Transcriptomic analysis on the effects of melatonin in gastrointestinal carcinomas

Lu Ao (lukey@fjmu.edu.cn)
Fujian Medical University https://orcid.org/0000-0001-7378-4967
Li Li
Fujian Medical University
Huaqin Sun
Fujian Medical University
Huxing Chen
Fujian Medical University
Yawei Li
Fujian Medical University
Haiyan Huang
Fujian Medical University
Xianlong Wang
Fujian Medical University
Zheng Guo
Fujian Medical University
Ruixiang Zhou
Fujian Medical University

Research article

Keywords: melatonin, gastrointestinal carcinomas, cell lines, differentially expressed genes, reverse, functional enrichment analysis

Posted Date: October 25th, 2019

DOI: https://doi.org/10.21203/rs.2.16442/v1

Version of Record: A version of this preprint was published on July 20th, 2020. See the published version at https://doi.org/10.1186/s12876-020-01383-z.

Abstract

Background : Melatonin has been shown with anticancer property and therapeutic potential for tumors. However, there lacks a systematic study on the molecular pathways of melatonin and its antitumor effects in gastrointestinal carcinomas.

Methods : Using the gene expression profiles of four cancer cell lines from three types of gastrointestinal carcinomas before and after melatonin treatment, including gastric carcinoma (GC), colorectal carcinoma (CRC) and hepatocellular carcinoma (HCC), differentially expressed genes (DEGs) and biological pathways influenced by melatonin were identified.

Results : There were 17 pathways commonly altered by melatonin in the three cancer types, including FoxO signaling pathways enriched by the upregulated DEGs and cell cycle signaling pathways enriched by the downregulated DEGs, confirmed the dual role of melatonin to tumor growth, pro-apoptosis and anti-proliferation. DEGs upregulated in the three types of cancer tissues but reversely downregulated by melatonin were commonly enriched in RNA transport, spliceosome and cell cycle signaling pathways, which indicate that melatonin might exert antitumor effects through these pathways. Our results further showed that melatonin can downregulate the expression levels of 5-FU resistance-related genes, such as thymidylate synthase in GC and ATR , CHEK1 , BAX and MYC in CRC, suggesting that melatonin might increase the sensitivity of 5-FU in GC and CRC patients.

Conclusions : Melatonin exerts the effects of pro-apoptosis and anti-proliferation on gastrointestinal carcinomas, and might increase the sensitivity of 5-FU in GC and CRC patients.

Background

Melatonin (*N*-acetyl–5-methoxytryptamine), a hormone secreted by the pineal gland and gastrointestinal tract during night and daytime, plays a key role in circadian rhythms(1), antioxidant activities(2, 3) as well as immune system regulation(4, 5). It has been reported that the melatonin concentration in the gastrointestinal tract tissues is 100–400 fold higher than that in plasma and liver is the main site for melatonin metabolism(6, 7). Recently, decreased melatonin levels have been demonstrated to be correlated with increased cancer risk. A large number of studies have reported that melatonin has anticancer effects on numerous types of tumors, such as liver(8, 9), colon(10), breast(11) and ovarian(12) cancers. These studies mainly highlight its dual role in tumor cells: pro-apoptosis and anti-proliferation, which are the two goals in the control of tumor growth. However, these *in vitro* studies only used tumor cell lines for a particular cancer type, and there lacks a systematic study to elucidate the global responsive pathways and the antitumor effects of melatonin's actions across multiple tumor types.

Recently, there is an increasing interest in exploring the clinical application of melatonin in cancer therapy. Many studies suggested that melatonin treatment is useful in enhancing the efficacy of some chemotherapeutic drugs and controlling the progression of cancers(13–16). For example, Lin et al.(17) found that melatonin synergistically promoted the sorafenib-induced apoptosis in hepatocellular

carcinoma cell lines. Moreover, many studies demonstrated that melatonin is beneficial to reduce the side effects of chemotherapeutic drugs(18–21). Lissoni et al.(20) found that melatonin attenuates the negative consequences of cisplatin in advanced non-small cell lung cancer patients. Therefore, it is worth to investigate the molecular mechanism of melatonin administration in aiding against different types of tumors.

Gene expression profiling provides an opportunity for researchers to investigate these potential effects of melatonin systematically on cancer development and therapeutic response. Gastric carcinoma (GC), colorectal carcinoma (CRC) and hepatocellular carcinoma (HCC) are three common malignant tumors in the digestive system, all with high morbidity and mortality across the world. In this study, our aim was to characterize the common biological signaling pathways altered by melatonin on the three types of gastrointestinal carcinomas with genome-wide expression data and further investigate the relationship between these pathways and the antitumor effect and synergistic drug response of melatonin.

We measured gene expression profiles of four tumor cell lines for the three cancer types treated with melatonin and analyzed differentially expressed genes (DEGs) between the treatment and control groups. Functional enrichment analyses showed that the DEGs after melatonin treatment in the three cancers were enriched in 17 common pathways, such as FoxO and ErbB signaling pathways enriched by the upregulated DEGs, and cell cycle signaling pathways enriched by the downregulated DEGs, confirmed its dual role in controlling tumor growth. We further found that the DEGs upregulated in tumor tissues but downregulated by melatonin in the cell lines were all enriched in RNA transport, spliceosome and cell cycle signaling pathways, which might be the potential targets for cancer therapy. We further compared the DEGs with 5-fluorouracil (5-FU) resistance-related genes in GC and CRC and found that melatonin might enhance the efficacy of 5-FU through downregulating the expression levels of resistance-related genes, such as thymidylate synthase (TS) in GC patients and ATR, CHEK1, BAX and MYC in CRC patients. Our study is helpful to gain a comprehensive understanding of the effects of melatonin on gastrointestinal carcinomas.

Methods

Cell culture and reagents

The gastric adenocarcinoma cell line HGC–27 and colorectal adenocarcinoma cell line HCT–8 cells were grown in Roswell Park Memorial Institute (RPMI) 1640 medium (Hyclone, Logan, UT, USA.). The human hepatocellular carcinoma cell lines HepG2 and Huh–7 were cultured in Dulbecco's modified Eagle's medium (DMEM) (Hyclone, Logan, UT, USA.). All the cells were supplemented with 10% fetal bovine serum and maintained at 37 °C in 5% CO_2. Cells were seeded in 9.6 cm^2 culture dishes at a density of 1 × 10^6 cells/well.

Cell viability assays

GC cell line HGC–27 and CRC cell line HCT–8 were seeded into 96-well plates containing 100 μl medium at a density of 1,000 cells/well. After 24 hours incubation, cells were changed with fresh medium containing 0 (1% ethanol as control was added), 1, 2, 3, 4 or 5 mmol/L melatonin for 24 h, 48 h or 72 h. After the treatment, medium was discarded carefully and solution containing 20 μl MTS (CellTiter 96® AQueous One Solution Cell Proliferation Assay; Promega, Madison, WI, USA) and 80 μl serum free medium was added to each well and incubated for 2 hours. Then the optical densities was measured at 490 nm with a microplate reader (Synergy HT; BioTek Instruments Inc., Winooski, VT, USA).

RNA extraction and microarray expression analysis

The four tumor cell lines treated with 2.5 mmol/L melatonin for 24 h served as the treatment group and the rest cells cultured with ethanol served as the controls at the same time. RNA from the treatment group and the control group was extracted using the RNeasy Mini kit (Qiagen, Germany). The quality of RNA was measured using an Agilent 2100 Bioanalyzer (Agilent, USA). The fragmented cRNA for DNA microarray analysis was prepared according to the manufacturer's instructions, then hybridized to customized Affymetrix GeneChip® PrimeView™ Human Gene Expression Array, which includes 49495 probe sets representing 19042 genes. Arrays were scanned with Affymetrix Genechip™ Scanner 30007G. Each sample had three biological replicates. Expression profiling data measured in our study are available in the Gene Expression Omnibus repository (GEO accession number: GSE132119).

Data pre-processing of expression data

Gene expression profiles of GC, CRC and HCC tumors and the corresponding normal samples used in this study were downloaded from GEO. The details of each dataset were shown in Table 1. The Robust Multi-array Average algorithm(22) were used to normalize the raw expression data. Probe-set IDs were mapped to Entrez gene IDs with their corresponding platform files. The expression value of a gene which was mapped to multiple probes was defined as the arithmetic mean of the expression values of those probes. Data were log2 transformed. Subsequent analysis was performed in R version 3.1.1.

Identification of DEGs

The Student's *t*-test was used to select DEGs between the treated and control cancer cell lines or between the cancerous and normal tissue samples. Because Student's *t*-test biases towards genes with low expression levels in small size samples, *i.e.* the cancer cell line datasets here, the reproducibility-based pairwise difference (PD)(29) was combined to detect DEGs between the treatment group and the control group of the cell line datasets.

Reproducibility evaluation of two DEG lists

For two DEG lists sharing k common genes, of which s genes had the same dysregulation directions (both upregulated or downregulated in the two DEG lists), the consistency score was calculated as s/k. The probability of observing at least s of k DEGs with the same dysregulation directions by chance can be evaluated using the cumulative binomial distribution model as follows(30),

[Please see supplementary files for formula.]

in which p_e is the probability of one gene having the same dysregulation direction in two DEG lists by random chance (here, = 0.5). The consistency score is considered significant for $p < 0.01$.

Statistical analysis

A directed regulatory network of protein-protein interaction by linking DEGs of CRC cancer cell line HCT–8 with 85 genes related with 5-FU resistance in CRC(31, 32) was constructed in the SIGnaling Network Open Resource (SIGNOR)(33) database. The expression levels of 5-FU resistance-related genes are positively associated with the degree of drug resistance.

Functional enrichment analysis was performed based on the Kyoto Encyclopedia of Genes and Genomes(34). The hypergeometric distribution model was used to determine biological pathways that were significantly enriched with DEGs(35). The Benjamini and Hochberg procedure (BH) was used to adjust the *p*-values to control the False Discovery Rate (FDR) and the statistical significance was set as FDR < 10%.

Results

Melatonin inhibited cell growth of HGC–27 and HCT–8 cells

The flowchart was described in Figure 1. GC cell line HGC–27 and CRC cell line HCT–8 were treated with 0, 1, 2, 3, 4 or 5 mmol/L melatonin for 24 h, 48 h or 72 h, respectively. Cell viability was assessed by MTS assay. The results revealed that melatonin inhibited the growth of HGC–27 and HCT–8 in a dose and time-dependent manner (Figure 2). The melatonin concentrations of 50% inhibition of cell viability were 1.98 mmol/L and 8.82 mmol/L, respectively, for HGC–27 and HCT–8 for the 24 h treatment. At present, the consensus of melatonin concentration and exposure time for inhibiting the cell viability in HepG2 and Huh–7 cell lines is 1 mmol/L and 24 h, respectively(9, 13, 36, 37). Based on these results, we selected 2.5 mmol/L and 24 h to treat the four tumor cell lines in the following experiments.

Identification and functional analysis of dysregulated genes in cancer cell lines treated by melatonin

To provide a comprehensive overview of the common biological signaling pathways altered by melatonin on three cancer types, we first detected the DEGs in the cancer cell lines due to the melatonin treatment. Using Student's *t*-test with 5% FDR control, 6,236 DEGs were detected in the HGC–27 cell lines; if the reproducibility-based PD with a consistency threshold of 90% was used, 7,287 DEGs were detected. A total of 5,265 DEGs were commonly detected by the two methods, all of which were with the same dysregulated directions. Then the two DEGs lists were combined and a full list of 7,898 DEGs of HGC–27 were obtained. Similarly, 6,363, 10,282 and 7,815 DEGs were detected in the HCT–8, Huh–7 and HepG2 cells, respectively (detailed information shown in Supplementary Table S1).

With a 10% FDR control, 4,114, 3,242, 4,673 and 3,837 upregulated DEGs of the four cell lines were enriched in 23, 44, 29 and 42 biological pathways, respectively (shown in Supplementary Table S2). There were 10 common pathways, including FoxO, ErbB and lysosome signaling pathways (Figure 3). The FoxO family genes play a crucial role in tumor suppression by upregulating their target genes involved in apoptosis(38). Our results also suggest that melatonin might enhance the apoptosis of tumor cells through the activation of FoxO signaling pathway(14).

Similarly, 3,784, 3,121, 5,609 and 3,978 downregulated DEGs of the four cell lines were enriched in 10, 14, 11 and 12 biological pathways, respectively (shown in Supplementary Table S3). There were 7 common pathways, including pyrimidine metabolism, DNA replication and cell cycle signaling pathways (Figure 3). These results further support the view that melatonin reduces the cell cycle of tumor to control tumor growth(8, 13, 39).

Comparison between the dysregulated genes in cancer tissues and those reversed by melatonin

To explore the potential anticancer effects of melatonin, we compared the DEGs found in cancer cell lines with those in cancer tissues. Using Student's *t*-test with 1% FDR control, 3,278 and 7,459 DEGs were identified between GC cancerous and normal samples in GSE27342 and GSE63089, respectively. A total of 3,068 DEGs with the same dysregulation directions in the two datasets were selected as dysregulated genes in the state of GC. Among the 1,475 upregulated genes, 603 DEGs were downregulated in the HGC–27 cell lines treated by melatonin, and enriched in 5 biological pathways with 10% FDR control. Among the 1,593 downregulated genes, 334 DEGs were upregulated by melatonin, which were enriched in 9 biological pathways (Supplementary Table S4).

Similarly, 3,336 DEGs with the same dysregulation directions in dataset GSE8671 and dataset GSE23878 were identified in CRC tumors using Student's *t*-test with 1% FDR control. Among the 1,317 upregulated and 2,019 downregulated genes in CRC tumors, 605 and 425 DEGs were reversely downregulated and upregulated in the HCT–8 cell lines treated by melatonin, respectively, which were enriched in 7 and 30 biological pathways. Moreover, 4,257 DEGs with the same dysregulation directions in dataset GSE14520 and dataset GSE39791 were identified in HCC tumors using Student's *t*-test with 1% FDR control. Among the 2,865 upregulated genes, 1,136 and 868 DEGs were downregulated, respectively, in the Huh–7 and HepG2 cell lines treated by melatonin, while among the 1,392 downregulated gens, 355 and 271 DEGs were upregulated, respectively. The functional enrichment analysis results were shown in Supplementary Tables S4 and S5.

Interestingly, there were 4 common pathways enriched by those DEGs which were upregulated in three cancers but downregulated in all four cell lines treated by melatonin, including ribosome biogenesis in eukaryotes, RNA transport, spliceosome and cell cycle signaling pathways. These results suggest that melatonin might exert antitumor effects through these pathways.

Comparison with the genes related with 5-FU resistance in GC and CRC

Because 5-fluorouracil (5-FU) is a routine chemotherapeutic agent of DNA damage in GC and CRC, we further investigated whether DEGs altered by melatonin are associated with 5-FU resistance.

Recently, we have developed a signature consisting of two gene pairs which could robustly predict the prognosis of GC patients treated with 5-FU-based chemotherapy(40). Using Student's *t*-test with 5% FDR control, 1,969 DEGs were identified between 88 patients with high-risk and 35 patients with low-risk of resistance to 5-FU-based regimens. Among the 871 downregulated genes in the resistant high-risk GC patients compared with the low-risk patients, 234 DEGs were upregulated in the HGC–27 cell lines treated by melatonin. Meanwhile, among the 1,098 upregulated genes in the resistance high-risk GC patients, 520 DEGs were downregulated in the HGC–27 cell lines treated by melatonin, which were enriched in 12 biological pathways with 10% FDR control (Supplementary Table S6). The pyrimidine metabolism pathway, which is responsible for the metabolism of 5-FU, was included, and the thymidylate synthase (TS) gene involved in the pathway was downregulated by melatonin. It has been reported that 5-FU exerts its anticancer effects through inhibition of TS to disrupt DNA synthesis and repair, resulting in lethal DNA damage(41). Zembutsu et al. have revealed that there is an inverse relationship between mRNA levels of TS and 5-FU sensitivity in a panel of cancer cell lines, including GC cell lines(42).

For CRC tumors, we investigated the relationship by analyzing the protein-protein interaction network. A directed regulatory network included 136 DEGs in the HCT–8 cell lines after melatonin treatment and 37 genes related with 5-FU resistance in CRC was shown in Figure 4. Four resistance-related genes (ATR, CHEK1, MYC and BAX) were the hubs with the largest degrees in the network (all≥11), of which the expression levels were downregulated by melatonin. The ATR-CHEK1 pathway is known to be responsible for DNA damage during cell cycle. It has been reported that inhibition the ATR-CHEK1 pathway could enhance the efficacy of DNA damage agents in variety of carcinomas, ciplastin in CRC, gemcitabine in pancreatic cancer(43) and cytosine arabinoside in Refractory Acute Leukemias(44), and reverse the radioresistance in oral squamous cell carcinoma cells(45).

In conclusion, our results suggest that melatonin could enhance the efficacy of 5-FU in GC and CRC patients.

Discussion

By performing a global analysis of gene expression profiles of four cancer cell lines across three types of gastrointestinal carcinomas, our study systematically uncovered the genes and pathways commonly altered by melatonin for the first time and confirmed its dual role in tumor cells: pro-apoptosis and anti-proliferation. Moreover, comparison of the DEG between tumor tissues and melatonin-treated cancer cell lines indicated that melatonin might exert antitumor effects through RNA transport, spliceosome and cell cycle signaling pathways. By comparing DEGs of melatonin with 5-FU-resistance related genes, we found that melatonin could increase the sensitivity of 5-FU by downregulating the expression level of resistance-related genes, such as TS in GC patients and ATR, CHEK1, MYC and BAX in CRC patients.

It is reported that melatonin can activate the MAPK cascades(39, 46, 47). In line with these studies, the upregulated genes by melatonin in HGC–27, HCT–8 and Huh–7 cell lines were significantly enriched in the MAPK pathway with 10% FDR control. With a loosen 5% *p*-value, the upregulated genes by melatonin in the HepG2 cell lines were also enriched the pathway. Genes MAP3K2, MAP3K7, MAP3K18, MAPK8 and MAPK9 in the pathway, which were responsible for DNA damage or apoptosis(48), were all upregulated by melatonin treatment in four cancer cell lines. The results also supported the dual role of melatonin in tumor cells.

Melatonin has been showed to increase the efficiency of cisplatin in ovarian cancer cell lines(49), 5-FU in CRC cells(50), sorafenib in HCC cells(14, 17). Our results indicated that melatonin may improve the chemotherapeutic effect of 5-FU in GC and CRC patients. The treatment by the combination of 5-FU and melatonin may obtain better therapeutic benefits for GC and CRC patients than 5-FU alone, which might be a good solution for patients with tumor insensitive or acquired-resistant to conventional 5-FU based chemotherapy. Therefore, in consideration of its low toxicity, it's worth to investigate the combination of melatonin with chemotherapeutic agents in aiding cancer patients against different types of tumors. Besides, we are aware that our study is carried out *in vitro* and the concentration of melatonin used in this study is hardly reached in humans. The proper dose and way of melatonin administration in clinic cancer therapy need be further investigated.

Conclusions

Our study systematically uncovered the genes and pathways commonly altered by melatonin for the first time and confirmed its dual role in tumor cells: pro-apoptosis and anti-proliferation. Our results further indicated that melatonin might increase the sensitivity of 5-FU in GC and CRC.

List Of Abbreviations

GC: gastric carcinoma; CRC: colorectal carcinoma; HCC: hepatocellular carcinoma; 5-FU:5-fluorouracil; DEGs: differentially expressed genes; FDR: False Discovery Rate.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Expression profiling data measured in our study are available in the Gene Expression Omnibus repository (GEO accession number: GSE132119).

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China (Grant Nos. 81602738 and 81872396), young and middle-aged backbone training project in the health system of Fujian province (Grant No. 2017-ZQN–56), outstanding youth scientific research personnel training program in Fujian Province University (Grant No. 2017B018), joint funds for the innovation of science and Technology of Fujian province (Grant No: 2018Y9065) and the Nursery Research Fund Project of Fujian Medical University (2015MP006).

Authors' contributions

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. LA, ZG and RXZ conceived and supervised the study. LL and HXC performed the cell line experiment and acquired the data. LA, HQS and YWL searched the data and participated in the statistical analysis. LA and LL drafted of the manuscript. HQS and HYH interpreted the results and drew the figures. XLW helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We thank professor Changming Huang of Fujian Medical University Union Hospital for kindly providing the HGC–27 cells, professor Xinhua Liao of Fujian Medical University for kindly providing the HCT–8 cells and professor Xu Lin of Fujian Medical University for kindly providing the Huh–7 and HepG2 cells.

References

1.Dominguez-Rodriguez A, Abreu-Gonzalez P, Sanchez-Sanchez JJ, Kaski JC, Reiter RJ. Melatonin and circadian biology in human cardiovascular disease. Journal of pineal research. 2010;49(1):14–22. Epub 2010/06/12.

2.Garcia JJ, Lopez-Pingarron L, Almeida-Souza P, Tres A, Escudero P, Garcia-Gil FA, et al. Protective effects of melatonin in reducing oxidative stress and in preserving the fluidity of biological membranes: a review. Journal of pineal research. 2014;56(3):225–37. Epub 2014/02/28.

3.Tan DX, Manchester LC, Hardeland R, Lopez-Burillo S, Mayo JC, Sainz RM, et al. Melatonin: a hormone, a tissue factor, an autocoid, a paracoid, and an antioxidant vitamin. Journal of pineal research. 2003;34(1):75–8. Epub 2002/12/18.

4.Volt H, Garcia JA, Doerrier C, Diaz-Casado ME, Guerra-Librero A, Lopez LC, et al. Same molecule but different expression: aging and sepsis trigger NLRP3 inflammasome activation, a target of melatonin. Journal of pineal research. 2016;60(2):193–205. Epub 2015/12/19.

5.Santello FH, Frare EO, Caetano LC, AlonsoToldo MP, do Prado JC, Jr. Melatonin enhances pro-inflammatory cytokine levels and protects against Chagas disease. Journal of pineal research. 2008;45(1):79–85. Epub 2008/02/21.

6.Konturek SJ, Konturek PC, Brzozowska I, Pawlik M, Sliwowski Z, Czesnikiewicz-Guzik M, et al. Localization and biological activities of melatonin in intact and diseased gastrointestinal tract (GIT). Journal of physiology and pharmacology: an official journal of the Polish Physiological Society. 2007;58(3):381–405. Epub 2007/10/12.

7.Bubenik GA. Thirty four years since the discovery of gastrointestinal melatonin. Journal of physiology and pharmacology: an official journal of the Polish Physiological Society. 2008;59 Suppl 2:33–51. Epub 2008/10/01.

8.Martin-Renedo J, Mauriz JL, Jorquera F, Ruiz-Andres O, Gonzalez P, Gonzalez-Gallego J. Melatonin induces cell cycle arrest and apoptosis in hepatocarcinoma HepG2 cell line. Journal of pineal research. 2008;45(4):532–40. Epub 2008/11/18.

9.Carbajo-Pescador S, Garcia-Palomo A, Martin-Renedo J, Piva M, Gonzalez-Gallego J, Mauriz JL. Melatonin modulation of intracellular signaling pathways in hepatocarcinoma HepG2 cell line: role of the MT1 receptor. Journal of pineal research. 2011;51(4):463–71. Epub 2011/07/02.

10.Farriol M, Venereo Y, Orta X, Castellanos JM, Segovia-Silvestre T. In vitro effects of melatonin on cell proliferation in a colon adenocarcinoma line. Journal of applied toxicology: JAT. 2000;20(1):21–4. Epub 2000/01/21.

11.Hill SM, Blask DE. Effects of the pineal hormone melatonin on the proliferation and morphological characteristics of human breast cancer cells (MCF–7) in culture. Cancer research. 1988;48(21):6121–6. Epub 1988/11/01.

12.Petranka J, Baldwin W, Biermann J, Jayadev S, Barrett JC, Murphy E. The oncostatic action of melatonin in an ovarian carcinoma cell line. Journal of pineal research. 1999;26(3):129–36. Epub 1999/05/08.

13.Carbajo-Pescador S, Martin-Renedo J, Garcia-Palomo A, Tunon MJ, Mauriz JL, Gonzalez-Gallego J. Changes in the expression of melatonin receptors induced by melatonin treatment in hepatocarcinoma HepG2 cells. Journal of pineal research. 2009;47(4):330–8. Epub 2009/10/13.

14.Prieto-Dominguez N, Ordonez R, Fernandez A, Mendez-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. Journal of pineal research. 2016;61(3):396–407. Epub 2016/08/04.

15.Lissoni P, Paolorossi F, Tancini G, Ardizzoia A, Barni S, Brivio F, et al. A phase II study of tamoxifen plus melatonin in metastatic solid tumour patients. British journal of cancer. 1996;74(9):1466–8. Epub 1996/11/01.

16.Pariente R, Pariente JA, Rodriguez AB, Espino J. Melatonin sensitizes human cervical cancer HeLa cells to cisplatin-induced cytotoxicity and apoptosis: effects on oxidative stress and DNA fragmentation. Journal of pineal research. 2016;60(1):55–64. Epub 2015/10/16.

17.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. Journal of pineal research. 2017;62(3). Epub 2017/02/09.

18.Hara M, Yoshida M, Nishijima H, Yokosuka M, Iigo M, Ohtani-Kaneko R, et al. Melatonin, a pineal secretory product with antioxidant properties, protects against cisplatin-induced nephrotoxicity in rats. Journal of pineal research. 2001;30(3):129–38. Epub 2001/04/24.

19.Lopez-Gonzalez MA, Guerrero JM, Rojas F, Delgado F. Ototoxicity caused by cisplatin is ameliorated by melatonin and other antioxidants. Journal of pineal research. 2000;28(2):73–80. Epub 2000/03/10.

20.Lissoni P, Paolorossi F, Ardizzoia A, Barni S, Chilelli M, Mancuso M, et al. A randomized study of chemotherapy with cisplatin plus etoposide versus chemoendocrine therapy with cisplatin, etoposide and the pineal hormone melatonin as a first-line treatment of advanced non-small cell lung cancer patients in a poor clinical state. Journal of pineal research. 1997;23(1):15–9. Epub 1997/08/01.

21.Lissoni P, Barni S, Mandala M, Ardizzoia A, Paolorossi F, Vaghi M, et al. Decreased toxicity and increased efficacy of cancer chemotherapy using the pineal hormone melatonin in metastatic solid tumour patients with poor clinical status. Eur J Cancer. 1999;35(12):1688–92. Epub 2000/02/16.

22.Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics. 2003;4(2):249–64. Epub 2003/08/20.

23.Cui J, Chen Y, Chou WC, Sun L, Chen L, Suo J, et al. An integrated transcriptomic and computational analysis for biomarker identification in gastric cancer. Nucleic acids research. 2011;39(4):1197–207. Epub 2010/10/23.

24.Zhang X, Ni Z, Duan Z, Xin Z, Wang H, Tan J, et al. Overexpression of E2F mRNAs associated with gastric cancer progression identified by the transcription factor and miRNA co-regulatory network analysis. PloS one. 2015;10(2):e0116979. Epub 2015/02/04.

25.Sabates-Bellver J, Van der Flier LG, de Palo M, Cattaneo E, Maake C, Rehrauer H, et al. Transcriptome profile of human colorectal adenomas. Molecular cancer research: MCR. 2007;5(12):1263–75. Epub 2008/01/04.

26.Uddin S, Ahmed M, Hussain A, Abubaker J, Al-Sanea N, AbdulJabbar A, et al. Genome-wide expression analysis of Middle Eastern colorectal cancer reveals FOXM1 as a novel target for cancer therapy. The American journal of pathology. 2011;178(2):537–47. Epub 2011/02/02.

27.Roessler S, Jia HL, Budhu A, Forgues M, Ye QH, Lee JS, et al. A unique metastasis gene signature enables prediction of tumor relapse in early-stage hepatocellular carcinoma patients. Cancer research. 2010;70(24):10202–12. Epub 2010/12/17.

28.Kim JH, Sohn BH, Lee HS, Kim SB, Yoo JE, Park YY, et al. Genomic predictors for recurrence patterns of hepatocellular carcinoma: model derivation and validation. PLoS medicine. 2014;11(12):e1001770. Epub 2014/12/24.

29.Ao L, Yan H, Zheng T, Wang H, Tong M, Guan Q, et al. Identification of reproducible drug-resistance-related dysregulated genes in small-scale cancer cell line experiments. Scientific reports. 2015;5:11895. Epub 2015/07/16.

30.Bahn AK. Application of binomial distribution to medicine: comparison of one sample proportion to an expected proportion (for small samples). Evaluation of a new treatment. Evaluation of a risk factor. Journal of the American Medical Women's Association. 1969;24(12):957–66. Epub 1969/12/01.

31.Soong R, Diasio RB. Advances and challenges in fluoropyrimidine pharmacogenomics and pharmacogenetics. Pharmacogenomics. 2005;6(8):835–47. Epub 2005/11/22.

32.Tan WL, Bhattacharya B, Loh M, Balasubramanian I, Akram M, Dong D, et al. Low cytosine triphosphate synthase 2 expression renders resistance to 5-fluorouracil in colorectal cancer. Cancer biology & therapy. 2011;11(6):599–608. Epub 2011/03/08.

33.Lo Surdo P, Calderone A, Cesareni G, Perfetto L. SIGNOR: A Database of Causal Relationships Between Biological Entities-A Short Guide to Searching and Browsing. Current protocols in bioinformatics. 2017;58:8 23 1–8 16. Epub 2017/06/28.

34.Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic acids research. 2000;28(1):27–30. Epub 1999/12/11.

35.Belanger BF, Williams WJ, Yin TC. A flexible renewal process simulator for neural spike trains. IEEE transactions on bio-medical engineering. 1976;23(3):262–6. Epub 1976/05/01.

36.Colombo J, Maciel JM, Ferreira LC, RF DAS, Zuccari DA. Effects of melatonin on HIF–1alpha and VEGF expression and on the invasive properties of hepatocarcinoma cells. Oncology letters. 2016;12(1):231–7. Epub 2016/06/28.

37.Prieto-Dominguez N, Mendez-Blanco C, Carbajo-Pescador S, Fondevila F, Garcia-Palomo A, Gonzalez-Gallego J, et al. Melatonin enhances sorafenib actions in human hepatocarcinoma cells by inhibiting mTORC1/p70S6K/HIF–1alpha and hypoxia-mediated mitophagy. Oncotarget. 2017;8(53):91402–14. Epub 2017/12/07.

38.Carbajo-Pescador S, Steinmetz C, Kashyap A, Lorenz S, Mauriz JL, Heise M, et al. Melatonin induces transcriptional regulation of Bim by FoxO3a in HepG2 cells. British journal of cancer. 2013;108(2):442–9. Epub 2012/12/22.

39.Mediavilla MD, Sanchez-Barcelo EJ, Tan DX, Manchester L, Reiter RJ. Basic mechanisms involved in the anti-cancer effects of melatonin. Current medicinal chemistry. 2010;17(36):4462–81. Epub 2010/11/11.

40.Li X, Cai H, Zheng W, Tong M, Li H, Ao L, et al. An individualized prognostic signature for gastric cancer patients treated with 5-Fluorouracil-based chemotherapy and distinct multi-omics characteristics of prognostic groups. Oncotarget. 2016;7(8):8743–55. Epub 2016/02/04.

41.Longley DB, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. Nature reviews Cancer. 2003;3(5):330–8. Epub 2003/05/02.

42.Zembutsu H, Ohnishi Y, Tsunoda T, Furukawa Y, Katagiri T, Ueyama Y, et al. Genome-wide cDNA microarray screening to correlate gene expression profiles with sensitivity of 85 human cancer xenografts to anticancer drugs. Cancer research. 2002;62(2):518–27. Epub 2002/01/26.

43.Venkatesha VA, Parsels LA, Parsels JD, Zhao L, Zabludoff SD, Simeone DM, et al. Sensitization of pancreatic cancer stem cells to gemcitabine by Chk1 inhibition. Neoplasia. 2012;14(6):519–25. Epub 2012/07/13.

44.Karp JE, Thomas BM, Greer JM, Sorge C, Gore SD, Pratz KW, et al. Phase I and pharmacologic trial of cytosine arabinoside with the selective checkpoint 1 inhibitor Sch 900776 in refractory acute leukemias. Clinical cancer research: an official journal of the American Association for Cancer Research. 2012;18(24):6723–31. Epub 2012/10/25.

45.Sankunny M, Parikh RA, Lewis DW, Gooding WE, Saunders WS, Gollin SM. Targeted inhibition of ATR or CHEK1 reverses radioresistance in oral squamous cell carcinoma cells with distal chromosome arm 11q loss. Genes, chromosomes & cancer. 2014;53(2):129–43. Epub 2013/12/12.

46.Chan AS, Lai FP, Lo RK, Voyno-Yasenetskaya TA, Stanbridge EJ, Wong YH. Melatonin mt1 and MT2 receptors stimulate c-Jun N-terminal kinase via pertussis toxin-sensitive and -insensitive G proteins. Cellular signalling. 2002;14(3):249–57. Epub 2002/01/29.

47.Hazlerigg DG, Thompson M, Hastings MH, Morgan PJ. Regulation of mitogen-activated protein kinase in the pars tuberalis of the ovine pituitary: interactions between melatonin, insulin-like growth factor–1,

and forskolin. Endocrinology. 1996;137(1):210–8. Epub 1996/01/01.

48.Raman M, Chen W, Cobb MH. Differential regulation and properties of MAPKs. Oncogene. 2007;26(22):3100–12. Epub 2007/05/15.

49.Futagami M, Sato S, Sakamoto T, Yokoyama Y, Saito Y. Effects of melatonin on the proliferation and cis-diamminedichloroplatinum (CDDP) sensitivity of cultured human ovarian cancer cells. Gynecologic oncology. 2001;82(3):544–9. Epub 2001/08/25.

50.Gao Y, Xiao X, Zhang C, Yu W, Guo W, Zhang Z, et al. Melatonin synergizes the chemotherapeutic effect of 5-fluorouracil in colon cancer by suppressing PI3K/AKT and NF-kappaB/iNOS signaling pathways. Journal of pineal research. 2017;62(2). Epub 2016/11/20.

Tables

Table 1. Datasets of cancer and normal samples for three types of gastrointestinal carcinomas

	GEO Accession	Platform	Normal Samples	Cancer Samples	References
GC	GSE27342	GPL5175	80	80	(23),
	GSE63089	GPL5175	45	45	(24),
CRC	GSE8671	GPL570	32	32	(25),
	GSE23878	GPL570	24	35	(26),
HCC	GSE14520	GPL3921	220	225	(27),
	GSE39791	GPL10558	72	72	(28),

Figures

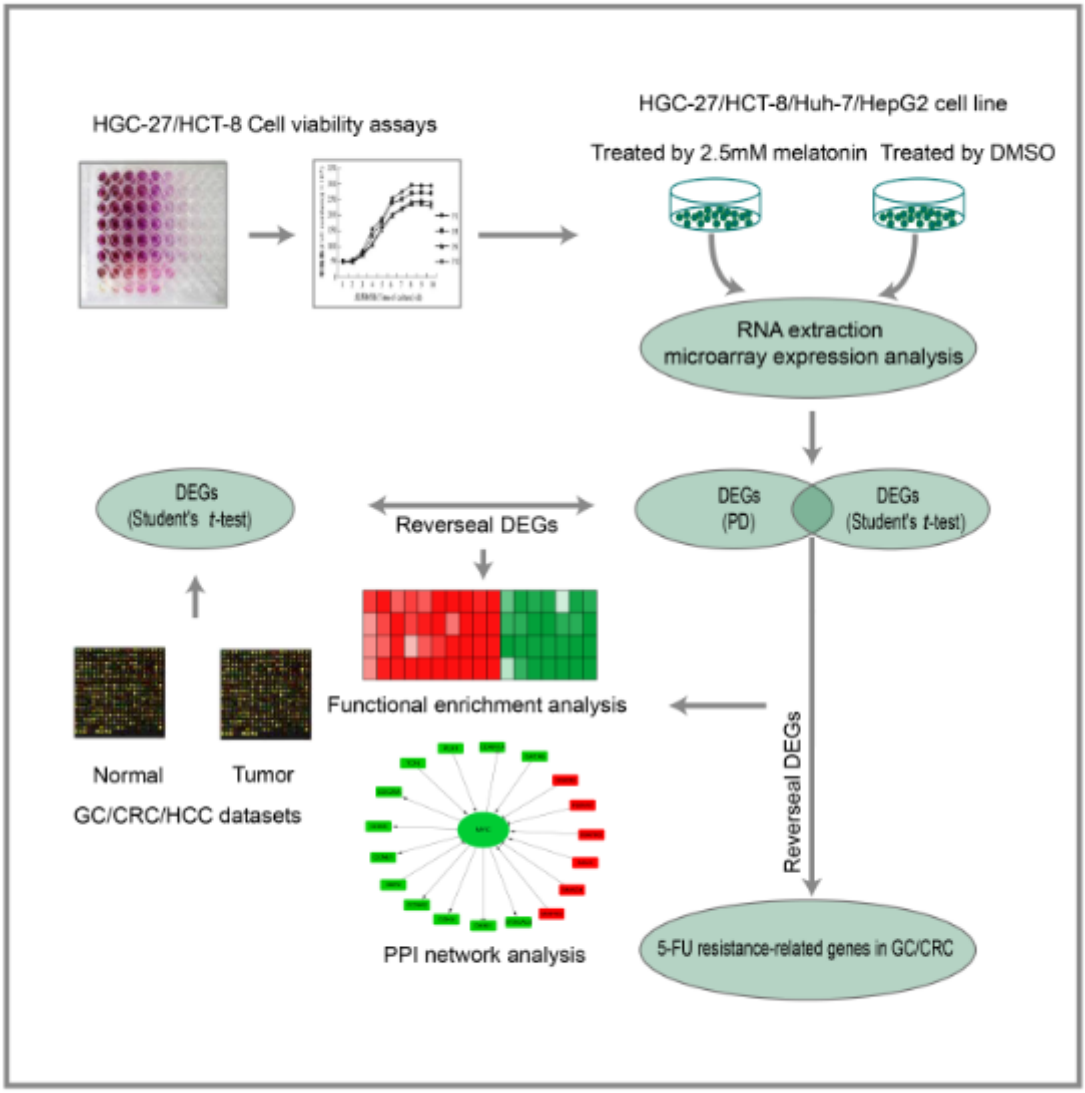

Figure 1

The flowchart of this study. Four cancer cell lines (HGC-27, HCT-8, Huh-7 and HepG2) across three types of gastrointestinal carcinomas (GC, CRC and HCC) were treated by melatonin four 24 hours and performed by DNA microarray analysis. The DEGs by melatonin treatment detected by Student's t-test and the reproducibility-based PD were combined to investigate the common biological signaling pathways altered by melatonin. The DEGs detected between tumor and normal tissues but reversed by melatonin in cancer cell lines were used to explore the potential anticancer effects of melatonin. The 5-FU resistance-related genes in GC and CRC but reversed by melatonin in cancer cell lines were used to explore the potential of melatonin to increase the sensitivity of 5-FU in GC and CRC.

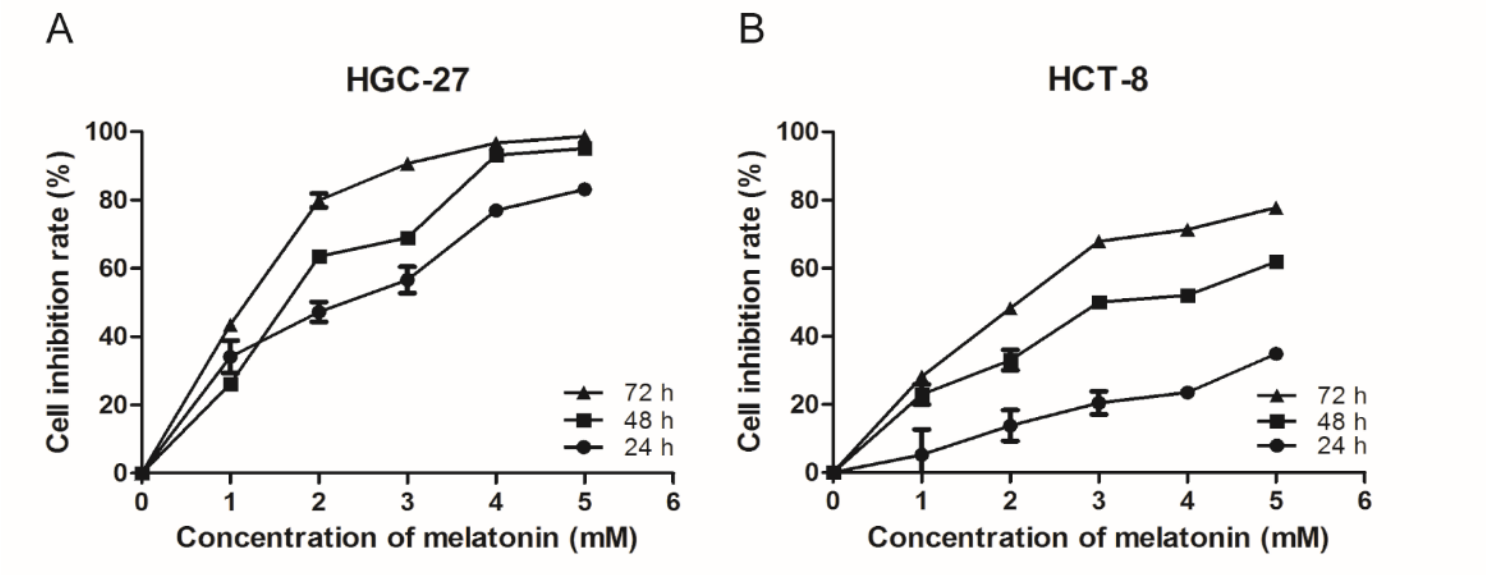

Figure 2

Effect of melatonin on cell growth in HGC-27 and HCT-8 cells. The antitumor effect of melatonin on GC cell line HGC-27 (A) and CRC cell line HCT-8 (B). The cells were treated with melatonin (0, 1, 2, 3, 4, 5 mmol/L) for 24 h, 48 h and 72 h, respectively. Cell viability was assessed by MTS assay.

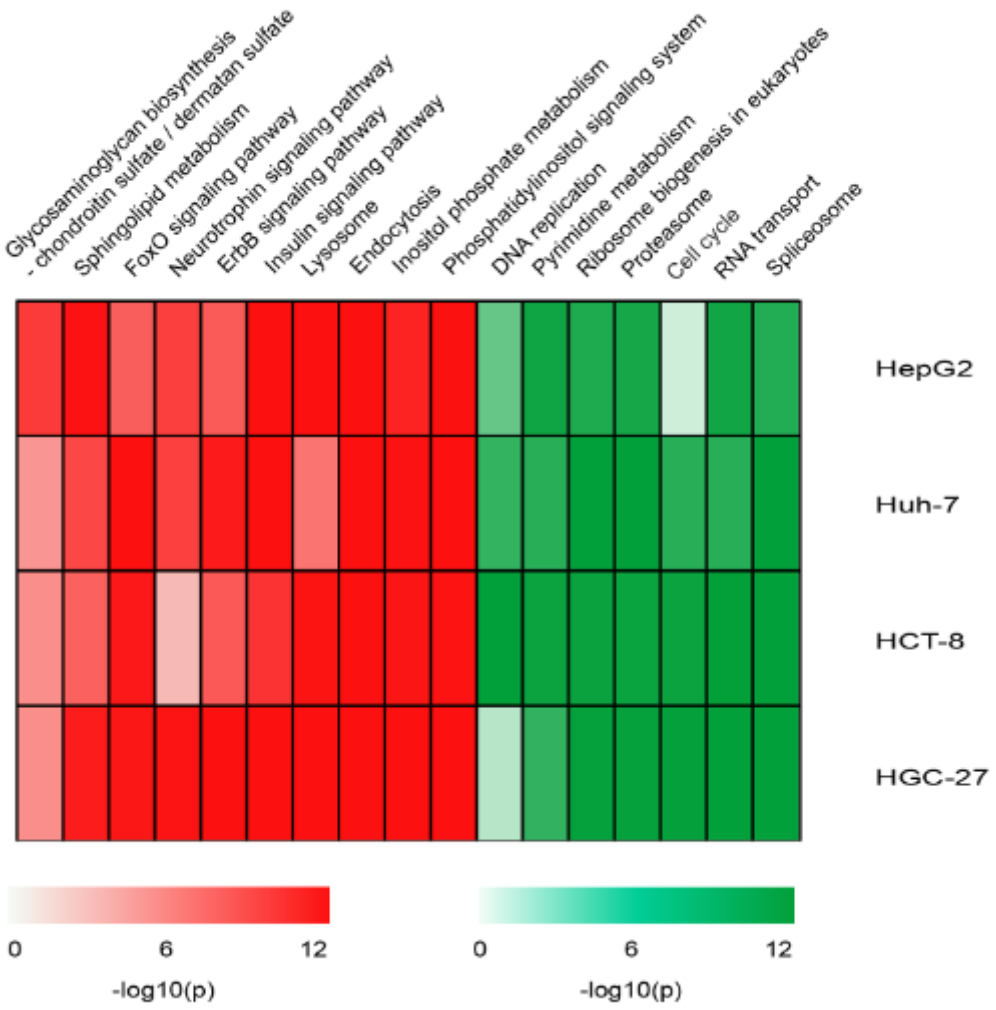

Figure 3

The common KEGG pathways significantly enriched by the upregulated and downregulated DEGs in four cancer cell lines treated by melatonin. The common KEGG pathways significantly enriched by the upregulated (Red) and downregulated (Green) DEGs in four cancer cell lines treated by melatonin. All p values of the KEGG pathway were adjusted by Benjamini and Hochberg ($p<0.1$). -log10(p) was used to generate the heat map.

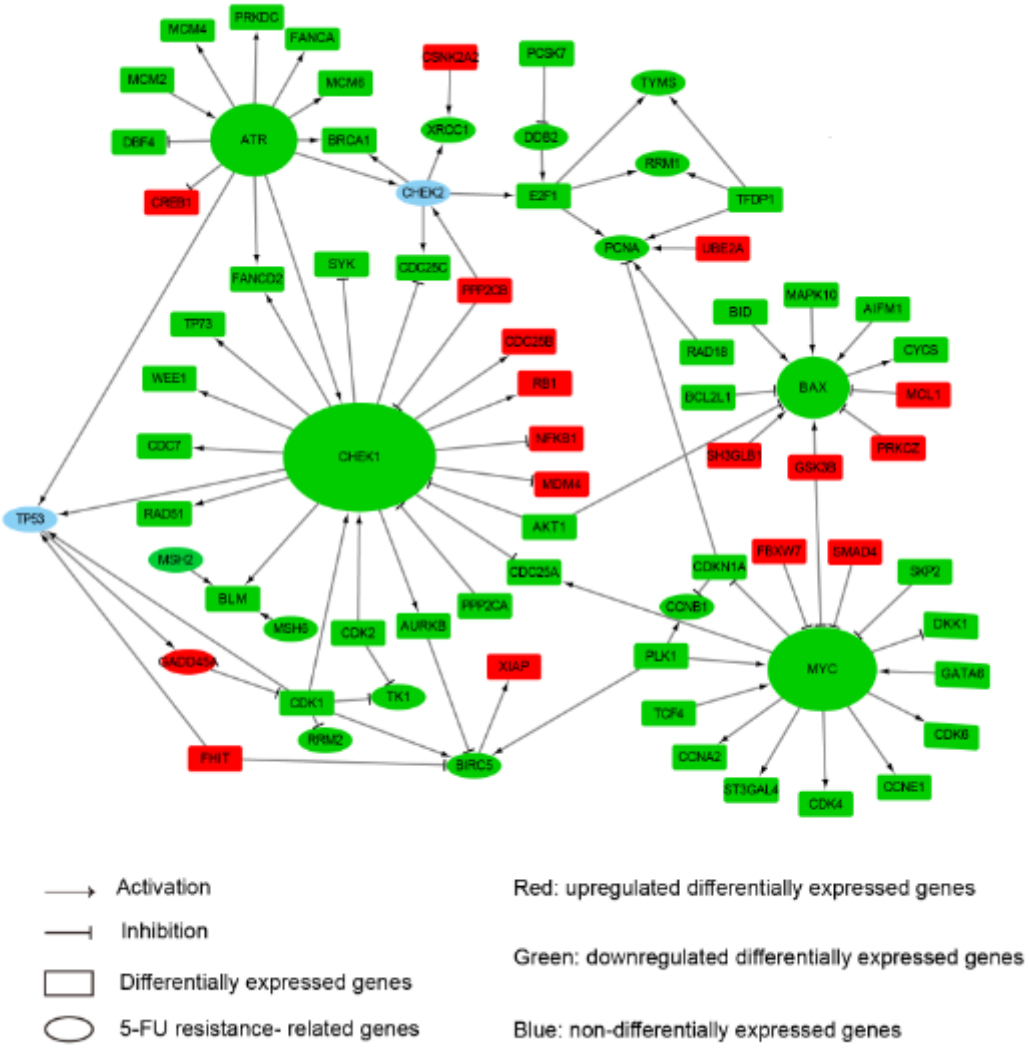

Figure 4

The protein-protein interaction network between DEGs of HCT-8 and 5-FU resistance-related genes in CRC. The network was consisted of DEGs of HCT-8 treated by melatonin and 5-FU resistance-related genes in CRC. The node shapes represent the types of genes. Ellipse, 5-FU resistance-related genes, of which overexpression are positively related with 5-FU resistance. Rectangle, DEGs of HCT-8 after melatonin treatment. The node colors indicate genes upregulated (Red), downregulated (Green) or non (Blue) - differentially expressed in HCT-8 after melatonin treatment.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTables.docx
- MethodsFormula.docx