Int J Mol Sci, 2022; 23(24): 15585.

[13] Morimoto Y, Tamura R, Ohara K, Kosugi K, Oishi Y, Kuranari Y, Yoshida K, Toda M. Prognostic significance of VEGF receptors expression on the tumor cells in skull base chordoma. J Neurooncol, 2019; 144(1): 65-77.

[14] West HJ, McCleland M, Cappuzzo F, Reck M, Mok TS, Jotte RM, Nishio M, Kim E, Morris S, Zou W, Shames D, Das Thakur M, Shankar G, Socinski MA. Clinical efficacy of atezolizumab plus bevacizumab and chemotherapy in KRAS-mutated non-small cell lung cancer with STK11, KEAP1, or TP53 comutations: subgroup results from the phase III IMpower150 trial. J Immunother Cancer, 2022; 10(2): e003027.

[15] Shepherd JH, Ballman K, Polley MC, Campbell JD, Fan C, Selitsky S, Fernandez-Martinez A, Parker JS, Hoadley KA, Hu Z, Li Y, Soloway MG, Spears PA, Singh B, Tolaney SM, Somlo G, Port ER, Ma C, Kuzma C, Mamounas E, Golshan M, Bellon JR, Collyar D, Hahn OM, Hudis CA, Winer EP, Partridge A, Hyslop T, Carey LA, Perou CM, Sikov WM. CALGB 40603 (Alliance): Long-Term Outcomes and Genomic Correlates of Response and Survival After Neoadjuvant Chemotherapy With or Without Carboplatin and Bevacizumab in Triple-Negative Breast Cancer. J Clin Oncol, 2022; 40(12): 1323-1334.

[16] Shen Y, Wang X, Lu J, Salfenmoser M, Wirsik NM, Schleussner N, Imle A, Freire Valls A, Radhakrishnan P, Liang J, Wang G, Muley T, Schneider M, Ruiz de Almodovar C, Diz-Muñoz A, Schmidt T. Reduction of Liver Metastasis Stiffness Improves Response to Bevacizumab in Metastatic Colorectal Cancer. Cancer Cell, 2020; 37(6): 800-817.e7.

[17] Li W, Zhou C, Yu L, Hou Z, Liu H, Kong L, Xu Y, He J, Lan J, Ou Q, Fang Y, Lu Z, Wu X, Pan Z, Peng J, Lin J. Tumor-derived lactate promotes resistance to bevacizumab treatment by facilitating autophagy enhancer protein RUBCNL expression through histone H3 lysine 18 lactylation (H3K18la) in colorectal cancer. Autophagy, 2024; 20(1): 114-130.

[18] Taheri FH, Hassani M, Sharifzadeh Z, Behdani M, Abdoli S, Sayadi M, Bagherzadeh K, Arashkia A, Abolhassani M. Tuning spacer length improves the functionality of the nanobody-based VEGFR2 CAR T cell. BMC Biotechnol, 2024; 24(1): 1.

[19] Luck R, Urban S, Karakatsani A, Harde E, Sambandan S, Nicholson L, Haverkamp S, Mann R, Martin-Villalba A, Schuman EM, Acker-Palmer A, Ruiz de Almodóvar C. VEGF/VEGFR2 signaling regulates hippocampal axon branching during development. Elife, 2019; 8: e49818.

[20] Hill A, Beitelshees M, Pfeifer BA. Vaccine Delivery and Immune Response Basics. Methods Mol Biol, 2021; 2183: 1-8.

[21] Weissig V. From Olive Oil Emulsions to COVID-19 Vaccines: Liposomes Came First. Methods Mol Biol, 2023; 2622: 1-19.

[22] Zhao Z, Ma X, Zhang R, Hu F, Zhang T, Liu Y, Han MH, You F, Yang Y, Zheng W. A novel liposome-polymer hybrid nanoparticles delivering a multi-epitope self-replication DNA vaccine and its preliminary immune evaluation in experimental animals. Nanomedicine, 2021; 35: 102338.

[23] Luo L, Luo Z, Wang L, Hu Y, Zhang J, Yin H, You J. Liposome Vaccine for Active Regulation of Cellular and Humoral Immunity. Mol Pharm, 2023; 20(11): 5668-5681.

[24] Valentin A, Bergamaschi C, Rosati M, Angel M, Burns R, Agarwal M, Gergen J, Petsch B, Oostvogels L, Loeliger E, Chew KW, Deeks SG, Mullins JI, Pavlakis GN, Felber BK. Comparative immunogenicity of an mRNA/LNP and a DNA vaccine targeting HIV gag conserved elements in macaques. Front Immunol, 2022; 13: 945706.

[25] Wang HJ, Ran HF, Yin Y, Xu XG, Jiang BX, Yu SQ, Chen YJ, Ren HJ, Feng S, Zhang JF, Chen Y, Xue Q, Xu XY. Catalpol improves impaired neurovascular unit in ischemic stroke rats via enhancing VEGF-PI3K/AKT and VEGF-MEK1/2/ERK1/2 signaling. Acta Pharmacol Sin, 2022; 43(7): 1670-1685.

[26] Zhang WW, Wang X, Xie P, Yuan ST, Liu QH. Anthrax lethal toxin suppresses high glucose induced VEGF over secretion through a post-translational mechanism. Int J Ophthalmol, 2015; 8(3): 453-8.

[27] Xu C, Zhong W, Zhang H, Jiang J, Zhou H. Gap26 inhibited angiogenesis through the β-catenin-VE-cadherin-VEGFR2-Erk signaling pathway. Life Sci, 2023; 328: 121836.

[28] Zhang S, Fu Z, Wei J, Guo J, Liu M, Du K. Peroxiredoxin 2 is involved in vasculogenic mimicry formation by targeting VEGFR2 activation in colorectal cancer. Med Oncol, 2015; 32(1): 414.