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RESEARCH

Targeting NAD metabolism regulates extracellular adenosine levels to improve the cytotoxicity of CD8+ effector T cells in the tumor microenvironment of gastric cancer

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Abstract

Purpose Nicotinamide adenine dinucleotide (NAD+) is closely related to the pathogenesis of tumors. However, the effect of NAD+ metabolism of gastric cancer (GC) cells on immune cells remains unexplained. We targeted nicotinamide phosphoribosyltransferase (NAMPT), a rate-limiting enzyme in the NAD+ synthesis salvage pathway, to observe its effect in the immune microenvironment.

Methods NAMPT of GC cell lines was inhibited by using the small molecule inhibitor (FK866) and short hairpin RNA (shRNA). CCK-8 test and flow cytometry were performed to detect cell viability and apoptosis. Immunofluorescence was used to observe changes in mitochondrial membrane potential (MMP).The transfected GC cells (AGS) and patient-derived organoids (PDOs) were cocultured with activated PBMCs, followed by flow cytometric analysis (FCA) for cytokines and inhibitory marker. The level of NAD and ATP of GC cells (AGS & MKN45) was tested combined with NMN and CD39 inhibitor.

Results Targeting NAD+ by FK866 obviously reduced MMP, which ultimately inhibited proliferation and increased the apoptosis of GC cells. NAMPT silencing reduced intracellular NAD and ATP , further decreased extracellular adenosine. Meawhile, the cytokines of CD8+T cells were significantly increased after cocultured with transfected AGS, and the expression of PD-1 was distinctly decreased. NMN reversed the effect of shNAMPT and enhanced the immunosuppression. Consistent results were obtained by coculturing PBMCs with PDOs.

Conclusion Restraining the function of NAMPT resulted in the functional improvement of effector CD8+ T cells by decreasing extracellular adenosine levels and inducing apoptosis of GC cells simultaneously. Therefore, this study demonstrates that NAMPT can be an effective target for gastric cancer immunotherapy.

Keywords Gastric cancer · NAD · NAMPT · Tumor microenvironment · ATP-adenosine axis · Organoids

Abbreviations

GC	Gastric cancer
NAD	Nicotinamide adenine dinucleotide
NAMPT	Nicotinamide phosphoribosyltransferase
shRNA	Short hairpin RNA
FCA	Flow cytometric analysis
OXPHOS	Oxidative phosphorylation
TCA	Tricarboxylic acid cycle
MMP	Mitochondrial membrane potential
TILs	Tumor-infiltrating lymphocytes
TME	Tumor microenvironment
PDOs	Patient-derived organoids
NMN	Nicotinamide mononucleotide

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Introduction

Gastric cancer (GC) ranked 5th in global incidence and 4th in mortality, with more than one million new cases and an estimated 769,000 deaths in 2020 (Sung et al. 2021). Despite improvements in technology and equipment, especially the

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