Histol Histopathol (2024) 39: 1159-1165

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORIGINAL ARTICLE Open Access

Targeting PEAK1 sensitizes anaplastic thyroid carcinoma cells harboring BRAF^{V600E} to Vemurafenib by Bim upregulation

Qiuhan Wang[1], Fengyun Hao[2], Liang Ning[3] and Chong Sun[4]

[1]Department of Nuclear Medicine, [2]Department of Pathology, [3]Department of Thyroid Surgery and [4]Department of Spine Surgery, the Affiliated Hospital of Qingdao University, Qingdao, Shandong, PR China

Summary. Pseudopodium-enriched atypical kinase 1 (PEAK1) has been demonstrated to be upregulated in human malignancies and cells. Enhanced PEAK1 expression facilitates tumor cell survival and chemoresistance. However, the role of PEAK1 inhibition to anaplastic thyroid carcinoma cell (ATC) and vemurafenib resistance is still unknown. Here, we observed that targeting PEAK1 inhibited cell viability and colony formation, but not cell apoptosis in both of the 8505C and Hth74 cells *in vitro*. Targeting PEAK1 sensitized 8505C and Hth74 cells to Vemurafenib by inducing cell apoptosis, and thereby decreasing cell viability. Mechanistically, Vemurafenib treatment upregulated PEAK1 expression. Combined PEAK1 depletion and Vemurafenib treatment upregulated Bim expression. Targeting PEAK1 sensitized Vemurafenib-induced apoptosis by upregulating Bim. In conclusion, Vemurafenib resistance in ATC cells harboring BRAF^{V600E} is associated with PEAK1 activation, resulting in the inhibition of pro-apoptotic Bim protein. Therefore, targeting PEAK1 may be an effective strategy to sensitize ATC harboring BRAF^{V600E} to Vemurafenib.

Key words: Anaplastic thyroid carcinoma, BRAF inhibitors, Vemurafenib, Apoptosis, Chemoresistance, Pseudopodium-enriched atypical kinase 1

Introduction

Anaplastic thyroid carcinoma (ATC) is a rare form of undifferentiated thyroid carcinoma, which represents about 1-2% of all thyroidal malignancies with an overall survival from 3 to 5 months and a one year survival rate of 20% in most of the cases (Lee et al., 2016; Molinaro et al., 2017). ATC is commonly derived from a differentiated tumor such as papillary thyroid cancer (PTC) or follicular thyroid cancer (FTC), which precedes or coexists with approximately 50% of ATCs. ATC typically harbors several oncogenic mutations, most commonly in the MAPK pathway (Ragazzi et al., 2014). The harbor activating BRAF^{V600E} mutation is the most prevalent genetic alteration in PTC (36-86%) and ATC (20-25%). BRAF^{V600E} mutations are an early and common driver mutation in PTC. Thus effective treatment of ATC through BRAF^{V600E} would meet an urgent clinical need.

Vemurafenib is the BRAF^{V600E} inhibitor, an effective and well-tolerated treatment strategy in advanced PTC patients harboring the BRAF^{V600E} mutation (Poulikakos et al., 2011; Dadu et al., 2015). BRAF inhibitors may yield short-term clinical benefits in these patients because of the resistance to Vemurafenib (Bollag et al., 2010; Montero-Conde et al., 2013; Boussemart et al., 2014; Jeong et al., 2019). However, resistance to Vemurafenib inhibition can be achieved by combined therapeutic modalities for MAPK pathway inhibition (Robert et al., 2015). In a mouse model, Vemurafenib inhibited growth of human ATC cells with BRAF^{V600E} mutation (Nehs et al., 2012). In 8505C, an ATC cell line model, 8505C had poor treatment response with Vemurafenib alone, but increased effect with Vemurafenib in combination with MAPK inhibitors (Ayroldi et al., 2018).

Vemurafenib can induce apoptotic cell death mediated by caspase-3, suggesting that the addition of a procaspase-3 activator could enhance anticancer effects (Adams and Cory, 2007). Vemurafenib also enhanced anti-apoptotic Mcl-1 and suppressed pro-apoptotic Bim (Bauer et al., 2017). Therefore, pro-apoptotic protein activation would improve the efficacy of Vemurafenib and counteract the resistance to Vemurafenib.

Enforced PEAK1 expression was reported to

Corresponding Author: Fengyun Hao, the Affiliated Hospital of Qingdao University, No 16, Jiangsu Road,Qingdao, Shandong, 266003 PR China. e-mail: haofengyun5166209@qdu.edu.cn or Chong Sun, the affiliated hospital of Qingdao University, No 16, Jiangsu Road, Qingdao, Shandong, 266003 PR China e-mail: sunchongjz@qduhospital.cn
www.hh.um.es. DOI: 10.14670/HH-18-705

promote invasion in pancreatic ductal epithelial cells through enhanced JAK1/Stat3 signaling (Strnadel et al., 2017). In basal breast cancer cells, PEAK1 upregulation activates ERK1/2 and Stat3 signals, and decreases cell viability; On the contrary, PEAK1 downregulation inactivates ERK1/2 and Stat3 signals, and increases cell viability (Tactacan et al., 2015). A recent study found that collagen-mediated activation of DDR1 induced PEAK1, SRC, and PYK2 signals in pancreatic cancer cells, resulting in collagen-induced chemoresistance and tumor progression (Aguilera et al., 2017). These accumulating data indicate that PEAK1 regulates chemoresistance. Therefore, PEAK1 is possibly a new therapeutic target for cancer. However, the role and mechanisms of PEAK1 on $BRAF^{V600E}$ inhibitor resistance in ATC cells is unknown. In the present study, we examined the role and mechanisms of PEAK1 inhibition on the sensitivity of ATC cells to Vemurafenib.

Materials and methods

Cell culture

The $BRAF^{V600E}$ mutant ATC cell line 8505C and Hth74 were obtained from European Collection of Cell Cultures. The two cell lines were cultured in complete RPMI 1640 medium containing 10% fetal bovine serum, and authenticated and detected for *Mycoplasma* before use, and the identity of the cell lines was verified by short tandem repeat (STR) analysis. A monolayer of 50-70% confluent cells was used in all of the assays.

siRNA (Small interfering RNA) transfection

siRNA oligonucleotides targeting Pseudopodium Enriched Atypical Kinase 1 (PEAK1 siRNA) were from Sigma (SASI_Hs02_00357289). siRNA guide sequences targeting BIM (Bim siRNA) were obtained from CST (Cell Signaling Technology, Shanghai, China). Nontargeting siRNA was purchased from Dharmacon and served as a negative control. Cells were seeded 24h before transfection at 50-60% confluence in growth medium without antibiotics. 100 nM siRNAs were then transfected into culture's 8505C and Hth74 cells using Lipofectamine 2000 reagent (Invitrogen, Shanghai, China) following the manufacturer's instructions. The cells were harvested 24-72h after transfection for Western blot analyses. To get the stable PEAK1 siRNA/NC siRNA colony, 8505C and Hth74 cells were transfected into PEAK1 siRNA/NC siRNA for 24h, then selected by puromycin (1 μg/mL) for 7 days.

Drug treatments

Vemurafenib was dissolved in absolute dimethyl sulfoxide (DMSO, vehicle) (Sigma, USA) to achieve a stock concentration of 10 mM for *in vitro* assays. 8505C or Hth74 cells were treated for 0-72h in the presence of 0.2% FBS DMEM at final 2% DMSO with: 0.1,0.5,1, 2.5, 5 or 10 μM of Vemurafenib.

Western blot assay

Cells were lysed with 100 μL Triton X-100 lysis buffer and clarified by centrifugation at 12,500 rpm for 30 minutes. Protein concentrations were measured according to the manufacturer's protocol (Bio-Rad). Cell lysates containing 30 μg total protein were analyzed using the standard Western blotting methods. The primary antibodies were obtained from Cell Signaling Technologies: PEAK1, Bim and α-Tubuline. The secondary antibody (anti-rabbit IgG) was from Santa Cruz Biotechnology. Equal protein sample loading was monitored using a α-Tubuline antibody. Protein bands were detected using chemiluminescence reagents.

MTT assay

The 8505C and Hth74 cells were plated at a density of 5000 cells/well in a 96-well plate and treated the next day with PEAK1siRNA or NS siRNA (100 nmol/L) for 1-5 days. To explore the effect of Vemurafenib (Selleck Chemicals, Houston, TX) on the cell viability, cells were plated on a 96-well plate (5000 cells/well) to determine the dose-response after 3 days of Vemurafenib or vehicle (DMSO) treatment. To explore the effect of PEAK1siRNA or NS siRNA on Vemurafenib sensitivity, the cells were plated at a density of 5000 cells/well in a 96-well plate and treated the next day with PEAK1 siRNA or NS siRNA (100 nmol/L) for 16h, then treated with a series of concentrations of Vemurafenib or vehicle (DMSO) for 3 days. To explore the effect of Bim on Vemurafenib sensitivity, the stable PEAK1 siRNA/NC siRNA colonies were plated at a density of 5000 cells/well in a 96-well plate and treated the next day with Bim siRNA or NS siRNA (100 nmol/L) for 16h, then treated with a series of concentrations of Vemurafenib or vehicle (DMSO) for 3 days. MTT was added at the end point to determine cell viability.

Colony formation assay

The stable PEAK1 siRNA or NS siRNA transfected cells (1×10^3) were seeded into 6-well plates and cultured for 7 days. After 7 days, cells were fixed and stained with MayGrunwald-Giemsa. The number of colonies were counted and reported in graphs.

Apoptosis analyses

Cell apoptosis in early and late stages was detected using an Annexin V-FITC apoptosis detection kit from Bio-Vision (Mountain View, USA) according to the manufacturer's protocol. Briefly, 3×10^5 8505C and Hth74 cells were seeded into 6-well plates and treated the next day with PEAK1 siRNA or NS siRNA (100 nmol/L) for 3 days. Furthermore, 8505C or Hth74 cells

(3×10^5) were seeded into 6-well plates and treated the next day with PEAK1 siRNA or Bim siRNA or NS siRNA (100 nmol/L) for 16h, then exposed to Vemurafenib for 3 days. In addition, the stable PEAK1siRNA or NS siRNA transfected cells (3×10^5) were seeded into 6-well plates and treated the next day with Bim siRNA or NS siRNA (100 nmol/L) for 16h, then exposed to Vemurafenib for 3 days. Then the cells were collected and flow cytometry was performed. All samples were assayed in triplicate.

Statistical analysis

The Chi-square test or Student's t test was used for analysis of the significance of each corresponding group.

$p<0.05$ was considered statistically significant. All data were analyzed using the SPSS22.0 software.

Results

Targeting PEAK1 prevents viability in ATC cells in vitro

PEAK1 siRNA (100 nM) or NC siRNA (NC) (100 nM) was transfected into 8505C and Hth74 cells for 48h, the knockdown efficiency was determined by western blot assay (Fig. 1A). We confirmed that the PEAK1 protein expression showed a significant decrease in the two cells, respectively. No significant change was shown in NC siRNA transfected cells. To determine the biologic effect of targeting PEAK1 on cell viability, we conducted the MTT assay. Targeting PEAK1 significantly inhibited cell viability in both of the cells (Fig. 1B,C). We then performed colony formation assays to evaluate the effectiveness of targeting PEAK1 on cell growth. We found that the cells transfected with PEAK1 siRNA produced fewer colonies compared with the NC siRNA-transfected cells (Fig. 1D).

Targeting PEAK1 did not affect apoptosis, but increased the sensitivity of ATC cells to Vemurafenib in vitro

To determine the mechanism by which targeting PEAK1 reduced viability, we first evaluated the PEAK1 siRNA-induced apoptosis in the two cells by Annexin V/PI staining and evaluated by flow cytometric analysis. The results showed that targeting PEAK1 for 72h did not significantly increase cell apoptosis (Fig. 2A).

We first determined the sensitivity of the 8505C and Hth74 cells to Vemurafenib. 8505C and Hth74 cells were exposed to 0.1, 0.5, 1, 2.5, 5 or 10 μM of Vemurafenib for 72h. The results showed the IC50 (drug concentration eliciting 50% of the maximum inhibition) was 2.4 μM for 8505C cells and 1.6 μM for Hth74 cells by MTT assay (Fig. 2B).

To assess the effect of PEAK1 inhibition on Vemurafenib response, the 8505C and Hth74 cells were transfected with PEAK1 siRNA or NC siRNA, then treated with1 μM of Vemurafenib for 72h. The results showed that the combined PEAK1 siRNA and Vemurafenib treatment significantly decreased cell viability in 8505C and Hth74 cells by MTT assay, respectively (Fig. 2B).

Targeting PEAK1 improves cell response to Vemurafenib -induced apoptosis

8505C and Hth74 cells is relatively resistant to Vemurafenib by MTT assay, we further investigated whether Vemurafenib could activate PEAK1 expression,

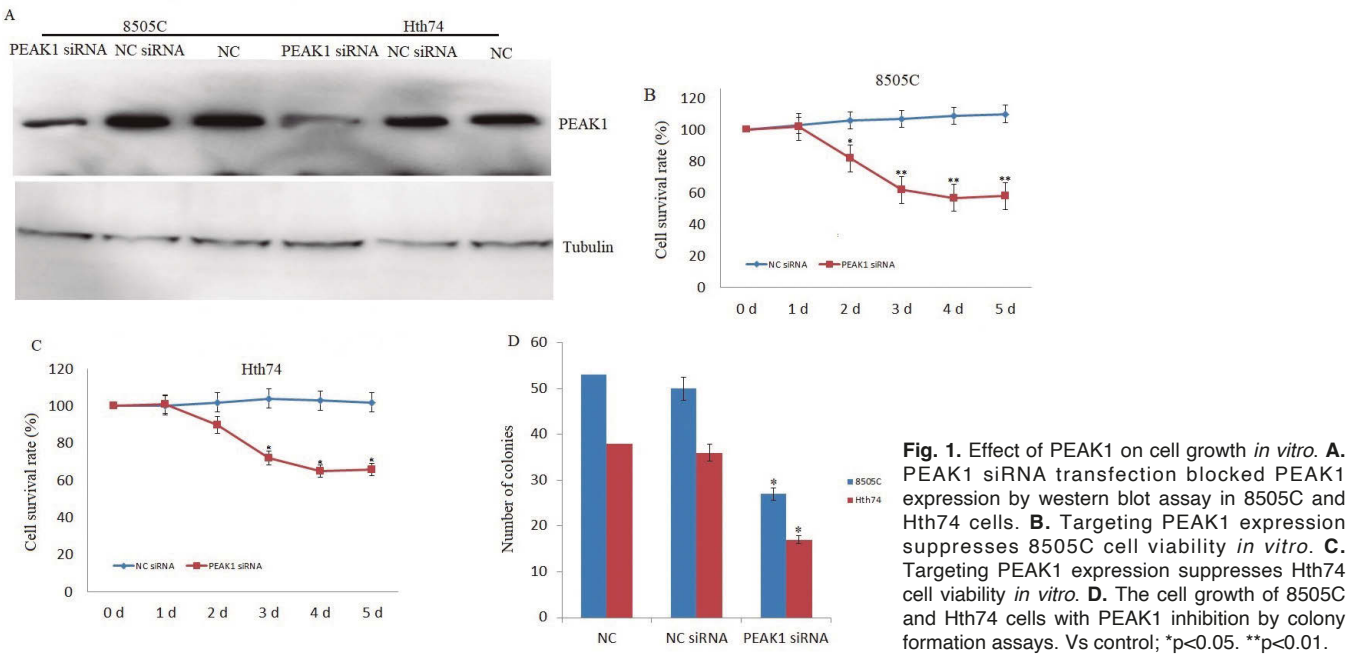

Fig. 1. Effect of PEAK1 on cell growth *in vitro*. **A.** PEAK1 siRNA transfection blocked PEAK1 expression by western blot assay in 8505C and Hth74 cells. **B.** Targeting PEAK1 expression suppresses 8505C cell viability *in vitro*. **C.** Targeting PEAK1 expression suppresses Hth74 cell viability *in vitro*. **D.** The cell growth of 8505C and Hth74 cells with PEAK1 inhibition by colony formation assays. Vs control; *p<0.05. **p<0.01.

which results in Vemurafenib resistance in both of the cells. For these experiments, 8505C and Hth74 cells were exposed to 1.0 μM Vemurafenib for 2, 6, 8, 12, 24, 48 and 72h. PEAK1 protein was detected by Western blot assay. PEAK1 expression was enhanced from 6-12h in 8505C and Hth74 cells (Fig. 3A). Targeting PEAK1 sensitizes both cells to Vemurafenib by inhibiting cell viability. We next determined whether PEAK1 inhibition improves Vemurafenib -induced apoptosis. 8505C and Hth74 cells were depleted of PEAK1 by siRNA treatment and allowed to be exposed to Vemurafenib (1.0μM) for 72h. We observed that Vemurafenib treatment induced fewer apoptotic cells in 8505C and Hth74 cells, but PEAK1 inhibition markedly enhanced Vemurafenib -induced cell apoptosis (Fig. 3C), and inhibited PEAK1 expression in Vemurafenib treated cells (Fig. 3B). These data suggest that targeting PEAK1 sensitizes *BRAF*-mutated cells to Vemurafenib treatment by inducing cell apoptosis.

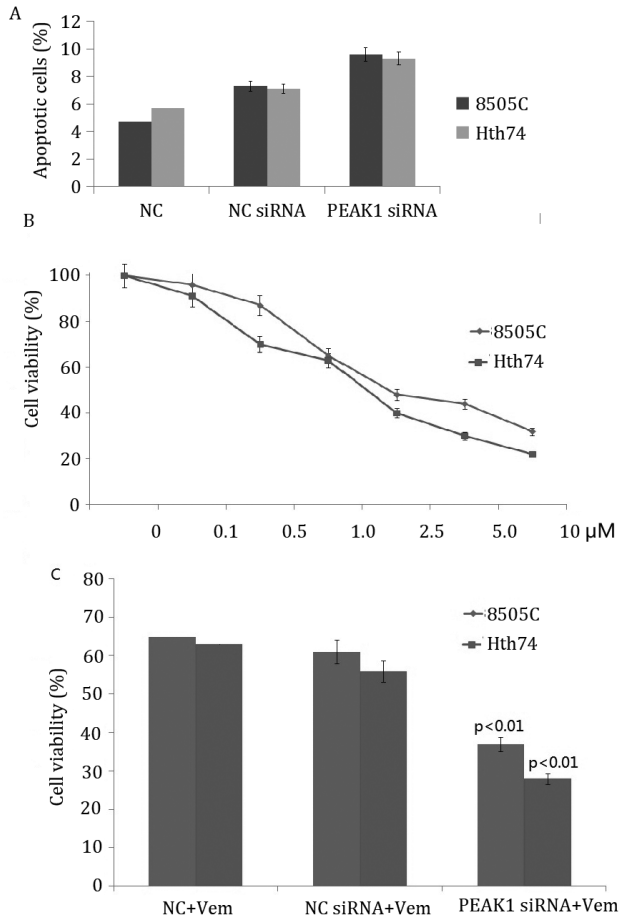

Fig. 2. Targeting PEAK1 did not affect apoptosis, but increased the sensitivity of ATC cells to vemurafenib *in vitro*. 8505C and Hth74 cells were transfected with PEAK1 siRNA or control siRNA for 72h. **A.** Cell apoptosis was detected by Annexin V/PI staining and evaluated by flow cytometric analysis. **B.** 8505C and Hth74 cells were exposed to 0.1, 0.5, 1, 2.5, 5 or 10 μM of vemurafenib for 72h, cell viability was detected by MTT assay. **C.** 8505C and Hth74 cells were transfected with PEAK1 siRNA or control siRNA, then treated with 1 μM of vemurafenib for 72h, cell viability was detected by MTT assay.

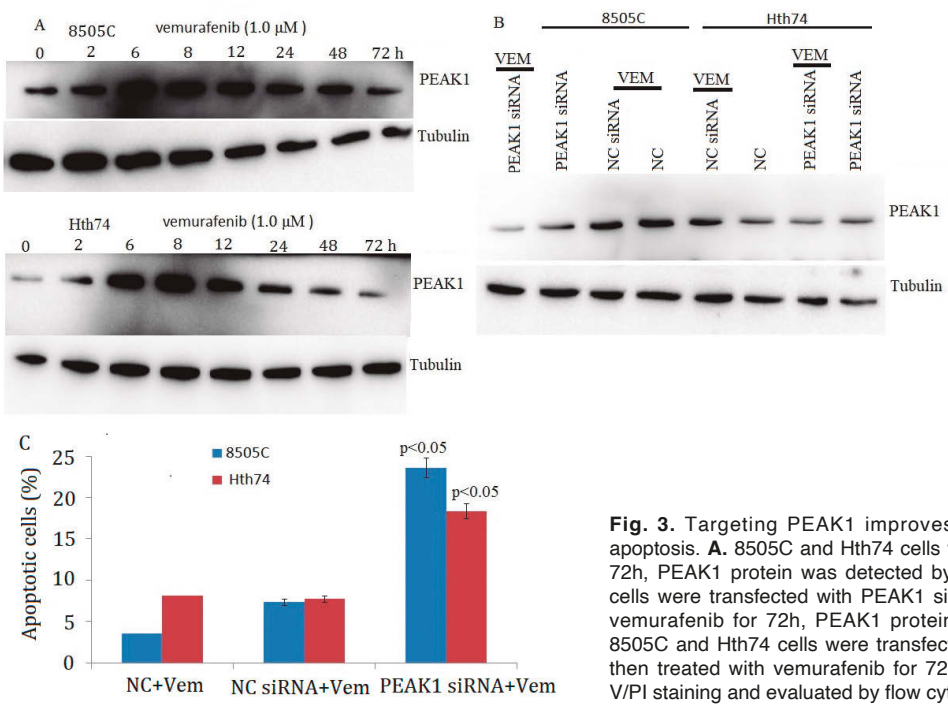

Fig. 3. Targeting PEAK1 improves cell response to vemurafenib -induced apoptosis. **A.** 8505C and Hth74 cells were exposed to 1 μM of vemurafenib for 0-72h, PEAK1 protein was detected by Western blot assay. **B.** 8505C and Hth74 cells were transfected with PEAK1 siRNA or its control siRNA, then treated with vemurafenib for 72h, PEAK1 protein was detected by Western blot assay. **C.** 8505C and Hth74 cells were transfected with PEAK1 siRNA or its control siRNA, then treated with vemurafenib for 72h. Cell apoptosis was detected by Annexin V/PI staining and evaluated by flow cytometric analysis.

Targeting PEAK1 sensitized Vemurafenib induced apoptosis by upregulating Bim

Vemurafenib upregulated Bim, which contributed to Vemurafenib -induced apoptosis in $BRAF^{V600E}$ mutant melanoma cells. In our study, slight upregulation of Bim expression was found after Vemurafenib (1.0 μM) treatments for 72h in both 8505C and Hth74 cells (Fig. 4A,B) but transfection with PEAK1 siRNA significantly promoted Vemurafenib-induced Bim protein expression in both cells (Fig. 4A,B).

PEAK1 deletion alone or Vemurafenib (1.0 μM) alone have a lesser effect on cell apoptosis in both of the cells. And combined PEAK1 deletion and Vemurafenib systematically increased the pro-apoptotic effect. We therefore determined whether Bim upregulation was related to the systematical effect. Transfection with Bim siRNA significantly inhibited the upregulation of Bim in both of the cells with combined PEAK1 deletion and Vemurafenib(1.0 μM) treatment for 72h (Fig. 4C).

We next examined the effects of the deletion of Bim alone or the combined deletion on cell apoptosis. Deletion of Bim attenuated PEAK1 deletion in combination with Vemurafenib (1.0 μM) induced cell apoptosis in both of the cells by flow cytometric assay (Fig. 4D).

Discussion

$BRAF^{V600E}$ mutation is a frequent event in ATC, a 20-25% mutation rate. Vemurafenib was developed as a low molecular weight molecule for the inhibition of the mutated serine threonine kinase BRAF, and it selectively binds to the ATP-binding site of $BRAF^{V600E}$ kinase and inhibits its activity. Vemurafenib inhibits tumor proliferation and oncogenic BRAF signaling through the MAPK pathway. Clinical studies have demonstrated that Vemurafenib improved progression-free survival in patients with $BRAF^{V600E}$ mutant melanoma by inhibiting MAPK signals, but primary resistance and development of secondary resistance to Vemurafenib resulted in only a transient initial response (Prahallad et al., 2012), the reasons of this disparity remain unclear. Several studies have tried to unravel the molecular basis

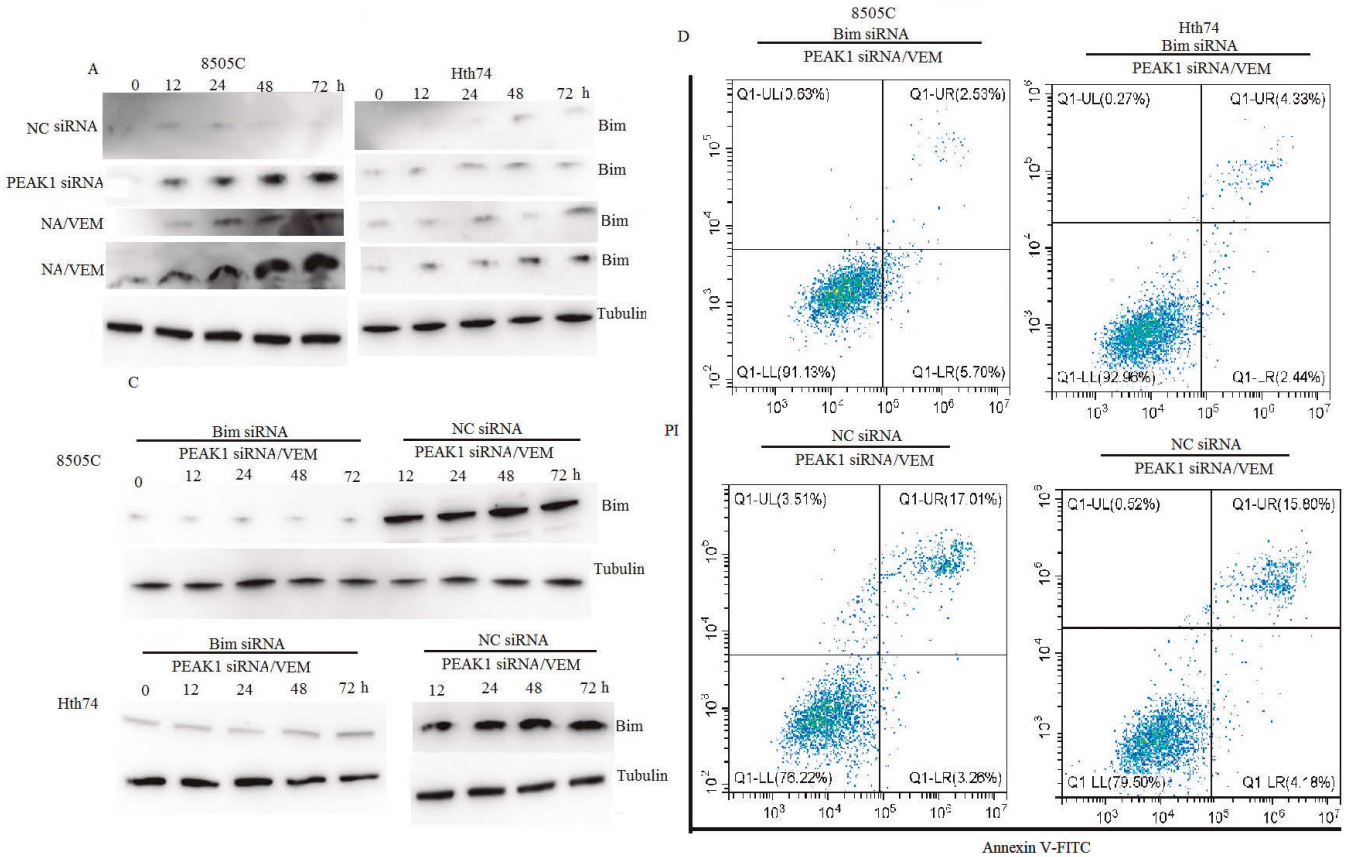

Fig. 4. Targeting PEAK1 improves vemurafenib -induced apoptosis by upregulation of Bim expression. **A, B.** 8505C and Hth74 cells were transfected with PEAK1 siRNA or/and treated with vemurafenib for 12-72h, Bim protein was detected in both of the cells by Western blot assay. **C.** NC siRNA or PEAK1 siRNA transfected 8505C and Hth74 cells were transfected with Bim siRNA or/and treated with vemurafenib for 12-72h. Bim protein was detected in both of the cells by Western blot assay. **D.** NC siRNA or PEAK1 siRNA transfected 8505C and Hth74 cells were transfected with Bim siRNA, then treated with vemurafenib for 72h. Cell apoptis was detected by Annexin V/PI staining and evaluated by flow cytometric analysis.

of chemoresistance to Vemurafenib. Interest has centered on the role of reactivation of cell proliferation and anti-apoptotic pathways (Vin et al., 2013; Benito-Jardón et al., 2019). However, the precise mechanism involved in Vemurafenib resistance by apoptotic signaling inhibition needs to be elucidated for ATC.

PEAK1 is overexpressed in many human malignancies (Strnadel et al., 2017; Ding et al., 2018). Previously, enhanced PEAK1 expression was reported to promote tumor cell proliferation and invasiveness in pancreatic ductal epithelial cell (Tactacan et al., 2015), HMVECs and HUVECs (Wang et al., 2018) and MEFs (Zheng et al., 2013). In our study, we used 8505C and Hth74 cells to investigate the role of PEAK1 *in vitro*. The results demonstrated that targeting PEAK1 inhibited cell viability and decreased the number of colonies in 8505C and Hth74 cells *in vitro*. We further studied the mechanism of targeting PEAK1 on cell viability inhibition. We observed that targeting PEAK1 alone did not significantly affect cell apoptosis in both cells. This means that the inhibition of cell viability by targeting PEAK1 alone is not achieved by promoting cell apoptosis, but may be achieved through other pathways. Targeting PEAK1 alone was reported to reduce cell cycle and cell cycle associated proteins (Strnadel et al., 2017). Further research is needed to determine whether targeting PEAK1 inhibits cell viability by affecting the cell cycle.

PEAK1 overexpression is reported to be related with collagen-mediated chemoresistance in pancreatic cancer cells (Aguilera et al., 2017). Kelber et al. (2012) and Fujimura et al. (2014) reported that targeting PEAK1 sensitized the PDAC cells to the current first-line chemotherapy gemcitabine. PEAK1 was over-expressed in the resistant PDAC cells, but in the sensitive PDAC cells, PEAK1 was down-expressed, and gemcitabine treatment increased PEAK1 expression in the sensitive PDAC cells (Fujimura et al., 2014), suggesting that required PEAK1 expression may be related to gemcitabine resistance. In our study, high PEAK1 expression was observed in 8505C and Hth74 cells, which were resistant to Vemurafenib treatment, whereas targeting PEAK1 restored sensitivity to Vemurafenib in 8505C and Hth74 cells. These data indicated that PEAK1 may be a target to reduce Vemurafenib resistance in ATC cells. Although targeting PEAK1 expression reduced Vemurafenib resistance, the underlying mechanisms are unknown.

In our study, targeting PEAK1 alone or Vemurafenib alone inhibited cell viability and colony formation, but did not induce apoptotis. However the combined PEAK1 inhibition and Vemurafenib markedly enhanced cell apoptosis, suggesting that targeting PEAK1 sensitizes ATC cells to Vemurafenib treatment by inducing cell apoptosis. The proapoptotic BH3-only Bim plays a key role in the control of apoptosis and in the initiation of the apoptotic pathways (Adams and Cory, 2007; Mukhopadhyay et al., 2014). In the present study *in vitro*, targeting PEAK1 alone or Vemurafenib treatment alone did not significantly upregulate Bim expression in both of the cells. But the combined PEAK1 inhibition and Vemurafenib markedly upregulated Bim expression in both of the cells. Our findings reported here that targeting Bim inhibited Vemurafenib -induced cell apoptosis in presence of PEAK1 inhibition *in vitro*. These results indicate that targeting PEAK1 sensitized ATC cells to Vemurafenib through Bim upregulation.

In summary, our study demonstrates that targeting PEAK1 in combination with Vemurafenib treatment increases the Vemurafenib sensitivity. Targeting PEAK1 can activate Bim expression to neutralize Vemurafenib resistance. Therefore, targeting PEAK1 is a new strategy to treat BRAF-mutated advanced thyroid cancer patients that are either primarily insensitive to Vemurafenib or those are have developed resistance against BRAF inhibition.

Acknowledgements. Not applicable

Funding Statement. Youth Scientific Research Fund of Qingdao University (N0 2021026).

Patient consent for publication. Not applicable

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

Author contributions. NL, HFY and SC conducted the research, analyzed the data and wrote the manuscript. wqh reviewed the final manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate. The study was approved by the Biomedical Research Ethics Committee of the Affiliated Hospital of Qingdao University.

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

References

Adams J.M. and Cory S. (2007). The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene 26, 1324-1337.

Aguilera K.Y., Huang H., Du W., Hagopian M.M., Wang Z., Hinz S., Hwang T.H., Wang H., Fleming J.B., Castrillon D.H., Ren X., Ding K. and Brekken R.A. (2017). Inhibition of discoidin domain receptor 1 reduces collagen-mediated tumorigenicity in pancreatic ductal adenocarcinoma. Mol. Cancer Ther. 16, 2473-2485.

Ayroldi E., Petrillo M.G., Marchetti M.C., Cannarile L., Ronchetti S., Ricci E., Cari L., Avenia N., Moretti S., Puxeddu E. and Riccardi C. (2018). Long glucocorticoid-induced leucine zipper regulates human thyroid cancer cell proliferation. Cell Death Dis. 9, 305.

Bauer D., Werth F., Nguyen H.A., Kiecker F. and Eberle J. (2017). Critical role of reactive oxygen species (ROS) for synergistic enhancement of apoptosis by vemurafenib and the potassium channel inhibitor TRAM-34 in melanoma cells. Cell Death Dis. 8, e2594.

Benito-Jardón L., Díaz-Martínez M., Arellano-Sánchez N., Vaquero-Morales P., Esparís-Ogando A. and Teixidó J. (2019). Resistance to MAPK inhibitors in melanoma involves activation of the IGF1R-MEK5-Erk5 pathway. Cancer Res. 79, 2244-2256.

Bollag G., Hirth P., Tsai J., Zhang J., Ibrahim P.N., Cho H., Spevak W., Zhang C., Zhang Y., Habets G., Burton E.A., Wong B., Tsang G.,

West B.L., Powell B., Shellooe R., Marimuthu A., Nguyen H., Zhang K.Y., Artis D.R., Schlessinger J., Su F., Higgins B., Iyer R., D'Andrea K., Koehler A., Stumm M., Lin P.S., Lee R.J., Grippo J., Puzanov I., Kim K.B., Ribas A., McArthur G.A., Sosman J.A., Chapman P.B., Flaherty K.T., Xu X., Nathanson K.L. and Nolop K. (2010). Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. Nature 467, 596-599.

Boussemart L., Malka-Mahieu H., Girault I., Allard D., Hemmingsson O., Tomasic G., Thomas M., Basmadjian C., Ribeiro N., Thuaud F., Mateus C., Routier E., Kamsu-Kom N., Agoussi S., Eggermont A.M., Désaubry L., Robert C. and Vagner S. (2014). eIF4F is a nexus of resistance to anti-BRAF and anti-MEK cancer therapies. Nature 513, 105-109.

Dadu R., Shah K., Busaidy N.L., Waguespack S.G., Habra M.A., Ying A.K., Hu M.I., Bassett R., Jimenez C., Sherman S.I. and Cabanillas M.E. (2015). Efficacy and tolerability of vemurafenib in patients with $BRAF^{V600E}$-positive papillary thyroid cancer: M.D. Anderson Cancer Center off label experience. J. Clin. Endocrinol. Metab. 100, E77-81.

Ding C., Tang W., Fan X., Wang X., Wu H., Xu H., Xu W., Gao W. and Wu G. (2018). Overexpression of PEAK1 contributes to epithelial-mesenchymal transition and tumor metastasis in lung cancer through modulating ERK1/2 and JAK2 signaling. Cell Death Dis. 9, 802.

Fujimura K., Wright T., Strnadel J., Kaushal S., Metildi C., Lowy A.M., Bouvet M., Kelber J.A. and Klemke R.L. (2014). A hypusine-eIF5A-PEAK1 switch regulates the pathogenesis of pancreatic cancer. Cancer Res. 74, 6671-6681.

Jeong J.H, Oh J.M., Jeong S.Y., Lee S.W., Lee J. and Ahn B.C. (2019). Combination treatment with the BRAFV600E inhibitor vemurafenib and the BH3 mimetic navitoclax for BRAF-Mutant thyroid carcinoma. Thyroid 29, 540-548.

Kelber J.A., Reno T., Kaushal S., Metildi C., Wright T., Stoletov K., Weems J.M., Park F.D., Mose E., Wang Y., Hoffman R.M., Lowy A.M., Bouvet M. and Klemke R.L. (2012). KRas induces a Src/PEAK1/ErbB2 kinase amplification loop that drives metastatic growth and therapy resistance in pancreatic cancer. Cancer Res. 72, 2554-2564.

Lee D.Y., Won J.K., Choi H.S., Park do J., Jung K.C., Sung M.W., Kim K.H., Hah J.H. and Park Y.J. (2016). Recurrence and survival after gross total removal of resectable undifferentiated or poorly differentiated thyroid carcinoma. Thyroid 26, 1259-1268.

Molinaro E., Romei C., Biagini A., Sabini E., Agate L., Mazzeo S., Materazzi G., Sellari-Franceschini S., Ribechini A., Torregrossa L., Basolo F., Vitti P. and Elisei R. (2017). Anaplastic thyroid carcinoma: from clinicopathology to genetics and advanced therapies. Nat. Rev. Endocrinol. 13, 644-660.

Montero-Conde C., Ruiz-Llorente S., Dominguez J.M., Knauf J.A., Viale A., Sherman E.J., Ryder M., Ghossein R.A., Rosen N. and Fagin J.A. (2013). Relief of feedback inhibition of HER3 transcription by RAF and MEK inhibitors attenuates their antitumor effects in BRAF-mutant thyroid carcinomas. Cancer Discov. 3, 520-533.

Mukhopadhyay S., Panda P.K., Sinha N., Das D.N. and Bhutia S.K. (2014). Autophagy and apoptosis: where do they meet? Apoptosis 19, 555-566.

Nehs M.A., Nucera C., Nagarkatti S.S., Sadow P.M., Morales-Garcia D., Hodin R.A. and Parangi S.(2012). Late intervention with anti-$BRAF^{V600E}$ therapy induces tumor regression in an orthotopic mouse model of human anaplastic thyroid cancer. Endocrinology 153, 985-994.

Poulikakos P.I., Persaud Y., Janakiraman M., Kong X., Ng C., Moriceau G., Shi H., Atefi M., Titz B., Gabay M.T., Salton M., Dahlman K.B., Tadi M., Wargo J.A., Flaherty K.T., Kelley M.C., Misteli T., Chapman P.B., Sosman J.A., Graeber T.G., Ribas A., Lo R.S., Rosen N. and Solit D.B. (2011). RAF inhibitor resistance is mediated by dimerization of aberrantly spliced $BRAF^{V600E}$. Nature 480, 387-390.

Prahallad A., Sun C., Huang S., Di Nicolantonio F., Salazar R., Zecchin D., Beijersbergen R.L., Bardelli A. and Bernards R. (2012). Unresponsiveness of colon cancer to $BRAF^{V600E}$ inhibition through feedback activation of EGFR. Nature 483, 100-103.

Ragazzi M., Ciarrocchi A., Sancisi V., Gandolfi G., Bisagni A. and Piana S. (2014). Update on anaplastic thyroid carcinoma: Morphological, molecular, and genetic features of the most aggressive thyroid cancer. Int. J. Endocrinol. 2014, 790834.

Robert C., Karaszewska B., Schachter J., Rutkowski P., Mackiewicz A., Stroiakovski D., Lichinitser M., Dummer R., Grange F., Mortier L., Chiarion-Sileni V., Drucis K., Krajsova I., Hauschild A., Lorigan P., Wolter P., Long G.V., Flaherty K., Nathan P., Ribas A., Martin A.M., Sun P., Crist W., Legos J., Rubin S.D., Little S.M. and Schadendorf D. (2015). Improved overall survival in melanoma with combined dabrafenib and trametinib. N. Engl. J. Med. 372, 30-39.

Strnadel J., Choi S., Fujimura K., Wang H., Zhang W., Wyse M., Wright T., Gross E., Peinado C., Park H.W., Bui J., Kelber J., Bouvet M., Guan K.L. and Klemke R.L. (2017). eIF5A-PEAK1 signaling regulates YAP1/TAZ protein expression and pancreatic cancer cell growth. Cancer Res. 77, 1997-2007.

Tactacan C.M., Phua Y.W., Liu L., Zhang L., Humphrey E.S., Cowley M., Pinese M., Biankin A.V. and Daly R.J. (2015). The pseudokinase SgK223 promotes invasion of pancreatic ductal epithelial cells through JAK1/Stat3 signaling. Mol. Cancer 14, 139.

Vin H., Ojeda S.S., Ching G., Leung M.L., Chitsazzadeh V., Dwyer D.W., Adelmann C.H., Restrepo M., Richards K.N., Stewart L.R., Du L., Ferguson S.B., Chakravarti D., Ehrenreiter K., Baccarini M., Ruggieri R., Curry J.L., Kim K.B., Ciurea A.M., Duvic M., Prieto V.G., Ullrich S.E., Dalby K.N., Flores E.R. and Tsai K.Y. (2013). BRAF inhibitors suppress apoptosis through off-target inhibition of JNK signaling. Elife 2, e00969.

Wang H., Lapek J., Fujimura K., Strnadel J., Liu B., Gonzalez D.J., Zhang W., Watson F., Yu V., Liu C., Melo C.M., Miller Y.I., Elliott K.C., Cheresh D.A. and Klemke R.L. (2018). Pseudopodium-enriched atypical kinase 1 mediates angiogenesis by modulating GATA2-dependent VEGFR2 transcription. Cell Discov. 4, 26.

Zheng Y., Zhang C., Croucher D.R., Soliman M.A., St-Denis N., Pasculescu A., Taylor L., Tate S.A., Hardy W.R., Colwill K., Dai A.Y., Bagshaw R., Dennis J.W., Gingras A.-C., Daly R.J. and Pawson T. (2013). Temporal regulation of EGF signalling networks by the scaffold protein Shc1. Nature 499, 166-171.

Accepted January 8, 2024