

ORIGINAL ARTICLE

HTR3A is correlated with unfavorable histology and promotes proliferation through ERK phosphorylation in lung adenocarcinoma

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Abstract

Lung cancer is the leading cause of cancer death around the world. Adenocarcinoma is the most common histological type and has various histologic subtypes: lepidic, acinar, papillary, solid, and invasive mucinous adenocarcinoma. Histologic subtypes are related to invasiveness of tumors; eg, lepidic subtype is less invasive than acinar/papillary subtype. HTR3A is the main subunit of 5-hydroxytryptamine 3 (5-HT₃) receptors, which are the only ligand-gated ion channels in seven families of 5-HT receptors. Although 5-HT₃ receptor is expressed mainly throughout the central and peripheral nervous systems, some papers report the effect of 5-HT₃ receptors on tumor cells, including lung cancer. However, whether HTR3A correlates with histopathological findings such as the histologic subtypes or the distribution in an individual sample remains unclear. Immunohistochemically, we revealed that the higher expression level of HTR3A was detected in acinar, papillary, and solid adenocarcinoma than in adenocarcinoma in situ and lepidic adenocarcinoma; the former was a more aggressive subtype than the latter. We also showed the relationship between HTR3A expression and Ki-67 positivity, widely used as a proliferation marker. Moreover, we generated HTR3A-knockdown lung adenocarcinoma cells and showed that the HTR3A knockdown attenuated proliferation by ERK phosphorylation. Our results indicated that HTR3A expression was related to proliferation in lung adenocarcinoma, by means of both in vitro and immunohistochemical assays on clinical samples. We showed the therapeutic potential of a 5-HT₃ receptor antagonist, tropisetron, for the treatment of lung adenocarcinoma.

KEYWORDS

ERK phosphorylation, HTR3A, immunohistochemistry, lung adenocarcinoma, proliferation

Abbreviations: 5-HT, 5-hydroxytryptamine; AIS, adenocarcinoma in situ; EGFR, epidermal growth factor receptor; FFPE, formalin-fixed paraffin-embedded; H-score, histological score; HTR, 5-hydroxytryptamine receptor; LI, labeling index.

Tone and Tahara contributed equally.

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1 | INTRODUCTION

Lung cancer is the leading cause of cancer death around the world. Non-small-cell lung carcinoma accounts for 85% of lung cancer, and adenocarcinoma is the most common histological type.¹ Lung adenocarcinoma has various histologic subtypes, such as lepidic, acinar, papillary, micropapillary, solid, and invasive mucinous adenocarcinoma. Histologic subtypes are a prognostic factor; lepidic adenocarcinoma has good prognosis, acinar and papillary adenocarcinoma have intermediate prognosis, and micropapillary, solid, and invasive mucinous adenocarcinoma have poor prognosis.² Moreover, most lung adenocarcinomas show a mixture of different histologic patterns. The combination of histologic subtypes is important for prognosis.³

5-Hydroxytryptamine receptors can be divided into seven families. 5-HT₃ receptors are the only ligand-gated ion channels and all other 5-HT receptors are G protein-coupled receptors.⁴ 5-HT₃ receptors are composed of five subunits, HTR3A-E. It appears that only the HTR3A subunits form functional homopentameric channels. All other subunit subtypes must heteropentamerize with HTR3A subunits to form functional channels.⁵ Although 5-HT₃ receptor is expressed mainly throughout the central and peripheral nervous systems, some papers report the effect of 5-HT₃ receptors on tumor cells. Lee et al⁶ reported that 5-HT₃ receptor antagonists are related to antitumor mechanisms in lung cancer. Tang et al⁷ reported that downregulation of HTR3A expression exerts an anticancer activity against cell growth in colorectal carcinoma cells. However, whether HTR3A correlates with histopathological findings, such as the histologic subtypes or the distribution in an individual sample, remains unclear.

In this study, we found that HTR3A was preferentially expressed in lung adenocarcinoma with aggressive histological subtypes by RNA sequencing and revealed that HTR3A expression was related to histologic subtypes in lung adenocarcinoma. By immunohistochemical analysis of 63 lung adenocarcinoma cases, we showed the higher expression level of HTR3A was detected in acinar, papillary, and solid adenocarcinoma than in AIS and lepidic adenocarcinoma; the former was a more aggressive subtype than the latter. Moreover, we generated HTR3A-knockdown lung adenocarcinoma cells and showed that the HTR3A knockdown attenuated proliferation through ERK phosphorylation. We analyzed the therapeutic potential of a 5-HT₃ receptor antagonist, tropisetron, for the treatment of lung adenocarcinoma.

2 | MATERIALS AND METHODS

2.1 | Patients

We examined 93 cases undergoing surgery for adenocarcinoma of the lung at Osaka University Hospital from 2014 to 2018. Of 93 cases, 30 were subjected to RNA sequencing and 63 were

subjected to immunohistochemistry. No prior therapy was given in any case. Histologic subtypes were classified according to WHO criteria.⁸ Resected specimens were fixed in 10% formalin and processed for paraffin embedding. Specimens were stored at room temperature in a dark room. Specimens for evaluation were sectioned at 4 μ m thickness and stained with H&E. In 63 cases subjected to immunohistochemistry, we evaluated *EGFR* and *KRAS* mutations. We carried out immunohistochemistry using EGFR E746-A750del (1:100, number 2085; Cell Signaling Technology) and EGFR L858R (1:100, number 3197; Cell Signaling Technology) as primary Abs, based on the criteria shown in our previous paper.⁹ We undertook mutational analysis for *KRAS* mutation. In more than 95% of cases of *KRAS*-mutated lung cancer, *KRAS* missense mutations are found in codons 12 and 13 in exon 2.¹⁰ Therefore, we examined the sequences of *KRAS* exon 2. Genomic DNA from paraffin-embedded tissue was extracted and purified. Exon 2 of *KRAS* was amplified using PCR primers.¹¹ Sequence analysis was then carried out. The study was approved by the Ethical Review Board of the Graduate School of Medicine, Osaka University (No. 16293).

2.2 | RNA sequencing of FFPE samples

We divided 30 cases into two groups. The first group had 20 cases and was composed of relatively indolent histological subtypes, such as lepidic, acinar, and papillary (good-prognosis group). The second group had 10 cases and was composed of relatively aggressive subtypes, such as micropapillary and solid (poor-prognosis group). We compared the levels of gene expression profiles between two groups. We prepared five 10- μ m-thick FFPE sections from each case and subjected them to RNA sequencing analysis. Total RNA was extracted from the cells using the miRNeasy FFPE Kit (Qiagen), in accordance with the manufacturer's protocol. Next-generation sequencing library preparation was carried out using the TruSeq RNA Access Library Prep Kit (Illumina), in accordance with the manufacturer's instructions. Sequencing was undertaken on an Illumina HiSeq 2500 platform in 75-base single-end mode with Illumina Casava 1.8.2 software for base calling. Sequenced reads were mapped to the human reference genome sequence (hg19) using TopHat version 2.0.13, in combination with Bowtie 2 version 2.2.3 and SAMtools version 0.1.19. Fragments per kilobase of exon per million mapped fragments were calculated using Cufflinks version 2.2.1. Raw data were deposited in the NCBI Gene Expression Omnibus database (GSE148801).

2.3 | Immunohistochemistry and evaluation

We examined 63 cases of lung adenocarcinoma. The histological subtypes were AIS (n = 12), lepidic (n = 7), acinar (n = 6), papillary (n = 18), and solid adenocarcinoma (n = 20; Table 1).

TABLE 1 Correlation between expression of HTR3A and clinicopathologic features of patients with lung adenocarcinoma

	Patients, n	H-score of HTR3A ^a	P value
T stage			
pTis and pT1	50	48.32 ± 3.20	.027000
≥pT2	13	63.27 ± 7.91	
Lymphovascular invasion			
-	47	44.43 ± 3.21	.000030
+	16	71.91 ± 5.15	
N stage			
pN0	50	48.6 ± 3.27	.030000
pN1 and pN2	8	65.2 ± 5.53	
Recurrence			
-	50	46.75 ± 3.25	.038000
+	9	61.67 ± 6.31	
Disease-related death			
-	55	48.54 ± 3.14	.280000
+	4	55.75 ± 9.02	
Smoking status			
Brinkman index ^b <800	40	45.5 ± 3.76	.022000
Brinkman index ≥800	21	58.24 ± 4.41	
EGFR mutation			
-	51	52.88 ± 3.46	.170000
+	12	45.13 ± 6.93	
KRAS mutation			
-	61	51.58 ± 3.21	.380000
+	2	46 ± 6.36	

Abbreviations: H-score, histological score.

^aData are represented as mean ± SE.

^bBrinkman index was calculated as the number of cigarettes smoked per day multiplied by the duration of smoking (y).

Immunohistochemical staining was carried out using the Autostainer Link 48 (Agilent Technologies) according to the manufacturer's instructions. In terms of HTR3A, staining intensity (0, 1+, 2+, or 3+) was determined for each sample independently by two pathologists (ST and EM) and H-score was calculated using the following formula: (1 × [% tumor cells of 1+] + 2 × [% tumor cells of 2+] + 3 × [% tumor cells of 3+]). We also measured the Ki-67 LI, ie, the fraction of Ki-67-positive tumor cells.

2.4 | Cell lines

The human lung adenocarcinoma cell lines A549, HCC827, H1792, and H1650 were obtained from ATCC. A549 cells were cultured in DMEM, and HCC827, H1792, and H1650 were cultured in RPMI-1640. These media were supplemented with 10% FBS (Biosera) and cells were cultured in a humidified 5% CO₂ incubator at 37°C.

2.5 | Antibodies

An Ab against HTR3A (LS-B2042; LifeSpan BioSciences) was used for immunoblotting (1:1000) and immunohistochemistry (1:200) analyses. An Ab against Ki-67 (M7240; Agilent Technologies) was used for immunohistochemistry (1:100). Antibodies for immunoblotting against ERK1/2 (1:1000; no. 4695), and phospho-ERK1/2 (Thr202/Tyr204) (1:2000; no. 4370) were obtained from Cell Signaling Technology. Antibodies against β-actin (1:1000; 13E5, HRP conjugate, no. 5125) was also purchased from Cell Signaling Technology.

2.6 | Immunoblotting

Cells were lysed in buffer containing 10 mmol/L HEPES, 10 mmol/L KCl, 1 mmol/L EDTA, 1 mmol/L DTT, and 0.1% Nonidet P-40. Electrophoresis was carried out in 5%-20% gradient

TABLE 2 Genes identified by RNA sequencing

Gene symbol	Fold change ^a	Gene name	Entrez Gene
VTRNA1-1	35.268	Vault RNA 1-1	56664
HTR3A	9.004	5-Hydroxytryptamine (serotonin) receptor 3A, ionotropic	3359
CEACAM5	7.543	Carcinoembryonic antigen-related cell adhesion molecule 5	1048
MUC16	6.956	Mucin 16, cell surface associated	94025
SPINK1	6.048	Serine peptidase inhibitor, Kazal type 1	6690
FGG	5.785	Fibrinogen gamma chain	2266

^aFold change was obtained by dividing the average of the value of the poor-prognosis group by that of the good-prognosis group. Top 6 genes, with fold change of more than 5, are listed.

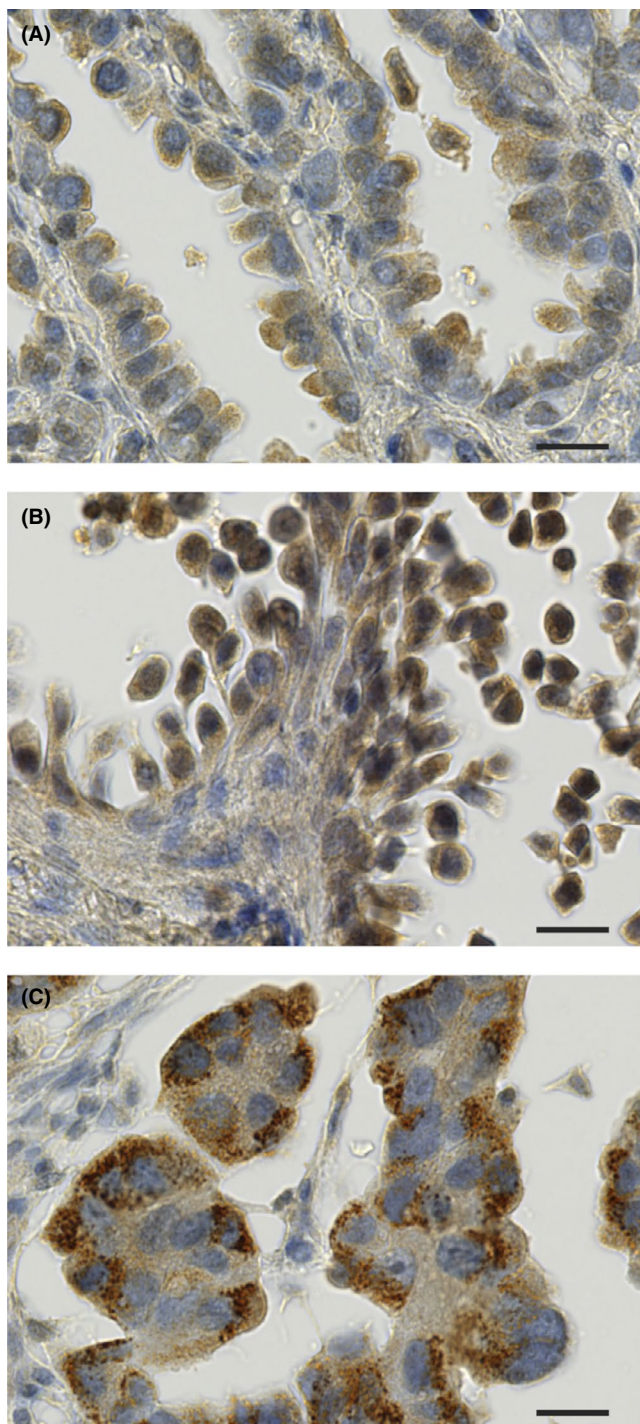


FIGURE 1 Varying immunohistochemical staining intensities representing HTR3A expression in lung adenocarcinoma. A-C, Representative immunohistochemistry images of lung adenocarcinoma are shown with intensity scores of weak (1+, A), moderate (2+, B) and strong (3+, C). Scale bar = 20 μ m (A-C)

SDS-polyacrylamide gels (ATTO), and proteins were transferred to PVDF membranes (Merck). We used the primary Ab and it was detected using an HRP-conjugated anti-rabbit IgG (H + L chain) (1:7000; MBL). We quantified the results using ImageJ version 1.52 (<https://imagej.nih.gov/ij/>).

2.7 | Generation of HTR3A-knockdown cells using siRNA-mediated silencing

A549 and H1792 cells (1×10^5) seeded into 6-well culture plates (Greiner Bio-One) were transfected with HTR3A-targeting siRNA (Silencer Select s7050, s7051, and s7052; Thermo Fisher Scientific) or nontargeting control siRNA (AM4611; Thermo Fisher Scientific) using Lipofectamine RNAiMAX Reagent (Thermo Fisher Scientific) at a final concentration of 50 nmol/L. Cells were subjected to the immunoblotting analysis and proliferation assays 72 and 24 hours after siRNA transfection, respectively.

2.8 | Proliferation assay

To evaluate proliferation, HTR3A-knockdown cells and control cells were seeded at 4×10^4 per well in 12-well culture plates and cultured for 3 days in a humidified 5% CO₂ incubator at 37°C. Cells were counted on days 2 and 3 using the Muse Cell Analyzer (Merck).

2.9 | Effect of 5-HT₃ receptor antagonist on lung adenocarcinoma cells

We evaluated the effect of a representative 5-HT₃ receptor antagonist, tropisetron, on A549 and H1792. Cells were seeded at 4×10^4 per well in 12-well plates and incubated for 24 hours. Next, 40 or 80 μ mol/L (A549) and 80 μ M (H1792) tropisetron hydrochloride (Tocris Bioscience) was added and cells were incubated for 72 hours (A549) and 96 hours (H1792). Cell proliferation was assayed by the Muse Cell Analyzer.

2.10 | Effect of 5-HT on lung adenocarcinoma cells

We evaluated the effect of 5-HT on A549 and H1792 cells. Cells were seeded at 3×10^3 (A549) and 2.5×10^3 (H1792) per well in 96-well plates and incubated for 3 hours. Next, 0, 0.3, 0.6, 1.25, or 2.5 μ mol/L 5-HT hydrochloride (Tokyo Chemical Industry) was added and cells were incubated for 48 hours (A549) and 24 hours (H1792). Cell proliferation was assayed by the Premix WST-1 Cell Proliferation Assay System (Takara) according to the manufacturer's instructions. The absorbance was measured at 450 and 690 nm using an SH-9000 laboratory microplate reader (Hitachi).

2.11 | Statistical analyses

Statistical analyses were undertaken using JMP Pro 14 software (SAS Institute). Results are shown as mean \pm SE. Differences in results were determined using Student's *t* test, the Wilcoxon signed-rank test, and ANOVA, followed by Dunnett's test. *P* values less than .05 were considered to indicate statistical significance.

3 | RESULTS

3.1 | High expression of HTR3A in the poor-prognosis group

To identify factors that were related to prognosis of lung adenocarcinoma, we analyzed the gene expression profiles of good-prognosis and poor-prognosis groups of lung adenocarcinoma using RNA sequencing. As shown in Table 2, HTR3A, the main subunit of 5-HT3 receptors, was more highly expressed in the poor-prognosis group than the good-prognosis group. This finding suggests that HTR3A expression is involved in the malignant potential of lung adenocarcinoma.

3.2 | Expression of HTR3A with immunohistochemical analysis

Lung adenocarcinoma cells expressed HTR3A not only in their plasma membrane, but also in the cytoplasm and nucleus. The expression level varied among cases. The typical staining patterns are shown in Figure 1: weak in Figure 1A, moderate in Figure 1B, and strong in Figure 1C. In cases with strong HTR3A expression, we found a dot-like staining pattern in the cytoplasm (Figure 1C).

3.3 | Association between HTR3A expression and clinicopathologic features

The clinicopathologic features of 63 cases of lung adenocarcinoma are shown in Table 1. Invasive size (T stage), lymphovascular invasion, lymph node metastasis, recurrence, and smoking were significantly correlated with HTR3A expression. Thus, high expression of HTR3A contributes to the aggressiveness of lung adenocarcinoma.

3.4 | Association between HTR3A expression and histologic subtypes

The associations between HTR3A expression level (H-score) and histologic subtypes were evaluated (Table 3). The expression of

HTR3A was weakly detected in AIS and lepidic adenocarcinoma (Figure 2A) and strongly in acinar, papillary, and solid adenocarcinoma (Figure 2B-D).

We examined the relationship between the expression of HTR3A and Ki-67, widely used as a proliferation marker. The rank order of Ki-67 LI was as follows: solid adenocarcinoma > acinar/papillary adenocarcinoma > AIS and lepidic adenocarcinoma (Table 3). We found a significant correlation between H-score of HTR3A and Ki-67 LI both in total cases and in acinar/papillary adenocarcinoma cases (Figure 2E).

Lung adenocarcinoma is often composed of a mixture of histologic subtypes. In fact, 22 cases showed the mixture of lepidic subtype and acinar/papillary subtype (Figure 3A; boxed area with solid line is lepidic subtype, boxed area with dotted line is papillary subtype). Then we compared HTR3A expression levels between an area of lepidic subtype (Figure 3B) and another of acinar/papillary subtype (Figure 3C) in an individual case. The H-score of the acinar/papillary area was significantly higher than that of the lepidic area (Figure 3D). In addition, we found that HTR3A expression in the micropapillary pattern tended to be higher than in surrounding tumor cells (Figure S1).

3.5 | HTR3A is expressed in lung adenocarcinoma cells

We assessed HTR3A expression in A549, HCC827, H1792, and H1650 cells. HTR3A expression was detected in all of these cell lines and was highest in A549, followed by H1792 (Figure 4A). Thus, we selected A549 and H1792 cells for further investigations.

3.6 | Involvement of HTR3A in proliferation of lung adenocarcinoma cells

We transfected A549 and H1792 cells with three individual siRNA duplexes specific for HTR3A (siHTR3A #1, #2, and #3), or a non-targeting control siRNA (siControl), and confirmed the decrease in HTR3A protein expression in HTR3A-knockdown cells (Figure 4B). We found that, in comparison with control cells, the proliferation of HTR3A-knockdown cells was attenuated (Figure 4C).

TABLE 3 Correlation between expression of HTR3A and histologic subtypes in patients with lung adenocarcinoma

Histologic subtype	Patients, n	H-score of HTR3A ^a	Ki-67 LI (%) ^a
AIS and lepidic adenocarcinoma	19	33.37 ± 4.38	3.68 ± 0.47
Acinar/ papillary adenocarcinoma	24	58.17 ± 4.69 ^b	11.23 ± 1.96 ^b
Solid adenocarcinoma	20	60.15 ± 5.05 ^b	20.75 ± 3.30 ^b

Abbreviations: AIS, adenocarcinoma in situ; H-score, histological score; LI, labeling index.

^aData are shown as mean ± SE.

^bP < .01 when compared with lepidic adenocarcinoma (Dunnett's test).

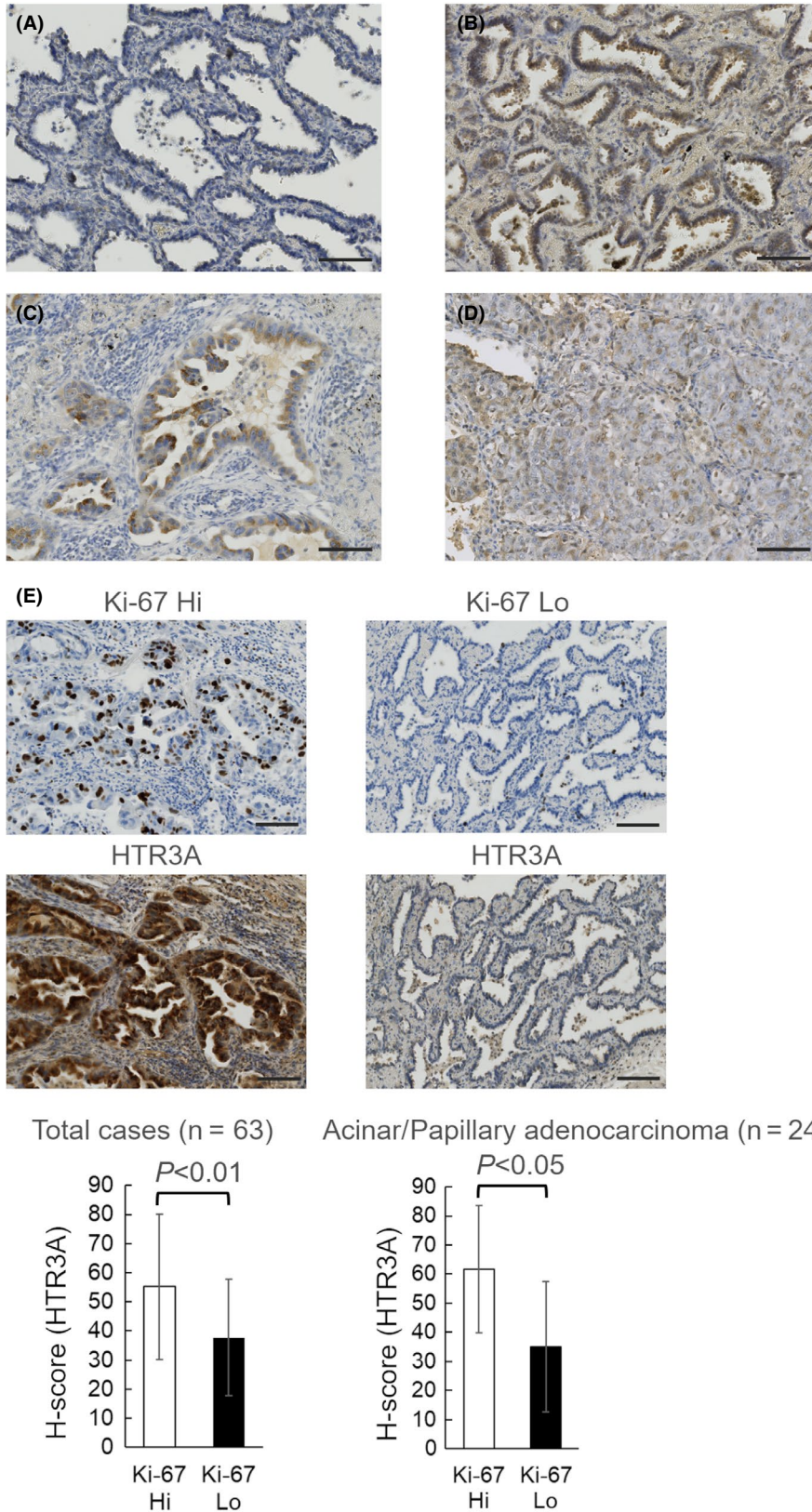


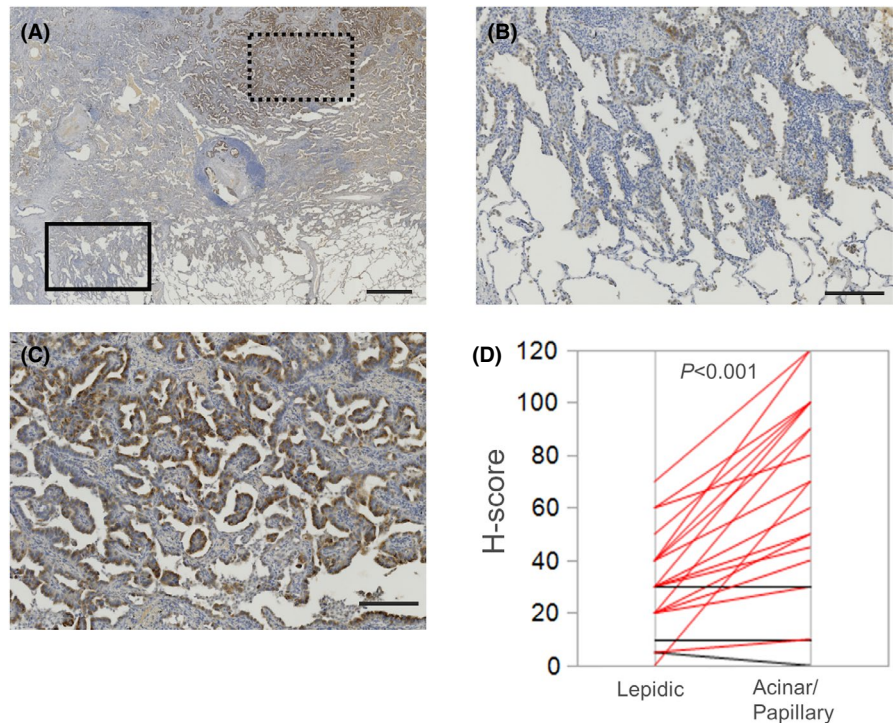
FIGURE 2 HTR3A expression in various histologic subtypes of lung adenocarcinoma and the relationship between HTR3A and Ki-67 expression. A-D, Typical images of immunohistochemistry of HTR3A in a case with lepidic adenocarcinoma (A), acinar adenocarcinoma (B), papillary adenocarcinoma (C), and solid adenocarcinoma (D), respectively. E, Typical images of the immunohistochemistry of HTR3A and Ki-67. Upper images and lower images indicate the same area. The left graph shows the difference in H-scores of HTR3A in total cases ($n = 63$) between high expression of Ki-67 ($\geq 5\%$) and low expression ($< 5\%$). The right graph shows the difference in acinar/papillary adenocarcinoma cases ($n = 24$) between high expression of Ki-67 ($\geq 5\%$) and low expression ($< 5\%$). Scale bar = 100 μm

3.7 | Involvement of HTR3A in activation of ERK-dependent signaling

Serine/threonine kinase ERK1/2 are members of the MAPK superfamily, which mediate cell proliferation. Activation of 5-HT

receptors on the cell surface initiates the ERK signaling cascade in the brain.¹² Therefore, we hypothesized that HTR3A promotes proliferation through activation of ERK1/2. Immunoblotting with phosphospecific Abs against ERK1/2 indicated that HTR3A-knockdown cells showed significantly lower levels of ERK1/2 phosphorylation

FIGURE 3 Varying HTR3A expression in an individual sample of lung adenocarcinoma with mixed histologic subtypes. A-C, Immunohistochemistry of HTR3A in a case with both lepidic and papillary areas (A). The boxed area with solid line (lepidic area) is enlarged in (B), and the area with dotted line (papillary area) is enlarged in (C). D, The comparison of H-scores of lepidic areas and acinar/papillary areas in 22 cases with both. Each line represents the H-score of the lepidic area and acinar/papillary area in each case, and the data show a significant increase in acinar/papillary area from 18 of 22 cases (red lines). Black line indicates no differential or decreased expression in other samples. We undertook the Wilcoxon signed-rank test and show the *P* value. Scale bar = 1 mm (A), 200 μ m (B, C)



(Figure 4D). This result indicates that HTR3A enhances proliferation through ERK1/2 phosphorylation. Moreover, we showed that tropisetron attenuated both proliferation and ERK1/2 phosphorylation (Figure 4E).

3.8 | Effect of 5-HT in proliferation and ERK-dependent signaling table

5-Hydroxytryptamine shows a growth stimulatory effect on several types of carcinoma.¹³ We confirmed the correlation between 5-HT and proliferation in lung adenocarcinoma cells. 5-Hydroxytryptamine promoted proliferation and ERK1/2 phosphorylation in A549 and H1792 cells (Figure 4F). This result indicates that the interactions between 5-HT and HTR3A promote proliferation through ERK1/2 phosphorylation.

4 | DISCUSSION

HTR3A is the main component of 5-HT₃ receptor. HTR3A or 5-HT₃ receptor is related to cancer progression in some types of cancer including lung carcinoma.^{6,7} To date, no studies have compared HTR3A expression in histologic subtypes of lung adenocarcinoma. In the present study, we identified HTR3A, which was predominantly expressed in the poor-prognosis group, and found that invasive size

(T stage), lymphovascular invasion, lymph node metastasis, recurrence, and smoking were significantly correlated with HTR3A expression. Thus, high expression of HTR3A contributes to the aggressiveness of lung adenocarcinoma. Lowery et al¹⁴ report that cigarette smoking increases the plasma 5-HT/catecholamine concentration. Our result indicated that, in lung cancer cells, smoking might be relevant to serotonin signaling.

Lung adenocarcinoma has various histologic subtypes. In the immunohistochemical study, the expression level of HTR3A in acinar, papillary, and solid adenocarcinoma was higher than AIS and lepidic adenocarcinoma. The expression level of Ki-67 was similar to HTR3A between histologic subtypes; the high expression of HTR3A was related to that of Ki-67. We considered that HTR3A was related to proliferation in lung adenocarcinoma. Moreover, we used 22 cases with both lepidic and acinar/papillary areas. In the case with both lepidic and acinar/papillary areas, the lepidic area indicates low proliferation and the acinar/papillary area indicates high proliferation. The expression of HTR3A in the acinar/papillary area was significantly higher than that of lepidic area. Additionally, we found that the tendency for HTR3A expression in the micropapillary pattern, which is extremely aggressive, was higher than that in surrounding tumor cells. These results indicated that the expression of HTR3A was high in the aggressive area in an individual sample.

As HTR3A is the main subunit of 5-HT₃ receptors, which are the ligand-gated ion channels, we first thought that HTR3A Ab was stained in the cell membrane. However, in immunohistochemical analysis, we often found that HTR3A Ab was stained in the cytoplasm and nucleus. According to the subcellular localization database (<https://compartments.jensenlab.org/Search>), HTR3A is surely expressed not only the cell membrane but also the cytoplasm and

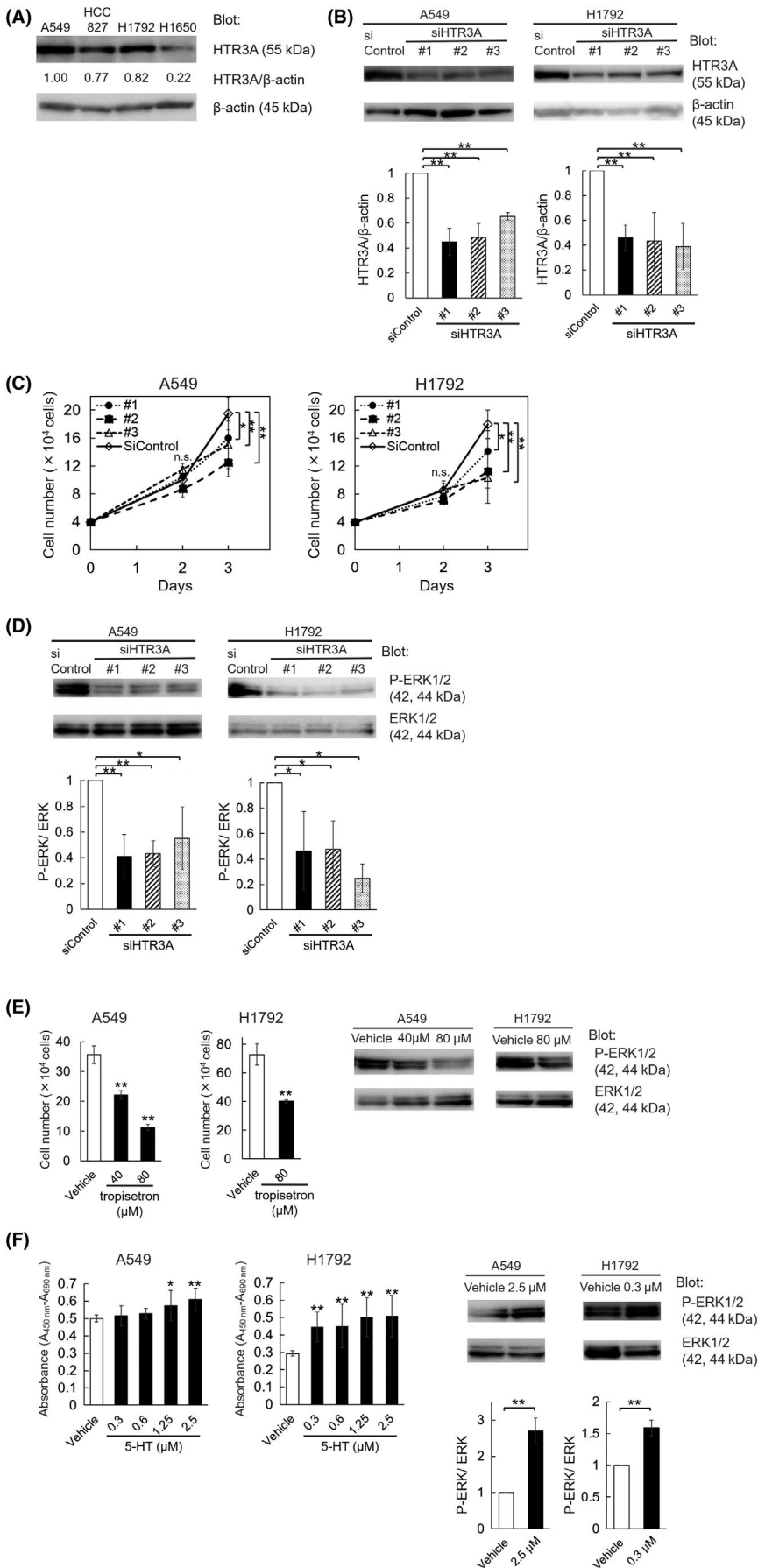


FIGURE 4 Relationship between HTR3A expression and the proliferation through ERK1/2 phosphorylation of lung adenocarcinoma cells. A, HTR3A protein levels in A549, HCC827, H1792, and H1650 cells. We quantified the results using ImageJ version 1.52. HTR3A/ β -actin quotient of A549 is expressed as 1. The relative quotient of HCC827, H1792, and H1650 is presented as the ratio to that of A549. B, Confirmation of HTR3A knockdown in siHTR3A #1, #2, and #3 A549 and H1792 cells. Equal protein loading is confirmed by quantifying β -actin (input control). We quantified HTR3A expression using ImageJ version 1.52. HTR3A/ β -actin quotient of control cells is expressed as 1. The relative quotient of HTR3A-knockdown cells is presented as the ratio to that of control cells. C, Proliferation assay by enumerating HTR3A-knockdown cells and control cells on days 2 and 3. D, ERK1/2 and P-ERK1/2 protein levels in HTR3A-knockdown cell and control cell lysates are determined by immunoblotting. We quantified the results using ImageJ version 1.52. P-ERK/ERK quotient of control cells is expressed as 1. The relative quotient of HTR3A-knockdown cells is presented as the ratio to that of control cells. E, Effect of tropisetron on the proliferation (left) and ERK1/2 phosphorylation (right) on lung adenocarcinoma cells. F, Effect of 5-HT on the proliferation (left) and ERK1/2 phosphorylation (right) on lung adenocarcinoma cells. We quantified the results using ImageJ version 1.52. P-ERK/ERK quotient of vehicle cells is expressed as 1. Data are shown as mean \pm SE. Asterisks indicate a significant difference between control cells and HTR3A-knockdown cells as analyzed by Dunnett's test (* P < .05, ** P < .01) (B-D). Asterisks indicate a significant difference between vehicle cells and 5-HT or tropisetron treated cells as analyzed by Student's t test (* P < .05, ** P < .01) (E, F). n.s., not significant

nucleus. Further investigation is necessary to investigate the function of HTR3A expressed in cytoplasm and nucleus.

Moreover, we showed that using lung adenocarcinoma cells, HTR3A played important roles in proliferation by means of functional assays using HTR3A-knockdown cells and tropisetron. We showed that ERK1/2 phosphorylation was defective in HTR3A-knockdown cells and tropisetron treated cells. Extracellular regulated kinase 1/2 is activated by phosphorylation at Thr202/Tyr204 and Thr185/Tyr187, which leads to the regulation of a large variety of processes including cell adhesion, cell cycle progression, cell migration, cell survival, differentiation, metabolism, proliferation, and transcription.¹⁵ The defective proliferation in HTR3A-knockdown cells and tropisetron treated cells could be due to the lack of ERK1/2 activation. HTR3A appears to regulate cancer proliferation through the ERK1/2-dependent pathway.

We showed the effect of 5-HT on proliferation and ERK1/2 phosphorylation in lung adenocarcinoma cells. This result supported that the interactions between 5-HT and HTR3A were essential for proliferation through ERK1/2 phosphorylation. However, it is not clear lung adenocarcinoma cells obtain 5-HT. Further research is required to investigate the underlying mechanisms.

Collectively, we showed that HTR3A was related to proliferation by means of both in vitro and immunohistochemical assays on clinical samples. This is, to our knowledge, the first report that HTR3A expression is related to the histologic subtype in lung adenocarcinoma. We found that HTR3A promoted proliferation through ERK1/2 phosphorylation in lung adenocarcinoma cells. We showed the therapeutic potential of a 5-HT₃ receptor antagonist, tropisetron, for the treatment of lung adenocarcinoma.

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DISCLOSURE

The authors have no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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