ORIGINAL CONTRIBUTION

EXPERIMENTAL ONCOLOGY

https://doi.org/10.15407/exp-oncology.2024.04.358

L. ZHU [1, 2], B-C. AHN [1, 2, 3, 4, *]

[1] Department of Nuclear Medicine, School of Medicine, Kyungpook National University, Daegu, South Korea
[2] Cardiovascular Research Institute, Kyungpook National University, Daegu, South Korea
[3] Department of Nuclear Medicine, Kyungpook National University Hospital, Daegu, South Korea
[4] BK21 FOUR KNU Convergence Educational Program of Biomedical Sciences for Creative Future Talents, Department of Biomedical Sciences, School of Medicine, Kyungpook National University, Daegu, South Korea

* Correspondence: Email: abc2000@knu.ac.kr

NATURAL KILLER CELL-DERIVED EXOSOME MIMETICS AS NATURAL NANOCARRIERS FOR *IN VITRO* DELIVERY OF CHEMOTHERAPEUTICS TO THYROID CANCER CELLS

Background. Exosomes have become a potential field of nanotechnology for the treatment and identification of many disorders. However, the generation of exosomes is a difficult, time-consuming, and low-yielding procedure. At the same time, exosome mimetics (EM) resemble exosomes in their characteristics but have higher production yields. The **aim** of this study was to produce natural killer (NK) cell-derived EM (NKEM) loaded with sorafenib and test their killing ability against thyroid cancer cell lines. **Materials and Methods**. Sorafenib was loaded into NKEM by mixing sorafenib with NK cells during NKEM production (NKEM-S). Then, these two types of nanoparticles were characterized with nanoparticle tracking analysis (NTA) to measure their sizes. In addition, the cellular uptake and *in vitro* killing effect of NKEM-S on thyroid cancer cell lines were investigated using confocal laser microscopy and bioluminescence imaging (BLI) techniques. **Results**. The uptake of NKEM and NKEM-S by the thyroid cancer cells was observed. Moreover, BLI confirmed the killing and anti-proliferation effect of NKEM-S on two thyroid cancer cell lines. Especially important, the NKEM-S demonstrated a desirable killing effect even for anaplastic thyroid cancer (ATC) cells. **Conclusion**. Sorafenib-loaded NKEM showed the ability to kill thyroid cancer cells *in vitro*, even against ATC. This provides a new opportunity for drug delivery systems and thyroid cancer treatment.

Keywords: thyroid cancer, exosome mimetics, natural killer cells, immunotherapy, drug delivery system.

Thyroid cancer has attracted more and more attention due to its rapid increase in incidence, and numerous improvements in treatment have been applied over the past few decades, including the development of molecularly targeted drugs and advancements [1, 2]. In general, thyroid cancers

Citation: Zhu L, Ahn B-C. Natural killer cell-derived exosome mimetics as natural nanocarriers for *in vitro* delivery of chemotherapeutics to thyroid cancer cells. *Exp Oncol.* 2024; 46(4): 358-367. https://doi.org/10.15407/exp-oncology.2024.04.358

can be categorized based on the cells and divided into differentiated thyroid cancer (DTC) including papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC), anaplastic thyroid cancer (ATC), and medullary thyroid cancer (MTC) [3]. Fortunately, more than 90% of thyroid cancer cases belong to the DTC, which has an excellent prognosis. In contrast, as one of the most fatal malignancies, ATC has worse prognosis (overall 1-year survival rate of about 20% and mean survival of less than 6 months) [4]. Although various therapeutic strategies, including surgery [4, 5], chemotherapy [2, 6], radiotherapy [7, 8], and thyroid stimulating hormone (TSH) inhibition therapy [9], have been used, none of them has been successful in improving the survival of ATC patients [10]. Sorafenib is known to kill tumors by inhibiting the proliferation and angiogenesis of tumor cells [11, 12] and was approved by the FDA for the treatment of hepatocellular carcinoma, advanced renal cell carcinoma, and DTCs [13, 14]. Although sorafenib is widely used for treating DTCs, however, it is not used for treating ATC. The development of an effective sorafenib delivery system to tumor cells may provide an opportunity for the treatment of ATC.

It is particularly motivating to load agents into the drug delivery system (DDS) to improve therapeutic effectiveness by increasing their accumulation in the tumor and minimizing off-target effects [15—23]. However, the presence of biological barriers reduces the interaction of nanoparticles with their targets, which reduces their biological utility and clinical translation [24, 25]. In addition, the cycle time of DDS is constrained due to the quick clearance by monocytes/macrophages or the reticuloendothelial system (RES) [26—28]. In comparison with artificial nanocarriers, exosomes or small extracellular vesicles revealed less clearance by the immune system, owing to their inherent existence in the body [29]. For instance, it has been demonstrated that exosomes from CD47-overexpressing human foreskin fibroblasts reduce phagocytosis-mediated clearance by monocytes and macrophages and increase the absorption by cancer cells [30]. Numerous oncological and non-oncological diseases have been diagnosed and treated using exosome technology [15, 29, 31—36]. In addition, the biological activities mediated by exosomes, such as immunomodulation, induction of apoptosis, and enhancement of proliferation, are largely under the control of these proteins and cargoes [15, 29]. However, the low production rate of exosomes may demand the consumption of huge amount of medium for their large-scale production [37—41].

Exosome mimetics (EM), which function biologically similarly to exosomes while having higher yields, are emerging as a new generation of bioinspired-nanoscale DDS [42]. Previous research has used cell extrusion to create mesenchymal stem cell-derived EM, and these nanoparticles still have the exosome-like ability to deliver chemotherapeutic drugs in high yields [42—45]. Notably, EM have several advantages over currently used synthetic systems. First of all, the bio-originated membrane of EM occurs to accelerate the internalization of the drug [15, 29, 42]. Secondly, the nanoscale EM size makes it easier to diffuse into the tumor tissue and extravasate into the blood vessels [16, 31, 43]. Thirdly, EM share biological and morphological similarities with exosomes. In our previous study, we generated NK-92MI cell-derived exosomes and demonstrated the therapeutic applicability of such nanoparticles for the treatment of melanoma cells [46]. In view of the above characteristics, EM instead of exosomes from NK cells may present an opportunity for the development of safer and more effective DDS. In the current study, we aimed to develop a method for loading sorafenib into NKEM and test the killing effect of the sorafenib-loaded NKEM (NKEM-S) against thyroid cancer cell lines *in vitro*.

Materials and Methods

Cell lines. Human PTC cell line K1, purchased from Sigma-Aldrich, USA, was maintained in DMEM high-glucose medium (HyClone, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin/streptomycin 100X solution (HyClone). Human ATC cell line BHT101, purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Germany), was maintained in DMEM high-glucose medium supplemented with 20% fetal bovine serum and 1% penicillin/streptomycin 100X solution. Human NK cell line NK-92MI, purchased from the American Type Culture Collection, USA, was cultured in CellGenix GMP SCGM medium (CellGenix, Germany) supplemented with 2% human serum (Sigma-Aldrich) and 1% penicillin/streptomycin 100X

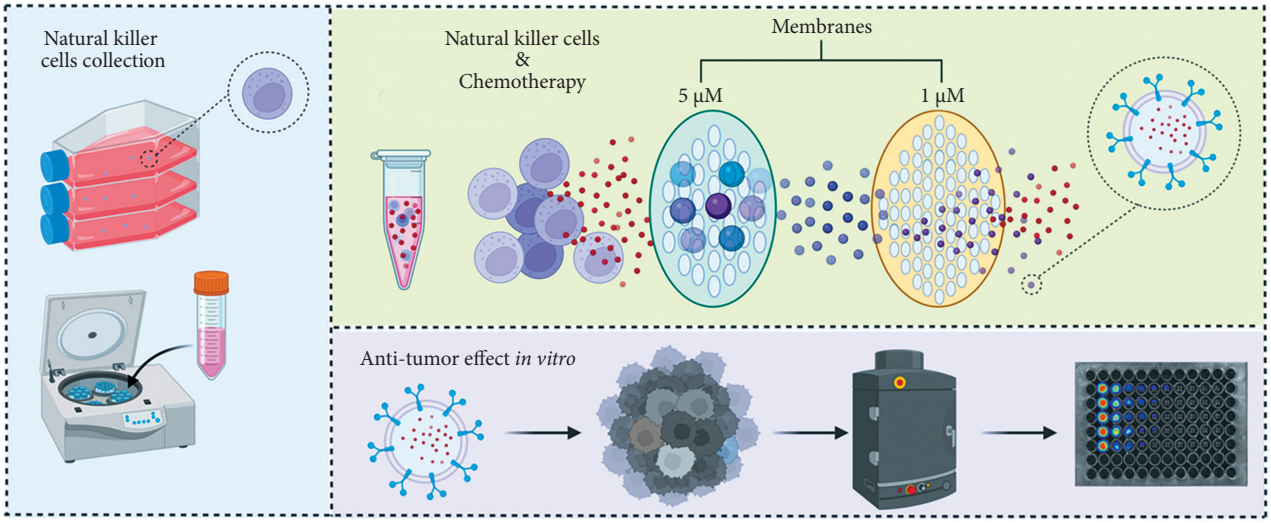

Fig. 1. Flowchart of experimental steps. Experimental steps included cell culture of natural killer cells, centrifugation, production of exosome mimics, loading of sorafenib, and cytotoxic effects on thyroid cancer cell lines

solution. The cancer cell lines were cultured and transfected with an enhanced firefly luciferase (effluc) gene. Established stable expression of the effluc gene was confirmed via the addition of the substrate D-luciferin and using the IVIS Lumina III imaging system (Perkin-Elmer, USA); the cells were referred to as K1/F and BHT101/F cells.

Generation and characterization of sorafenib-loaded NKEM. NKEM/NKEM-S were prepared by adjusting the protocol in previous studies [41, 47, 48], NK-92MI cells were suspended in a medium supplemented with sorafenib (50 ng/mL) and extruded using a mini-extruder (Avanti Polar Lipid, USA). In detail, NKEM/NKEM-S were produced by squeezing out live NK-92MI cells through 5 μm and 2 μm membranes (Nuclepore, Whatman, Inc., USA) without or with sorafenib. Then, the NKEM/NKEM-S were filtered through 0.22 μm filters, purified by ultracentrifugation at 100,000 × g for 1 h at 4 °C (Beckman Coulter, Brea, CA, USA), and then washed with PBS to obtain NKEM and NKEM-S. The morphologies of NKEM and NKEM-S were evaluated with a nanoparticle tracking analysis (NTA) (NanoSight LM10 instrument, Malvern Panalytical, UK).

Cellular uptake assay. NKEM and NKEM-S were labelled with DiI (Thermo Fisher Scientific, Waltham, MA, USA), a fluorescent lipophilic dye, and incubated with ATC cell line (BHT101/F cells) for 3 h. The cancer cells without NKEM were referred to as the blank. After incubation, the samples were washed, fixed, treated with Hoechst dye (Thermo Fisher Scientific), and covered with Vectashield mounting medium (Vector Laboratories, USA). The samples were examined using a confocal laser microscope (Zeiss, LSM, Germany).

In vitro cytotoxicity of NKEM-S. The toxicity of NKEM-S to the DTC cell line (K1/F) and ATC cell line (BHT101/F) was evaluated with bioluminescence imaging (BLI) at various concentrations at 24 and 48 h in a dose-dependent manner by using the IVIS Lumina III imaging system. The flows of the experiment are summarized in Fig. 1.

Statistical analysis. All data were expressed as the mean ± standard deviation (SD), and statistical significance was determined using GraphPad Prism 5 (GraphPad Software Inc., USA). A value of $p < 0.05$ was considered statistically significant.

Results

Characterization of NKEM-S. Fig. 2, *a* outlines the detailed schematic of the NKEM-S production process. NKEM-S had a small size distribution, with an average particle diameter of 201.9 nm, as revealed by NTA. Drug loading resulted in no significant change in the size of the nanoparticles (NKEM-S) (Fig. 2, *b*).

Cellular uptake. The drug loaded into NKEM should be delivered into the target cells to achieve therapeutic effects; therefore, the interaction between cancer cells and nanoparticles was assessed. NKEM-S were labelled with DiI and incubated with BHT101/F cells. As shown in Fig. 3, NKEM and NKEM-S were taken up by ATC cells.

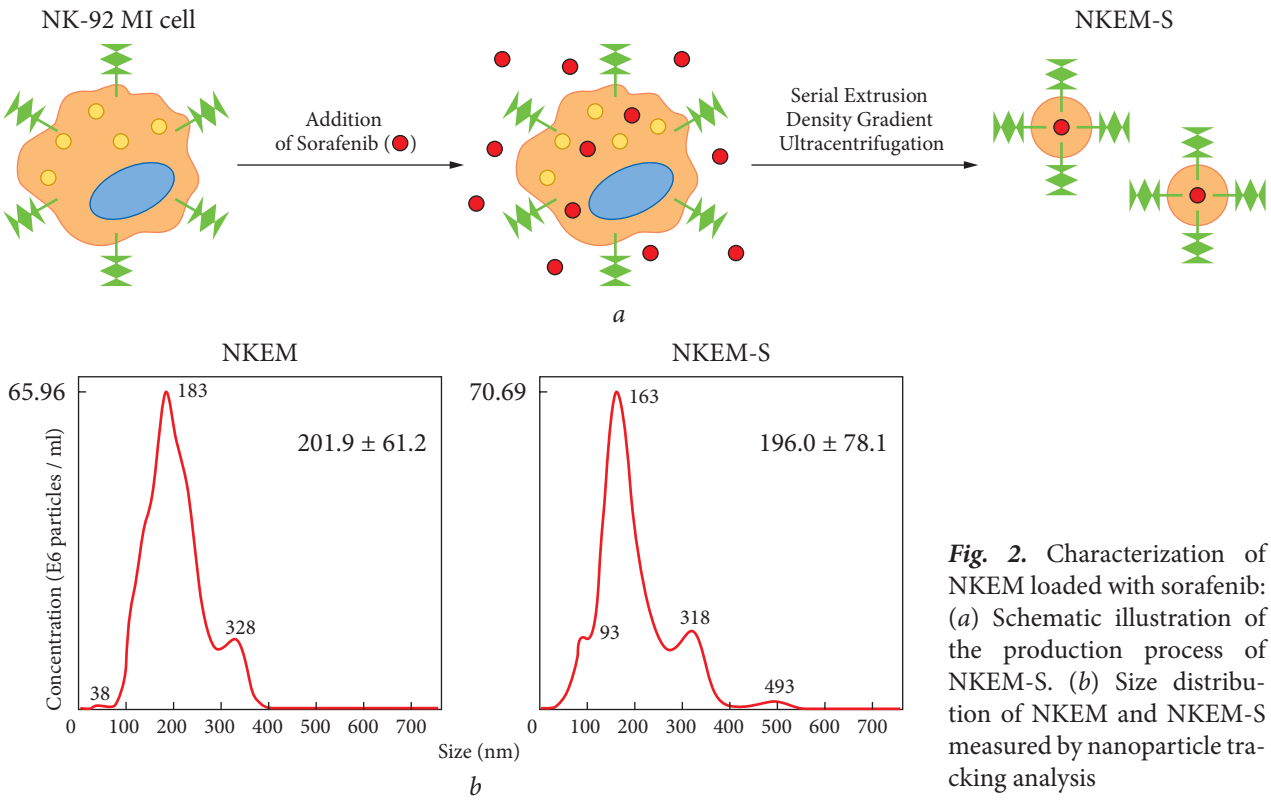

Fig. 2. Characterization of NKEM loaded with sorafenib: (*a*) Schematic illustration of the production process of NKEM-S. (*b*) Size distribution of NKEM and NKEM-S measured by nanoparticle tracking analysis

In vitro antiproliferative and killing effects of NKEM-S against the thyroid cancer cells. The killing effects of NKEM-S against K1/F and BHT101/F cells were evaluated by BLI (Fig. 4). The cancer cells were cultured with NKEM-S of various concentrations and subjected to BLI for 24 and 48 h. Analysis of the reporter gene signal in K1/F and BHT101/F cells (Fig. 4, *a*, *b*) indicated a dose- and time-dependent cytotoxicity of NKEM-S. The quantitative analysis of the data showed that NKEM-S exhibited significant cytotoxicity to both thyroid cancer cell lines. Even the ATC cell line (BHT101/F) was less sensitive to NKEM-S as compared with the PTC cell line (K1/F) (Fig. 4, *c*, *d*).

Discussion

Extracellular vesicles have been exploited by DDS for many years [38, 47, 49—51]. For instance, exosomes derived from milk were used for treating cancer in combination with paclitaxel and doxorubicin [41, 52—55], and curcumin-encapsulated exosomes derived from milk were shown to be resistant to intestinal digestion and exhibited enhanced intestinal permeability [54]. In addition to the chemical agents, exosome-mediated delivery of siRNA, miRNA, and shRNA has been reported [20, 53, 56, 57]. In comparison with artificial nanoparticles such as liposomes, exosomes offer advantages in terms of better functions and longer circulation time, which may be due to their natural origin [22, 25, 42, 58]. However, for the use of exosomes as a DDS, it is important to address several limitations in exosome preparation procedures such as low production yield, expensive and difficult purification process, differences in characters and functions of exosomes produced by different protocols [28]. According to previous studies, exosomes generated from NK-92MI cells display an anti-tumor ability, suggestive of their application for the delivery of chemotherapeutics to tumor therapy [17—19, 21, 23]. For example, Han et al. investigated NK-derived exosome-embedded paclitaxel (PTX-NK-exos), and the drug-loaded exosomes effectively inhibited the proliferation and induced apoptosis in breast cancer cells [21].

EM have been recently developed to overcome the limitations of exosomes [59, 60]. For example, the large-scale production of EM was feasible through the direct extrusion of cells via microfilters; and the characteristics and bio-functions of EM were similar to those of exosomes [40, 41, 61]. Gho et al. [41] produced doxorubicin-loaded EM by the breakdown of macrophages and confirmed

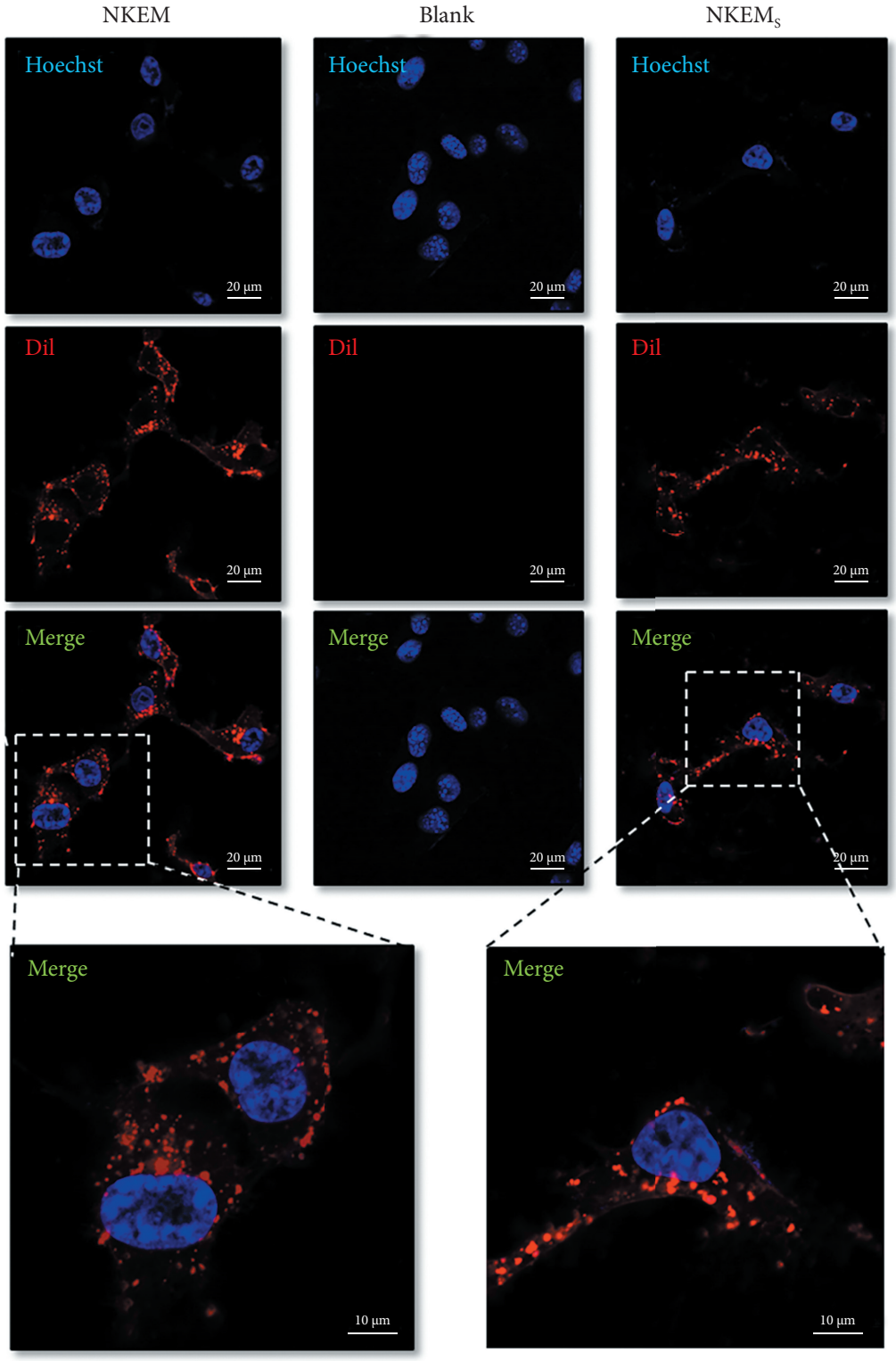

Fig. 3. Accumulation of NKEM/NKEM-S in BHT101/F cells

their antitumor effects following systemic injection. Furthermore, EM from pancreatic β-cells have been developed and applied for the treatment of diabetes [62]. Not only cells but also gram-negative bacteria have been used for generating EM, which exhibited antibacterial and antitumor responses [37, 38] at both *in vitro* and *in vivo* studies. The results of a recent study are similar to our results in that the combination of exosomes and sorafenib improved the targeting ability of the drug, reduced toxic effects on normal cells, allowed for sustained drug release, and indicated the antitumor impact

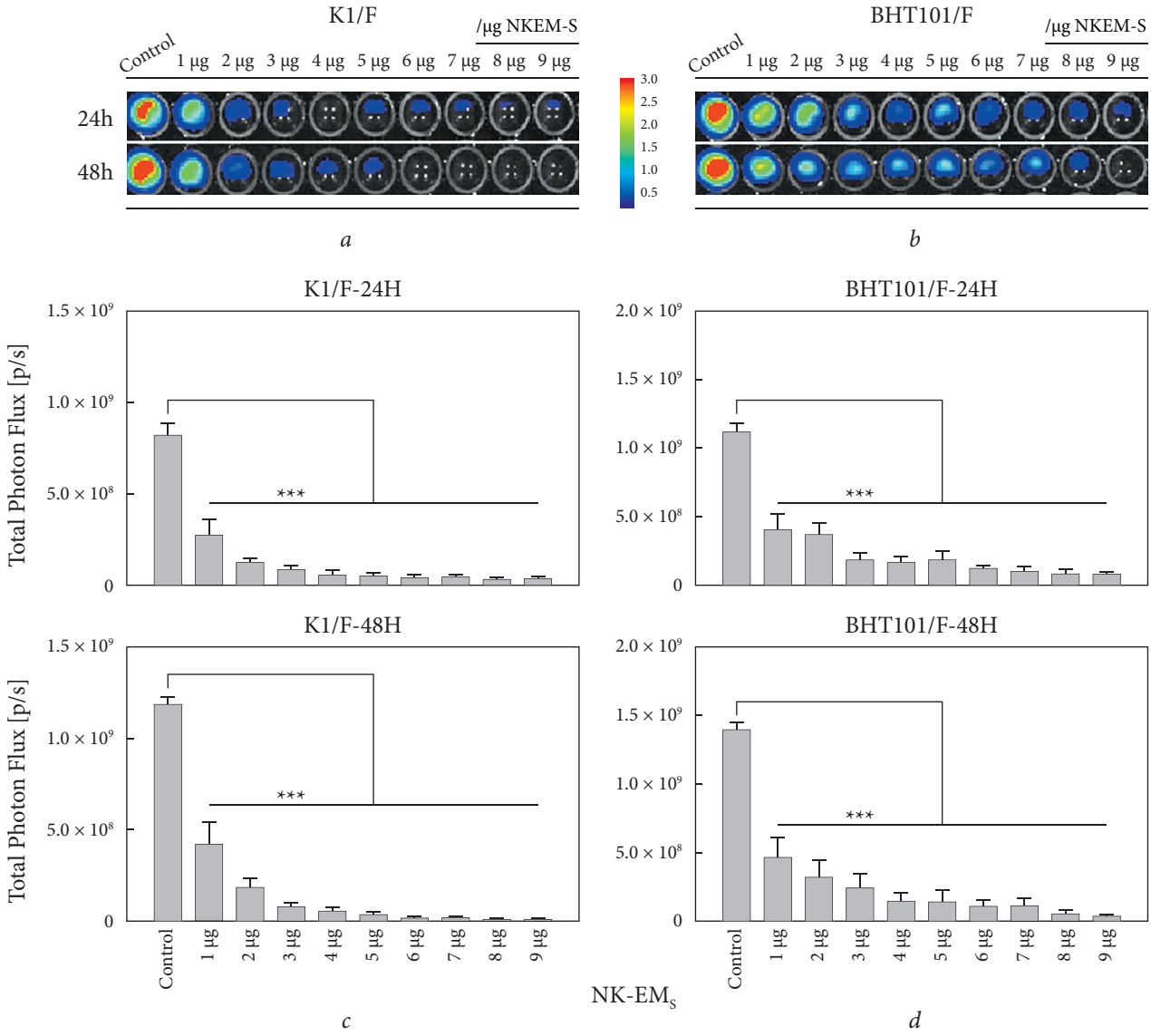

Fig. 4. *In vitro* antiproliferative ability of NKEM-S to K1/F and BHT101/F cells assessed by BLI. The killing effect of NKEM-S on K1/F cells was shown in (*a*, *c*), and BHT101/F cells showed lower sensitivity to NKEM-S compared to K1/F cells (*b*, *d*). Experiments were performed at least in triplicates, and mean ± SD was plotted, *** $p < 0.001$ (by Student's *t*-test)

on the breast cancer cells [63]. Even the exosomes from the NK cells have been widely explored, while the EMs derived from NK cells have not been applied for the DDS in cancer therapy especially for thyroid cancer. In the present study, sorafenib, widely used as a targeted therapeutic agent for various cancers including thyroid cancers, was loaded into NKEM and applied to treat DTC and ATC cells. As shown in Fig. 2, *b*, both NKEM and NKEM-S were spherical and had a size of approximately 100 nm. Although many drug loading methods such as incubation, saponification, permeabilities, and sonication have been reported, it remains unclear whether these loading strategies disrupt the integrity, stability, function, and loading efficiency of nanovesicles [39, 41, 64], which is a direction for subsequent research.

DDS used for therapeutic application should be able to deliver the incorporated therapeutic agents to the target site and avoid RES identification, especially macrophages that may consume foreign bodies by phagocytosis [38, 39, 64]. Several synthetic drug delivery systems, including liposomes, gold nanoparticles, and polymeric nanoparticles, have been developed and used in preclinical and clinical applications [40]. Although these preparations have shown promising results, cell-produced DDS can better avoid RES recognition, across en-

dothelial barriers, and provide better options for future experimental studies [65, 66]. In the current study, we developed a sorafenib-loaded NKEM and demonstrated its efficient delivery into thyroid cancer cells as shown in Fig. 3. In addition, the *in vitro* killing ability of NKEM-S against two thyroid cancer cell lines was also confirmed (Fig. 4), but the lack of in vivo experiments was the one limitation of this study. Nevertheless, NKG2D-equipped nanoparticles had promising applications in tumor-targeting ability in mice models of colon cancer [67]. Meanwhile, the expression of NKG2D in the NK membrane has been confirmed by several studies [68, 69]. These results show that nanoparticles from NK cells can inherit the cell with membrane components, exhibit good biosafety, and have a tumor-centric biodistribution in *in vivo* experiments. Therefore, even though no *in vivo* experiments were performed on NKEM in this study, the application of NKEM to the oncology treatment including thyroid cancer, remains promising.

To sum up, we successfully loaded sorafenib into NKEM, and the nanoparticles exhibited higher *in vitro* killing ability against thyroid cancer cells, even the ATC cell line. Results of the study suggest that NKEM-S may serve as auspicious nanoparticles for DDS and become therapeutic agents for the treatment of ATC.

Author contributions

Conceptualization: Liya Zhu & Byeong-Cheol Ahn; Data curation: Liya Zhu; Funding acquisition: Byeong-Cheol Ahn; Project administration: Byeong-Cheol Ahn; Writing the original draft: Liya Zhu; and Writing, editing, and review: Liya Zhu & Byeong-Cheol Ahn, and supervision: Byeong-Cheol Ahn.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT), Grant/Award Number: NRF-2022R1A2C2005057.

REFERENCES

1. Araque KA, Gubbi S, Klubo-Gwiezdzinska J. Updates on the management of thyroid cancer. *Horm Metab Res*. 2020;52(8):562-577. https://doi.org/10.1055/a-1089-7870
2. Oh JM, Ahn BC. Molecular mechanisms of radioactive iodine refractoriness in differentiated thyroid cancer: Impaired sodium iodide symporter (NIS) expression owing to altered signaling pathway activity and intracellular localization of NIS. *Theranostics*. 2021; 11(13):6251-6277. https://doi.org/10.7150/thno.57689
3. Fagin JA, Wells Jr. SA. Biologic and clinical perspectives on thyroid cancer. *N Engl J Med*. 2016;375(11):1054-1067. https://doi.org/10.1056/NEJMra1501993
4. Bible KC, Kebebew E., Brierley J, et al. 2021 American Thyroid Association guidelines for management of patients with anaplastic thyroid cancer. *Thyroid*. 2021;31(3):337-386. https://doi.org/10.1089/thy.2020.0944
5. Tuccilli C, Baldini E, Sorrenti S, et al. CTLA-4 and PD-1 ligand gene expression in epithelial thyroid cancers. *Int J Endocrinol*. 2018;2018:1742951. https://doi.org/10.1155/2018/1742951
6. Subbiah V, Kreitman RJ, Wainberg ZA, et al. Dabrafenib and Trametinib treatment in patients with locally advanced or metastatic BRAF V600-mutant anaplastic thyroid cancer. *J Clin Oncol*. 2018;36(1):7-13. https://doi.org/10.1200/JCO.2017.73.6785.
7. Lee CH, Jung JH, Son SH, et al. Risk factors for radioactive iodine-avid metastatic lymph nodes on post I-131 ablation SPECT/CT in low- or intermediate-risk groups of papillary thyroid cancer. *PLoS One*. 2018;13(8):e0202644. https://doi.org/10.1371/journal.pone.0202644
8. Jeong JH, Kong EJ, Jeong SY, et al. Clinical outcomes of low-dose and high-dose postoperative radioiodine therapy in patients with intermediate-risk differentiated thyroid cancer. *Nucl Med Commun*. 2017;38(3):228-233. https://doi.org/10.1097/MNM.0000000000000636
9. Liang JJ, Feng WJ, Li R, et al. Analysis of the value and safety of thyroid-stimulating hormone in the clinical efficacy of patients with thyroid cancer. *World J Clin Cases*. 2023;11(5):1058-1067. https://doi.org/10.12998/wjcc.v11.i5.1058
10. Zhu L, Li XJ, Kalimuthu S, et al. Natural killer cell (NK-92MI)-based therapy for pulmonary metastasis of anaplastic thyroid cancer in a nude mouse model. *Front Immunol*. 2017;8:816. https://doi.org/10.3389/fimmu.2017.00816
11. Gounder MM, Mahoney MR, Van Tine BA, et al. Sorafenib for advanced and refractory desmoid tumors. *N Engl J Med*. 2018;379(25):2417-2428. https://doi.org/10.1056/NEJMoa1805052
12. Sumbly V, Landry I, Sneed C, et al. Leukemic stem cells and advances in hematopoietic stem cell transplantation for acute myeloid leukemia: a narrative review of clinical trials. *Stem Cell Investig*. 2022;9:10. https://doi.org/10.21037/sci-2022-044

13. Feng F, Jiang Q, Jia H, et al. Which is the best combination of TACE and Sorafenib for advanced hepatocellular carcinoma treatment? A systematic review and network meta-analysis. *Pharmacol Res*. 2018. https://doi.org/10.1016/j.phrs.2018.06.021
14. Thomas L, Lai SY, Dong W, et al. Sorafenib in metastatic thyroid cancer: a systematic review. *Oncologist*. 2014;19(3):251-258. https://doi.org/10.1634/theoncologist.2013-0362
15. Rehman FU, Liu Y, Zheng M, Shi B. Exosomes based strategies for brain drug delivery. *Biomaterials*. 2022;293:121949. https://doi.org/10.1016/j.biomaterials.2022.121949
16. Mondal J, Pillarisetti S, Junnuthula V, et al. Hybrid exosomes, exosome-like nanovesicles and engineered exosomes for therapeutic applications. *J Control Release*. 2022; 353:1127-1149. https://doi.org/10.1016/j.jconrel.2022.12.027
17. Aarsund M, Nyman TA, Stensland ME, et al. Isolation of a cytolytic subpopulation of extracellular vesicles derived from NK cells containing NKG7 and cytolytic proteins. *Front Immunol*. 2022;13:977353. https://doi.org/10.3389/fimmu.2022.977353
18. Aarsund M, Segers FM, Wu Y, et al. Comparison of characteristics and tumor targeting properties of extracellular vesicles derived from primary NK cells or NK-cell lines stimulated with IL-15 or IL-12/15/18. *Cancer Immunol Immunother*. 2022;71(9):2227-2238. https://doi.org/10.1007/s00262-022-03161-0
19. Enomoto Y, Li P, Jenkins LM, et al. Cytokine-enhanced cytolytic activity of exosomes from NK Cells. *Cancer Gene Ther*. 2022;29(6):734-749. https://doi.org/10.1038/s41417-021-00352-2
20. Fabbri M. Natural Killer Cell-derived vesicular miRNAs: a new anticancer approach? *Cancer Res*. 2020;80(1):17-22. https://doi.org/10.1158/0008-5472.CAN-19-1450
21. Han D, Wang K, Zhang T, et al. Natural killer cell-derived exosome-entrapped paclitaxel can enhance its anti-tumor effect. *Eur Rev Med Pharmacol Sci*. 2020;24(10):5703-5713. https://doi.org/10.26355/eurrev_202005_21362
22. Wang G, Hu W, Chen H, et al. Cocktail strategy based on NK cell-derived exosomes and their biomimetic nanoparticles for dual tumor therapy. *Cancers (Basel)*. 2019;11(10):1560. https://doi.org/10.3390/cancers11101560
23. Wu J, Wu D, Wu G, et al. Scale-out production of extracellular vesicles derived from natural killer cells via mechanical stimulation in a seesaw-motion bioreactor for cancer therapy. *Biofabrication*. 2022; 14(4). https://doi.org/10.1088/1758-5090/ac7eeb
24. Zhao M, van Straten D, Broekman MLD, et al. Nanocarrier-based drug combination therapy for glioblastoma. *Theranostics*. 2020;10(3):1355-1372. https://doi.org/10.7150/thno.38147
25. Rehman FU, Liu Y, Zheng Y, Shi B. Exosomes based strategies for brain drug delivery. *Biomaterials*. 2023;293:121949. https://doi.org/10.1016/j.biomaterials.2022.121949
26. Lakhal S, Wood MJ. Exosome nanotechnology: an emerging paradigm shift in drug delivery: exploitation of exosome nanovesicles for systemic *in vivo* delivery of RNAi heralds new horizons for drug delivery across biological barriers. *Bioessays*. 2011;33(10):737-741. https://doi.org/10.1002/bies.201100076
27. Kim SM, Kim HS. Engineering of extracellular vesicles as drug delivery vehicles. *Stem Cell Investig*. 2017;4:74. https://doi.org/10.21037/sci.2017.08.07
28. Kim OY, Lee J, Gho YS. Extracellular vesicle mimetics: Novel alternatives to extracellular vesicle-based theranostics, drug delivery, and vaccines. *Semin Cell Dev Biol*. 2017;67:74-82. https://doi.org/10.1016/j.semcdb.2016.12.001
29. Sun JX, Xu JZ, An Y, et al. Future in precise surgery: Fluorescence-guided surgery using EVs derived fluorescence contrast agent. *J Control Release*. 2022;353:832-841. https://doi.org/10.1016/j.jconrel.2022.12.013
30. Shao J, Zaro J, Shen Y. Advances in exosome-based drug delivery and tumor targeting: from tissue distribution to intracellular fate. *Int J Nanomedicine*. 2020;15:9355-9371. https://doi.org/10.2147/IJN.S281890
31. Zheng X, Sun K, Liu Y, et al. Resveratrol-loaded macrophage exosomes alleviate multiple sclerosis through targeting microglia. *J Control Release*. 2022;353:675-684. https://doi.org/10.1016/j.jconrel.2022.12.026
32. Li J, Li J, Peng Y, et al. Dendritic cell derived exosomes loaded neoantigens for personalized cancer immunotherapies. *J Control Release*. 2022;353:423-433. https://doi.org/10.1016/j.jconrel.2022.11.053
33. Jia R, Cui K, Li Z, et al. NK cell-derived exosomes improved lung injury in mouse model of Pseudomonas aeruginosa lung infection. *J Physiol Sc*i. 2020;70(1):50. https://doi.org/10.1186/s12576-020-00776-9
34. Li D, Wang Y, Jin X, et al. NK cell-derived exosomes carry miR-207 and alleviate depression-like symptoms in mice. *J Neuroinflammation*. 2020;17(1):126. https://doi.org/10.1186/s12974-020-01787-4
35. Wang L, Wang Y, Quan J. Exosomal miR-223 derived from natural killer cells inhibits hepatic stellate cell activation by suppressing autophagy. *Mol Med*. 2020;26(1):81. https://doi.org/10.1186/s10020-020-00207-w
36. Federici C, Shahaj E, Cecchetti S, et al. Natural-Killer-derived extracellular vesicles: immune sensors and interactors. *Front Immunol*. 2020;11:262. https://doi.org/10.3389/fimmu.2020.00262
37. Kim OY, Park HT, Dinh NTH, et al. Bacterial outer membrane vesicles suppress tumor by interferon-gamma-mediated antitumor response. *Nat Commun*. 2017;8(1):626. https://doi.org/10.1038/s41467-017-00729-8.
38. Kim OY, Choi SJ, Jang SC, et al. Bacterial protoplast-derived nanovesicles as vaccine delivery system against bacterial infection. *Nano Lett*. 2015;15(1):266-274. https://doi.org/10.1021/nl503508h
39. Kim OY, Dinh NT, Park HT, et al. Bacterial protoplast-derived nanovesicles for tumor targeted delivery of chemotherapeutics. *Biomaterials.* 2017;113:68-79. https://doi.org/10.1016/j.biomaterials.2016.10.037

40. Goh WJ, Zou S, Ong WY, et al. Bioinspired cell-derived nanovesicles versus exosomes as drug delivery systems: a cost-effective alternative. *Sci Rep*. 2017;7(1):14322. https://doi.org/10.1038/s41598-017-14725-x
41. Jang SC, Kim OY, Yoon CM, et al. Bioinspired exosome-mimetic nanovesicles for targeted delivery of chemotherapeutics to malignant tumors. *ACS Nano*. 2013;7(9):7698-7710. https://doi.org/10.1021/nn402232g
42. Liu H, Deng S, Han L, et al. Mesenchymal stem cells, exosomes and exosome-mimics as smart drug carriers for targeted cancer therapy. *Colloids Surf B Biointerfaces*. 2022;209(Pt 1):112163. https://doi.org/10.1016/j.colsurfb.2021.112163
43. Wang J, Li M, Jin L, et al. Exosome mimetics derived from bone marrow mesenchymal stem cells deliver doxorubicin to osteosarcoma *in vitro* and in vivo. *Drug Deliv*. 2022;29(1):3291-3303. https://doi.org/10.1080/10717544.2022.2141921
44. Zhang W, Wang L, Guo H, et al. Dapagliflozin-loaded exosome mimetics facilitate diabetic wound healing by HIF-1alpha-mediated enhancement of angiogenesis. *Adv Healthc Mater*. 2022:e2202751. https://doi.org/10.1002/adhm.202202751
45. Rajendran RL, Gangadaran P, Kwack MH, et al. Engineered extracellular vesicle mimetics from macrophage promotes hair growth in mice and promotes human hair follicle growth. *Exp Cell Res*. 2021;409(1):112887. https://doi.org/10.1016/j.yexcr.2021.112887
46. Zhu L, Kalimuthu S, Gangadaran P, et al. Exosomes derived from Natural Killer Cells exert therapeutic effect in melanoma. *Theranostics*. 2017;7(10):2732-2745. https://doi.org/10.7150/thno.18752
47. Kalimuthu S, Gangadaran P, Rajendran RL, et al. A new approach for loading anticancer drugs into mesenchymal stem cell-derived exosome mimetics for cancer therapy. *Front Pharmacol*. 2018;9:1116. https://doi.org/10.3389/fphar.2018.01116.
48. Gangadaran P, Hong CM, Oh JM, et al. *In vivo* non-invasive imaging of radio-labeled exosome-mimetics derived from red blood cells in mice. *Front Pharmacol*. 2018;9:817. https://doi.org/10.3389/fphar.2018.00817.
49. Gangadaran P, Rajendran RL, Lee HW, et al. Extracellular vesicles from mesenchymal stem cells activates VEGF receptors and accelerates recovery of hindlimb ischemia. *J Control Release*. 2017;264:112-126. https://doi.org/:10.1016/j.jconrel.2017.08.022.
50. Kalimuthu S, Gangadaran P, Li XJ, et al. *in vivo* therapeutic potential of mesenchymal stem cell-derived extracellular vesicles with optical imaging reporter in tumor mice model. *Sci Rep*. 2016;6:30418. https://doi.org/10.1038/srep30418.
51. Imai T, Takahashi Y, Nishikawa M, et al. Macrophage-dependent clearance of systemically administered B16BL6-derived exosomes from the blood circulation in mice. *J Extracell Vesicles*. 2015;4:26238. https://doi.org/10.3402/jev.v4.26238
52. Vadevoo SMP, Kim JE, Gunassekaran GR, et al. IL4 receptor-targeted proapoptotic peptide blocks tumor growth and metastasis by enhancing antitumor immunity. *Mol Cancer Ther*. 2017;16(12):2803-2816. https://doi.org/10.1158/1535-7163.MCT-17-0339
53. Munagala R, Aqil F, Jeyabalan J, Gupta RC. Bovine milk-derived exosomes for drug delivery. *Cancer Let*t. 2016;371(1):48-61. https://doi.org/10.1016/j.canlet.2015.10.020
54. Vashisht M, Rani P, Onteru SK, Singh D. Curcumin encapsulated in milk exosomes resists human digestion and possesses enhanced intestinal permeability *in vitro*. *Appl Biochem Biotechnol*. 2017;183(3):993-1007. https://doi.org/10.1007/s12010-017-2478-4.
55. Haney MJ, Klyachko NL, Zhao Y, et al. Exosomes as drug delivery vehicles for Parkinson's disease therapy. *J Control Release*. 2015;207:18-30. https://doi.org/10.1016/j.jconrel.2015.03.033
56. Xin H, Li Y, Buller B, et al. Exosome-mediated transfer of miR-133b from multipotent mesenchymal stromal cells to neural cells contributes to neurite outgrowth. *Stem Cells*. 2012;30(7):1556-1564. https://doi.org/:10.1002/stem.1129
57. Katakowski M, Buller B, Zheng X, et al. Exosomes from marrow stromal cells expressing miR-146b inhibit glioma growth. *Cancer Lett.* 2013;335(1):201-204. https://doi.org/10.1016/j.canlet.2013.02.019
58. Sun D, Zhuang X, Xiang X, et al. A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. *Mol Ther*. 2010;18(9):1606-1614. https://doi.org/10.1038/mt.2010.105
59. Hu CM, Fang RH, Wang KC, et al. Nanoparticle biointerfacing by platelet membrane cloaking. *Nature.* 2015;526(7571):118-121. https://doi.org/10.1038/nature15373
60. Wei X, Ying M, Dehaini D, et al. Nanoparticle functionalization with platelet membrane enables multifactored biological targeting and detection of atherosclerosis. *ACS Nano*. 2017;12(1):109-116. https://doi.org/10.1021/acsnano.7b07720
61. Molinaro R, Corbo C, Martinez JO, et al. Biomimetic proteolipid vesicles for targeting inflamed tissues. *Nat Mater.* 2016;15(9):1037-1046. https://doi.org/10.1038/nmat4644
62. Oh K, Kim SR, Kim DK, et al. *In vivo* differentiation of therapeutic insulin-producing cells from bone marrow cells via extracellular vesicle-mimetic nanovesicles. *ACS Nano*. 2015;9(12):11718-11727. https://doi.org/10.1021/acsnano.5b02997

63. Hashemi ZS, Ghavami M, Kiaie SH, et al. Novel delivery of sorafenib by natural killer cell-derived exosomes-enhanced apoptosis in triple-negative breast cancer. *Nanomedicine (Lond)*. 2023;18(5):437-453. https://doi.org/10.2217/nnm-2022-0237
64. Tan S, Wu T, Zhang D, Zhang Z. Cell or cell membrane-based drug delivery systems. *Theranostics*. 2015;5(8):863-881. https://doi.org/10.7150/thno.11852
65. Yuan D, Zhao Y, Banks WA, et al. Macrophage exosomes as natural nanocarriers for protein delivery to inflamed brain. *Biomaterials*. 2017;142:1-12. https://doi.org/10.1016/j.biomaterials.2017.07.011
66. Essola JM, Zhang M, Yang H, et al. Exosome regulation of immune response mechanism: Pros and cons in immunotherapy. *Bioact Mater*. 2024;32:124-146. https://doi.org/10.1016/j.bioactmat.2023.09.018
67. Tan L, Han S, Ding S, et al. Chitosan nanoparticle-based delivery of fused NKG2D-IL-21 gene suppresses colon cancer growth in mice. *Int J Nanomedicine*. 2017;12:3095-3107. https://doi.org/10.2147/IJN.S128032
68. Wu D, Shou X, Zhang Y, et al. Cell membrane-encapsulated magnetic nanoparticles for enhancing natural killer cell-mediated cancer immunotherapy. *Nanomedicine*. 2021;32:102333. https://doi.org/10.1016/j.nano.2020.102333
69. Pitchaimani A, Nguyen TDT, Marasini R, et al. Biomimetic Natural Killer membrane camouflaged polymeric nanoparticle for targeted bioimaging. *Adv Funct Mater*. 2019;29(4):1806817. https://doi.org/10.1002/adfm.201806817

Submitted: April 12, 2024

Л. Жу [1, 2], *Б-К. Ан* [1, 2, 3, 4]

[1] Відділення медичної радіології, медичний факультет, Національний університет Кьюнгпоок, Дейгу, Республіка Корея
[2] Інститут серцево-судинної хірургії, Національний університет Кьюнгпоок, Дейгу, Республіка Корея
[3] Відділення медичної радіології, госпіталь Національний університет Кьюнгпоок, Дейгу, Республіка Корея
[4] Освітня програма з біомедичних наук для обдарованої молоді, медичний факультет, Національний університет Кьюнгпоок, Дейгу, Республіка Корея

ЕКЗОСОМОМІМЕТИКИ З КЛІТИН — ПРИРОДНИХ КІЛЕРІВ ЯК ПРИРОДНІ НАНОНОСІЇ ДЛЯ ДОСТАВКИ *IN VITRO* ХІМІОТЕРАПЕВТИЧНИХ ЗАСОБІВ ДО КЛІТИН РАКУ ЩИТОПОДІБНОЇ ЗАЛОЗИ

Стан питання. Дослідження екзосом як один з розділів нанотехнології має перспективи для діагностики та лікування різноманітних захворювань. Однак, одержання екзосом є досить непростим, займає багато часу, і має невисокий вихід кінцевого продукту. Екзосомоміметики (ЕМ) нагадують екзосоми за своїми характеристиками, але їх можна одержати із досить високим виходом. **Мета** дослідження полягала в одержанні ЕМ з клітин — природних кілерів (ПК) — ПКЕМ, навантажених сорафенібом та вивченні їхньої цитотоксичної здатності щодо клітин ліній раку щитоподібної залози. **Матеріали та методи**. ПКЕМ, навантажені сорафенібом (ПКЕМ-С), одержували змішуванням сорафенібу з ПК під час продукування ПКЕМ. ПКЕМ та ПКЕМ-С характеризували шляхом аналізу траєкторій руху наночастинок для визначення їхніх розмірів. Визначали також поглинання цих наночастинок клітинами раку щитоподібної залози *in vitro* та їхню цитотоксичну здатність щодо цих клітин. Використовували методи конфокальної лазерної мікроскопії та біолюмінесцентної візуалізації. **Результати**. Клітини раку щитоподібної залози поглинали ПКЕМ та ПКЕМ-С. Методом біолюмінесцентної візуалізації підтверджено цитотоксичний та антипроліферативний ефекти ПКЕМ-С у відношенні клітин двох ліній раку щитоподібної залози *in vitro*. Що особливо важливо, ПКЕМ-С демонстрували цитотоксичну дію на клітини анапластичного раку щитоподібної залози. **Висновки**. Навантажені сорафенібом ПКЕМ здатні спричиняти загибель клітин раку щитоподібної залози, особливо анапластичного раку, *in vitro*. Це відкриває нові можливості систем доставки лікарських засобів для лікування хворих на рак щитоподібної залози.

Ключові слова: рак щитоподібної залози, екзосомоміметики, природні кілери, імунотерапія, системи доставки лікарських засобів.