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Perspective

Mild photothermal therapy for cancer cell modulation: A transformative approach to regulate cancer cell phenotype and enhance therapeutic outcomes

1. EMT in cancer metastasis and chemoresistance

Cancer metastasis is contingent on the epithelial-mesenchymal transition (EMT) of cancer cells. During EMT, epithelial cancer cells transform into a mesenchymal phenotype, which endows cancer cells with enhanced migratory and invasive abilities, promoting their dissemination throughout the body. EMT also contributes significantly to chemoresistance, allowing cancer cells to survive and metastasize even after chemotherapy [1]. Therefore, targeting EMT has emerged as a promising therapeutic strategy to prevent cancer metastasis and improve the effectiveness of conventional anticancer drugs. However, translating EMT-targeted compounds into clinical practice has faced challenges due to their limited cellular specificity and therapeutic efficacy. This is because EMT process involves numerous signaling pathways and intricate interactions within the tumor microenvironment. These factors complicate efforts to achieve a sustained therapeutic impact, as interference with one pathway may be counteracted by compensatory mechanisms within others, ultimately reducing long-term efficacy. Another limitation of current compounds is that they largely aim to prevent EMT initiation rather than targeting cancer cells that have already transitioned through the EMT process. This overlooks cancer cells that have undergone EMT. Hence, therapeutic approaches that can effectively and specifically eliminate mesenchymal-type cancer cells with high metastatic potential and enhanced drug resistance may hold the key to revolutionizing cancer treatment.

2. Mild photothermal therapy (PTT) modulates cancer cell phenotype by reversing EMT

Implementing PTT as a clinical approach for treating cancer metastasis is currently hindered by two primary obstacles [2]. First, achieving uniform loading of photo-absorbers across the entire tumor, especially at the tumor margins where mesenchymal-type metastatic cells are typically concentrated, remains a significant challenge. Second, conventional PTT typically requires high local temperatures (above 50 °C) to achieve thermal ablation of cancer cells, which necessitates the use of high-intensity lasers. This risks collateral damage to surrounding healthy tissues, potentially leading to adverse side effects such as skin damage, inflammation, and scar formation. Together, these limitations highlight the need for alternative PTT approaches that can effectively target EMT-induced metastatic cancer cells without the risks associated with high-temperature ablation.

In response to these challenges, we proposed an alternative solution: applying a mild photothermal effect (at 42–45 °C) through a minimal dosage of photo-absorbers and low intensity laser illumination to reverse EMT, thereby inhibiting cancer metastasis and sensitizing cancer cells to chemotherapeutic agents, avoiding the drawbacks associated with high-temperature photothermal ablation (i.e., traditional PTT) [3–5]. This transformative approach leverages mild, non-ablative heat to modulate cancer cell phenotype, reversing the EMT and regulating phenotype-related properties critical to metastasis and chemoresistance. Specifically, by targeting cancer cell surface markers closely associated with EMT, such as CD146 and CD44, which are commonly overexpressed in metastatic and mesenchymal-type cancer cells, mild PTT triggers a shift back to an epithelial phenotype for these types of cancer cells. EMT reversal, marked by reduced mesenchymal marker expression (N-cadherin and vimentin) and increased epithelial marker expression (E-cadherin and cytokeratin), results in the reorganization of the actin cytoskeleton, which reduces cell motility, effectively impairing the cells' ability to migrate and invade surrounding tissues. Moreover, the mild PTT induced EMT reversal sensitizes the cancer cells to chemotherapeutic agents, overcoming one of the primary challenges in cancer treatment. Instead of focusing solely on ablating cancer cells, our approach redefines the therapeutic objectives of PTT by emphasizing targeted modulation of cellular processes that contribute to metastasis and chemoresistance. This non-lethal alternative not only expands the therapeutic window of PTT but also holds potential for integration with other treatment modalities such as surgical resection and traditional chemotherapy, paving the way for a more versatile and clinically applicable strategy in cancer management.

Mechanistically, we showed that EMT reversal occurs through the molecular pathways involving CD146 and CD44 receptors. In mesenchymal cancer cells, transmembrane proteins CD146 and CD44 play a critical role in upregulating RhoA signaling by recruiting Rho-guanine-nucleotide-dissociation-inhibitory factor α (RhoGDIα), a key inhibitor of RhoA, through their interactions with the phosphorylated forms of ezrin, radixin, and moesin proteins (p-ERM). These interactions disrupt the inhibitory complex between RhoGDIα and guanosine diphosphate (GDP)-RhoA, effectively releasing GDP-RhoA from its bound state. The dissociation of GDP-RhoA from RhoGDIα allows for the subsequent exchange

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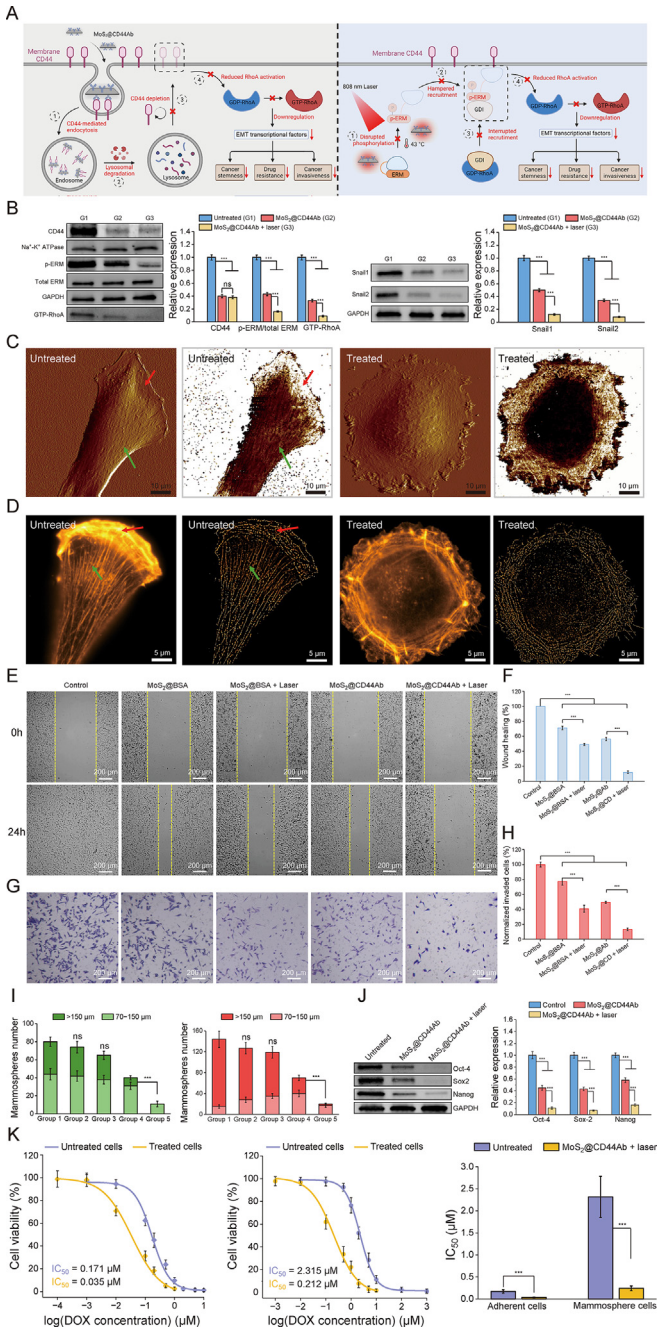

Fig. 1. Mild photothermal therapy (PTT) for cancer cell modulation. (A) Schematic illustrating the molecular mechanism of mild PTT induced epithelial-mesenchymal transition (EMT) reversal. (B) Western blot analysis of cell membrane CD44, cytoplasmic phosphorylated forms of ezrin, radixin, and moesin proteins (p-ERM), total ERM, guanosine triphosphate (GTP)-RhoA, EMT transcriptional factors of Snail and Slug in cancer cells after nanoparticle and mild PTT treatment. The 60% decrease of membrane CD44 can be attributed to the cell uptake of CD44-antibody modified nanoparticle system, depleting membrane CD44 into lysosomal degradation pathway. The downregulation of CD44 leads to a consistent 60% reduction of p-ERM, RhoA activation, EMT transcriptional factor (Snail and Slug). Following the mild PTT, p-ERM and GTP-RhoA showed a larger decrease (80%–90%), leading to further downregulation of EMT transcriptional factors ($n = 3$). (C) Nanomechanical atomic force microscopy (AFM) images of untreated and mild PTT treated mesenchymal cancer cells. In untreated cells, green arrows indicate actin stress fibers aligned with the migration direction, while red arrows highlight the lamellipodium at the front edge of cell movement. (D) Wide-field and direct stochastic optical reconstruction microscopy (dSTORM) images of untreated and mild PTT treated cancer cells. (E, F) Two-dimensional (2D) scratch assay evaluating the migratory ability of cancer cells. After the mild PTT, the cancer cells almost completely stopped their migration. (G, H) 3D invasion assay assessing the invasive ability of cancer cells. After the mild PTT, the cancer cell invasion was significantly inhibited by 90%. (I) Quantification of the mammospheres under 2D (left) and 3D (right) cell culturing models. The number and size of mammospheres from both 2D- and 3D-cultured cells were remarkably diminished after the mild PTT ($n = 6$). (J) Western blot analyses of cancer stemness markers. The cancer stemness markers expression showed a 40%–60% decrease after the nanoparticle internalization and an 80%–90% decrease after the mild PTT, consistent with the molecular mechanism studies of mild PTT induced EMT reversal ($n = 3$). (K) Dose-dependent cytotoxicity induced by chemotherapeutic drug doxorubicin (DOX) and half-maximal drug inhibitory concentration (IC_{50}) values on cancer adherent and mammosphere cells with or without mild PTT treatment. After mild PTT, both 2D- and 3D-cultured cancer cells exhibited heightened sensitivity to DOX. Specifically, 2D-cultured adherent cells demonstrated a five-fold increase in sensitivity to DOX-induced cytotoxicity, while 3D-cultured mammosphere cells showed an even more pronounced response, with a ten-fold increase in DOX sensitivity ($n = 3$). Data is presented as mean ± standard deviation (SD), one-way analysis of variance (ANOVA). $^{***}P < 0.001$. ns: no significant difference. Reprinted from Ref. [3] with permission. GDP: guanosine diphosphate; RhoGDIα: rho-guanine-nucleotide-dissociation-inhibitory factor α; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; Ab: antibody; BSA: bovine serum albumin.

of GDP for guanosine triphosphate (GTP), thereby activating RhoA to its GTP-bound form (GTP-RhoA). This activation triggers downstream signaling pathways upregulating cytoskeletal dynamics, cell migration, cancer cell stemness, and drug-resistance. Upon cellular uptake of CD146-or CD44-antibody modified nanoparticle delivery systems, transmembrane CD146/CD44 is downregulated by a lysosomal-degradation pathway, which reduces the availability of membrane binding sites for RhoGDIα. In tandem, mild hyperthermia triggered by near-infrared laser irradiation disrupts the phosphorylation of ERM proteins, further impairing the structural "bridges" that link RhoGDIα to residual transmembrane CD146/CD44 (Fig. 1A). Together, both factors lead to a downregulation of RhoA activity and its downstream EMT transcription processes (Fig. 1B). Consequently, this disruption effectively converts mesenchymal-type cancer cells to an epithelial phenotype, reducing their invasiveness, drug resistance, and cancer stem-like properties.

3. Mild PTT induced EMT reversal inhibits cancer metastasis

To investigate how mild PTT induced EMT reversal affects the cancer metastasis, the single cell morphology, cytoskeleton organization and collective cancer cell motilities were analyzed. We employed nano-mechanical atomic force microscopy (AFM) and direct stochastic optical reconstruction microscopy (dSTORM) to visualize the single cell structure. AFM, a live-cell imaging technique, allows simultaneous imaging of cell morphology, surface topography, and elastic modulus of single cells (Fig. 1C). In untreated cells, AFM revealed two characteristic features of mesenchymal-type cells: 1) dorsal stress fibers along the cell's long axis, visible as high-modulus regions explicitly correlated in both modulus and topography images, and 2) lamellipodia at the leading edge, with high modulus owing to the dense actin network beneath the membrane. Untreated cells exhibited an elongated shape with leading and trailing edges, showing an average modulus of 46 kPa due to the presence of dorsal stress fibers. After mild PTT, these dorsal stress fibers disappeared, and the cells transitioned to a rounded morphology with a lower aspect ratio. The modulus of treated cells dropped significantly to approximately 5 kPa, indicating the collapse of actin structures critical for maintaining cell stiffness. Additionally, a flat, thin membrane with an underlying circumferential actin network formed around the cell periphery, replacing the previously polarized lamellipodia structure. AFM analysis demonstrated that EMT reversal induced by mild PTT disrupts the actin cytoskeleton at both structural and mechanical levels, effectively halting the cell migration. For more detailed imaging of the actin organization within the cell's ventral region, a

super-resolution tool, dSTORM was applied. It uses total internal reflection fluorescence (TIRF) illumination, which eliminates background fluorescence and allows for precise quantification of ventral stress fibers. dSTORM imaging confirmed the AFM findings, where in untreated cells, lamellipodia formed a dense actin network at the leading edge, while ventral stress fibers aligned along the cell's long axis, enabling the cell's migratory capability. However, in mild PTT treated cells, dSTORM revealed an absence of ventral stress fibers, replaced by a circumferential actin network around the cell periphery (Fig. 1D). Furthermore, collective cell migration and invasive capacity were assessed using a two-dimensional (2D) scratch assay and a 3D invasion assay. In the scratch assay, a small gap was created on the cell monolayer and cell migration was assessed by measuring the percentage of gap closure over a specified time period. The untreated cells fully closed the gap within 24 h, highlighting the robust migratory characteristic of mesenchymal cancer cells. Notably, cells subjected to mild PTT showed a dramatic reduction in migration, demonstrating only 10.9% gap closure within the same 24-h period (Figs. 1E and F). In the 3D invasion assay, cells moved through a Matrigel-coated transwell insert, mimicking the cell invasion across the extracellular matrix. Only 10% of treated cells crossed the matrix within 24 h, compared to 100% for untreated cells (Figs. 1G and H). Together, these results demonstrated that mild PTT effectively halts cancer cell migration and invasion. These findings from single cell cytoskeleton organization and collective cell movement consistently underscore and validate the efficacy of mild PTT in significantly impeding cancer metastasis.

4. Mild PTT induced EMT reversal attenuates cancer chemoresistance

The reversal of EMT holds significant promise for addressing cancer stem-like cells (CSCs), the key contributors to chemoresistance and tumor recurrence. EMT is strongly associated with CSC traits, and its reversal significantly reduces cancer cell stemness, thereby enhancing their sensitivity to chemotherapy. In our study, CD44-targeted MoS_2 nanosheets and mild PTT were employed to promote a process termed “photothermal differentiation”, where CSCs were shifted from a stem-like, drug-resistant, mesenchymal phenotype to a more differentiated, drug-sensitive, epithelial state. A well-established measurement for cancer cell stemness and self-renewal capacity is their ability to form mammospheres, which serves as an indicator of tumor regenerative potential. Mild PTT notably reduced both the number and size of mammospheres, suggesting a sharp decrease in cancer cell stemness (Fig. 1I). This reduction was further confirmed at the molecular level through decreased expression of CSC markers of Oct-4, Sox2, and Nanog (Fig. 1J). Mild PTT diminished the self-renewal potential of cancer cells, enhancing their responsiveness to chemotherapeutic agents. After mild PTT treatment, 2D-cultured adherent cells exhibited a fivefold increase in chemotherapeutic drug sensitivity, while 3D-cultured mammosphere cells showed a tenfold increase in responsiveness (Fig. 1K). By reversing EMT, mild PTT provides a promising strategy to modulate CSC properties, thereby complementing conventional therapies in overcoming chemoresistance and reducing tumor recurrence.

5. Perspectives of mild PTT in cancer treatment

Mild PTT is emerging as a promising adjunctive treatment that enhances current cancer therapies, such as surgery and chemotherapy. Operating at sub-lethal temperatures, mild PTT targets residual cancer cells at tumor margins after surgery, thereby reducing the risk of recurrence and metastasis. By using functionalized nanoparticles to bind to cancer cell markers, mild PTT selectively delivers controlled heat upon near-infrared irradiation, inducing localized mild hyperthermia. This treatment disrupts cancer cell structures, particularly actin fibers, which are essential for cell movement and invasiveness, thereby reducing cancer cell motility and limiting their ability to migrate and form secondary tumors. This selective targeting minimizes damage to surrounding healthy tissue, making mild PTT a valuable option for complex anatomical regions and improving long-term patient outcomes. In addition to physically targeting residual cancer cells, mild PTT has the potential to “prime” these cells, making them more susceptible to conventional cancer therapies, where sub-lethal thermal doses can weaken the defense mechanisms of cancer cells, enhancing their sensitivity to chemotherapeutic drugs. This sensitization effect can be particularly beneficial in tumors that are otherwise resistant to chemotherapy, where mild PTT can disrupt cancer cell homeostasis and reduce their ability to withstand drug-induced apoptosis. Thus, integrating mild PTT into treatment regimens offers a multifaceted approach, combining mechanical, biochemical, and thermal influences to more effectively manage cancer cell populations that may otherwise contribute to recurrence.

Exploring the combination of mild PTT with immunotherapy introduces another compelling direction for future research. Mild PTT, by reversing EMT and reducing cancer cell stemness, could reprogram the tumor microenvironment to make it more responsive to immune system attacks. By influencing the mechanical and biochemical properties of the tumor's surrounding tissues, mild PTT can disrupt pro-cancerous interactions between tumor cells and their microenvironment. This disruption may enhance the immune system's ability to recognize and target cancer cells, making mild PTT an exciting candidate for combination with immunotherapies, such as immune checkpoint inhibitors and adaptive T cell therapies. This sophisticated approach could increase the prospect of long-term cancer remission and metastasis prevention, offering promising potential for improved management of metastatic and treatment-resistant cancers. Additionally, implementing mild PTT in early-stage, pre-malignant lesions may provide a preventive strategy, intercepting cancer progression at its onset and limiting its evolution into invasive disease. By addressing cancer cells at such an early stage, mild PTT could expand its therapeutic impact beyond traditional treatment settings, making it a valuable tool for cancer interception and prevention. As research progresses, optimizing mild PTT will require continued innovation in nanoparticle design and delivery systems. Engineered nanoparticles that are biocompatible, degradable, and capable of selectively targeting tumor cells will be instrumental in ensuring the safety and efficacy of mild PTT in clinical applications. Furthermore, advances in multi-functional nanoparticles, those capable of targeting multiple cancer markers, will enhance the specificity of mild PTT in treating heterogeneous tumors, reducing off-target effects and increasing therapeutic precision.

Notably, our foundational work highlights the potential of mild PTT to strategically modulate EMT as a minimally invasive method to precisely regulate cancer cell phenotype in metastasis and chemoresistance. As research continues, mild PTT could become an integral part of the cancer therapy landscape, offering a novel and complementary approach for managing metastatic and treatment-resistant cancers. With ongoing refinements in nanoparticle design, new marker discovery, therapeutic combinations, and clinical protocols, mild PTT could ultimately serve as a transformative step in the fight against cancer, advancing the paradigm of personalized and precise oncology.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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References

[1] J. Yang, P. Antin, G. Berx, et al., Guidelines and definitions for research on epithelial–mesenchymal transition, Nat. Rev. Mol. Cell Biol. 21 (2020) 341–352.
[2] X. Deng, Z. Shao, Y. Zhao, Solutions to the drawbacks of photothermal and photodynamic cancer therapy, Adv. Sci. 8 (2021), 2002504.
[3] J. Liu, S. Smith, C. Wang, Photothermal attenuation of cancer cell stemness, chemoresistance, and migration using CD44-targeted MoS_2 nanosheets, Nano Lett. 23 (2023) 1989–1999.
[4] J. Liu, S. Smith, C. Wang, Reversing the epithelial-mesenchymal transition in metastatic cancer cells using CD146-targeted black phosphorus nanosheets and a mild photothermal treatment, ACS Nano 16 (2022) 3208–3220.
[5] J. Liu, L. Kang, S. Smith, et al., Transmembrane MUC18 targeted polydopamine nanoparticles and a mild photothermal effect synergistically disrupt actin cytoskeleton and migration of cancer cells, Nano Lett. 21 (2021) 9609–9618.

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