PTP4A2 regulates Sorafenib resistance via activating autophagy in hepatocellular carcinoma

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Research

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Abstract

Background: To investigate the function and the potential mechanism of PTP4A2 in the progression of hepatocellular carcinoma (HCC).

Method: We analyzed PTP4A2 expression in patients with HCC and evaluated the relevance with clinical features. To investigate the role of PTP4A2 on the proliferation, apoptosis, autophagy and sorafenib sensitivity of HCC cells. Then miRNA's targeted regulation mechanism for PTP4A2 and the downstream target mechanism were explored.

Results: Here, we found that PTP4A2 expression increased significantly in HCC tissues, and report that PTP4A2 is an independent prognostic factor for HCC patients in the first time. Silencing of PTP4A2 decreased cell proliferation and autophagy, and increased cell apoptosis and sorafenib chemosensitivity, while the effect of PTP4A2 overexpression was opposite. In addition, miR-425 was found to negatively regulate PTP4A2. Mechanismly, the upregulation of PTP4A2 activated autophagy by targeting the RAB24. Overexpression of RAB24 re-enhances PTP4A2-silenced HCC cells proliferation and autophagy. Upregulation of RAB24 by PTP4A2 is partially dependent on PTP4A2 promotes HDAC1 accumulation in the cytoplasm of HCC cells and epigenetically up-regulates RAB24 transcription.

Conclusion: This study confirmed that PTP4A2 is an oncogene in HCC, and antagonizing PTP4A2 may be a potential strategy of treatment to increase HCC cells' Sorafenib sensitivity.

1. Background

Hepatocellular carcinoma (HCC) is the fifth major reason of cancer-related death in the world and one of the commonest cancers worldwide [1][2]. Currently, most HCC patients are diagnosed in the advanced stage when surgical resection has little effect [3]. The 5-year survival rate of HCC patients remains less than 20 % [4] [5, 6] [7]. In 2007, the US Food and Drug Administration (FDA) approved sorafenib as a first-line targeted drug for unresectable HCC disease. However, Sorafenib treatment is not effective for a certain number of HCC patients. Although sorafenib is beneficial to about 30% of patients, it usually causes acquired resistance within 6 months [7, 8]. This indicates that there are primary and acquired HCC sorafenib cells. However, the various factors that cause sorafenib resistance have not been thoroughly investigated.

Autophagy, through its established role in maintaining genome stability, has been considered as a mechanism for multi-stage inhibition of tumor formation, elimination of endogenous sources of ROS (Reactive oxygen species), the maintenance of bioenergetic functions, degradation of oncogenic proteins and induction of immunresponse mechanisms against malignant transformations [9]. Studies have shown that therapy of Sorafenib can enhance the autophagy of HCC cells [10-12]. Shi et al. revealed that apoptosis and autophagy of HCC cell were induced by Sorafenib through up-regulation of IRE1 signal involving ER(endoplasmic reticulum) stress [10]. In addition, studies have shown that through activity of the Akt pathway or inhibition of mTORC1, Sorafenib can induce autophagy [11, 12]. Obviously, autophagy

is responsible for coordinating the HCC's adaptive response to sorafenib. However, the role of autophagy in sorafenib-induced HCC resistance is unclear.

Protein tyrosine phosphatase (PTP) is a general term for a large class of enzymes, which have changeable effects on signal transduction [13, 14]. The functions of three closely related small PTPs (PTP4A1, PTP4A2 and PTP4P3) in tumor cells have attracted our attention, including promoting tumor migration, invasion and metastasis [15-18]. Recently, a growing body of research has shown that, in several types of malignant tumors, the up-regulation of PTP4A2 expression was strongly related to cancer progression [19-21]. Currently, the participation of PTP4A2 and its significance of clinic/prognosis in HCC are uncertain. Here we discuss PTP4A2 expression in HCC and its potential function in the HCC pathogenesis and investigate the influence on autophagy mediated by PTP4A2 on the resistance of HCC to sorafenib.

2. Materials And Methods

The detailed methods were described in the Supplementary Materials and Methods, including specimens and patients, Cell culture, Immunohistochemistry analysis, Quantitative real-time PCR, Western blot, vector construction and retroviral infection, CCK-8 assay, Cell apoptosis assays, Colony formation assay, Dual-luciferase experiment, Xenograft mouse model.

3. Result

The up-regulation of PTP4A2 was correlated with poorer prognosis of HCC.

To investigate the expression of the PTP4A2, we measured the protein and mRNA expression levels of PTP4A2 in seven HCC cell lines (7721, 7402, 7701, 97H, 7703, 97L, huh7) and one human normal hepatocellular cell line (LO-2), and twelve fresh HCC tissues (T) with their paired adjacent normal-tissues (ANTs). A relatively higher expression of PTP4A2 was found in HCC tissues than the matched ANTs (Fig. 1B). Furthermore, all seven HCC cell lines showed heightened PTP4A2 protein and mRNA expression compared with LO-2 (Fig. 1A), indicating that PTP4A2 expression is up-regulated in HCC. Next, the expression of PTP4A2 was measured in 245 paraffin-embedded HCC tissues by IHC. Compared with ANTs, HCC tissues also exhibited higher protein expression levels of PTP4A2 and the four categories of the intensity of PTP4A2 IHC staining were depicted in Fig.1B. Analyses of the results indicated that a higher expression of PTP4A2 was strongly correlated with Tumor size and increased TNM stage (Fig. 1C and table 1). The Kaplan-Meier analysis showed that the group of PTP4A2-high-expression had both poorer overall survival (OS) and progression-free survival (PFS) time than the group of PTP4A2-low-expression (log-rank test, $P < 0.001$, Fig. 1C). The prognostic value of PTP4A2 expression was analyzed by univariate analysis model and multivariate Cox regression against OS and PFS additionally (OS: HR, 2.772, 95% confidence interval (CI), 1.398–5.497; $P = 0.004$, PFS: HR, 2.005, 95%CI, 0.952–4.223; $P = 0.011$, respectively; Table 2 and 3). Kaplan–Meier survival analyses of published HCC data sets from

TCGA (The Cancer Genome Atlas) revealed the same relationship (Fig. 1C). In summary, these results suggest that PTP4A2 may act a prognostic biomarker for HCC.

PTP4A2 regulates cellular proliferation, apoptosis, and drug resistance of HCC.

Based on the find that PTP4A2 expressed highly in HCC, and the expression of PTP4A2 is connected with patients' prognosis, we proceed to explore the impact of PTP4A2 on cellular proliferation and apoptosis in HCC cells. PTP4A2 was knocked down in 7721 and 7701 cells (which is high-expression of PTP4A2) by shRNA (Fig. 2A) and PTP4A2 was overexpressed in 7402 cells (which is low-expression of PTP4A2) by an overexpression plasmid (Fig. 2A). Silencing PTP4A2 expression in 7721 and 7701 reduced cellular proliferation (Fig. 2B and C) and promoted cellular apoptosis (Fig. 2D). Overexpression of PTP4A2 promoted proliferation (Fig. 2B and C) and reduced apoptosis (Fig. 2D) in 7402. To determine whether PTP4A2 can induce Sorafenib resistance in HCC cell, the IC50 values of sorafenib was detected in 7721, 7701 and 7402 cells by CCK-8 assay. Compare to cells with low expression of PTP4A2, high expression group have a higher resistance to sorafenib (Fig. 2E). To explore whether PTP4A2 takes effect in Sorafenib-induced HCC cytotoxicity, we detected that sorafenib-induced apoptosis was promoted in 7721 after knocking down PTP4A2, and PTP4A2 overexpression in 7402 cells reduced Sorafenib-induced apoptosis (Fig. 2F). These data suggest that PTP4A2 has an effect on the proliferation, apoptosis and drug resistance of HCC cells.

Upregulation of PTP4A2 enhanced the activity of autophagy.

Analyses of PTP4A2-regulated gene signatures via GSEA and GO analyses showed that PTP4A2 high expression was positively related to an enrichment of autophagy gene signatures (Fig. 3A). Genes upregulated by PTP4A2-overexpress showed pathway enrichment for the GO terms "autophagy " and "autophagosome assembly" (Fig. 3A). These results suggested that PTP4A2 contributes to the activation of autophagy. Thus, we examined whether PTP4A2 regulated autophagy-associated proteins in HCC cell lines. Western blot analysis showed that LC3B and Atg7 expression was reduced while p62 expression level was increased after PTP4A2 knockdown. The opposite resulted was observed after PTP4A2 overexpressing, which further indicates that PTP4A2 is involved in autophagy (Fig. 3B). Of note, silencing PTP4A2 potentially inhibit autophagic flux to produce autophagosomes/autolysosomes without autophagy inducers, while PTP4A2 overexpression significantly promotes autophagy-related genes' expression and formation of autophagosome (Fig. 3C).

MiR-425 is a negative regulator of PTP4A2

It is well-known that microRNA (miRNA) plays a crucial role in protein regulation. To clarify the mechanisms of regulating the expression of PTP4A2 in HCC cells, We first overlapped the prediction algorithm of MICRORNA and the results of miRNA analysis of significantly down-regulated miRNA in HCC (Fig. 4A). And we singled out miR-425 as a potential regulator. In addition, one study has suggested miR-425 can predict response to sorafenib therapy in patients with HCC [22]. The quantitative RT-PCR analyses revealed that miR-425 was indeed downregulated in all seven HCC cell lines compared with LO2

(Fig. 4B). Furthermore, the expression of miR-425 was lower in HCC cells with high PTP4A2 expression (7721 and 7701) and raised in low (7402, 7703, 97H, 97L, huh7; Fig. 4B), showing a negative relationship between miR-425 and PTP4A2. In addition, the PTP4A2 expression was repressed by miR-425 mimics in 7721 and 7701 cells, and PTP4A2 expression was enhanced by anti-miR-425 in 7402 cells (Fig. 4C), suggesting that the expression of PTP4A2 was regulated by miR-425 negatively. And the dual-luciferase experiments further confirm that miR-425 can regulate PTP4A2 directly (Fig. 4D and 4E). Moreover, there was an inverse relation between miR-425 and PTP4A2 expression ($r = -0.7162$, $P < 0.0001$, Fig. 4E). The above data shows that miR-425 acts as a negative regulator of PTP4A2.

MiR-425 regulates cellular proliferation, apoptosis and autophagy in HCC

The above data has shown that PTP4A2 can regulate cellular multiplication, apoptosis and autophagy in HCC, and miR-425 has a direct regulatory effect on the expression of PTP4A2. Therefore, we speculate whether miR-425 also can regulate the cellular proliferation, apoptosis and autophagy of HCC. Transfection of 7721 and 7701 cells with miR-425 mimics decreased cellular proliferation and risen apoptosis of cells, while silencing miR-425 with anti-miR-425 in 7402 cells had an inverse effect (Fig. 4F and 4G). LC3B expression was downregulated and p62 was upregulated after overexpressing miR-425 in 7721 cells and LC3B expression was upregulated and p62 was downregulated after miR-425 silencing in 7402 cells (Fig. 4H). Next, to confirm the relevance of miR-425 and autophagy, we detected autophagy flux through the mRF-GFP-LC3 reporter gene. It is worth noting that in the absence of autophagy inducers, miR-425 overexpression can effectively inhibit autophagy flux to produce autophagosomes and/or autolysosomes, while silencing miR-425 significantly promotes autophagy-related genes' expression and the formation of autophagosome. (Fig. 4I).

The recovery of PTP4A2 expression can counteract miR-425 mediated sorafenib sensitivity

To explore whether miR-425 promotes the chemosensitivity of sorafenib in HCC cells by inhibiting the expression of PTP4A2, we co-transfected the cells. By co-transfection with the plasmid of PTP4A2 overexpression, PTP4A2 expression was reduced in 7721 cells transfected with miR-425 mimic. Similarly, by co-transfection with PTP4A2 silencing plasmid, the expression of PTP4A2 was restored in 7402 cells transfected with miR-425 inhibitor (Fig. 5A). Compared to miR-425 mimics, co-transfection with miR-425 mimics and PTP4A2 overexpression plasmid can partially attenuate Sorafenib-induced 7721 cell cytotoxicity and promote Sorafenib-induced apoptosis (Fig. 5B). However, compared with the miR-425 inhibitor, 7402 cells co-transfected with miR-425 inhibitor and PTP4A2 knockdown vector partially restore the cytotoxicity induced by sorafenib and inhibit cell apoptosis (Fig. 5C). In addition, co-transfection with miR-425 mimics and overexpression plasmid of PTP4A2 restored the decrease in markers of autophagy caused by miR-425 mimics in 7721 cells, and co-transfection with miR-425 inhibitor and PTP4A2 knockdown vector compromised induction of autophagy caused by miR-425 inhibitor in 7402 cells (Fig. 5D and E). The above data indicate that miR-425 directly regulates PTP4A2 expression to increase the chemosensitivity of sorafenib in HCC cells.

Effect of PTP4A2 on Sorafenib chemosensitivity in HCC in vivo

In order to further confirm the effect of PTP4A2 required for HCC tumor growth *in vivo*, we established a model of xenograft mouse to verify PTP4A2 influence on the sorafenib treatment sensitivity of HCC cells. Inhibition of PTP4A2 expression with sh-PTP4A2 plasmid or miR-425 overexpression lentivirus can reduce the tumorigenicity of 7721 cells and increase the cellular toxicity of Sorafenib in the subcutaneous cancer model (Fig. 6A and C). Up-regulation of PTP4A2 expression with overexpression plasmid or anti-miR-425 lentivirus increased the tumorigenicity of 7402 cells and reduced the cytotoxicity of sorafenib in the model (Fig. 6B and D). In addition, to verify the effect of PTP4A2 on tumor cell apoptosis, we conducted a TUNEL test. The results showed that inhibiting PTP4A2 expression groups can significantly increase the apoptosis of 7721 cells and raise the apoptosis of subcutaneous tumor cells induced by sorafenib (Figure 6E). Up-regulating the expression of PTP4A2 groups can both reduce the apoptosis of 7402 cells and the apoptosis induced by sorafenib (in subcutaneous tumors (Figure 6F).

PTP4A2 regulates the expression of RAB24 in HCC cells

In an effort to identify latent downstream targets regulated by PTP4A2 in suppressing autophagy in HCC cells, we used a Human Autophagy RT² Profiler PCR Array containing 89 autophagy-related genes to compared the different mRNA expression profiles between 7721-shPTP4A2 cells and control 7721-shC cells. In Fig. 7A, we listed three up-regulated genes (UVRAG, RAB24 and RGS19) and four down-regulated genes (HDAC6, TNF, ATG4D and DRAM2) for conditional classification based on the folding change of mRNA level more than twice. The seven targets were validated by a western blot assay (Fig. 7A). We found that RAB24 expression significantly decreased in 7721-PTP4A2-shRNA-1 cells, but the other six genes (ie UVRAG, RGS19, HDAC6, TNF, ATG4D and DRAM2) did not show the expression of differential protein. In addition, we found a positive correlation between PTP4A2 and RAB24 expression through IHC in HCC tissue(Fig. 7B, Left). Therefore, RAB24 was selected from the up-regulated genes for further research.

Overexpression of RAB24 restores PTP4A2-silenced HCC cells proliferation and autophagy

In order to determine whether RAB24 is involved in PTP4A2-induced HCC cell proliferation and autophagy, we performed a rescue experiment. We overexpressed RAB24 in PTP4A2-silenced 7721 cells (Fig. 7B, Right). CCK-8 assay and colony formation assay showed that after RAB24 overexpressed, the suppressed proliferation capacities of PTP4A2-silenced 7721 cells were rescued (Fig. 7C). At the same time, the impaired autophagy of 7721 cells silenced by PTP4A2 was also significantly enhanced. (Fig. 7D).

PTP4A2 promotes HDAC1 accumulation in the cytoplasm of HCC cells and epigenetically up-regulates RAB24 transcription

Subsequently, we investigated the potential mechanisms by which PTP4A2 up-regulates RAB24 in HCC cells. In the present study, we first observed that ectopic overexpression of PTP4A2 in HCC cells could largely increase both the mRNA expression and the RAB24 gene's transcriptional activity (Fig. 7E). These data indicate that PTP4A2 can indirectly regulate the transcription level of RAB24. Histone modification is

an important epigenetic mechanism that regulates gene expression (eg: histone acetylation and/or methylation), and plays an important role in cancer genesis [20, 21]. Next, we explored whether histone acetylation or methylation status were affected by PTP4A2 in RAB24 promoter. The results suggested that the forced PTP4A2 expression dramatically increased the acetylation levels of histones H2AK5, H2BK12 and H3K9/14 on the RAB24 promoter (Fig. 7F, left), whereas the histone methylation levels on the RAB24 promoter did not change (Fig. 7F, right). Furthermore, although ectopic overexpression of PTP4A2 in HCC cells did not change the protein levels of HDAC1, HDAC2 and P300, the trans-localization of HDAC1 but not HDAC2 or P300 from the cell nucleus to cytoplasm was dramatically risen (Fig. 7G). These data provided evidence that PTP4A2 could epigenetically up-regulate RAB24 gene expression by inhibiting the nuclear localization of HDAC1 to bind to the RAB24 promoter in HCC cells.

4. Discussion

Currently, HCC patients with the same tumor clinical/pathological stage often show considerable variability in recurrence and metastasis of tumor. Therefore, it will be meaningful to find new biomarkers that can provide additional staging information to help select treatments and optimize treatment outcomes. Recent studies have noted that the expression of PTP4A2 is upregulated in several cancer types such as breast, colon and pancreatic cancer [19, 20, 21]. Consistent with these studies, we reported for the first time that PTP4A2 expression was markedly upregulated in HCC tissues. In addition, PTP4A2 increases the proliferation of HCC cells and reduces their apoptosis, indicating that PTP4A2 acts as an oncogene in HCC cells. Moreover, our analysis of clinic suggested that HCC patients with high-PTP4A2-expression were remarkably associated with the progression of the disease, indicating that PTP4A2 may be a potential biomarker for evaluating the prognosis of these patients.

Interestingly, we discovered that the upregulation of PTP4A2 was correlated with the activation of the autophagy. Basic autophagy plays a role in suppressing cancer by maintaining the genomic stability in normal cells and during cancer treatment (including radiation, chemotherapy and target therapy), and usually activates autophagy [23, 24]. Research shows that sorafenib treatment can enhance HCC cell autophagy [10-12]. Based on the above research, we found that silencing PTP4A2 can inhibit autophagy and then increase the Sorafenib chemosensitivity in 7721 cells. Meanwhile, the activity of autophagy can be restrained by up-regulated expression of PTP4A2, and also can suppress the Sorafenib chemosensitivity in 7721 cells. All results suggested that autophagy mediated by PTP4A2 may be a novel sensitive target for sorafenib chemotherapy in HCC cells.

Through regulating critical oncogenes or tumor suppressors, miRNA also exerts significant effects on tumor progression [25-27]. Here we find that PTP4A2 is negatively regulated by miR-425. MiR-425 is a highly conserved 23-nucleotide miRNA found on human chromosome 3. We found that up-regulated the expression of miR-425 can suppress cellular proliferation and autophagy, and raise cell apoptosis in HCC cells. The functional recovery of PTP4A2 further confirmed the existence of the "miR-425-PTP4A2-autophagy" axis. Experiments also indicated that miR-425 acts as an anti-oncogene in HCC cells. We

confirmed that miR-425 was capable of increasing sensitivity to Sorafenib in HCC cells by suppressing the PTP4A2–autophagy-axis *in vivo*.

To better understand downstream genes involved in PTP4A2 and HCC cell proliferation and autophagy, a Human Autophagy RT² Profiler PCR Array containing 89 autophagy-related genes was used to compare mRNA expression profiles between 7721-shControl cells and 7721-shPTP4A2 cells. We found and verified that RAB24 acts a PTP4A2 downstream target in HCC cells, which is mainly located in the ER-cis-Golgi membrane and late endosomal structure. To determine if RAB24 is a downstream target involving in PTP4A2-induced HCC cell regulate proliferation and autophagy, a rescue experiment was carried out. After upregulating RAB24 expression in HCC 7721 cells that stably inhibited PTP4A2, it successfully enhanced cell proliferation and autophagy. The above data indicate that RAB24 is a pivotal downstream target of PTP4A2, and PTP4A2 may regulate HCC cell proliferation and autophagy by regulating RAB24.

However, the molecular mechanism by which PTP4A2 regulates RAB24 expression has not been elucidated so far. Abnormally epigenetic regulation of certain cancer-related genes is an important molecular element in cancer development and/or progression[28-30]. As it is known, histone acetylation is a key regulator of gene expression and plays an critical role in tumorigenesis [31].

We further observed that after enforced expression of PTP4A2 in HCC cells, the acetylation levels of histone H2AK5, H2BK12 and H3K9/14 on the promoter of RAB24 were substantially increased concurrent with an apparent translocation of HDAC1 to cytoplasm and a less enrichment of HDAC1 on the RAB24 promoter. These data demonstrate that PTP4A2 may attenuate the transcription of RAB24 in HCC cells by inhibiting the nuclear localization of HDAC1 and epigenetically up-regulate RAB24 gene expression.

To sum up, our report describes the high expression of PTP4A2 in HCC tissues for the first time, and also an indicator of poor prognosis for HCC patients. Stimulating or antagonizing the autophagy axis of PTP4A2-RAB24 can increase or reduce the production capacity of HCC cells. Inhibition of PTP4A2 expression or overexpression of miR-425 can raise the chemosensitivity of sorafenib in HCC cells. Our results demonstrate that PTP4A2 may be a prognostic biomarker and an operable target of HCC chemosensitization.

Abbreviations

HCC, hepatocellular carcinoma; PTP, Protein tyrosine phosphates; CCK8, Cell Counting Kit-8; IHC, Immunohistochemistry; PI, propidium iodide; PVDF, polyvinylidene difluoride.

Declarations

Competing interests

The authors declare that there is no conflict of interests.

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Author contributions

DLC and YH designed research; DLC, YH and MZ performed experiments; DLC, YH, MZ performed data analysis; YG, ZSZ, WY, DX and SY contributed to the guidance of experiments; DLC and YH wrote the manuscript; JXZ and DLC edited and revised manuscript. All authors approved the final version of the manuscript.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Consent for publication

Consent to publish has been obtained from all authors.

Ethics approval and consent to participate

All aspects of this study were approved by the medical ethics committee of Sun Yat-Sen University Cancer Center. All animal studies were performed with approval from the Institutional Animal Care and Use Committee of Sun Yat-Sen University.

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Figures

Figure 1

Figure 1

Over-expression of PTP4A2 is correlated with poor patient survival. (A) The level of PTP4A2 protein and mRNA examined by Western blotting and q-PCR in 7 human HCC cell lines (i.e., 7721, 7402, 7701, 97H, 7703, huh7 and 97L) and one immortalized primary hepatocellular epithelial cell line Lo-2. Among 12 HCC cases, increased expression of PTP4A2 was detected via western blotting in 10 pairs of HCC tissues compared with the matched non-cancerous liver tissues and the mRNA expression of PTP4A2 was significantly up-regulated in 10/12 pairs of HCC tissues based on q-PCR. The expression levels were normalised to those of GAPDH. (N, adjacent normal liver tissue; T, HCC tissue.); (B) The representative images show different staining intensities of PTP4A2: (a) negative staining, (b) weak staining, (c) moderate staining, and (d) strong staining (original magnification, x200 and x400). (C) Kaplan–Meier curves of the OS and PFS of HCC patients with high or low PTP4A2 expression: 245 cases in our Sun Yat-sen University Cancer Center (SYSUCC) cohort (Left panel), and 356 cases in The Cancer Genome Atlas (TCGA) data sets (Right panel) The P value was computed by the log-rank test. **, $P < 0.01$.

Figure 2

Figure 2

PTP4A2 regulates cell proliferation, apoptosis, and drug resistance in HCC cells. (A) Western blotting reveals that PTP4A2 was efficiently knocked down by the treatment of PTP4A2-shRNA-1 or PTP4A2-shRNA-2 in 7721 and 7701 HCC cells and PTP4A2 was efficiently overexpressed in 7402 cells. (B) The cell growth rate of HCC cells between shPTP4A2 and shControl and between OE-PTP4A2 and vector by CCK-8 kit. * $P < 0.05$, ** $P < 0.01$ by Student's t-test. (C) Representative images of decreased foci formation in

monolayer culture induced by PTP4A2 silenced and PTP4A2 overexpression in HCC cells. Data are the means $\pm$ s.d. of three independent experiments. **P<0.01 by Student's t-test. (D) Apoptosis of 7721, 7701 and 7402 cells with modulation of PTP4A2 expression was detected by flow cytometric analysis of annexin V and propidium iodide staining. (E) IC50 values of sorafenib in 7721, 7701 and 7402 cells determined by CCK-8 kit (* P <0.05). (F) The effect of knockdown or overexpression of PTP4A2 on cell proliferation was examined in 7721, 7701 and 7402 HCC cells. CCK-8 was used to detect cell proliferation ability, and propidium iodide and annexin V staining were used to detect apoptosis.

Figure 3

Figure 3

Upregulation of PTP4A2 enhanced the activity of autophagy. (A) GSEA and GO analyses revealed that high expression of PTP4A2 was positively correlated with an enrichment of autophagy gene signatures. (B) Protein expression of p62, Atg7 and LC3-I/II after intervention on PTP4A2 expression was detected by Western blot analysis. (C) Detection of the autophagic flux with the mRFP-GFP-LC3 reporter, which allows differentiation between autophagosomes (GFP + /RFP +, yellow puncta) and autolysosomes (GFP - /RFP +, red puncta). Confocal microscopy images merged with GFP, RFP and DAPI (blue) fluorescence of representative cells were acquired, and the percentages of cells showing accumulation of yellow or red puncta were calculated.

Figure 4

Figure 4

MiR-425 is a negative regulator of PTP4A2. (A) Illustration of screening miRNAs regulating PTP4A2: Method #1 used the prediction algorithm of MICRORNA. ORG identified 23 miRNAs, which hold potential as PTP4A2 suppressors. Method#2 used miRNA profiling and identified 17 miRNAs (from Targetscan), which were downregulated significantly in HCC. Then, we overlapped the results of two methods and singled out miR-425 as a potential regulator. (B) Quantitative RT-PCR assay of miR-425 expression in

seven HCC cell lines. (C) Expression of miR-425 was detected in 7721, 7701 and 7402 cells by q-PCR after transfection with miR-425 mimics or anti-miR-425, respectively (Up). PTP4A2 protein expression was analyzed by Western blot analysis after in 7721, 7701 and 7402 cells after modulation of miR-425 expression (Down). (D) Predicted binding sites for miR-425 within the PTP4A2 3'UTR (from targets can.org). (E) Relative luciferase activity from HEK293T cells co-transfected with miR-425 mimics and pMIR-PTP4A2-3'UTR-WT, pMIR-PTP4A2-3'UTR-MUT1, or pMIR- PTP4A2-3'UTR-MUT2 (Left). Luciferase activity was detected 48 hours after transfection .Negative correlation between PTP4A2 and miR-425 expression in 28 pairs of human primary HCC tissues and their corresponding adjacent noncancerous HCC tissues.****, $P < 0.0001$ (Right). (F) The effects of miR-425 mimics or inhibitors on apoptosis of 7721, 7701 or 7402 cells were assessed through annexin V staining and flow cytometry analysis. (G) 7721 and 7701 cells were transfected with miR-425 mimics, and 7402 cells with miR-425 inhibitors and the CCK-8 method was used to detect cell proliferation. (H) Protein expression of p62 and LC3-I/II was detected by Western blot after intervention on miR-245 expression. (I) Detection of the autophagic flux with the mRFP-GFP-LC3 reporter in 7721 cells with miR-425 mimics, and 7402 cells with miR-425 inhibitors.

Figure 5

Figure 5

Restoration of PTP4A2 expression counteracts miR-425-mediated Sorafenib sensitivity. (A) Protein expression of PTP4A2 was detected by Western blot after 7721 cells transfected with miR-425 mimics by co-transfection with a PTP4A2 overexpression plasmid and 7402 cells transfected with miR-425 inhibitor by co-transfection with a PTP4A2 silencing plasmid. (B) and (C) CCK-8 was used to determine treatment effect on cell proliferation and flow cytometry was used to detect cell apoptosis. (D) and (E) Detection of

the autophagic flux with the mRFP-GFP-LC3 reporter. ****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

Figure 6

Figure 6

Effects of PTP4A2 and miR-425 on Sorafenib chemosensitivity in 7721 and 7402 cells in nude mice. (A) and (B) Tumor tissues isolated from indicated mice at day 35 posttransplant. (C) and (D), The tumor

volumes were calculated at day 35 posttransplant. (E) and (F), Tumor cells apoptosis was tested by TUNEL assay. ****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

Figure 7

PTP4A2 regulates the expression of RAB24 in HCC cells. (A) The seven genes, UVRAG, RAB24, RGS19, HDAC6, TNF, ATG4D and DRAM2), were examined >2-fold mRNA differential expression in 7721-MAEL-shRNA-1 cells compared with that in 7721-shControl cells by using a Human Autophagy RT2 Profiler PCR

Array. Silence of PTP4A2 by shRNA-1 in 7721 cells substantially downregulated RAB24 expression as examined by western blotting. (B) Overexpression of PTP4A2 and high-level expression of RAB24 was examined by IHC in a HCC case; scale bar, 100 μ m. Western blot shows that RAB24 was decreased in 7721-PTP4A2-shRNA-1 cells. (C) CCK-8 kit and colony formation assays show that overexpression of RAB24 substantially increased the proliferation abilities of 7721-PTP4A2-shRNA-1 cells. Data are the means $\pm$ s.d. of three independent experiments; scale bar, 100 μ m. * P <0.05, ** P <0.01 by Student's t-test. (D) Detection of the autophagic flux with the mRFP-GFP-LC3 reporter in 7721-PTP4A2-shRNA-1 cells. (E) Both mRNA expression levels (left) and transcriptional activity (right) of the RAB24 was significantly increased after overexpression of PTP4A2 in HCC cells, as examined by q-PCR and the Dual-Luciferase Reporter assays. (F) ChIP assays demonstrated that enforced expression of PTP4A2 in the 7721 cell line significantly increased the acetylation levels at histone H2AK5, H2BK12 and H3K9/14 on the RAB24 promoter (P <0.01), whereas the tri-methylation levels at histone H3K4, H3K9 and H3K27 on the RAB24 promoter were not altered. (G) HDAC1 and HDAC2 protein levels did not differ between 7721-vector and 7721-PTP4A2 cells. Western blotting demonstrates that the amount of cytoplasmic HDAC1 increased, whereas nuclear HDAC1 decreased in 7721-PTP4A2 cells compared with 7721-vector cells. No difference of HDAC2 or P300 expression was examined between 7721-PTP4A2 and 7721-vector cells.

Supplementary Files

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