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Original Article

Melatonin combined with sorafenib synergistically inhibit the invasive ability through targeting metastasis-associated protein 2 expression in human renal cancer cells

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Abstract

Objectives: Renal cell carcinoma (RCC) was the most common and lethal urological malignancy with the dismal outcome when distant metastasis. Melatonin was known as a potential oncostatic agent against several types of malignancy and sorafenib had been considered as an agent to treat RCC, but the synergistic effects of melatonin and sorafenib on human RCC have not been elucidated. **Materials and Methods:** Human renal cancer cell lines (Caki-1 and ACHN) were treated with melatonin combined with sorafenib were detected the cell growth and cell cycle by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide assay and flow cytometry. The ability of cell migration/invasion was performed with *in vitro* migration and invasion assay. The proteins and mRNA expression of metastasis-associated protein 2 (MTA2) from the RCC cells were measured by quantitative reverse transcription-polymerase chain reaction and western blotting. Clinical significance of MTA2 in RCC tissues was analyzed from The Cancer Genome Atlas database by using TISIDB software. **Results:** Our results showed that melatonin combined with sorafenib, sorafenib or melatonin-treated alone did not induce the cytotoxic effects or cell cycle arrest in human RCC cells and HK2 cells. Additionally, cotreatment with melatonin and sorafenib synergistically reduced migration and invasion in human Caki-1 and ACHN cells through synergistically suppression of MTA2 expression. Bioinformatics analysis showed that MTA2 expression significantly correlated with overall survival ($P < 0.002$), tumor grade ($P < 0.001$) and tumor stage ($P < 0.001$) in human RCC. **Conclusion:** Our results demonstrated that concomitantly used melatonin and sorafenib could significantly reduce the abilities of migration and invasion of RCC cells through inhibiting MTA2. We considered that this novel promising combination strategy towards the treatment of RCC, but further studies are warranted.

Keywords: *Melatonin, Metastasis-associated protein 2, Renal cell carcinoma, Sorafenib*

Introduction

Renal cell carcinoma (RCC), which accounting for most of the new cases of kidney cancer, increased in incidence by 2.4% per year and with a limited median survival time and overall survival when metastasis [1,2]. Therapies to treat RCC including surgery and immunotherapy, but the efficacy was limited when RCC was diagnosed with distant metastasis [3]. Recently, inhibitors of tyrosine kinase and mTOR inhibitors to target against RCC had led to better survival but the outcome of advanced stage or distant metastasis of RCC remained to be improved [4].

Of these novel target therapies, sorafenib, which had been known as a multi-kinase inhibitor with anti-angiogenic and proapoptotic activities to improve the outcomes in hepatocellular carcinoma patients [5], and had been demonstrated to be effective against multiple cancer cells through inducing apoptosis in bladder cancer cells [6], synergistically with benofuroxann derivative in lung carcinoma [7], and with fusariotoxin enniatin B in cervical cancer [8]. In addition, sorafenib was proved to be effective in treating advanced RCC with improvement of progression-free

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survival [9] and showed comparable therapeutic efficacy to other target therapies in advanced RCC [10,11]. When treating RCC, sorafenib could being synergistically used with other agents, such as vascular endothelial growth factor inhibitor, to improve survival and quality of life of patients [12].

Mounting evidence had demonstrated that melatonin (N-acetyl-5-methoxytrypamine), a molecule with diverse functions including antioxidant, and-inflammation, pro-apoptosis and even anti-invasion and anti-migration [13], having beneficial application in the treatment of a myriad of tumors, including nasopharyngeal carcinoma, osteosarcoma, cervical cancer and RCC [14-17]. Furthermore, co-treatment with melatonin could enhance the abilities of other chemotherapeutic agents, including sorafenib, to suppress the carcinogenesis of hepatocellular carcinoma, pancreatic cancer, and acute myeloid leukemia through mediating the generation of reactive oxygen species (ROS), whose accumulation could lead to apoptosis [18-21].

As distant metastasis required dysregulation of cytoskeleton and cell-environment interactions to disseminate from the primary origin to distant sites and metastasis-associated protein 2 (MTA2), being a member of the metastasis tumor-associated family of transcriptional regulators, was known to play an important role in the process of tumorigenesis including distant metastasis [22-25]. Evidence had shown that overexpression of MTA2 was related to increased abilities of tumor invasion, metastasis, and poor prognosis in multiple human cancers [23-26]. We recently found that increased expression of MTA2 was associated with RCC grade and independently predicted poor overall survival; while the abilities of migration, invasion, and metastasis decreased markedly of RCC cells after downregulation of MTA2 [27].

Taken these studies together, it had been shown that individual use of melatonin and sorafenib alone could retard the progression of RCC and that concomitant use could inhibit several kinds of cancers, but to the best of our knowledge, the effects of concomitant use of melatonin and sorafenib on the tumorigenesis of RCC remained unclear. Therefore, we investigate the mechanisms of melatonin and sorafenib on the tumorigenesis as well as the role of modulating the expression of MTA2 of RCC cells.

Materials and methods

Chemical reagents and antibody

Melatonin (Product Number: 461326; purify ≥98%), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) (Product Number: M2128), dimethyl sulfoxide (DMSO, Product Number: D2650) and propidium iodide (PI) (Product Number: P4170) were purchased from Sigma (St. Louis, MO, USA). Sorafenib (BAY 43-9006) was purchased from Selleck Chemicals LLC (Houston, USA) and dissolved in DMSO solution at final concentration at 100 mM. Melatonin was dissolved in ddH_2O at stock concentration of 1 M and sorafenib was dissolved in DMSO solution at and stored at −20°C. Specific primary antibodies against MTA2 (sc-55566), β-actin (sc-69879), and second antibodies against goat anti-mouse IgG-HRP (sc-2302) and goat anti-rabbit IgG-HRP (sc-2357) antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Cell culture

Human renal cancer cells lines Caki-1 and ACHN were purchased from the American Type Culture Collection (ATCC, Manassas, USA). Both RCC cells were cultured in MEM medium. Normal proximal tubular HK2 cells were cultured in DMEM-F12 medium supplemented with 10% fetal bovine serum (FBS) (Hyclone, USA) and 1% penicillin/streptomycin followed by humidified atmosphere containing 5% CO_2 at 37°C. The cell culture passages number of Caki-1 was below 30, whereas for the ACHN cells not more than 40 passages number was used. Each cell culture passage was duplicate in further experiment.

Cell viability assessment

HK2 and RCC cells were treated with melatonin (4 mM), sorafenib (2.5 and 5 μM) or a combination of both drugs for 24 h by using the MTT assay. After treatment for 24 h, culture medium was removed and then cells were added with 0.5 mg/mL MTT for 4 h. Then isopropanol was added to dissolved the blue formazan crystals and was detected at 570 nm using an enzyme-linked immunosorbent assay plate reader.

Propidium iodide staining by flow cytometry analysis

Cell cycle assay was performed as described in a previous report [28]. RCC cell (5×10^5/well) were added with melatonin (4 mM), sorafenib (2.5 and 5 μM) or a combination of both drug for 24 h were detected with PI staining by flow cytometry, then fixed with 70% ice ethanol for 2 days. These fixed cells were stained with PI reagent for 20 min. Cell DNA content was measured through flow cytometry by using the Muse Cell Analyzer (Merck Millipore, Burlington, MA, USA).

In vitro migration and invasion assay

Using a 24-well modified Boyden chamber containing the 8-μm pore size of the membrane (Millipore) were determined with cell migrated ability by cell migration assay. Precoated 5% Matrigel matrix on membrane filter inserts for cell invasion assay. RCC cells (4×10^5/well) are seeded in the top of the insert in MEM medium (serum free), while medium containing 10% FBS are placed in the 24-well below and incubated for 16 h. The migrate cells were determined by using a microscope at × 200. The fourth fields were counted for each filter, and each sample was assayed in triplicate.

Western blotting

Cells were washed with phosphate-buffered saline and resuspended in lysis reagent with 1% NP40 and proteinase/phosphatase inhibitor cocktails (Roche Molecular Biochemicals). Then quantified the protein concentration was detected using the Bradford method. Total proteins (20 μg) were separated using 10%–12% sodium dodecyl-sulfate polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride (PVDF) membrane. Membranes incubated with 5% nonfat milk buffer (20 mM Tris-HCl, pH 7.6, 150 mM NaCl, and 0.1% Tween 20) for 1 h. Then, PVDF membranes were incubated with MTA2 and β-actin antibodies at 4°C overnight. Next days washed the membrane for twice and incubated with horseradish peroxidase-linked

anti-mouse or anti-rabbit antibodies. Antibody-bound protein bands were detected using highly sensitive Immunoblot Western Chemiluminescent HRP Substrate (Millipore, Billerica, MA, USA), and photographed with an LAS-4000 mini luminescent image analyzer.

Quantitative reverse transcription-polymerase chain reaction analysis

Total RNA was isolated using TRIzol reagents (Invitrogen, USA), and 0.5 μg RNA was used to synthesize cDNA with a GoScript™ Reverse Transcription Mix, Oligo (dT) (A2791, Promega, Wisconsin, USA). cDNA was performed with a GoTaq® Green Master Mix (M7122 Promega, Wisconsin, USA). Primer sequences for MTA2: Forward, 5'-GTTCTGGCAATACGGCGAGT-3' and reverse, 5'-CTTCGGCTGAATGCACAAAGA-3'. GAPDH: Forward, 5'-CATCATCCCTGCCTCTACTG-3' and reverse, 5'-GCCTGCTTCACCACCTTC-3'. GAPDH served as an internal control. All quantitative reverse transcription-polymerase chain reaction (qRT-PCR) reactions were run in triplicate and normalised by the internal control products of GAPDH and $2^{-\Delta CT}$ method was used to calculate the relative expression levels.

Bioinformatic databases

The TISIDB (http://cis.hku.hk/TISIDB), an online database containing RNA expression information, tumor stage, tumor grade and overall survival data from the The Cancer Genome Atlas (TCGA) database were used for analysis and assessment of expression of MTA2 of human RCC patients.

Statistical analysis

Each experiment was repeated at least three times. Results are presented as the mean ± standard error. Statistical analyses and comparisons were made using GraphPad Prism 4 (GraphPAD Software Inc. San Diego, CA, USA) and SPSS (version 18.0). Significance was defined at the $P < 0.05$ or 0.01 levels.

Results

Effect of melatonin combined with sorafenib on cell growth in human Renal cell carcinoma cells and in normal HK2 cells

The chemical structure of melatonin and sorafenib is shown in Figure 1a. In this study, using the concentration of melatonin and sorafenib as previously reports [29,30]. To test the potential antitumor effect of melatonin combined with sorafenib on human two RCC cell lines Caki-1, ACHN, normal proximal tubule epithelial cell lines HK2. Treatment with melatonin (4 mM) or melatonin (4 mM) combined with sorafenib (2.5 and 5 μM) for 24 h we showed that melatonin (4 mM), or melatonin (4 mM) combined with sorafenib (2.5 and 5 μM) were not affect cell growth rates of human HK2 cells [Figure 1b]. Likewise, not affect the cell growth of both Caki-1 and ACHN cells in these combine treatment [Figure 1c and d]. The IC50 value of melatonin and sorafenib treated in Caki-1 cells with an IC50 of 6.23 ± 1.2 mM and 7.62 ± 2.5 μM at 24 h; ACHN cells with an IC50 of 6.84 ± 1.5 mM and 8.59 ± 3.1 μM at 24 h. Based on these observations, melatonin combined with sorafenib at these concentrations did not exhibit significant cytotoxicity.

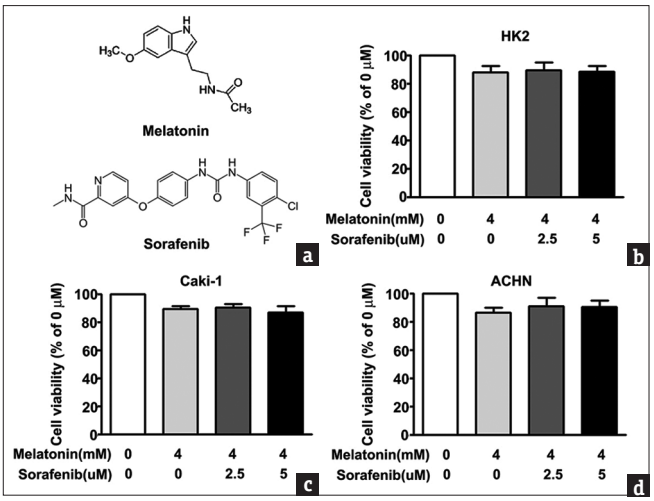

Figure 1: Effect of melatonin combined with sorafenib on cell growth in human renal cell carcinoma cells cells and normal cells. (a) Structure of melatonin and sorafenib. (B-D) Treatment with melatonin (4 mM), or both melatonin (4 mM) and sorafenib (2.5 and 5 μM) for 24 h was detected with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide assay. These data are presented as the mean ± standard deviation of three independent experiments

Melatonin combined with sorafenib not induce cell cycle arrest of human RCC cells

To examine the effect of melatonin combined with sorafenib on the human RCC cell cycle were detected with PI staining by flow cytometry. As shown in Figure 2, our results showed that treated only with melatonin (4 mM) in RCC cells, the cell cycle phase were not significantly changed. Similar results in melatonin (4 mM) combined with sorafenib (2.5 and 5 μM)-treated Caki-1 and ACHN cells These results suggest that the melatonin combined with sorafenib did not influence on cell growth and cell cycle distribution in both RCC cells.

Melatonin combined with sorafenib suppressed migration and invasion ability of renal cell carcinoma cells

From our results showed that melatonin, sorafenib or a combination of both drugs not influences on RCC cell viability. Next, to examine the effect of melatonin combined with sorafenib on cell migration and invasion in human Caki-1 and ACHN cells. We found that treatment with melatonin (4 mM) alone reduced cell migration and invasion in Caki-1 and ACHN cells [Figure 3]. Cotreatment of melatonin (4 mM) or sorafenib (2.5 or 5 μM) had a greater inhibitory effect on migrate and invasive abilities in Caki-1 and ACHN cells, compared with treatment with melatonin alone [Figure 3]. This results suggested that melatonin combined with sorafenib inhibit metastasis of human RCC cells.

Metastasis-associated protein 2 expression was required for migration/invasion inhibition by melatonin combined with sorafenib, and high expression of metastasis-associated protein 2 is closely associated with poor prognosis of renal cell carcinoma

Previous studies have shown that MTA2 involved in RCC cell migration and invasion [27]. Our results found that downregulation of MTA2 in melatonin-treated RCC cells, cotreatment of melatonin (4 mM) and sorafenib

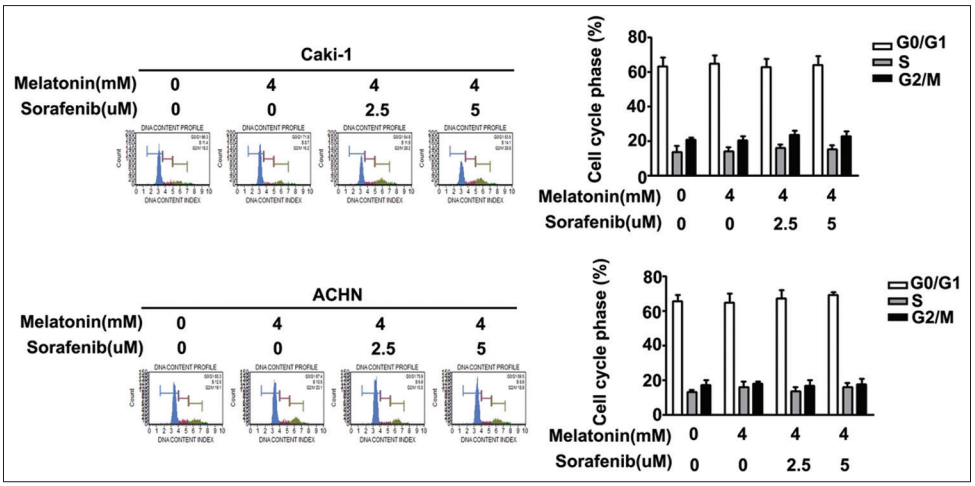

Figure 2: Effect of melatonin combined with sorafenib on regulation of cell cycle in human renal cell carcinoma cells cells. Treatment with melatonin (4 mM), or both melatonin (4 mM) and sorafenib (2.5 and 5 μM) for 24 h was analyzed with propidium iodide stining by flow cytometry. These data are presented as the mean ± standard deviation of three independent experiments

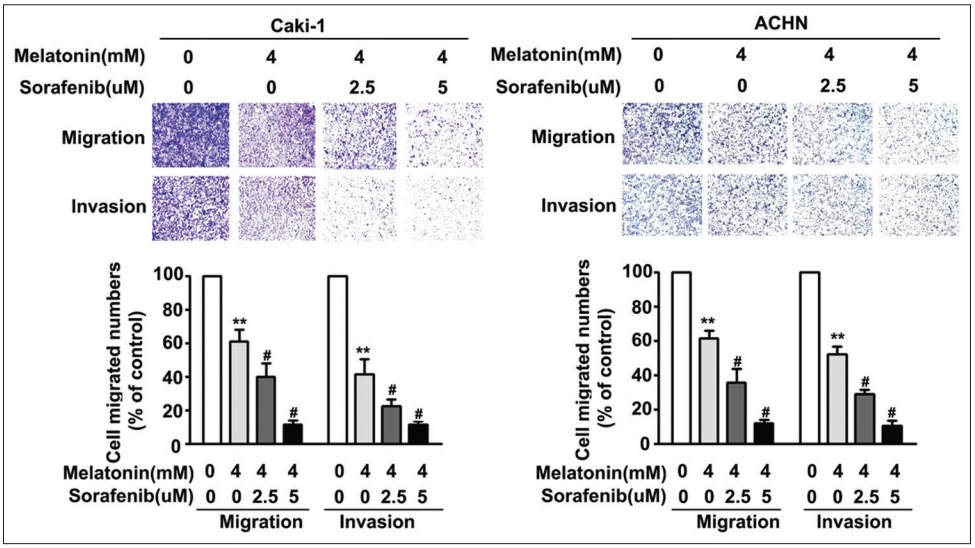

Figure 3: Impact of melatonin combined with sorafenib on cell migration and invasion in human renal cell carcinoma cells cells. Caki-1 and ACHN cells were treated with melatonin (4 mM), or both melatonin (4 mM) and Sorafenib (2.5 and 5 μM) for 24 h. The cell migrated numbers was detected by using cell migration and matrigel-based invasion assay. The numbers of migrate or invaded cell were counted, and shown in the histogram in the lower panel. These data are presented as the mean ± standard deviation of three independent experiments. $^{**}P < 0.01$ in comparison to the control cells. $^{\#}P < 0.01$ in comparison to the melatonin-treated cell

(2.5 or 5 μM) significantly inhibited the protein [Figure 4a, left panel: Caki-1; right panel: ACHN] and mRNA [Figure 4b, left panel: Caki-1; right panel: ACHN] expression of MTA2 by using western blotting and qRT-PCR assay. Next, we examined the expression of MTA2 in RCC tumor tissues from TCGA database using TISIDB software. Moreover, the MTA2 expression was significantly higher in the high risk group of RCC tissues and increased with increasing tumor grade [Figure 4c, $R = 0.173$, $P = 6.5 \times 10^{-5}$) and tumor stage [Figure 4d, $R = 0.161$, $P = 0.000198$] according to the analysis results from the TISIDB software. To further clarify whether MTA2 expression was correlated with the overall survival of patients with RCC by using Kaplan–Meier survival analyses, to find that high MTA2 expression in RCC patients had a significantly lower survival rate compared with low MTA2 expression in RCC patients [$P < 0.001$, Figure 4e]. These results demonstrated that MTA2 could be a prognostic marker for RCC patients and it's involved in regulation of RCC cell metastasis.

Discussion

In this study, we found that (1) enhanced expression of MTA2 was correlated with higher grade and poor survival of RCC patients; (2) melatonin alone or concomitant with sorafenib could inhibit RCC cells' invasive and migratory abilities with no cytotoxicity of RCC; (3) concomitant treatment of RCC with melatonin and sorafenib could inhibit the protein and mRNA expression of MTA2, which indicated poor prognosis when highly expressed of human RCC [Figure 5].

Over the past decades, the incidence of kidney cancer had increased steadily over the world due to improved imaging

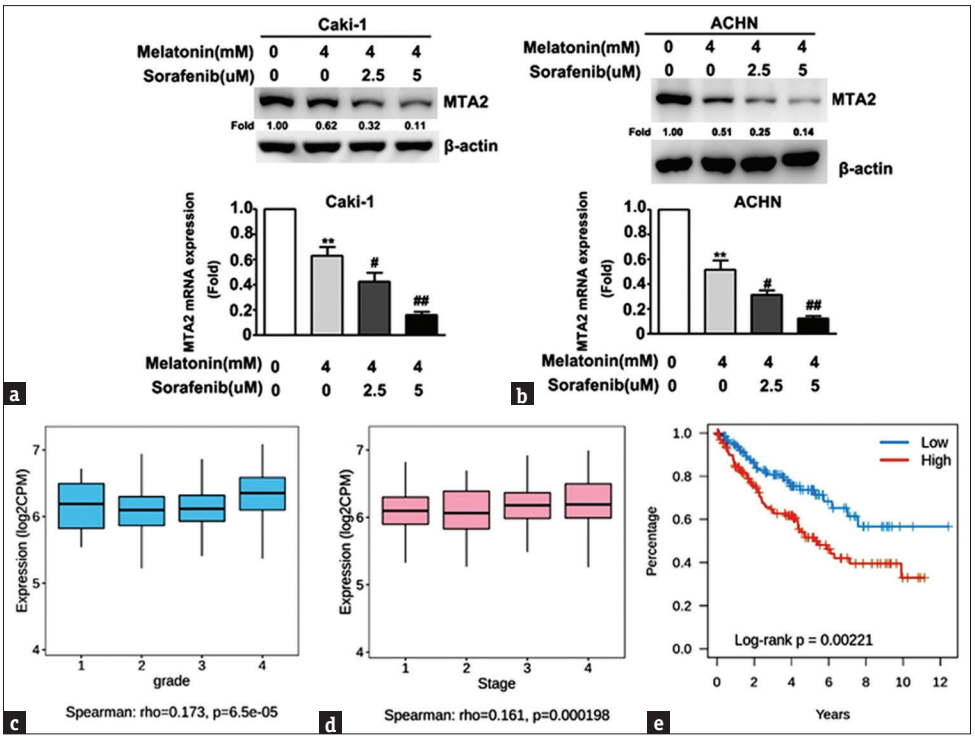

Figure 4: Effect of melatonin combined with sorafenib on metastasis-associated protein 2 expression in human renal cell carcinoma cells cells and clinical significance of metastasis-associated protein 2 of human renal cell carcinoma cells tissues. Caki-1 and ACHN cells were treated with melatonin (4 mM), or both melatonin (4 mM) and Sorafenib (2.5 and 5 μM) for 24 h, then extracted the total protein and quantification. The protein expression of metastasis-associated protein 2 were detected with western blotting, β-actin as the internal control. (a) The protein expression of metastasis-associated protein 2 were detected with western blotting, (b) The mRNA expression of metastasis-associated protein 2 was analyzed using quantitative reverse transcription-polymerase chain reaction assay. GAPDH as the internal control. (c) Tumor grade and (d) tumor stage for renal cell carcinoma cells patient were analyzed using TISIDB from The Cancer Genome Atlas database. (e) Overall survival for patients with renal cell carcinoma cells patients were measured using Kaplan–Meier analysis from The Cancer Genome Atlas database. These data are presented as the mean ± standard deviation of three independent experiments. $^{**}P < 0.01$ in comparison to the control cells. $^{\#}P < 0.01$ in comparison to the melatonin-treated cell

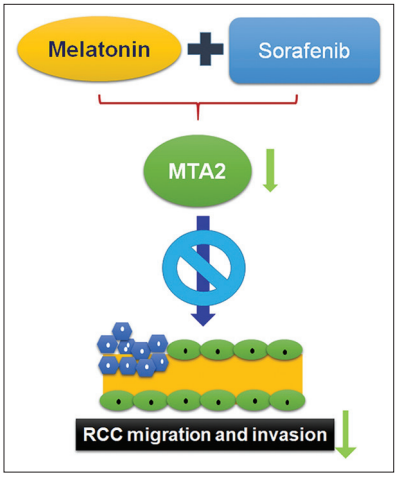

Figure 5: A graphic representation of melatonin combined with sorafenib inhibits the metastasis of human renal cell carcinoma cells by targeting metastasis-associated protein 2 expression

techniques, but the outcome was dismal when there was distant metastasis [31,32]. Recently, in addition to those traditional treatments including surgical and chemotherapy, regimen to treat metastatic RCC had included agents as sorafenib, sunitinib, and temsirolimus to target the signaling pathways of carcinogenesis of RCC [33]. As a novel cancer progression-related protein, MTA2 had been proved to inactivate p53 that leads to growth arrest and apoptosis of cancer cells [34] and linked with poor prognosis of several types of cancers [24-26,35]. Over-expression of MTA2 had been independently portended tumor invasion, metastasis, and poorer long-term survival in patients with esophageal squamous cells carcinoma [35]. Moreover, the expression of MTA2 was positively correlated with tumor invasion, lymph nodes metastasis in gastric cancer [24], which indicated its role of up-regulation to modulate the process of carcinogenesis. As the expression of MTA2 down-regulated, the abilities of invasion and migration of multiple cancers, including colorectal cancer [23], gastric cancer [24], lung cancer [25], and breast cancer [26] could be inhibited. Recently, we found that higher expression of MTA2 could mediate the expression of matrix metalloproteinase-9 (MMP-9) and was an independent prognostic factor for overall survival in RCC [27], which indicated that MTA2 over-expression could be a marker indicative of worse prognosis.

Sorafenib, an oral multi-tyrosine kinase inhibitor, which could affect the downstream activation of multiple signaling pathways, had been approved for the treatment of several types of cancers, including hepatocellular carcinoma, bladder cancer, and advanced RCC [5,6,36,37]. In addition, sorafenib was reported to decrease the viability of RCC cells by causing apoptotic cell death by activating transcription factor 4 pathways through the CCAAT/enhancer-binding

protein-homologous protein and p53 upregulated modulator of apoptosis [38]. Moreover, co-treatment of sorafenib with perifosine, an Akt and a PI3K inhibitor, or metformin could individually increase the chemosensitivity of sorafenib to induce apoptosis and inhibit the metastatic abilities of bladder cancer or hepatocellular carcinoma [6,39]. Combined treatment of benzofuroxan derivative and sorafenib showed stronger cytotoxicity and induced apoptosis [7]. We previously found that combination treatment with fisetin and sorafenib could exert greater cytotoxicity in cervical cancer through activating apoptosis with the results of decreasing tumor growth through the ERK signaling pathway than either agent used alone [40]. Taken together, we knew that sorafenib alone or in combination with other agents could enhance the effects of cytotoxicity against a myriad of cancer cells.

From the literature, in addition to its physiological roles as synchronizing circadian rhythms, antioxidant, and inflammation [13], melatonin had been known to protect against cancer by several processes, including enhancing the effects of chemotherapeutic agents by modulating the expression and phosphorylation of their targets or regulating cell death associated mechanisms such as apoptosis or autophagy [41,42]. There were studies showing the inhibitory abilities of melatonin on tumor growth and metastasis [14-17]. Melatonin could sensitize cisplatin, a kind of chemotherapy agent to induce caspase-9 dependent mitochondrial apoptosis through the JNK/Parkin/mitophagy pathway to treat cervical cancer [16]. By inhibiting CC motif chemokine ligand 24 gene expression, melatonin was demonstrated to suppress the abilities of invasion and migration via inhibiting the JNK pathway of osteosarcoma cells [15]. Of nasopharyngeal carcinoma, melatonin could inhibit metastasis by modulating the expression of MMP-9 through the transcription factor specificity protein-1 and the JNK/MAPK signaling pathway [14]. Furthermore, melatonin was shown to suppress the ability of motility of RCC by down-regulating expression of MMP-9 [17], which indicated its role to counteract tumor metastasis through modulating the remodeling of extracellular matrix. In this study, we similarly found that melatonin alone could inhibit the abilities of invasion and migration as well as the expression of MTA2 of RCC cells. To examine the role of MTA2 in the presence with melatonin and sorafenib-treated RCC cells, using the transfection of MTA2 plasmid in melatonin combined with sorafenib-treated RCC cells and clarify the metastatic role and molecular mechanism is need to further investigation.

Evidence had shown that the combination of melatonin with other agents such as thapsigargin or Kahweol could enhance the process of apoptosis of RCC cells through upregulation of CCAATenhancer-binding protein homologous protein expression or p53-upregulated modulator of apoptosis [43,44]. Furthermore, melatonin could augment the sorafenib-induced cytotoxicity as apoptosis and increased expression of ROS along with mitochondrial membrane depolarization of hepatocellular carcinoma [19,20]. Of pancreatic adenocarcinoma and acute myeloid leukemia, co-treatment of melatonin and sorafenib could synergistically induce apoptosis or enhance the accumulation of mitochondrial-mediated ROS with the results of enhancing oxidative stress and ultimately cancer cell death [18,21]. Take these studies together, it showed that co-treatment with melatonin and other chemotherapeutic agents including sorafenib could suppress the process of carcinogenesis. In this study, we additionally demonstrated that combined treatments of melatonin and sorafenib could markedly augment the inhibition of metastasis potential as well as the expression of MTA2 of RCC, which indicated concomitant use of melatonin and sorafenib could serve as a potential chemotherapeutic strategy to improve the clinical outcomes of RCC. Therefore, other important key molecules for melatonin combined with sorafenib regulate the transcription of MTA2 in RCC cell migration and invasion, which will be a focus in detailing the molecular mechanism and requires further investigation.

Metastatic RCC remained as an incurable disease in the vast majority of patients and given that the biology of RCC is complex with the results of multiple treatment decisions based on the many mechanisms that regulated tumor-cell metabolism, cell-cycle or immunological environments and that systemic therapies for advanced RCC were associated with a variety of toxicities, such as sorafenib with nephrotoxicity presented as hypertension, proteinuria or acute interstitial nephritis, novel strategy with the combination of immunomodulatory agents was considered due to favorable toxicity and durable responses [43,44]. In this study, although we demonstrated that melatonin and sorafenib could synergistically inhibit the metastasis potential of RCC through down-regulated MTA2 expression, the detailed signaling pathways to regulate the expression of MTA2 as well as the targets MTA2 acted on needed to be elucidated in future. Moreover, *in vivo* study should be conducted to demonstrate the beneficial synergistic effects of melatonin and sorafenib on RCC.

Conclusion

Taken together, our study indicated that a combination of melatonin and sorafenib exert better promising synergistic effects than either agent used alone against RCC, and this synergism is based on inhibiting the expression of MTA2. Thus, combined treatment with melatonin and sorafenib represents a novel therapeutic strategy for further clinical developments in RCC.

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Conflicts of interest

There are no conflicts of interest.

References

1. Palumbo C, Pecoraro A, Knipper S, Rosiello G, Luzzago S, Deuker M, et al. Contemporary age-adjusted incidence and mortality rates of renal cell carcinoma: Analysis according to gender, race, stage, grade, and histology. Eur Urol Focus 2021;7:644-52.
2. Ssaad AM, Gad MM, Al-Husseini MJ, Ruhban IA, Sonbol MB, Ho TH. Trends in renal-cell carcinoma incidence and mortality in the United

States in the last 2 decades: A SEER-based study. Clin Genitourin Cancer 2019;17:46-57.e5.

3. Volpe A, Cadeddu JA, Cestari A, Gill IS, Jewett MA, Joniau S, et al. Contemporary management of small renal masses. Eur Urol 2011;60:501-15.
4. Chiong E, Tay MH, Tan MH, Kumar S, Sim HG, Teh BT, et al. Management of kidney cancer in Asia: Resource-stratified guidelines from the Asian Oncology Summit 2012. Lancet Oncol 2012;13:e482-91.
5. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378-90.
6. Amantini C, Morelli MB, Santoni M, Soriani A, Cardinali C, Farfariello V, et al. Sorafenib induces cathepsin B-mediated apoptosis of bladder cancer cells by regulating the Akt/PTEN pathway. The Akt inhibitor, perifosine, enhances the sorafenib-induced cytotoxicity against bladder cancer cells. Oncoscience 2015;2:395-409.
7. Teixeira SF, Alexandre de Azevedo R, Salomon MA, Jorge SD, Levy D, Bydlowski SP, et al. Synergistic anti-tumor effects of the combination of a benzofuroxan derivate and sorafenib on NCI-H460 human large cell lung carcinoma cells. Biomed Pharmacother 2014;68:1015-22.
8. Dornetshuber-Fleiss R, Heilos D, Mohr T, Richter L, Süssmuth RD, Zlesak M, et al. The naturally born fusariotoxin enniatin B and sorafenib exert synergistic activity against cervical cancer *in vitro* and *in vivo*. Biochem Pharmacol 2015;93:318-31.
9. Kane RC, Farrell AT, Saber H, Tang S, Williams G, Jee JM, et al. Sorafenib for the treatment of advanced renal cell carcinoma. Clin Cancer Res 2006;12:7271-8.
10. Huang HF, Fan XR, Ji ZG. The effectiveness of sorafenib over other targeted agents in the second-line treatment of metastatic renal cell carcinoma: A meta-analysis. Pathol Oncol Res 2019;25:1497-503.
11. Zhang HL, Sheng XN, Li XS, Wang HK, Chi ZH, He ZS, et al. Sorafenib versus sunitinib as first-line treatment agents in Chinese patients with metastatic renal cell carcinoma: The largest multicenter retrospective analysis of survival and prognostic factors. BMC Cancer 2017;17:16.
12. Wang Q, Qin J, Liu X. Analysis of efficacy of sorafenib combined with vascular endothelial growth factor inhibitor on renal cell carcinoma. J BUON 2019;24:1638-43.
13. Reiter RJ, Rosales-Corral SA, Tan DX, Acuna-Castroviejo D, Qin L, Yang SF, et al. Melatonin, a full service anti-cancer agent: Inhibition of initiation, progression and metastasis. Int J Mol Sci 2017;18:E843.
14. Ho HY, Lin CW, Chien MH, Reiter RJ, Su SC, Hsieh YH, et al. Melatonin suppresses TPA-induced metastasis by downregulating matrix metalloproteinase-9 expression through JNK/SP-1 signaling in nasopharyngeal carcinoma. J Pineal Res 2016;61:479-92.
15. Lu KH, Su SC, Lin CW, Hsieh YH, Lin YC, Chien MH, et al. Melatonin attenuates osteosarcoma cell invasion by suppression of C-C motif chemokine ligand 24 through inhibition of the c-Jun N-terminal kinase pathway. J Pineal Res 2018;65:e12507.
16. Chen L, Liu L, Li Y, Gao J. Melatonin increases human cervical cancer HeLa cells apoptosis induced by cisplatin via inhibition of JNK/Parkin/mitophagy axis. *In Vitro* Cell Dev Biol Anim 2018;54:1-10.
17. Lin YW, Lee LM, Lee WJ, Chu CY, Tan P, Yang YC, et al. Melatonin inhibits MMP-9 transactivation and renal cell carcinoma metastasis by suppressing Akt-MAPKs pathway and NF-κB DNA-binding activity. J Pineal Res 2016;60:277-90.
18. Fang Z, Jung KH, Yan HH, Kim SJ, Rumman M, Park JH, et al. Melatonin synergizes with sorafenib to suppress pancreatic cancer via melatonin receptor and PDGFR-β/STAT3 pathway. Cell Physiol Biochem 2018;47:1751-68.
19. Lin S, Hoffmann K, Gao C, Petrulionis M, Herr I, Schemmer P. Melatonin promotes sorafenib-induced apoptosis through synergistic activation of JNK/c-jun pathway in human hepatocellular carcinoma. J Pineal Res 2017;62:e12398.
20. Prieto-Domínguez N, Ordóñez R, Fernández A, Méndez-Blanco C, Baulies A, Garcia-Ruiz C, et al. Melatonin-induced increase in sensitivity of human hepatocellular carcinoma cells to sorafenib is associated with reactive oxygen species production and mitophagy. J Pineal Res 2016;61:396-407.
21. Tian T, Li J, Li Y, Lu YX, Tang YL, Wang H, et al. Melatonin enhances sorafenib-induced cytotoxicity in FLT3-ITD acute myeloid leukemia cells by redox modification. Theranostics 2019;9:3768-79.
22. Zhu CJ, Zhang S, Liang Y, Li W. Elicitation of metastasis associated protein 2 expression in the phagocytosis by murine testicular Sertoli cells. Biochem Biophys Res Commun 2014;445:667-72.
23. Zhou J, Zhan S, Tan W, Cheng R, Gong H, Zhu Q. P300 binds to and acetylates MTA2 to promote colorectal cancer cells growth. Biochem Biophys Res Commun 2014;444:387-90.
24. Zhou C, Ji J, Cai Q, Shi M, Chen X, Yu Y, et al. MTA2 promotes gastric cancer cells invasion and is transcriptionally regulated by Sp1. Mol Cancer 2013;12:102.
25. Zhang B, Zhang H, Shen G. Metastasis-associated protein 2 (MTA2) promotes the metastasis of non-small-cell lung cancer through the inhibition of the cell adhesion molecule Ep-CAM and E-cadherin. Jpn J Clin Oncol 2015;45:755-66.
26. Covington KR, Brusco L, Barone I, Tsimelzon A, Selever J, Corona-Rodriguez A, et al. Metastasis tumor-associated protein 2 enhances metastatic behavior and is associated with poor outcomes in estrogen receptor-negative breast cancer. Breast Cancer Res Treat 2013;141:375-84.
27. Chen YS, Hung TW, Su SC, Lin CL, Yang SF, Lee CC, et al. MTA2 as a potential biomarker and its involvement in metastatic progression of human renal cancer by miR-133b targeting MMP-9. Cancers (Basel) 2019;11:E1851.
28. Hsieh YH, Hsu WH, Yang SF, Liu CJ, Lu KH, Wang PH, et al. Potential antimetastatic effect of timosaponin AIII against human osteosarcoma cells through regulating the Integrin/FAK/Cofilin axis. Pharmaceuticals (Basel) 2021;14:260.
29. Akbarzadeh M, Movassaghpour AA, Ghanbari H, Kheirandish M, Fathi Maroufi N, Rahbarghazi R, et al. The potential therapeutic effect of melatonin on human ovarian cancer by inhibition of invasion and migration of cancer stem cells. Sci Rep 2017;7:17062.
30. Wu MH, Chiu YF, Wu WJ, Wu PL, Lin CY, Lin CL, et al. Synergistic antimetastatic effect of cotreatment with licochalcone A and sorafenib on human hepatocellular carcinoma cells through the inactivation of MKK4/JNK and uPA expression. Environ Toxicol 2018;33:1237-44.
31. Arabsalmani M, Mohammadian-Hafshejani A, Ghoncheh M, Hadadian F, Towhidi F, Vafaee K, et al. Incidence and mortality of kidney cancers, and human development index in Asia; a matter of concern. J Nephropathol 2017;6:30-42.
32. Znaor A, Lortet-Tieulent J, Laversanne M, Jemal A, Bray F. International variations and trends in renal cell carcinoma incidence and mortality. Eur Urol 2015;67:519-30.
33. Heng DY. The next 10 years: Challenges for the future and overcoming resistance to targeted therapies for renal cell carcinoma. Can Urol Assoc J 2016;10:S256-8.
34. Toh Y, Nicolson GL. The role of the MTA family and their encoded proteins in human cancers: Molecular functions and clinical implications. Clin Exp Metastasis 2009;26:215-27.
35. Liu YP, Shan BE, Wang XL, Ma L. Correlation between MTA2 overexpression and tumour progression in esophageal squamous cell carcinoma. Exp Ther Med 2012;3:745-9.
36. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: A phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol 2009;10:25-34.
37. García-Echeverría C. Protein and lipid kinase inhibitors as targeted

anticancer agents of the Ras/Raf/MEK and PI3K/PKB pathways. Purinergic Signal 2009;5:117-25.

38. Wang Q, Wu G, Che X, Li Q, Zhang Z, Tang Q. Sorafenib induces renal cell carcinoma apoptosis *via* upregulating activating transcription factor 4. Pharmazie 2018;73:156-60.
39. Hsieh SC, Tsai JP, Yang SF, Tang MJ, Hsieh YH. Metformin inhibits the invasion of human hepatocellular carcinoma cells and enhances the chemosensitivity to sorafenib through a downregulation of the ERK/JNK-mediated NF-κB-dependent pathway that reduces uPA and MMP-9 expression. Amino Acids 2014;46:2809-22.
40. Lin MT, Lin CL, Lin TY, Cheng CW, Yang SF, Lin CL, et al. Synergistic effect of fisetin combined with sorafenib in human cervical cancer HeLa cells through activation of death receptor-5 mediated caspase-8/caspase-3 and the mitochondria-dependent apoptotic pathway. Tumour Biol 2016;37:6987-96.
41. Reiter RJ. Mechanisms of cancer inhibition by melatonin. J Pineal Res 2004;37:213-4.
42. Wang Y, Wang P, Zheng X, Du X. Therapeutic strategies of melatonin in cancer patients: A systematic review and meta-analysis. Onco Targets Ther 2018;11:7895-908.
43. Min KJ, Kim HS, Park EJ, Kwon TK. Melatonin enhances thapsigargin-induced apoptosis through reactive oxygen species-mediated upregulation of CCAAT-enhancer-binding protein homologous protein in human renal cancer cells. J Pineal Res 2012;53:99-106.
44. Um HJ, Park JW, Kwon TK. Melatonin sensitizes Caki renal cancer cells to kahweol-induced apoptosis through CHOP-mediated up-regulation of PUMA. J Pineal Res 2011;50:359-66.