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OK-432 suppresses lymphorrhea after urological tumorectomy by regulating Akt/NFκB mediated VEGFC/VEGFR-3 signaling

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Abstract: *Lymphorrhea following retroperitoneal surgery is a challenging complication. This study aimed at exploring the efficiency, mechanisms of immunotherapeutic agent OK-432 in urological cancer-associated lymphorrhea. Intracavitary administration of OK-432 suppressed lymphorrhea after urological tumorectomy. In malignancy-related lymphorrhea, researches on OK-432 was in high-profile. Expressions of VEGFC increased in infiltrating urothelial cancer compared to superficial tumor and was correlated to immune cell infiltration. OK-432 impaired T24 cells viability and anchorage-independent growth by inhibiting Akt/NFκB-mediated VEGFC expression, while facilitated migration and tube formation of lymphatic endothelial cells by enhancing Akt/NFκB-mediated VEGFR-3 expression. OK-432 is an alternative for the lymphorrhea after urological tumorectomy by promoting VEGFR-3 mediated-lymphatic endothelial cells migration and impairing viability of cancer cells through down-regulating expression of VEGFC, and both of which were Akt/NFκB signaling dependent.*

Keywords: *Picibanil, Urothelial Carcinoma, Lymphorrhagia, Vascular Endothelial Growth Factor, Immunotherapy*

1. Introduction

Lymphorrhea following abdominal or retroperitoneal surgery is a rare but challenging complication, which is defined as the leakage of milky appearing fluid rich in triglycerides caused by the injury of lymph [1, 2]. The main etiology includes malignancy, cirrhosis, infection, inflammatory, surgery and trauma [3]. Treatment of lymphorrhea relies on decreasing chyle flow, supplementing medium-chain triglycerides through high-protein and low-fat diet, adequate rest and drainage [4]. Continuous intravenous administration of somatostatin was the early reported conservative treatment for postsurgical lymphorrhea [5]. In some refractory cases, entirely retroperitoneal coverage with fibrin glue and Vicryl mesh is alternative [6].

Lymphatic dissection and excision are indispensable procedures in most malignancy operations. OK-432 is a preparation incubated with lyophilized low-virulent Streptococcus pyogenes (Group A) and benzyl penicillin. It has been used as immunotherapeutic agents effectively in many types of malignancies by inducing intense local inflammation that promotes the release of various cytokines such as tumor necrosis factor (TNF), interferon gamma (IFN-γ) and interleukins (ILs). Therefore, it was used to prevent seroma formation and reduce drainage magnitude [7]. OK-432 sclerotherapy was applied to the treatment of refractory lymphorrhea after gastrectomy for early gastric and reduce seroma formation after axillary lymphadenectomy for breast cancer [1, 7]. The drainage performance of lymphatic vessels is influenced by vascular permeability and pumping activity, which were affected by vascular endothelial growth factor C (VEGFC)/vascular endothelial growth factor receptor 3 (VEGFR-3) signaling axis as well as several inflammatory mediators such as nuclear factor (NF)-κB, TNF-α, IL-1β [8, 9].

Metastasis is the leading cause of cancer-associated mortality, and both blood (angiogenesis) and lymphatic vasculatures (lymph angiogenesis) are essential structures for mediating distal metastasis [10]. Epithelial cancer cells escape from apoptosis in the absence of attachment to extracellular matrix

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(ECM), which may lead to cellular survival in lymphatic systems to form metastasis [11]. This anchorage-independent growth is invoked by activation of phosphoinositide 3-kinases (PI3K)/Akt signaling [12]. When treating lymphorrhea following tumorectomy, we hope the selected topical therapy may not lead to unintentional tumor spread. For urological cancers, inspiring results have been observed in preventing recurrence and progression of non-muscle-invasive bladder cancer (NMIBC) through intravesical administering of OK-432 [13]. Intracavitary administration of OK-432 significantly impaired malignant ascites in advanced renal cell carcinoma [14].

In our work, two patients with refractory lymphatic leak after surgeries for upper urological cancers were successfully treated by intracavitary administration of OK-432. Furthermore, we explored related mechanisms on antineoplastic activity in urothelial tumor as well as lymphangial repair effects of OK-432.

2. Materials and methods

2.1. Reagents

OK-432 (Sapylin, 1KE equals 100 μg of dried streptococci) was from Sinopharm Group Luya (Shandong) pharmaceutical company. Primary antibodies were as follows: rabbit anti-human VEGFC (1:1000, Cell Signaling Technology, #2445), VEGFR-3 (1:1000, Abcam, ab27278), rabbit anti-human phospho-Akt (Ser473) (1:1000, Cell Signaling Technology, #4060), rabbit anti-human phospho-NFκB p65 (Ser536) (1:000, Cell Signaling Technology, #3033), mouse anti-human β-Actin (1:1000, Proteintech, 66009-1-Ig) was served as loading control. Human VEGFC enzyme-linked immunosorbent assay (ELISA) Kit (Elabscience, E-EL-H1600c) was applied to detect concentrations of secretory VEGFC in the culture solutions. SC79 (Selleck, #S7863) was used as the activator of PI3K/Akt pathway[15].

2.2. Management of postsurgical lymphorrhea by OK-432

Case 1: An 80-year-old Chinese female with tumor in her pelvic part of left ureter received radical nephroureterectomy, and postoperative pathological indicated invasive urothelial carcinoma in left ureteral (Grade II). She suffered lymphatic postoperative leak and attempted diet therapy for 1 month with limited efficiency (about 100ml drainage flow within 24 h then). She was informed with the possible advantages and disadvantages of OK-432 sclerotherapy and approved it. After excluding allergy to penicillin, she received 1 KE OK-432 dissolved in 10 mL NS intracavitary administration through retroperitoneal drainage tube, and 2KE in 20 mL NS retroperitoneal infusion 4 days later.

Case 2: A 54-year-old Chinese female with a left retroperitoneal mass accompany with tumor thrombus across left renal vein to inferior vena cava, and postoperative pathological indicated clear cell carcinoma in the left kidney. She received left retroperitoneal tumorectomy as well as embolectomy and suffered lymphatic leak since the 10th day (about 200 ml drainage flow within 24 h) after operation. She was informed with the possible advantages and disadvantages of OK-432 sclerotherapy and approved it. After excluding penicillin allergy, she received 2 KE OK-432 dissolved in 20 ml NS intracavitary administration through retroperitoneal drainage tube at the 11th day after operation.

2.3. Literature analysis

Publications on OK-432, tumor and lymphorrhea were evaluated. Web of Science was used for literature retrieve with the time span from 1900 to 2019 (totally 120 years) and search language was set to auto. The databases (time span) involved were as follows: Web of Science Core Collection (1900 to 2019), Chinese Science Citation DatabaseSM (1989 to 2019), KCI-Korean Journal Database (1980 to 2019), MEDLINE® (1950 to 2019), Russian Science Citation Index (2005 to 2019), SciELO Citation Index (2002 to 2019). Retrieval strategy of publications on OK-432 and tumor was: TS=(((Picibanil) OR Sapylin) OR OK-432) AND TS=((((neoplasm) OR cancer) OR malignancy) OR tumor). Retrieval strategy of publications on lymphorrhea and OK-432 was: TS=(((((chylous fistula) OR lymphorrhea) OR lymphatic leakage) OR chyle leakage) OR chyloretroperitoneum) AND TS=(((Picibanil) OR Sapylin) OR OK-432). Retrieval strategy of publications on OK-432 and tumor and lymphorrhea was: TS=(((Picibanil) OR Sapylin) OR OK-432) AND TS=((((neoplasm) OR cancer) OR malignancy) OR tumor) AND TS=(((((chylous fistula) OR lymphorrhea) OR lymphatic leakage) OR chyle leakage) OR chyloretroperitoneum). Retrieval strategy of publications on OK-432 and tumor was: TS=(((Picibanil)

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OR Sapylin) OR OK-432) AND TS=((((neoplasm) OR cancer) OR malignancy) OR tumor). Retrieval strategy of publications on lymphorrhea and OK-432 was: TS=(((((chylous fistula) OR lymphorrhea) OR lymphatic leakage) OR chyle leakage) OR chyloretroperitoneum) AND TS=(((Picibanil) OR Sapylin) OR OK-432). Retrieval strategy of publications on OK-432 and tumor and lymphorrhea was: TS=(((Picibanil) OR Sapylin) OR OK-432) AND TS=((((neoplasm) OR cancer) OR malignancy) OR tumor) AND TS=(((((chylous fistula) OR lymphorrhea) OR lymphatic leakage) OR chyle leakage) OR chyloretroperitoneum). Publication years and times cited were analyzed. (TS meant topic)

2.4. Bioinformatics analysis

Differential expressions of VEGFC between infiltrating bladder urothelial carcinoma and superficial bladder cancer was online analyzed through www.oncomine.org. Correlation between VEGFC expression and tumor-associated immune cells infiltration was analyzed through cistrome.shinyapps.io/timer. The filter was set as Gene: VEGFC, Cancer Type: BLCA (bladder urothelial carcinoma). Distribution of VEGFC or VEGFR-3 (FLT4) expression in bladder urothelial carcinoma across immune subtypes was retrieved from the online integrated database TISIDB (http://cis.hku.hk/TISIDB/index.php).

2.5. Cell culture

Human lymphatic endothelial cells (Sciencell, #2010) were cultured in Endothelial Cell Medium (ScienCell, #1001) supplemented with 5% fetal bovine serum (FBS, Biological Industries). Bladder urothelial carcinoma T24 cells (American Type Culture Collection, RRID: CVCL_0554) were cultured in RPMI 1640 medium (Gibco) supplemented with 10% FBS and maintained at 37°C supplied with 5% CO_2 atmosphere. The cell lines have been authenticated by the Short Tandem Repeats identification and tested for mycoplasma contamination.

2.6. Cell viability assay

Cell viability assay was assessed with the Cell Counting Kit-8 (CCK-8, Dojindo). T24 cells (1×10^4 per well) were seeded in 96-well plates overnight and treated with the indicated concentrations (0 KE/mL, 0.125 KE/mL, 0.25 KE/mL, 0.5 KE/mL, 1 KE/mL, 2 KE/mL) of OK-432 for 24 h, 48 h and 72 h respectively. At each time point, 10 μL of CCK-8 solution was added into each well and incubated at 37 ℃ for 2 h. The absorbance at 450 nm was measured on a microplate reader.

2.7. Detection of detachment-induced apoptosis

To establish detachment-induced cancer cells, T24 cells were suspension cultured in ultra-low attachment 6-well plates (Corning) with or without OK-432 (0.25 KE/mL) for 48 h. Apoptosis of the cells were detected using the FITC-AnnexinV apoptosis detection kit (BD Biosciences). Suspended cells were collected and incubated with FITC/propidium iodide (PI) for 15 min in the dark at room temperature, and the apoptosis index was determined by using a flow cytometer (Beckman Coulter).

2.8. Transwell migration assay

Cell migration assay was performed using the 24-well Transwell plate with 8.0-μm pore polycarbonate membrane inserts (Corning). After treated with 0.125 KE/mL OK-432 for 48 h, homogeneous single lymphatic endothelial cell suspensions were added to the upper chambers with corresponding dispositions and 500 μl complete medium was added into the lower chambers. After incubation for 24 h at 37°C, migrated cells were fixed with ice-cold methanol and stained with 0.1% crystal violet for 15 min at room temperature. Migrated cells were counted in random fields with an inverted phase contrast microscope at 100× magnification.

2.9. Tube formation assay

Matrigel was prepared using a 1:1 ratio with growth factor reduced Matrigel (BD Biosciences) Matrigel and serum-free medium. Endothelial cells were respectively resuspended in parental T24 cells medium, T24 cells treated with OK-432 (0.25 KE/mL) conditioned medium, T24 cells treated with OK-432 (0.25 KE/mL) combining with SC79 (30 nM) conditioned medium. About 1×10^4 resuspended endothelial cells with each condition were then seeded on Matrigel coated 96-well plates for 8 h.

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Tubule formation was inspected and counted under an inverted light microscope.

2.10. Western blotting

Total cellular protein was isolated by using RIPA lysis buffer (Beyotime), separated on SDS-PAGE gels and transferred onto PVDF membranes (Merck Millipore). The membranes were blocked by 5% non-fat milk in Tris-buffered saline with Tween-20 and then incubated with primary antibodies overnight at 4°C, and then incubated with corresponding secondary antibodies (Proteintech). Protein bands were visualized with enhanced chemiluminescence (Beyotime) and analyzed by Image J software (NIH, Bethesda, MD, USA).

2.11. ELISA for VEGFC

The T24 cells were treated with OK-432 (0.25 KE/mL), OK-432 (0.25 KE/mL) combining with SC79 (30 nM) and isometric dimethyl sulfoxide (DMSO, as control) for 48 h. Culture solutions were centrifugated at 1000×g for 20 min and collected for detection. The ELISA was conducted in accordance with the manufacturer's instructions. The absorption values were measured at 450 nm on a multi-well plate reader.

2.12. Statistical analysis

Data were expressed as average ± standard deviation (*SD*). Statistical analyses were performed using the SPSS software with the two-tailed Student's *t* test between two groups, and the means of more than two groups were compared using one-way analysis of variance (*ANOVA*) with post-hoc *Tukey*'s test. Experiments were performed in triplicates and $*p < 0.05$ was considered to indicate a statistically significant result.

3. Results

3.1. Regional administration of OK-432 suppressed lymphorrhea after urological tumorectomy

In case 1, after receiving intracavitary administration of OK-432 for 1 KE through retroperitoneal drainage tube (**left blue arrow in Fig. 1A**), the volume of drainage transiently increased while rapidly decreased. When the patient received the other 2 KE of OK-432 administration 4 days later (**right blue arrow in Fig. 1A**), provisionally increased but subsequently remarkably decreased drainage was observed. The drainage tube was pulled out 9 days after the first administration of OK-432. The patient did not complain of special discomfort during the treatment. In case 2, after receiving intracavitary administration of OK-432 for 2 KE through retroperitoneal drainage tube (**red arrow in Fig. 1B**), the volume of drainage temporarily increased for 1 day and promptly decreased. The drainage tube was pulled out 4 days after the administration of OK-432. The patient did not complain of special discomfort during the treatment.

3.2. Researches on OK-432 in malignancy-related lymphorrhea was in small quantity but high-profile

According to the results of literature retrieve, from 1972 to 2019, there were 1861 publications about OK-432 on tumors altogether, and the highest-yield was in the year of 1989 with 103 publications (**Fig. 1C**). From 1989 to 2016, there were 20 publications about OK-432 on lymphorrhea altogether, and the highest-yield was in the year of 2008 with 4 publications (**Fig. 1D**). From 1993 to 2017, there were only 15 publications about OK-432 on malignancy-related altogether, and the highest-yield was in the year of 2008 with 3 publications (**Fig. 1E**). According to the citation analysis of the related publications, the amounts of citations presented an overall upward trend. However, the growth speed of citations about OK-432 on tumors is descending recently (**Fig. 1F**), while the growth speeds of citations about OK-432 on lymphorrhea (**Fig. 1G**) or malignancy-related lymphorrhea (**Fig. 1H**) are both rising generally. All these indicated that studies about OK-432 on malignancy-related lymphorrhea rather than on tumor alone is becoming more and more concerned.

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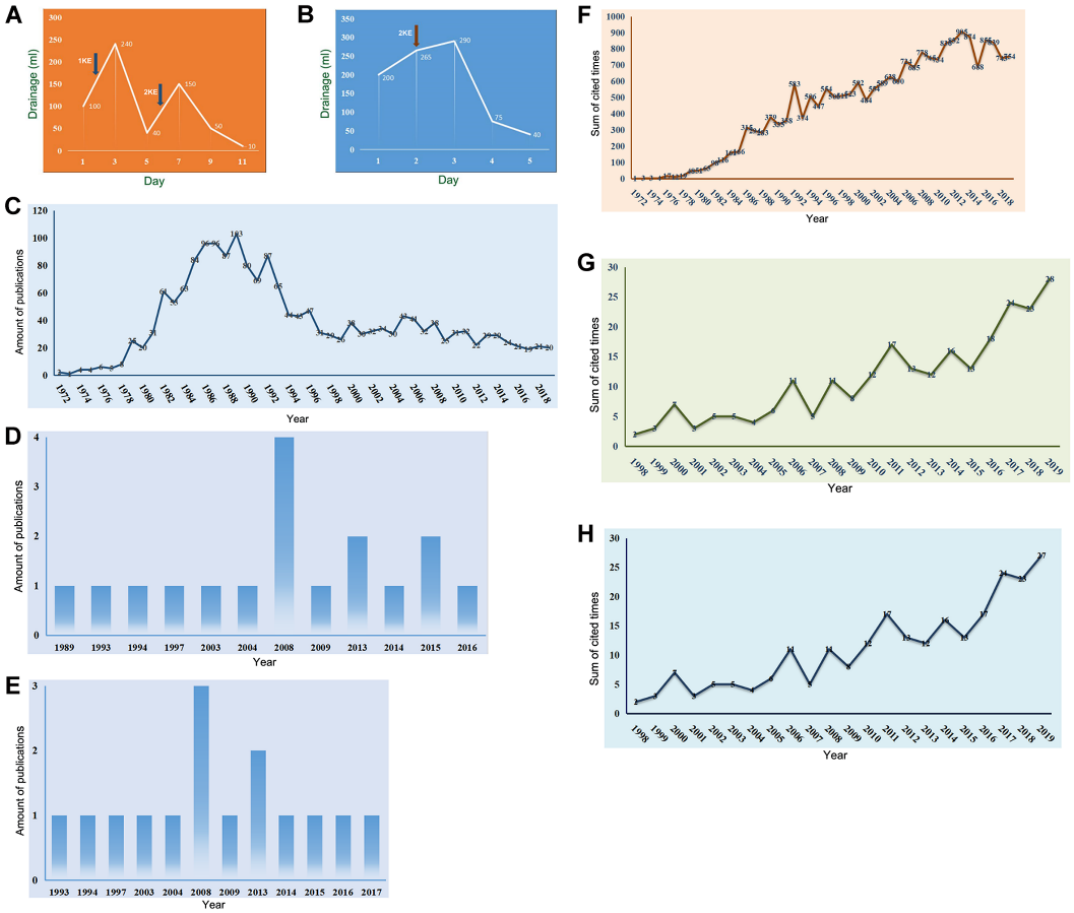

In both Case 1 ***(A)*** *and Case 2* ***(B)****, OK-432 treatment dramatically whittled chyle flow following temporarily increased drainage, and the curves represent the volumes (ml) of drainage in different days and the arrows indicate intracavitary administration of OK-432. Yearly amounts of publications regarding OK-432 on tumors* ***(C)****, OK-432 on lymphorrhea* ***(D)*** *and malignancy-related lymphorrhea* ***(E)****. Citation trends of the publications regarding OK-432 on tumors* ***(F)****, OK-432 on lymphorrhea* ***(G)*** *and malignancy-related lymphorrhea* ***(H)****.*

Figure 1: Case report and literature analysis of OK-432 in lymphorrhea after urological tumorectomy.

3.3. Expression of VEGFC increased in infiltrating bladder urothelial cancer and was correlated to immune cell infiltration

According to the analysis among 6 datasets through ONCOMINE, expressions of VEGFC significantly increased in infiltrating bladder urothelial carcinoma when compared to superficial bladder cancer (**Fig. 2A**). While based on the comprehensive analysis of tumor-infiltrating immune cells through TIMER, expression of VEGFC in bladder cancer was markedly corelated to the infiltrations of B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils and dendritic cells (**Fig. 2B**). In addition, on the basis of TISIDB database, expressions of VEGFC (**Fig. 2C**) and its ligand VEGFR-3 (FLT4, **Fig. 2D**) were significantly correlated with different immune subtypes in bladder urothelial cancer.

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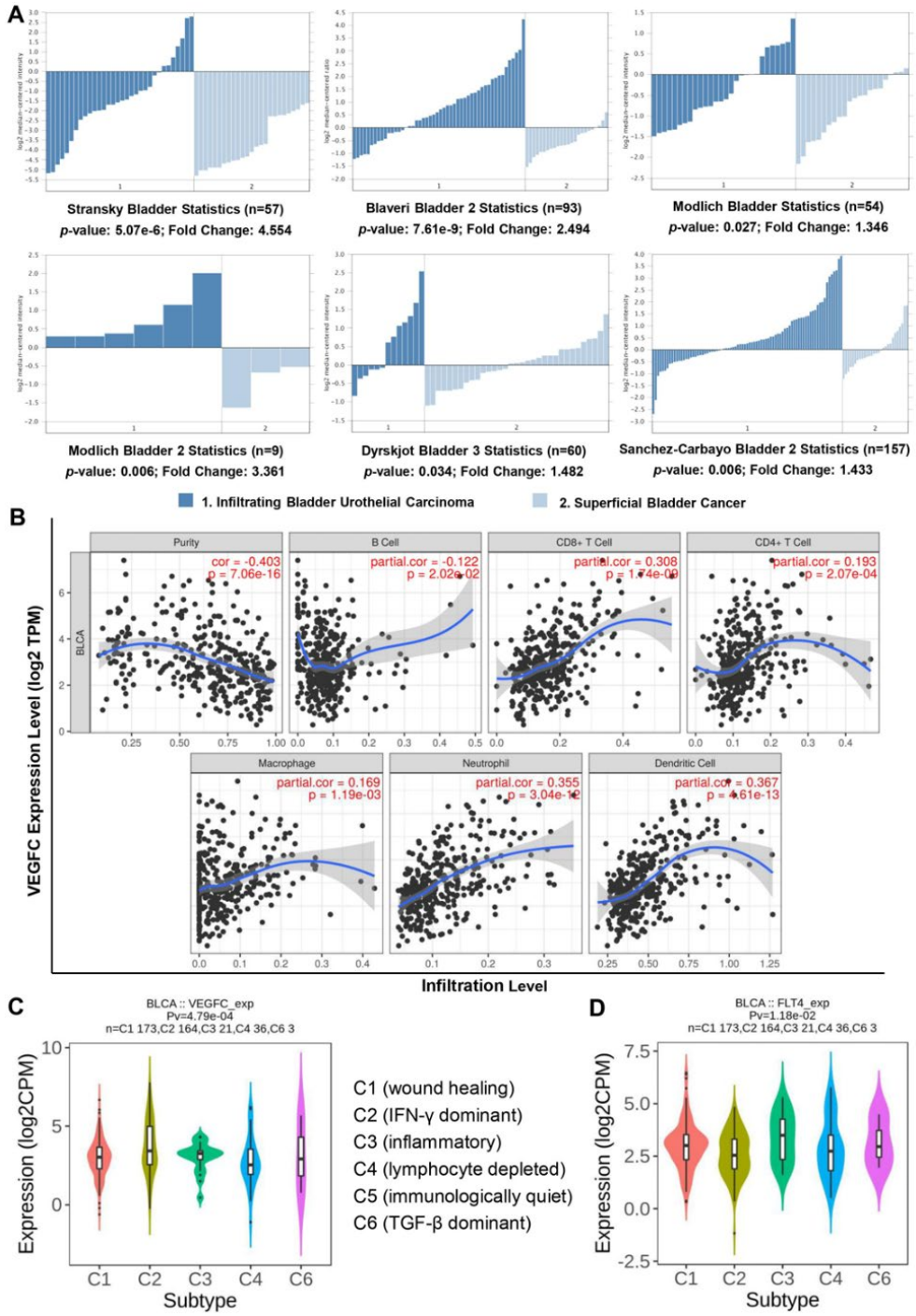

(A) Expressions of VEGFC increased in infiltrating bladder urothelial carcinoma when compared to superficial bladder cancer; data analysis were respectively performed through ONCOMINE among 6 datasets: Nat Genet 2006/12/01, Clin Cancer Res 2005/06/01, Clin Cancer Res 2004/05/15, Clin Cancer Res 2004/05/15, Cancer Res 2004/06/01, J Clin Oncol 2006/02/10. (B) Expression of VEGFC in bladder cancer was corelated to the infiltrations of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils and dendritic cells; data comprehensive analysis of tumor-infiltrating immune cells was performed through TIMER. (C) According to the TISIDB database, VEGFC expression in bladder urothelial cancer was significantly correlated with different immune subtypes. (D) According to the TISIDB database, VEGFR-3 (FLT4) expression in bladder urothelial cancer was significantly correlated with different immune subtypes.

Figure 2: Bioinformatics analysis of VEGFC/VEGFR-3 in bladder urothelial carcinoma.

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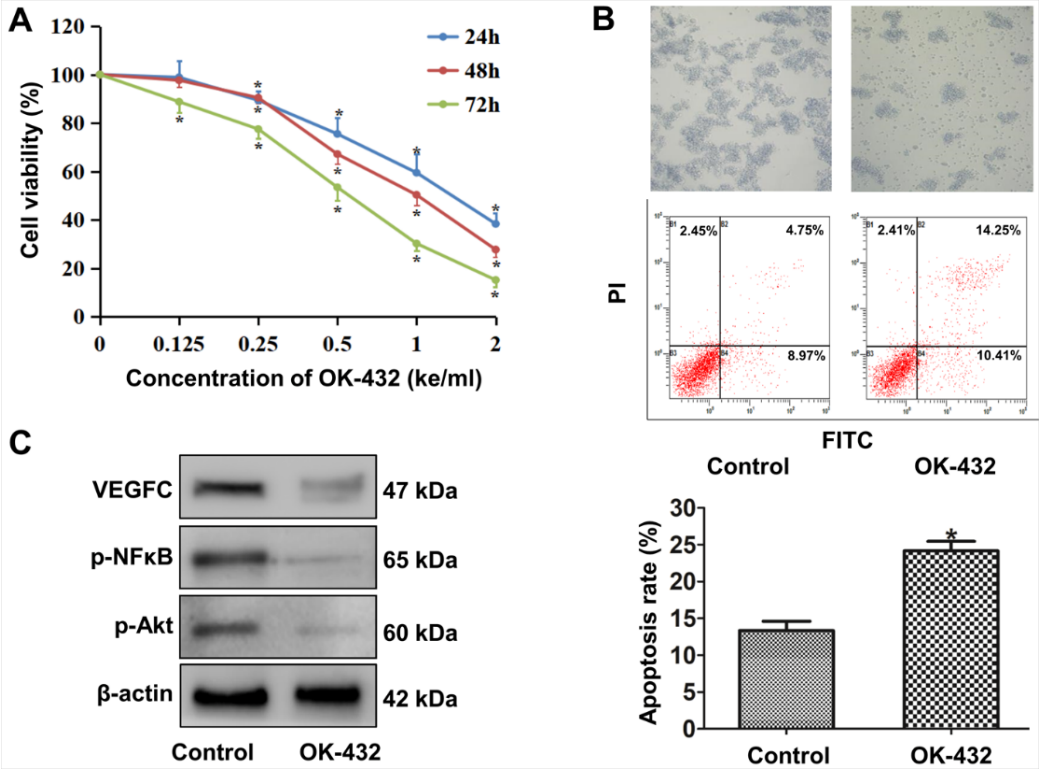
3.4. OK-432 impaired viability, anchorage-independent growth of bladder urothelial carcinoma cells by inhibiting Akt/NFκB/VEGFC cascade

The CCK-8 assay showed that OK-432 resulted in a remarkable decrease in cell viability of T24 cells in a dose- and time-dependent manner (**Fig. 3A**). Flow cytometry revealed that OK-432 significantly promoted detachment-induced apoptosis, which indicated the weakened anchorage-independent growth of T24 cells (**Fig. 3B**). For western blotting, when treated with 0.25 KE/mL OK-432 for 48 h, prominently declined expressions of p-Akt, p-NFκB and VEGFC proteins were observed in T24 cells (**Fig. 3C**).



*OK-432 impaired viability (A) and promoted detachment of apoptosis (B) of T24 cells by inhibiting protein expressions of p-Akt, p-NFκB and VEGFC (C). *p < 0.05.*

Figure 3: OK-432 impaired viability, anchorage-independent growth of bladder urothelial carcinoma cells by inhibiting Akt/NFκB/VEGFC cascade.

3.5. OK-432 facilitated migration of lymphatic endothelial cells by activating Akt/NFκB/VEGFR-3 cascade

Transwell migration assay demonstrated that when treated with OK-432, remarkably enhanced cell migration was observed in lymphatic endothelial cells (**Fig. 4A**). In accordance with this, significantly boosted expressions of p-Akt, p-NFκB and VEGFR-3 proteins in lymphatic endothelial cells for western blotting assay (**Fig. 4B**).

3.6. OK-432 inhibited lymphangiogenesis of bladder urothelial carcinoma cells by attenuating Akt/NFκB/VEGFC cascade

Tube formation assay demonstrated that OK-432 obviously inhibited lymphangiogenesis capability (tube formation of lymphatic endothelial cells) of T24 cells, which could be partly reversed by PI3K/Akt pathway activator SC79 (**Fig. 4C**). Western blotting illustrated that significantly enhanced expressions of p-Akt, p-NFκB proteins in OK-432 treated T24 cells, while the effects were partly reversed by SC79 treatment (**Fig. 4D**). ELISA assay manifested that secretory VEGFC of T24 cells was suppressed by OK-432, which could be partly reversed by SC79 (**Fig. 4E**).

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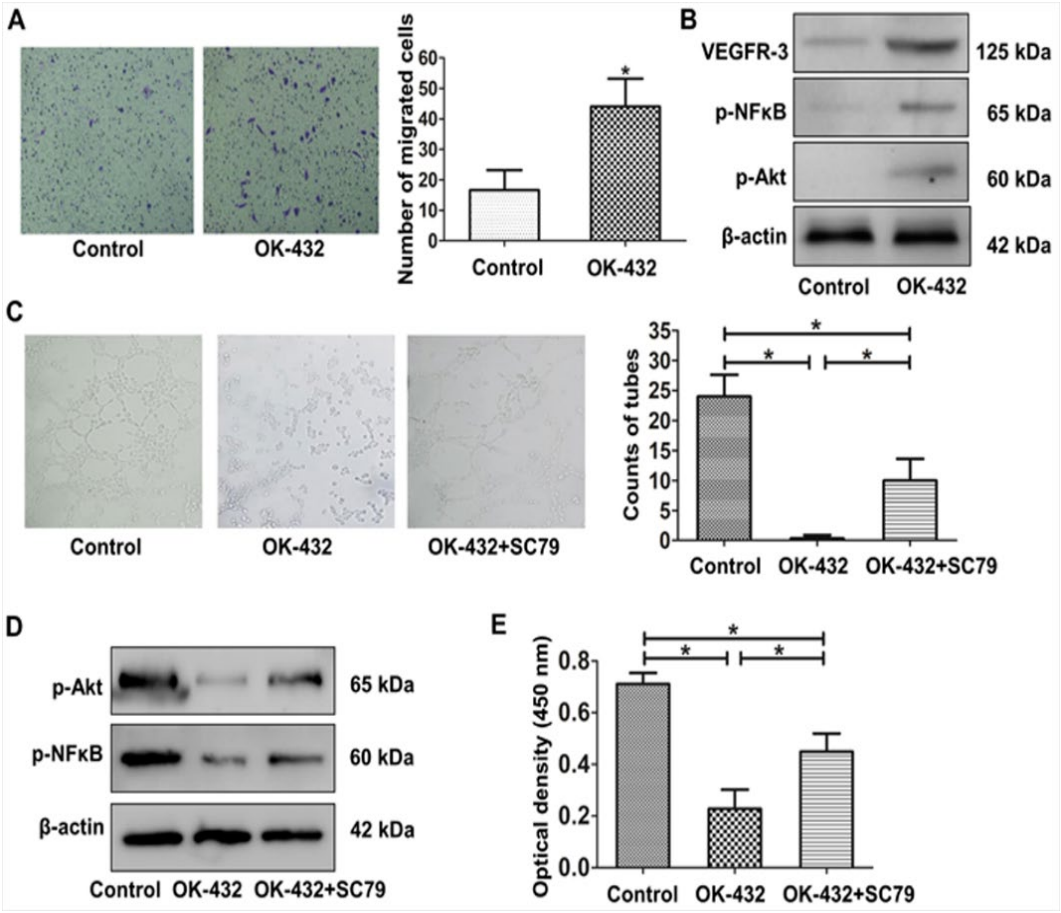

*OK-432 facilitated cell migration (original magnification ×100) of lymphatic endothelial cells (A) by impairing protein expressions of p-Akt, p-NFκB, VEGFR-3 (B). OK-432 inhibited tube formation assay (original magnification ×200) detected lymphangiogenesis capability (C), western blotting assay detected protein expressions of p-Akt and p-NFκB (D), ELISA detected secretory VEGFC (E) in T24 cells, and all the effects were reversed by PI3K/Akt pathway activator SC79 (C-E). *p < 0.05.*

Figure 4: OK-432 facilitated migration of lymphatic endothelial cells and inhibited lymphangiogenesis of bladder urothelial carcinoma cells by regulating Akt/NFκB/VEGFC cascade.

4. Discussion

Although lymphorrhea following tumorectomy is an infrequent complication, literature analysis revealed that related work has been persistently focused. As an immunotherapeutic agent, OK-432 elicits antitumor effects by activating some immunocompetent cells and inducing cytokines like ILs, TNF-α, and IFN-γ [16]. Temporarily increased drainage after OK-432 administration might result from inflammatory reaction-induced effusion. The aspirates from cystic lymphangiomas after injection of OK-432 exhibited a marked elevation in IL-6, IL-8, VEGF, and TGF-β1 levels after OK-432 injection, while IL-6, IL-8, TNF-α, and IFN-γ levels were elevated in the culture supernatants of the mononuclear cells cultured with OK-432 [17]. IL-1a, IL-6, transforming growth factor (TGF)-β1 and VEGF were increased in drainage fluids treated with OK-432 after modified radical mastectomy. After treated with the drainage fluids, significantly enhanced proliferation, migration and angiogenesis of human umbilical vein endothelial cells (HUVECs) were observed [18]. Additionally, treating lymphatic malformations with OK-432, lymphatic endothelial cells whittled mesenchymal status after OK-432 administration, which might induce dilated vessel constriction as the mechanism of sclerotherapy [19]. Furthermore, lymphangiogenesis related VEGFR-3 in pleural fluids was up-regulated following OK-432 pleurodesis in fetal chylothorax [20]. Macrophages-derived lipocalin-2 was verified to induce proliferation and tube formation of lymphatic endothelial cells in a PI3K/Akt-dependent manner and subsequently induce VEGFC, which functioned as an autocrine signal stimulating the receptor VEGFR-3 [21]. In our present work, OK-432 facilitated migration of lymphatic endothelial cells by blocking expression of VEGFR-3, which indicated that VEGFR-3 pathway was implicated in the

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OK-432 mediated lymphatic repair.

Overexpression of mitogen-activated protein kinase (MAPK) 4 activates the mechanistic target of rapamycin complex 2 (mTORC2), phosphorylation of Akt at Serine 473 and full activation, leading to the transition of epithelial cells to non-anchored growth. And MAPK4 was found correlated with decreased overall survival in bladder cancer [22]. In the present study, OK-432 activated Akt/NFκB signaling to facilitate anchorage-independent growth of bladder cancer cells, which may protect patients from tumor cell spread during intracavitary therapies. OK-432 synergized with IFN-γ to confer dendritic cells (DCs) mature by activating MAPK and NFκB pathway, which subsequently induced strong cytotoxic lymphocyte (CTL) and NK cell response against tumor cells [23]. The tumor-killing activity triggered by OK-432 stimulated DCs mature and NK cells depended on TNF-related apoptosis-inducing ligand (TRAIL) and Fas ligand (FasL) pathways [24]. In bladder cancer, OK-432 suppressed cell proliferation and metastasis through tumor associated macrophages [25]. The gram-negative bacterial antigen lipopolysaccharide (LPS) could cause severe inflammation reaction and be responsible for the progression of colorectal carcinoma by increasing NFκB binding to the promoter of VEGFR-3[26]. Similarly, lymphangiogenesis of gallbladder carcinoma was promoted by TNF-α triggered enhanced NFκB combining to the promoter of VEGFC [27].

Our work revealed that OK-432 remitted viability, anchorage-independent growth and tube formation of lymphatic endothelial cells in bladder urothelial carcinoma by inhibiting Akt/NFκB signaling triggered overexpression of VEGFC. Interestingly, OK-432 accelerated migration of lymphatic endothelial cell by activating Akt/NFκB/VEGFR-3 signaling, which might theoretically facilitate lymphatic vessel repair to reduce lymphorrhea. As increased VEGFC expressions were demonstrated in infiltrating bladder urothelial cancer and might be correlated to immune cell infiltration, OK-432 is supposed to be a promising locally antineoplastic immunotherapy as well as sclerotherapy for urothelial cancer. Therefore, OK-432 sclerotherapy is an alternative for lymphorrhea after urological tumorectomy, and more clinical trials as well as further mechanism exploration deserve to be carried out.

5. Conclusions

In summary, OK-432 is an alternative immunotherapy for the lymphorrhea after urological tumorectomy by promoting VEGFR-3 mediated-lymphatic endothelial cells migration and impairing viability of cancer cells through down-regulating expression of VEGFC, and both of which were Akt/NFκB signaling dependent.

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