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The Effect of Cisplatin on *GATA2* and *GATA6* Gene Expression in Head and Neck Cancer Cell Lines

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Abstract

Objective: Head and neck squamous cell carcinoma (HNSCC) constitutes over 90% of malignancies in the head and neck region and remains a significant clinical burden due to high mortality and resistance to therapy. Cisplatin is a commonly used chemotherapeutic agent in HNSCC treatment; however, its effectiveness is often limited by resistance. This study aimed to evaluate the impact of cisplatin on *GATA2* and *GATA6* expression in two HNSCC cell lines.

Materials and Methods: The HNSCC cell lines, HSC3 and SCC47, were exposed to varying concentrations of cisplatin to assess cytotoxic effects, with cell viability evaluated using the MTS assay. Based on the results, the half-maximal inhibitory concentrations (IC_{50}) were determined as 2.18 µM for HSC3 and 5.6 µM for SCC47 at 48 hours post-treatment. Subsequent experiments involved treating each cell line with its corresponding IC_{50} dose for 48 hours. Total RNA was then isolated using the TRIzol reagent, and complementary DNA (cDNA) was synthesized for downstream analysis. Quantification of *GATA2* and *GATA6* gene expression was performed via quantitative PCR (qPCR) using TaqMan probes, with *ACTB* as the housekeeping gene. Relative gene expression levels of *GATA2* and *GATA 6* were calculated using the comparative ΔΔCt method.

Results: *GATA6* expression was significantly upregulated (approximately 3-fold) following cisplatin treatment, whereas *GATA2* levels remained unchanged compared to untreated controls in HSC3 cells. In contrast, SCC47 cells showed a modest increase in both *GATA2* and *GATA6* expression; however, these changes did not reach statistical significance.

Conclusion: Cisplatin modulates the expression of *GATA2* and *GATA6* in a cell line-dependent manner in HNSCC. The observed upregulation of *GATA6* in the more cisplatin-sensitive HSC3 line may be associated with treatment response. However, this association remains correlative, and further functional studies are required to establish causality. These preliminary findings warrant additional investigation to clarify whether *GATA2* and *GATA6* could serve as potential biomarkers or therapeutic targets in cisplatin-treated HNSCC.

Keywords: HNSCC, cisplatin, *GATA2*, *GATA6*, gene expression

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INTRODUCTION

Accounting for over 90% of malignancies in the head and neck region, head and neck squamous cell carcinoma (HNSCC) poses a substantial global burden, marked by high morbidity and mortality rates. According to 2022 data, approximately 946,456 new cases and over 482,001 deaths occur annually (1).

The most frequently affected anatomical regions include the oral cavity, larynx, and pharynx. The development of HNSCC is commonly associated with factors such as alcohol and tobacco consumption, exposure to environmental carcinogens, and high-risk human papillomavirus (HPV) infections (2). In the treatment of head and neck cancers, surgical resection is frequently complemented by radiotherapy and chemotherapy (3). Among the most common chemotherapeutic agents used in these combination therapies is cisplatin. Cisplatin functions by forming intra-cellular DNA cross-links, which subsequently block replication and trigger apoptosis (4). However, while cisplatin demonstrates efficacy in some patients, a significant proportion develop either primary or acquired resistance to the treatment (5,6). Elucidating these resistance mechanisms is crucial for the development of targeted therapies.

GATA transcription factors are a family of zinc finger DNA-binding proteins that regulate the development of various tissues by modulating gene transcription, either through activation or repression. This tightly coordinated regulation enables GATA factors to couple cellular differentiation with the cessation of proliferation and the enhancement of cell survival. Given their critical roles in maintaining tissue homeostasis, it is not surprising that dysregulation of GATA genes has been implicated in the pathogenesis of several human cancers (7).

In recent years, transcription factors have been extensively investigated in cancer biology due to their critical roles in regulating processes such as cell proliferation, differentiation, metastasis, and drug resistance (8). In this context, the GATA family of transcription factors has garnered significant attention due to its wide-ranging biological functions, spanning embryonic development, regulation of immune responses, and involvement in cancer pathogenesis (9). This family comprises six members, *GATA1* through *GATA6*, each characterized by tissue-specific expression patterns and functions (10).

While *GATA2* is classically known for its crucial role in the differentiation and maintenance of hematopoietic cells (11), recent reports indicate its expression in epithelial-derived tumors and its potential to regulate cell proliferation and metastasis (12). *GATA6* is expressed in epithelial tissues such as the digestive system, lung, and pancreas, where it can act as either a tumor suppressor or a tumor promoter in tumor development (13,14).

The effects of GATA family members on head and neck cancer are not yet fully understood. However, preliminary data suggest that alterations in the expression levels of these genes may influence tumor cell behaviors, including proliferation, invasion, and drug response (15). This study investigated the effect of cisplatin treatment on the expression levels of *GATA2* and *GATA6* genes in two distinct HNSCC cell lines, namely HSC3 and SCC47. This research is expected to contribute to the understanding of whether *GATA2* and *GATA6* are associated with treatment response in head and neck cancers and may support their future consideration as potential biomarkers.

MATERIALS AND METHODS

Cell Culture

The HNSCC cell lines, HSC3 and SCC47, were acquired from the American Type Culture Collection (ATCC, Manassas, VA, USA). These cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM; high glycose) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich, St. Louis, MO, USA) and 1% penicillin-streptomycin (Invitrogen, Carlsbad, CA, USA). Cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2. Routine screening for mycoplasma contamination was performed on all cell lines using a PCR-based detection kit (Mycoplasma PCR Detection Kit; Applied Biological Materials, Richmond, BC, Canada).

Cytotoxicity Assay

Cytotoxicity of cisplatin was evaluated using the MTS assay. HSC3 and SCC47 cells were seeded into 96-well plates at a density of 2500 cells per well and incubated overnight to allow for cell adhesion. After that cells were treated with varying concentrations of cisplatin (0–20 μM). Untreated cells served as negative controls, while wells containing medium only were used as blanks. Following incubation periods of 24 to 72 hours, 12 μL of MTS reagent (CellTiter 96® AQueous One Solution; Promega, Madison, WI, USA) was added to each well, followed by 2 hours of incubation in the dark at 37°C. Absorbance was measured at 490 nm using microplate reader,

RNA Isolation After Cisplatin Treatment

HSC3 and SCC47 cells were seeded into 6-well plates at a density of 80,000 cells per well and incubated overnight to allow for adhesion. The next day, cells were treated with cisplatin at their respective IC_{50} concentrations and incubated for 48 hours. Following treatment, cells were harvested by trypsinization and centrifugation. Total

RNA was extracted from the resulting cell pellets using TRIzol™ reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA concentration and purity were measured using a NanoPhotometer (Implen, Munich, Germany).

Quantitative Real-Time PCR (qPCR)

Complementary DNA (cDNA) synthesis was performed using 1000 ng of total RNA with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA), following the manufacturer's instructions. Quantitative real-time PCR (qPCR) was subsequently conducted on the StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using TaqMan® probes specific for *GATA2* and *GATA6*. β-actin (ACTB) served as the endogenous control for normalization. All reactions were carried out in technical triplicates, and relative gene expression levels were determined using the comparative ΔΔCt method.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism, version 8.0 (GraphPad Software, San Diego, CA, USA). Results are presented as mean ± standard deviation (SD). A p-value <0.05 was considered statistically significant.

RESULTS

Cisplatin-Induced Cytotoxicity in HSC3 and SCC47 Cells

Cisplatin treatment induced a dose- and time-dependent reduction in cell viability in both HSC3 and SCC47

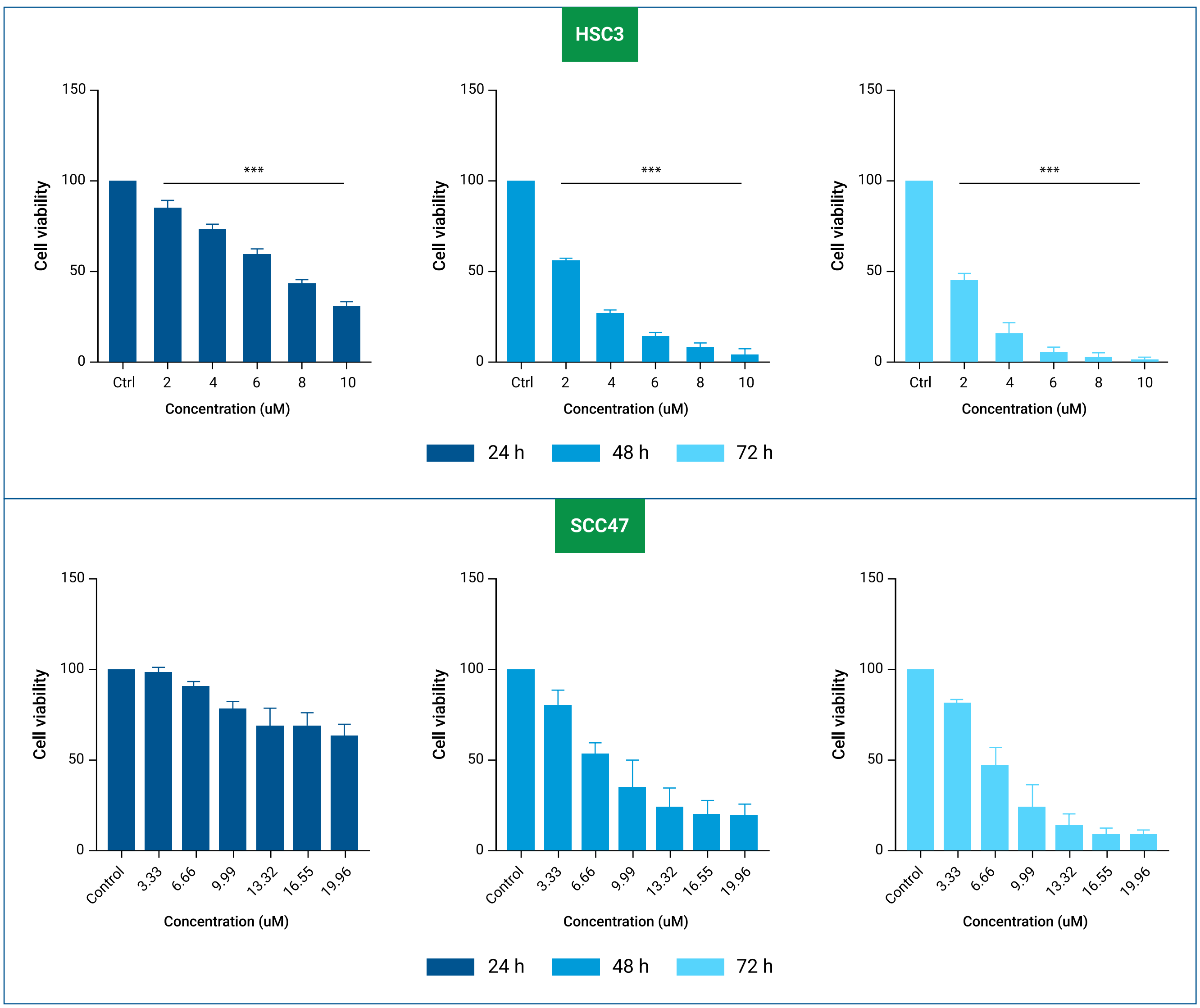

Figure 1. Effect of cisplatin on cell viability in HSC3 and SCC47 cells. Cells were treated with increasing concentrations of cisplatin (0–20 μM) for 24, 48, and 72 hours, and cell viability was measured using the MTS assay. Data represent the mean ± SD of three independent biological replicates (p<0.05, p<0.01, p<0.001).

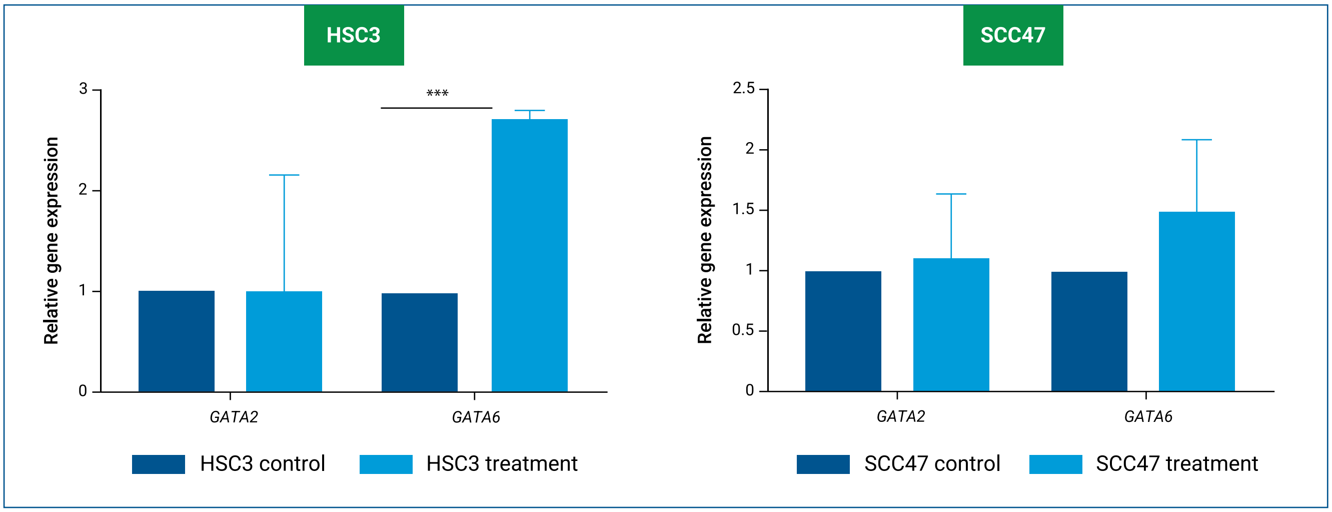

Figure 2. Relative expression of *GATA2* and *GATA6* following cisplatin treatment in HSC3 and SCC47 cells. HSC3 cells were treated with cisplatin at 2.18 μM for 48 hours, whereas SCC47 cells were treated at 5.6 μM for 48 hours (corresponding to their respective IC_{50} values). Gene expression was quantified by qRT-PCR, normalized to ACTB, and calculated using the $2^{-\Delta\Delta Ct}$ method. Data represent the mean ± SD from three independent biological replicates, each performed in technical triplicates. Statistical significance was determined using an unpaired two-tailed Student's *t*-test compared with untreated controls ($p<0.05$, $p<0.01$, $p<0.001$).

HNSCC cell lines. In HSC3 cells, significant cytotoxicity was observed at concentrations of ≥4 μM. Specifically, at 10 μM, cell viability decreased to below 20% after 72 hours of treatment ($p<0.001$) (Figure 1). The half-maximal inhibitory concentration (IC_{50}) of cisplatin in HSC3 cells was calculated as 2.18 μM at 48 hours. For SCC47 cells, a more gradual decline in viability was observed, with the IC_{50} determined to be 5.6 μM at 48 hours. At the highest tested concentration (19.98 μM), cell viability dropped below 15% after 72 hours (Figure 1).

Differential Effects of Cisplatin on *GATA2* and *GATA6* Expression in HSC3 and SCC47

To assess the impact of cisplatin, gene expression analysis was performed 48 hours after treatment with the respective IC_{50} doses, revealing distinct expression patterns of *GATA2* and *GATA6* between the two cell lines. In HSC3 cells, *GATA6* expression was markedly upregulated, showing an approximately 3-fold increase compared to untreated controls, while *GATA2* levels remained largely unchanged (Figure 2). In contrast, SCC47 cells exhibited a slight increase in both *GATA2* and *GATA6* expression following cisplatin exposure; however, these changes were not statistically significant (Figure 2).

DISCUSSION

In this study, we demonstrated that cisplatin induces a time and dose-dependent cytotoxic effect on the HNSCC cell lines HSC3 and SCC47, with IC_{50} values of 2.18 μM and 5.6 μM, respectively. These results align with previous reports highlighting variability in cisplatin sensitivity among HNSCC cell lines. This variability may be attributed to intrinsic molecular factors such as p53 status, DNA repair capacity, apoptotic threshold, and HPV status. Notably, SCC47 is HPV-positive, whereas HSC3 is HPV-negative, a difference that may contribute to their differential responses to cisplatin (16).

Beyond its direct cytotoxic effects, cisplatin modulated the expression of *GATA2* and *GATA6*, transcription factors involved in cell differentiation, proliferation, and stress response (17). In HSC3 cells, *GATA2* expression remained largely unchanged following cisplatin exposure, while *GATA6* was significantly upregulated, suggesting a possible role in the cellular response to DNA damage. In SCC47 cells, both *GATA2* and *GATA6* showed a modest increase in expression; however, these changes did not reach statistical significance. This may reflect a less pronounced transcriptional response or differences in regulatory sensitivity compared to HSC3.

These findings demonstrate that cisplatin reduces cell viability in a cell line-dependent manner and differentially modulates the expression of key transcription factors. Notably, *GATA6* exhibited a substantial increase in the more cisplatin-sensitive HSC3 cells, suggesting a possible role in stress adaptation or the cellular response to DNA damage. An alternative interpretation is that *GATA6* upregulation may reflect the activation of pro-apoptotic pathways or an attempt to drive differentiation, thereby sensitizing HSC3 cells to cisplatin. Based on this, we propose two testable hypotheses: first, *GATA6* may facilitate the transcription of apoptotic regulators in response to cisplatin-induced DNA damage, lowering the apoptotic threshold in HSC3. Second, *GATA6* may promote partial differentiation programs that reduce cellular plasticity and survival capacity, thereby enhancing cisplatin cytotoxicity. In contrast, *GATA2* expression remained unchanged in HSC3, while both *GATA2* and *GATA6* showed modest, non-significant upregulation in SCC47 cells. These distinct expression patterns point to a cell-specific transcriptional response to cisplatin and underscore the potential of *GATA2* and *GATA6* as biomarkers or therapeutic targets in HNSCC.

Previous studies have implicated *GATA2* in both tumor suppression and progression, contingent on the cellular context. Notably, previous research in colorectal cancer has shown that high *GATA2* expression is significantly correlated with poor disease-free survival and increased recurrence risk, highlighting its potential role as a prognostic biomarker (18). In contrast to findings in colorectal cancer, reduced expression of the hematopoietic transcription factor *GATA2* has been associated with poor prognosis in hepatocellular carcinoma (HCC) patients following surgical resection (19). This suggests that the prognostic role of *GATA2* may vary depending on tumor type and tissue context.

GATA6 is a member of the evolutionarily conserved GATA transcription factor family, which regulates gene expression by binding to GATA-specific motifs located within promoter regions (20). *GATA6* has been implicated in many cancer types, exhibiting context-dependent functions. In certain malignancies, such as gastric, colorectal, and breast cancers, as well as cutaneous T-cell lymphoma, it acts as an oncogenic driver, contributing to tumor progression (21-24).

In line with our findings that cisplatin induces differential *GATA6* expression in HNSCC, recent studies in oral squamous cell carcinoma (OSCC) provide mechanistic insights into *GATA6*'s oncogenic roles. Notably, *GATA6* has been shown to bind the *FN1* promoter and upregulate fibronectin-1 expression, thereby promoting proliferation, invasion, and migration in OSCC models; these effects were reversed upon *FN1* overexpression following *GATA6* knockdown (25).

Conversely, in other tumor types, including astrocytoma and HCC, *GATA6* has been shown to exert tumor-suppressive effects (26,27). Collectively, our findings indicate that *GATA2* and *GATA6* show differential expression in response to cisplatin in a gene and cell line-dependent manner. While these observations highlight a potential association between GATA factors and treatment sensitivity, further functional validation is required before they can be considered reliable biomarkers or therapeutic targets in HNSCC.

This study has several limitations. Only two HNSCC cell lines (one HPV-positive and one HPV-negative) were analyzed, which limits generalizability, particularly given the known differences in p53 pathway status. The study is correlative and lacks functional validation, and although ACTB was used as a housekeeping gene, inclusion of additional reference genes would strengthen the qPCR analyses. Finally, the mechanisms underlying GATA modulation remain unclear and warrant further investigation.

Ethical Approval: N:A:

Informed Consent: N.A.

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REFERENCES

1 Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229-63. [CrossRef]

2 Sun Z, Sun X, Chen Z, Du J, Wu Y. Head and neck squamous cell carcinoma: risk factors, molecular alterations, immunology and peptide vaccines. Int J Pept Res Ther. 2022;28(1):19. [CrossRef]

3 Liu Y, Zhang N, Wen Y, Wen J. Head and neck cancer: pathogenesis and targeted therapy. MedComm (2020). 2024;5(9):e702. [CrossRef]

4 Kanno Y, Chen CY, Lee HL, Chiou JF, Chen YJ. Molecular mechanisms of chemotherapy resistance in head and neck cancers. Front Oncol. 2021;11:640392. [CrossRef]

5 Yu W, Chen Y, Putluri N, Coarfa C, Robertson MJ, Putluri V, et al. Acquisition of cisplatin resistance shifts head and neck squamous cell carcinoma metabolism toward neutralization of oxidative stress. Cancers (Basel). 2020;12(6):1670. [CrossRef]

6 Robinson AM, Rathore R, Redlich NJ, Adkins DR, VanArsdale T, Van Tine BA, et al. Cisplatin exposure causes c-Myc-dependent resistance to CDK4/6 inhibition in HPV-negative head and neck squamous cell carcinoma. Cell Death Dis. 2019;10(11):867. [CrossRef]

7 Zheng R, Blobel GA. GATA transcription factors and cancer. Genes Cancer. 2010;1(12):1178-88. [CrossRef]

8 Vishnoi K, Viswakarma N, Rana A, Rana B. Transcription factors in cancer development and therapy. Cancers (Basel). 2020;12(8):2296. [CrossRef]

9 Lentjes MH, Niessen HE, Akiyama Y, de Bruïne AP, Melotte V, van Engeland M. The emerging role of GATA transcription factors in development and disease. Expert Rev Mol Med. 2016;18:e3. [CrossRef]

10 Shu J, Zhang K, Zhang M, Yao A, Shao S, Du F, et al. GATA family members as inducers for cellular reprogramming to pluripotency. Cell Res. 2015;25(2):169-80. [CrossRef]

11 Peters IJA, de Pater E, Zhang W. The role of *GATA2* in adult hematopoiesis and cell fate determination. Front Cell Dev Biol. 2023;11:1250827. [CrossRef]

12 Lin H, Hu P, Zhang H, Deng Y, Yang Z, Zhang L. *GATA2*-mediated transcriptional activation of Notch3 promotes pancreatic cancer liver metastasis. Mol Cells. 2022;45(5):329-42. [CrossRef]

13 Ma M, An J, Jiang T, Xie K. GATA6 in pancreatic cancer initiation and progression. Genes Dis. 2024;12(2):101353. [CrossRef]

14 Ju W, Lin L, Zhang Q, Lv X, Teng S, Hong Y, et al. *GATA6* inhibits the biological function of non-small cell lung cancer by modulating glucose metabolism. J Cancer Res Clin Oncol. 2024;150(3):126. [CrossRef]

15 Lin L, Bass AJ, Lockwood WW, Wang Z, Silvers AL, Thomas DG, et al. Activation of GATA binding protein 6 (*GATA6*) sustains oncogenic lineage-survival in esophageal adenocarcinoma. Proc Natl Acad Sci U S A. 2012;109(11):4251-6. [CrossRef]

16 Aggarwal N, Yadav J, Thakur K, Bibban R, Chhokar A, Tripathi T, et al. Human papillomavirus infection in head and neck squamous cell carcinomas: transcriptional triggers and changed disease patterns. Front Cell Infect Microbiol. 2020;10:537650. [CrossRef]

17 Rong L, Liu J, Qi Y, Graham AM, Parmacek MS, Li S. GATA-6 promotes cell survival by up-regulating BMP-2 expression during embryonic stem cell differentiation. Mol Biol Cell. 2012;23(18):3754-63. [CrossRef]

18 Chen L, Jiang B, Wang Z, Liu M, Ma Y, Yang H, et al. Expression and prognostic significance of GATA-binding protein 2 in colorectal cancer. Med Oncol. 2013;30(2):498. [CrossRef]

19 Li YW, Wang JX, Yin X, Qiu SJ, Wu H, Liao R, et al. Decreased expression of GATA2 promoted proliferation, migration and invasion of HepG2 *in vitro* and correlated with poor prognosis of hepatocellular carcinoma. PLoS One. 2014;9(1):e87505. [CrossRef]

20 Ayanbule F, Belaguli NS, Berger DH. GATA factors in gastrointestinal malignancy. World J Surg. 2011;35(8):1757-65. [CrossRef]

21 Deng X, Jiang P, Chen J, Li J, Li D, He Y, et al. *GATA6* promotes epithelial-mesenchymal transition and metastasis through MUC1/β-catenin pathway in cholangiocarcinoma. Cell Death Dis. 2020;11(10):860. [CrossRef]

22 Kamijo H, Miyagaki T, Shishido-Takahashi N, Nakajima R, Oka T, Suga H, et al. Aberrant CD137 ligand expression induced by *GATA6* overexpression promotes tumor progression in cutaneous T-cell lymphoma. Blood. 2018;132(18):1922-35. [CrossRef]

23 Song Y, Tian T, Fu X, Wang W, Li S, Shi T, et al. *GATA6* is overexpressed in breast cancer and promotes breast cancer cell epithelial-mesenchymal transition by upregulating slug expression. Exp Mol Pathol. 2015;99(3):617-27. [CrossRef]

24 Sulahian R, Casey F, Shen J, Qian ZR, Shin H, Ogino S, et al. An integrative analysis reveals functional targets of *GATA6* transcriptional regulation in gastric cancer. Oncogene. 2014;33(49):5637-48. [CrossRef]

25 Zhai J, Luo G. *GATA6* induced FN1 activation promotes the proliferation, invasion and migration of oral squamous cell carcinoma cells. Mol Med Rep. 2022;25(3):102. [CrossRef]

26 Kamnasaran D, Qian B, Hawkins C, Stanford WL, Guha A. *GATA6* is an astrocytoma tumor suppressor gene identified by gene trapping of mouse glioma model. Proc Natl Acad Sci U S A. 2007;104(19):8053-8. [CrossRef]

27 Tan HW, Leung CO, Chan KK, Ho DW, Leung MS, Wong CM, et al. Deregulated *GATA6* modulates stem cell-like properties and metabolic phenotype in hepatocellular carcinoma. Int J Cancer. 2019;145(7):1860-73. [CrossRef]