Nanoparticle-based combination drug delivery systems for effective cancer treatment

He, Y.

Chapter 1

General introduction

General introduction

Cancer is the most lethal disease worldwide, claiming the lives of nearly 196 million people in 2021. Breast cancer had the highest incidence, with around 2.26 million cases, while skin cancer ranked fifth with around 1.2 million cases worldwide in 2021 [1]. These malignancies are associated with high incidence and aggressiveness. As a result, the search for treatments for malignant tumors has been a major focus of research in the field of cancer. Traditional cancer treatments, such as surgery, radiotherapy and systemic therapy, are often associated with severe damage, toxicity and drug resistance. Certain types of skin cancer located in delicate areas may not be amenable to surgical intervention. The rapid development of nanotechnology has spurred novel ideas, particularly within the realm of nanomedicine. The so-called nanomedicine is the combination of medicine and nanotechnology, using the characteristics of nanomaterials to study the characteristics of living bodies and discover new phenomena and laws from them, providing new theories and methods for human health and disease diagnosis and treatment. As nanomedicine research progresses, the unique physicochemical as well as biological properties of novel nanomaterials are being explored, and new diagnostic and therapeutic techniques are gradually breaking down the barriers of traditional medical technology, becoming one of the frontier technologies at the intersection of life and materials.

Nanomedicine Research Areas

Currently, nanomedicine research primarily focuses on developing nanoparticles (NPs) with detection, diagnostic, or therapeutic functions leveraging their unique properties. The unique properties of various types of nanomedicine make them a promising solution for treating numerous diseases [2]. For example, certain rare earth elements with optical, magnetic, or radioactive properties are utilized for imaging techniques such as optical imaging, MRI, CT imaging, and radionuclide imaging. These techniques greatly enhance the accuracy of disease detection and diagnosis, paving the way for more effective treatment strategies [3]. Notably, NPs possess the ability to encapsulate chemotherapeutic drugs and imaging agents, enabling precise control and monitoring of drug delivery [4,5]. This targeted drug delivery approach can minimize the side effects of chemotherapy and enhance its efficacy by concentrating the drug at the tumor site while reducing exposure to healthy cells [6,7]. Currently, many types of NPs have been approved by the FDA for use in the treatment of diseases, and a proportion of NPs are in clinical trials. Although nanomedicine has revolutionized treatment strategies for many diseases,

including cancer, the disease process is regulated by multiple mechanisms, and challenges such as drug insolubility and side effects persist. Thus, current research in nanomedicine primarily focuses on developing nanomaterials that can meet diverse medical purposes.

A variety of nanomaterials are under current study for diverse applications These materials encompass organic, inorganic, and hybrid nanoparticles [8]. Organic nanoparticles, such as polymeric nanoparticles, liposomes, and dendrimers, are composed of carbon-based materials. They offer tunable pores, low toxicity, biodegradability, and biocompatibility, making them suitable carriers for incorporating inorganic nanoparticles, chemotherapeutic drugs, or targeting structures with modified properties while retaining their original properties. Inorganic nanoparticles, typically composed of metals, metal oxides, rare-earth metals, or semiconductors, find wide-ranging applications in imaging, sensing, and catalysis. Examples include magnetic nanoparticles, quantum dots, and rare-earth metal nanoparticles. Rare-earth metal nanoparticles, a subset of inorganic NPs, are often doped into NPs as rare-earth ions. They are utilized for bioimaging, labeling, and detection due to their unique optical properties, photostability, low toxicity, and ability to convert near-infrared (NIR) photons into ultraviolet or visible light [9,10]. CaF_2, a fluorite crystal, exhibits good biocompatibility, low toxicity, and excellent optical properties. It finds application in various optical devices and serves as a fluorescent imaging probe and bioimaging contrast agent when doped into target NPs [11,12]. For example, Yu *et al*. utilized the optical properties of Ce, Gd , Nd and CaF_2 to synthesize CaF_2 : Ce, Gd, Nd NPs, which are not only biocompatible and optically transparent, but also produce deeper tissue penetration at 808 nm excitation, demonstrating the appeal of multimodal imaging probes [13].

Hybrid NPs are composed of both organic and inorganic materials, utilizing a nanomaterials platform. Through techniques such as surface modification, integration, and coupling technologies, these NPs are engineered to possess multifunctional capabilities including targeting, diagnosis (imaging) and treatment. They are poised to play a pivotal role in advancing the integration of precision diagnosis and treatment in the future. Currently, organic NPs are frequently used as carriers in synthesizing NPs with novel functions. These functions can involve the incorporation of inorganic NPs, chemotherapeutic drugs or targeting structures that undergo physical or chemical modifications while preserving their inherent properties [14]. Moreover, PLGA, phospholipids, and polyethylene glycols have been approved for use by the FDA [15]. Consequently, polymer-based NPs are gaining increasing attention in various areas of drug delivery, drug targeting and medical imaging. An overview diagram of the different types of nanoparticle applications is

depicted in Figure 1.

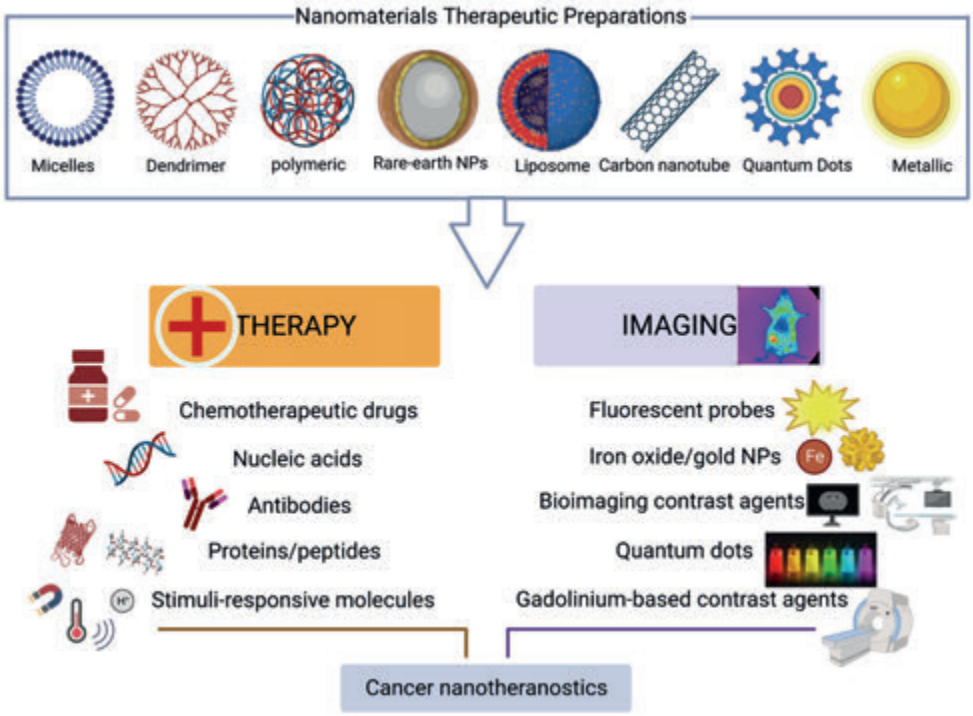

Figure 1. Nanomaterials based on metals, polymers, semiconductor quantum dots, carbon-based materials and liposomes for cancer treatment and therapy

Prolonging drug transport, controlling drug release, and targeting drugs to specific cells are major challenges in current clinical research in nanomedicine. Conventional drugs can induce serious side effects by affecting organs and tissues throughout the body. Nanomedicine enhances traditional cancer therapies by transitioning from systemic absorption of high drug doses to the delivering anti-cancer or immunotherapeutic drugs directly to cancer cells, the tumor microenvironment or the immune system, thereby reducing side effects significantly [16]. Scientists have explored multifunctional nanoparticle systems capable of drug delivery, carrying targeting ligands, and controlling drug release, leveraging the characteristics of various of NPs mentioned earlier [17]. NPs functionalized with ligands such as folate receptors, nucleic acid adaptors, low-density lipoprotein receptors, glucose transporters, and transferrin receptors have been used for drug delivery to tumor cells [18-20]. For example, based on the characteristic overexpression of folate receptors on the surface of cancer cells, Mazen *et al*. modified folate onto the surface of polyethylene glycolated PLGA-5- Fluorouracil

NPs and then acted on colon and breast cancers, resulting in a 4-fold reduction in IC_{50} [21]. While free growth hormone-releasing factor (peptide) is reported to be undetectable after 1.5 hours of subcutaneous injection, NPs carrying growth hormone-releasing factor are reported to release growth hormone-releasing factor slowly and continuously for up to 24 h [22]. Notably, the uptake and transport of NPs *in vivo* are greatly influenced by their size, and NPs with particle sizes below 70 nm can freely cross cell membranes into capillaries and are readily metabolized and cleared out of the body by the kidneys. Particle larger than 500 nm are typically cleared through phagocytosis by the liver and renal circulation. To avoid rapid clearance of the drug during transport, NPs in the particle size range of 100−500 nm are generally preferred. While the availability of NPs has enhanced drug bioavailability, further customization of NPs remains a major focus of current research.

Exploration and application of Nanomedicine in breast cancer

Breast cancer is characterized by the abnormal proliferation and growth of breast cells, triggered by various carcinogenic factors [23]. As the disease advances, cancer cells progressively infiltrate the breast tissue and may metastasize to nearby lymph nodes or other organs. Studies have reported a direct correlation between the development of breast cancer and abnormal secretion of estrone and estradiol. Because breast cancer cells have large amounts of estrogen receptor alpha (ERα) and epidermal growth factor 2 (ERBB2) on their surface when the receptors are activated, the proliferation of breast cancer cells is also activated and begins to grow uncontrollably [24]. Historically, breast cancer treatment primarily involved surgery accompanied by chemotherapy or radiotherapy. For example, depending on the characteristics of breast cancer, anti-ERBB2 (e.g. trastuzumab) [25] and small molecule tyrosine kinase inhibitors (e.g. lapatinib) that target ERBB2 therapy [26] are widely used in the treatment of breast cancer. Although effective in preventing the spread of malignant breast tumors, chemotherapy and radiotherapy often induce severe side effects, significantly impacting patients' quality of life. Hence, there is an urgent need to explore novel treatment options aimed at mitigating these side effects. The advent of nanomedicine has brought new hope to the treatment of breast cancer.

Among the most significant advantages of nanomedicine in breast cancer treatment is its capacity for actively targeting cancer cells. Breast cancer cells are known to overexpress specific receptors on their surfaces, such as folate receptors [27], HER2 receptor, estrogen receptor, low-density lipoprotein receptors [28], glucose

transporters [29], and transferrin receptors [30]. Consequently, researchers have used corresponding ligands as target molecules for delivering drugs to these cancer cells (Fig. 2). For example, Sahar *et al*. demonstrated that targeting Dox-PLGA copolymer NPs to folate receptors effectively minimized the side effects of chemotherapeutic agents while exhibiting potent anti-tumor properties [31]. Such targeted approaches can mitigate the side effects of chemotherapy and enhance treatment efficacy. In addition to active targeting, passive targeting can be achieved by modulating the tumor microenvironment through various means, including enhanced permeation and retention (EPR) effects (Fig. 2), enzyme sensitivity, heat sensitivity, or pH sensitivity. For example, Docetaxel-Incorporated Albumin-Lipid NPs that accumulate at the tumor site through the EPR effect have shown better anti-tumor efficacy in animal models compared to injections or tablets [32].

Targeting the tumor microenvironment primarily exploits the aberrant vascular system, extracellular matrix (ECM), and tumor-associated immune system. The virtually uncontrolled vascular proliferation in tumor tissue renders it a prime target for numerous nanoparticles designed to inhibit tumor angiogenesis. For example, Sengupta *et al*. used doxorubicin (DOX) as a core to form nanocells by attaching an anti-angiogenic agent (combretastatin) to the surface of PLGA. This approach effectively inhibit angiogenesis and tumor growth, while also addressing side effects such as cardiotoxicity caused by DOX [33]. Main components of the ECM, such as hyaluronic acid, collagen, and various enzymes (e.g., matrix metalloproteinases, hyaluronidase), promote cancer proliferation, invasion, and angiogenesis. The main components of ECM, hyaluronic acid, collagen, and various enzymes (e.g., matrix metalloproteinases, hyaluronidase), are able to promote cancer proliferation, invasion and angiogenesis. NPs can be designed to target these components, as demonstrated by the attachment of hyaluronic acid to the surface of epirubicin-based NPs by electrostatic adsorption. This modification enhances tumor penetration and the chemotherapeutic effect of epirubicin [34].

Therapies that target the immune system, such as cancer vaccine therapies, immune checkpoint blockade therapies, and immune system modulator therapies, are also rapidly advancing in the field of cancer treatment [35]. In light of these therapeutic approaches, scientists have devised novel nanoplatforms that not only offer superior efficacy compared to conventional therapies but also enhance the bioavailability of drugs [36,37]. The application of nanomedicine in cancer is promising and developing rapidly, with numerous studies on NPs relevant to the diagnosis and treatment of cancer already reaching the clinical research stage. For example, the combination of the polyethylene glycolated liposome doxorubicin (Caelyx®) and paclitaxel has demonstrated excellent anti-cancer

effects and reduced the risk of side effects in the clinical treatment of breast cancer (NCT03221881) [38]. The FDA approved liposomes of doxorubicin for the clinical treatment of cancer in 1995 [39]. In conclusion, nanomedicine has improved the cure rates of cancer by addressing the toxicity and lack of specificity of chemotherapeutic drugs.

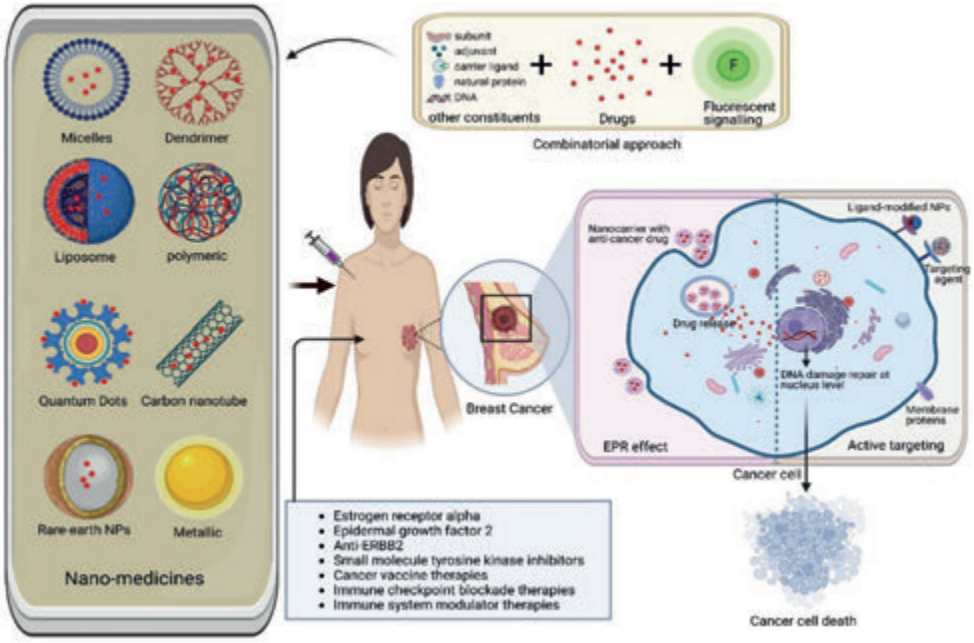

Figure 2. The application of Nanomedicine in breast cancer.

Research and applications of lipids in cancer therapy

Lipids are essential components of cells. They are involved in a variety of cellular activities, such as membrane synthesis, regulation of intracellular signalling, and energy production, immune response, apoptosis, and redox homeostasis, by providing energy to living cells [40]. The rapid proliferation of cancer cells requires large amounts of energy and nutrients, often leading to competition with healthy cells for these resources [41]. In order to meet the energy needs of cancer cells, they are forced to change their lipid metabolic profile, i.e. “reprogrammed”. Intensive studies on lipid metabolism have revealed that abnormal lipid metabolism in cancer, interactions between the tumor microenvironment (TEM) and lipid metabolism, and the activation of related oncogenic signaling pathways are the main factors contributing to the proliferation and spread of malignant tumors. This

reprogramming of lipid metabolism in tumors can regulate various pro-tumorigenic functions. Additionally, lipids play a critical role in the expression of immunogenic functions in immune-related cells within the TEM [42]. Therefore, the exploration of lipids' role in cancer therapy is increasingly becoming a focal point in cancer treatment.

Different types of lipids play distinct roles in tumor progression. Specific studies have revealed that certain lipids exhibit cancer-promoting effect. For example, *in vivo*, endogenous sphingolipids (such as sphingomyelin), synthesized from palmitic acid and serine or through the cleavage of fatty acid residues from ceramide, regulate cell proliferation, migration, and invasion via phosphorylation reactions [43,44]. Hiu *et al*. showed that exogenous palmitic acid (PA) promotes melanoma proliferation and invasion by activating the Akt signaling pathway [45]. However, not all lipids promote the proliferation and metastasis of tumor cells. Many studies have shown that polyunsaturated fatty acids (PUFAs) can inhibit tumor development. For example, Zhang *et al*. found that PUFAs can directly inhibit colon cancer proliferation by inhibiting the production of pro-inflammatory cytokines and enhancing the production of anti-inflammatory lipoxins [46]. Li *et al*. showed the beneficial effects of n-3 polyunsaturated fatty acids in inhibiting the proliferation of prostate cancer cells in a mouse model [47]. Furthermore, a clinical follow-up study showed that individuals with a higher intake of n-3 polyunsaturated fatty acids have a lower risk of developing breast cancer [48].

Lipid-based therapies have shown great potential in breast cancer management, with research focusing on various lipid-based formulations. Specifically, liposomal formulations of chemotherapeutic agents and lipid-based NPs for targeted drug delivery, and immunomodulatory agents, have demonstrated significant promise in this regard. For example, liposomal doxorubicin (Doxil) is a liposomal formulation of the chemotherapeutic agent doxorubicin [49]. Doxil has been shown to have improved pharmacokinetics and reduced toxicity compared to free doxorubicin, making it a promising treatment option for breast cancer [50]. In addition to liposomal formulations of chemotherapeutic agents, lipids have also been explored as potential targeted drug delivery vehicles. Lipids can be modified to include targeting moieties such as antibodies or peptides, which can specifically bind to receptors overexpressed on the surface of breast cancer cells [51,52]. Moreover, lipids have potential as immunomodulatory agents in breast cancer therapy. Lipid-based nanoparticles can deliver immunomodulatory agents such as cytokines and immunostimulatory nucleic acids to breast cancer cells, stimulating the immune system to recognize and eliminate cancer cells. For example, saturated fatty acid palmitic acid, encapsulated in PLGA NPs, shows promising anti-tumor and

immunomodulatory effects in breast cancer treatment [53]. In conclusion, lipids play an important role in the progression of breast cancer, and exploring the role of lipids in cancer therapy is becoming a focal point in the treatment of breast cancer.

Exploration and application of Nanomedicine in skin cancer

The emergence of nanomedicine has also brought new hope for the treatment of skin cancer. According to the latest data, breast cancer and skin cancer (melanoma and squamous cell carcinoma of the skin) are among the types of cancer that have seen the fastest rise in new cases in recent years [23]. Skin cancer encompasses three main types: basal cell carcinoma, cutaneous squamous cell carcinoma (cSCC), and melanoma (Fig. 3). Of these, cSCC consists of malignant proliferating cells that can rapidly invade the dermis from the epidermis and metastasize to other parts of the body or lymph nodes. Melanoma, being highly metastatic and aggressive, can be fatal and is often treated as a systemic disease. Treatment of skin cancer is often influenced by factors such as its location, lesion distribution, and migration extent [54]. Surgical procedures (such as curettage, laser treatment or dermabrasion) primarily target single visible lesions, but they often come with side effects like severe disfigurement, edema, ulceration, or scarring. In cases where surgery is not feasible, radiotherapy, chemotherapy, or immunomodulatory drugs are administered orally, parenterally, or topically. While oral and parenteral drugs may lead to systemic side effects, topical administration through the skin is gaining attention for its ability to directly target the tumor site, bypassing first-pass metabolism [55]. However, the cure rate for topical administration is low, and prolonged use may induce severe inflammation or systemic toxicity [56]. There is an urgent need to explore ways to reduce or eliminate the serious side effects associated with conventional therapies. To this end, scientists are exploring how the unique properties of nanocarriers can be used to slow drug release, enhance drug targeting, prolong drug retention in the tumor, and thereby reduce drug dose and toxicity. It has been reported that NPs carrying chemotherapeutic drugs can penetrate the skin and remain at the tumor site through sweat glands, sebaceous glands, hair follicles [57], and follicular sebaceous glands [58], which would improve the specificity and therapeutic efficacy of the drug [59,60]. Various nanocarriers have been developed based on the characteristics of inorganic and organic nanocarriers that effectively deliver drugs and genes and act on the tumor microenvironment, immunomodulation, and tumors (Fig. 3). Although the use of nanomedicines in skin cancer has been widely explored, the clinical application of nanomedicine-based topical therapies continues to progress. Overall, nanomedicine presents a promising strategy for improving the treatment of skin

cancer, particularly for patients unsuitable for traditional treatments.

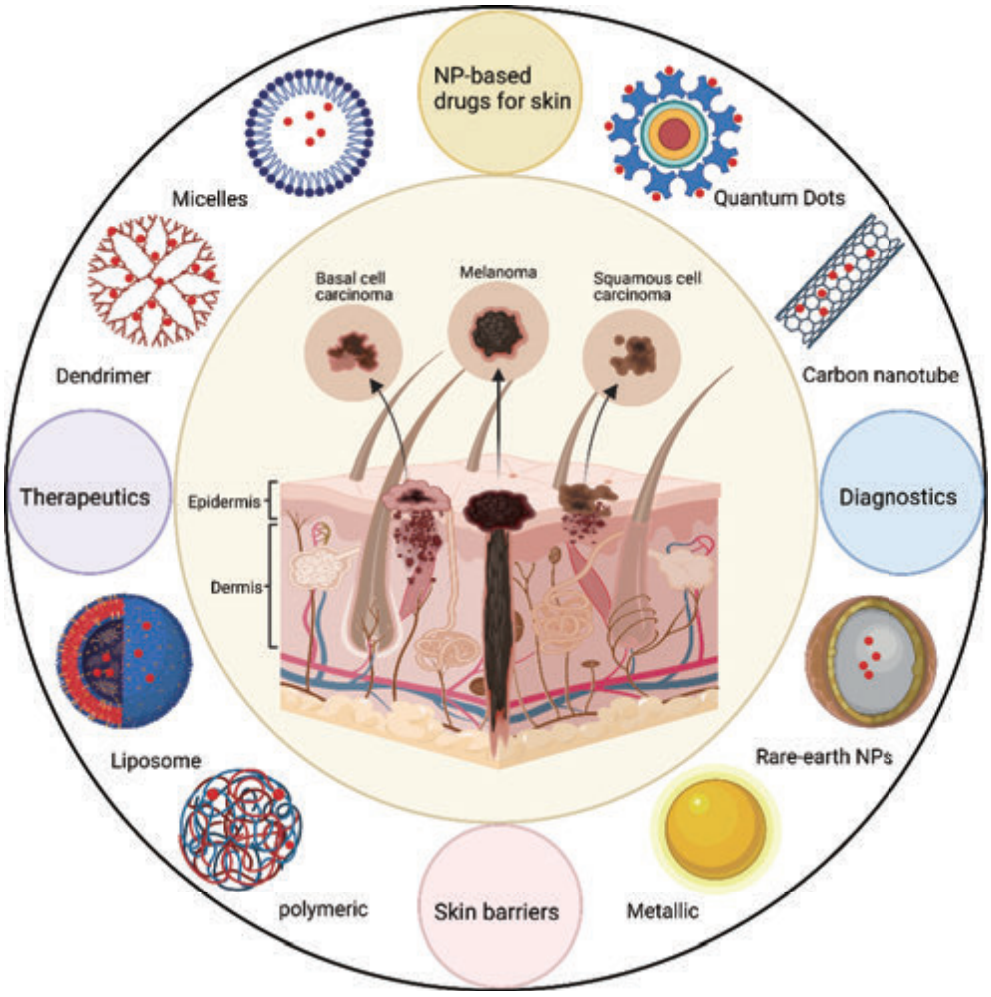

Figure 3. The application of Nanomedicine in skin cancer.

Aims of this thesis

While various approaches to cancer treatment exist, research combining multiple treatment modalities, such as targeting the tumor immune microenvironment with the assistance of lipids, chemotherapy and nanomedicine, is relatively rare. Moreover, obtaining accurate information about drug distribution *in vivo* remains challenging. Although many studies target TAMs and CAFs, there is a significant gap in research focusing on reversing M2-TAMs to M1-TAMs and inhibiting CAFs

activity. Therefore, this thesis explored different innovative strategies in combination with chemotherapy. The first aim of the thesis is to track the distribution of drugs *in vivo* by exploiting the imaging capabilities of rare-earth doped nanoparticles (RENPs) and combining them with DOX@PLGA NPs to construct a real-time monitoring chemotherapy platform with tracking and therapeutic capabilities. The second aim of this thesis is to screen lipids that possess the ability to convert M2-TAM to M1-TAM, and to overcome the limitations of lipid insolubility in water and the severe side effects caused by doxorubicin chemotherapy, by using biodegradable PLGA to encapsulate the lipids and doxorubicin. The use of nanomedicine aims to improve the biocompatibility and bioavailability of lipids and chemotherapeutic drugs and explore the impact of lipids on the tumor immune microenvironment *in vivo*. Finally, to evaluate the efficacy of nanomedicines in skin cancer treatment, the thesis constructs a full-thickness model (FTM) of melanoma and cutaneous squamous cell carcinoma (cSCC) containing CAFs. The FTM allows for targeted exploration of new methods for skin cancer treatment without interference from non-target cells and provides a solid foundation for future research.

Outline of this thesis

This introduction introduces the background of this thesis topic in detail. It begins with a brief introduction to the current problems facing cancer treatment and the promise that the advent of nanomedicine holds for cancer treatment, giving an initial insight into the strategies used to treat cancer using nanomedicine. This is followed by an analysis of the research areas in nanomedicine, the classification of NPs and the main issues in current clinical research in nanomedicine, to give the reader a better understanding of the applications of different types of NPs. We then detail the current dilemmas facing the treatment of breast and skin cancers, and the exploration and application of nanomedicine in breast and skin cancers. Finally, we detail the exploration and application of lipids in the treatment of cancer.

In **chapter 2** we developed a real-time monitoring chemotherapy platform capable of diagnosis and therapy by combining PLGA NPs loaded with chemotherapeutic drugs (doxorubicin) and CaF_2:Y, Nd NPs. The physical properties, drug release rate, and NIR-II imaging capabilities were evaluated through experimental data analysis. In addition, the NPs were combined with EGF in order to enhance the targeting ability of the NPs on breast cancer cells.

To gain a more comprehensive and detailed understanding of the role of TAM in the tumor microenvironment (TME) in regulating anti-tumor and tumor promotion

and the progress of research on nano-drugs targeting TAM and TEM, we have conducted a thorough review of relevant literature. We analyze the current status of nano-drugs targeting tumor-associated macrophages and the passive and active TAM targeting strategies for tumor treatment potential in **chapter 3**. This analysis serves as the foundation for designing and studying nanoparticles tailored to modulate the TME, with a focus on combined chemotherapy for cancer treatment.

Tumor-promoting M2-type macrophages and anti-tumor M1-type macrophages in the tumor microenvironment play an inverse role in tumor proliferation and migration. In light of this, we propose a therapeutic strategy aimed at reversing M-2 type macrophages to M1 type macrophages in **chapter 4**. In this study, we focus on the role of the natural lipids including docosahexaenoic acid (DHA), ceramide (Cer), sphingomyelin (SM) and palmitic acid (PA) on the proliferation, migration and invasion of breast cancer cells. Furthermore, we analyze their effects on the M2-TEM phenotype and the proliferation and migration of lipid-treated M2-TEM cells against 4T1 cells. Among them, PA and Cer exhibited the ability to induce the reversal of M2-TEM to M1-TEM, effectively suppressing tumor cell growth.

To address the challenges arising from the poor water solubility and cell permeability of PA, as well as the severe side effects associated with DOX, the **chapter 5** proposes employing PLGA NPs as carriers. These PLGA NPs are designed to encapsulate PA and/or DOX, aiming to explore the potential of PA as a standalone therapeutic agent or in combination with DOX for eradicating breast tumors. The chapter discusses the efficacy of these NPs in inhibiting breast tumor growth and proliferation using a 4T1 breast tumor model. Furthermore, it delves into an in-depth analysis of the impact of PA-encapsulated NPs on repolarizing M2 macrophages to the M1 phenotype *in vitro* and their role in immune regulation within the tumor microenvironment *in vivo*.

Cancer-associated fibroblasts (CAFs) are a crucial component of the tumor microenvironment and play a significant role in the proliferation and migration of skin cancer. The emergence of nano-drug delivery systems has introduced novel approaches for the treatment of skin cancer. Among these, topical drug delivery offers advantages such as bypassing the first-pass effect in the liver and enhancing local therapeutic effects. In **chapter 6**, full-thickness model (FTM) containing CAFs and either melanoma or cutaneous squamous cell carcinoma (cSCC) is used as a skin cancer model. The aim is to investigate the efficacy of nano-delivery systems in penetrating the tumor skin tissue to reach the tumor microenvironment and achieve anti-tumor effects. Specifically, two topical drug delivery methods, intradermal injection, and topical application, are compared to assess their effectiveness.

The research findings are summarized and discussed in **chapter 7**. Additionally, the paragraph explores the challenges and opportunities for this line of research in the future of cancer treatment.

References

1. Ferlay, J.; Colombet, M.; Soerjomataram, I.; Parkin, D.M.; Pineros, M.; Znaor, A.; Bray, F. Cancer statistics for the year 2020: An overview. Int J Cancer 2021, 10.1002/ijc.33588, doi:10.1002/ijc.33588.
2. Chen, G.; Roy, I.; Yang, C.; Prasad, P.N. Nanochemistry and Nanomedicine for Nanoparticle-based Diagnostics and Therapy. Chemical Reviews 2016, 116, 2826-2885, doi:10.1021/acs.chemrev.5b00148.
3. Giese, E.C. Rare Earth Elements: therapeutic and diagnostic applications in modern medicine. Clinical and Medical Reports 2018, 2, doi:10.15761/cmr.1000139.
4. Siddique, S.; Chow, J.C.L. Recent Advances in Functionalized Nanoparticles in Cancer Theranostics. Nanomaterials (Basel) 2022, 12, doi:10.3390/nano12162826.
5. Gavas, S.; Quazi, S.; Karpinski, T.M. Nanoparticles for Cancer Therapy: Current Progress and Challenges. Nanoscale Res Lett 2021, 16, 173, doi:10.1186/s11671-021-03628-6.
6. Yao, Y.; Zhou, Y.; Liu, L.; Xu, Y.; Chen, Q.; Wang, Y.; Wu, S.; Deng, Y.; Zhang, J.; Shao, A. Nanoparticle-Based Drug Delivery in Cancer Therapy and Its Role in Overcoming Drug Resistance. Front Mol Biosci 2020, 7, 193, doi:10.3389/fmolb.2020.00193.
7. Shen, S.; Wang, S.; Zheng, R.; Zhu, X.; Jiang, X.; Fu, D.; Yang, W. Magnetic nanoparticle clusters for photothermal therapy with near-infrared irradiation. Biomaterials 2015, 39, 67-74, doi:https://doi.org/10.1016/j.biomaterials.2014.10.064.
8. Park, W.; Shin, H.; Choi, B.; Rhim, W.-K.; Na, K.; Keun Han, D. Advanced hybrid nanomaterials for biomedical applications. Progress in Materials Science 2020, 114, doi:10.1016/j.pmatsci.2020.100686.
9. Gu, M.; Li, W.; Jiang, L.; Li, X. Recent progress of rare earth doped hydroxyapatite nanoparticles: Luminescence properties, synthesis and biomedical applications. Acta Biomaterialia 2022, 148, 22-43, doi:https://doi.org/10.1016/j.actbio.2022.06.006.
10. Lyu, L.; Cheong, H.; Ai, X.; Zhang, W.; Li, J.; Yang, H.; Lin, J.; Xing, B. Near-infrared light-mediated rare-earth nanocrystals: recent advances in improving photon conversion and alleviating the thermal effect. NPG Asia Materials 2018, 10, 685-702, doi:10.1038/s41427-018-0065-y.
11. Yu, Z.; Eich, C.; Cruz, L.J. Recent Advances in Rare-Earth-Doped Nanoparticles for NIR-II Imaging and Cancer Theranostics. Front Chem 2020, 8, 496, doi:10.3389/fchem.2020.00496.
12. Dubey, V., Som, S., & Kumar, V. (Eds.). Luminescent Materials in Display and Biomedical Applications. CRC Press. 2020, doi.org/10.1201/9780429025334, doi:doi.org/10.1201/9780429025334.
13. Yu, Z.; He, Y.; Schomann, T.; Wu, K.; Hao, Y.; Suidgeest, E.; Zhang, H.; Eich, C.; Cruz, L.J. Rare-Earth-Metal (Nd (3+), Ce (3+) and Gd (3+))-Doped CaF (2): Nanoparticles for Multimodal Imaging in Biomedical Applications. Pharmaceutics 2022, 14, doi:10.3390/pharmaceutics14122796.
14. Zielinska, A.; Carreiro, F.; Oliveira, A.M.; Neves, A.; Pires, B.; Venkatesh, D.N.; Durazzo, A.; Lucarini, M.; Eder, P.; Silva, A.M., *et al*. Polymeric Nanoparticles:

Production, Characterization, Toxicology and Ecotoxicology. Molecules 2020, 25, doi:10.3390/molecules25163731.

15. Lin, Y.C.; Gao, M.Y.; Wu, Y.J.; Fang, Y.P. Lipid-enveloped PLGA as a hybrid carrier for sustained delivering camptothecin in ovarian cancer. IET Nanobiotechnology 2017, 11, 797-802, doi:10.1049/iet-nbt.2016.0141.
16. Wei, G.; Wang, Y.; Yang, G.; Wang, Y.; Ju, R. Recent progress in nanomedicine for enhanced cancer chemotherapy. Theranostics 2021, 11, 6370-6392, doi:10.7150/thno.57828.
17. Mitchell, M.J.; Billingsley, M.M.; Haley, R.M.; Wechsler, M.E.; Peppas, N.A.; Langer, R. Engineering precision nanoparticles for drug delivery. Nat Rev Drug Discov 2021, 20, 101-124, doi:10.1038/s41573-020-0090-8.
18. He, Y.; de Araujo Junior, R.F.; Cruz, L.J.; Eich, C. Functionalized Nanoparticles Targeting Tumor-Associated Macrophages as Cancer Therapy. Pharmaceutics 2021, 13, 50, doi:10.3390/pharmaceutics13101670.
19. Zhao, Z.; Ukidve, A.; Kim, J.; Mitragotri, S. Targeting Strategies for Tissue-Specific Drug Delivery. Cell 2020, 181, 151-167, doi:10.1016/j.cell.2020.02.001.
20. Shukla, A.; Maiti, P. Nanomedicine and versatile therapies for cancer treatment. MedComm (2020) 2022, 3, e163, doi:10.1002/mco2.163.
21. El-Hammadi, M.M.; Delgado, A.V.; Melguizo, C.; Prados, J.C.; Arias, J.L. Folic acid-decorated and PEGylated PLGA nanoparticles for improving the antitumour activity of 5-fluorouracil. Int J Pharm 2017, 516, 61-70, doi:10.1016/j.ijpharm.2016.11.012.
22. Bowers, C.Y.; Granda, R.; Mohan, S.; Kuipers, J.; Baylink, D.; Veldhuis, J.D. Sustained elevation of pulsatile growth hormone (GH) secretion and insulin-like growth factor I (IGF-I), IGF-binding protein-3 (IGFBP-3), and IGFBP-5 concentrations during 30-day continuous subcutaneous infusion of GH-releasing peptide-2 in older men and women. J Clin Endocrinol Metab 2004, 89, 2290-2300, doi:10.1210/jc.2003-031799.
23. Siegel, R.L.; Miller, K.D.; Fuchs, H.E.; Jemal, A. Cancer statistics, 2022. CA Cancer J Clin 2022, 72, 7-33, doi:10.3322/caac.21708.
24. Waks, A.G.; Winer, E.P. Breast Cancer Treatment: A Review. Jama 2019, 321, 288-300, doi:10.1001/jama.2018.19323.
25. Xia, W.; Gerard, C.M.; Liu, L.; Baudson, N.M.; Ory, T.L.; Spector, N.L. Combining lapatinib (GW572016), a small molecule inhibitor of ErbB1 and ErbB2 tyrosine kinases, with therapeutic anti-ErbB2 antibodies enhances apoptosis of ErbB2-overexpressing breast cancer cells. Oncogene 2005, 24, 6213-6221.
26. Chen, F.L.; Xia, W.; Spector, N.L. Acquired resistance to small molecule ErbB2 tyrosine kinase inhibitors. Clin Cancer Res 2008, 14, 6730-6734, doi:10.1158/1078-0432.CCR-08-0581.
27. Assaraf, Y.G.; Leamon, C.P.; Reddy, J.A. The folate receptor as a rational therapeutic target for personalized cancer treatment. Drug Resist Updat 2014, 17, 89-95, doi:10.1016/j.drup.2014.10.002.
28. Guan, X.; Liu, Z.; Zhao, Z.; Zhang, X.; Tao, S.; Yuan, B.; Zhang, J.; Wang, D.; Liu, Q.; Ding, Y. Emerging roles of low-density lipoprotein in the development and treatment of breast cancer. Lipids Health Dis 2019, 18, 137, doi:10.1186/s12944-019-1075-7.

29. Shin, E.; Koo, J.S. Glucose Metabolism and Glucose Transporters in Breast Cancer. Front Cell Dev Biol 2021, 9, 728759, doi:10.3389/fcell.2021.728759.

30. Bellotti, E.; Cascone, M.G.; Barbani, N.; Rossin, D.; Rastaldo, R.; Giachino, C.; Cristallini, C. Targeting Cancer Cells Overexpressing Folate Receptors with New Terpolymer-Based Nanocapsules: Toward a Novel Targeted DNA Delivery System for Cancer Therapy. Biomedicines 2021, 9, doi:10.3390/biomedicines9091275.

31. Helmy, S.A.; El-Mofty, S.; El Gayar, A.M.; El-Sherbiny, I.M.; El-Far, Y.M. Novel doxorubicin / folate-targeted trans-ferulic acid-loaded PLGA nanoparticles combination: In-vivo superiority over standard chemotherapeutic regimen for breast cancer treatment. Biomed Pharmacother 2022, 145, 112376, doi:10.1016/j.biopha.2021.112376.

32. Gao, H.; Cao, S.; Yang, Z.; Zhang, S.; Zhang, Q.; Jiang, X. Preparation,-Characterization and Anti-Glioma Effects of Docetaxel-Incorporated Albumin-Lipid Nanoparticles. J Biomed Nanotechnol 2015, 11, 2137-2147, doi:10.1166/jbn.2015.2076.

33. Sengupta, S.; Eavarone, D.; Capila, I.; Zhao, G.; Watson, N.; Kiziltepe, T.; Sasisekharan, R. Temporal targeting of tumour cells and neovasculature with a nanoscale delivery system. Nature 2005, 436, 568-572, doi:10.1038/nature03794.

34. Chen, E.; Han, S.; Song, B.; Xu, L.; Yuan, H.; Liang, M.; Sun, Y. Mechanism Investigation of Hyaluronidase-Combined Multistage Nanoparticles for Solid Tumor Penetration and Antitumor Effect. Int J Nanomedicine 2020, 15, 6311-6324, doi:10.2147/IJN.S257164.

35. Yan, S.; Luo, Z.; Li, Z.; Wang, Y.; Tao, J.; Gong, C.; Liu, X. Improving Cancer Immunotherapy Outcomes Using Biomaterials. Angew Chem Int Ed Engl 2020, 59, 17332-17343, doi:10.1002/anie.202002780.

36. Burnett, M.; Abuetabh, Y.; Wronski, A.; Shen, F.; Persad, S.; Leng, R.; Eisenstat, D.; Sergi, C. Graphene Oxide Nanoparticles Induce Apoptosis in wild-type and CRISPR/Cas9-IGF/IGFBP3 knocked-out Osteosarcoma Cells. J Cancer 2020, 11, 5007-5023, doi:10.7150/jca.46464.

37. Dianzani, C.; Monge, C.; Miglio, G.; Serpe, L.; Martina, K.; Cangemi, L.; Ferraris, C.; Mioletti, S.; Osella, S.; Gigliotti, C.L., *et al*. Nanoemulsions as Delivery Systems for Poly-Chemotherapy Aiming at Melanoma Treatment. Cancers (Basel) 2020, 12, doi:10.3390/cancers12051198.

38. Rigatos, S.K.; Tsavdaridis, D.; Athanasiadis, A.; Stathopoulos, J.G.; Stathopoulos, G.P. Paclitaxel and liposomal doxorubicin (Caelyx) combination in advanced breast cancer patients: a phase II study. Oncol Rep 2003, 10, 1817-1819.

39. Pillai*, G. Nanomedicines for Cancer Therapy: An Update of FDA Approved and Those under Various Stages of Development. Symbiosis 2014.

40. Butler, L.M.; Perone, Y.; Dehairs, J.; Lupien, L.E.; de Laat, V.; Talebi, A.; Loda, M.; Kinlaw, W.B.; Swinnen, J.V. Lipids and cancer: Emerging roles in pathogenesis, diagnosis and therapeutic intervention. Advanced drug delivery reviews 2020, 159, 245-293.

41. Beloribi-Djefaflia, S.; Vasseur, S.; Guillaumond, F. Lipid metabolic reprogramming in cancer cells. Oncogenesis 2016, 5, e189, doi:10.1038/oncsis.2015.49.

42. Fu, Y.; Zou, T.; Shen, X.; Nelson, P.J.; Li, J.; Wu, C.; Yang, J.; Zheng, Y.;

Bruns, C.; Zhao, Y., *et al*. Lipid metabolism in cancer progression and therapeutic strategies. MedComm (2020) 2021, 2, 27-59, doi:10.1002/mco2.27.

43. Ogretmen, B. Sphingolipid metabolism in cancer signalling and therapy. Nat Rev Cancer 2018, 18, 33-50, doi:10.1038/nrc.2017.96.

44. Pyne, N.J.; Pyne, S. Sphingosine 1-phosphate and cancer. Nature Reviews Cancer 2010, 10, 489-503.

45. Kwan, H.Y.; Fu, X.; Liu, B.; Chao, X.; Chan, C.L.; Cao, H.; Su, T.; Tse, A.K.W.; Fong, W.F.; Yu, Z.-L. Subcutaneous adipocytes promote melanoma cell growth by activating the Akt signaling pathway. Journal of Biological Chemistry 2014, 289, 30525-30537.

46. Zhang, C.; Yu, H.; Ni, X.; Shen, S.; Das, U.N. Growth inhibitory effect of polyunsaturated fatty acids (PUFAs) on colon cancer cells via their growth inhibitory metabolites and fatty acid composition changes. PLoS One 2015, 10, e0123256.

47. Li, J.; Gu, Z.; Pan, Y.; Wang, S.; Chen, H.; Zhang, H.; Chen, W.; Chen, Y.Q. Dietary supplementation of α-linolenic acid induced conversion of n-3 LCPUFAs and reduced prostate cancer growth in a mouse model. Lipids in Health and Disease 2017, 16, 1-9.

48. Murff, H.J.; Shu, X.O.; Li, H.; Yang, G.; Wu, X.; Cai, H.; Wen, W.; Gao, Y.T.; Zheng, W. Dietary polyunsaturated fatty acids and breast cancer risk in Chinese women: a prospective cohort study. International Journal of Cancer 2011, 128, 1434-1441.

49. O'Brien, M.E.R.; Wigler, N.; Inbar, M.; Rosso, R.; Grischke, E.; Santoro, A.; Catane, R.; Kieback, D.G.; Tomczak, P.; Ackland, S.P., *et al*. Reduced cardiotoxicity and comparable efficacy in a phase IIItrial of pegylated liposomal doxorubicin HCl (CAELYX™/Doxil®) versus conventional doxorubicin forfirst-line treatment of metastatic breast cancer. Annals of Oncology 2004, 15, 440-449, doi:10.1093/annonc/mdh097.

50. Gabizon, A.; Shmeeda, H.; Barenholz, Y. Pharmacokinetics of pegylated liposomal Doxorubicin: review of animal and human studies. Clin Pharmacokinet 2003, 42, 419-436, doi:10.2165/00003088-200342050-00002.

51. Allen, T.M.; Cullis, P.R. Liposomal drug delivery systems: from concept to clinical applications. Adv Drug Deliv Rev 2013, 65, 36-48, doi:10.1016/j.addr.2012.09.037.

52. Peer, D.; Karp, J.M.; Hong, S.; Farokhzad, O.C.; Margalit, R.; Langer, R. Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol 2007, 2, 751-760, doi:10.1038/nnano.2007.387.

53. He, Y.; de Araújo Júnior, R.F.; Cavalcante, R.S.; Yu, Z.; Schomann, T.; Gu, Z.; Eich, C.; Cruz, L.J. Effective breast cancer therapy based on palmitic acid-loaded PLGA nanoparticles. Biomaterials Advances 2023, 145, doi:10.1016/j.bioadv.2022.213270.

54. Krishnan, V.; Mitragotri, S. Nanoparticles for topical drug delivery: Potential for skin cancer treatment. Advanced Drug Delivery Reviews 2020, 153, 87-108.

55. Palmer, B.C.; DeLouise, L.A. Nanoparticle-enabled transdermal drug delivery systems for enhanced dose control and tissue targeting. Molecules 2016, 21, 1719.

56. Lebwohl, M.; Shumack, S.; Gold, L.S.; Melgaard, A.; Larsson, T.; Tyring, S.K.

Long-term follow-up study of ingenol mebutate gel for the treatment of actinic keratoses. JAMA dermatology 2013, 149, 666-670.

57. ademann, J.; Richter, H.; Teichmann, A.; Otberg, N.; Blume-Peytavi, U.; Luengo, J.; Weiß, B.; Schaefer, U.F.; Lehr, C.-M.; Wepf, R., *et al*. Nanoparticles – An efficient carrier for drug delivery into the hair follicles. European Journal of Pharmaceutics and Biopharmaceutics 2007, 66, 159-164, doi:https://doi.org/10.1016/j.ejpb.2006.10.019.

58. Knorr, F.; Lademann, J.; Patzelt, A.; Sterry, W.; Blume-Peytavi, U.; Vogt, A. Follicular transport route – Research progress and future perspectives. European Journal of Pharmaceutics and Biopharmaceutics 2009, 71, 173-180, doi:https://doi.org/10.1016/j.ejpb.2008.11.001.

59. Alvarez-Román, R.; Naik, A.; Kalia, Y.N.; Guy, R.H.; Fessi, H. Skin penetration and distribution of polymeric nanoparticles. J Control Release 2004, 99, 53-62, doi:10.1016/j.jconrel.2004.06.015.

60. Fang, C.L.; Aljuffali, I.A.; Li, Y.C.; Fang, J.Y. Delivery and targeting of nanoparticles into hair follicles. Ther Deliv 2014, 5, 991-1006, doi:10.4155/tde.14.61.