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Hydroxychloroquine Modulates m6A RNA Methylation in Prostate Cancer Cells

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ABSTRACT

Prostate cancer ranks as the second most prevalent cancer in men globally. One of the evolving subjects of investigation in prostate cancer is the role of N6-methyladenosine (m6A) modifications. Hydroxychloroquine (HCQ), an autophagy inhibitor, was shown to be promising in enhancing the response to chemotherapy in prostate cancer. The interplay between autophagy and m6A is an emerging area of research. However, the relationship between m6A modifications and HCQ remains unclear. The objective of this study was to examine the effect of HCQ on the regulation of m6A methylation in prostate cancer. Initially, the cytotoxic effect of HCQ on LNCaP and PC3 cells was evaluated. The IC_{50} values for each cell were calculated. Finally, m6A levels in HCQ-treated and untreated cells were determined using m6A RNA methylation quantification kit. HCQ showed a significant dose- and time-dependent reduction in cell viability. Following HCQ treatment, a statistically significant decrease in m6A levels was observed: from 0.050±0.001% to 0.013±0.02% in PC3 cells and from 0.039±0.001% to 0.016±0.01% in LNCaP cells. The study unveils for the first time that HCQ affects m6A methylation in prostate cancer. The impact of autophagy inhibitor HCQ on m6A modifications introduces a novel dimension to its potential mechanisms of action.

Keywords: Prostate cancer, Hydroxychloroquine, Autophagy, RNA modifications, N6-methyladenosine (m6A).

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Introduction

Prostate cancer is the second most common type of cancer in men worldwide, after lung cancer. The global incidence and mortality rates of prostate cancer display a correlation with advancing age, with the average age at diagnosis exceeding fifty [1]. Androgen receptor (AR) signaling plays pivotal roles in prostate development, maintenance, and the progression of prostate cancer. The standard treatment for advanced prostate cancer involves a combination of androgen deprivation therapy with anti-androgens and the administration of docetaxel. In most cases, androgen deprivation therapy may lose effectiveness, ultimately leading to the emergence of a life-threatening form of prostate cancer known as castration-resistant prostate cancer [2].

N6-methyladenosine (m6A) modifications represent the most prevalent type of modification in mammalian mRNAs when compared to other forms of RNA modifications. These modifications have been shown to play significant roles in tissue development, cell regeneration, differentiation, DNA damage response, and the development of cancer [3]. m6A modifications in mRNA or some non-coding RNAs such as long non-coding RNA (lncRNA) also play an important role in many cellular processes such as spermatogenesis, T-cell homeostasis, pluripotency and reprogramming [4,5]. In RNAs, m6A is typically located in stop codons, 3'UTR regions, and internal long exons. Following the discovery that m6A is the primary substrate of the fat mass and obesity-associated protein (FTO) in a dynamic and reversible manner, m6A modifications have garnered considerable attention [4,5].

m6A modifications in mammals are subject to dynamic and reversible regulation by a group of regulatory proteins. These modifications are introduced to RNA by the methyltransferases METTL3 and METTL14 ("writers") and removed by FTO or ALKBH5 ("erasers"). The recognition of m6A occurs in the nucleus through YTHDC1, HNRNPA2B1, and IGF2BP1/2/3, while in the cytoplasm, it is facilitated by YTHDF1/2/3, YTHDC2, eIF3, and IGF2BP1/2/3 ("readers") [6]. Dysregulation of these regulator proteins has been linked to a number of pathophysiological processes in many disorders, including cancer [7]. METTL14 has been shown to suppress the metastatic potential of cancer cells in hepatocellular carcinoma [8]. Alterations in m6A writer and eraser proteins have been shown to be crucial for self-renewal and tumorigenesis in glioblastoma stem cells [9]. In addition, it has been suggested that FTO may have an oncogenic function by affecting the expression of tumor suppressor genes and oncogenes in AML and squamous cell lung cancer [10]. The prevalence of m6A-related genes is high in prostate cancer. These genes have been shown to play multiple roles in the development, progression, and metastases of prostate cancer [11,12]. Several research studies have also suggested that the regulation

of m6A could be a mechanism of resistance in the treatment of metastatic prostate cancer [13,14].

Hydroxychloroquine (HCQ) is a lysosomotropic amine traditionally used in the treatment of malaria and rheumatoid arthritis. As a derivative of chloroquine (CQ), HCQ has demonstrated the capability to inhibit autophagy. It has emerged as one of the most widely used autophagy inhibitors in both preclinical and clinical settings [15,16]. Although HCQ and CQ differ by only one hydroxyl group, the addition of this hydroxyl group results in a significant reduction in toxicity [17]. The inhibition of autophagy by HCQ enhances the response to chemotherapy in the majority of cases among prostate cancer patients [18,19]. The combination of HCQ with other agents could increase the cytotoxicity in cancer cells [20]. Autophagy levels rise due to castration resistance, but HCQ treatment has been found to reduce it [21]. Numerous clinical studies have been conducted and continue to explore the effects of using HCQ alone or in combination with other anticancer agents in various types of cancer [22–25]

The significance of m6A in prostate cancer and the highly effective nature of HCQ in this cancer type underscore the need for a study that delves into the connection between these two factors. Upon conducting a literature search, it became apparent that no such study currently exists. Consequently, we have devised a research plan aimed at investigating the impact of HCQ on m6A levels in prostate carcinoma cells LNCaP and PC3.

Materials and Method

Cell Culture and Preparation of Hydroxychloroquine

The human prostate cancer cell lines PC3, LNCaP, and healthy prostate cell line PNT1A were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were grown in Roswell Park Memorial Institute-1640 medium (RPMI-1640, Thermo Fisher Scientific, MA, USA) containing high glucose, supplemented with 10% fetal bovine serum (FBS, Thermo Fisher Scientific, MA, USA) and 100 U/mL penicillin (Sigma-Aldrich, St Louis, MO, USA), and 100 µg/mL streptomycin (Sigma-Aldrich, St Louis, MO, USA). The cultures were maintained at 37 °C in a humidified incubator (Thermoscientific, MA, USA) with 5% CO_2.

In order to create a stock solution of 5 mM HCQ (TRC, Toronto, Canada), 5 mg of the compound was dissolved in 2300 µL of water. Subsequent dilutions for use in the experiments were prepared using RPMI-1640 (Thermo Fisher Scientific, MA, USA).

Cell Viability Assay

The effect of HCQ on the viability of prostate cancer cells PC3 and LNCaP, as well as normal prostate cells PNT1A, was assessed through the WST-1 assay (Roche Applied Science, Indianapolis, IN, USA). In brief, PC3, LNCaP, and PNT1A cells were seeded in 96-well culture plates at a density of 5×10^3 cells per well. Once the cells adhered, they were exposed to increasing concentrations of HCQ for 24, 48, and 72 hours. Subsequently, 10 µL of WST-1 reagent (Roche Applied Science, Indianapolis, IN, USA) was added to each well at the end of each time point, and the cells were incubated at 37°C for 2 hours in the dark. After incubation, the cells were analyzed using a microplate reader at a wavelength of 450 nm (Thermoscientific, MA, USA). The half-maximal inhibitory concentration (IC_{50}) value of HCQ for each cancer cell line was determined using the linear regression model. Each experiment was conducted three times for each cell line.

Isolation of Total RNA

Total RNA from PC3 and LNCaP cells treated and untreated with IC_{50} of HCQ was extracted using RNeasy Mini Kit (Qiagen, Valencia, CA, USA). The isolation procedure was carried out following the manufacturer's instructions. In summary, the cells were pelleted in a centrifuge and subsequently treated with the provided kit buffers in the specified sequence. The samples were then processed through the column and treated with deoxyribonuclease I. After a series of washing steps, the RNAs were collected in a sterile microcentrifuge tube using the elution solution. The purity and concentration of the RNAs were determined using Nanodrop (Thermoscientific, MA, USA). To assess RNA quality, the samples were electrophoresed on a 1% formaldehyde agarose gel, and the quality was confirmed by observing the presence of distinct 18S and 28S bands.

Determination of m6A Level

The commercial m6A RNA methylation quantification kit (Abcam, USA) was used in accordance with the manufacturer's instructions to measure the level of m6A in the total RNAs of treated and untreated cells. Briefly, 200 ng of RNA were seeded into each well, then a capture antibody solution was added to capture m6A RNAs. After the washing steps, a detection antibody solution was used to obtain signals. The colorimetric measurement of the m6A levels was then performed by reading each well's absorbance at a wavelength of 450 nm using a microplate reader (Thermoscientific, USA).

Statistical Analysis

GraphPad Prism 9.1.0 (La Jolla, CA, ABD) was utilized to perform statistical analysis. The data were displayed as the mean standard deviation of three independent experiments. Statistical differences between groups were analyzed considering sample distribution. m6A test results were analyzed by Student’s t-test. Two-way analysis of variance (ANOVA) followed by Tukey’s test was carried out for multiple comparisons of WST-1 data.

Results

Effect of HCQ on Cell Proliferation

Increasing HCQ concentrations (10, 20, 40, 60, 80 and 100 µM) were administered to LNCaP and PC3 cells for 24, 48 and 72 hours to examine the sensitivity of each cell type to the growth-inhibiting effects of HCQ. The data from WST-1 assay indicated that HCQ inhibited the growth of LNCaP and PC3 cells in a dose- and time-dependent

manner. For PC3 cells, the viability of cells was statistically significantly reduced ($p<0.05$) at 80 µM and 100 µM of HCQ after 24 hours incubation, whereas significantly reduced viability was observed at 40 µM and higher concentrations for 48 hours treatment. Concentrations above 20 µM statistically significantly decreased the viability when treated for 72 hours (Figure 1a). Regarding LNCaP cells, cellular viability was statistically significantly decreased ($p<0.05$) at 40 µM and higher concentrations during 24 and 48-hour treatments. All HCQ concentrations statistically significantly reduced viability when applied for 72 hours (Figure 1b). The cytotoxic effect of HCQ on healthy cells was determined using PNT1A normal prostate epithelial cells. The results of the WST-1 assay revealed that HCQ did not have a statistically significant effect on viability up to 100 µM treatment for 48 and 72 hours (Figure 1c).

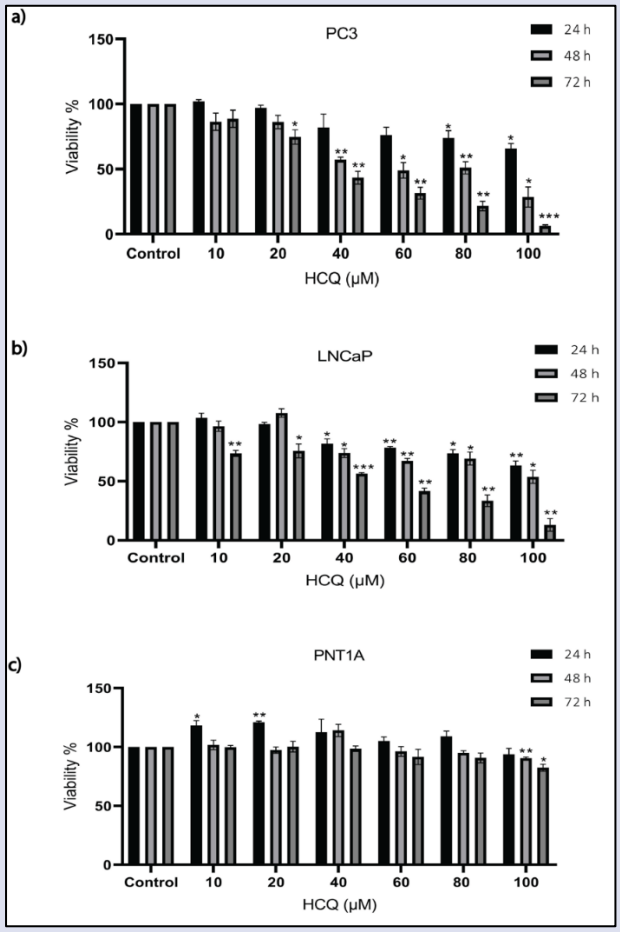

Figure 1: Effects of hydroxychloroquine on a) PC3, b) LNCaP, and c) PNT1A cell viability (*P < 0.05, **P < 0.01, *** P < 0.005)

Based on the outcomes of the cell viability assay by WST-1, the IC_{50} value of HCQ for each cancer cell line was calculated to determine the concentration at which the drug would be applied to cells in the m6Aassay. The calculation was carried out using the linear regression model. IC_{50} values for each time point and cell line were given in Table 1. Subsequent experiments were conducted utilizing the determined IC_{50} values. PNT1A cells were exclusively employed for evaluating the cytotoxicity of HCQ.

Table 1: IC_{50} values of hydroxychloroquine for PC3 and LNCaP cells

	IC_{50} (µM)	
	PC3	LNCaP
24hr	284,7	133,2
48hr	67,9	105,7
72hr	44,9	52,0

Alteration in m6A Levels Following HCQ Treatment

The purity and quality of the isolated RNAs from PC3 and LNCaP cells were verified by the presence of distinct 28S and 18S ribosomal RNA (rRNA) bands, as observed on a 1% agarose gel. These bands were quantified using the ImageJ software program to analyze their intensities and served as internal RNA standards. The presence of these distinct rRNA bands, which are major components, validated and confirmed the integrity of the RNA samples and ensured that there was no degradation of the products. Additionally, our results achieved the generally accepted 28S:18S rRNA ratio of 2:1 for mammalian rRNA, indicating good RNA quality [26]. To assess the change in m6A levels in the samples, a commercial m6A RNA methylation quantification kit was employed. The cells were treated with the IC_{50} concentrations of the drug for 72 hours. Thus, PC3 cells were treated with 44,9 µM of HCQ whereas LNCaP cells were treated with 52 µM of HCQ. Following the m6A quantification assay and the determination of optical densities, m6A% values were calculated in accordance with the manufacturer's protocol. Statistical analysis of the data demonstrated that the m6A level of both PC3 and LNCaP cells was statistically significantly reduced upon treatment with HCQ ($p<0.05$) (Figure 2). The m6A level of PC3 cells, which was 0.050±0.001% in the control group, decreased to 0.013±0.02% in the HCQ-treated group. On the other hand, the m6A level of LNCaP cells, which was 0.039±0.001% in the control group, reduced to 0.016±0.01% in the HCQ-treated group. This represented a 2.4-fold decrease in m6A levels for LNCaP cells and a 3.9-fold decrease for PC3 cells.

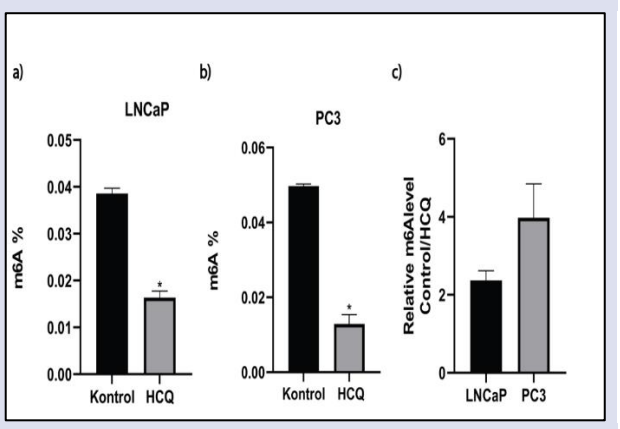

Figure 2: m6A percentage in a) PC3 and b) LNCaP cells after treatment with hydroxychloroquine (*P < 0.0001). c) Relative m6A level of control cells to PC3 and LNCaP cells after hydroxychloroquine treatment

Discussion

Given the absence of evidence regarding the relationship between HCQ and m6A, it was aimed to investigate this association using two distinct subtypes of prostate carcinoma cells. Androgen dependency is a crucial characteristic of prostate carcinoma that significantly influences treatment outcomes [2]. In this study, PC3 and LNCaP cell lines were employed, representing androgen-independent and androgen-dependent human prostate cancer cell lines, respectively. PC3 cells are derived from bone metastases and display highly aggressive behavior, which contrasts with the behavior seen in most clinical cases of prostate cancer. Conversely, LNCaP cells originate from lymph node metastases and exhibit a biologically slower behavior, consistent with most cases observed in clinical settings [27,28]. Because of these differences and the varying effects of drugs on the subtypes, both cell lines were used in this study to provide a comprehensive approach.

Over the past few decades, Hydroxychloroquine (HCQ) has emerged as a compelling candidate for cancer therapy, showcasing its remarkable capacity to impede the process of autophagy. This unique characteristic has brought HCQ to the forefront of research in the field of oncology, where it has garnered significant attention for its potential anti-cancer properties across a range of cancer types, including prostate cancer [15,16]. It influences how lysosomes break down proteins by altering the pH level and encourages the accumulation of LC3 and P62 proteins in autophagy bodies [29,30]. The inhibition of autophagy by HCQ has been shown to sensitize tumors to previously resistant treatment regimens or enhance the response to existing treatments [18,19]. In a study investigating the effect of the combination of HCQ with an apoptosis inhibitor on prostate cancer cells, it was shown that the combination of the drugs increased cytotoxicity in prostate cancer *in vitro* and *in vivo.* The modulation of reactive oxygen species was suggested as a potential mechanism for the increased cytotoxicity due to autophagy inhibition [20]. In an *in vivo* study, researchers have shown that HCQ also inhibits autophagy in castration-resistant prostate cancer cells and stated that cellular apoptosis is induced as a result of inhibition [21]. Numerous investigations have delved into the potential applications of HCQ in the realm of cancer, with a particular emphasis on its connection to the intricate autophagy process. However, despite the substantial body of research in this area, there remains a notable dearth of studies scrutinizing the interplay between HCQ and the m6A mechanisms, which are critical in the regulation of gene expression and post-transcriptional modifications [4,5]. Considering this research gap, our study aimed to investigate this effect in prostate cancer cells. At first, the effect of HCQ on cellular proliferation was investigated and the concentration to be used for the m6A test was determined. WST-1 assay data revealed that HCQ had dose- and time-dependent anti-proliferative effects on both PC3 and LNCaP cells. Importantly, it was found to have no toxic effect on normal prostate cells, indicating that HCQ could be safely used in the study. The IC_{50} concentration of the drug was determined for both cell lines and found to be fairly close to each other as, 44,9 µM for PC3 and 52 µM for LNCaP cells when treated for 72 hours. Consequently, these concentrations of HCQ were applied to the cells for the specified time period before conducting the m6A assay.

Within the context of prostate cancer, the study of m6A-related genes has gained substantial attention, primarily due to their pivotal roles in numerous aspects of cancer, encompassing the initiation of the disease, its relentless progression, and the critical influence they exert on responses to therapeutic interventions. [11–13,31]. Dysregulated m6A modification machinery has been found to impact the expression of genes associated with cell migration, invasion, epithelial-to-mesenchymal transition, leading to enhanced metastatic potential, and genes linked to drug metabolism, DNA repair, and cellular survival, contributing to resistance to therapeutic interventions [11,12]. Furthermore, altered m6A modifications in genes regulating AR expression and activity can influence AR mRNA stability and translation efficiency, thereby affecting AR signaling and contributing to prostate cancer progression. Studies have shown that m6A methylases METTL3 and METTL14 are upregulated in prostate cancer, and their overexpression promotes cancer cell proliferation and invasion. Additionally, the downregulation of the demethylase FTO has been observed, correlating with increased tumor growth and aggressiveness [13,14]. Altered expression of the m6A reader YTHDF2 has also been reported, with its loss being associated with poor patient survival. These findings suggest that m6A levels are elevated in prostate cancer. These findings collectively point to a pronounced elevation in m6A levels within the context of prostate cancer [11,12]. This emerging understanding underscores the complexity of the m6A regulatory network and its profound impact on the clinical landscape, offering a promising avenue for further investigations into the molecular intricacies of the disease and the development of potential therapeutic strategies. When LNCaP and PC3 cells were treated with HCQ, a decrease in the total m6A level was observed. Specifically, the m6A level exhibited a 2.4-fold decrease in LNCaP cells, while a 3.9-fold decline was observed in PC3 cells. This outcome implies that one of the anti-carcinogenic mechanisms of action of HCQ in prostate cancer may involve the disruption of m6A regulations. It's even conceivable that the m6A modification machinery is more dysregulated in PC3 cells compared to LNCaP cells. The fact that HCQ had a more pronounced effect on PC3 cells may suggest that it interferes with m6A modifications in genes associated with cell movement, invasion, DNA repair, or cell survival, as opposed to genes controlling AR regulation.

Autophagy and m6A (N6-methyladenosine) are two distinct biological processes that play important roles in cellular function and regulation. Intriguingly, the interface between autophagy and m6A has garnered attention, as

various m6A writers and erasers have been identified as pivotal players in regulating autophagy-related processes [32,33]. For example, several m6A writers and erasers have been found to regulate autophagy-related processes. Inhibition of the m6A writer METTL3 was shown to promote autophagy and enhance cellular response to nutrient deprivation [34,35], while the inhibition or downregulation of the m6A erasers ALKBH5 and FTO were found to inhibit autophagy under specific conditions [36,37]. m6A modification on specific mRNAs such as ATG5 or ATG7 can affect their stability and translation, thereby modulating the levels of autophagy-related proteins [36]. Additionally, m6A modification has been linked to the regulation of the autophagy-related kinase ULK1, a key initiator of the autophagy process [38,39]. The intricate crosstalk between autophagy and m6A modifications continues to be an evolving area of research, with the precise underlying mechanisms still unraveling [32,33]. Although the precise mechanisms underlying the interplay between autophagy and m6A are still being elucidated, these findings suggest that m6A modification can influence autophagy and, in turn, autophagy-related processes can impact the m6A landscape.

HCQ, in this context, emerges as a notable player. HCQ has demonstrated considerable promise as an autophagy inhibitor, a property that has been substantiated in preclinical studies and some clinical trials [22–25]. However, it has not been extensively explored in the context of m6A modifications until now. This dual role of HCQ, as an autophagy modulator and its potential connection to m6A modifications, exemplifies the multifaceted nature of this drug and its significance in the broader landscape of cancer and cellular regulation research. As the exploration of these interrelated processes unfolds, it promises to open up novel avenues for therapeutic interventions and a deeper understanding of the intricate biology governing both normal and diseased cellular states. Our results reveal that the m6A level in prostate cancer cells is significantly reduced when treated with HCQ. This observation could suggest that autophagy is inhibited due to m6A modifications, or it could mean that the m6A level changes because of autophagy inhibition. In either case, these findings hint at a new dimension for research involving HCQ. It's crucial to recognize that the field of m6A research is continuously evolving. To gain a comprehensive understanding of the intricate connection between HCQ and m6A modification in cancer biology, extensive research, including investigations into m6A regulatory proteins, is necessary. These studies will provide insights into the magnitude and functional implications of this relationship, offering valuable contributions to cancer research.

Conflicts of Interest

There are no conflicts of interest in this work.

References

[1] Rawla P., Epidemiology of Prostate Cancer, *World J. Oncol.*,10(2) (2019) 63–89.

[2] Kyprianou N., English H.F., Isaacs J.T., Programmed cell death during regression of PC-82 human prostate cancer following androgen ablation, *Cancer Res.*, 50(12) (1990) 3748–53.

[3] Xiang Y., Laurent B., Hsu C.H., Nachtergaele S., Lu Z., Sheng W., Xu C., Chen H., Ouyang J., Wang S., Ling D., Hsu P.H., Zou L., Jambhekar A., He C., Shi Y., Corrigendum: RNA m6A methylation regulates the ultraviolet-induced DNA damage response, *Nature*, 552(7685) (2017) 430.

[4] Aguilo F., Zhang F., Sancho A., Fidalgo M., Cecilia S.D., Vashisht A., Lee D.F., Chen C.H., Rengasamy M., Andino B., Jahouh F., Roman A., Krig S.R., Wang R., Zhang W., Wohlschlegel J.A., Wang J., Walsh M.J., Coordination of m(6)A mRNA Methylation and Gene Transcription by ZFP217 Regulates Pluripotency and Reprogramming, *Cell Stem Cell.*, 17(6) (2015) 689–704.

[5] [5] Lin Z., Hsu P.J., Xing X., Fang J., Lu Z., Zou Q., Zhang K.J., Zhang X., Zhou Y., Zhang T., Zhang Y., Song W., Jia G., Yang X., He C., Tong M.H., Mettl3-/Mettl14-mediated mRNA N6-methyladenosine modulates murine spermatogenesis, *Cell Res.*, 27(10) (2017) 1216–30.

[6] Luo J., Liu H., Luan S., He C., Li Z., Aberrant Regulation of mRNA m6A Modification in Cancer Development, *Int. J. Mol. Sci.*, 19(9) (2018) 2515.

[7] Fitzsimmons C.M., Batista P.J., It's complicated… m6A-dependent regulation of gene expression in cancer, *Biochimica Et Biophysica Acta Bba - Gene Regul Mech.*, 1862(3)(2019) 382–93.

[8] Ma J., Yang F., Zhou C., Liu F., Yuan J., Wang F., Wang T., Xu Q., Zhou W., Sun S., METTL14 suppresses the metastatic potential of hepatocellular carcinoma by modulating N6-methyladenosine-dependent primary MicroRNA processing, *Hepatology.*, 65(2) (2017) 529–43.

[9] Cui Q., Shi H., Ye P., Li L., Qu Q., Sun G., Sun G., Lu Z., Huang Y., Yang C.G., Riggs A.D., He C., Shi Y., m6A RNA Methylation Regulates the Self-Renewal and Tumorigenesis of Glioblastoma Stem Cells, *Cell Reports*, 18(11) (2017) 2622–34.

[10] Li Z., Weng H., Su R., Weng X., Zuo Z., Li C., Huang H., Nachtergaele S., Dong L., Hu C., Qin X., Tang L., Wang Y., Hong G.M., Huang H., Wang X., Chen P., Gurbuxani S., Arnovitz S., Li Y., Li S., Strong J., Neilly M.B., Larson R.A., Jiang X., Zhang P., Jin J., He C., Chen J., FTO Plays an Oncogenic Role in Acute Myeloid Leukemia as a N 6-Methyladenosine RNA Demethylase, *Cancer Cell*, 31(1) (2017) 127–41.

[11] Li J., Xie H., Ying Y., Chen H., Yan H., He L., Xu M., Xu X., Liang Z., Liu B., Wang X., Zheng X., Xie L., YTHDF2 mediates the mRNA degradation of the tumor suppressors to induce AKT phosphorylation in N6-methyladenosine-dependent way in prostate cancer, *Mol. Cancer*, 19(1) (2020) 152.

[12] Chen Y., Pan C., Wang X., Xu D., Ma Y., Hu J., Chen P., Xiang Z., Rao Q., Han X., Silencing of METTL3 effectively hinders invasion and metastasis of prostate cancer cells, *Theranostics*, 11(16) (2021) 7640–57.

[13] Cotter K.A., Gallon J., Uebersax N., Rubin P., Meyer K.D., Piscuoglio S., Jaffrey S.R., Rubin M.A., Mapping of m6A and Its Regulatory Targets in Prostate Cancer Reveals a METTL3-Low Induction of Therapy Resistance, *Mol Cancer Res.,* 19(8) (2021) 1398–411.

[14] Zhang F., Chen F., Wang C., Zhou F., The functional roles of m6A modification in prostate cancer, *Proteom Clin Appl.*, (2023) e2200108.

[15] Farrow J.M., Yang J.C., Evans C.P., Autophagy as a modulator and target in prostate cancer, *Nat. Rev. Urol.*, 11(9) (2014) 508–16.

[16] Sotelo J., Briceño E., López-González M.A., Adding chloroquine to conventional treatment for glioblastoma multiforme: a randomized, double-blind, placebo-controlled trial, *Ann Intern Med.,* 144(5) (2006) 337–43.

[17] Ben-Zvi I., Kivity S., Langevitz P., Shoenfeld Y., Hydroxychloroquine: From Malaria to Autoimmunity, *Clin. Rev. Allerg. Immu.*, 42(2) (2012) 145–53.

[18] Carew J.S., Kelly K.R., Nawrocki S.T., Autophagy as a target for cancer therapy: new developments, *Cancer Management Res*, 4 (2012) 357–65.

[19] Levy J.M.M., Thompson J.C., Griesinger A.M., Amani V., Donson A.M., Birks D.K., Morgan M.J., Mirsky D.M., Handler M.H., Foreman N.K., Thorburn A., Autophagy Inhibition Improves Chemosensitivity in BRAFV600E Brain Tumors, *Cancer Discov*, 4(7) (2014) 773–80.

[20] Saleem A., Dvorzhinski D., Santanam U., Mathew R., Bray K., Stein M., White E., DiPaola R.S., Effect of dual inhibition of apoptosis and autophagy in prostate cancer, *Prostate*, 72(12) (2012) 1374–81.

[21] Ling S.T., Deng C.L., Huang L., Yao Q.S., Liu C., Sun C.T., Wang L., Yang Y., Gong X.X., Chen C.B., Hydroxychloroquine Blocks Autophagy and Promotes Apoptosis of the Prostate after Castration in Rats, *Urol Int.*, 104(11–12) (2019) 968–74.

[22] Rosenfeld M.R., Ye X., Supko J.G., Desideri S., Grossman S.A., Brem S., Mikkelson T., Wang D., Chang Y.C., Hu J., McAfee Q., Fisher J., Troxel A.B., Piao S., Heitjan D.F., Tan K.S., Pontiggia L., O'Dwyer P.J., Davis L.E., Amaravadi R.K., A phase I/II trial of hydroxychloroquine in conjunction with radiation therapy and concurrent and adjuvant temozolomide in patients with newly diagnosed glioblastoma multiforme, *Autophagy*, 10(8) (2014) 1359–68.

[23] Rangwala R., Leone R., Chang Y.C., Fecher L.A., Schuchter L.M., Kramer A., Tan K.S., Heitjan D.F., Rodgers G., Gallagher M., Piao S., Troxel A.B., Evans T.L., DeMichele A.M., Nathanson K.L., O'Dwyer P.J., Kaiser J., Pontiggia L., Davis L.E., Amaravadi R.K., Phase I trial of hydroxychloroquine with dose-intense temozolomide in patients with advanced solid tumors and melanoma, *Autophagy*, 10(8) (2014) 1369–79.

[24] Vogl D.T., Stadtmauer E.A., Tan K.S., Heitjan D.F., Davis L.E., Pontiggia L., Rangwala R., Piao S., Chang Y.C., Scott E.C., Paul T.M., Nichols C.W., Porter D.L., Kaplan J., Mallon G., Bradner J.E., Amaravadi R.K., Combined autophagy and proteasome inhibition, *Autophagy*, 10(8) (2014) 1380–90.

[25] Mahalingam D., Mita M., Sarantopoulos J., Wood L., Amaravadi R.K., Davis L.E., Mita A.C., Curiel T.J., Espitia C.M., Nawrocki S.T., Giles F.J., Carew J.S., Combined autophagy and HDAC inhibition, *Autophagy*, 10(8) (2014) 1403–14.

[26] Tuma R.S., Beaudet M.P., Jin X., Jones L.J., Cheung C.Y., Yue S., Singer V.L., Characterization of SYBR Gold Nucleic Acid Gel Stain: A Dye Optimized for Use with 300-nm Ultraviolet Transilluminators, *Anal Biochem.*, 268(2) (1999) 278–88.

[27] Horoszewicz J.S., Leong S.S., Kawinski E., Karr J.P., Rosenthal H., Chu T.M., Mirand E.A., Murphy G.P., LNCaP model of human prostatic carcinoma, *Cancer Res.*, 43(4) (1983)1 809–18.

[28] Bokhoven A.V., Varella-Garcia M., Korch C., Johannes W.U., Smith E.E., Miller H.L., Nordeen S.K., Miller G.J., Lucia M.S., Molecular characterization of human prostate carcinoma cell lines, *Prostate*, 57(3)(2003)205–25.

[29] Solomon V.R., Lee H., Chloroquine and its analogs: A new promise of an old drug for effective and safe cancer therapies, *Eur. J. Pharmacol.*, 625(1–3) (2009) 220–33.

[30] Kumano M., Furukawa J., Shiota M., Zardan A., Zhang F., Beraldi E., Wiedmann R.M., Fazli L., Zoubeidi A., Gleave M.E., Cotargeting Stress-Activated Hsp27 and Autophagy as a Combinatorial Strategy to Amplify Endoplasmic Reticular Stress in Prostate Cancer, *Mol. Cancer Ther.*, 11(8) (2012) 1661–71.

[31] Eide T., Ramberg H., Glackin C., Tindall D., Taskén K.A., TWIST1, A novel androgen-regulated gene, is a target for NKX3-1 in prostate cancer cells, *Cancer Cell Int.*, 13(1) (2013) 4–4.

[32] Liang J., Sun J., Zhang W., Wang X., Xu Y., Peng Y., Zhang L., Xiong W., Liu Y., Liu H., Novel Insights into The Roles of N6-methyladenosine (m6A) Modification and Autophagy in Human Diseases, *Int. J. Biol. Sci.*, 19(2) (2023) 705–20.

[33] Jo H., Shim K., Jeoung D., Roles of RNA Methylations in Cancer Progression, Autophagy, and Anticancer Drug Resistance, *Int. J. Mol. Sci.*, 24(4) (2023) 4225.

[34] Lin Z., Niu Y., Wan A., Chen D., Liang H., Chen X., Sun L., Zhan S., Chen L., Cheng C., Zhang X., Bu X., He W., Wan G., RNA m6 A methylation regulates sorafenib resistance in liver cancer through FOXO3-mediated autophagy, *Embo J.*, 39(12) (2019) e103181.

[35] Liu S., Li Q., Li G., Zhang Q., Zhuo L., Han X., Zhang M., Chen X., Pan T., Yan L., Jin T., Wang J., Lv Q., Sui X., Xie T., The mechanism of m6A methyltransferase METTL3-mediated autophagy in reversing gefitinib resistance in NSCLC cells by β-elemene, *Cell Death Dis.*, 11(11) (2020)

[36] Wang X., Wu R., Liu Y., Zhao Y., Bi Z., Yao Y., Liu Q., Shi H., Wang F., Wang Y., m 6 A mRNA methylation controls autophagy and adipogenesis by targeting Atg5 and Atg7. Autophagy., 16(7) (2019) 1221–35.

[37] Xu Y., Zhou J., Li L., Yang W., Zhang Z., Zhang K., Ma K., Xie H., Zhang Z., Cai L., Gong Y., Gong K., FTO-mediated autophagy promotes progression of clear cell renal cell carcinoma via regulating SIK2 mRNA stability, *Int. J. Biol. Sci.*, 18(15) (2022) 5943–62.

[38] Jin S., Zhang X., Miao Y., Liang P., Zhu K., She Y., Wu Y., Liu D.A., Huang J., Ren J., Cui J., m6A RNA modification controls autophagy through upregulating ULK1 protein abundance, *Cell Res.*, 28(9) (2018) 955–7.

[39] Zhang Y., Gao L., Wang W., Zhang T., Dong F., Ding W., M6A demethylase fat mass and obesity-associated protein regulates cisplatin resistance of gastric cancer by modulating autophagy activation through ULK1, *Cancer Sci.*, 113(9) (2022) 3085–96.