# 中文摘要

## PFKP 对人宫颈癌细胞生物学行为和有氧糖酵解影响的研究


**研究目的：**

宫颈癌是一个全球性的公共卫生问题，严重威胁女性健康。在许多发展中国家，宫颈癌的发病率和死亡率仍然居高不下，因此仍然需要探索新的治疗方法。

有氧糖酵解作为肿瘤细胞的典型特征之一，通过消耗葡萄糖，从而产生乳酸和 ATP。ATP 和代谢过程中生成的中间产物 6-磷酸葡萄糖与丙酮酸可以促进细胞的合成代谢，进而促进肿瘤细胞增殖；同时有氧糖酵解产生的乳酸可以破坏细胞外基质，促进肿瘤细胞的侵袭和迁移。近年来血小板型磷酸果糖激酶（The platelet isoform of phosphofructokinase，PFKP）作为肿瘤细胞有氧糖酵解的关键限速酶之一受到广泛关注。有研究表明 PFKP 不仅可以引起葡萄糖代谢的变化，也可影响多个与糖酵解相关的基因的表达，提示 PFKP 高表达可以促进肿瘤细胞糖酵解的进行。在神经细胞瘤、前列腺癌、肝癌、乳腺癌、肾癌等多种实体瘤中已经发现 PFKP mRNA 表达上调，还有研究表明 PFKP 高表达与患者的生存预后负相关，然而 PFKP 在宫颈癌中的作用及机制尚不明确。

因此本文拟采用基因转染方法探究 PFKP 对人宫颈癌细胞生物学行为和有氧糖酵解的影响，为宫颈癌的治疗提供实验依据。

**研究方法：**

（1）分析 PFKP 在人宫颈癌中的表达情况及其对患者预后的影响：利用 Gene Expression Profiling Interactive Analysis（GEPIA）数据库中的数据，分析宫颈癌和正常宫颈组织中 PFKP mRNA 的表达水平；利用 The Human Protein Atlas（HPA）数据库分析宫颈癌与正常宫颈组织中 PFKP 的蛋白表达情况；通过 Kaplan-Meier plotter 数据库在线分析 PFKP 和宫颈癌患者预后的相关性；

（2）探究 PFKP 对人宫颈癌细胞生物学行为的影响：首先利用 Western blot 检测 MS751、HeLa、SiHa 和 C-33 A 四种人宫颈癌细胞系中 PFKP 的蛋白表达水平；然后利用 siRNA 对高表达 PFKP 的细胞系进行 PFKP 基因的沉默，对低表达

PFKP 的细胞系进行 PFKP 的转染，使其过表达 PFKP；MTS 法检测 PFKP 沉默或过表达对人宫颈癌细胞增殖的影响；Annexin V-FITC/PI 双染法检测 PFKP 沉默或过表达对细胞凋亡的影响；Transwell 小室法和伤口愈合实验分别检测 PFKP 沉默或过表达后对人宫颈癌细胞侵袭与迁移能力的影响；利用 Western blot 检测 PFKP 沉默或过表达对人宫颈癌细胞干性相关蛋白 c-Myc、Sox2 和 Nanog 表达水平的影响；

（3）探究 PFKP 对人宫颈癌细胞有氧糖酵解的影响：采用葡萄糖试剂盒与乳酸测试盒分别检测 PFKP 沉默或过表达对人宫颈癌细胞葡萄糖摄取和乳酸生成的影响。

**研究结果：**

（1）GEPIA 和 HPA 的分析结果显示，人宫颈癌组织中 PFKP 的 mRNA 和蛋白表达水平显著高于正常宫颈组织（$P < 0.05$）；Kaplan-Meier plotter 分析结果显示 PFKP 高表达的宫颈癌患者的生存率显著低于 PFKP 低表达的宫颈癌患者（$P < 0.05$）；

（2）Western blot 结果显示，在检测的四种人宫颈癌细胞系中，PFKP 在 MS751 细胞中表达最高，之后依次为 HeLa 细胞、SiHa 细胞和 C-33 A 细胞；Western blot 结果显示，利用 siRNA 沉默 PFKP 后，MS751 细胞的 PFKP 蛋白水平显著降低（$P < 0.05$），对 C-33 A 细胞与 SiHa 细胞进行 PFKP 过表达后，PFKP 的蛋白表达显著提高（$P < 0.05$）；MTS 结果提示，与对照组相比，沉默 PFKP 显著抑制 MS751 细胞的增殖（$P < 0.01$），而过表达 PFKP 对 C-33 A 细胞与 SiHa 细胞的增殖具有显著的促进作用（$P < 0.05$）；Annexin V-FITC/PI 双染法结果表明沉默和过表达 PFKP 对细胞凋亡没有明显的影响；Transwell 检测结果提示，沉默 PFKP 基因可以显著降低 MS751 细胞的侵袭能力（$P < 0.05$），过表达 PFKP 则可以显著促进 C-33 A 细胞与 SiHa 细胞的侵袭能力（$P < 0.01$）；伤口愈合实验结果显示，沉默 PFKP 基因可以抑制 MS751 细胞的迁移能力（$P < 0.01$），过表达 PFKP 可以显著提高 C-33 A 细胞与 SiHa 细胞的迁移能力（$P < 0.05$）；Western blot 结果显示，沉默 PFKP 基因后，干性相关基因 c-Myc、Sox2 和 Nanog 的蛋白表达也随之显著降低（$P < 0.01$），对 C-33 A 细胞与 SiHa 细胞进行 PFKP 过表达后，

和对照组 OE-NC 相比，c-Myc、Sox2 和 Nanog 三种干性相关基因的蛋白表达水平升高（$P < 0.05$）；

（3）葡萄糖测试盒与乳酸测试盒检测结果提示，沉默 PFKP 会显著减少 MS751 细胞的葡萄糖摄取量和乳酸生成（$P < 0.01$），过表达 PFKP 可以显著促进 C-33 A 细胞与 SiHa 细胞的葡萄糖摄取和乳酸生成（$P < 0.01$）。

**结论：**

（1）人宫颈癌组织中 PFKP mRNA 和蛋白表达水平显著高于正常宫颈组织，且与宫颈癌患者的生存期负相关；

（2）PFKP 沉默可以显著抑制人宫颈癌细胞的增殖、侵袭和迁移，减弱细胞干性；PFKP 过表达可以显著促进人宫颈癌细胞增殖、侵袭和迁移，增强细胞干性；

（3）PFKP 沉默可以抑制人宫颈癌细胞有氧糖酵解，降低细胞的葡萄糖摄取和乳酸的产生；PFKP 过表达可以促进人宫颈癌细胞有氧糖酵解，提高细胞的葡萄糖摄取和乳酸的产生。

**关键词：**

宫颈癌，PFKP，基因转染，有氧糖酵解

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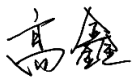
作者签名：  　指导教师签名： 

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# Abstract


## The effects of PFKP on the biological behavior and aerobic glycolysis of human cervical cancer cells

**Aims:**

Cervical cancer is a global public health problem, which seriously threatens women's health. The incidence and mortality of cervical cancer remain high in many developing countries, and new treatments still need to be explored.

Aerobic glycolysis, one of the characteristic features of tumor cells, produces lactic acid and ATP by consuming glucose. ATP and the metabolic intermediates glucose-6-phosphate and pyruvate promote cell synthesis, which in turn promotes tumor cell proliferation, while lactic acid can destroy extracellular matrix, promote the invasion and migration of tumor cells. In recent years, the platelet isoform of phosphofructokinase (PFKP) has been widely known as one of the key rate-limiting enzymes of tumor aerobic glycolysis. Some studies have shown that PFKP can cause changes in glucose metabolism and affect the expression of several genes related to glycolysis metabolism, suggesting that high expression of PFKP can promote glycolysis in tumor cells. The up-regulation of PFKP mRNA expression was found in neurocytoma, prostate cancer, liver cancer, breast cancer, kidney cancer and other solid tumors. However, the effects and mechanism of PFKP in cervical cancer remain unclear.

The aim of this study was to investigate the effects of PFKP on biological behavior and aerobic glycolysis of human cervical cancer cells by gene transfection, and to provide experimental basis for the treatment of cervical cancer.

**Methods:**

(1)To detect the expression of PFKP in human cervical cancer and its impact on the prognosis of patients: using the data from Gene Expression Profiling Interactive Analysis (GEPIA) database, the expression levels of PFKP mRNA in cervical cancer

and normal cervical tissues were analyzed; The Human Protein Atlas (HPA) database was used to analyze the protein expression of PFKP in cervical cancer and normal cervical tissues, and the Kaplan-Meier plotter database was used to analyze the correlation between PFKP and the prognosis of cervical cancer patients.

(2)To investigate the effect of PFKP on the biological behavior of human cervical cancer cells: Western blot was used to detect the protein expression of PFKP in MS751, Hela, Siha and C-33 A cell lines. Then the cell lines with high expression of PFKP was silenced by siRNA, and the cell lines with low expression of PFKP were transfected with PFKP plasmid to overexpress PFKP gene. The effect of PFKP silencing or overexpression on the proliferation of human cervical cancer cells was detected by MTS, and the effect of PFKP silencing or overexpression on the apoptosis of human cervical cancer cells were detected by Annexin V-FITC/PI double staining. The effects of PFKP silencing or overexpression on the invasion and migration of human cervical cancer cells were detected by Transwell chamber assay and wound healing assay respectively. Western blot was used to detect the effect of PFKP silencing or overexpression on the expression of c-Myc, Sox2 and Nanog in human cervical cancer cells.

(3)To investigate the effect of PFKP on aerobic glycolysis in human cervical cancer cells: the effects of PFKP silencing or overexpression on glucose uptake and lactate production of human cervical cancer cells were detected by glucose kit and lactate kit, respectively.

**Results:**

(1)The results of GEPIA and HPA showed that the expression of PFKP in cervical carcinoma tissues was significantly higher than in normal tissues. Kaplan-Meier plotter results showed that the survival rate of patients with high PFKP expression was significantly lower than patients that with low PFKP expression.

(2)The expression of PFKP was the highest in MS751 cells, followed by HeLa cells, SiHa cells and C-33 A cells. After silencing PFKP by siRNA, the protein level of

PFKP in MS751 cells was significantly decreased ($P < 0.05$), and the protein level of PFKP in C-33 A cells and SiHa cells were significantly increased after PFKP overexpression ($P < 0.05$). Compared with the control group, the proliferation of MS751 cells was significantly inhibited by PFKP silencing ($P < 0.01$), while the proliferation of C-33 A cells and SiHa cells were significantly promoted by PFKP overexpression ($P < 0.05$). The results of Annexin V-FITC/PI double staining showed that silencing and overexpression of PFKP have no significant effect on apoptosis. The results of Transwell analysis suggested that the invasive ability of MS751 cells was significantly reduced by PFKP silencing ($P < 0.05$), the invasive ability of C-33 A cells and SiHa cells were significantly enhanced by PFKP overexpression ($P < 0.01$). The wound healing experiment showed that the migration ability of MS751 cells could be inhibited by PFKP silencing ($P < 0.01$), the migration ability of C-33 A cells and SiHa cells were significantly increased by PFKP overexpression ($P < 0.05$). Western blot results showed that by silencing PFKP gene, the protein expression of stem-related genes c-Myc, Sox2 and Nanog also were decreased significantly ($P < 0.01$), and after PFKP overexpression in C-33 A cells and SiHa cells, compared with OE-NC of control group, the protein expression levels of c-Myc, Sox2 and Nanog were increased ($P < 0.05$)

(3)The results of lactate test and Glucose test showed that lactate production and glucose uptake in MS751 cells were significantly reduced by PFKP silencing, while lactate production and glucose uptake in C-33 A and SiHa cells were increased significantly by PFKP overexpression ($P < 0.01$).

**Conclusion:**

(1)The expression level of PFKP mRNA and protein in human cervical cancer tissues was significantly higher than that in normal cervical tissues, and negatively correlated with the prognosis of patients with cervical cancer;

(2)The proliferation, invasion and migration of human cervical cancer cells were significantly inhibited and the stemness of human cervical cancer cell was weaken by

PFKP silencing; the proliferation, invasion and migration of human cervical cancer cells were significantly promoted and the stemness of human cervical cancer cell was enhanced by PFKP overexpression;

(3)The aerobic glycolysis and glucose uptake and lactate production in human cervical cancer cells were inhibited by PFKP silencing, The aerobic glycolysis and increase glucose uptake and lactate production in human cervical cancer cells were promoted by PFKP overexpression.

**Keywords:**

Cervical cancer，PFKP，Gene transfection，Aerobic glycolysis

# 目录

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