Transcriptional Regulation of the Colorectal Cancer Stem Cell Markers, *Nanog and Oct4*, Induced by a Thermodynamic-Based Therapy Approach

Zakieh Ghorbani
Islamic Azad University Marvdasht
Mansour Heidari
Tehran University of Medical Sciences
Mojtaba Jafarinia
Islamic Azad University Marvdasht
Mahdi Rohani
Pasteur Institute of Iran
Abolfazl Akbari (akbariia2006@gmail.com)
Iran University of Medical Sciences: Tehran University of Medical Sciences https://orcid.org/0000-0002-2151-4639

Research

Keywords: Cancer stem cell, Cell proliferation, Colorectal cancer, Gene expression, Hyperthermia, Photodynamic therapy

Posted Date: September 14th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-856001/v1

Abstract

Background: Cancer stem cells (CSC) play a crucial role in tumorigenesis, recurrence, metastasis, and chemoresistanc. Some studies suggest that hyperthermia and photodynamic therapy (PDT) may be effective for cancer treatment, particularly when combined with other therapeutic approaches. However, the results are conflicting. Our aim was to evaluate the effect of hyperthermia combined with PDT on colorectal CSC viability and the gene expression of the CSC markers.

Results: Cell viability decrased by PDT (P=0.015) and the combination therapy (P=0.006) but not hyperthermia alone (P=0.4) compared to control. Gene expression of CSC markers significantly decreased in all therapies.

Conclusion: Hyperthermia combined with PDT was more efficient in eliminating tumors than hyperthermia or PDT alone.

Background

Colorectal cancer (CRC) is the third most commonly detected cancer with 1.8 million cases worldwide in 2018 [1–3]. Currently, conventional treatments for CRC include surgical removal of tumors, radiation therapy, and chemotherapy [4]. Despite advances in these treatments, CRC has shown resistance to these treatments alone [5]. Several lines of evidence suggest that a subpopulation of CRC cells, named cancer stem cells (CSCs), contribute to resistance to radiation and chemotherapy and therefore, lead to failure of the cancer treatments [6, 7] [8]. It has also been shown that colorectal CSCs have the capacity of tumorigenesis, recurrence and metastasis [9]. To overcome the drawbacks of conventional therapies, several studies have focused on the novel and combined strategies, including combination of ionizing radiation, hyperthermia and photodynamic therapy (PDT) [10–12].

Hyperthermia (also commonly known as thermal therapy or thermotherapy) occurs when an individual's body temperature (local, regional or whole body) increases [13]. Hyperthermia has been found to be highly effective in CRC therapy specially when combined with chemotherapy, radiotherapy, or immunotherapy [14, 15]. The previous study demonstrated that mild hypothermia or fever-range hyperthermia had inhibitory effect on viability/proliferation in glioblastoma, prostate, lung, breast cancer cell lines [16]. However, the other study has found that hyperthermia alone had no significant effect on breast cancer cells but its combination with radiotherapy has been useful for apoptosis [17]. Here, there is the gap in our knowledge and why we are doing this study on hyperthermia effects.

Therapy with light (i.e. PDT) is a non-invasive strategy that can also be effective as a supporting treatment for various types of cancer and malignant tumors [18]. It use a photosensitizer which becomes activated by laser in a specific wavelength and reacts with oxygen to generate reactive oxidant species (ROS) in target tissues, leading to cell death [19, 20]. ROS can unbalance redox system as well as damage DNA and protein. Moreover, PDT can dysregulate mitochondrial activity, autophagy, and CSCs dormancy [21]. One of the most studied photosensitizer is curcumin (Cur) which has numerous

therapeutic properties. An increasing body of evidence suggests that Cur might an appropriate photosensitizer for PDT [22]. A previous study has showed that curcumin based PDT can not effect on apoptosis in melanoma [23]. To deliver the photosensitizer to the malignant tumor, gold nanoparticles (GNPs) have been widely used due to their biocompatibility and low toxicity [24].

Given that some studies showed limited an somewhat conflicting role for hyperthermia and PDT alone in treating malignant tumor, we aimed to investigate whether hyperthermia and Cur-GNPs-mediated PDT alone and in combination could effect on the cell viability and the expression of cancer stem cell markers in CRC.

Results

Cell morphology assay

Hyperthermia and PDT had no impact Using an inverted microscope, The results showed that the hyperthermia and PDT have no effect on cell morphology.

Hyperthermia decreased expression of CSC marker but did not affect cell viability.

Hyperthermia did not decrease cell viability of HT-29 cells compared to the control (37°C) (Fig. 1a). However, expression of *Nanog* gene was significantly decreased in the HT-29 cells treated with hyperthermia at 42°C ($P = 0.038$), but not at 43°C ($P > 0.05$). Additionally, there was no significant difference in *Oct4* gene expression between hyperthermia group and the control (Fig. 1b).

PDT decreased cancer cell viability and downregulated expression of CSC markers.

The cell viability of HT-29 was significantly decreased by Cur-GNPs-mediated PDT in a dose-dependent manner (Fig. 2a). *Nanog* and *Oct4* gene expression also decreased ($P < 0.05$) (Fig. 2b).

Combination of PDT and hyperthermia was more effective in downregulating gene expression of CSC markers.

Combination of hyperthemia and PDT was more effective in reducing cell viability than hyperthermia or PDT alone (compared to hyperthermia; $P < 0.01$, compared to PDT; $P < 0.05$), (Fig. 3a). Moreover, the combination of PDT and hyperthermia downregulated the gene expression of *Nanog* and *Oct4* ($P < 0.05$) (Fig. 3b).

Discussion

In our study, PDT but not hyperthermia was effective in reducing cell viability. Both treatments significantly reduced gene expression of CSC markers. However, combination of hyperthermia and PDT

was more effective than hyperthermia and PDT alone.

The previous study has shown that hyperthermia exposure downregulated the stemness-related genes such as *Abcg2* and *Nanog* in colon cancer cell lines [26]. These results showed that hyperthermia might contribute to chemosensitization of CRC.

Hyperthermia has been shown to enhance the effectiveness of chemotherapy and radiation treatment [27]. A previous study have revealed that hyperthermia reduced the number of CSCs cells and downregulated expression of CSC markers such as *Nanog* [6, 26]. We found similar results in our study where hyperthermia significantly reduced expression of *Nanog*, a CSC marker, but the cell viability remained the same. Although evidence has suggested the effectiveness of hyperthermia in cancer treatment, temperature elevation alone cannot remove cancer cells [28]. On the other hand, hyperthermia can inhibit repair of chemotherapy- or radiotherapy-induced DNA damage [29] and activate extracellular heat shock proteins (HSPs) leading to the antitumor immune system from the heat-treated necrotic tumor cells [30]. Thereby, a series of events have been revealed to cause cell death. After mild hyperthermia, some cellular functions can be recovered and some subpopulations of cells may be resistant to hyperthermic-induced cell death [31]. Thus, inability to reduce cell viability may be due to the rate of temperature or short time hyperthermia exposure.

Next, we use PDT to teat HT-29 cells. We found that expression of CSC markers as well as the cell viability were significantly decreased. This finding is similar to previous studies who reported a beneficial role for PDT in treating several types of cancer including including glioblastomas, lung, esophageal, bladder, colorectal and nasopharyngeal carcinomas [32]. PDT-generated ROS can change the activity of CSC markers *Oct4* and *Sox2* [21]. Furthermore, ROS attacks DNA causing point mutations in key genes such as *Ras* [33] and *p53* [34] which regulate several pathways in the cell including regulate proliferation, differentiation and apoptosis [35]. In addition, ROS binds to lipids and produces free radicals and peroxides that can damage cell membranes and change mitochondrial permeability leading to apoptosis [36].

Lastly, we used a combination of hyperthermia, and PDT. Cell viability and CSC marker expression was significantly lower comared to hyperthermia, PDT or the control. Previous studies have also reported a decrease in cell viability and increased DNA fragmentation and ROS production [37, 38]. The combination therapy induced the mitochondrial activation and increased the *Caspases-9, -3* and poly ADP-ribose polymerase (*PARP*) expression leading to apoptosis [39]. In one study, authors showed that hyperthermia increased efficacy of PDT on gastric cancer cells through elevated ROS production leading to enhanced PDT-induced cytotoxicity [37]. The increased treatment efficiency in the combination therapy may be due to downregulation of proliferation-related genes, increased ROS production and mitochondrial-dependent apoptosis [40].

Our study showed the anti-cancer effects of combined hyperthermia and PDT *in vitro* system. Although we believe that cell lines studies will support clinical applications, for prospective studies, it is suggested more investigation on several CRC cell lines, using various irradiation dose and animal models.

In brief, we provided an important evidence of the effectiveness of hyperthermia combined with PDT in decreasing cell viability via regulating stemness-related pathways in CRC. However, the underlying molecular mechanisms of the combination of hyperthermia and PDT is still unknown. Future studies should focus on understanding the mechanism of cancer treatment via combination therapy.

Conclusion

Our results suggest that PDT in combination with hyperthermia may be more effective in treating and eliminate primary and metastatic tumors including colorecta CSC.

Materials And Methods

In vitro cytotoxicity evaluation of Cur-GNPs

Synthesized and characterized gold nanoparticles (GNP) coated with Cur (Cur-GNPs) [25] were generously gifted by Seyed Mohammad amini (Radiation Biology Research Center, Iran University of Medical Sciences, Tehran, Iran).

The colon carcinoma (HT-29) cell line was purchased from National Bank of Pasteur Institute (Iran) and cultured in RPMI-1640 cell growth medium supplemented with penicillin (100U/ml), streptomycin (100µg/ml), and 10% FBS. A standard humidified atmosphere (95% air & 5% CO2 at 37 °C) was provided for continuous cell culture.

In order to evaluate Cur-GNPs cytotoxicity, 10^4 cells/well HT-29 cells were seeded into each well of a 96-well plate. After 24h, culture medium containing a known concentration of Cur-GNPs was added and were incubated for an additional 24h. Using the same procedure, Cur dark cytotoxicity was examined by 1h incubation. For photodynamic studies, we added fresh culture medium containing Cur-GNPs (128mg/ml) and Cur (6.4µg/ml) to cells, and they were incubated for 1h. Then, the culture medium was replaced and exposed to the 150mW (15.7mW/cm^2) laser (Thor International Ltd, Amersham, Bucks, UK) for 2 minutes. The real-time PCR and MTT tests were completed after 24 h incubation.

In vitro treatment with hyperthermia, PDT and combination treatment

The cell lines was cultured in RPMI-1640 supplemented with 10% of fetal bovine serum (FBS) (Gibco, USA) and incubated at 37 °C in 5% CO_2. The medium was replaced every 2 days until the cells reached 80-90% confluence. The cells were incubated in culture medium supplemented with 10% FBS in an incubator preheated to 42 and 43 ˚C for 2 hours. Control groups were incubated at 37˚C for 2 hours, as well. After hyperthermia treatment, the cells were incubated at 37˚C for 2 hours prior to analysis.

For PDT treatment, first the cells were seeded in 96-well plates and incubated with 5 µM solution of Cur-GNPs for 24 h. Cur-GNPs culture medium was then removed and the plate was washed with 350 µL PBS per well. Irradiation was accomplished at room temperature with a LED-based illumination device (PDT

EDL-1; Hamamatsu Photonics K.K., Hamamatsu, Japan). The expriments were adjusted by low-power laser (32 mW 630 nm diode laser, 0.5 J/cm2, continuous mode for 360 seconds). Following irradiation, fresh culture medium lacking phenol red was added, cells were incubated at 37 °C for 24 h. After the irradiation, cells were enriched by fresh medium and incubated for 24 h. Then, cell viability was evaluated by MTT assay.

For combination therapy with both hyperthermia and PDT, a cell group was simultaneously exposed to hyperthermia and PDT with the aforementioned conditions. In order to minimize interference with the optical sensitizer and maximize the effect of the laser, the cell culture medium was enriched with low FBS (% 1). The preheated cells at 42 and 43 ˚C for 2 hours, were cultured in the presence of Cur-GNPs and treated with low-power laser. Appropriate control groups were used in all experiments. The cells with no treatment were considered as control. Then, MTT test was performed to evaluate cell viability.

Cell morphology

To evaluate the effect of the hyperthermia and PDT on the cellular phenotype, the cells were evaluated using an inverted microscope.

Cell viability assay

After treatments, the cell viability was determined by MTT [3-(4, 5-dimethylthiazol-2-Yl)-2, 5-diphenyltetrazolium bromide] (BioIDEA,Iran) assay. For MTT assay, HT-29 cells were seeded in 96-well plates a day prior to the experiment. The cell viability was evaluated after hyperthermia exposure. Then, the culture medium was aspirated and 10µl MTT solution with a final concentration of 0.5 mg/mL was added. Afterward, 3 hours of incubation at 37°C, MTT solution was aspirated and 100µl well DMSO was added to each well. After 30 min incubation at 37 °C, the absorbance ($^{570}/_{630}$ nm) was measured using a microplate reader (**BioTek**, USA).

RNA extraction and cDNA synthesis

Total RNA was extracted from cells using high pure RNA isolation kit (Roche, Germany) according to the manufacturer’s instructions. The concentration of RNA was quantified using Nano Drop™ Lite Spectrophotometer (Thermo Fisher Scientific, USA). A 2% agarose gel electrophoresis was used to assess quality of RNA. Subsequently, cDNA was synthesized from the purified total RNA using a PrimeScript RT reagent Kit (Takara, Japan) according to the manufacturer's protocol.

Primers designed for quantitative Real-Time PCR

The GeneRunner softwarewas was used to design the primers for amplification of interested genes.Additionally, the primer specificity was confirmed by Primer-BLAST (www.ncbi.nlm.nih.gov/tools/primer-blast). The sequences of primers used in the current study are listed in Table 1. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as internal control for normalization (Table 1).

Table 1. Primers designed for amplification of interested genes.

DNA fragment name	Primer name	Primer sequence	Product length
OCT4	F	5´ CTTGAATCCCGAATGGAAAGGG 3´	164
	R	5´ GTGTATATCCCAGGGTGATCCTC 3´	
NANOG	F	5´ TTTGTGGGCCTGAAGAAAACT 3´	116
	R	5´ AGGGCTGTCCTGAATAAGCAG 3´	
GAPDH	F	5´ CACCAGGGCTGCTTTTAAC 3´	190
	R	5´ ATCTCGCTCCTGGAAGAT 3´	

Quantitative Real-Time PCR

To analyze mRNA levels, q-RT PCR was performed using SYBR® Premix Ex Taq™ II (Takara, Japan) and the ABI7500 system (Applied Biosystems; Thermo Fisher Scientific). The reaction system included 10 μLSYBR_ *Premix Ex Taq*_II (2x), 0.4 μL ROX dye, 2 μL template cDNA, 0.4 μL each primer, and 6.8 μL RNase-free water. The q-RT PCR reactions were performed in the following cycling conditions: The initial denaturation step 95 °C for 30 sec, then 40 cycles of 95 °C for 5 sec and 60 °C for 30 sec. All experiments were repeated at least three times.

Statistical analysis

The SPSS 19.0 (SPSS, Inc., Chicago, IL, USA) software was used for statistical analysis. Kruskal Wallis test was used to compare the experiments. Gene expression data drived from real-time PCR were analyzed using GraphPad Prism 7.0 software. Data were presented as mean ± SEM. The p-values <0.05 were considered statistically significant.

Abbreviations

Photodynamic therapy (PDT)**,** Gold nanoparticles coated with curcumin (Cur-GNPs), Colorectal cancer (CRC), Reactive oxidant species (ROS)

Declarations

Availability of data and materials

The data sets used and analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgements

Not applicable.

Funding

This work was supported by Grant No. 97-03-49-13165 from Iran University of Medical Sciences.

Author contributions

ZGh and AA contributed to study design and conception. MH and MR assisted to experimental investigations. MR and MJ assisted with analysis of the data. ZGh prepared the manuscript which AA, MH and MJ significantly revised it. All authors read and approved the final manuscript.

Availability of data and materials

The data that support the findings of this study are available on request

from the corresponding author.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Akbari A, Mobini GR, Maghsoudi R, Akhtari J, et al. Modulation of transforming growth factor-β signaling transducers in colon adenocarcinoma cells induced by staphylococcal enterotoxin B. J Gastrointest Canc. 2016;13(1):909-14. https://doi.org/10.3892/mmr.2015.4596.
2. Emami SS, Akbari A, Zare A-A, Agah S, et al. MicroRNA expression levels and histopathological features of colorectal cancer. J Gastrointest Canc. 2019;50(2):276-84. https://doi.org/10.1007/s12029-018-0055-x.
3. Rawla P, Sunkara T, Barsouk AJPg. Epidemiology of colorectal cancer: Incidence, mortality, survival, and risk factors. Prz Gastroenterol. 2019;14(2):89. http://doi.org/10.5114/pg.2018.81072.
4. Takimoto R, Kamigaki T, Okada S, Matsuda E, et al. Prognostic factors for colorectal cancer patients treated with combination of immune-cell therapy and first-line chemotherapy: a retrospective study. Anticancer Res. 2019;39(8):4525-32. https://doi.org/10.21873/anticanres.13629.

5. Kim J-E, Shin J-Y, Cho M-H. Magnetic nanoparticles: an update of application for drug delivery and possible toxic effects. Archives of toxicology. Arch Toxicol. 2012;86(5):685-700. https://doi.org/10.1007/s00204-011-0773-3.
6. Medema JP. Cancer stem cells: the challenges ahead. Nat Cell Biol. 2013;15(4):338. https://doi.org/10.1038/ncb2717.
7. Mirzaei A, Madjd Z, Kadijani AA, Alinaghi S, et al. Cancer Stem cell's potential clinical implications. Iranian Journal of Cancer Prevention. Int J Cancer Manag. 2017;10(1). https://doi.org/10.17795/ijcp-5897.
8. Cojoc M, Mäbert K, Muders MH, Dubrovska A. A role for cancer stem cells in therapy resistance: cellular and molecular mechanisms. Semin Cancer Biol: Elsevier. 2015. https://doi.org/10.1016/j.semcancer.2014.06.004.
9. Zhu P, Fan Z. Cancer stem cells and tumorigenesis. Biophys Rep. 2018;4(4):178-88. https://doi.org/10.1007/s41048-018-0062-2.
10. Kolosnjaj-Tabi J, Wilhelm C. Magnetic nanoparticles in cancer therapy: how can thermal approaches help?. Nanomedicine. 2017. https://doi.org/10.2217/nnm-2017-0014.
11. Mirzaei A, Madjd Z, Kadijani AA, Tavakoli-Yaraki M, et al. Evaluation of circulating cellular DCLK1 protein, as the most promising colorectal cancer stem cell marker, using immunoassay based methods. Cancer Biomark. 2016;17(3):301-11. https://doi.org/10.3233/CBM-160642.
12. Rentsch M, Schiergens T, Khandoga A, Werner J. Surgery for Colorectal Cancer-Trends, Developments, and Future Perspectives. Visc Med. 2016;32(3):184-91. https://doi.org/10.1159/000446490.
13. Song X, Kim HC, Kim SY, Basse P, et al. Hyperthermia-enhanced TRAIL-and mapatumumab-induced apoptotic death is mediated through mitochondria in human colon cancer cells. J Cell Biochem. 2012;113(5):1547-58. https://doi.org/10.1002/jcb.24023.
14. Lutgens L, van der Zee J, Pijls-Johannesma M, De Haas-Kock DF, et al. Combined use of hyperthermia and radiation therapy for treating locally advanced cervix carcinoma. Cochrane Database Syst Rev. 2010(3). https://doi.org/10.1002/14651858.CD006377.
15. Sadhukha T, Niu L, Wiedmann TS, Panyam J. Effective elimination of cancer stem cells by magnetic hyperthermia. Mol Pharm. 2013;10(4):1432-41. https://doi.org/10.1021/mp400015b.
16. Kalamida D, Karagounis IV, Mitrakas A, Kalamida S, et al. Fever-range hyperthermia vs. hypothermia effect on cancer cell viability, proliferation and HSP90 expression. PLoS One. 2015;10(1):e0116021. https://doi.org/10.1371/journal.pone.0116021.
17. Hadi F, Tavakkol S, Laurent S, Pirhajati V, et al. Combinatorial effects of radiofrequency hyperthermia and radiotherapy in the presence of magneto-plasmonic nanoparticles on MCF-7 breast cancer cells. J Cell Physiol. 2019;234(11):20028-35. https://doi.org/10.1002/jcp.28599.
18. Agostinis P, Berg K, Cengel KA, Foster TH, et al. Photodynamic therapy of cancer: an update. CA Cancer J Clin. 2011;61(4):250-81. https://doi.org/10.3322/caac.20114.

19. dos Santos AlF, de Almeida DRQ, Terra LF, Baptista McS, et al. Photodynamic therapy in cancer treatment-an update review. J Cancer Metastasis Treat. 2019;5. https://doi.org/10.20517/2394-4722.2018.83.
20. Kwiatkowski S, Knap B, Przystupski D, Saczko J, et al. Photodynamic therapy–mechanisms, photosensitizers and combinations. Biomed Pharmacother. 2018;106:1098-107. https://doi.org/10.1016/j.biopha.2018.07.049.
21. Zhang Z-J, Wang K-P, Mo J-G, Xiong L, Wen YJ. Photodynamic therapy regulates fate of cancer stem cells through reactive oxygen species. World J Stem Cells. 2020;12(7):562. https://doi.org/10.4252/wjsc.v12.i7.562.
22. Kazantzis K, Koutsonikoli K, Mavroidi B, Zachariadis M, et al. Curcumin derivatives as photosensitizers in photodynamic therapy: photophysical properties and in vitro studies with prostate cancer cells. Photochem Photobiol Sci. 2020;19(2):193-206. https://doi.org/10.1039/c9pp00375d.
23. Szlasa W, Supplitt S, Drąg-Zalesińska M, Przystupski D, et al. Effects of curcumin based PDT on the viability and the organization of actin in melanotic (A375) and amelanotic melanoma (C32)–in vitro studies. Biomed Pharmacother. 2020;132:110883. https://doi.org/10.1016/j.biopha.2020.110883.
24. Tiwari PM, Vig K, Dennis VA, Singh SR. Functionalized gold nanoparticles and their biomedical applications. Nanomaterials. 2011;1(1):31-63. https://doi.org/10.3390/nano1010031.
25. Shaabani E, Amini SM, Kharrazi S, Tajerian RJ. Curcumin coated gold nanoparticles: synthesis, characterization, cytotoxicity, antioxidant activity and its comparison with citrate coated gold nanoparticles. Nanomed J. 2017;4(2):115-25. https://doi.org/10.22038/NMJ.2017.8413.
26. Gao F, Ye Y, Zhang Y, Yang J. Water bath hyperthermia reduces stemness of colon cancer cells. Clin Biochem. 2013;46(16-17):1747-50. https://doi.org/10.1016/j.clinbiochem.2013.08.023.
27. Moyer HR, Delman KA. The role of hyperthermia in optimizing tumor response to regional therapy. International Journal of Hyperthermia. Int J Hyperthermia. 2008;24(3):251-61. https://doi.org/10.1080/02656730701772480.
28. Chung H-J, Lee H-K, Kwon KB, Kim H-J, Hong S-T. Transferrin as a thermosensitizer in radiofrequency hyperthermia for cancer treatment. scientific reports. 2018;8(1):1-11. https://doi.org/10.1038/s41598-018-31232-9.
29. Pelicci PG, Dalton P, Orecchia R. Heating cancer stem cells to reduce tumor relapse. Breast Cancer Res. 2011;13(3):305. https://doi.org/10.1186/bcr2847.
30. Lin F-C, Hsu C-H, Lin Y-Y. Nano-therapeutic cancer immunotherapy using hyperthermia-induced heat shock proteins: insights from mathematical modeling. Int J Nanomedicine. 2018;13:3529. https://doi.org/doi:10.1186/bcr2847.
31. Sadhukha T, Niu L, Wiedmann TS, Panyam J. Effective elimination of cancer stem cells by magnetic hyperthermia. Mol Pharm. 2013;10(4):1432-41. https://doi.org/10.1021/mp400015b.
32. El-Daly SM, Abba ML, Gamal-Eldeen AM. The role of microRNAs in photodynamic therapy of cancer. Eur J Med Chem. 2017;142:550-5. https://doi.org/10.1016/j.ejmech.2017.10.011.

33. Ozsvari B, Sotgia F, Lisanti MP. A new mutation-independent approach to cancer therapy: Inhibiting oncogenic RAS and MYC, by targeting mitochondrial biogenesis. Aging. 2017;9(10):2098. https://doi.org/10.18632/aging.101304.
34. Shen Y-A, Lin C-H, Chi W-H, Wang C-Y, et al. Resveratrol impedes the stemness, epithelial-mesenchymal transition, and metabolic reprogramming of cancer stem cells in nasopharyngeal carcinoma through p53 activation. Evid Based Complement Alternat Med. 2013;2013. https://doi.org/10.1155/2013/590393.
35. Li H, Zhang J, Tong JHM, Chan AWH, et al. Targeting the oncogenic p53 mutants in colorectal cancer and other solid tumors. Int J Mol Sci. 2019;20(23):5999. https://doi.org/10.3390/ijms20235999.
36. Yang B, Liu H, Yang H, Chen W, et al. Combinatorial photochemotherapy on liver cancer stem cells with organoplatinum (ii) metallacage-based nanoparticles. J Mater Chem B. 2019;7(42):6476-87. https://doi.org/10.1039/c9tb01299k.
37. Kurokawa H, Ito H, Terasaki M, Matsui H. Hyperthermia enhances photodynamic therapy by regulation of HCP1 and ABCG2 expressions via high level ROS generation. Sci Rep. 2019;9(1):1-8. https://doi.org/10.1038/s41598-018-38460-z.
38. Xu J, Gao J, Wei QJ. Combination of photodynamic therapy with radiotherapy for cancer treatment. Journal of Nanomaterials. 2016;2016. https://doi.org/10.1155/2016/8507924.
39. Lu C-H, Kuo Y-Y, Lin G-B, Chen W-T, Chao C-Y. Application of non-invasive low-intensity pulsed electric field with thermal cycling-hyperthermia for synergistically enhanced anticancer effect of chlorogenic acid on PANC-1 cells. PLoS One. 2020;15(1):e0222126. https://doi.org/10.1371/journal.pone.0222126.
40. Noghreiyan AV, Imanparast A, Ara ES, Soudmand S, et al. In-vitro investigation of cold atmospheric plasma induced photodynamic effect by Indocyanine green and Protoporphyrin IX. Photodiagnosis Photodyn Ther. 2020;31:101822. https://doi.org/10.1016/j.pdpdt.2020.101822.

Figures

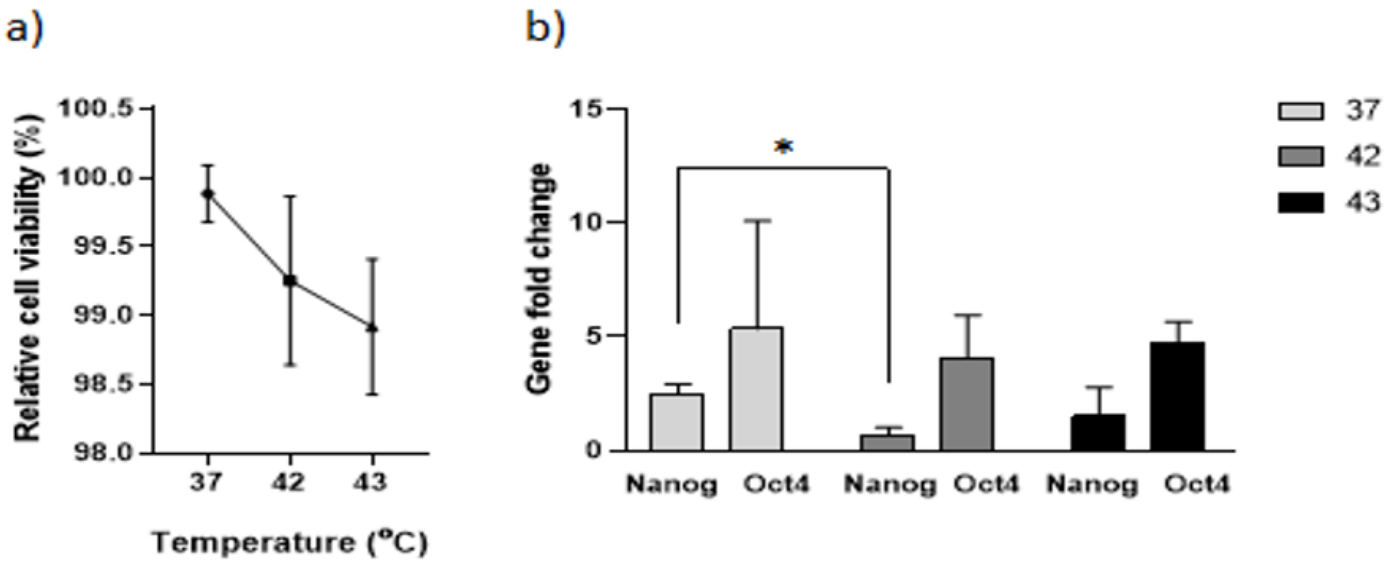

Figure 1

a) Cell viability after treatment with hyperthermia. b) Effect of hyperthermia on expression of Nanog and Oct4 measured by qRT-PCR in HT-29 cell line. *p < 0.05.

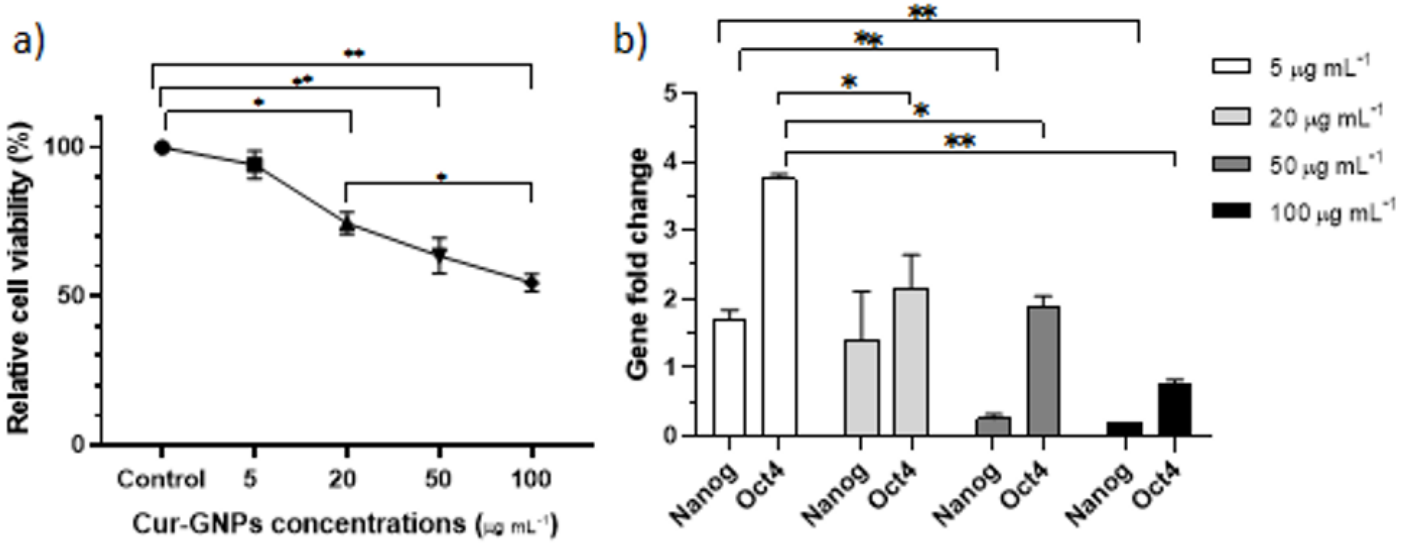

Figure 2

a) Cell viability after treatment with PDT. b) Relative gene expression of Nanog and Oct4 induced by PDT with various concentrations of Cur-GNPs. *p < 0.05, **p < 0.01, ***p < 0.001.

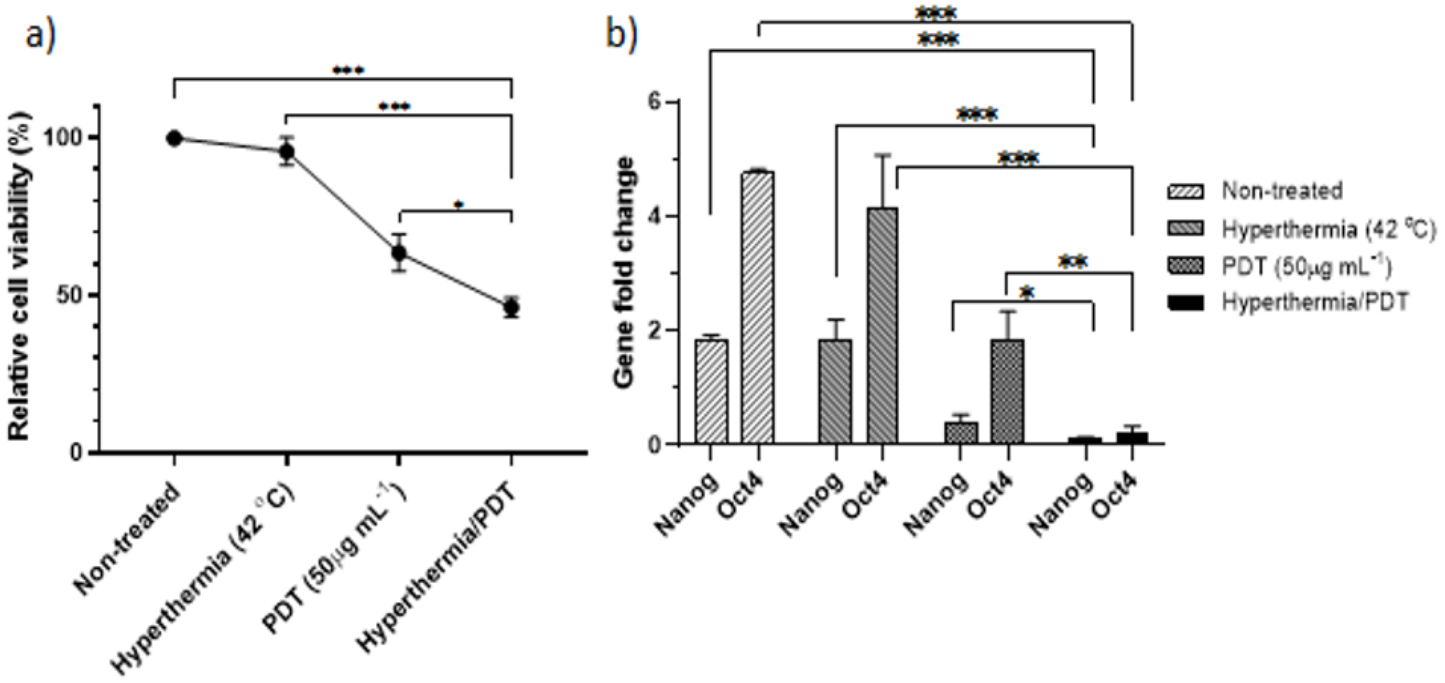

Figure 3

a) Cell viability after treatment with a combination of PDT and hyperthermia b) Relative effect of PDT and hyperthermia combination therapy on gene expression of Nanog and Oct4. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.