The Downregulation of EIF3a Contributes to Vemurafenib Resistance in Melanoma By Activating ERK Via PPP2R1B

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Research

Keywords: melanoma, vemurafenib resistance, eIF3a, ERK, PPP2R1B

Posted Date: May 17th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-498841/v1>

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Abstract

Background: Vemurafenib, a BRAF V600E inhibitor, provides therapeutic benefits for melanoma patients, but the frequently emergence of drug resistance remains a challenge. Understanding the mechanisms behind vemurafenib resistance may generate novel therapeutic strategy for melanoma patients.

Methods: KEGG analysis for eIF3a-mediated pathway and identified the KRAS pathway was significantly enriched after silencing eIF3a. The role of eIF3a in vemurafenib chemosensitivity were investigated by CCK8, and clonogenic assay. To explore the underlying molecular mechanisms of eIF3a on regulating ERK, we performed a series of overexpression and knockdown experiments. In addition, luciferase assay and RIP assay were used to demonstrate the translation regulation between eIF3a and PPP2R1B. The association between eIF3a and PPP2R1B mRNA expression was analyzed using the TCGA datasets.

Results: we demonstrated that eIF3a, a translational regulatory protein, was an important mediator involved in vemurafenib resistance. EIF3a expression was significantly lower in vemurafenib-resistant A375 melanoma cells (A375R) compared with the parental A375 cells. Overexpression of eIF3a enhanced the sensitivity of BRAF inhibitor by downregulating p-ERK expression. We further revealed that eIF3a controls ERK activity through regulating the phosphatase PPP2R1B expression via translation mechanism, thus determining the sensitivity of vemurafenib in melanoma cells. In addition, the positive relationship between eIF3a and PPP2R1B was also demonstrated in tumor samples from HPA and TCGA database.

Conclusion: eIF3a is a potential prognostic predictor for vemurafenib therapy, which may provide new strategy for predicting vemurafenib responses in clinical treatment.

Background

Melanoma is an aggressive cancer with incidence rapidly increasing [1]. About 60% of melanoma patients harbored BRAF kinase mutation (BRAFFV600E), which activates the MAPK pathway and contributes to the immortalization feature of cancer [2]. Selective BRAF inhibitors (BRAFi), such as vemurafenib, have been approved for clinical use and significantly improved progression-free survival (PFS) and overall survival (OS) of the melanoma patients with BRAF mutant compared with the traditional chemotherapy dacarbazine [3-5]. However, the universal emergence of resistance after vemurafenib treatment limited its application in clinic [6-9]. Multiple mechanisms involved in vemurafenib resistance were categorized [8], however, these mechanisms do not fully characterize the causes of vemurafenib resistance, and there is a lack of effective targets and strategies for overcoming clinical vemurafenib resistance, which encourages further research [10].

The eukaryotic translation initiation factor 3a (eIF3a), the largest subunit of eIF3 complex, plays an important role in joining and recruiting mRNA to the ribosome [11-13]. The eIF3a expression is associated with physiological and pathological processes by regulating cell cycle, apoptosis, differentiation and fibrosis [12, 14-16]. Many researchers reported that eIF3a played an important role in the occurrence and

development of tumors, and elevation of eIF3a favored to maintaining the malignant phenotype [17, 18]. Recent reports suggested that eIF3a could mediate glycolytic metabolism and the level of anti-eIF3A autoantibody in serum could act as a potential diagnostic marker for hepatocellular carcinoma [19, 20]. Moreover, there is a great deal of researches suggesting that eIF3a can affect patient prognosis and treatments responses, such as the cancer patients with high eIF3a level had better relapse-free and overall survival than those with low eIF3a expression [21, 22]. Previous study including our own supported the notion that knockdown of eIF3a reduced cellular response to cisplatin by regulating the expressions of DNA repair proteins in lung cancer [23], nasopharyngeal carcinoma [24], and ovarian cancer [25]. Tumia R, *et al* also reported that the high eIF3a expression increased radiotherapy and chemotherapy response in breast, gastric, lung, and ovarian cancer patients [26], further suggesting that eIF3a may affect patient responses to treatments.

Here, this study reported for the first time that eIF3a expression is positively associated with vemurafenib sensitivity in melanoma, and this effect by eIF3a is mediated through regulating the translation of protein phosphatase PPP2R1B that inhibits the phosphorylation of ERK. Furthermore, the relationship between eIF3a and PPP2R1B was detected in cancer patient samples. These findings not only reveal a novel role of eIF3a in the resistance of vemurafenib and present the eIF3a/PPP2R1B/ERK as a new regulatory pathway in cancer, but also may provide a novel biomarker for predicting the vemurafenib response.

Materials And Methods

Cell lines and culture

The human melanoma cell lines A375, SK-28, and HEK293T were cultured in DMEM medium. The vemurafenib-resistant A375 cells (A375R) were cultured in DMEM medium with 2 μ M vemurafenib. All cell culture media were supplemented with 10% fetal bovine serum, 100 units/mL penicillin and 100 μ g/mL streptomycin. All cell lines were maintained at 37°C in a humidified atmosphere containing 5% CO₂/95% air.

Reagents and antibodies

Vemurafenib, dabrafenib and L-mimosine were purchased from Selleck (Shanghai, China). ERK inhibitor SCH772984 was purchased from TOPSCIENCE (Shanghai, China). Antibodies used in immunoblotting: eIF3a and PPP2R1B were purchased from Abcam. ERK and p-ERK (Thr202/Tyr204) were purchased from Cell Signaling Technologies. Anti-Flag and anti-HA was purchased from MBL. β -action and β -Tubulin was purchased from Proteintech.

siRNA and plasmid transfection

siRNA targeting eIF3a and PPP2R1B was purchased from RiboBio (Guangzhou, China). PPP2R1B plasmid was purchased from Gene (Shanghai, China). Transfection of siRNA was carried out according to the manufacturer's protocol. Briefly, cells in exponential phase of growth were plated in six-well tissue

culture plates and then transfected with siRNA using lipofectamine RNAiMax (Invitrogen) reagent and OPTI-MEM medium. Transfection of the plasmid was carried out using lipofectamine 2000 (Invitrogen) reagent according to the manufacturer's protocol.

Western blot analysis

Cells were lysed at ice for 30 minutes in RIPA supplemented with a protease inhibitor cocktail (Biotool), followed by centrifugation at $12,000 \times g$ for 15 minutes at 4 °C. The protein concentration of the supernatant was determined by BCA assay (Beyotime Biotechnology, Shanghai, China). Proteins (20-30 µg) were resolved by SDS-PAGE and then transferred to PVDF membrane (0.22 µm, Merck Millipore). After blocking with skim milk, the PVDF membranes were incubated with the respective antibodies in 5% BSA at 4 °C overnight, followed by incubation with a secondary antibody at room temperature for 1h. The protein signals were detected by an enhanced chemiluminescence kit.

Clonogenic assay

Cells were plated in 6-well tissue culture plates (1000 cells per well) and exposed for indicated treatment at 37°C in a humidified atmosphere containing 5% CO₂/95% air. At the end of incubation, cells were fixed with 4% paraformaldehyde and stained with crystal violet for 30 min, washed with PBS, and then the colonies were photographed and counted.

Acquisition and analysis of GEO data

Gene expression from GSE118239 was extracted from the NCBI Gene Expression Omnibus (GEO) database and analyzed using the R2: Genomics Analysis and Visualization Platform (<http://r2.amc.nl>). Normalized log2 transformed gene expression data were downloaded from the R2 platform to a Microsoft Excel spreadsheet for additional analysis.

Cell viability assay

The cell viability was measured using a CCK8 (Bimake, Shanghai, China) reagent. Briefly, 2×10^3 cells/well were planted in 96-well-plate and treatment with various drugs concentrations for indicated durations. After treatment, 10 µL CCK8 reagent was added into each well and incubated for 1h. The absorbance was read at 450nm wavelength.

Quantitative real-time PCR

Total RNAs were isolated from cells using the Trizol reagent (Takara) and 1st strand cDNA was synthesized using PrimeScript RT Reagent Kit (Perfect real time) (Takara). Real time PCR was performed using SYBR Premix Ex Tap (Takara), and was run on LC480. For quantification of gene expression, the $2^{-\Delta\Delta Ct}$ method was used. The expression level of actin was used for normalization.

Luciferase Reporter Assay

To analyze PPP2R1B translation activity, the 293T cells were co-transfected with the PPP2R1B-containing luciferase reporter plasmid after silencing eIF3a. Cells were harvested and the luciferase activities were measured using Promega's Luciferase Assay System (Promega, Madison, Wisconsin).

RNA Immunoprecipitation (RIP)

The RIP assay was performed according to the manufacturer's instructions using EZMagna RIP Kit (Millipore, Billerica, MA). In briefly, 293T cell lysates were incubated with anti-eIF3a antibodies or anti-IgG with rotation at 4°C overnight. Next, the expression of PPP2R1B mRNAs was determined by qRT-PCR.

Statistical analysis

All experiments were performed at least three times. For measurement of CCK8 assays statistical analyses were performed using the two-tailed Student's *t*-test in GraphPad Prism software. *p* values < 0.05 were considered statistically significant.

Results

eIF3a is associated with vemurafenib sensitivity

To explore the potential function of eIF3a in cancer therapy, gene set enrichment analysis (GSEA) was used for pathway enrichment analysis. KEGG analysis showed that the KRAS signaling pathway was significantly enriched after knockdown of eIF3a (**Figure 1a**). It has been reported that reactivation of RAS is associated with vemurafenib resistance [27]. Based on this viewpoint, we hypothesize that eIF3a may be involved in vemurafenib resistance. To test this hypothesis, we firstly compared the level of eIF3a in A375 and A375R cells, which is less responsive to vemurafenib than A375 cells (**Figure 1b**), and found that there is a significant downregulation of eIF3a in A375R cells (**Figure 1c**). We further found that knockdown of eIF3a by siRNA decreased the responses of human melanoma cells to vemurafenib, as demonstrated by CCK8 assay and colony formation assay (**Figure 1d-e**). Furthermore, overexpression of eIF3a rendered A375R cells more sensitive to vemurafenib (Fig. 1f). To further support the idea that eIF3a is associated with vemurafenib sensitivity, A375 cells were transfected with different amounts of siRNA, followed by vemurafenib treatment. Fig.1g-h showed that the level of eIF3a was positively correlated with the sensitivity of vemurafenib. These data suggest that the reduction of eIF3a in melanoma may result in vemurafenib resistance.

In addition, we also evaluated the effect of eIF3a on the anti-tumor activity of dabrafenib, another BRAF inhibitor approved by the FDA to treat melanoma [28]. Consistent with the results obtained with vemurafenib, silencing of eIF3a also reduced the cytotoxicity of dabrafenib (**Figure 2a-b**), and the expression of eIF3a was positively correlated with the toxicity of dabrafenib (**Figure 2c-d**). These experiments demonstrated the involvement of eIF3a in regulating the sensitivity of dabrafenib in the human melanoma cells.

eIF3a affects the sensitivity of vemurafenib by activating ERK signaling

Next, we investigated the molecular mechanism involved in the regulation of eIF3a in sensitivity to vemurafenib. Figure 1a shows that inhibiting eIF3a activates the RAS signaling pathway. It's well known that ERK is the classical downstream of RAS signaling pathway, and the aberrant activation of ERK plays a central role in vemurafenib resistance [29]. Consistently, we also verify that the A375R cells harbored higher p-ERK and lower eIF3a compared with A375 cells (**Figure 3a**). Therefore, we next determined whether eIF3a regulates the sensitivity of vemurafenib by modulating the activity of ERK. As expected, inhibiting eIF3a by either siRNA or L-mimosine, a small molecule inhibitor of eIF3a, significantly increased the phosphorylation of ERK in melanoma cells (**Figure 3b-c**). Figure 3d-e further demonstrated the negative correlation between the expressions of eIF3a and p-ERK. Moreover, the regulation of eIF3a on p-ERK was also observed in non-small cell lung cancer and breast cancer cell lines (**Figure S1**). The ectopic expression of eIF3a resulted in a reduction in phosphorylation of ERK (**Figure 3f**). To further validate the effect of eIF3a on the activation of ERK, we re-introduced eIF3a into eIF3a knockdown cells by transfecting an eIF3a overexpression plasmid, and then measured p-ERK. As shown in figure 3g, the p-ERK was increased in the cells when eIF3a expression was knocked down, and eIF3a overexpression blocked the up-regulation of p-ERK in the cells subjected to silencing of eIF3a expression. Furthermore, knockdown of eIF3a in A375 cells could mitigate the suppression effects of vemurafenib on p-ERK, which revealed that vemurafenib-induced MAP kinase signaling blocking was attenuated by silencing eIF3a (**Figure 3h**). And by combination with an ERK inhibitor, the insensitivity to vemurafenib after knockdown of eIF3a was overcome (**Figure 3i-j**). These results indicated that the loss of eIF3a contributed to the activation of ERK, in turn conferring BRAF inhibitor resistance.

eIF3a suppresses ERK activity through upregulating the expression of PPP2R1B

We next sought to investigate the molecular mechanism underlying eIF3a knockdown-mediated the activation of ERK. It has been reported that the MAPK pathway can be dephosphorylated by numerous protein phosphatases, for example, reduction of dual specificity phosphatase 4 (DUSP4) and dual specificity phosphatase 6 (DUSP6) results in activation of the MAP kinase pathway [30-32]. Therefore, we wondered whether there was a phosphatase involved in regulating eIF3a knockdown-mediated ERK activation. In order to assess this hypothesis, we firstly measured the expressions of proteins phosphatases after silencing eIF3a by mass spectrometry. Among those examined, PPP2R1B, a regulatory subunit of the PP2A complex [33], was decreased in eIF3a-knockdown cells compared with that in control cells (**Figure 4a**).

Consistent with the results of proteomics, knockdown or inhibition of eIF3a by siRNA or small-molecule inhibitor caused a decrease in PPP2R1B and an increase in p-ERK (**Figure 4b-c**), and overexpression of eIF3a resulted in PPP2R1B elevation (**Figure 4d**). Figure 4e further demonstrated that eIF3a could regulate the expressions of PPP2R1B. In addition, the regulation of eIF3a on PPP2R1B protein levels was also determined in non-small cell lung cancer cells (**Figure S2**). Next, we examined whether there is a regulatory effect between PPP2R1B and ERK. As shown in figure 4f, silencing of PPP2R1B caused a significant increase in p-ERK, and overexpression of PPP2R1B induced a remarkable reduction in p-ERK (**Figure 4g**). To further determine whether PPP2R1B mediates the regulation of eIF3a in the activity of

ERK, we overexpressed PPP2R1B after silencing eIF3a. Figure 4h shows that re-introduction of PPP2R1B reversed the upregulation of p-ERK by eIF3a deficiency.

We further investigated the functional role of PPP2R1B in vemurafenib resistance, and found that PPP2R1B knockdown reduced the sensitivity of vemurafenib in A375 cells, and overexpression of PPP2R1B significantly enhanced the anti-tumor effect of vemurafenib (**Figure S3a-b**). Furthermore, ectopic expression of PPP2R1B in cells silenced eIF3a partially restored sensitivity to vemurafenib (**Figure S3c**). These findings support that eIF3a controls the action of ERK through regulating the PPP2R1B expression, thus determining the sensitivity of vemurafenib in melanoma cells.

eIF3a up-regulates the expression of PPP2R1B by promoting its translation

Next, we wanted to explore how eIF3a affects the expression of PPP2R1B. We further examined the mRNA level of PPP2R1B after knockdown or overexpression of eIF3a. The results showed that silencing or overexpression of eIF3a did not change the mRNA level of PPP2R1B (**Figure 5a-b**), indicating that eIF3a regulates PPP2R1B at the post-transcription level. Given that eIF3a is a translation regulator, we then examined whether eIF3a controls the expression of PPP2R1B through regulating its translation. Figure 5c indicates that the mRNA of PPP2R1B could bind to the eIF3a protein. In addition, luciferase assay shows silencing of eIF3a inhibited PPP2R1B translation (**Figure 5d**). Furthermore, HCQ, an autophagy inhibitor, or MG132, a proteasome inhibitor, had no effect on the down-regulation of PPP2R1B in the cells with eIF3a knockdown (**Figure 5e-f**). These results indicate that eIF3a could regulate the translation of PPP2R1B.

The association of eIF3a and PPP2R1B is validated in melanoma patient samples

To further validate the association between eIF3a and PPP2R1B in human melanoma patient samples, we first assessed protein expression levels of eIF3a and PPP2R1B by the Human Protein Atlas (<https://www.proteinatlas.org/>). The results showed that the expression of eIF3a was positively related to that of PPP2R1B in melanoma patients (**Figure 6a**). We further analyzed the correlation between eIF3a and PPP2R1B by TCGA database. Consistently, the strong positive correlation between eIF3a and PPP2R1B was observed in melanoma, lung cancer and breast cancer (**Figure 6b-d**). In addition, we also analyzed the relationship between eIF3a and PPP2R1B in primary, metastasis and uveal melanoma by using the TIMER web server (<https://cistrome.shinyapps.io/timer/>), and found the expression of eIF3a was positively associated with PPP2R1B (**Figure 6e**).

Discussion

Acquired resistance to vemurafenib is a major hurdle in the management of melanoma, thereby, it is necessary and imperative to explore the mechanism behind vemurafenib resistance and develop more effective therapeutic strategies for BRAF(V600E) mutation melanoma patients. In this study, we revealed for the first time the potentially important role of eIF3a in targeted therapy. We found that eIF3a was downregulated in vemurafenib-resistant A375 cells, and its depletion significantly decreased the cellular

response to BRAF inhibitors in melanoma cells. We further elucidated that eIF3a modulated the sensitivity of vemurafenib by promoting the translation of PPP2R1B, and PPP2R1B further resulted in decreased ERK activity by dephosphorylation, indicating that eIF3a/PPP2R1B/ERK axis was a key mediator of melanoma resistance to vemurafenib (**Figure 7**). The positive relationship between eIF3a and PPP2R1B was also demonstrated in the melanoma tissue. These results suggested that eIF3a may be a predictor to evaluate the efficacy of BRAF inhibitors

As a key regulator in cancer, eIF3a is highly expressed in multiple cancers and is relevant to the sensitivity of DNA damage-induced therapy such as platinum [25] and ionizing radiation [26]. Our previous study demonstrated that eIF3a could increase the sensitivity of platinum-based chemotherapy in ovarian cancer and non-small cell lung cancer, and the patients with high level of eIF3a have a better prognosis [23, 25].

Activation of ERK is critical for cell proliferation and differentiation, and even impact the response of cancer cells to chemotherapies and targeted therapies [34]. In addition, ERK also could regulate the metabolic processes of cancer cell, such as glucose metabolism and fatty acid metabolism, and promote cancer cell invasion and migration [35]. As a critical mechanism significantly affecting the cancer pathogenesis, the regulation of ERK has been intensively investigated. Previous studies showed that activation levels of ERK can be regulated by multiple proteins or miRNA, including BOP1, VRK3, and miR-30 [36, 37]. Therefore, a better understanding of ERK-mediated drug resistance may be helpful for deepening anti-tumor mechanism studies. Here, we demonstrated that the reduction of eIF3a caused ERK activation, as evidence by increased ERK phosphorylation, thus leading to vemurafenib resistance, which may help provide novel strategy to rescue the drug sensitivity for anti-cancer drug development.

To illustrate the regulatory mechanism of eIF3a in the activation of ERK, we performed proteomics technology by mass spectrometry to identify eIF3a interacting proteins. PPP2R1B, a phosphatase, was shown to be decreased in cells subjected to eIF3a knockdown. We demonstrated that eIF3a knockdown or overexpression decreased or increased the protein expression of PPP2R1B, respectively. In addition, consistent with other phosphatases of ERK, PPP2R1B knockdown in melanoma cell lines led to increased ERK phosphorylation and mediated the negative regulation of eIF3a in vemurafenib sensitivity. As a tumor-suppressor factor, the mutations and alterations of PPP2R1B have been found in human cancers, including colon cancer, lung cancer and cervical cancer, and involved in the chemotherapy sensitivity [38-40]. Previous studies have reported that silencing PPP2R1B enhanced 5-FU resistance [41], and overexpress of PPP2R1B leads to tongue squamous cell carcinoma and esophageal cancers cells more sensitivity to docetaxel and cisplatin respectively by acting as an AKT phosphatase [33, 42]. In this study, we found that PPP2R1B influenced the vemurafenib sensitivity by inhibiting ERK phosphorylation, and ectopic expression of PPP2R1B restored cell sensitivity to vemurafenib. Our study suggested that ERK may be a novel protein target of PPP2R1B phosphatase and PPP2R1B may be a potential target for developing novel strategies against vemurafenib resistance.

Lastly, we also found strong correlations in cancer tissue between eIF3a and PPP2R1B from HPA database and TCGA database, further supporting our findings in cells. In addition, the interaction between

eIF3a and PPP2R1B was also found in lung cancer and breast cancer tissue, which suggested that the mechanism is universal. As the importance of vemurafenib in clinical application, investigating the regulatory axis of eIF3a-PPP2R1B-ERK is crucial and worthwhile. Moreover, our findings highlight eIF3a as a promising biomarker for melanoma or other cancers to predict the therapeutic effect of vemurafenib.

Taken together, our results revealed that the eIF3a-PPP2R1B-ERK axis may contribute to the resistance of vemurafenib, which not only added new insights about vemurafenib resistance, but also provided new clues on how to predict the response treatment to vemurafenib.

Conclusion

In summary, we clarified the potential role of eIF3a in vemurafenib resistance in melanoma for the first time. We further revealed that eIF3a controls ERK activity through regulating the phosphatase PPP2R1B expression via translation mechanism, thus determining the sensitivity of vemurafenib in melanoma cells. This study may provide new strategy for predicting vemurafenib responses in clinical treatment.

Abbreviations

eIF3a: eukaryotic translation initiation factor 3a

ERK: p44/42 MAPK (Erk1/2)

PPP2R1B: Protein phosphatase 2 scaffold subunit Abeta

RIP: RNA Immunoprecipitation

TCGA: The Cancer Genome Atlas

GEO: Gene Expression Omnibus

qPCR: Quantitative real-time PCR

Declarations

Ethics approval and consent to participate: The study was approved by the ethical review committee of Xiangya Hospital, Central South University.

Consent for publication: Not applicable

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

Funding: This work was supported by National Key Research and Development Program of China (2016YFC1306900), National Natural Science Foundation of China (81874327), Key Research and Development Program of Hunan Province (2019SK2251), Innovation and Research Project of Development and Reform Committee of Hunan Province (2019-875), Project Program of National Clinical Research Center for Geriatric Disorders (Xiangya Hospital, 2020LNJJ02) and Science and Technology Program of Changsha (kh2003010).

Author contributions: ZQL and YC designed, conceived the study, and revised the manuscript. SLJ, ZBW and TZ performed the experiments and analyzed data. HHZ provided experiment platform. SLJ drafted the manuscript. CL, TJ, CL and JFF provided technological support in the experiments.

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Figures

Figure 1

eIF3a is associated with vemurafenib sensitivity. a. Enrichment plots from gene set enrichment analysis (GSEA) of eIF3a in KRAS signaling pathway. b. A375 and A375R cells were treated with a series of concentrations of vemurafenib for 72h, and the cell survival was measured by the CCK-8 assay. *p<0.05,

****p<0.01, t-test.** c. The expression of eIF3a in A375 cells and A375R cells was measured by western blot. β -actin was used as a loading control. d. A375 and SK-28 cells were transfected with the nontargeting RNA or eIF3a siRNA and then treated with vemurafenib for 72h, and cell viability was measured by the CCK-8 assay. *p<0.05, **p<0.01, t-test. e. A375 cells were transfected with eIF3a siRNA and treated with vemurafenib, and cell proliferation was measured by the colony formation assay. *p<0.05, **p<0.01, t-test. f. A375R cells transfected with the empty vector or Flag-eIF3a plasmid and then treated with vemurafenib for 72h, and cell viability was measured by the CCK-8 assay. *p<0.05, **p<0.01, t-test. g-h. A375 and SK-28 cells were transfected with a non-targeting siRNA or an eIF3a siRNA with the indicated concentrations, then treated with vemurafenib for 72h, and cell viability was measured by the CCK-8 assay. *p<0.05, **p<0.01, t-test.

Figure 2

eIF3a was also associated with in dabrafenib sensitivity. a-b. A375 and SK-28 cells were transfected with the nontargeting RNA or eIF3a siRNA and then treated with dabrafenib for 72h, and cell viability was measured by the CCK-8 assay. *p<0.05, **p<0.01, telF3a affects the sensitivity of vemurafenib by reactivation of ERK signaling. a. The level of eIF3a, p-ERK and ERK in A375 cells and A375R cells were measured by western blot. b. A375 and SK-28 cells were transfected with a non-targeting siRNA or an eIF3a siRNA, the expressions of eIF3a, p-ERK and ERK were measured by western blot. c. A375 or SK-28 cells were treated with eIF3a inhibitor L-mimosine with the indicated concentrations for 24h, the expressions of eIF3a, p-ERK and ERK were measured by western blot. d. A375 and SK-28 cells were transfected with a non-targeting siRNA or an eIF3a siRNA with the indicated concentrations, the expressions of eIF3a, p-ERK and ERK were measured by western blot. e. HEK293T cells were transfected

with a non-targeting siRNA or an eIF3a siRNA, the expressions of eIF3a, p-ERK and ERK were measured by western blot. f. HEK293T cells were transfected with a control plasmid or a flag-eIF3a plasmid, the expressions of eIF3a, p-ERK and ERK were measured by western blot. g. HET293T cells were transfected with a non-targeting siRNA or an eIF3a siRNA, followed by transfection with a Flag-eIF3a expression plasmid. The expressions of Flag, eIF3a, p-ERK and ERK were measured by western blot. h. A375 cells were transfected with the nontargeting RNA or eIF3a siRNA and then treated with vemurafenib. The expressions of eIF3a, p-ERK and ERK were measured by western blot. i. SK-28 cells were transfected with a non-targeting siRNA or an eIF3a siRNA, then treated with vemurafenib, or combined with ERK inhibitor. The cell viability was measured by the CCK-8 assay. * $p < 0.05$, ** $p < 0.01$, t-test. c. A375 cells were transfected with a non-targeting siRNA or an eIF3a siRNA with the indicated concentrations, the expression of eIF3a was measured by western blot. β -tubulin was used as a loading control. d. A375 and SK-28 cells were transfected with a non-targeting siRNA or an eIF3a siRNA with the indicated concentrations, then treated with dabrafenib for 72h, and cell viability was measured by the CCK-8 assay. * $p < 0.05$, ** $p < 0.01$, t-test.

Figure 3

eIF3a affects the sensitivity of vemurafenib by reactivation of ERK signaling. a. The level of eIF3a, p-ERK and ERK in A375 cells and A375R cells were measured by western blot. b. A375 and SK-28 cells were transfected with a non-targeting siRNA or an eIF3a siRNA, the expressions of eIF3a, p-ERK and ERK were

measured by western blot. c. A375 or SK-28 cells were treated with eIF3a inhibitor L-mimosine with the indicated concentrations for 24h, the expressions of eIF3a, p-ERK and ERK were measured by western blot. d. A375 and SK-28 cells were transfected with a non-targeting siRNA or an eIF3a siRNA with the indicated concentrations, the expressions of eIF3a, p-ERK and ERK were measured by western blot. e. HEK293T cells were transfected with a non-targeting siRNA or an eIF3a siRNA, the expressions of eIF3a, p-ERK and ERK were measured by western blot. f. HEK293T cells were transfected with a control plasmid or a flag-eIF3a plasmid, the expressions of eIF3a, p-ERK and ERK were measured by western blot. g. HET293T cells were transfected with a non-targeting siRNA or an eIF3a siRNA, followed by transfection with a Flag-eIF3a expression plasmid. The expressions of Flag, eIF3a, p-ERK and ERK were measured by western blot. h. A375 cells were transfected with the nontargeting RNA or eIF3a siRNA and then treated with vemurafenib. The expressions of eIF3a, p-ERK and ERK were measured by western blot. i. SK-28 cells were transfected with a non-targeting siRNA or an eIF3a siRNA, then treated with vemurafenib, or combined with ERK inhibitor. The cell viability was measured by the CCK-8 assay. *p<0.05, **p<0.01, t-test.

Figure 4

eIF3a regulates ERK activity through controlling expression of PPP2R1B. a. Three differential expression of protein phosphatases were found after silencing eIF3a by mass spectrometry. b. A375 and SK-28 cells were transfected with a non-targeting siRNA or an eIF3a siRNA, the expressions of eIF3a, PPP2R1B, p-ERK and ERK were measured by western blot. c. A375 or SK-28 cells were treated with eIF3a inhibitor L-m with the indicated concentrations for 24h, the expressions of eIF3a, PPP2R1B, p-ERK and ERK were measured by western blot. d. HEK293T cells were transfected with a control plasmid or a flag-eIF3a plasmid, the expressions of flag, eIF3a, PPP2R1B, p-ERK and ERK were measured by western blot. e. A375 cells were transfected with a non-targeting siRNA or an eIF3a siRNA with the indicated concentrations, the expressions of eIF3a, PPP2R1B and p-ERK were measured by western blot. f. A375 and SK-28 cells were transfected with non-targeting siRNA or PPP2R1B siRNA, the expressions of eIF3a, PPP2R1B, p-ERK and ERK were measured by western blot. g. A375 and A375R cells were transfected with a control plasmid or a HA-PPP2R1B plasmid, the expressions of eIF3a, PPP2R1B, p-ERK and ERK were measured by western blot. h. SK-28 or HEK293T cells were transfected with a non-targeting siRNA or an eIF3a siRNA, followed by transfection with a HA-PPP2R1B plasmid. The expressions of eIF3a, HA, PPP2R1B, p-ERK and ERK were measured by western blot.

Figure 5

eIF3a could regulate the translation of PPP2R1B. **a.** A375 cells were transfected with a non-targeting siRNA or an eIF3a siRNA, the mRNA of eIF3a and PPP2R1B were measured by q-PCR. **b.** HEK293T cells were transfected with a control plasmid or a flag-eIF3a plasmid, the mRNA of eIF3a and PPP2R1B were measured by q-PCR. **c.** RIP assay indicating that PPP2R1B mRNA could bind to eIF3a protein. **d.** Luciferase assay shows that the translation activity of PPP2R1B in the control or eIF3a knockdown

HEK293T cells. e. A375 cells were transfected with a non-targeting siRNA or an eIF3a siRNA, followed by treatment with 20μM HCQ for 24h. The expressions of eIF3a, LC3 and PPP2R1B were measured by western blot. f. A375 cells were transfected with a non-targeting siRNA or an eIF3a siRNA, followed by treatment with 20μM MG132 for 4h. The expressions of eIF3a and PPP2R1B were measured by western blot.

Figure 6

Association of eIF3a and PPP2R1B expression in melanoma patients. a. IHC analyses of eIF3a and PPP2R1B levels of melanoma tissues on the HPA database. b. Pearson correlation analyses of the mRNA levels of eIF3a and PPP2R1B in melanoma (Data from TCGA). c. Pearson correlation analyses of the mRNA levels of eIF3a and PPP2R1B in lung cancer. d. Pearson correlation analyses of the mRNA levels of eIF3a and PPP2R1B in breast cancer. The correlation coefficient (R) and P value (by two-sided t-test) were indicated. e. Pearson correlation analyses of the mRNA levels of eIF3a and PPP2R1B in primary, metastasis, uveal melanoma. TPM represents transcripts per million.

Figure 7

Model for the role of eIF3a-PPP2R1B-ERK axis in regulation of the BRAF inhibitor resistance. In normal BRAF mutation cells, the BRAF inhibitor could suppress the activity of ERK and inhibited cell proliferation. When eIF3a is depleted, the translation of PPP2R1B was inhibited, which results in the persistence of p-ERK, leading to the occurrence of drug-resistance.

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