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A Novel Report of Serotonin Receptors Antagonists Inducing Apoptosis in Human Lung Carcinoma Cells

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Abstract

Background: lung cancer is emerging as a leading cause of death in the world. Non-small-cell lung cancer (NSCLC) is one of the major subtypes of LC. A recent study shows the nervous system involved in the progress of cancer. Serotonin as a neurotransmitter can affect cancer development by its receptors (such as 5HTR2A and 5HTR3A).

Methods: Two human NSCLC cell lines (QUDB and A549) and one normal cell line (HFLF) were treated by four doses of ketanserin and tropisetron for 48 h and cell viability was determined by MTT assay. The detection and quantification of apoptosis and its differentiation from necrosis were carried out by using Annexin-V-FLUOS Staining. Subsequently, the nuclear morphology of cells was assessed by mixed dye fluorescent staining. Caspase assay was conducted to measure the caspase activity after treatment with two drugs. Finally, the gene expression pattern of *5HTR2A* and *5HTR3A* genes before and after treatment was studied by Real-time quantitative reverse transcription-polymerase chain reaction.

Results: Treatment with ketanserin (100 $\mu moll^{-1}$in QUDB and 50 $\mu moll^{-1}$ in A549) and tropisetron (100 $\mu moll^{-1}$in both NSCLC cell lines) significantly inhibited the proliferation of human lung cancer cells and induced apoptosis. Expression analysis indicated that *5HTR2A* and *5HTR3A* genes have been expressed in human NSCLC cell lines.

Conclusions: ketanserin and tropisetron are responsible for inducing apoptosis in human lung cancer cells and by doing some more studies, these two FDA-approved drugs may be used for the treatment of these tumor cells.

Keywords: 5HTR2A, 5HTR3A, NSCLC, QUDB, HFLF, Tropisetron, Ketanserin

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Introduction

Lung cancer is the leading cause of cancer-related death worldwide [1]. ***Lung cancer*** is traditionally divided ***into*** two ***main types:*** Small-cell-lung cancer (SCLC*)* and non-small-cell lung cancer (NSCLC). Approximately 75% of lung tumors are NSCLC which consists of squamous cell carcinoma, adenocarcinoma, and large cell carcinoma [2].

Neurotransmitters have been recognized as cell mediators of the nervous system, but recent studies have provided new evidence that neurotransmitters play serious roles in the immune system and sometimes act as a regulator for the migration of tumor cells and leukocytes[3-5]. Numerous studies have shown their functions in the incidence and progression of some cancers, which are mediated by their different types of receptors [6,7].

Serotonin or 5-hydroxytryptamine (5-HT) as a neurotransmitter has various functions in the brain including mood state, anxiety, emotion, sexual behavior, memory, regulation of circadian rhythm, sleep-wakefulness, appetite, and many others [8]. In addition to its famous functions, serotonin is a mitogenic factor for a wide range of normal cells in culture such as lung fibroblasts, renal mesangial cells, vascular smooth muscle cells, hepatocytes, etc [9-11]. It has important biological functions that are mediated via 5-HTR1 to 5-HTR7. All its receptors except 5HTR3 are members of the seven-transmembrane domain G protein-coupled receptor family, while 5HTR3 is an ionotropic receptor belonging to the Cys-loop superfamily of pentameric proteins [12,13].

The existence of serotonin receptors on the peripheral blood mononuclear cells (PBMC) and nervous system represents the role of serotonin in connection with the Central nervous system (CNS) and immune system. Thus, interrupting this relation can lead to occurring the symptoms of cancer. In a dose-dependent state, the effect of serotonin on B-lymphocyte proliferation and hepatocytes has been observed [14].

Current chemotherapy treatments describe the cancer patient survival rates that have remained mostly unchanged over the past three decades. Also, experimental documents showed that conventional chemotherapy has less effect on human cancer cells. This resistance to chemotherapeutics in humans is combined with unselective cytotoxicity that mostly has bad effects on the normal cell, leading to dose restriction and requiring supportive treatment [15]. This article recommended that alternative perceptual and practical approaches are required to treat human cancer. However, the potential of serotonin receptors has not been investigated in the treatment of lung cancer, yet.

In the previous work, we illustrated the presence of these two serotonin receptors (5HTR2A and 5HTR3A) on PBMC of lung cancer patients and the control group. our results showed that both receptors had higher mRNA expression in lung cancer patients compared with the control group [16]. Tropisetron and ketanserin are selective antagonists of 5HTR3A and 5HTR2A, respectively, and both of them are FDA-approved drugs and used as an antihypertensive and efficacious agent in counteracting chemotherapy-induced emesis, respectively [17,18].

Based on our previous study, we hypothesized the presence of 5HTR3A and 5HTR2A on QUDB, HFLF, and A549 cell lines and by using 5HTR2A and 5HTR3A antagonists might induce apoptosis in these NSCLC cell lines.

Materials and Methods

Chemicals

Tropisetron and ketanserin were purchased from Sigma Chemical Company (USA). For in-vitro studies, tropisetron and ketanserin powder form as selective serotonin receptors antagonist dissolved in ethanol to create a stock solution.

Cell culture

Two NSCLC cell lines (QU-DB and A549) and one normal cell line (HFLF-PI5) as control applied in our study, were purchased from the National Cell Bank of Iran (Pasteur Institute of Iran, Tehran). They were preserved and treated based on the guidelines for care and use of experimental cells of the Ethics Committee of the National Institute of Genetic Engineering and Biotechnology. The cells were cultured in Dulbecco's minimum essential medium (DMEM) supplemented with 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). Cells were incubated at 37°C in a humidified chamber supplemented with 5% CO_2. Cells in the logarithmic growth phase (~90% confluence) were selected for the experiments. The $8\times10^5$of each cell line was treated with tropisetron and ketanserin at various concentrations ranging from 10, 25, 50, and 100 $\mu moll^{-1}$. Figure1 shows the Morphology of Cell lines cultured in the appropriate medium.

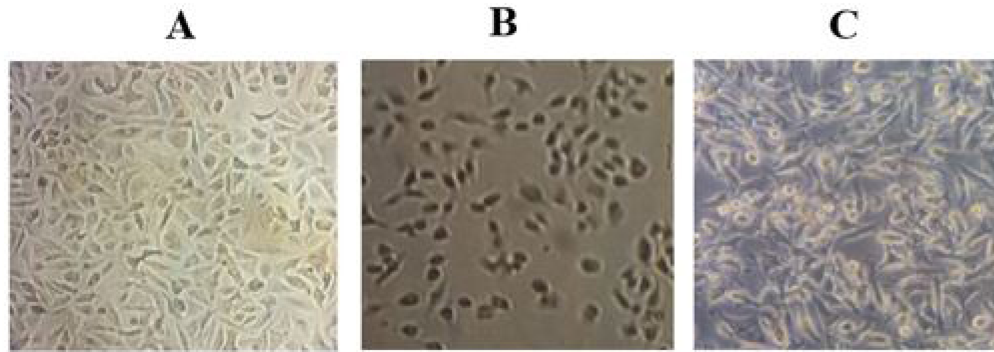

Figure 1: Culture of lung cancer cell lines QUDB, A549, and HFLF in the appropriate medium

A: Morphology of HFLF Cell line cultured in RPMI 1640. B: Morphology of QUDB cell line cultured in DMEM.C: Morphology of A549 cell line cultured in DMEM

Cell viability evaluation by MTT assay

MTT Cell Proliferation Assay was performed to detect oxidative and reductive enzymes and to determine the effective dosage of tropisetron and ketanserin. The cells were seeded at 10^4 cells/well in triplicate in a 96 well plate. They were treated with different doses of tropisetron and ketanserin (10, 25, 50, and 100 $\mu moll^{-1}$) following a 48 -hour of incubation at 37ºC in 5% CO_2 and 95% air. Approximately, 10µl of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (sigma-USA) (MTT reagent) was added to each well. After 4 hours, the supernatant was removed from each and 60 µl DMSO (sigma-USA) along with glycine buffer was added to each. The absorbance was measured at 570 nm by an ELISA plate reader (ELX800™, USA). This test was repeated three times in triplicate form.

Quantitative real-time polymerase chain reaction

The analysis of *5HTR2A* and *5HTR3A* gene expression was performed using q-RT PCR. The total cellular RNA was extracted by High Pure RNA Isolation Kit (Roche, Germany) based on its instruction. To normalize RNA concentration, we assigned its absorbance by using NanoDrop 2000 instrument (Wilmington, USA) at 260 nm, and then for each reaction of cDNA synthesis, we used a constant concentration RNA (70 ng/µl). To obtain cDNA, total mRNA was reverse- transcribed into first-strand cDNA at 42ºC for 1 hour using Oligo (dt) primer and Revert Aid First Strand cDNA Synthesis Kit (Fermentas, USA).

Specific primer pairs for *5HTR2A*, *5HTR3A* (Metabion, Germany) that had been designed before in our group, were used to amplify the gene sequences[16]. The expression levels of the mRNAs were determined by using LightCycler® 480 SYBR Green I Master, with B-actin as an internal control. Real-time PCR was performed on a Corbett RotorGene 6000 Real-Time PCR Instrument (QIAGEN). The results of the qRT-PCR analysis were determined based on the threshold cycle (Ct), and the relative expression levels were calculated using the Linreg software, after normalizing the expression with the internal control gene

Flow cytometry

In the early stage of apoptosis, changes occur at the cell surface. For instance, phosphatidylserine (PS) from the inner part of the plasma membrane is translocated to the outer layer in which PS becomes exposed to the external surface of the cell. Quantitative analysis of PS on the outer leaflet of the apoptotic cell membrane was performed by Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide kit (Roche) according to the manufacturer's protocol. Briefly, cells (5×10^5 cells) were seeded in a 6-well plate and treated with tropisetron and ketanserin (10, 25, 50 and 100 $\mu moll^{-1}$), then incubated for 72 h. Afterward, cultured cells were harvested by trypsinization, and cells were washed twice with phosphate-buffered saline (PBS) and resuspended in Annexin V-binding buffer. The cell suspension was then incubated with 5 µl of Annexin V (AV)-FITC and incubated for 10 min at 4˚C in the dark. After adding 10 µl of propidium iodide (PI) and incubating for another 10 min at 4˚C in the dark, the cells were read by a FACS can flow cytometer (BD Biosciences, San Jose, CA, USA). The fraction of cell population in different quadrants, representing living (AV^-PI^-), early apoptotic (AV^+PI^-), late apoptotic or necrotic cells (AV^+PI^+; the latter can additionally have the AV^-PI^+ phenotype). The data was analyzed by using quadrant statistics.

Immunofluorescence staining

Briefly, QU-DB, A549, and HFLFcells (2×10^{5}) were seeded on a coverslip in a 6-well plate, incubated with tropisetron or ketanserin at their appropriate amount of two drugs. Coverslips were removed from the plate and washed with 1x PBS buffer and treated with 10 µl/ml of Acridine orange (10 mg/ml) and Ethidium bromide (10 mg/ml). After incubation for 5 min, the unbound dye was removed using 1x PBS buffer. The cells were examined under a fluorescence microscope (Germany) and representative fields were captured at 40x magnification.

Acridine orange permeates all cells and makes the nuclei appear green. Ethidium bromide is only taken up by cells when cytoplasmic membrane integrity is lost and stains the nucleus red. Ethidium bromide also dominates over Acridine orange. Thus, live cells showed a normal green nucleus; early apoptotic cells had yellow color nucleus with condensed or fragmented chromatin; late apoptotic cells exposed condensed and fragmented red chromatin; cells that have died from direct necrosis had a structurally normal orange nucleus. At least, they were observed and photographed by an invert immunofluorescence microscope.

Statistical analysis

Statistical correlation of data was checked for significance by ANOVA and Student's t-test. P>0.05 was considered to indicate a statistically significant variation between controls and treated cells at different concentrations in MTT assay, mixed dye staining test, and flow cytometry. The experiments were performed in triplicate.

Results

Tropisetron and ketanserin suppress the growth of QUDB and A549 cells

Our results suggest that tropisetron and ketanserin induce apoptosis in QUDB and A549 cells (*Figure 2).* We subsequently, used MTT assay to determine the concentration of administration, accurately. First, by MTT assay, we confirmed that tropisetron and ketanserin suppress the growth of QUDB and A549 in a dose-dependent manner and cause significant changes at a concentration of 50 and 100 µmoll^{-1}in both NSCLC cell lines.

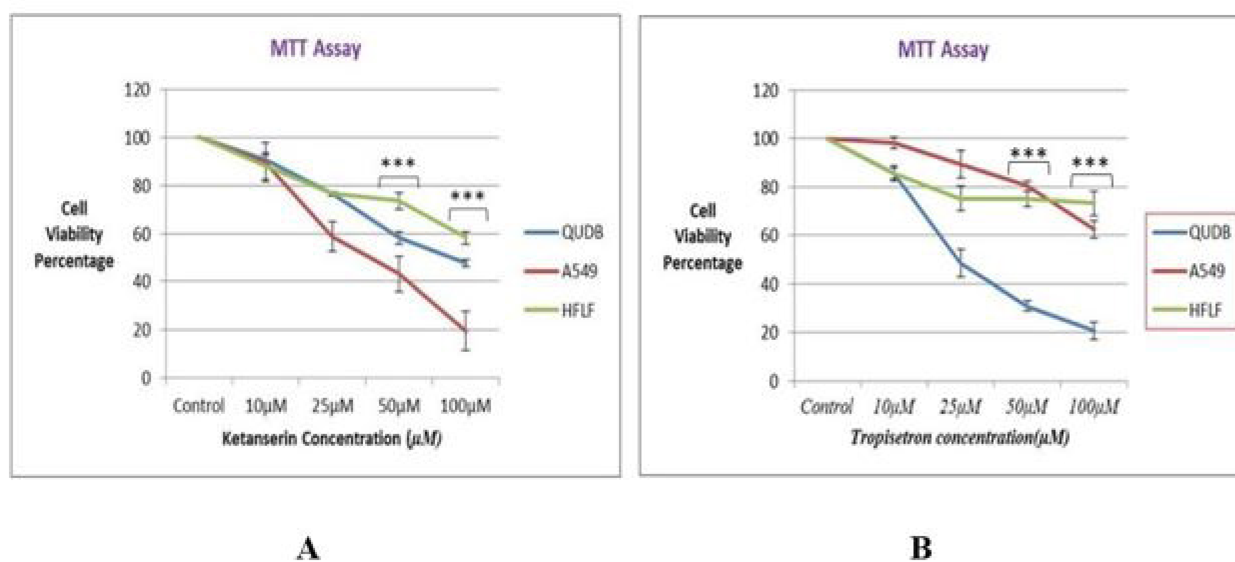

Figure 2: Cell viability by the MTT assay

Cell viability of control or treated **QUDB, A549, and HFLF** was assessed 48 h post-treated by the MTT assay (n = 3. ***p < 0.001). A: Detection of Cell viability in QUDB, A549, and HFLF cell lines after treatment with ketanserin. B: Detection of Cell viability in QUDB, A549, and HFLF cell lines after treatment with tropisetron

Induction of lung cancer cells apoptosis by treatment with ketanserin and tropisetron

By Flow cytometry analysis, we demonstrated that treatment with ketanserin (100 $\mu moll^{-1}$in QUDB and 50 $\mu moll^{-1}$ in A549) and tropisetron (100 $\mu moll^{-1}$in both NSCLC cell lines) significantly inhibited the proliferation of both human lung cancer cells and induced apoptosis in them (*Figure 3*). *But* there were considerable numbers of apoptotic cells at each concentration after treatment with ketanserin.

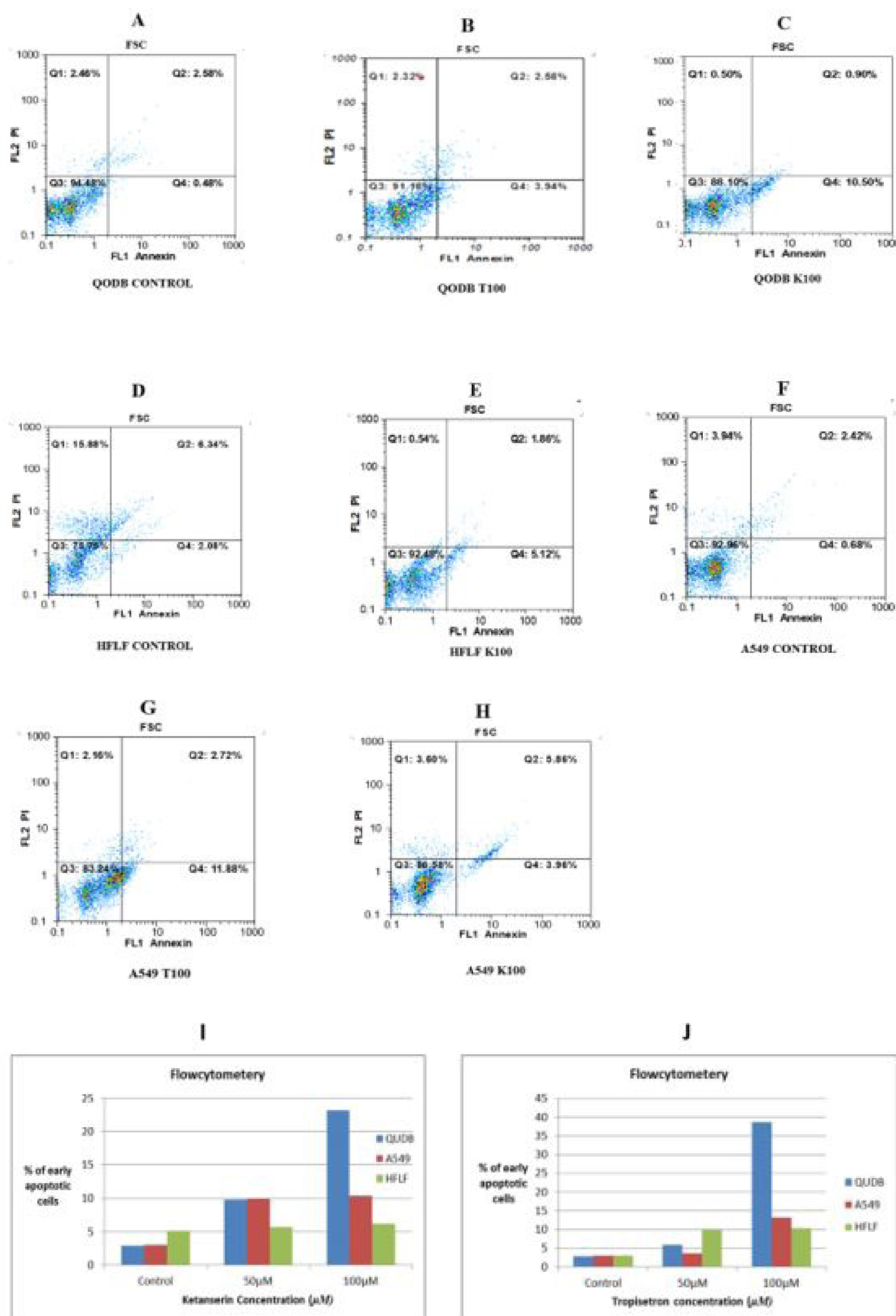

Figure 3: Detection of apoptosis and necrosis in lung cancer cell lines QUDB, A549, and HFLF after treatment with tropisetron and ketanserin by flow cytometry

Apoptotic cells in the control of QUDB cell lines were revealed by the annexin-V-FITC (FL1; displayed on the x-axis) propidium iodide (FL2; y-axis) staining and subsequent flow cytometry analysis. Q1: Percentage of necrotic cells, Q2: Percentage of late-phase apoptotic cells, Q3: Percentage of viable cells, Q4: percentage of early phase apoptotic cells. Figure 3A-3H represents the output results of *FloMax* software A-C) Dispersion graph of flow cytometric analysis in QUDB cells and after treatment with100 µM concentrations of tropisetron and ketanserin. D-E) Dispersion graph of flow cytometric analysis in HFLF cells and after treatment with 100 µM concentration of ketanserin. F-H) Dispersion graph of flow cytometric analysis in A549 cells and after treatment with 100 µM concentration of tropisetron and ketanserin. Figure 3I and Figure 3J represent the comparison percentage of early apoptotic cells treated with different doses of tropisetron and ketanserin.

Gene Expression Study of receptors

HTR2A and HTR3A were expressed by QUDB, A549 and HFLF cell lines. There is no significant difference in gene expression of the receptors before and after cell lines drug treatment.

Morphological features of lung apoptosis in cell lines after treatment with tropisetron and ketanserin

To elucidate the apoptosis induced by treatment with tropisetron and ketanserin in QUDB, A549, and HFLF cells, a simple method based on microscopic observations of cells stained with Acridine orange/Ethidium bromide (AO/EB) was performed. AO/EB staining revealed a uniform green nucleus in all cells that were not exposed to tropisetron and ketanserin. However, morphological features of apoptosis were observed in these cell lines after treatment with tropisetron and ketanserin for 48 h. Early apoptotic features including chromatin condensation, membrane blebbing, fragmented nuclei, large size cytoplasmic and membrane vacuoles, together with a complete loss of membrane integrity, were prominently observed in two NSCLC (QUDB and A549) cell lines. Yellow fluorescence was revealed as early apoptotic cells, whereas red fluorescence showed late apoptosis. They were predominantly observed in tropisetron and ketanserin-treated QUDB and A549 cells. Necrosis (characterized by a structurally normal orange nucleus) was also observed in these cell lines after 48 h treatment of tropisetron and ketanserin (*Figure 4, Figure 5*). Furthermore, expression analysis indicated that *5HTR2A* and *5HTR3A* genes have been expressed in two human NSCLC (QUDB and A549) and HFLF cell lines.

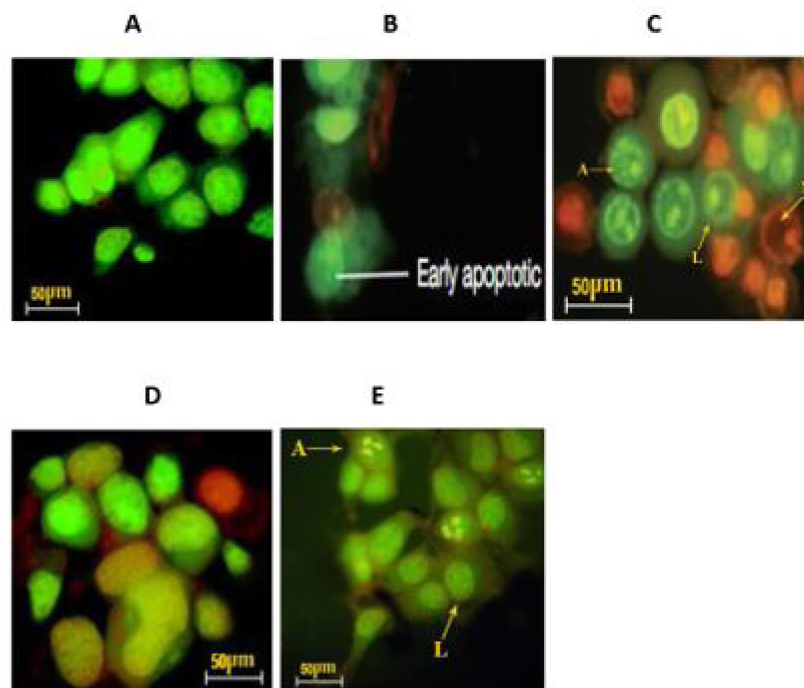

Figure 4: Detection of apoptosis in lung cancer cell lines QUDB after treatment with tropisetron and ketanserin by immunofluorescence staining

Morphological changes in treated QUDB cell compared to control cells (untreated), assessed by acridine orange and ethidium bromide staining and immunofluorescence microscopy. A: control cells that have a completely green nucleus, B: QUDB Cell treated with 50 µmoll-1 tropisetron, C: QUDB Cell treated with 100 µmoll-1 tropisetron , which is the most apoptotic cells, D: QUDB Cell treated with 50 µmoll-1 ketanserin, E: QUDB Cell treated with 100 µmoll-1 ketanserin , which again show the highest percentage of apoptotic cells.(L: Living cells, A: Apoptotic cells; cell membrane blabbing, N: Apoptotic cells; chromatin condensation)

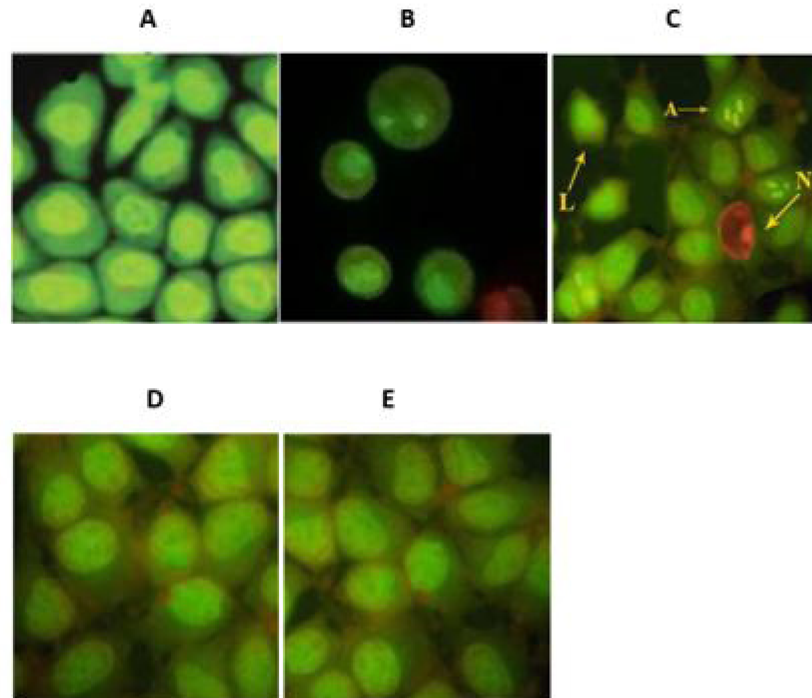

Figure 5: Detection of apoptosis in lung cancer cell lines A549after treatment with tropisetron and ketanserin by immunofluorescence staining

Morphological changes in treated A549 cell compared to control cells (untreated), assessed by acridine orange and ethidium bromide staining and immunofluorescence microscopy. A: control cells that have a completely green nucleus, B: A549 Cell treated with 50 µmoll-1 tropisetron, C: A549 Cell treated with 100 µmoll-1 tropisetron , which is the most apoptotic cells, D: A549 Cell treated with 50 µmoll-1 ketanserin, E: A549 Cell treated with 100 µmoll-1 ketanserin , which again show the highest percentage of apoptotic cells.(L: Living cells, A: Apoptotic cells; cell membrane blabbing, N: Apoptotic cells; chromatin condensation)

Discussion

Our present results revealed that tropisetron and ketanserin probably act as tumor suppressors in human cancers. Our data implicated combined treatment with these two drugs and chemotherapeutic drugs might effectively enhance chemotherapy-induced cytotoxicity in NSCLC cancer cells, which had tremendous clinical significance. We speculated that tropisetron and ketanserin mainly play key roles in apoptosis of NSCLC cancer cells.

Serotonin is a neurotransmitter, which is known as a growth factor for several types of non -tumoral cells and exhibits a growth stimulatory effect on a variety of cell types, including bovine placental cells, and takes part in cell proliferation in different tumors [19].

TPH (Tryptophan hydroxylase) and AADC (Aromatic L-amino acid decarboxylase) are two enzymes that are involved in the synthesis of serotonin from tryptophan. AADC is extremely expressed in human small cell lung cancer cells tissues. Studies show that Serotonin plays a role in the growth of several cancers such as colorectal, hepatocellular, prostate, breast, and small cell lung cancer cells through its receptors (1A, 1B, 2A, and 2B) [20].

5HTR2A is expressed in several tissues and has a variety of functions, including atrial fibroblast proliferation via mitogen-activated protein kinase (MAPK) pathway and migration of aortic muscle cells by activation of PLC and PKC pathways[10]. 5HTR3A affects the potassium voltage-gated channel, permits actions that are sensitive to Na^{+}, and plays important role in the regulation of Na^{+} /K^{+} movement in some immune cells [12,13]. According to the Kegg pathway databases, both HTR2A and ketanserin, in addition to the neuroactive ligand-receptor interaction pathway and serotonergic synapse, also play a role in the calcium signaling pathway. The calcium signaling pathway is one of the known pathways in lung cancer and increases tumor proliferation [21]. (Figure 6)

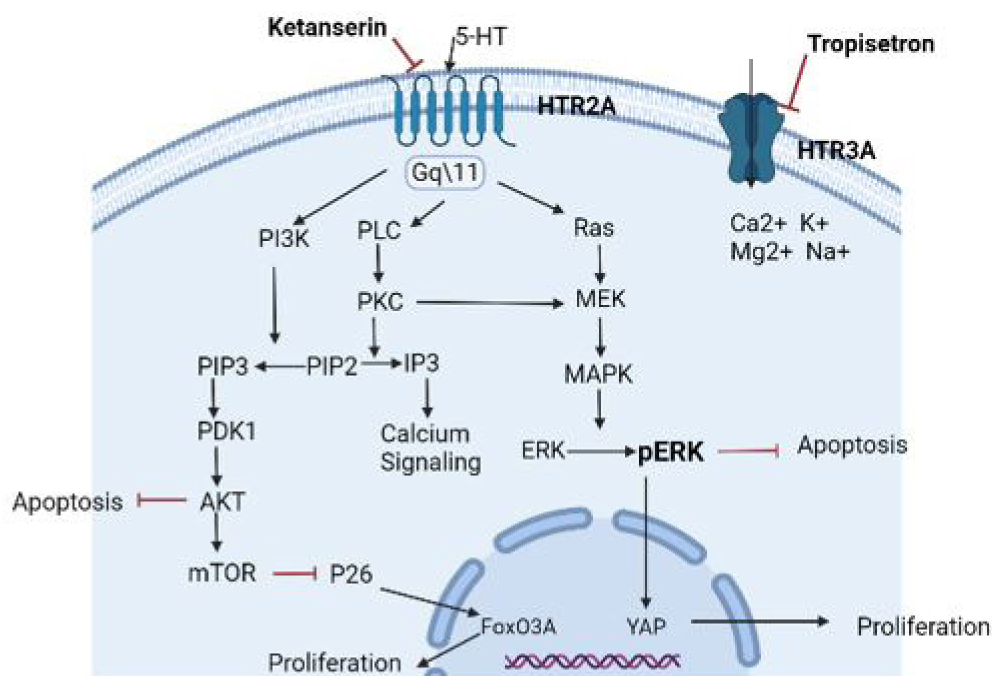

Figure 6: Schematic illustration of the role of HTR_{2A} signaling pathway on cancer. Serotonin signaling promotes tumor proliferation and inhibits apoptosis of tumor cells via HTR2A and activates MAPK and PI3K/Akt signaling. Ketanserin as the antagonist of HTR2A can block this pathway

Role of MAPK and Nrf2 pathways in ketanserin-elicitted attenuation of cigarette smoke-induced (CSM)=IL-8 production in human bronchial epithelial cells. They demonstrated that CSM-induced IL=8 was mediated by 5HTR2A, via activation of p38, ERK1/2 MAPK, and Nrf2 signaling pathways. Their results provide evidence for a novel antioxidative and anti-inflammatory role of ketanserin in CSM-induced airway inflammation [22]. Inhibiting the 5HT3 receptors with antagonists, such as ondansetron and tropisetron, disrupts TNF-α and IL-1β production, suggesting that these receptors may activate the p38/MAPK pathway [23, 24]. In vitro and Immunohistochemical assays on clinical samples revealed HTR3A increased proliferation through ERK1/2 phosphorylation in lung adenocarcinoma cells. ERK1/2 phosphorylation was defective in tropisetron-treated cells as a 5-HT3 receptor antagonist [25].

There is considerable evidence in the literature that the MAP kinases, the classic Erk1 and Erk2 (p42/44), and the related stress-activated kinases, Jnk and p38 MAP kinase, which have been implicated as key regulators of cell proliferation, can be activated in response to hypoxic stress [26].

A high dose of 5HT increased the growth of small cell lung cancer cells grafted in athymic nude mice whereas a low dose inhibited tumor growth. This observation led to the hypothesis that high doses of 5HT exert a direct mitogenic effect on tumor cells whereas low doses of 5HT reduce tumor growth by decreasing the oxygen tension and blood supply to the tumors [27].

Many studies which used serotonin ligands in therapeutics are mostly targeting the 5HTR2A and 5HTR3A, but to date, the alteration of serotonin receptors expression in cancer disease has not been explored sufficiently [28, 29].

In the previous work, we demonstrated the presence of these two serotonin receptors (5HTR2A and 5HTR3A) on PBMC of lung cancer patients and the control group. Our results showed that both receptors overexpressed in lung cancer patients compared to the control group [16].

Study Showed 5HTR3A is one of the fifteen differentially expressed immune-related genes (DEIRGs) identified from analysis databases for the evaluation of prognostic factors to forecast the survival of patients with NSCLC. In this research reported mRNA expression level HTR3a was significantly highly expressed in NSCLC tissues than in normal tissues [30].

In the recent study, our results showed that tropisetron and ketanserin suppress the growth of QUDB and A549 cells in a dose-dependent manner and they cause significant changes at concentrations of 50-100 $\mu moll^{-1}$ tropisetron and in 100 $\mu moll^{-1}$ ketanserin.

5-HT exerts a positive growth effect on MCF-7 cells, through the 5HTR2A, which is fully expressed in this cell line [14]. The present study shows incubation of Human breast cans cer cell lineages MCF-7 with 10 μM 5-HT promoted cell growth rate, an effect that was prevented by ketanserin as the 5-HTR2A antagonist. Their results revealed that serotonin interferes with breast cancer cells' proliferation and metabolism through 5-HTR2A by Jak1/STAT3 and adenylyl cyclase/PKA signaling pathways [31].

5HTR1B and *5HTR2A* are not expressed in either pHMEC (primary Human Mammary Epithelial Cells) or established breast cancer cell lines, which implies that expression in tumor specimens represents the presence of stromal or vascular elements, which typically express *5HTR1B* and *5HTR2A* in smooth muscle cells [32].

Immunohistochemical analysis to examine the expression of serotonin (5HT) receptor subtypes 1A, 1B, 2B, and 4 in a tissue microarray containing tumor specimens from patients with breast cancer Shows that all four serotonin receptors exhibited different expression patterns in breast cancer specimens [12].

5HTR2A and *5HTR3A* on PBMC were overexpressed in breast cancer patients compared to the control group and *5HTR2A* and *5HTR3A* were expressed in MCF-7 breast cani cer cell lines [33, 34]. Moreover, we found that tropisetron and ketanserin inhibited lung cancer cell survival and promoted them to apoptosis.

5-HT2A receptor antagonists impede hepatic stellate cell activation and facilitate apoptosis. They declared that serotonin plays an important role in the pathogenesis of hepatic inflammation and fibrosis, and therefore may represent a novel target for the prevention and treatment of hepatic fibrosis [35].

5-HT levels and expression of tryptophan hydroxylase 1 (TPH1) were significantly upregulated in colorectal tumor tissues, colorectal cancer mouse models, and colorectal cancer cell lines compared with normal colorectal tissues or epithelial cell lines.5-HT enhanced NLRP3 inflammasome activation in THP-1 cells and immortalized bone marrow-derived macrophages (iBMDM) via HTR3A. HTR3A antagonist tropisetron reduce tumor progression in colorectal cancer mouse model [36].

Clonogenic survival of colon carcinoma HCT116 cells treated with 5-HT and the selective 5-HTR antagonists ketanserin (5-HT2A) and ondansetron (5-HT3) after exposure to ionizing radiation and irradiated cell-conditioned medium (ICCM) was examined. Western blot results showed that the relative protein levels of these target receptors were similar. The findings display a role of serotonin in the increase of cell death following exposure to ionizing radiation in colon cancer cells [37].

5-HTR1 and 5-HTR2 appear to be the principal ree ceptors relevant to the human pulmonary arteries. Under most experimental conditions, stimulation of the 5HTR1 causes vasodilation, and the 5-HTR2A often mediates vasospasm in the puli monary circulation [12-14].

The addition of the 5HTR2A antagonist, ketanserin (0.1M), abrogated the enhanced growth response observed in the cells from chronically hypoxic animals induced with 5-HT and 0.2% serum for the hypoxic cells, which was not significantly different from normoxic cells. In contrast, neither the 5-HTR2B inhibitor SDZ SER 082 (1 M) nor the 5-HTR2B inhibitor RS102221 (1 M) had any effect on reducing the proliferation of cells in the presence of 5-HT and 0.2% serum for the hypoxic cells, which was significantly different, p0.05, from normoxic cells. [38].

Research on animal models shows the potential anti-neoplastic drug of tropisetron in lung cancer. Tumor sizes significantly reduced in tropisetron treated mice in comparison with control. Mice that received tropisetron showed significantly higher levels of IFN-γ, E-cadherin, and necrotic cells and lower levels of IL-4, and Ki-67 [39].

Taken together, our results indicated that tropisetron and ketanserin function as tumor suppressors in NSCLC cancer cells and suggested that these two drugs might be a potential therapeutic target for NSCLC. Tropisetron and ketanserin may impact apoptosis and tumor proliferation by affecting the downstream signalling pathways of receptors. In the face of significant progress in understanding the lung cancer signaling network, effective therapies remain limited and NSCLC often leads to relapse and death due to tumor heterogeneity, insufficient disruption of oncogenic pathways, metastasis, drug-induced toxicity, and drug resistance. In the future, new and supplementary approaches are required for the treatment of lung cancer. Therefore, identification of the molecular pathogenesis of lung cancer is essential.

Conflict of Interest

Conflict of Interest: None

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