Research Article

World Journal of Hematology and Oncology

Synergistic Anti-disease Effect of Inhibition of Histone Deacetylase and leaguer of the Glycolytic Pathway

Mathew O*

Department of surgery, Harvard University, USA

***Corresponding author**

Olivia Mathew, Department of surgery, Harvard University, USA,
Email: olivia1307@gmail.com

Received Date: 5-Aug-2022
Accepted Date: 7-Aug-2022
Published Date: 8-Sep-2022

1.Abstract

Anaplastic Thyroid Cancer (ATC) is characterized by a advanced chance of epigenetic changes; these do more frequently than inheritable mutations. Studies using preclinical models have reported that a combination of N-hydroxy- 7-(2- naphthylthio) heptanomide (HNHA) and 2-deoxy- D- glucose(2DG) plays a pivotal part in cancer stem cell- suchlike cells in ATC. This study aimed to probe whether combinatorial remedy with HNHA and 2DG promotes excrescence repression via caspase fractionalization and cell cycle arrest in ATC. ATC cell lines 8505C and SNU 80, insulated from the current case, were treated with HNHA and 2DG alone or in combination, and cell viability was determined via the MTT assay. Synergisticanti-cancer goods of combinatorial remedy on the cell cycle and intracellular signaling pathways were assessed via inflow cytometry and immunoblot analyses. An ATC cell line- deduced xenograft model was used to examineanti-tumor exertion in vivo. Combinatorial remedy with HNHA and 2DG synergistically reduced the viability of ATC cells and significantly convinced apoptotic cell death, apparent from caspase- 3 fractionalization. likewise, combinatorial remedy downregulatedanti-apoptotic factors. therefore, combinatorial remedy significantly suppressed excrescence volume in ATC cell xenografts, compared to HNHA or 2DG alone.

The present results show that combinatorial remedy with HNHA and 2DG is more effective than treatment with HNHA or 2DG alone in ATC, thereby suggesting a new remedial approach for ATC, including cancer stem- suchlike cells.

2. Keywords

Anaplastic thyroid cancer; Cancer stem- suchlike cell;Anti-apoptosis; Apoptosis; Metabolic stress; Histone deacetylase inhibition

3. Introduction

The thyroid is a gland in the neck, which generally secretes thyroid hormones; they play pivotal places in regulating normal metabolism. Thyroid cancer comprises 4 major types papillary, follicular, medullary, and anaplastic [1]. Thyroid cancer accounts for further than 80 of total endocrine-affiliated melanoma and is the most common endocrine malice, with an adding worldwide prevalence [2]. Thyroid cancer can be well- discerned, inadequately- discerned, and anaplastic grounded on cell isolation characteristics and its food of the follicular cell phenotype. discerned Thyroid Cancer (DTC) is the most common type, counting for further than 90 of all thyroid lymphomas. DTC comprises papillary and follicular histological subtypes [3, 4]. still, inadequately- discerned Anaplastic Thyroid Cancer (ATC) has a poor prognostic owing to chemotherapeutic resistance and aggressiveness [5], with a total median survival of several months [6]. presently, new targeted curatives have successfully increased the life of cancer cases. Kinase impediments have been suggested for treating radioactive iodine(RAI)-refractory DTC cases with metastatic, fleetly progressive, and/ or imminently hanging complaint, which can not be controlled locally using indispensable approaches [7]. still, this has not been observed among cases with advanced cancer subtypes. Treatment of progressive metastatic cancer frequently yields limited benefits; hence, new remedial approaches for cases at a high threat of cancer- related mortality are justified [1]. Recent studies have reported motes and mechanisms nearly associated with poor clinical issues in advanced thyroid cancer [8, 9]. Among these mechanisms, the synergisticanti-cancer effect of the glucose catabolism 2- deoxyglucose (2- DG) and histone deacetylase asset (HDACI)- convinced repression of cancer stem cells (CSCs) could be considered one of the probable reasons for poor clinical issues [10]. Other than typical mutations, epigenetic silencing of excrescence suppressor genes frequently dysregulate tumorigenic signaling pathways [11]. Histone acetyl- transferase (chapeau) and HDACs beget the acetylation and deacetylation, independently, of lysine remainders in histone tails, therefore regulating the commerce of transcriptional complexes for DNA [12]. Accordingly, chapeau and HDAC reclamation constitute a crucial element in dynamic

gene regulation in cellular proliferation and isolation during carcinogenesis [13]. also, a hallmark of rapid-fire excrescence growth is the shift from mitochondrial respiration to aerobic glycolysis (also known as the Warburg effect) [14, 15], although aerobic glycolysis is ineffective in fulfilling the energy and biomass conditions associated with rapid-fire excrescence growth [16]. The unique metabolic attributes of cancer cells enable curatives targeting metabolic pathways in cancer cells. Pharmacotherapeutic agents targeting glucose catabolism by 2- deoxyglucose (2- DG) have been reported, with varied efficacity, in numerous subtypes of solid excrescences [17-20].

This study aimed to interpret the medium underpinning the synergisticanti-cancer effect of inhibition of histone deacetylase and leaguer of glycolysis by N- hydroxy- 7-(2-naphthylthio) heptanomide(HNHA) and 2DG in anaplastic thyroid cancer stem cells.

4. Materials and Methods

4.1. Patients/Tissue Specimens

Fresh excrescences were resected from primary thyroid cancer and metastatic spots in cases with biochemically and histologically proven aggressive RAI-refractory papillary thyroid cancer, who were treated at the Thyroid Cancer Center, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea. farther protocol and details are described in our former papers [21, 22].

4.2. Excrescence Cell insulation and Primary Culture

On the day after resection, the excrescences were placed in normal saline with antifungal and antibiotic agents and transported to the laboratory. The protocol and details are showed in our former papers [21, 22]. The exploration protocol was approved by the Institutional Review Board of the Thyroid Cancer Center, Gangnam Severance Hospital, Yonsei University College of Medicine (IRB Protocol 3 – 2016-0076).

4.3. Cell Culture

ATC cell lines 8505C, SNU- 80, and GSA1 were attained from the European Collection of Cell societies (ECACC, Salisbury, UK) or the Korea Cell Line Bank (Seoul National University, Seoul, Korea) or via excrescence cell insulation from the case (at the Thyroid Cancer Center, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea) cultivating in RPMI- 1640 medium with 10 FBS (Table 1). Mycoplasma impurity was always checked with the Lookout Mycoplasma PCR Discovery tackle (Sigma- Aldrich; MP0035). Cell lines were authenticated via short tandem reprise profiling/ karyotyping/ isoenzyme analysis [22].

4.4. Cell Viability Assay

Cell viability was determined using the 3-(4, 5- dimethylthiazol-2-yl)- 2, 5- diphenyl tetrazolium platitude (MTT) assay. farther details are given in our former composition [22].

4.5. Immunoblot Analysis

Equal quantities of protein (20 μg) were separated electrophoretically on 8 – 10 SDS- polyacrylamide gels and electro- transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, MA, USA). The membranes were latterly blocked and incubated with the following primary antibodies overnight at 4 °Canti-Ki-67 (Abcam), anti-cyclin D1 (Santa Cruz Biotechnology, Dallas, TX, USA), anti-p21 (Santa Cruz Biotechnology), anti-Bcl-2 (Santa Cruz Biotechnology), anti-caspase-3 (Santa Cruz Biotechnology), andanti-β-actin(Santa Cruz Biotechnology). farther protocols and details are described in our former composition [21].

4.6. Flow Cytometry Analysis of the Cell Cycle

Cells were treated with 2DG and HNHA alone or in combination in RPMI- 1640 medium containing 10 FBS for 40 h, gathered via trypsinization, and fixed with 70 ethanol. Protocol is given in our former papers [21, 22]. 4.7. mortal ATC Cell Xenograft Model

mortal ATC cells (2.0 106 cells/ mouse) were dressed in vitro and also fitted subcutaneously into the upper left hand of womanish BALB/ c raw mice. After 7 d, excrescence- bearing mice were grouped aimlessly(n = 10/ group) and treated with 25 mg/ kg HNHA(intraperitoneally) alone, 500 mg/ kg 2DG(p.o.) alone, or a combination of6.5 mg/ kg HNHA and 95 mg/ kg 2DG, formerly every 2 d for 10

12 injections. Excrescence size was measured every alternate day, using calipers. Excrescence volume was estimated using the following formula L × S/ 2(where L is the longest periphery and S is the shortest periphery). creatures were maintained under specific pathogen-free conditions. All trials were approved by the Beast Experiment Committee of Yonsei University(21- 23).

5. Results

5.1. SynergisticAnti-Cancer goods of Combinatorial Administration of 2DG and HNHA Were More Effective Than Those of 2DG or HNHA Administration Alone

The synergistic goods of the excrescence repression via inhibition of histone deacetylase and leaguer of the glycolytic pathway were more effective using a combination of HNHA and 2DG than using HNHA or 2DG alone in ATC cells. We assayed for cell viability in 8505C, SNU 80, and GSA1 cells upon treatment with either HNHA or 2DG alone and a combination of both, via an MTT assay (Figure 1A, B and C). The combination of 2DG and HNHA suppressed cell proliferation more effectively than either agent used alone (Figure 1A, B, and C).

5.2. Synergistic goods of 2DG and HNHA on Cancer Cell Proliferation in Ptient- Drived ATCs

To assess the synergistic anticancer goods of a combination of 2DG and HNHA on ATC, we assayed 8505C, GSA1, and SNU 80 cells, and case- deduced ATCs (Figure 2, Information regarding ATC, attained from the ECACC or the Korea Cell Line

Bank or excrescence cell insulation from the current case); cell proliferation was assessed in the presence and absence of these composites.

IC50 was the smallest for the combination of 2DG and HNHA among all treatment groups for 8505C, SNU 80, and GSA1 cells (Table 1).

Combinatorial treatment with 2DG and HNHA most significantly convinced thesub-G0G1 cells, thereby converting cell death in 8505C, SNU 80, and GSA1 cells (Figure 3A, B, and C). The synergistic effect of 2DG and HNHA most potently increased thesub-G0G1 population, therefore converting apoptosis, cell cycle arrest, and reduction in the viability of 8505C, SNU 80, and GSA1 cells.

Together, these data indicate that inhibition of histone deacetylase and leaguer of the glycolytic pathway upon treatment with a combination of HNHA and 2DG effectively suppresses ATC cells.

5.3. Combinatorial Teatment with 2DG and HNHA Iduced Aoptosis and Cell Cycle Arrest in ATC Cells

Immunoblot analyses of protein situations in 8505C, SNU 80, and GSA1 cell lines indicated that combinatorial administration of 2DG and HNHA most prominently increased p21 situations, a well- known cell cycle arrest protein, and reduced cyclin D1 situations, which is a positive cell cycle controller, compared with responses to either agent administered alone (Figure 4A). specially, proliferation (Ki- 67) andanti-apoptotic (Bcl- 2) labels were most prominently suppressed upon combinatorial treatment with 2DG and HNHA. Apoptotic labels(adhered-caspase 3) were most upregulated upon combinatorial treatment with 2DG and HNHA compared with groups treated with either agent alone.

Together, the results suggest that combinatorial administration of 2DG and HNHA effectively suppresses ATC function.

5.4. Combinatorial Administration of HNHA and 2DG Prominently Reduced Excrescence Size in a Xenograft Model

To estimate the synergistic anticancer effect of combinatorial administration of 2DG and HNHA in vivo, we generated a mouse xenograft excrescence model with 8505C, SNU 80, and case- deduced ATC, GSA1 cells (Figure 4B- J). Each agent used alone didn't prominently suppress 8505C, SNU 80, and GSA1 cell xenograft excrescences; still, combinatorial administration of 2DG and HNHA prominently redounded in excrescence repression (Figure 5B, E, and H). also, there was no substantiation for systemic toxin or treatment- related mortality in any group. Mouse body weight wasn't significantly told by treatment with sorafenib, lenvatinib, or HNHA (Figure 5D, G and J). Combinatorial treatment with 2DG and HNHA significantly dropped excrescence volumes in comparison with each agent used alone (Figure 5C, F and I). Consequently, combinatorial treatment with 2DG and HNHA had potent anticancer goods in ATC and in the ATC xenograft model.

Accordingly, these results propose a implicit new remedial approach to treat cases at a high threat of cancer- related mortality.

6. Discussion

Thyroid cancer is the most common endocrine-affiliated malice [24], with an increased worldwide prevalence, including Korea [25]. Age- grounded cancer prevalence rates during 2011 were81.0 per,000(27.9 in men,134.1 in women) in agreement with the data curated in the Korea National Cancer Incidence Database. The prevalence of thyroid cancer increases by23.3 per time in both men and women, and thyroid cancer has been the most common cancer among women in Korea, since 2009 [26]. High- resolution ultrasonography may have contributed to early discovery of asymptomatic small thyroid nodes [27]. Accordingly, the size portion of linked thyroid cancers has dropped [28, 29]. The induction in multitudinous surgically treated cases of thyroid cancer were substantially due to induction in cancers measuring 1 cm or lower. Accordingly, increased evaluation of thyroid cancer via neck ultrasonography and treatment in the early phase may reduce thyroid cancer- related mortality. Unfortunately, still, ATC is still one of the most treatment- resistant cancers [30]. thus, a new clinical approach is warranted for treating ATC. This study suggests a promising new treatment strategy for some intractable conditions in future. Synergistic anticancer goods of combinatorial treatment with medicines inhibitingnon-overlapping cancer pathways is a reasonable approach to suppress cancer cell proliferation. also, boluses of two medicines could be lower than those of individual medicines and increase the remedial efficacity with minimal side goods.

2DG and HDACIs can access the blood- brain hedge, a prerequisite for glioma treatment, and are also well-permitted by cases [10]. The present results show that HDACIs similar as HNHA forcefully synergize with 2- DG, performing in cancer cell death. In addition to HDAC inhibition, medicine campaigners are anticipated to block glucose metabolism. Glycosylation of necessary proteins similar as recap factors could be oppressively told by the absence of glucose [31] or by blocking glycolysis, using 2- DG [31]. In an energy-deficient state, the reduced ATP- to- AMP rate stimulates the first cellular energy detector, the excrescence suppressor LKB1 [32]. Stimulation of AMP- protein kinase results in a global reduction in restatement and in cell size by precluding the phosphorylation of downstream effectors similar as rapamycin [33]. 2- DG inactivates the transcriptional exertion of Sp1 by affecting its O- GlcNAcylation situations [34]. Since multitudinous recap factors are reformed by O- GlcNAc, O-GlcNAcylation of other recap factors can lead to the regulation of gene expression in response to glucose.

Histone revision constitutes a rather old conception of epigenetic regulation. Among some other types of histone revision, histone deacetylation is deregulated in multitudinous cancers. A current study reported that that HDAC1, HDAC2, and HDAC3 are upregulated in RCC [35]. also, certain reports

suggest that overexpression of class I HDACs, in particular HDAC1, serves as a cancer marker associated with poor prognostic [36]. HDAC impediments rear gene silencing by inhibiting HDAC exertion, thereby allowing for histone acetylation. Preclinical studies have proved the eventuality of HDACI in treating thyroid cancer. HNHA reportedly alters cell cycle- regulating proteins, particularly CDK4, 6, cyclin D1, p53, p21, and related apoptotic proteins and significantly inhibits the growth of thyroid cancer in vitro and in vivo [23].

This study showed the synergisticanti-cancer effect of 2-DG, an asset of the glycolytic pathway, and HNHA, an asset of HDAC, performing in the induction of apoptosis in ATC cells. Epigenetic silencing of excrescence suppressor genes results in the dysregulation of tumorigenesis. specially, the present results indicate that combinatorial treatment with 2DG and HNHA constitutes a potentially effective, new clinical approach for ATC

References

1. Sherman SI. Thyroid carcinoma. Lancet. 2003; 361(9356): 501-511.

2. Nikiforov YE. Thyroid carcinoma: molecular pathways and therapeutic targets. Mod Pathol. 2008;21 Suppl 2: S37-43.

3. Schlumberger MJ. Papillary and follicular thyroid carcinoma. N Engl J Med. 1998; 338(5): 297-306.

4. Soares P, Lima J, Preto A, et al. Genetic alterations in poorly differentiated and undifferentiated thyroid carcinomas. Curr Genomics. 2011; 12(8): 609-617.

5. Smallridge RC. Approach to the patient with anaplastic thyroid carcinoma. J Clin Endocrinol Metab. 2012; 97(8): 2566-2572.

6. Gullu BE, Celik O, Gazioglu N, Kadioglu P. Thyroid cancer is the most common cancer associated with acromegaly. Pituitary. 2010; 13(3): 242-248.

7. Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. 2016; 26(1): 1-133.

8. Bernet V, Smallridge R. New therapeutic options for advanced forms of thyroid cancer. Expert Opin Emerg Drugs. 2014; 19(2): 225-241.

9. Ma R, Minsky N, Morshed SA, Davies TF. Stemness in human thyroid cancers and derived cell lines: the role of asymmetrically dividing cancer stem cells resistant to chemotherapy. J Clin Endocrinol Metab. 2014; 99(3): E400-409.

10. Egler V, Korur S, Failly M, et al. Histone deacetylase inhibition and blockade of the glycolytic pathway synergistically induce glioblastoma cell death. Clin Cancer Res. 2008; 14(10): 3132-3140.

11. Kazanets A, Shorstova T, Hilmi K, Marques M, Witcher M. Epigenetic silencing of tumor suppressor genes: Paradigms, puzzles, and potential. Biochim Biophys Acta. 2016; 1865(2): 275-288.

12. de Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. Histone deacetylases (HDACs): characterization of the classical HDAC family. Biochem J. 2003; 370(Pt 3): 737-749.

13. Cress WD, Seto E. Histone deacetylases, transcriptional control, and cancer. J Cell Physiol. 2000; 184(1): 1-16.

14. Lu J, Tan M, Cai Q. The Warburg effect in tumor progression: mitochondrial oxidative metabolism as an anti-metastasis mechanism. Cancer Lett. 2015; 356(2 Pt A): 156-164.

15. Xu XD, Shao SX, Jiang HP, et al. Warburg effect or reverse Warburg effect? A review of cancer metabolism. Oncol Res Treat. 2015; 38(3): 117-122.

16. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science. 2009; 324(5930): 1029-1033.

17. Maschek G, Savaraj N, Priebe W, et al. 2-deoxy-D-glucose increases the efficacy of adriamycin and paclitaxel in human osteosarcoma and non-small cell lung cancers in vivo. Cancer Res. 2004; 64(1): 31-34.

18. Mohanti BK, Rath GK, Anantha N, et al. Improving cancer radiotherapy with 2-deoxy-D-glucose: phase I/II clinical trials on human cerebral gliomas. Int J Radiat Oncol Biol Phys. 1996; 35(1): 103-111.

19. Zhang D, Li J, Wang F, Hu J, Wang S, Sun Y. 2-Deoxy-D-glucose targeting of glucose metabolism in cancer cells as a potential therapy. Cancer Lett. 2014; 355(2): 176-183.

20. Cheong JH, Park ES, Liang J, et al. Dual inhibition of tumor energy pathway by 2-deoxyglucose and metformin is effective against a broad spectrum of preclinical cancer models. Mol Cancer Ther. 2011;

10(12): 2350-2362.

21. Kweon SS, Shin MH, Chung IJ, Kim YJ, Choi JS. Thyroid cancer is the most common cancer in women, based on the data from population-based cancer registries, South Korea. Jpn J Clin Oncol. 2013; 43(10): 1039-1046.

22. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010; 127(12): 2893-2917.

23. Jung KW, Won YJ, Kong HJ, Oh CM, Lee DH, Lee JS. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2011. Cancer Res Treat. 2014; 46(2): 109-123.

24. Kang HW, No JH, Chung JH, et al. Prevalence, clinical and ultrasonographic characteristics of thyroid incidentalomas. Thyroid. 2004; 14(1): 29-33.

25. Davies L, Welch HG. Current thyroid cancer trends in the United States. JAMA Otolaryngol Head Neck Surg. 2014; 140(4): 317-322.

26. Han MA, Choi KS, Lee HY, Kim Y, Jun JK, Park EC. Current status of thyroid cancer screening in Korea: results from a nationwide interview survey. Asian Pac J Cancer Prev. 2011; 12(7): 1657-1663.

27. Keutgen XM, Sadowski SM, Kebebew E. Management of anaplastic thyroid cancer. Gland Surg. 2015; 4(1): 44-51.

28. Han I, Kudlow JE. Reduced O glycosylation of Sp1 is associated with increased proteasome susceptibility. Mol Cell Biol. 1997; 17(5): 2550-2558.

29. Shaw RJ, Bardeesy N, Manning BD, et al. The LKB1 tumor suppressor negatively regulates mTOR signaling. Cancer Cell. 2004; 6(1): 91-99.

30. Dennis PB, Jaeschke A, Saitoh M, Fowler B, Kozma SC, Thomas G. Mammalian TOR: a homeostatic ATP sensor. Science. 2001; 294(5544): 1102-1105.

31. Kang HT, Ju JW, Cho JW, Hwang ES. Down-regulation of Sp1 activity through modulation of O-glycosylation by treatment with a low glucose mimetic, 2-deoxyglucose. J Biol Chem. 2003; 278(51): 51223-51231.

32. Fritzsche FR, Weichert W, Roske A, et al. Class I histone deacetylases 1, 2 and 3 are highly expressed in renal cell cancer. BMC Cancer. 2008; 8: 381.

33. Barneda-Zahonero B, Parra M. Histone deacetylases and cancer. Mol Oncol. 2012; 6(6): 579-589.

34. Kim SM, Park KC, Jeon JY, et al. Potential anti-cancer effect of N-hydroxy-7-(2-naphthylthio) heptanomide (HNHA), a novel histone deacetylase inhibitor, for the treatment of thyroid cancer. BMC Cancer. 2015; 15: 1003.