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Upregulation of MAPK Pathway Genes: A Potential Driver of Gastric Carcinogenesis

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Abstract: This study focuses on oncogene-driven mechanisms in gastric cancer, employing a murine gastric cancer model to investigate the molecular processes underlying tumor initiation and progression. By comparing gene expression profiles between normal and cancerous tissues, key genes will be identified and their roles in gastric cancer advancement, as well as their associated regulatory networks, will be elucidated. Furthermore, gene-editing technologies will be utilized to functionally validate candidate oncogenes and determine their specific contributions to tumorigenesis. Ultimately, this research indicates that the activation of mechanism of PI3K/Akt pathway-related genes can increase gastric carcinogenesis.

1. Introduction

Gastric cancer is one of the most prevalent malignancies worldwide, particularly in East Asian countries such as China, Japan, and South Korea[1]. It remains a leading cause of cancer-related death globally, posing a major threat to public health. The development of gastric cancer is a complex, multistep process involving the dysregulation of multiple genes and signaling pathways. In this case, the pathway of the chromogenic activated protein kinase (MAPK pathway) is one of the hot spots in the current research of the proliferation, invasion, transfer and treatment of gastric cancer. The MAPK pathway is an important intracellular signal transduction network, which converts extracellular stimuli into intracellular responses through the tertiary kinase cascade (MAPKKK-MAPKK-MAPK). There are four main branches in mammals: extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), p38 MAPK and ERK5.[2] Among them, the ERK pathway mainly mediates growth factor signals and regulates cell proliferation and differentiation. The JNK and p38 pathways are involved in the stress response, inflammation and apoptosis processes.[3] In gastric cancer, the abnormal activation of the MAPK pathway is mainly driven by factors such as receptor tyrosine kinase (RTK) mutations, RAS/RAF gene variations, and epigenetic changes.[4]

Genetically engineered mouse models (GEMMs) have become powerful tools for studying the molecular mechanisms of gastric cancer[5]. By overexpressing oncogenes or knocking out tumor suppressors in gastric tissue, these models mimic human tumor development and provide valuable insights into the functional roles of specific genes and pathways.

Recent advances in molecular biology have identified several key oncogenes, such as c-MYC, KRAS, and HER, which are frequently overexpressed or mutated in gastric cancer.[6] These

oncogenes activate downstream pathways like PI3K/AKT and RAS/MAPK, which enhance cell proliferation, survival, and metastasis. For example, c-MYC overexpression accelerates the cell cycle, while KRAS mutations sustain uncontrolled growth. HER2 amplification is also linked to poor clinical outcomes. Spondin 2 (SPON2) induces the epithelial-mesenchymal transition (EMT) by activating the MAPK/ERK1/2 pathway and accelerates the metastasis of gastric cancer. Chemerin up-regulates VEGF and matrix metalloproteinases (MMPs) through ERK1/2 phosphorylation, enhancing tumor invasion ability.[7] In addition, epigenetic regulations such as miR-592 and long non-coding RNA CASC2 can also affect the progression of gastric cancer by targeting key molecules of the MAPK pathway.[8] There is cross-regulation between the Myc and MAPK pathways: ERK1/2 can promote the degradation of Myc by phosphorylating the Thr58 site, while Myc enhances the invasion ability of tumor cells by activating the MEK/ERK pathway. Together, these alterations form a complex oncogenic signaling network.

Previous studies have shown that changes in MAPK pathway related genes have the potential possibility of inducing gastric cancer[ref]. From this perspective, this study observed the changes of classic genes in the MAPK pathway and studied the possibility of inducing gastric cancer through biological models, which provides certain reference significance for the study of the related molecular mechanisms of gastric cancer.

2. Materials and Methods

2.1 Genotyping of Mice and Polymerase Chain Reaction (PCR)

Genotyping of mice is critical for establishing genetic models and studying gene function. PCR offers a rapid and accurate method of identifying mouse genotypes, providing a foundation for further genetic research. For the research on MAPK pathways, this study selected genes related to the MAPK pathway and designed primers as shown in the following table 1.

Table 1 Primer sequence information related to the MAPK pathway

Primer	Sequence(5' to 3')
Vegfa-H-F	AGGGCAGAATCATCACGAAGT
Vegfa-H-R	AGGGTCTCGATTGGATGGCA
Vegfb-H-F	GAGATGTCCCTGGAAGAACACA
Vegfb-H-R	GAGTGGGATGGGTGATGTCAG
Map3k14-H-F	AAAATGGCCCGTGTGTGTTG
Map3k14-H-R	GCCGAGTGGAGACTCATCC
Mapk1-H-F	TCTGGAGCAGTATTACGACCC
Mapk1-H-R	CTGGCTGGAATCTAGCAGTCT
Map3k7-H-F	ATTGTAGAGCTTCGGCAGTTATC
Map3k7-H-R	CTGTAAACACCAACTCATTGCG
Map4k1-H-F	TACAGCCACCGCTCTTTGATG
Map4k1-H-F	TGCCTTTTTCCTTCAGTCGGG
Vegfa-M-F	CTGCCGTCCGATTGAGACC
Vegfa-M-R	CCCCTCCTTGTACCACTGTC
Vegfb-M-F	GCCAGACAGGGTTGCCATAC
Vegfb-M-R	GGAGTGGGATGGATGATGTCAG
Map3k14-M-F	TGTGGGAAGTGGGAGATCCTA
Map3k14-M-R	GGCTGAACTCTTGGCTATTCTCA
Mapk1-M-F	CAGGTGTTCGACGTAGGGC
Mapk1-M-R	TCTGGTGCTCAAAAGGACTGA
Map3k7-M-F	CGGATGAGCCGTTACAGTATC
Map3k7-M-R	ACTCCAAGCGTTTAATAGTGTCG
Map4k1-M-F	CCCATTCTTATGTGGGGCATT
Map4k1-M-F	TGGAAGAGCACCGACTTTGC

Six genes were involved in this study, namely Vegfa, Vegfb, Map3k14, Mapk1, Map3k7 and

Map4k1..

Lysis Buffer: Prepare 5 × Lysis Buffer A (5 g NaOH and 0.12 g EDTA in 400 mL distilled water) and 5 × Neutralization Buffer B (9.69 g Tris base in 400 mL distilled water, pH adjusted to 5.5). Dilute 1:5 before use. Other Reagents: 2 × Taq DNA polymerase, DNA templates, RNase-free H_2O.

DNA Extraction via Alkaline Lysis: Tail tips (~0.5 cm) were collected and placed in 1.5 mL centrifuge tubes. Add 100 µL of Lysis Buffer A and incubate at 98 ℃ for 1 hour, shaking every 20 minutes. After incubation, add an equal volume of Neutralization Buffer B. Centrifuge at room temperature for 2 minutes and collect the supernatant as a DNA template.

PCR Amplification: Prepare a 15 µL reaction mix containing: 7.5 µL 2× Taq polymerase, 0.5 µL of each primer, 1 µL DNA template, and 5.5 µL RNase-free water.PCR Conditions: 95 ℃ for 5 min; then 35 cycles of 95 ℃ for 30 s, 58 ℃ for 30 s, and 72 ℃ for 40 s; final extension at 72 ℃ for 2 min.

2.2 Agarose Gel Electrophoresis

Agarose gel electrophoresis is used to separate and visualize nucleic acid fragments. It allows verification of PCR product size and integrity, facilitating downstream gene analysis.

Equipment: Electrophoresis system, gel tray, microwave oven, UV transilluminator, micropipettes and tips, electronic balance.

Reagents: Agarose powder, electrophoresis buffer (TAE or TBE), DNA samples, loading buffer.

Gel Preparation: We prepare a 2% agarose gel by dissolving agarose in an electrophoresis buffer with microwave heating. We cool the melted agarose to 50–60 ℃, pour it into a mold, insert a comb, and allow it to solidify.

Sample Loading: We place the gel into the electrophoresis tank and add a running buffer. We mix DNA samples with loading buffer and load them into wells.

Electrophoresis: We performed electrophoresis by running at 120 V for 40 minutes. After electrophoresis, we placed the gel on a UV transilluminator for observation. We documented the results using a gel imaging system and compared them to a DNA ladder.

2.3 Reverse Transcription and Quantitative Real-Time PCR (qPCR)

Reverse transcription and real-time qPCR are used to quantify gene expression and assess transcriptional activity under different conditions. These techniques provide precise data on gene regulation and function, particularly in disease contexts.

Reagents: Reverse transcription kit, SYBR Green qPCR kit, RNA samples, gene-specific primers, RNase-free water.

Equipment: Thermal cycler, microcentrifuge tubes, pipettes, and tips.

cDNA Synthesis: We prepare a 10 µL reverse transcription reaction containing: 2 µL 5× PrimeScript Buffer, 0.5 µL PrimeScript RT Enzyme Mix, 0.5 µL Random 6-mers, 0.5 µL Oligo(dT) primer, 500 ng total RNA, and RNase-free water to 10 µL. Incubate the reaction at 37°C for 15 min and then denature the enzyme at 85 ℃ for 10 s. Lastly, we dilute the resulting cDNA 1:20 for qPCR.

qPCR: We prepare a 10 µL qPCR reaction containing: 5 µL 2× SYBR Premix, 0.2 µL of each primer, 0.2 µL ROX reference dye, 1 µL cDNA, and 3.4 µL RNase-free water. We then load the qPCR reaction into a qPCR instrument and perform the reaction with the established program.

2.4 Hematoxylin and Eosin (H&E) Staining

H&E staining is a standard histological method used to examine tissue morphology. It highlights cellular structures and overall tissue architecture, providing a basis for pathological diagnosis and evaluation.

Reagents: Absolute ethanol (Sangon Biotech, A500737), xylene (Shanghai Pharmaceutical, 10023418), PBS buffer (Gibco, 10010-023), hematoxylin solution (Beyotime, C0107), eosin solution (Beyotime, C0109), neutral resin (Shanghai Pharmaceutical, 10004160).

Deparaffinization: We immerse paraffin-embedded tissue sections in xylene I and II for 10 minutes each.

Rehydration: We sequentially immerse slides in 100% ethanol I and II followed by 95%, 90%, 80%, and 70% ethanol for 3 minutes each.

Rinsing: We rinse the slides in running tap water for 5 minutes twice.

Hematoxylin Staining: We apply a hematoxylin solution for 3–5 minutes and then rinse under running water for 10 minutes.

Eosin Staining: We apply an eosin solution for 30 seconds and follow with a 10-minute rinse under running water.

Dehydration and Clearing: We dehydrate the sections in graded ethanol (70–100%) for 2 minutes each and treat with xylene I and II for 3 minutes each.

Mounting: We seal the slides with neutral resin and cover slips for long-term preservation.

3. Result

3.1 Genotyping of Mice

The genotypes of the mice were successfully identified by alkaline lysis of tail tip tissue to extract DNA, PCR amplification and agarose gel electrophoresis. As shown by the above DNA electrophoresis bands, the tail genotypes of the experimental group and the control group showed negative and positive ATP4b-Cre gene and Myc-related gene, respectively, which proved that the genotype of the experimental mice was the required genotype for the experiment. The correct mouse model provides a basis for subsequent experiments and proves the validity of the subsequent experimental data. (figure 1)

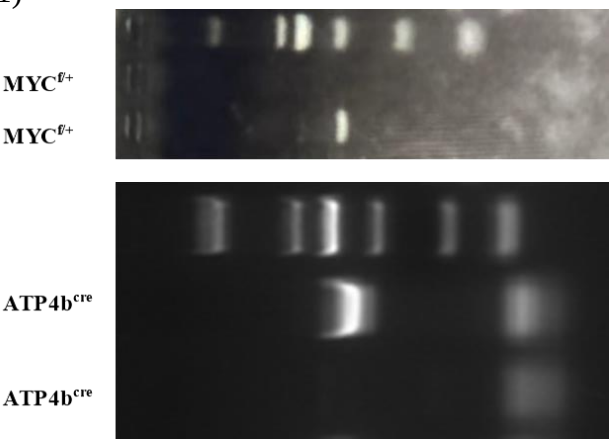

Fig.1 PCR image shows the genotyping of mice of interest.

3.2 qPCR Analysis

Reverse transcription and real-time PCR techniques were used to quantify the expression levels of specific genes in gastric cancer tissues and normal gastric tissues. We selected several oncogenes and key genes of signaling pathways closely related to the occurrence and development of gastric cancer as research objects. The above figure shows the expression of Vegfa, Vegfb, Map3k14, Mapk1, Map3k7 and Map4k1 in SGC 7901 cell line and mice (Student's t-test: *P < 0.05, **P < 0.01, ***P < 0.001.). The expression of six genes was significantly increased, among which Mapk1 was the most

significantly increased in mice and SGC 7901 cell line. Meanwhile, the increase of Vegfb gene was least significant in mice and SGC 7901 cell line. These results indicate that there is a certain relationship between the occurrence of gastric cancer and the expression of six MAPK pathway related genes, and the expression correlation of Mapk series genes may be higher than that of Vegfa series genes. (figure 2)

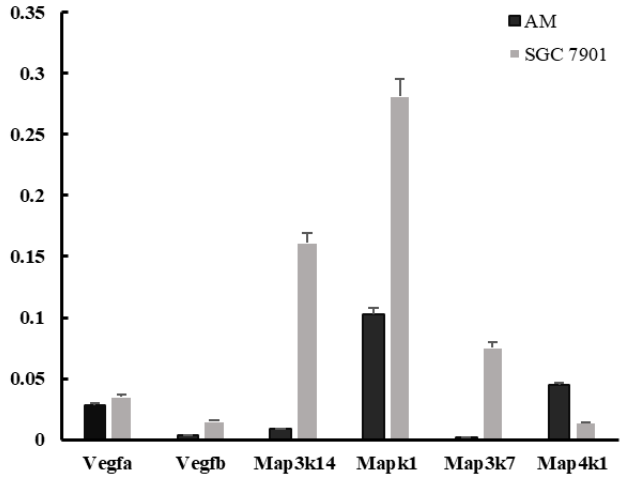

Fig.2 RT-qPCR analysis of MAPK pathway target genes.

3.3 Histological Analysis of Gastric Tissue Sections

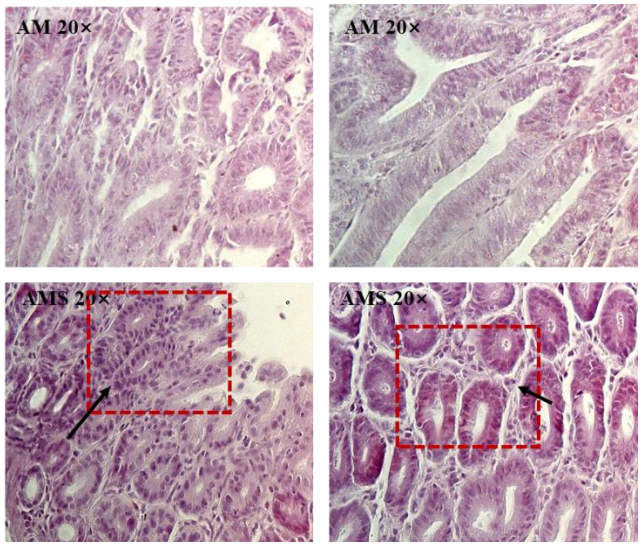

Fig.3 Representative hematoxylin and eosin stained images of gastric sections from AM and AMS mice. (The framed part represents the tissue cells that have developed cancer)

Hematoxylin and eosin (H&E) staining revealed distinct morphological differences between normal and tumor gastric tissues. Normal tissue sections exhibited characteristic glandular structures, orderly cell arrangement, and normal nuclear-to-cytoplasmic ratios, with no apparent pathological changes. In contrast, gastric cancer tissues showed disrupted glandular architecture, disordered cellular organization, enlarged and hyperchromatic nuclei, and altered nuclear-to-cytoplasmic ratios, indicating cellular atypia and proliferative activity. Furthermore, stromal proliferation and inflammatory cell infiltration were observed surrounding the tumor tissue. These histological features are indicative of malignancy and align with the typical characteristics of gastric carcinoma. The

comparison with normal tissues highlights the significant morphological alterations in tumor tissues and further confirms the successful establishment of the gastric cancer model.(figure 3)

3.4 Summary of Findings

Collectively, genotyping results validated the successful generation of genetically engineered mouse models. The qPCR findings revealed altered expression of key oncogenes and signaling molecules in gastric cancer tissues, providing molecular insights into tumorigenesis. The histological observations corroborated these findings at the tissue level, showcasing hallmark features of malignant transformation. Together, these experimental results provide consistent and complementary evidence supporting the hypothesis that oncogene activation drives molecular signaling changes contributing to gastric cancer progression in this mouse model.

4. Conclusion

This study investigated the molecular mechanisms driven by oncogenes in a mouse model of gastric cancer. We successfully constructed a gastric cancer model by establishing Setd2 knockout and Atp4b-Cre transgenic mice and examined key molecular events during tumor development.

Genotyping confirmed the successful modification of target genes, laying the foundation for further research. The qPCR results showed that the expressions of the six genes related to the MAPK pathway were significantly increased, among which Mapk1 was most significantly expressed in the mouse and SGC 7901 cell lines. The expression of the Vegfb gene was not obvious in mouse and SGC 7901 cell lines. These results suggest that the occurrence of gastric cancer is related to the expression of six MAPK pathway-related genes to a certain extent.

These findings support the critical role of oncogenes and downstream signaling pathways in gastric tumorigenesis. However, limitations remain, including the small sample size and the use of a mouse model, which may differ from human gastric cancer in key aspects. Furthermore, only a limited number of genes and pathways were analyzed in this study.

In summary, our study provides valuable insight into the oncogene-driven molecular alterations in gastric cancer and highlights potential targets for diagnosis and therapy.

References

[1] Arnold M, Sierra MS, Laversanne M, et al. Global patterns and trends in gastric cancer. Cancer Epidemiol Biomarkers Prev, 2017, 26(1): 188-198.

[2] Anauate AC, Leal MF, Calcagno DQ, et al. The Complex Network between MYC Oncogene and microRNAs in Gastric Cancer: An Overview. Int J Mol Sci. 2020;21(5):1782. Published 2020 Mar 5. doi:10.3390/ijms21051782

[3] Zhou, X., Guo, Z., Pan, Y. et al. PRSS23-eIF4E-c-Myc axis promotes gastric tumorigenesis and progression. Oncogene 44, 1167–1181 (2025). https://doi.org/10.1038/s41388-025-03294-3

[4] Mulè, Patrizia et al. "WNT Oncogenic Transcription Requires MYC Suppression of Lysosomal Activity and EPCAM Stabilization in Gastric Tumors." Gastroenterology vol. 167,5 (2024): 903-918. doi:10.1053/j.gastro.2024.06.029

[5] Kim J, Lee J. Molecular pathogenesis of gastric cancer: where are we now? Mol Cells, 2015, 38(6): 467-472.

[6] Park Y, Shin YK, Kim J, et al. Genomic landscape of gastric carcinoma. Cancer J, 2016, 22(4): 231-238.

[7] Guan, Xiaoqing et al. "Dual inhibition of MYC and SLC39A10 by a novel natural product STAT3 inhibitor derived from Chaetomium globosum suppresses tumor growth and metastasis in gastric cancer." Pharmacological research vol. 189 (2023): 106703. doi:10.1016/j.phrs.2023.106703

[8] Liao X, Qian X, Zhang Z, et al. ARV-825 Demonstrates Antitumor Activity in Gastric Cancer via MYC-Targets and G2M-Checkpoint Signaling Pathways. Front Oncol. 2021; 11: 753119. Published 2021 Oct 18. doi: 10.3389/fonc. 2021. 753119