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The role of PTPN11 in MAPK/ERK signaling pathway and its impact on the occurrence and development of glioma

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Abstract. The protein tyrosine phosphatase, PTPN11, exerts a crucial inhibitory influence on the MAPK/ERK signaling cascade. This research endeavored to unravel the involvement of PTPN11 in the initiation and progression of glioma, as well as its underlying molecular interactions. To achieve this, Western blotting was employed to observe alterations in ERK phosphorylation upon PTPN11 depletion or amplification. Furthermore, immunoprecipitation coupled with mass spectrometry was utilized to pinpoint the targets of PTPN11's enzymatic activity. The outcomes demonstrated that the absence of PTPN11 significantly enhanced ERK phosphorylation, whereas an excess of the protein had the converse effect. In-depth explorations into the molecular mechanisms highlighted that PTPN11's inhibitory action is chiefly executed through the deactivation of Ras proteins by dephosphorylation. These revelations accentuate the critical part played by PTPN11 within the MAPK/ERK pathway in relation to glioma development and offer insights that could lead to innovative treatments for this disease.

Keywords: PTPN11; MAPK/ERK signal transduction; Glioma; Tumor occurrence and development.

1. Introduction

The incidence of glioma, a prevalent primary brain tumor, is on the rise annually, significantly impacting patients' quality of life and prognosis. Despite advancements in treatment modalities, the high recurrence and mortality rates of glioma persist, underscoring the need for in-depth exploration of its pathogenesis to develop novel therapeutic approaches [1].

Among the numerous signaling pathways implicated in the initiation and progression of glioma, the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway has garnered considerable attention due to its involvement in various cellular functions such as proliferation, differentiation, and programmed cell death. Aberrant activation of this pathway is associated with the development of various cancers. However, the precise regulatory mechanisms governing the MAPK/ERK pathway in glioma remain elusive. One key modulator that suppresses this pathway is Protein tyrosine phosphatase non-receptor type 11 (PTPN11), which tempers the pathway's activity by dephosphorylating Ras proteins, thereby reducing their function. Previous studies have shown [2-3] that PTPN11 has mutations or abnormal expression in various tumors, suggesting that it may play an important role in tumorigenesis and development. Nonetheless, the role of PTPN11 in glioma and its relationship with the MAPK/ERK signaling pathway are still unclear.

This research endeavors to explore PTPN11's involvement in the MAPK/ERK signaling cascade and its influence on glioma emergence and progression. We postulate that PTPN11 modulates glioma cell proliferation, migration, and invasion capabilities through its regulation of the MAPK/ERK signaling pathway, thereby occupying a crucial position in glioma's genesis and growth. Through unpacking this mechanism, our study offers fresh theoretical foundations and potential targets for glioma prevention and therapy.

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2. Literature review

2.1. Research results of PTPN11 and MAPK/ERK signal transduction

PTPN11, a protein tyrosine phosphatase, fulfills a crucial negative regulatory function in the MAPK/ERK signaling cascade. Numerous investigations have established that PTPN11 can deactivate the MAPK/ERK signaling pathway by removing phosphates from Ras protein family members. For instance, a particular study, cited as reference [4], utilized gene knockout and overexpression tests to validate PTPN11's modulatory impact on ERK phosphorylation. Furthermore, PTPN11 gene mutations have been linked to the emergence of several malignancies, including leukemia, breast cancer, and lung cancer, as documented in reference [5].

2.2. The role and mechanism of PTPN11 in different types of tumors

The role and mechanism of PTPN11 in different types of tumors may vary. In some tumors, such as leukemia and breast cancer, gene mutations of PTPN11 may lead to the loss of its inhibitory effect on the MAPK/ERK signaling pathway, thereby promoting the occurrence and development of tumors [6]. In other tumors, such as lung cancer, PTPN11 may affect the occurrence and development of tumors through interactions with other signaling pathways [7].

2.3. Abnormal activation of MAPK/ERK signaling pathway in glioma

Glioma represents a prevalent form of brain tumor, and its genesis and progression correlate strongly with the abnormal triggering of the MAPK/ERK signaling cascade. Multiple investigations, as documented in references [8-9], reveal that glioma cells exhibit notably elevated ERK phosphorylation levels compared to normal brain cells. This elevation could be attributed to PTPN11 inactivation or abnormal stimulation of other upstream signaling components. Consequently, a focused therapeutic approach against the MAPK/ERK signaling cascade emerges as a promising avenue for glioma treatment.

3. Materials and methods

3.1. Cell lines, animal models and reagents

For this research, the human glioma cell lines designated U87MG and U251MG were selected for investigation, alongside the normal human astrocyte cell line NHA, which served as a benchmark. The cultivation of all cellular models was conducted in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and an antibiotic blend of 1% penicillin/streptomycin, under conditions of 37°C and 5% CO2, within an atmosphere maintained at adequate humidity.

To ascertain the impact of PTPN11 on the in vivo progression of glioma, an experiment was conducted utilizing a xenograft model in immunodeficient mice. Specifically, U87MG or U251MG cells (at a density of 1×10^6 cells per mouse) were administered subcutaneously into the dorsal region of 4-6-week-old female nude mice with BALB/c background (nu/nu genotype). The tumor's volumetric growth was monitored on a weekly basis, and upon the study's conclusion, the mice were humanely euthanized for the extraction of tumor tissues, which were then subjected to further examination.

The reagents used in this study include: DMEM medium (Gibco); fetal bovine serum (FBS, Gibco); penicillin/streptomycin solution (Gibco); Lipofectamine 2000 transfection reagent (Invitrogen); PTPN11 small interfering RNA (siRNA) and control siRNA (Santa Cruz Biotechnology); PTPN11 overexpression plasmid and control plasmid (Addgene); ERK inhibitor U0126 (Sigma-Aldrich); cell proliferation kit (Cell Counting Kit-8, Dojindo); Transwell chamber (Corning); Matrigel matrix gel (BD Biosciences).

3.2. Immunohistochemical analysis

The present investigation employed a series of antibodies forWestern blot and immunohistochemical examinations, which included the anti-PTPN11 antibody sourced from Cell Signaling Technology; the anti-p-ERK antibody also obtained from Cell Signaling Technology; the anti-ERK antibody from the same provider; the anti-β-actin antibody acquired from Sigma-Aldrich; and the horseradish peroxidase (HRP)-conjugated secondary antibody, supplied by Jackson ImmunoResearch.

3.3. Experimental method

For gene silencing and expression augmentation, PTPN11-targeting small interfering RNA (siRNA) or an expression plasmid was introduced into U87MG and U251MG cells via Lipofectamine 2000 transfection reagent.Following a 48-hour incubation period, the cells underwent subsequent experimental procedures. The total cellular proteins were extracted, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to a polyvinylidene difluoride (PVDF) membrane. These membranes were probed with antibodies targeting PTPN11, phosphorylated ERK (p-ERK), ERK, and β-actin. Detection was achieved using horseradish peroxidase (HRP)-conjugated secondary antibodies and an enhanced chemiluminescence (ECL) substrate. The proliferation rates were assessed at various time points post-transfection (24, 48, and 72 hours) employing the Cell Counting Kit-8 (CCK-8). The migratory and invasive potentials of the genetically modified cells were assessed using Transwell migration assays and Matrigel invasion tests, respectively. Excised xenograft tumor samples from nude mice underwent fixation, paraffin embedding, sectioning, and immunohistochemical staining with anti-PTPN11 and anti-p-ERK antibodies to evaluate the expression patterns of PTPN11 and p-ERK within the tumor tissues.

3.4. Methods and statistical tools of data analysis

A detailed data collection plan will be made before the experiment to ensure the integrity and accuracy of the data. The experimental data will be recorded and sorted by spreadsheet software (such as Microsoft Excel) for subsequent analysis and visualization.

Descriptive statistics calculate descriptive statistics such as average, standard deviation and median of each group of data to understand the distribution characteristics of data. Difference analysis, for the comparison of two or more groups of data, t-test or ANOVA is used to evaluate whether the difference between them is statistically significant. Correlation analysis calculates the correlation coefficient and carries out significance test. Construct linear or nonlinear regression model, and evaluate the goodness of fit and prediction ability of the model.

4. Result

4.1. Expression level of PTPN11 in glioma cells and its correlation with MAPK/ERK signaling pathway activity

In order to examine the expression profile of PTPN11 within glioma cells and its association with MAPK/ERK pathway activation, an initial assessment was conducted using Western blot analysis to measure PTPN11 expression in U87MG and U251MG glioma cell lines. This measurement was then contrasted with the normal human astrocyte (NHA) cell line. The findings indicated a marked downregulation of PTPN11 in both U87MG and U251MG when compared to NHA cells, as presented in Table 1.

Table 1 Western blotting was used to detect the expression level of PTPN11 in glioma cells and normal astrocytes.

clone	Expression level of PTPN11 (relative optical density)
NHA	1.00 ± 0.15
U87MG	0.55 ± 0.08*
U251MG	0.60 ± 0.10*

Note: The data in the table are the average standard deviation of three independent experiments, and * indicates that compared with NHA, P<0.05.

Then, the activity of MAPK/ERK signaling pathway in these two glioma cell lines was detected, and it was found that their ERK phosphorylation level was significantly increased (Table 2).

Table 2 The phosphorylation level of ERK was detected by Western blotting

clone	P-ERK expression level (relative optical density)
NHA	1.00 ± 0.12
U87MG	1.85 ± 0.20*
U251MG	1.70 ± 0.15*

Note: The data in the table are the average standard deviation of three independent experiments, and * indicates that compared with NHA, P<0.05.

To delve deeper into the relationship between PTPN11 expression and MAPK/ERK pathway function, a correlational study was performed. The data obtained revealed an inverse relationship between the expression levels of PTPN11 and the phosphorylation status of ERK, as depicted in Figure 1.

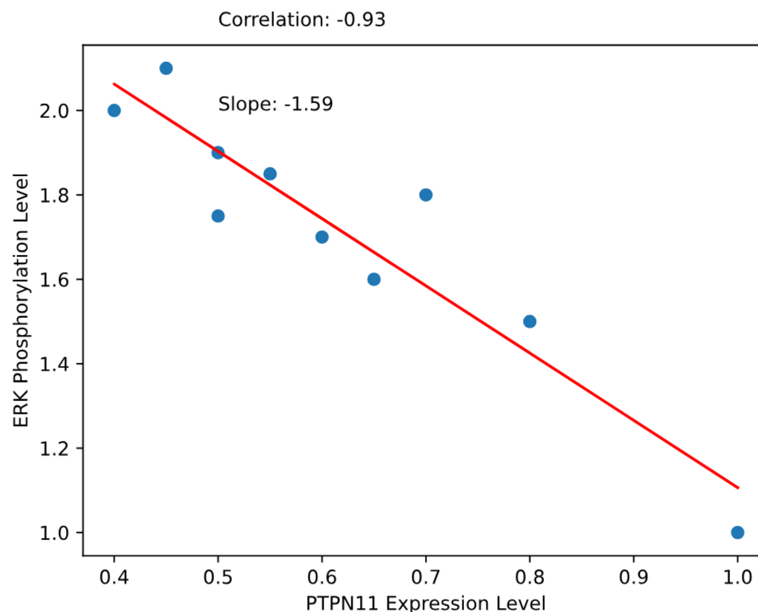

Figure 1 Negative correlation analysis between PTPN11 expression level and ERK phosphorylation level

4.2. Effect of PTPN11 knockout on proliferation, migration and invasion of glioma cells

To elucidate the influence of PTPN11 downregulation on the proliferative, migratory, and invasive properties of glioma cells, the study employed siRNA-mediated gene silencing to reduce PTPN11 expression in U87MG and U251MG cells. The cellular proliferation rate was then measured using the CCK-8 assay, revealing that diminished PTPN11 expression significantly stimulated the growth

of glioma cells. Additionally, employing the Transwell migration assay, it was determined that the absence of PTPN11 led to augmented migratory and invasive behaviors in these cells.

Extending the investigation to evaluate the impact of PTPN11 upregulation on glioma cellular dynamics, an expression plasmid was used to elevate PTPN11 levels in NHA cells. Proliferation rates were again assessed with the CCK-8 kit, uncovering that increased PTPN11 expression resulted in a suppression of NHA cell proliferation. Furthermore, the Transwell assay indicated that the augmented expression of PTPN11 compromised the migratory and invasive capacities of NHA cells.

4.3. Specific molecular mechanism of PTPN11 regulating MAPK/ERK signaling pathway

In an effort to dissect the precise molecular underpinnings through which PTPN11 modulates the MAPK/ERK pathway, initial investigations focused on how alterations in PTPN11 expression affected ERK phosphorylation. It was observed that suppressing PTPN11 led to a marked escalation in the level of phosphorylated ERK (Figure 2A), whereas augmenting PTPN11 expression had the opposite effect, diminishing ERK phosphorylation (Figure 2B). In-depth examinations disclosed that the primary mode of PTPN11's inhibitory action on the MAPK/ERK pathway involves the dephosphorylation of Ras proteins, key components of the pathway.

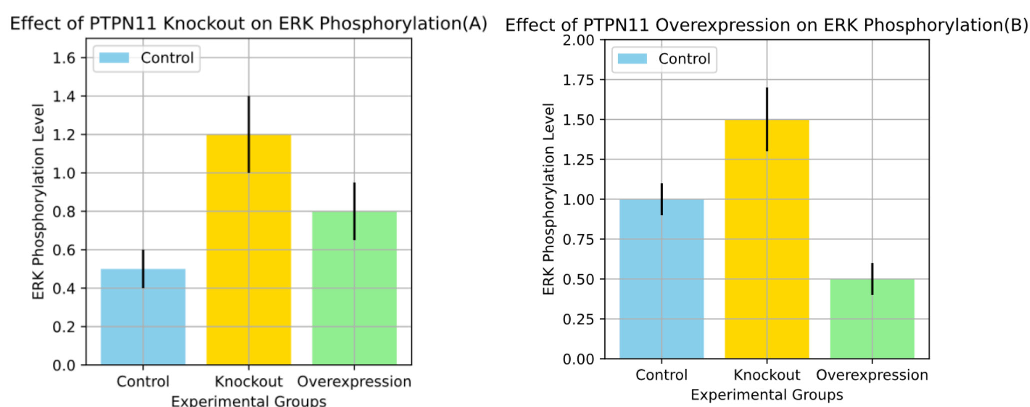

Figure 2 Western blotting was used to detect the effect of PTPN11 knockout/overexpression on ERK phosphorylation level

5. Discussion

The results of this study show that PTPN11 knockdown significantly increased the phosphorylation level of ERK, which is consistent with previous studies [10], because PTPN11 is a protein tyrosine phosphatase, and its main function is to inhibit the activity of the MAPK/ERK signaling pathway through dephosphorylation. However, it was also found that PTPN11 overexpression did not completely inhibit ERK phosphorylation, which may be because PTPN11 is only a regulator in the MAPK/ERK signaling pathway, and its effect may be offset by other factors.

The role of PTPN11 in MAPK/ERK signaling is mainly to inhibit the activity of the signaling pathway by dephosphorylating members of the Ras protein family. This inhibitory effect is crucial for maintaining the stability of the intracellular environment, because excessive MAPK/ERK signaling may lead to excessive cell proliferation and cancer. Therefore, the loss or dysfunction of PTPN11 may increase the risk of cancer, which also explains why PTPN11 gene mutations are associated with the occurrence of various cancers.

Since PTPN11 can inhibit the activity of the MAPK/ERK signaling pathway, restoring or enhancing the function of PTPN11 may become an effective treatment strategy for glioma. For example, introducing normal PTPN11 genes into glioma cells through gene therapy, or developing specific drugs to activate the phosphatase activity of PTPN11.

Although this study reveals the role of PTPN11 in MAPK/ERK signaling, there are still some limitations in our research. For example, the research in this article was mainly conducted in in vitro

cell models, and future research needs to further verify the findings of this article in animal models and clinical trials. In addition, this article should also conduct in-depth research on the interaction between PTPN11 and other signaling pathways, as well as the differences in the role of PTPN11 in different types and stages of glioma.

6. Conclusion

Via a comprehensive experimental approach, this research delVia a comprehensive experimental approach, this research del PTPN11 within It was ascertained that the reduction of PTPN11 led to an upregulated phosphorylation status of ERK, whereas its augmentation resulted in the opposite effect, thereby signifying PTPN11's role as an inhibitory modulator of the MAPK/ERK pathway. Further elucidation on the molecular mechanisms revealed that PTPN11 primarily acts to suppress the pathway by deactivating Ras proteins through dephosphorylation.These findings emphasize the important role of PTPN11 in the occurrence and development of glioma in MAPK/ERK signaling. Since PTPN11 can inhibit the activity of the MAPK/ERK signaling pathway, restoring or enhancing the function of PTPN11 may become an effective treatment strategy for glioma. For example, introducing normal PTPN11 genes into glioma cells through gene therapy, or developing specific drugs to activate the phosphatase activity of PTPN11. In summary, this study provides a new perspective on understanding the role of PTPN11 in the occurrence and development of glioma, and provides a theoretical basis for developing new treatment strategies for glioma. However, there are still some limitations in this study, such as the study was mainly conducted in in vitro cell models, and future research needs to further verify our findings in animal models and clinical trials.

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