Review

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Nanodrug-Mediated Thermotherapy of Cancer Stem-Like Cells

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Cancer stem-like cells (CSCs) are rare subpopulations of cancer cells that are resistant to conventional chemotherapy and radiotherapy and contribute to cancer metastases and tumor recurrence. Therefore, it is of significance to develop an effective therapy to eliminate the CSCs. Cancer thermotherapy realized by depositing heat into tumor in a minimally invasive way is a promising alternative to the conventional therapies for cancer treatment. However, this method is limited by its inability to target CSCs, potentially allowing the CSCs to survive and re-initiate tumor growth. More recently, nanodrug-mediated thermotherapy has been explored to selectively eliminate CSCs and specifically deposit heat in tumor to spare healthy tissue. Here, we provide a brief overview of the targeting moieties and nanoplatforms used in developing nanodrug-mediated thermotherapy of cancer with particular emphasis on the CSCs, as well as the challenges and potential directions for future research in this emerging field.

Keywords: Cancer Stem-Like Cell, Nanoparticle, Thermotherapy, Targeting, Drug Delivery.

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1. INTRODUCTION

Cancer stem-like cells (CSCs) are rare subpopulations of cancer cells that can differentiate into the multiple types of resident cells in tumor necessary for the tumor to grow.[1–5] There has been mounting evidence that CSCs may contribute to post-treatment cancer recurrence, due to their high resistance to conventional chemo and radiotherapies.[1–6] Furthermore, CSCs have been shown to correlate with malignant tumor progression and metastasis in human patients.[7,8] Therefore, the development of an effective therapy capable of targeting and eradicating CSCs is very much in need and clinically relevant for eliminating cancer as a major healthcare problem.

Thermotherapy involving the use of superphysiological temperatures to burn and destroy tumor is attracting more and more attention as a promising alternative to the conventional approaches for cancer treatment.[9–13] These superphysiological temperatures can be achieved by delivering and converting energies carried in electromagnetic (e.g., radiofrequency, microwave, and laser) or ultrasound waves into heat in tumor.[13–15] Because some of these waves can penetrate deep into the human body, thermotherapy can be performed minimally invasively in an office-based setting with a much shorter recovery time compared to conventional radical surgical interventions.

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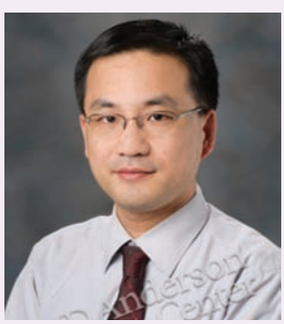

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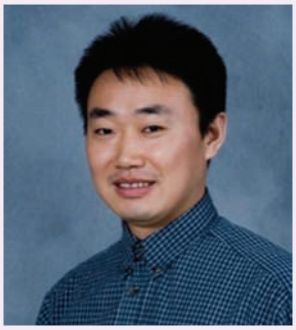

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However, thermotherapy alone carries the risk of damage to healthy tissues because of non-specific heating and thermal diffusion. Moreover, it is limited by the inability to selectively target CSCs in tumor, potentially allowing the rare CSCs to survive and cause cancer relapse.

More recently, nanodrug-mediated thermotherapy has been explored to improve the safety and efficacy of conventional thermotherapy.[16–20] This combinational therapy could be designed to achieve specific release of chemotherapeutic drugs (including small molecular drugs, siRNAs, microRNAs, and/or plasmid DNAs) and deposition of heat in tumor simultaneously by utilizing the capability of passive targeting of nanomaterials (20–150 nm) as a result of the enhanced permeability and retention (EPR) of tumor (as compared to normal tissue) vasculature and by modifying the nanomaterials with moieties to actively target the tumor vasculature (Fig. 1). In addition, the synergistic effect between thermo and chemotherapy allows for a lower thermal threshold and drug dose for complete tumor destruction than that by thermo or chemotherapy alone. Moreover, by further modifying the nanodrug surface with appropriate moieties to specifically target the CSCs, this combinational therapy could be further designed to deliver drug and deposit heat in the CSCs to specifically destroy them (Fig. 1).

In this review, we provide a concise literature survey of the targeting moieties on nanodrugs and the platform of nanoparticles that have been used for the combinational thermotherapy with particular emphasis on destroying the CSCs, followed by a brief discussion of the potential challenges and approaches for overcoming the challenges to further advance this exciting field.

2. MOIETIES USED FOR TARGETING CSCs

Advances in our knowledge of the properties of CSCs have made it possible to specifically target and eradicate CSCs using nanoscale drug delivery systems. Since the CSCs contribute to cancer recurrence and metastasis,[21,22] delivery of therapeutic agents to specifically target and destroy them is of great importance for cancer therapy. In the past decade, both antibodies and hyaluronic acid (HA) have been used to modify nanoparticles for targeting CSCs (Fig. 2(A) and Table I).

2.1. Antibodies

Antibodies can bind with their corresponding antigens with extremely high affinity and specificity.[23] Therefore, nanoparticles surface-decorated with an antibody (or ligand) of a specific antigen (or receptor) on CSCs can be

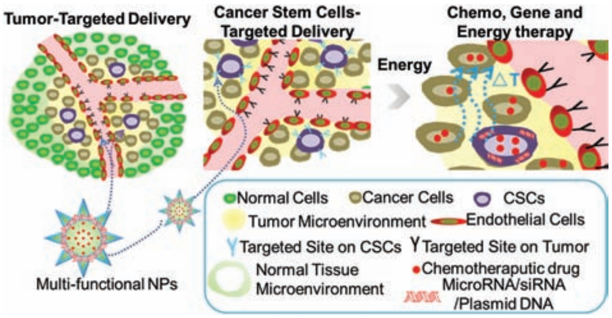

Figure 1. A schematic illustration of nanodrug-mediated thermotherapy. Nanodrug is accumulated at tumor site as a result of the enhanced permeability and retention (EPR) of tumor vasculature. Moreover, nanodrug modified with appropriate targeting moieties could selectively bind to cancer stem-like cells (CSCs that are randomly drawn in the figure for illustration). As a result, it could be used to achieve controllable chemotherapeutic drug release and selective heat deposition in tumor simultaneously under external stimuli such as electromagnetic field or laser if the nanodrug contains materials (e.g., gold and thermally responsive polymers) that are responsive to the stimuli.

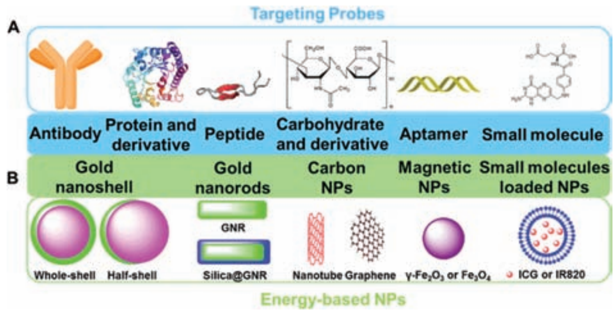

Figure 2. A schematic illustration of (A), targeting moieties that have been used in drug delivery and (B), nanoplatforms that have been used for cancer thermotherapy. GNR: gold nanorods; ICG: indocyanine green.

Table I. Summary of moieties used for targeting CSCs.

Moieties	Type	Types of CSCs Targeted
Antibodies	CD44 antibody	Bladder, breast, colon, gastric, glioma, medulloblastoma, leukemia, head, neck, osteosarcoma, ovarian, hepatocellular carcinoma, pancreatic, and prostate
	CD133 antibody	Hepatocellular, head, lung, colon, prostate, and pancreatic
	ABC transporter antibody	Lung, hepatocellular, and leukemia
	CD24 antibody	Colon
	CD20 antibody	Lymphoma, leukemia, and melanoma
	CD96 antibody	Acute myeloid leukemia
	CD117 antibody	Chronic myeloid leukemia
	EpCAM antibody	Hepatocellular, colon, and lung
glycosaminoglycan	Hyaluronic acid	Bladder, breast, colon, gastric, glioma, medulloblastoma, leukemia, head, neck, osteosarcoma, ovarian, hepatocellular carcinoma, pancreatic, and prostate

used to specifically deliver therapeutic agents into the CSCs via surface receptor-mediated endocytosis.

CD44 antibody. The surface glycoprotein CD44 is probably the most commonly used marker as a surface receptor for targeting CSCs.[24] CD44 has been shown to overexpress in bladder, breast, colon, gastric, glioma, medulloblastoma, leukemia, head, neck, osteosarcoma, ovarian, hepatocellular carcinoma, pancreatic and prostate CSCs.[25] Li et al. reported an anti-CD44 antibody-modified liposome for delivering doxorubicin into hepatocellular carcinoma CSCs.[26] This nanosystem is capable of producing a sevenfold higher drug accumulation in tumor compared to the corresponding free drug, which in turn inhibits the tumor growth more efficiently than the free drug *in vivo*.

CD133 antibody. CD133 is a 120 kDa transmembrane glycoprotein.[27] Recently, CD133 has also been used as a marker to identify several types of CSCs and its antibody has been used as a moiety for modifying nanodrug delivery systems to target CSCs. Dou et al. decorated anti-CD133 antibody on nanoparticles for targeted delivery to liver CSCs.[28] The use of this nanodrug delivery system resulted in significant improvement in therapeutic response in CD133 positive subpopulation. Besides drug delivery, CD133 antibody has been used to modify superparamagnetic iron oxide nanoparticles (SPIONs) for imaging CSCs.[29] The amount of SPIONs in CD133 positive cells was determined to be 1.7×10^{-13} mole iron (9.5 pg) or 7.0×10^{6} NPs per CSC.[29]

ABC (ATP-binding cassette) transporter antibody. The ABC transporters are transmembrane protein complexes that can pump various anti-cancer drugs out of cells and have been shown to contribute to the resistance of CSCs to chemotherapeutic drugs.[30] CSCs express high levels of ABC transporters and two typical genes that have been identified in CSCs are ABCB1 which encodes *p*-glycoprotein and the half-transporter ABCG2.[31–33] Therefore, ABC transporter proteins have been identified as potential therapeutic targets for CSCs.[34] Dou et al. investigated the therapeutic effect of anti-ABCG2 monoclonal antibody modified silver nanoparticles and vincristine on myeloma CSCs. They found that the anti-ABCG2 antibody could inhibit CSCs growth and prolonged the survival of myeloma-bearing mice.[35]

Other antibodies. CD24 is a family of 35–60 kDa variably glycosylated proteins and has been reported to overexpress on colorectal CSCs[36] although it is often negative on mammary CSCs. CD20 is an activated glycosylated phosphoprotein that is overexpressed in most B-cell lymphoma and leukemia cells and in a subpopulation of CSC-like melanoma cells.[37] In addition, CD96, known as Tactile (T cell-activated increased late expression), is a 160 kDa transmembrane glycoprotein. It has been shown that CD96 is a leukemic stem cell-specific marker in human acute myeloid leukemia. Therefore, nanodrug modified with anti-CD96 antibody may provide a potential target for leukemic stem cell-specific therapy.[38] CD117 is a transmembrane protein receptor encoded by the c-kit proto-oncogene and is the receptor for stem cell factor.[39] Although CD117 is used frequently as a marker for hematopoietic stem cells (HSCs), it is indicated that in chronic myeloid leukemia mice model induced by BCR-ABL oncogene, HSCs expressing BCR-ABL function as chronic myeloid leukemic stem cells because BCR-ABL expressing HSCs sorted as lineage^{-}Sca-1^{+}CD117^{+} cells could cause recipient mice to die of chronic myeloid leukemia.[40] Therefore, CD117 is a potential therapeutic target for chronic myeloid leukemic stem cells. Epithelial cell adhesion molecule (EpCAM) is also a commonly used tumor-associated antigen and is overexpressed on a wide range of epithelial cancer (carcinoma) cells.[41] Several kinds of EpCAM positive tumor cells have been confirmed with stem-like cell characters including hepatocellular carcinoma cells,[42] human colorectal cells,[43] and human lung tumor cells.[44] Gold NPs modified with EpCAM antibody have been used for siRNA delivery to retinoblastoma cells.[45] At present, it has not been incorporated in a nanodrug system for targeting CSCs.

2.2. Hyaluronic Acid

Hyaluronic acid (HA), also called hyaluronan or hyaluronate, has been used in drug delivery systems to target CSCs.[46] HA is a negatively charged, non-sulfated glycosaminoglycan (GAG) composed of alternating units of

N-acetyl-D-glucosamine and D-glucuronic acid.[47] CD44, the natural receptor of HA, is overexpressed on many types of CSCs.

As a hydrophilic polymer, HA has often been conjugated with a hydrophobic polymer to form an amphiphilic product for synthesizing nanoparticles. Choi et al. produced self-assembled HA NPs by chemically conjugating the hydrophobic 5b-cholanic acid to the HA backbone and the NPs were shown to be efficiently taken up by cancer cells overexpressing CD44.[48] To improve the stability of the HA NP-encapsulated drug in blood during circulation, Yoon et al. developed photo-crosslinked HA NPs without compromising its capability of targeting CD44-overexpressed cells.[49] Later, they further prepared tumor-targeting HA NPs for both photodynamic imaging and therapy by incorporating Ce6 into the NPs.[50] Under laser irradiation, Ce6 could be released from the NPs and induce singlet oxygen formation in tumor cells. Choi et al. modified HA with both hydrophobic molecules and Cy5.5 to generate core–shell NPs.[51] These NPs were found to accumulate in tumor efficiently *in vivo*. Below is a more detailed discussion of the various nanoplatforms used in combination with thermotherapy for treating cancer with particularly emphasis on targeting the CSCs.

3. NANODRUG PLATFORMS USED FOR COMBINATIONAL THERMALTHERAPY

Recently, nanodrug-mediated thermotherapy has attracted a lot of attention in part due to its ability to deposit heat specifically and locally in tumor to minimize damage to normal tissue. Below is an overview of different nanomaterials (both nanodrug and NPs without drug) developed for use in cancer thermotherapy with particular emphasis on destroying the CSCs (Fig. 2B).

3.1. Gold-Based Nanomaterial Platforms

Gold-based nanomaterials, which have much stronger absorption of the near-infrared (NIR, 700–900 nm) light than water as the major (~70%) substance in the human body, have been extensively explored for cancer thermotherapy.[14, 52–54] After specific delivery into tumor and NIR irradiation into the localized surface plasmon resonance (LSPR) bands of gold nanomaterials, the energy absorbed by the nanomaterials can be converted to and released as heat. Among different NP platforms, functionalized gold (Au) NPs excited by NIR are more stable and less toxic than that of commonly used fluorescence dyes.[55] In addition, the superb biocompatibility of gold has made gold NPs one of the best choices for non-invasive heat delivery into the human body.[56–58] Moreover, chemotherapeutic drugs have been encapsulated in or linked to the gold nanomaterials to develop multifunctional nanoplatforms.[56]

Gold nanorods (GNRs). GNRs for photothermal therapy was first demonstrated *in vitro* by the EI-Sayed group in 2006.[59] By conjugating GNRs with anti-EGFR (epidermal growth factor receptor) antibody, they also found that GNRs could be used for molecular imaging.[59] Since then, GNRs with different surface modifications have been extensively investigated for photothermal therapy.[60–63] Because the resonance of GNRs can be tuned by adjusting their aspect ratio, GNRs with suitable aspect ratios that absorb and scatter light strongly in the NIR region (700–900 nm) have been extensively studied for photothermal therapy. However, GNRs have relatively low specific surface area, resulting in limited drug carrying capacity. In order to resolve this problem, mesoporous silica-coated GNRs have been developed for improved drug encapsulation capacity.[64] This multi-functional nanoplatform consists of two parts: the inner core of gold for imaging and photothermal therapy and the outer mesoporous silica shell for drug delivery and chemotherapy. Moreover, this mesoporous silica shell is capable of preventing the GNRs from aggregation, rendering improved stability of the nanoparticulate system.

For therapy of CSCs, GNR-mediated photothermal therapy has been used by Xu et al. for selective destruction of mammary (MCF-7) CSCs.[65] They reported that polyelectrolyte conjugated GNRs could reduce the expression of stem cell genes and inhibit the formation of CSC-containing mammospheres from MCF-7 cells after near infrared laser irradiation. They further showed enhanced cellular uptake of the GNRs by CSCs compared to non-stem cancer cells,[65] which could explain the selective elimination of CSCs with the polyelectrolyte conjugated GNRs for photothermal therapy. After loading with salinomycin, a newly developed anti-breast CSC drug, synergistic inhibition of CSCs was observed for the drug and gold nanorod-mediated photothermal therapy *in vitro*. So far, no work has been reported on GNRs modified with a moiety to target CSCs. Future work in this direction is certainly warranted to further improve the safety and efficacy of GNR-mediated cancer thermotherapy.

Gold nanoshell. Gold nanoshells (GNSs) consist of a thin gold layer on silica, polystyrene, liposome, or other solid core NPs.[66–68] The thickness of the gold shell determines the resonant frequency of GNSs, ranging from that of the visible light to NIR.[69] GNSs and their photothermal capability were reported first by the Halas group in 1999.[70] Since then, various GNSs have been prepared. Tang et al. introduced gold shell on silica nanorattles that could be used for drug delivery.[71] Moreover, they conjugated transferrin on the GNSs to form a multifunctional nanoplatform that combines active targeting (by transferrin), passive targeting (due to nanoscale size), and remotely controlled photothermal therapy and chemotherapy.[72] Besides whole GNSs, half-GNS-based polymer NPs (Fig. 2(B)) were reported first by Yoo's group by depositing gold films on doxorubicin-loaded PLGA NPs. This nanoplatform also combines chemotherapy and photothermal therapy together.[73]

One of the first studies that sparked the interest in GNS-mediated photothermal therapy on CSC treatment was carried out by the Rosen research group, in which they showed that combining GNSs and radiation together could eliminate breast CSCs that are resistant to radiotherapy.[74] This combinational therapy compromised the cells' ability to repair DNA in response to the ionizing radiation on the cells, which enhanced destruction of tumor cells and decreased the CSC subpopulations among all cancer cells.

3.2. Carbon-Based Nanomaterial Platforms

Carbon nanotubes and graphenes (or graphene oxides) are the two commonly used carbon-based 3D and 2D nanoplatforms, respectively.

Carbon nanotubes. Carbon nanotubes (CNTs) can be single, double, or multi-walled (SWNT, DWNT or MWNT) and are another important family of nanomaterials for thermotherapy. Dai's group was the first to exploit their characteristics for photothermal therapy with an 808 nm laser.[75] Since then, CNTs with different modifications or drug delivery properties have been developed for CSC therapy. Peng et al. exploited the CD133 monoclonal antibody-modified SWNT in selectively targeting and eradicating $CD133^+$ glioblastoma stem-like cells under laser irradiation.[76] Torti's group showed that breast CSCs are highly resistant to conventional hyperthermia, but sensitive to CNT-mediated photothermal therapy.[77] The latter leads to reduced proliferative ability in CSCs to minimize the possibility of tumor recurrence.

Graphenes. Graphenes or graphene oxides as two-dimensional nanomaterials with unique properties have attracted much attention in the past few years for various biomedical applications including both drug delivery and photothermal therapy.[78–81] Akhavan et al. found that reduced graphene oxide of different sizes could induce different cyto- and geno-toxic effects on human mesenchymal stem cells.[82] Reduced graphene oxide with an average lateral dimension of 11 ± 4 nm exhibits strong toxicity to CSCs.[82] Robinson et al.[83] found that reduced graphene oxide in form of single layered sheet of ~20 nm in average lateral dimension has high NIR light absorbance and biocompatibility *in vitro* for photothermal therapy. So far, no study has been reported on thermotherapy combined with graphene-based drug delivery in the literature.

Table II. Summary of studies on nanoparticle-mediated thermotherapy of CSCs.

Heating source	NP type	Therapeutic drug/ other therapy	Targeting probe	Heating dose	CSC type	Efficacy	Ref
NIR (Photothermal)	GNR	Salinomycin	–	~3.2 W/cm² 8 min or 15 min (*In vitro*)	Breast (MCF-7)	Effective; Selective elimination of CSCs	[65]
	GNS	Radiation	–	42 °C 20 min (*In vitro*)	Breast (Primary)	Effective; Elimination of radio-resistant CSCs	[74]
	SWNT	–	Anti-CD133 antibody	2.0 W/cm^2 5 min (*In vivo*)	Glioblastoma (Primary)	Effective; Selectively eradication	[76]
	MWNT	–	–	3.0 W/cm^2 30 s (*In vivo*)	Breast (HMLER)	Effective; Tumor regression and long-term survival in mice	[77]
NIR (Photodynamic)	ICG	–	Anti-CD117 or CD96 antibody	12.5 J/cm^2 30 min (*In vivo*)	Leukemia (32D-p210-GFP)	Effective; Increase survival rate	[89]
Magnetic field (Thermal)	Fe_3O_4	–	–	6 kA/m; 386 kHz (*In vitro*)	Breast (MDA-MB-231)	Effective; Reduction of CSC population	[84]
	γ-Fe_2O_3	Paclitaxel	Anti-ABCG2 antibody	–	Multiple myeloma (RPMI 8226)	Effective; Inhibition of CSCs derived tumor	[91]

Notes: NPs: nanoparticles; GNR: gold nanorod; GNS: gold nanoshell; SWNT: single-walled carbon nanotube; MWNT-multi-walled carbon nanotube; and ICG: indocyanine green.

3.3. Magnetic Materials Based Nanodrug Platforms

Underneath an alternating magnetic field, magnetic NPs can generate heat[84] although it appears that the heat generation is not as effective as photothermal therapy. However, magnetic heat generation has its advantage in that the magnetic NPs may also be used for MRI imaging.[85] For example, iron oxide NPs have been used with MRI to track mesenchymal stem cells in lung metastases *in vivo*.[86] Furthermore, using magnetic NP-mediated hyperthermia to treat breast CSCs was reported in a recent study.[84] In addition to thermal effects, reactive oxygen species are detected during magnetic hyperthermia. Under these pleiotropic effects, effective eradication of CSCs was observed *in vitro*. Future work to understand the effect of magnetic hyperthermia on tumor growth and tumor recurrence *in vivo* is warranted.

3.4. Nanodrug Platform Loaded with Small Molecules for Heating

Indocyanine green or ICG is a near-infrared (NIR) fluorescence dye that has been approved by FDA for clinical use. It is a superior probe for live imaging and can strongly absorb NIR light to convert its energy into cytotoxic heat for tumor treatment.[87,88] However, free ICG is not stable with a half-life of only 2 to 4 min in circulation. Therefore, encapsulating ICG in NPs to extend its half-life in circulation has been investigated. It is worth noting that in addition to its thermal effects, ICG under NIR irradiation can also serve as an excellent photosensitizer for photodynamic therapy. ICG-loaded calcium phosphosilicate NPs modified with moiety that targets CD117 have been used to treat myeloid leukemia CSCs, leading to an impressive 29% leukemia-free survival.[89] Besides ICG, IR820 is another near-infrared fluorescence dye that has better stability compared to ICG.[34] When IR820 is encapsulated into ferritin nanocages, enhanced thermal therapy with high photothermal conversion efficiency can be achieved.[90]

4. OUTLOOK AND CONCLUSION

Due to their resistance to conventional therapies as well as their high tumorigenic potential, the difficulty of eliminating CSCs represents a major challenge to safely and effectively treat cancer. Therefore, the capability of destroying CSCs is crucial for cancer therapy and due to their rareness among a natural population of cancer cells *in vivo*, the development and identification of moieties to effectively target CSCs for further destruction is very much in need. Besides HA and antibodies, peptides, small molecules, aptamers, and other protein or carbohydrate-derived molecules may be developed and used as the moiety for targeting CSCs (Fig. 2(A)). These studies may also contribute to the technology advancement in imaging the CSCs to ascertain their complete eradication *in vivo*.

Although thermotherapy is promising as a minimally invasive alternative to conventional therapies and radical surgical intervention for cancer treatment, the lack of specificity to target CSCs requires its combination with nanodrugs of various nanoplatforms containing gold, carbon, magnetic materials, and/or small molecules for improved cancer treatment. It is worth noting that research on nanodrug-mediated thermotherapy of CSCs is still in its infancy as few nanoplatforms have been tested for CSCs (Table II). Nonetheless, the interesting findings from these limited studies suggest that this strategy holds great potential for augmented cancer therapy. By using NPs that are responsive to external stimuli such as laser and electromagnetic field for heating and anticancer drugs that are particularly potent to kill the CSCs, nanodrug-mediated thermotherapy could be used to achieve selective drug release and specific heat deposition in tumor and particularly in the CSCs (Fig. 3). This has the potential to kill all

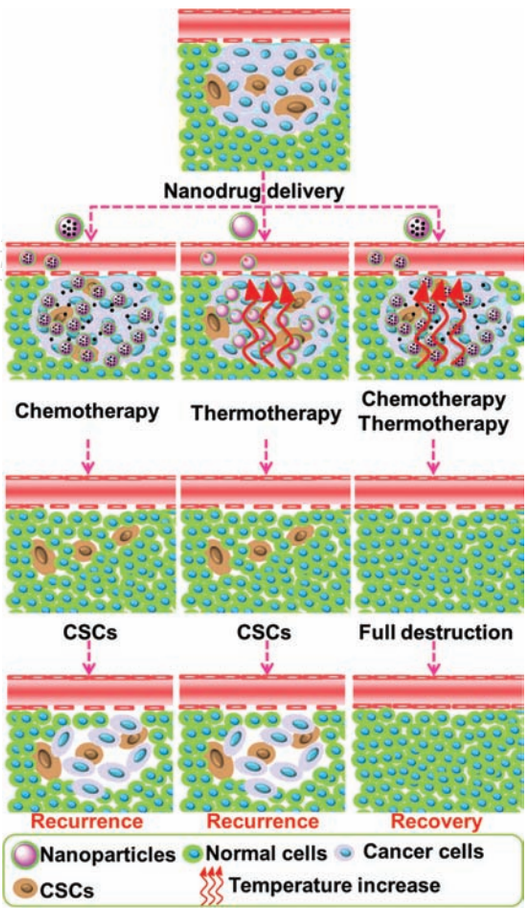

Figure 3. A schematic illustration of the resistance of cancer stem-like cells (CSCs) after chemotherapy alone, thermotherapy alone, and nanodrug-mediated thermotherapy. Chemotherapy or thermotherapy alone may destroy all non-stem cancer cells leaving behind some remnant CSCs that will reinitiate tumor growth. Nanodrug combined with thermotherapy could effectively kill all non-stem cancer cells and CSCs, leading to the eventual elimination of cancer with no recurrence.

cancer cells in the tumor with no cancer recurrence and minimal toxicity to normal tissue. However, the capability of monitoring the CSCs *in vivo* during and after treatment is very much in need to track and ensure complete eradication of the CSCs.

In conclusion, the combined treatment of nanodrug and thermotherapy shows great promise in improving the safety and efficacy of cancer treatment. Although it is challenging, future work should focus on developing multi-functional nanodrug capable of integrating localized, targeted, and/or combined thermo, chemo, and photodynamic therapies in one nanoplatform. This integrated nanoplatform together with the capability of tracking the CSCs *in vivo* holds great potential to target and completely destroy the CSCs–the root of cancer recurrence and metastasis.

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References and Notes

1. C. T. Jordan, M. L. Guzman, and M. Noble, *N. Engl. J. Med.* 355, 1253 (**2006**).
2. N. Y. Frank, T. Schatton, and M. H. Frank, *J. Clin. Invest.* 120, 41 (**2010**).
3. W. Jiang, J. Peng, Y. Zhang, W. C. Cho, and K. Jin, *Int. J. Mol. Sci.* 13, 16636 (**2012**).
4. W. Rao, S. Zhao, J. Yu, X. Lu, D. L. Zynger, and X. He, *Biomaterials* 35, 7762 (**2014**).
5. L. Vermeulen, F. de Sousa e Melo, D. J. Richel, and J. P. Medema, *Lancet. Oncol.* 13, e83 (**2012**).
6. A. Scopelliti, P. Cammareri, V. Catalano, V. Saladino, M. Todaro, and G. Stassi, *Expert. Opin. Biol. Ther.* 9, 1005 (**2009**).
7. M. Kucia, R. Reca, K. Miekus, J. Wanzeck, W. Wojakowski, A. Janowska-Wieczorek, J. Ratajczak, and M. Z. Ratajczak, *Stem. Cells* 23, 879 (**2005**).
8. P. C. Hermann, S. L. Huber, T. Herrler, A. Aicher, J. W. Ellwart, M. Guba, C. J. Bruns, and C. Heeschen, *Cell. Stem. Cell* 1, 313 (**2007**).
9. X. He and J. C. Bischof, *Crit. Rev. Biomed. Eng.* 31, 355 (**2003**).
10. M. H. Falk and R. D. Issels, *Int. J. Hyperthermia.* 17, 1 (**2001**).
11. K. F. Chu and D. E. Dupuy, *Nat. Rev. Cancer* 14, 199 (**2014**).
12. A. Januszewski and J. Stebbing, *Lancet. Oncol.* 15, 565 (**2014**).
13. X. He, *Open. Biomed. Eng. J.* 5, 47 (**2011**).
14. Z. Qin and J. C. Bischof, *Chem. Soc. Rev.* 41, 1191 (**2012**).
15. E. S. Day, J. G. Morton, and J. L. West, *J Biomech. Eng.* 131, 074001 (**2009**).
16. K. Gilstrap, X. Hu, X. Lu, and X. He, *Am. J. Cancer. Res.* 1, 508 (**2011**).
17. W. Rao, W. Zhang, I. Poventud-Fuentes, Y. Wang, Y. Lei, P. Agarwal, B. Weekes, C. Li, X. Lu, J. Yu, and X. He, *Acta. Biomater.* 10, 831 (**2014**).
18. C. D. Landon, J. Y. Park, D. Needham, and M. W. Dewhirst, *Open. Nanomed. J.* 3, 38 (**2011**).
19. M. M. Shenoi, N. B. Shah, R. J. Griffin, G. M. Vercellotti, and J. C. Bischof, *Nanomedicine.* 6, 545 (**2011**).
20. A. Sawdon, E. Weydemeyer, and C.-A. Peng, *J. Biomed. Nanotechnol.* 10, 1894 (**2014**).
21. T. Brabletz, A. Jung, S. Spaderna, F. Hlubek, and T. Kirchner, *Nat. Rev. Cancer* 5, 744 (**2005**).
22. G. P. Gupta and J. Massagué, *Cell* 127, 679 (**2006**).
23. G. Köhler and C. Milstein, *Nature* 256, 495 (**1975**).
24. A. Aruffo, I. Stamenkovic, M. Melnick, C. B. Underhill, and B. Seed, *Cell* 61, 1303 (**1990**).
25. T. Reya, S. J. Morrison, M. F. Clarke, and I. L. Weissman, *Nature* 414, 105 (**2001**).
26. L. Wang, W. Su, Z. Liu, M. Zhou, S. Chen, Y. Chen, D. Lu, Y. Liu, Y. Fan, Y. Zheng, Z. Han, D. Kong, J. C. Wu, R. Xiang, and Z. Li, *Biomaterials* 33, 5107 (**2012**).
27. D. Beier, P. Hau, M. Proescholdt, A. Lohmeier, J. Wischhusen, P. J. Oefner, L. Aigner, A. Brawanski, U. Bogdahn, and C. P. Beier, *Cancer Res.* 67, 4010 (**2007**).
28. C. Jin, Z. X. Yang, J. Y. Yang, H. M. Li, Y. He, J. Z. An, L. Bai, and K. F. Dou, *J Nanopart. Res.* 16 (**2013**).
29. L. F. Gamarra, L. F. Pavon, L. C. Marti, W. M. Pontuschka, J. B. Mamani, S. M. Carneiro, M. I. Camargo-Mathias, C. A. Moreira, and E. Amaro, *Nanomed.-Nanotechnol. Biol. Med.* 4, 330 (**2008**).
30. M. Dean, T. Fojo, and S. Bates, *Nat. Rev. Cancer* 5, 275 (**2005**).
31. M. Kim, H. Turnquist, J. Jackson, M. Sgagias, Y. Yan, M. Gong, M. Dean, J. G. Sharp, and K. Cowan, *Clin. Cancer. Res.* 8, 22 (**2002**).
32. K. Miyake, L. Mickley, T. Litman, Z. R. Zhan, R. Robey, B. Cristensen, M. Brangi, L. Greenberger, M. Dean, T. Fojo, and S. E. Bates, *Cancer. Res.* 59, 8 (**1999**).
33. L. A. Doyle, W. D. Yang, L. V. Abruzzo, T. Krogmann, Y. M. Gao, A. K. Rishi, and D. D. Ross, *P. Natl. Acad. Sci.* 95, 15665 (**1998**).
34. K. Chen, Y. H. Huang, and J. L. Chen, *Acta. Pharmacol. Sin.* 34, 732 (**2013**).
35. J. Dou, X. F. He, Y. J. Liu, Z. H. Huang, C. P. Yang, F. F. Shi, D. Y. Chen, and N. Gu, *J. Nanopart. Res.* 15, 2127 (**2013**).
36. T. M. Yeung, S. C. Gandhi, J. L. Wilding, R. Muschel, and W. F. Bodmer, *P. Natl. Acad. Sci.* 107, 3722 (**2010**).
37. S. E. Zabierowski and M. Herlyn, *J. Clin. Oncol.* 26, 2890 (**2008**).
38. N. Hosen, C. Y. Park, N. Tatsumi, Y. Oji, H. Sugiyama, M. Gramatzki, A. M. Krensky, and I. L. Weissman, *P. Natl. Acad. Sci.* 104, 11008 (**2007**).
39. V. C. Broudy, N. L. Lin, W. C. Liles, S. J. Corey, B. O'Laughlin, S. Mou, and D. Linnekin, *Blood* 94, 1979 (**1999**).
40. Y. Hu, S. Swerdlow, T. M. Duffy, R. Weinmann, F. Y. Lee, and S. Li, *P. Natl. Acad. Sci.* 103, 16870 (**2006**).
41. P. T. Went, A. Lugli, S. Meier, M. Bundi, M. Mirlacher, G. Sauter, and S. Dirnhofer, *Hum. Pathol.* 35, 122 (**2004**).
42. T. Yamashita, J. Ji, A. Budhu, M. Forgues, W. Yang, H. Y. Wang, H. Jia, Q. Ye, L. X. Qin, E. Wauthier, L. M. Reid, H. Minato, M. Honda, S. Kaneko, Z. Y. Tang, and X. W. Wang, *Gastroenterology.* 136, 1012 (**2009**).
43. P. Dalerba, S. J. Dylla, I. K. Park, R. Liu, X. Wang, R. W. Cho, T. Hoey, A. Gurney, E. H. Huang, D. M. Simeone, A. A. Shelton, G. Parmiani, C. Castelli, and M. F. Clarke, *P. Natl. Acad. Sci.* 104, 10158 (**2007**).
44. J. E. Visvader and G. J. Lindeman, *Nat. Rev. Cancer* 8, 755 (**2008**).
45. M. Mitra, M. Kandalam, J. Rangasamy, B. Shankar, U. K. Maheswari, S. Swaminathan, and S. Krishnakumar, *Mol. Vis.* 19, 1029 (**2013**).
46. P. Bulpitt and D. Aeschlimann, *J. Biomed. Mater. Res.* 47, 152 (**1999**).
47. P. M. Friedman, E. A. Mafong, A. N. Kauvar, and R. G. Geronemus, *Dermatol. Surg.* 28, 491 (**2002**).
48. K. YoungáChoi, K. HyunáMin, J. HeeáNa, I. ChanáKwon, and S. YoungáJeong, *J. Mater. Chem.* 19, 4102 (**2009**).
49. H. Y. Yoon, H. Koo, K. Y. Choi, I. Chan Kwon, K. Choi, J. H. Park, and K. Kim, *Biomaterials*. 34, 5273 (**2013**).

50. H. Y. Yoon, H. Koo, K. Y. Choi, S. J. Lee, K. Kim, I. C. Kwon, J. F. Leary, K. Park, S. H. Yuk, and J. H. Park, *Biomaterials*. 33, 3980 (**2012**).
51. K. Y. Choi, H. Chung, K. H. Min, H. Y. Yoon, K. Kim, J. H. Park, I. C. Kwon, and S. Y. Jeong, *Biomaterials*. 31, 106 (**2010**).
52. A. M. Gobin, E. M. Watkins, E. Quevedo, V. L. Colvin, and J. L. West, *Small*. 6, 745 (**2010**).
53. W. Cai, T. Gao, H. Hong, and J. Sun, *Nanotechnol. Sci. Appl.* 1, 17 (**2008**).
54. A. M. Pekkanen, M. R. Dewitt, and M. N. Rylander, *J. Biomed. Nanotechnol.* 10, 1677 (**2014**).
55. E. Boisselier and D. Astruc, *Chem. Soc. Rev.* 38, 1759 (**2009**).
56. S. S. Agasti, A. Chompoosor, C. C. You, P. Ghosh, C. K. Kim, and V. M. Rotello, *J. Am. Chem. Soc.* 131, 5728 (**2009**).
57. H. Park, J. Yang, J. Lee, S. Haam, I. H. Choi, and K. H. Yoo, *ACS. Nano* 3, 2919 (**2009**).
58. M. S. Yavuz, Y. Cheng, J. Chen, C. M. Cobley, Q. Zhang, M. Rycenga, J. Xie, C. Kim, K. H. Song, A. G. Schwartz, L. V. Wang, and Y. Xia, *Nat. Mater.* 8, 935 (**2009**).
59. X. Huang, I. H. El-Sayed, W. Qian, and M. A. El-Sayed, *J. Am. Chem. Soc.* 128, 2115 (**2006**).
60. X. Huang, P. K. Jain, I. H. El-Sayed, and M. A. El-Sayed, *Lasers Med. Sci.* 23, 217 (**2008**).
61. E. B. Dickerson, E. C. Dreaden, X. Huang, I. H. El-Sayed, H. Chu, S. Pushpanketh, J. F. McDonald, and M. A. El-Sayed, *Cancer Lett.* 269, 57 (**2008**).
62. T. Niidome, M. Yamagata, Y. Okamoto, Y. Akiyama, H. Takahashi, T. Kawano, Y. Katayama, and Y. Niidome, *J. Control. Release.* 114, 343 (**2006**).
63. J. L. Li, D. Day, and M. Gu, *Adv. Mater.* 20, 3866 (**2008**).
64. S. O. Obare, N. R. Jana, and C. J. Murphy, *Nano Lett.* 1, 601 (**2001**).
65. Y. Xu, J. Wang, X. Li, Y. Liu, L. Dai, X. Wu, and C. Chen, *Biomaterials* 35, 4667 (**2014**).
66. W. Shi, Y. Sahoo, M. T. Swihart, and P. Prasad, *Langmuir.* 21, 1610 (**2005**).
67. G. Wu, A. Mikhailovsky, H. A. Khant, C. Fu, W. Chiu, and J. A. Zasadzinski, *J. Am. Chem. Soc.* 130, 8175 (**2008**).
68. M. P. Melancon, W. Lu, Z. Yang, R. Zhang, Z. Cheng, A. M. Elliot, J. Stafford, T. Olson, J. Z. Zhang, and C. Li, *Mol. Cancer Ther.* 7, 1730 (**2008**).
69. A. Lin, L. Hirsch, M.-H. Lee, J. Barton, N. Halas, J. West, and R. Drezek, *Technol. Cancer. Res. Treat.* 3, 33 (**2004**).
70. R. D. Averitt, S. L. Westcott, and N. J. Halas, *J. Opt. Soc. Am. B.* 16, 1824 (**1999**).
71. H. Liu, D. Chen, L. Li, T. Liu, L. Tan, X. Wu, and F. Tang, *Angew. Chem. Int. Ed.* 123, 921 (**2011**).
72. H. Liu, T. Liu, X. Wu, L. Li, L. Tan, D. Chen, and F. Tang, *Adv. Mater.* 24, 755 (**2012**).
73. S. M. Lee, H. Park, J. W. Choi, Y. N. Park, C. O. Yun, and K. H. Yoo, *Angew. Chem. Int. Ed.* 50, 7581 (**2011**).
74. R. L. Atkinson, M. Zhang, P. Diagaradjane, S. Peddibhotla, A. Contreras, S. G. Hilsenbeck, W. A. Woodward, S. Krishnan, J. C. Chang, and J. M. Rosen, *Sci. Transl. Med.* 2, 55ra79 (**2010**).
75. N. W. S. Kam, M. O'Connell, J. A. Wisdom, and H. Dai, *Proc. Natl. Acad. Sci. USA* 102, 11600 (**2005**).
76. C. H. Wang, S. H. Chiou, C. P. Chou, Y. C. Chen, Y. J. Huang, and C. A. Peng, *Nanomed.-Nanotechnol. Biol. Med.* 7, 69 (**2011**).
77. A. R. Burke, R. N. Singh, D. L. Carroll, and J. Wood, *Biomaterials* 33, 2961 (**2012**).
78. Z. Liu, J. T. Robinson, S. M. Tabakman, K. Yang, and H. J. Dai, *Mater. Today* 14, 316 (**2011**).
79. Z. Liu, J. T. Robinson, X. Sun, and H. Dai, *J. Am. Chem. Soc.* 130, 10876 (**2008**).
80. X. Sun, Z. Liu, K. Welsher, J. T. Robinson, A. Goodwin, S. Zaric, and H. Dai, *Nano. Res.* 1, 203 (**2008**).
81. H. Dong, C. Dong, T. Ren, Y. Li, and D. Shi, *J. Biomed. Nanotechnol.* 10, 2086 (**2014**).
82. O. Akhavan, E. Ghaderi, and A. Akhavan, *Biomaterials* 33, 8017 (**2012**).
83. J. T. Robinson, S. M. Tabakman, Y. Liang, H. Wang, H. Sanchez Casalongue, D. Vinh, and H. Dai, *J. Am. Chem. Soc.* 133, 6825 (**2011**).
84. T. Sadhukha, L. Niu, T. S. Wiedmann, and J. Panyam, *Mol. Pharm.* 10, 1432 (**2013**).
85. H. Lee, E. Lee, D. K. Kim, N. K. Jang, Y. Y. Jeong, and S. Jon, *J. Am. Chem. Soc.* 128, 7383 (**2006**).
86. M. R. Loebinger, P. G. Kyrtatos, M. Turmaine, A. N. Price, Q. Pankhurst, M. F. Lythgoe, and S. M. Janes, *Cancer. Res.* 69, 8862 (**2009**).
87. M. Zheng, C. Yue, Y. Ma, P. Gong, P. Zhao, C. Zheng, Z. Sheng, P. Zhang, Z. Wang, and L. Cai, *ACS. nano.* 7, 2056 (**2013**).
88. J. Yu, D. Javier, M. A. Yaseen, N. Nitin, R. Richards-Kortum, B. Anvari, and M. S. Wong, *J. Am. Chem. Soc.* 132, 1929 (**2010**).
89. B. M. Barth, I. A. E, S. S. Shanmugavelandy, J. M. Kaiser, D. Crespo-Gonzalez, N. A. DiVittore, C. McGovern, T. M. Goff, N. R. Keasey, J. H. Adair, T. P. Loughran, Jr., D. F. Claxton, and M. Kester, *ACS. nano.* 5, 5325 (**2011**).
90. P. Huang, P. Rong, A. Jin, X. Yan, M. G. Zhang, J. Lin, H. Hu, Z. Wang, X. Yue, W. Li, G. Niu, W. Zeng, W. Wang, K. Zhou, and X. Chen, *Adv. Mater.* 26, 6401 (**2014**).
91. C. Yang, X. He, L. Song, X. Zhan, Y. Zhang, J. Dou, and N. Gu, *J. Biomed. Nanotechnol.* 10, 336 (**2014**).

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