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EDITORIAL

Cancer photodynamic therapy with chlorin e6-loaded, goat milk-derived extracellular vesicles: [^{18}F]FDG lights up the way

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Photodynamic therapy (PDT), a two-step process that utilizes light energy to excite a photosensitizer to generate harmful singlet oxygen, is an effective treatment approach for several types of cancer [1–3]. Compared to conventional cancer treatments, such as chemotherapy and surgery, PDT offers the advantages of non-invasiveness, localized therapy, and high spatiotemporal selectivity [4–6]. Therefore, PDT has the potential to minimize therapeutic side effects and allow repeated treatments without inducing drug resistance. However, the efficacy of this technique is heavily influenced by the penetration depth of external light source and the accumulation of photosensitizer at the target site [7, 8]. Clinical application of PDT is thus restricted to only superficial tumors in most cases.

Given these limitations, recent efforts have focused on exploring alternative excitation light sources with improved penetration depth and developing innovative nanocarriers for precise delivery of photosensitizers to tumors. Cerenkov luminescence (CL) is a blue-weighted light emission (250–600 nm) that originates from fast-charged particles as a result of the decay of positron emission tomography (PET) radionuclides, such as ^{18}F [9–11]. CL, being an internal excitation source, can increase the light fluence within the target tissue, thus overcoming the issue of light penetration [12]. Therefore, CL has the potential to locally excite photosensitizers and induce PDT in deep-seated tumors with high specificity.

The choice of photosensitizers and their precise delivery are important factors that affect the antitumor efficacy of CL-induced PDT [13, 14]. At present, amphiphilic chlorin e6 (Ce6) is a promising photosensitizer for PDT since its broad absorption (400 and 660 nm) matches well with the CL spectrum for optimal generation of singlet oxygen [15–17]. However, application of PDT using Ce6 is limited due to its hydrophobic nature, low bioavailability, and non-specific phototoxicity [18]. Thus, an efficient drug delivery system (DDS) is urgently needed to improve the delivery of Ce6 and yield high uptake in tumor cells [7]. Extracellular vesicles (EVs) are biologically-derived, liposome-like structures that have become increasingly utilized as a natural drug carrier owing to their simplicity of production and low levels of immunogenicity [19]. To protect from extracellular degradation, the therapeutic cargo is typically encapsulated in the lipid membrane bilayer of EVs [20]. Over the last decade, EVs have demonstrated excellent experimental results for drug delivery in autoimmune diseases, degenerative diseases, cardiovascular diseases, and many others [21–25]. Therefore, EVs serve as a good vehicle option for Ce6 photosensitizers to enhance their retention at tumor sites and reduce off-target accumulation.

In this issue of the *European Journal of Nuclear Medicine and Molecular Imaging*, Guo et al. reported a cleverly designed and well-executed study of a Ce6-loaded goat milk-derived extracellular vesicle (GEV) nanoplatform in conjunction with [^{18}F]FDG for real-time fluorescence imaging and CL-induced PDT [26]. This study represents a novel combination of using food-derived drug carrier materials and approved tumor-targeted radiotracers, which could overcome the limitations of traditional PDT methods and pave the path for clinical implementation. In this work, GEV was extracted by sequential ultracentrifugation and Ce6 photosensitizer was then loaded in GEV through co-incubation. The large size and unique fusogenic property of GEV@Ce6 could improve the tumor delivery efficiency via passive targeting. Importantly, GEV@Ce6 exhibited good stability, and the optical properties of Ce6 were not affected by GEV encapsulation, which is crucial for CL-induced fluorescence imaging and PDT.

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From in vitro studies, more GEV@Ce6 was taken up by 4T1 tumor cells compared to free Ce6, which implied that GEV encapsulation is necessary for enhanced delivery of Ce6. Unlike free Ce6, which showed considerable cytotoxicity in 4T1 tumor cells, GEV@Ce6, [^{18}F]FDG, and GEV showed no cytotoxicity, thus indicating the excellent biocompatibility of these components. After co-incubation of GEV@Ce6 and [^{18}F]FDG, the reactive oxygen species (ROS) level was substantially higher than that of the control group. As a result, 4T1 cell viability was markedly reduced due to the generation of cytotoxic ROS from CL-induced PDT. Furthermore, the spectral coupling of Ce6 and CL from [^{18}F]FDG could lead to fluorescence emission owing to Cerenkov radiation energy transfer (CRET). CRET refers to the transfer of CL energy from a radionuclide to a fluorescent receptor or photosensitizer. In this case, the solution containing both [^{18}F]FDG and GEV@Ce6 emitted the highest fluorescence intensity compared to the other groups. The relative radiance in the red-filtered images (> 620 nm) increased with the concentration of GEV@Ce6, while that of the blue-filtered images (< 520 nm) decreased, consistent with the absorbance of CL and emission by GEV@Ce6. However, CRET was not detected from the nanoplatform in the absence of [^{18}F]FDG. This finding suggests that fluorescence signal from CRET could be used to confirm the co-localization of the nanoplatform and radiotracer.

In vivo fluorescence imaging of 4T1 tumor-bearing mice showed that GEV@Ce6 could retain in the tumor tissue more effectively compared to free Ce6 via the enhanced permeability and retention effect. From PET/CT and CL images, sufficient CL signal was found at the tumor site due to the accumulation of tumor-avid [^{18}F]FDG. These results demonstrated the potential for CL-induced cancer theranostic application since both of the nanoparticle agent and radiotracer could be efficiently delivered to the tumors. Subsequently, studies of the combined antitumor effect showed that the 35-day survival rate of mice treated with GEV@Ce6 and [^{18}F]FDG was 40%, whereas mice in all other groups reached their endpoints (0%). Therefore, CL-induced PDT by GEV@Ce6 and [^{18}F]FDG could significantly suppress tumor growth and prolong overall survival time. Lastly, blood biochemistry and histochemistry analyses of major organs showed no significant systemic toxicity at 20 days after treatment, demonstrating the good safety profiles of the nanoplatform and combined therapy method.

In summary, this is an intriguing study where the authors have validated the use of a Ce6-loaded GEV nanosystem for [^{18}F]FDG CL-triggered PDT for cancer theranostics. They have harnessed the power of CL as a depth-independent, internal excitation source for light-responsive therapeutics. This is also the first report to describe the use of biocompatible GEV as a natural delivery vehicle that could circumvent the shortcomings of artificial DDS and insoluble photosensitizers, such as fast blood clearance and non-specific uptake. Moreover, the authors have maximized the co-localization of GEV@Ce6 and [^{18}F]FDG at the tumor site by injecting the radiotracer after high tumor accumulation of the nanoplatform was achieved. This strategy can effectively avoid possible liver damage as well as side effects from off-target retention in normal tissues. Note that these safety concerns often emerge when using nanoparticles loaded with both radionuclide and photosensitizer in a single platform. Therefore, the novel approach described herein certainly opens up new opportunities to develop promising and precise therapeutics for other types of cancer in the future. Overall, this report, which presents state-of-the-art advances with very encouraging results, is of high interest for the readership of EJNMMI.

Considerable effort has been made in recent years to expand the functions and applications of CL. For example, a Ce6-loaded hollow mesoporous silica nanoparticle with great drug loading capacity was intrinsically radiolabeled with an oxophilic ^{89}Zr radionuclide for CL-mediated PDT [27]. Interestingly, one group developed ^{89}Zr-labeled porphyrin-decorated magnetic nanoparticles to guide tumor delivery via an external magnetic field as well as provide treatment via CL-induced PDT [28]. In another example, transferrin-coated TiO_2 nanoparticles, in combination with PET radionuclides (e.g., ^{18}F and ^{64}Cu), were used to achieve CL-triggered PDT [29]. These studies have shown the importance of CL in improving the efficacy of cancer PDT. Thus, we expect to see follow-up studies that evaluate different combinations of radioisotopes and photosensitizers to further strengthen the CL light intensity and mitigate the deficiencies of traditional PDT.

The possibility of offtarget effects is always a challenge in any disease therapy. To this end, the current research aims to ensure the precise delivery of radionuclide and photosensitizer in order to decrease non-specific uptake [26]. First, EVs are present in a variety of peripheral biofluids, such as blood, urine, saliva, and milk [20]. Among these fluids, commercial goat milk is easily accessible and extraction of EVs is highly reproducible. Therefore, GEV is an ideal DDS with the capability to increase and extend Ce6 retention at tumor sites, while reducing the non-specific phototoxicity of Ce6. Second, [^{18}F]FDG is a common diagnostic radiotracer in clinical cancer applications. The utility of [^{18}F]FDG as an internal light source for PDT is favored by its ability to target various tumors, resulting in higher uptake and retention. These advantages make the combination of GEV@Ce6 and [^{18}F]FDG a superb approach for optimizing PDT efficacy and eliminating possible side effects.

Since the discovery of CL-induced PDT, more efforts should be made to amplify the CL light intensity and develop DDS with broad applicability. The intensity of CL produced by beta emitters is dependent on their particle energy [30].

In terms of PDT light source, it makes sense to choose a bright radioisotope with an intense CL emission for greater ROS generation and better therapeutic outcome. Instead of ^{18}F as mentioned above, ^{68}Ga is more suitable for clinical implementation, although their delivery method requires further exploration [31]. ^{90}Y is also a good alternative with higher CL intensity and longer half-life (64.2 h) compared to other radionuclides, but its biosafety profile could hamper the likelihood for clinical use [32]. In terms of EV delivery system, the current isolation methods need to be refined to avoid damages to EVs. All things considered, the strategy described herein has the potential to extend PDT to a wide variety of diseases [26], and we look forward to future developments in this exciting area.

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Declarations

Ethics approval and consent to participate This article does not contain any studies with human participants or animals performed by any of the authors.

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