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Review Article

The Impact of Melatonin and Ionizing Radiation on Autophagy in Cancer Cells

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Abstract

Autophagy is an intracellular process for the degradation and elimination of misfolded proteins and damaged organelles. The mechanism of autophagy involves the formation of autophagosomes for the degradation of cytoplasmic components; it fuses with lysosomes to be recycled. The process of autophagy often occurs during stressful conditions, including anoxia, poor nutrition, chemicals, and radiation. Herein, we summarize the biological mechanisms of autophagy, together with the role of autophagy in cancer. Subsequently, we discuss recent advances about the influences of melatonin and ionizing radiation on autophagy in cancer cells. The report specifically discusses the following publications: "Inhibiting MT2-TFE3-dependent autophagy enhances melatonin-induced apoptosis in tongue squamous cell carcinoma", "Melatonin and 5-fluorouracil co-suppress colon cancer stem cells by regulating cellular prion protein-Oct4 axis", "Melatonin enhances sorafenib actions in human hepatocarcinoma cells by inhibiting mTORC1/p70S6K/HIF-1α and hypoxia-mediated mitophagy", "Hyperbaric oxygen therapy sensitizes gastric cancer cells to melatonin-induced apoptosis through multiple pathways", "Inhibition of the autophagic response sensitizes lung cancer cells to radiation", "Role of autophagy in high linear energy transfer radiation-induced cytotoxicity to tumor cells", "Inhibiting autophagy with chloroquine enhances the anti-tumor effect of high-LET carbon ion irradiation through ER stress-related apoptosis" and "Suppression of PC-1/PrLZ sensitizes prostate cancer cells to ionizing radiation by attenuating DNA damage repair and inducing autophagic cell death". Autophagic activity has been documented in clinicopathological studies and is increased in patients with various types of cancer and is associated with worse outcomes. Further investigation of the biological mechanisms and the effects of autophagy on tumor initiation and progression should be exploited as an innovative autophagy-targeted therapeutic intervention so as to improve clinical outcomes of various cancer patients.

Keywords: *Melatonin; Ionizing Radiation; X-Ray Irradiation; Carbon Ion Irradiation; Autophagy; Cancer Cells*

Introduction

Autophagy is an intracellular process for the degradation and elimination of misfolded proteins and damaged organelles [1-3]. The mechanism of autophagy includes the formation of autophagosomes for the degradation of cytoplasmic components; it then fuses with lysosomes which digest and recycle the damaged components. The process of autophagy occurs during stressful conditions, including anoxia, poor nutrition, chemicals, and radiation [1,4]. The mechanism of autophagy is crucial for removing cells containing damaged components and defends cell organelles against toxins, to preserve cell metabolism and energy homeostasis, and to enhance cell survival [1].

Various studies indicates that autophagy increases the capability of the cell to adapt to different and potentially detrimental environments and confers apoptosis resistance in instances of increased oxidative stress, increased inflammatory response, reduced energy and

nutrients, and in cases of being exposed to cytotoxic agents, including ionizing radiation and chemotherapeutic agents [3,5,6]. Consequently, autophagy is involved in the process of cancer progression since it enhances the capability of neoplastic cells to cope with the unfavorable situation in the environment and plays critical roles in the resistance of these cells to radiotherapy and chemotherapy [7,8]. Understanding the interaction between apoptosis and autophagy in tumors is critical for identification of novel targets for cancer therapy and the promotion of therapeutic efficiency [4,9].

Melatonin (N-acetyl-5-methoxytryptamine), an indole synthesized by the pineal gland and many other tissues, is an endogenous inhibitor of tumors [10-20]. This molecule executes its anticancer effects in various types of cancer due to its pro-apoptotic, anti-proliferative, anti-cell differentiation and anti-angiogenic actions [21,22]. Melatonin treatment has been recommended as a strategy for inhibiting the progression of colitis-associated colon carcinogenesis (CACC) by targeting autophagy [23,24]. Melatonin also has anticancer effects that involve autophagy and the Nrf2 pathway. In hypoxia/reoxygenation situations, melatonin decreases autophagic activity and increases Nrf2 expression, resulting in choriocarcinoma cell apoptosis as seen in humans [25].

Radiation therapy is an important modality of cancer treatment. X-rays are commonly used photons in radiation therapy. X-rays are defined as low LET (Linear Energy Transfer) radiation which produce sparse ionization along their trajectories [26]. Failure of radiation therapy using photon irradiation is commonly related with metastasis [27]. There is an increasing application of high-LET charged particles such as protons and carbon ions in the treatment of cancer [28,29]. Carbon ions generate dense ionization along their trajectories, and induce unrepairable clustered DNA damage [29,30].

Since radiation impairs proteins and cytoplasmic organelles, it is anticipated that an integral or intensified autophagic flux would be crucial for cell recovery [31]. Inhibition of LC3 autophagosomal membrane protein expression sensitizes human endothelial cells to radiation [32]. The exploitation of novel agents targeting autophagy in the cancer cell plays an indispensable part in enhancing the efficacy of radiotherapy, with the eventual goal of improving survival [33].

In this review, we summarize the biological mechanisms of autophagy, together with the role of autophagy in cancer. Subsequently, we discuss recent advances related to the influence of melatonin and ionizing radiation on autophagy in cancer cells. In addition, we discuss how autophagy is a promising therapeutic target in cancer treatment.

The impact of melatonin on autophagy in cancer cells

Inhibiting MT2-TFE3-dependent autophagy enhances melatonin-induced apoptosis in tongue squamous cell carcinoma

In a study of Fan., *et al.* melatonin activated transcription factor E3 (TFE3) and increased TFE3 reporter activity. Inhibition of TFE3-dependent autophagy sensitized melatonin-induced apoptosis in tongue squamous cell carcinoma (TSCC) cells. An association between melatonin-induced TFE3-dependent autophagy and the melatonin membrane receptor was also detected [34]. These findings suggest that inhibition of melatonin membrane receptor-TFE3-dependent autophagy may enhance the therapeutic efficacy of melatonin in the treatment of TSCC [34].

Melatonin induces apoptosis in the Cal27 cells

Flow cytometry analysis following Annexin V-FITC and propidium iodide (PI) dual labeling was employed to identify the effects of melatonin treatment on apoptotic induction in Cal27 cells (human TSCC cell line). Cal27 cells were treated with melatonin at a concentration of 0.5, 1, or 2 mmol/L for 24 hours. The apoptotic rates of the melatonin-treated Cal27 cells rose to 7.0%, 21.0% and 32.2% of total cells, respectively, compared to 5.9% in the control samples. Both cleaved PARP (poly (ADP-ribose) polymerase) and the ratio of BAX to BCL-2 was elevated in Cal27 cells which were treated with the different concentrations of melatonin. Caspase-3 activity increased in a dose-dependent manner in Cal27 cells treated with melatonin at increasing concentrations. These results revealed that apoptosis was associated with the response of Cal27 cells to melatonin treatment [34].

Melatonin increases autophagic flux in the Cal27 cells

Fan., *et al*. established a Cal27 cell line that stably expressed the green fluorescent protein (GFP)-LC3 fusion gene. Fluorescence microscopy was employed to detect GFP-LC3 puncta. After treatment with various concentrations of melatonin for 24 hours, Cal27 cells exhibited more GFP-LC3 puncta than negative controls. Cal27 cells were transfected with tandem fluorescent mRFP-GFP-LC3B (tf-LC3), a unique marker that allows for the estimation of autophagic flux. Melatonin increased the number of red puncta, which indicated the formation of autolysosomes [34].

Bafilomycin A1 (Baf A1), an inhibitor of the lysosomal V-ATPase, brings about an accumulation of autophagosomes owing to a defect in the fusion between autophagosomes and lysosomes. Application of Baf A1 resulted in increased LC3-II expression in the cells treated with 2 mmol/L melatonin. These results revealed that melatonin induced increase of autophagic flux in TSCC cells [34].

Inhibition of melatonin-induced autophagy sensitizes Cal27 cells to melatonin-induced apoptotic cell death

Autophagy related 7 (ATG7) deficiency markedly interferes with autophagy [34]. Cal27 cells transfected with ATG7-siRNA resulted in a lower level of GFP-LC3 puncta accumulation and LC3-II expression after treatment with 2 mmol/L melatonin than those cells treated with the scrambled siRNA control, representing the involvement of ATG7 in the melatonin-mediated autophagy in Cal27 cells. Knockdown of ATG7 by siRNA enhanced expression of cleaved PARP-1. Melatonin markedly increased caspase-3 activity after inhibition of autophagy flux. These results indicate that inhibition of melatonin-induced autophagy sensitized Cal27 cells to melatonin-induced apoptotic cell death [34].

Melatonin induces nuclear translocation of TFE3 in the Cal27 cells

MiT/TFE proteins, which include microphthalmia-associated transcription factor (MITF), transcription factor E3 (TFE3) and transcription factor EB (TFEB), are major regulators of autophagy and lysosomal biogenesis [34]. TFE3 mRNA expression increased markedly after exposure to different concentrations of melatonin for 24 hours. Only TFE3 translocated into the nucleus following exposure to melatonin. Melatonin markedly increased the TFE3 luciferase activity and the mRNA of 14 genes, which include those encoding various subunits of the v-ATPase, lysosomal transmembrane proteins, lysosomal hydrolases and genes encoding proteins involved in the formation of autophagosomes and their degradation [34].

To explore the mechanism underlying the melatonin-induced TFE3 nuclear translocation, the phosphorylation status of TFE3 was assessed. Since Ser321 dephosphorylation is the primary event leading to TFE3 nuclear translocation, the level of TFE3 Ser321 phosphorylation was evaluated. Melatonin treatment caused a marked decrease in Ser321 phosphorylation of TFE3 [34].

In brief, melatonin induced TFE3 Ser321 dephosphorylation, then activated TFE3 nuclear translocation and increased TFE3 reporter activity, which led to autophagy-related gene expression and lysosomal biogenesis.

TFE3 mediates melatonin-induced autophagy in the Cal27 cells

Inhibition of TFE3 activity reduced expression of melatonin-induced TFE3-responsive genes. Moreover, the protein level of LC3 II/LC3 I was suppressed. In addition, TFE3-siRNA enhanced melatonin-induced caspase-3 activity. These results indicated that melatonin-induced autophagy increased melatonin-induced apoptosis in Cal27 cells by activating the TFE3 pathway and inhibiting the TFE3-dependent autophagy [34].

Melatonin membrane receptor 2 accounts for the dual roles of TFE3 pathway in the effects of melatonin

Melatonin receptor 1B (melatonin membrane receptor 2 or MT2) was identified in TSCC cells which included Cal27, SCC9 and Tca-8113 cells. Melatonin-induced enhancement of TFE3 dephosphorylation and nuclear translocation was partly reversed by luzindole (a melatonin receptor antagonist) and by MT2-siRNA. In addition, melatonin-induced enhancement in the transcriptional activity of TFE3 was inhibited by luzindole and by MT2-siRNA. Incubation of cells with luzindole or MT2-siRNA prior to the application of melatonin increased caspase-3 activity [34].

Targeting autophagy or TFE3 signaling enhances the antitumor effect of melatonin *in vivo*

A xenograft tumor model was established by subcutaneous inoculation of Cal27 cells into nude mice. Melatonin (100 mg/kg body weight) did not induce visible side effects or alterations in the body weight of the mice. Melatonin combined with hydroxychloroquine (HCQ) reduced the tumor size and weight significantly compared to melatonin alone. Western blot analysis revealed that the levels of cleaved PARP, the ratio of BAX to BCL-2, and caspase-3 activity increased with inhibition of autophagy [34].

After treatment with melatonin for 21 days, both the wild type (WT) tumor volume and tumor weight were inhibited. Nonetheless, melatonin administration resulted in a marked reduction in the TEF3 knockout (TFE3KO) tumor burden. In addition, Western blot analysis revealed the melatonin treatment led to more frequent apoptosis in the TFE3KO group than in the WT group [34].

TFE3 expression correlates positively with TSCC development and prognosis

TFE3 expression was significantly higher in poorly differentiated TSCC with a higher TNM stage. TFE3 expression was markedly elevated in samples from patients with advanced stages (III/IV) of TSCC compared to samples from those with early stage disease. Statistical analysis demonstrated that TSCC patients with higher TFE3 expression had a poorer overall survival rate than those with lower TFE3 expression. These findings substantiate the hypothesis that TFE3 may enhance TSCC progression. Therefore, TFE3 is an encouraging therapeutic target in human TSCC [34].

In brief, the melatonin-induced enhancement in TFE3-dependent autophagy is mediated via the melatonin membrane receptor in TSCC. Inhibition of autophagy may intensify the antitumor effect of melatonin. Furthermore, melatonin combined with autophagy inhibitors may exert synergistic effects on the inhibition of tumor development. These results demonstrate the association between apoptosis and melatonin-induced autophagy, which could give rise to further improvements of therapeutic strategies for TSCC.

Melatonin and 5-fluorouracil co-suppress colon cancer stem cells by regulating cellular prion protein-Oct4 axis

Cancer stem cells are a subpopulation of self-renewing cells and account for tumor development and treatment failure. Octamer-binding transcription factor 4 (Oct4) induces and preserves pluripotency. Oct4 gene knockdown inhibits tumor growth by inducing cancer stem cell-like apoptosis. Oct4 plays an essential role in colorectal cancer development which includes tumor initiation, metastasis, and chemoresistance [35]. Cellular prion protein (PrP^C) is involved in proliferation, apoptosis, invasion, metastasis, and chemoresistance. In addition, PrP^C is a candidate biomarker for colorectal adenoma-to-carcinoma progression [31]. In glioblastoma, the expression of Oct4, Sox2, and PrP^C was markedly elevated in neurospheres (stem-like condition), compared with monolayer (non-stem condition) [35].

Combined treatment with 5-FU and melatonin induces apoptosis of human colon cancer stem cells

The carboxyfluorescein diacetate succinimidyl ester (CFSE) assay revealed that combined treatment with 5-FU and melatonin markedly inhibited colon cancer stem cell proliferation compared to treatment with either 5-FU or melatonin alone. The expression of the anti-apoptotic protein, BCL2, was significantly suppressed in response to the combination therapy compared to the control and treatment with either 5-FU or melatonin alone [35]. On the contrary, the expression levels of pro-apoptotic proteins including BAX, cleaved caspase-3, and cleaved PARP-1 were markedly elevated. A flow cytometry assay of human cancer stem cells stained with PI and annexin V revealed that combination therapy with 5-FU and melatonin markedly increased cancer stem cell apoptosis [35]. These findings indicate that combined treatment with 5-FU and melatonin suppresses proliferation and promotes apoptosis of colon cancer stem cells.

PrP^C regulates colorectal cancer stem cell markers

The expression levels of PrP^C and the cancer stem cell markers Oct4, Nanog, Sox2 and ALDH1A1 were evaluated by Western blot in both normal and human colon cancer tissues. PrP^C and stem cell marker expression levels were significantly higher in colorectal cancer tissues than in normal tissues [35].

Expression levels of these proteins markedly increased in drug-resistant colorectal cancer cells (SNU-C5/5FUR and SNU-C5/OxalR) and cancer stem cells relative to the wild type (SNU-C5/WT). Expression levels of PrP^C, Oct4, Nanog, Sox2, and ALDH1A1 in human cancer stem cells treated with control shRNA (control) and PRNP (prion protein) shRNA (shPRNP) revealed that PrP^C silencing lowered colorectal cancer stem cell markers [35].

Combined treatment with chemotherapy and melatonin inhibits the expression of human colon cancer stem cell markers by inhibiting PrP^C expression

Human colon cancer stem cells were assessed with Western blot and flow cytometry. PrP^C expression level was significantly decreased in response to combined treatment with 5-FU and melatonin compared to those of the control, treatment with 5-FU alone, or treatment with melatonin alone. In addition, the expressions of Oct4, Nanog, Sox2, and ALDH1A1 were significantly reduced in response to the combined treatment with 5-FU and melatonin compared with those levels in the control and the treatment with 5-FU alone. These results reveal that combined treatment with 5-FU and melatonin inhibits cancer stem cell marker expression by suppressing PrP^C expression [35].

PrP^C protects against 5-FU and melatonin-mediated degradation of Oct4

PrP^C overexpression inhibited the Oct4 degradation induced by combined treatment with 5-FU and melatonin. On the other hand, the degradation of Nanog, Sox2, and ALDH1A1 were caused by 5-FU/melatonin co-treatment. Heat shock protein family A member 1-like (HSPA1L) stabilized PrP^C in colorectal cancer cells. HSPA1L expression was markedly elevated in PRNP-overexpressing cancer stem cells compared to the wild type. The immunoprecipitation assay revealed that HSPA1L binding to Oct4 markedly elevated in PRNP-overexpressing cancer stem cells compared to the wild type. On the contrary, HSPA1L binding to Nanog, Sox2, or ALDH1A1 did not significantly differ from the wild type. These findings indicate that PrP^C inhibits Oct4 degradation by increasing HSPA1L binding to Oct4 [35].

PrP^C and Oct4 regulate 5-FU- and melatonin-mediated autophagy in colon cancer stem cells

AMPK phosphorylation increased and mTOR phosphorylation decreased after combined treatment of human colon cancer stem cells with 5-FU and melatonin. PRNP overexpression inhibited these effects. Oct4 knockdown significantly enhanced AMPK phosphorylation compared to combined treatment with 5-FU and melatonin [35]. The expressions of LC3BII, ATG7, and Beclin 1 were markedly elevated in response to combined treatment of colon cancer stem cells with 5-FU and melatonin compared with the untreated cells. These effects were inhibited by PRNP overexpression. Oct4 knockdown markedly enhanced the expression levels of LC3BII, ATG7, and Beclin 1 compared with combined treatment. An autophagy detection assay revealed that PRNP overexpression significantly inhibited 5-FU and melatonin-mediated autophagy. These findings clarify that PrP^C and Oct4 play vital roles in 5-FU and autophagy in colon cancer stem cells [35].

Combined treatment with 5-FU and melatonin inhibits colon cancer progression by regulating PrP^C expression

Combined treatment with 5-FU and melatonin significantly decreased tumor volume compared to 5- FU alone, melatonin alone, PRNP + 5-FU + melatonin and the control. PRNP overexpression markedly increased tumor growth compared with co-treatment with 5-FU and melatonin. Oct4 expression decreased in response to combined treatment with 5-FU and melatonin, whereas PRNP overexpression blocked the inhibitory effect of Oct4 [35]. Immunofluorescence staining for proliferating cell nuclear antigen (PCNA) demonstrated that combined treatment with 5-FU and melatonin inhibited tumor cell proliferation in a xenograft model, but PRNP overexpression protected tumor cell proliferation from the inhibitory effects of 5-FU and melatonin. 5-FU and melatonin suppressed tumor-mediated angiogenesis in tumor tissues, while this effect was inhibited by PRNP overexpression. These findings imply that combined treatment with 5-FU and melatonin inhibits colon cancer development by reducing PrP^C expression [35].

In brief, these findings indicate that co-treatment with anticancer drug and melatonin is a potential therapy for colorectal cancer. Additionally, PrP^C preserves cancer stemness during tumor progression. Consequently, targeting the PrP^C-Oct4 axis may prove to be an effective treatment for colorectal cancer [35].

Melatonin enhances sorafenib actions in human hepatocarcinoma cells by inhibiting mTORC1/p70S6K/HIF-1α and hypoxia-mediated mitophagy

The multikinase inhibitor sorafenib is the approved agent for the treatments of advance hepatocellular carcinoma (HCC). Due to its antiangiogenic feature, long term sorafenib treatment reduces microvessel density and enhances tumor hypoxia, resulting in an imbalance between cell survival and death programs (autophagy, including mitophagy which is an autophagy form that controls mitochondrial homeostasis, and apoptosis) and contributing in drug resistance and poor outcome [36].

Combined treatment with sorafenib and an adjuvant agent to target the hypoxia-inducible factor-1α (HIF-1α) is regarded as a promising therapeutic approach to enhance the efficacy of sorafenib. Melatonin is a well-documented antitumor agent against various cancer types. Prieto-Dominguez., *et al*. evaluate the melatonin capability to sensitize HCC cells to sorafenib under hypoxia, aiming at HIF-1α and the mitophagy-related pathways [36].

Melatonin enhances sorafenib cytotoxicity under normoxic and hypoxic conditions

Viability of HepB3 cells was evaluated 48 hours after treatment with melatonin (1 and 2 mM) and/or sorafenib (2.5, 5 and 10 μM) under normoxia or hypoxia using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tertazolium bromide (MTT) assay. The minimal doses that significantly reduced Hep3B viability under normoxia was 5 μM sorafenib alone or 2 mM melatonin alone, while a dose of 10 μM sorafenib was required to exert comparative effects under hypoxia [36].

Administration of 2 mM melatonin, however, with each of the sorafenib concentrations tested synergistically increased sorafenib cytotoxicity in Hep3B cells. Accordingly, 2 mM melatonin and 5 μM sorafenib were chosen to perform additional experiments [36].

Combined treatment with sorafenib and melatonin reduces HIF-1α expression and viability of Hep3B cells

Hep3B cells incubated under normoxia or hypoxia were treated with sorafenib (5 μM) or melatonin (2 mM) for 24 hours. Melatonin inhibited HIF-1α protein expression more effectively than sorafenib. Combined treatment of melatonin and sorafenib markedly diminished HIF-1α protein expression [36].

To evaluate the HIF-1α involvement in the melatonin/sorafenib-induced Hep3B cell death under hypoxia, control and HIF-1α small interfering RNAs (siRNAs) were utilized. No statistically significant differences in Hep3B viability between control and HIF-1α siRNAs cells after treatments were observed. The result could be interpreted as a compensatory effect by which HIF-2α is upregulated when the HIF-1α expression is reduced [36].

To investigate the melatonin-mediated dynamic changes on HIF-1α expression, Hep3B cells were preincubated under hypoxia for 3 hour, after which melatonin was added for 1.5 and 3 hours. After removal of melatonin, cells were incubated again under hypoxia conditions for additional 1.5 and 3 hours to re-establish the initial hypoxic environment. Melatonin inhibited HIF-1α protein levels in a time-dependent manner, while after removal of melatonin, the HIF-1α levels were gradually restored [36].

Melatonin downregulates the mTOR complex 1 (mTORC1)/ribosomal protein S6 kinase beta-1 (p70S6K) pathway to inhibit HIF-1α protein synthesis

Hep3B cells were incubated under hypoxic condition for 0, 1, 3 and 6 hours with or without melatonin (2 mM). Melatonin significantly reduced the phosphorylation/activation state of mTORC1, its downstream kinase p70S6K, and its effector ribosomal protein S6 (RP-S6) in a time-dependent manner; these changes were associated with the reduction of HIF-1α under hypoxia. Furthermore, Hep3B cells exhibited a rise in mTOR complex 2 (mTORC2) and Akt phosphorylation after melatonin treatment with hypoxia [36].

mTORC2 and Akt phosphorylation levels were also enhanced by rapamycin treatment with hypoxia. However, rapamycin treatment with hypoxia decreased mTORC1, p70S6K, RP-S6 phosphorylation and consequently HIF-1α synthesis [36].

LY294002 downregulated Akt, mTORC1, mTORC2, p70S6K and RP-S6 phosphorylation both alone and in combination with melatonin. In addition, LY294002 decreased HIF-1α accumulation more intensely than melatonin [36].

Melatonin increases sensitivity to sorafenib through inhibition of hypoxia-induced mitophagy

Mitophagy, a particular form of autophagy, regulates mitochondrial homeostasis during various cellular stresses such as oxygen depletion. Hypoxia markedly induced the expression of BNIP3 and NIX, mitophagy mediators directly modulated by HIF-1α. Melatonin or sorafenib alone reduced expression of BNIP3 and NIX by 1-3 fold. Hep3B cells treated with both agents revealed much lower levels than those observed under normoxia [36].

The impact of BNIP3 gene-silencing on cell viability after administration of melatonin and sorafenib was evaluated. BNIP3 expression was nearly completely eliminated 48 hours after treatment. BNIP3 depletion resulted in enhanced melatonin and sorafenib sensitivity and decreased cell viability, indicating a cytoprotective role of hypoxia-related mitophagy in HCC cancer cells [36].

Hypoxia enhanced the autophagosome markers, sequestosome-1 (p62) and LC3-II, reaching a peak value at 12 hours, which diminished thereafter. Sorafenib delayed the peak until 24 hours and simultaneous administration of melatonin reduced p62 and LC3-II levels to those detected in normoxic cells [36]. In addition, the functional impact of melatonin on mitophagy was evaluated utilizing 5 μM bafilomycin A1 (Baf-A1), an inhibitor of autophagosome-lysosome fusion. When this process was inhibited, both p62 and LC3-II were markedly elevated. Combined treatment with sorafenib and melatonin significantly decreased p62 and LC3-II levels, even in the presence of Baf-A1 [36].

Twelve hours of oxygen deprivation induced organelle fusion which was demonstrated by co-localization of the mitochondrial marker Tom20 and the lysosomal marker LAMP2. Sorafenib delayed their interaction until 24 hours. The combination of melatonin and sorafenib completely eliminated the interaction. These results reveal that melatonin limits hypoxia-mediated autophagosome formation and subsequent mitophagy in HCC cells [36].

In brief, the results indicate that melatonin enhances the Hep3B cell sensitivity to sorafenib, blocking HIF-1α synthesis and inhibiting the cytoprotective mitophagy induced by the hypoxic microenvironment, an essential component of the multifactorial mechanisms responsible for the failure of chemotherapy.

Hyperbaric oxygen therapy sensitizes gastric cancer cells to melatonin-induced apoptosis through multiple pathways

Wei., *et al.* investigated the effects of combined administration of hyperbaric oxygen (HBO) and melatonin on the gastric adenocarcinoma cell line SGC7901 to elucidate the underlying mechanisms which may create new possibilities for adjuvant therapy of gastric cancer [37].

HBO effectively sensitize human gastric cancer cells to melatonin-induced apoptosis

By means of Hoechst 33258 staining, most of untreated SGC7901 cells (the control group) presented a normal shape with round intact nuclei. Compared with the control group, melatonin treatment alone, and the combined treatment with HBO and melatonin, significantly induced the apoptotic features of nuclei stained with Hoechst 33258, including apparent nucleus shrinkage with increased density, condensation and fragmentation. During the interval the cells were treated with a combination of HBO and melatonin, more apoptotic cells were found than those treated with melatonin alone [37].

DNA ladder assay utilizing gel electrophoresis revealed clear DNA fragments generated by apoptotic SGC7901 cells treated with a combination of HBO and melatonin. Flow cytometric analyses of the cell line SGC7901 also revealed an significantly elevated proportion of Annexin V^+ and PI^+ cells following treatment with combined HBO and melatonin compared to control cells or those treated with HBO or melatonin only [37].

Hoechst 33258 staining, DNA ladder assay and flow cytometric analysis reveal that HBO further enhances melatonin-induced apoptosis and indicate that HBO promotes a sensitizing effect on melatonin-induced apoptosis of gastric cancer cells [37].

HBO potentiates melatonin-mediated apoptosis through upregulation of p53 expression and downregulation of BCL-2/Bax ratio

Western blot assay revealed that p53 level enhanced significantly in the cells treated with HBO and melatonin compared to HBO or melatonin treatment alone. The assay also showed an increased Bcl-2/Bax ratio in HBO treated cells and a decreased Bcl-2/Bax ratio in melatonin treated cells. The ratio was markedly reduced in the cells with combined HBO and melatonin treatment compared to melatonin treatment alone [37].

The combination of HBO and melatonin enhances autophagy of gastric cancer cells

LC3 is a reliable marker of autophagy. Wei., *et al.* evaluated whether HBO and melatonin influenced the autophagy pathway by assessing LC3 expression. The results showed that LC3 expression was enhanced when gastric cancer cells were treated with HBO or melatonin alone. When gastric cancer cells were treated with both HBO and melatonin, LC3 expression was further enhanced. This finding indicated that HBO combined with melatonin raised autophagy of gastric cancer cells more effectively [37].

In brief, the findings indicate that HBO sensitizes human gastric cancer cells to melatonin-induced apoptosis through various complex molecular mechanisms. HBO may provide an innovative approach for further development of potential anti-gastric cancer therapeutics. The combination of HBO and melatonin could be a promising strategy for the treatment of advanced gastric cancer [37].

The impact of low LET and high LET radiation on autophagy in cancer cells

Inhibition of the autophagic response sensitizes lung cancer cells to radiation

Karagounis., *et al.* investigated the autophagosomal and lysosomal response to radiation in lung cancer cell lines by assessing the expression of the related proteins, together with the effect of pertinent gene silencing in radiosensitization. Additionally, tumor sensitization was assessed in *in vivo* autophagic gene silencing model after irradiation [33].

A549 (human alveolar basal epithelial adenocarcinoma cells) and NCI-H1299 (human non-small-cell lung carcinoma cells) cell lines were employed as *in vitro* cancer models. Exposure of cells to 2-9 Gy of radiation was performed utilizing a 6 MV photon beam of a Linear Accelerator. Radiosensitization of both cell types was then assessed after transfection with siRNAs targeting LC3A (the autophagosomal membrane-bound protein light chain 3A), LC3B (light chain 3B), TFEB (transcription factor EB) and LAMP2a (lysosome-associated membrane protein 2a). Cell viability was examined 7 days after ionizing radiation utilizing the AlamarBlue Cell Viability Reagent assay. Human lung carcinoma H129 cell line was stably transfected with small-hairpin RNA of the MAP1LC3A gene, and tumor radiosensitization in Athymic Nude- Foxn1^{nu} was assessed [33].

The effect of irradiation on LC3A autophagic flux

In A549 cells, 4 Gy of irradiation significantly depressed levels of membrane-bound LC3A-II that continued low for at least 7 days after irradiation. Confocal microscopy disclosed an increased autophagic flux as an early (2 days) response of A549 cells to radiation and demonstrated conspicuous LC3A/LAMP2a co-localization [33].

In H1299 cells, the membrane-bound LC3A-II form was unchanged at 2 days after irradiation, while a remarkable reduction was noted at 7 days. Confocal microscopy corroborated that initially the autophagic flux remained unchanged, which was followed by a conspicuous induction at 7 days (LC3A/LAMP2a co-localization) [33].

Aggresome assessment of H1299 cells revealed a lack of its accumulation in the cytoplasm. Levels of LC3A and p62 mRNA were increased at 2 days after irradiation and persisted at high levels subsequently [33].

The effect of irradiation on LC3B autophagic flux

The pattern of membrane-bound LC3B-II response to radiation was analogous to LC3A in both cell lines [33].

The effect of irradiation on lysosomal markers

TFEB expression was low in A549 cells but was markedly elevated 7 days later. Lysosomal protein LAMP2a rose after irradiation in the A549 cell. The cathepsin-D protein revealed an initial reduction at 2 days; it was re-established at 7 days. In H1299 cells, levels of TFEB mRNA were markedly elevated on day 2. LAMP2a continued stable and cathepsin-D was further enhanced 7 days after irradiation [33].

Radiosensitivity

In A549 cells, the radiation dose necessary to induce 50% growth inhibition at 7 days after irradiation was 6.2 Gy in control cells and was 1.9, 4.3, 3.2 and 2.5 Gy in cells with silenced LC3A, LC3B, TFEB and LAMP2a genes, respectively. In H1299 cells, the radiation dose required to induce 50% growth inhibition was 8.6 Gy in control cells and was 4.2, 6.5, 4.5 and 3.4 Gy when the LC3A, LC3B, TFEB and LAMP2a genes were silenced, respectively. Incubation of both A549 cells and H1299 cells with the autophagy inhibitors, chloroquine and bafilomycin, also led to increased sensitivity to radiation [33].

Xenografts

Karagounis., *et al.* established stable shLC3A-transfected H1299 cells (shH1299) for xenograft experiments. When the size of tumors reached 1 cm, they were irradiated with two consecutive fractions of 6 Gy. Wild-type H1299 cells persisted their growth and moved into an accelerated phase of growth 12 days later. The shH1299 tumors underwent a loss of growth up to 12 days after irradiation. The difference was statistically significant at all post-irradiation time points [33].

In brief, the capability of lung cancer cells to survive after irradiation of 4 Gy is based on their ability to maintain a functional autophagic flux. Abolition of this ability leads to an increased radiosensitivity. The discovery of biological agents that are capable of inhibiting the autophagic function of cancer cells may improve the tumor control probability of locally advanced lung cancer and may increase the overall survival rate of patients with metastatic lung cancer.

Role of autophagy in high linear energy transfer radiation-induced cytotoxicity to tumor cells

Jin., *et al.* investigated whether tumor cells undergo autophagy after exposure to high-LET carbon ion irradiation and explored the role of autophagy in high-LET radiation-induced cytotoxicity. Additionally, the correlation between autophagy and apoptosis and pertinent underlying mechanisms were investigated [38].

High-LET radiation induced autophagy effectively in tumor cells

The monodansylcadaverine (MDC) dye was utilized to evaluate levels of mature autophagic vesicle formation in HeLa cells following irradiation. The irradiated cells demonstrated numerous punctate staining of bright blue. Unirradiated cells revealed diffuse MDC staining instead of a punctate staining pattern. The re-distribution of green fluorescent protein (GFP)-LC3 from a diffuse cytosolic to a punctate autophagosome associated pattern was detected in HeLa, MCF-7, and MDA-MB-231 cells at 4 and 24 hours post-irradiation. The morphological findings indicated that the carbon ion irradiation effectively induced autophagy in the tumor cells [38].

Unirradiated cells exhibited a low level of LC3-II at all time points. Nevertheless, the level of LC3-II expression in the irradiated cells were enhanced markedly with the time interval post-irradiation, indicating the process of autophagy was enhanced in these cells [38].

Surveillance of the natural autophagic substrate p62 (sequestosome 1 or SQSTM1) was utilized to evaluate the autophagic flux. The study of Jin., *et al.* suggested that SQSTM1/ p62 was degraded in autolysosomes and the autophagic flux was activated after irradiation. The expression of other crucial proteins associated with autophagy under high-LET irradiation was observed as well. Atg5 expression progressively increased and reached a maximum, then decreased thereafter in HeLa and MCF-7 cells [38].

Autophagy level increased with LET and dose of carbon ions irradiation

Jin., *et al.* investigated the existence of acidic vesicular organelles in order to quantify the probable induction of autophagy. The autophagy levels in HeLa, MCF-7, and MDA-MB-231 cells exposed to the carbon ion irradiation with LETs of 13 and 75 keV/μm at doses of 2 Gy

or 5 Gy was evaluated at 24, 48 and 72 hours after irradiation. The results demonstrated that the autophagic rate of these cells increased with LET and dose at the time points under investigation after the carbon ion irradiations [38].

Carbon ions induced autophagy in tumor cells by depressing the PI3K/Akt pathway

The variation in expression level of serine/threonine kinase, mammalian target of rapamycin (mTOR) is associated with autophagic regulation mTOR and is modulated by the PI3K/Akt pathway [39]. HeLa cells were exposed to carbon ions with different LETs, i.e., 13 and 75 keV/μm. Levels of phosphorylation of PI3K/Akt-related proteins, phospho-Akt and phospho-p70S6K, were estimated in HeLa cells at 24 hours after irradiation. A greater reduction in the protein expression level was detected in HeLa cells after exposure to the carbon ions of 75 keV/μm than those exposure to 13 keV/μm [38].

Phosphorylation of the PI3K/Akt-related proteins were effectively inhibited by carbon ion irradiation. The cellular constituents of phospho-Akt and phospho-p70S6K proteins in HeLa cells irradiated with the relatively low LET carbon ions decreased to 90% and 68% of the comparable amounts in the unirradiated cells, respectively, and to 24% and 15% in the cells irradiated with the high-LET carbon ions. These results reveal that the carbon ion irradiations induced autophagy in HeLa cells by reducing the activation of the PI3K/Akt pathway, and this pathway was more effectively inhibited by the carbon ion beams with high LET than with relatively low LET [38].

Role of autophagy in radiosensitivity to high-LET radiation

Beclin 1 and Atg5 genes encode proteins that modulate autophagy. These two genes were knocked down with siRNA individually or as a combination of both genes. Twenty-four hours after transfection, HeLa cells were irradiated with the carbon ion beam of 75 keV/μm. Knockdown of Beclin 1 together with Atg5 inhibited the high-LET radiation-induced acidic vesicular organelle (AVO) formation significantly, whereas Beclin 1 or Atg5 knockdown alone had only a marginal effect. The survival fractions of the cells of irradiation + siRNA Beclin 1 and Atg5 were markedly lower than those of the other three groups (irradiation alone, irradiation + siRNA Beclin 1, irradiation + siRNA Atg5) [38].

Effect of pharmacological autophagy inhibition on cellular radiosensitivity to high-LET radiation

3-Methyladenine (3-MA) reduces autophagy by interrupting autophagosome formation through the inhibition of type III Phosphatidylinositol 3-kinases (PI-3K) [40]. HeLa, MDA- MB-231 and MCF-7 cells were irradiated with carbon ion beam alone or in combination with 3-MA so as to determine whether pharmacological inhibitor of autophagy pathways regulates cellular radiosensitivity. The percentages of acidic vesicular organelle (AVO)-harboring cells in the group of carbon ion irradiation with 3-MA decreased to 15.1%, 24.4% and 20.8%, compared with the group of irradiation alone in the three cell lines (20.3%, 35.1% and 25.5%), respectively [38].

The appearance of abundant autophagosomes in cells treated with chloroquine indicated an inhibition of autophagy. The levels of LC3-II expression accumulated in chloroquine pretreated HeLa, MDA-MB-231, and MCF-7 cells irradiated with the carbon ion beams. The survival of these three cell lines decreased significantly in the presence of 3-MA or chloroquine. These results showed that both 3-MA and chloroquine increased the radiosensitivity of the tumor cells to carbon ion irradiation [38].

Inhibition of autophagy enhanced high-LET radiation-induced apoptosis

HeLa and MDA-MB-231 cells pretreated with autophagy inhibitor, 3-MA, were irradiated with carbon ion beam of 75 keV/μm at 2 Gy. Thereafter, cellular apoptosis was quantified at 4 or 24 hours after irradiation. The apoptotic rates of HeLa cells were significantly higher in the group of carbon ion irradiation with 3-MA than in the cells exposed to radiation alone. Additionally, protein expression of cleaved caspase-3 increased in HeLa cells treated with combination of carbon ion irradiation and 3-MA. Flow cytometric measurements of DNA fragmentation in MDA-MB-231 cells at 24 hours after irradiation demonstrate that high-LET radiation increased the apoptotic rate of MDA-MB-231 cells. This effect was enhanced by combined treatment with carbon ion irradiation and chloroquine. These results revealed that inhibition of autophagy increased high-LET radiation-induced apoptosis in HeLa and MDA-MB-231 cell lines [38].

In brief, the results imply that targeting autophagy might enhance the effectiveness of heavy ion radiotherapy.

Inhibiting autophagy with chloroquine enhances the anti-tumor effect of high-LET carbon ion irradiation through ER stress-related apoptosis

Ionizing radiation leads to cell death by eliciting a sequence of physiological and biochemical effects inside the cells, such as apoptosis, DNA damage, cell cycle disorder and oxidative stress response [42]. Various physiological or pathological changes may induce an imbalance between the endoplasmic reticulum (ER) protein folding load and capability, resulting in the accumulation of unfolded or misfolded proteins in the ER lumen, a situation identified as ER stress. ER stress gives rise to a protective cellular stress response, recognized as the unfolded protein response (UPR) [43]. Earlier studies utilizing heavy-ion radiation revealed that high-LET carbon ion irradiation elicited the ER stress and mediated autophagy and apoptosis through the UPR down-stream signaling axis [41]. Zheng., *et al*. investigated the influence of autophagy and apoptosis on the radiosensitivity of tumor cells under conditions of ER stress following carbon ion irradiation [41].

Suppression of cell proliferation induced by carbon ion irradiation was enhanced by chloroquine

The results of the study revealed that combined treatment with chloroquine and radiation reduced the proliferative capacity of sarcoma S180 cells significantly compared with the untreated control. The suppressive effect of combined treatment with 4 Gy X-ray irradiation and chloroquine on cell proliferation was comparable to that resulted from the combined treatment with 2 Gy carbon ion irradiation and chloroquine [41].

The IRE1 signaling axis was required for activation of carbon ion radiation-induced autophagy in S180 cells

The immunoglobulin heavy chain binding protein (BiP) was up-regulated by carbon ion or X-ray irradiation and further enhanced by the combination of radiation and chloroquine in S180 cells. The level of inositol requiring enzyme 1 (IRE1) was raised by carbon ion radiation and further enhanced when the treatment was coupled with chloroquine. For both carbon ion and X-ray irradiation, the downstream JNK was increased in the combined treatment group. The expression level of Beclin-1 and p62 (SQSTM1) in S180 cells were also enhanced by chloroquine after carbon ion or X-ray irradiation [41].

Expression of C/EBP homologous protein (CHOP) further aggravated the apoptotic cell death

Carbon ion irradiation induced a markedly higher apoptosis rate than that caused by X-ray irradiation. At the same radiation dose of 2 Gy, the apoptosis rate caused by carbon ion irradiation combined with chloroquine was substantially higher than that induced by X-rays combined with chloroquine. Carbon ion irradiation brought about a rise in the expression of CHOP. On the contrary, X-rays did not generate the expression of CHOP. When CHOP was activated, carbon ion irradiation-induced apoptosis was significantly increased [41].

Carbon ion irradiation combined with chloroquine inhibited the growth of tumors

Tumor-bearing mice were treated with carbon ion irradiation (LET = 75 keV/lm) at a dose of 2 Gy and chloroquine (50 mg/kg/day). This treatment exhibited a notable decrease in tumor volume and weight compared with the control and radiation alone group. The irradiated tumors demonstrated softening of the tumor tissue, eosinophilic staining, and abnormal nuclear morphology. These findings were further aggravated in the combined treatment group [41].

Carbon ion irradiation induced ER stress and subsequently elicited autophagy via the IRE1 signaling pathway

Carbon ion-induced ER stress is involved in autophagy through the IRE1/JNK/p-Bcl-2/Beclin-1 signaling axis. Chloroquine suppressed autophagy and enhanced apoptosis of S180 cells growing *in vivo*. The proteins involved in the IRE1/p-JNK/p-Bcl-2 pathway were all increased by carbon ion irradiation combined with chloroquine. The expression of autophagy-related proteins Beclin-1 and p62 were markedly enhanced by this treatment [41].

Carbon ion irradiation combined with chloroquine significantly increased the apoptotic rate of tumor tissue cells

Radiation led to an obvious rise in TUNEL-positive tumor cells compared with the control. A markedly greater percentage of TUNEL-positive tumor cells was detected in the combined treatment group compared with the control. The combined treatment also brought about a marked enhancement in the expression of CHOP. Pro-apoptotic Bax in the combined treatment group was significantly expressed following treatment.

The study also revealed that high-LET carbon ion irradiation combined with chloroquine, enhances the anti-tumor activity of carbon ion irradiation by exacerbating ER stress-related apoptosis, which increases the radiosensitivity of tumor cells. Consequently, suppressing autophagy might be an encouraging therapeutic strategy in carbon ion radiotherapy due to exacerbation of ER stress-related apoptosis [41].

Suppression of PC-1/PrLZ sensitizes prostate cancer cells to ionizing radiation by attenuating DNA damage repair and inducing autophagic cell death

LNCaP cells are androgen-responsive, non-metastatic, and slightly tumorigenic prostate cancer cells while C4-2 cells are a subline derived from LNCaP cells that possess a more aggressive phenotype which includes androgen independence and osseous metastases [44]. C4-2 cells are more likely to develop radioresistance than parental LNCaP cells. The human prostate and colon gene-1 (PC-1, also known as PrLZ) is over-expressed in C4-2 cells in contrast to LNCaP cells [44].

PC-1/PrLZ is a member of the TPD52 protein family which includes members highly related with proliferation and progression of a variety of malignant tumors, including breast, lung and prostate cancer. PC-1/PrLZ is uniquely expressed in prostate tissues. PC-1/PrLZ is commonly overexpressed in advanced prostate cancer cells. This phenomenon is involved in malignant phenotypes highly associated with aggressive progression in prostate cancer [44]. Shang., *et al.* examined the probable role of PC-1/PrLZ for radioresistance in human prostate cancer cell lines [44].

PC-1/PrLZ expression was induced by ionizing radiation in prostate cancer cells

PC-1/PrLZ expression is enhanced in C4-2 and C4-2B cells following ionizing radiation, with the high expression continuing for at least 24 hours after 4-Gy of irradiation. Immunofluorescent staining analysis disclosed that endogenous PC-1/PrLZ localized mainly in the cytoplasm and weakly in the nuclei of C4-2 cells [44].

PC-1/PrLZ expression is correlated with radioresistance in prostate cancer cells

Both RT- PCR and Western blot analysis verified that PC-1/PrLZ expression was inhibited in C4-2 shPC-1 cells and enhanced in the PC-1/PrLZ-hypo-expressing LNCaP cells. The MTT assay and a clonogenic assay verified that shRNA-mediated suppression of PC-1/PrLZ expression (C4-2 shPC-1) markedly sensitized C4-2 cells to ionizing radiation. On the other hand, overexpression of PC-1/PrLZ in LNCaP cells markedly elevated radioresistance of LNCaP cells. These results reveal that PC-1/PrLZ expression influences radioresistance in prostate cancer cells [44].

Suppression of PC-1/PrLZ decreased DNA DBS repair capacity which induced the prolonged activation of the DNA damage response signal pathway

The phosphorylated H2AX (γH2AX) foci assay is a delicate technique for quantifying DNA DSBs. C4-2 shPC-1 cells present more sluggish DSBs repair compared with controls at 0.5 to 4 hours after irradiation [44]. DNA-PKcs and ataxia-telangiectasia mutated (ATM) are two fundamental enzymes of the non-homologous end joining (NHEJ) repair pathway. Shang., *et al.* estimated phosphorylation of these enzymes at specific sites that were crucial to DNA-PKcs and ATM repair activity. Suppression of PC-1/PrLZ expression noticeably protracted phosphorylation of DNA-PKcs and ATM at S2056 and S1981, respectively, in C4-2 prostate cancer cells after 10 Gy of irradiation (40). PC-1/ PrLZ-deficient C4-2 shPC-1 cells are more sensitive to irradiation; this finding may be caused by prolonged DNA damage repair [44].

Radiosensitization by suppressing PC-1/PrLZ is attributed to induction of enhanced autophagic cell death

Earlier studies indicate that autophagy also operates as a pro-death mechanism that is commonly activated in tumor cells treated with chemotherapy or radiotherapy [44]. Shang., *et al.* measured the accumulation of acidic vesicular organelles (AVO) after 4-Gy of gamma irradiation and detected that both ionizing radiation and PC-1/PrLZ silencing induced autophagy. Treatment of PC-1/PrLZ-deficient cells with ionizing radiation enhanced AVOs compared to control cells. After 4-Gy of irradiation, acridine orange-positive cells raised from 18.17% to 58.58% for C4-2 NC cells and 47.91% to 80.96% for C4-2 sh cells. These findings indicate that suppression of PC-1/PrLZ expression may radiosensitize prostate cancer cells by inducing autophagic cell death [44]. Consequently, PC-1/PrLZ is an innovative candidate involved in DNA DSB repair and radioresistance and targeting PC-1/PrLZ might offer an encouraging therapeutic strategy for radiosensitizing prostate cancer cells.

Conclusion

Under clinicopathological conditions, autophagic activity has been demonstrated to be increased in patients with non-small-cell lung carcinoma, colorectal carcinoma, urothelial cell carcinoma, endometrioid carcinoma, and gastric carcinoma and associated with worse outcomes.

Melatonin activates TFE3 and enhances TFE3 reporter activity. Suppression of TFE3-dependent autophagy sensitizes melatonin-induced apoptosis in tongue squamous cell carcinoma (TSCC) cells. A connection between melatonin-induced TFE3-dependent autophagy and the melatonin membrane receptor has been detected. The melatonin-induced enhancement in TFE3-dependent autophagy is mediated through the melatonin membrane receptor in TSCC. Suppression of autophagy may increase the antitumor effect of melatonin. Additionally, melatonin combined with autophagy inhibitors may exert synergistic effects on the suppression of tumor development.

PRNP overexpression markedly inhibits 5-FU- and melatonin-mediated autophagy. PrP^C and Oct4 modulate 5-FU- and melatonin-mediated autophagy in colon cancer stem cells. Melatonin enhances the Hep3B cell sensitivity to sorafenib, precluding HIF-1α synthesis to inhibit the cytoprotective mitophagy induced by the hypoxic microenvironment, an essential component of the multifactorial mechanisms responsible for the failure of chemotherapy.

Hyperbaric oxygen (HBO) sensitizes human gastric cancer cells to melatonin-induced apoptosis through various complex molecular mechanisms. The combination of HBO and melatonin enhances autophagy of gastric cancer cells. The combination of HBO and melatonin may be a promising strategy for the treatment of advanced gastric cancer.

The capability of lung cancer cells to survive after irradiation of 4 Gy is based on their ability to maintain a functional autophagic flux. Abolition of this ability leads to an increased radiosensitivity. The discovery of biological agents that inhibit the autophagic function of cancer cells may improve the tumor control probability of locally advanced lung cancer and may increase overall survival rate of patients with metastatic lung cancer.

The autophagy level in HeLa, MCF-7, and MDA-MB-231 cells increased with LET and dose of carbon ion irradiation. Inhibition of autophagy increased high-LET irradiation-induced apoptosis in HeLa and MDA-MB-231 cell lines. Accordingly, targeting autophagy might enhance the effectiveness of heavy ion radiotherapy.

Chloroquine, an autophagy inhibitor, combined with high-LET carbon ion irradiation, may enhance the anti-tumor effect of carbon ion irradiation through aggravating ER stress-related apoptosis, leading to an increase in radiosensitivity of sarcoma S180 cells. Thus, inhibiting autophagy may be a promising therapeutic strategy in carbon ion radiotherapy by aggravating ER stress-related apoptosis.

PC-1/PrLZ expression is associated with radioresistance in prostate cancer cells. Inhibition of PC-1/PrLZ decreased DNA DBS repair capacity. Radiosensitization due to inhibition of PC-1/PrLZ is ascribed to the induction of enhanced autophagic cell death. Therefore,

PC-1/PrLZ is an innovative candidate involved in DNA DSB repair and radioresistance and targeting PC-1/PrLZ may offer an encouraging therapeutic strategy for radiosensitizing prostate cancer cells.

Further investigation on the biological mechanism related to the effects of autophagy on tumor initiation and progression is the basis for exploiting innovative autophagy-targeted therapeutic intervention so as to improve clinical outcomes in a variety of cancer patients.

Conflict of Interest

We declare that there is no conflict of interest.

Bibliography

1. Yun CW and Lee SH. "The roles of autophagy in cancer". *International Journal of Molecular Sciences* 19.11 (2018): E3466.
2. Mizushima N. "Autophagy: Process and function". *Genes and Development* 21.22 (2007): 2861-2873.
3. Yonekawa T and Thorburn A. "Autophagy and cell death". *Essays in Biochemistry* 55 (2013): 105-117.
4. Fan T., *et al*. "Inhibiting MT2-TFE3-dependent autophagy enhances melatonin-induced apoptosis in tongue squamous cell carcinoma". *Journal of Pineal Research* 64.2 (2018): e12457.
5. Button RW., *et al*. "Dual PI-3 kinase/mTOR inhibition impairs autophagy flux and induces cell death independent of apoptosis and necroptosis". *Oncotarget* 7.5 (2016): 5157-5175.
6. Yeo SK., *et al*. "Autophagy differentially regulates distinct breast cancer stem-like cells in murine models via EGFR/Stat3 and Tgfbeta/Smad signaling". *Cancer Research* 76.11 (2016): 3397-3410.
7. Simstein R., *et al*. "Apoptosis, chemoresistance, and breast cancer: insights from the MCF-7 cell model system". *Experimental Biology and Medicine* 228.9 (2003): 995-1003.
8. Townson JL., *et al*. "The role of apoptosis in tumor progression and metastasis". *Current Molecular Medicine* 3.7 (2003): 631-642.
9. Guo W., *et al*. "Inhibiting autophagy increases epirubicin's cytotoxicity in breast cancer cells". *Cancer Science* 107.11 (2016): 1610-1621.
10. Vijayalaxmi., *et al*. "Melatonin: from basic research to cancer treatment clinics". *Journal of Clinical Oncology* 20.10 (2002): 2575-2601.
11. Cabrera J., *et al*. "Melatonin decreases cell proliferation and induces melanogenesis in human melanoma SK-MEL-1 cells". *Journal of Pineal Research* 49.1 (2010): 45-54.
12. Carbajo-Pescador S., *et al*. "Melatonin modulation of intracellular signaling pathways in hepatocarcinoma HepG2 cell line: role of the MT1 receptor". *Journal of Pineal Research* 51.4 (2011): 463-471.
13. Sanchez-Barcelo EJ., *et al*. "Melatonin uses in oncology: breast cancer prevention and reduction of the side effects of chemotherapy and radiation". *Expert Opinion on Investigational Drugs* 21 (2012): 819-831.
14. Cutando A., *et al*. "Role of melatonin in cancer treatment". *Anticancer Research* 32 (2012): 2747-2753.
15. Carbajo-Pescador S., *et al*. "Inhibition of VEGF expression through blockade of Hif1a and STAT3 signalling mediates the anti-angiogenic effect of melatonin in HepG2 liver cancer cells". *British Journal of Cancer* 109 (2013): 83-91.

16. Pariente R., *et al*. "Melatonin sensitizes human cervical cancer HeLa cells to cisplatin-induced cytotoxicity and apoptosis: effects on oxidative stress and DNA fragmentation". *Journal of Pineal Research* 60 (2016): 55-64.

17. Reiter RJ., *et al*. "Melatonin, a full service anti-cancer agent: inhibition of initiation, progression and metastasis". *International Journal of Molecular Sciences* 18 (2017): E843.

18. Gonzalez Gonzalez A., *et al*. "Clinical uses of melatonin: evaluation of human trials on cancer treatment". *Melatonin Research* 2 (2019): 47-69.

19. Mayo JC., *et al*. "Understanding the role of melatonin in cancer metabolism". *Melatonin Research* 2 (2019): 76-104.

20. Reiter RJ., *et al*. "Inhibition of mitochondrial pyruvate dehydrogenase kinase: a proposed mechanism by which melatonin causes cancer cells to overcome cytosolic glycolysis, reduce tumor biomass and reverse insensitivity to chemotherapy". *Melatonin Research* 2 (2019): 105-119.

21. Wei JY., *et al*. "Melatonin induces apoptosis of colorectal cancer cells through HDAC4 nuclear import mediated by CaMKII inactivation". *Journal of Pineal Research* 58 (2015): 429-438.

22. Bizzarri M., *et al*. "Molecular mechanisms of the proapoptotic actions of melatonin in cancer: a review". *Expert Opinion on Therapeutic Targets* 17 (2013): 1483- 1496.

23. Trivedi PP., *et al*. "Melatonin modulated autophagy and Nrf2 signaling pathways in mice with colitis-associated colon carcinogenesis". *Molecular Carcinogenesis* 55.3 (2016): 255-267.

24. Gil-Martin E., *et al*. "The emergence of melatonin in oncology: Focus on colorectal cancer". *Medicinal Research Reviews* 39 (2019): 2239-2285.

25. Sagrillo-Fagundes L., *et al*. "Melatonin: The smart molecule that differentially modulates autophagy in tumor and normal placental cells". *PLoS One* 14.1 (2019): e0202458.

26. Baskar R., *et al*. "Cancer and radiation therapy: current advances and future directions". *International Journal of Medical Sciences* 9 (2012): 193-199.

27. Su WH., *et al*. "Radiation-induced increase in cell migration and metastatic potential of cervical cancer cells operates via the K-Ras pathway". *The American Journal of Pathology* 180 (2012): 862-871.

28. Tsujii H and Kamada T. "A review of update clinical results of carbon ion radiotherapy". *Japanese Journal of Clinical Oncology* 42 (2012): 670-685.

29. Helm A., *et al*. "The influence of C-Ions and X-rays on human umbilical vein endothelial cells". *Frontiers in Oncology* 6 (2016): 5.

30. Hamada N., *et al*. "Recent advances in the biology of heavy-ion cancer therapy". *Journal of Radiation Research* 51 (2010): 365-383.

31. Zois CE and Koukourakis MI. "Radiation-induced autophagy in normal and cancer cells: towards novel cytoprotection and radio-sensitization policies?". *Autophagy* 5 (2009): 442-450.

32. Kalamida D., *et al*. "Important role of autophagy in endothelial cell response to ionizing radiation". *PLoS One* 9 (2014): e102408.

33. Karagounis IV., *et al*. "Repression of the autophagic response sensitises lung cancer cells to radiation and chemotherapy". *British Journal of Cancer* 115.3 (2016): 312-321.

34. Fan T., *et al*. "Inhibiting MT2-TFE3-dependent autophagy enhances melatonin-induced apoptosis in tongue squamous cell carcinoma". *Journal of Pineal Research* 64 (2018): e12457.

35. Lee JH., *et al*. "Melatonin and 5-fluorouracil co-suppress colon cancer stem cells by regulating cellular prion protein-Oct4 axis". *Journal of Pineal Research* 65 (2018): e12519.

36. Prieto-Dominguez N., *et al*. "Melatonin enhances sorafenib actions in human hepatocarcinoma cells by inhibiting mTORC1/p70S6K/HIF-1α and hypoxia-mediated mitophagy". *Oncotarget* 8 (2017): 91402-91414.

37. Wei X., *et al*. "Hyperbaric oxygen treatment sensitizes gastric cancer cells to melatonin-induced apoptosis through multiple pathways". *Journal of Cellular Biochemistry* 119 (2018): 6723-6731.

38. Jin X., *et al*. "Role of autophagy in high linear energy transfer radiation-induced cytotoxicity to tumor cells". *Cancer Science* 105 (2014): 770-778.

39. Wirawan E., *et al*. "Autophagy: for better or for worse". *Cell Research* 22.1 (2012): 43-61.

40. Wu YT., *et al*. "Dual role of 3-methyladenine in modulation of autophagy via different temporal patterns of inhibition on class I and III phosphoinositide 3-kinase". *The Journal of Biological Chemistry* 285 (2010): 10850-10861

41. Zheng X., *et al*. "Inhibiting autophagy with chloroquine enhances the anti-tumor effect of high-LET carbon ions via ER stress- related apoptosis". *Medical Oncology* 34 (2017): 25.

42. Anscher MS and Vujaskovic Z. "Mechanisms and potential targets for prevention and treatment of normal tissue injury after radiation therapy". *Seminars in Oncology* 32 (2 Suppl 3) (2005): S86-91.

43. Senft D and Ronai ZA. "UPR, autophagy, and mitochondria crosstalk underlies the ER stress response". *Trends in Biochemical Sciences* 40 (2015):141-148.

44. Shang ZF., *et al*. "Suppression of PC-1/PrLZ sensitizes prostate cancer cells to ionizing radiation by attenuating DNA damage repair and inducing autophagic cell death". *Oncotarget* 7 (2016): 62340-62351.

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