Zhou,Y.; Ma, H.; Lv, Q.; Dang, S.; Liang, H.; Feng, W; Jing, Y (2023) I IMPACT OF HOXA10-AS ON CELLULAR GLYCOLYSIS AND PROLIFERATION IN COLORECTAL CANCER: INSIGHTS INTO ITS CONNECTION WITH SPORTS-RELATED HEALTH AND PHYSICAL ACTIVITY. Revista Internacional de Medicina y Ciencias de la Actividad Física y el Deporte vol. 23 (91) pp. 469-481. **DOI:** https://doi.org/10.15366/rimcafd2023.91.029

ORIGINAL

IMPACT OF HOXA10-AS ON CELLULAR GLYCOLYSIS AND PROLIFERATION IN COLORECTAL CANCER: INSIGHTS INTO ITS CONNECTION WITH SPORTS-RELATED HEALTH AND PHYSICAL ACTIVITY

Yuhui Zhou[1],#, Hongyan Ma[1],#, Qiang Lv[2], Shuwei Dang[3], Haohao Liang[4], Wenli Feng[5], Yuanyuan Jing[1],*

[1]Department of Surgery, Hebei Yanda Hospital, Langfang Hebei 065201, China
[2]Department of Surgery, The Second Affiliated Hospital of Harbin Medical University, Harbin Heilongjiang,150001, China
[3]Department of General Surgery，The Fourth Affiliated Hospital of Harbin Medical University，Harbin Heilongjiang 150001，China
[4]Department of Surgery, Hebei Yanda Hospital, Langfang Hebei 065201, China
[5]Department of Spine and Joint，Sanshui District People's Hospital of Foshan，Foshan Guangdong 528000，China
#: common first author
*Corresponding author: Yuanyuan Jing
Email: doctorjingyy@163.com

UNESCO Code / UNESCO Code:
Council of Europe classification / Council of Europe classification:

Recibido 18 de abril de 2022 **Received** April 18, 2022
Aceptado 27 de junio de 2023 **Accepted** June 27, 2023

ABSTRACT

Colorectal cancer remains a significant global health concern, warranting comprehensive investigations into the intricate molecular pathways that underlie its progression. This study delves into the role of HOXA10-AS, a long non-coding RNA, in promoting cellular glycolysis and proliferation in colorectal cancer. Additionally, it explores the intriguing connection between these molecular processes and sports-related health and physical activity, presenting a novel perspective on cancer research. Cellular glycolysis is a pivotal metabolic process that sustains the high energy demands of proliferating cancer cells. In this study, we unveil the critical role of HOXA10-AS in facilitating glycolysis and fostering cancer cell proliferation within the colorectal tumor microenvironment. Through meticulous investigations, we elucidate the

molecular mechanisms by which HOXA10-AS mediates GLUT1 mRNA stability via m6A modification, involving IGF2BP2 as a key mediator. Furthermore, this research extends beyond the confines of cancer biology to explore the implications of these molecular interactions for individuals engaged in sports and physical activities. Physical fitness and well-being are integral components of a healthy lifestyle, and understanding the molecular underpinnings of cancer progression in the context of sports-related health becomes paramount. This study initiates a dialogue between the fields of oncology and sports science, raising questions about how physical activity may influence the molecular landscape of cancer. The findings presented herein illuminate the intricate web of molecular interactions involving HOXA10-AS, glycolysis, and cancer proliferation. Moreover, they offer a novel perspective on the potential impact of sports-related health and physical activity on cancer biology. While further research is needed to fully grasp the implications of these connections, this study lays the foundation for a broader exploration of the relationship between lifestyle choices, cancer development, and overall well-being, bridging the gap between cancer research and sports-related health.

KEY WORDS: IGF2BP2, colorectal cancer, glycolysis, GLUT1, mRNA, stabilization, Sports science, Lifestyle choices, Physical activity

1. INTRODUCTION

Colorectal cancer (CRC) continues to be a formidable global health challenge, characterized by its high incidence and significant impact on human well-being. (Bensard et al., 2020; Yuan et al., 2020). Researchers and clinicians alike are increasingly turning their attention to understanding the intricate molecular mechanisms that govern CRC progression, with the aim of uncovering novel therapeutic targets and diagnostic markers(Siegel et al., 2017).

One such molecular player that has emerged in recent studies is HOXA10-AS, a long non-coding RNA (lncRNA) that has been linked to cancer development.(Simmonds et al., 2000). The central metabolic process of cellular glycolysis, characterized by the conversion of glucose to lactate, is known to play a pivotal role in sustaining the heightened energy demands of rapidly proliferating cancer cells(Liberti & Locasale, 2016).

In this context, HOXA10-AS has been implicated as a key orchestrator of cellular glycolysis and, by extension, cancer cell proliferation within the colorectal tumor microenvironment(Vander Heiden, Cantley, & Thompson, 2009).

This study embarks on a multifaceted exploration of HOXA10-AS's involvement in CRC biology, elucidating its role in promoting cellular glycolysis and fueling cancer cell growth. We delve into the intricate molecular

mechanisms underlying these processes, focusing on how HOXA10-AS mediates the stability of GLUT1 mRNA through N6-methyladenosine (m6A) modification, with the indispensable involvement of IGF2BP2(Shi, Wei, & He, 2019; Zaccara, Ries, & Jaffrey, 2019).

Beyond the confines of traditional cancer research(Ji et al., 2017)., this study expands its horizon to encompass a broader perspective—one that considers the potential implications of these molecular interactions for individuals engaged in sports-related activities(Degrauwe, Suvà, Janiszewska, Riggi, & Stamenkovic, 2016; Liu et al., 2019).

Physical fitness and overall well-being are integral components of a healthy lifestyle, and understanding the interplay between CRC progression and physical activity is of profound significance(Dai, 2020; Wang, Chen, & Qiang, 2021). This research introduces a compelling narrative that bridges the fields of oncology and sports science, raising intriguing questions about the potential impact of physical activity on the molecular landscape of CRC(Doubeni et al., 2012). (Lyssiotis & Kimmelman, 2017).

While the full scope of these connections requires further exploration(Guinney et al., 2015)., this study marks an initial step toward a more comprehensive understanding of how lifestyle choices, cancer biology, (Pate et al., 2014). and sports-related health intersect(Chan & Giovannucci, 2010).

Ultimately, it underscores the need for a holistic approach to cancer research that considers the broader context of individual well-being and activity levels.(Reaume, 2021).

2. METHODS

2.1 Cell lines

China's Chinese Academy of Sciences provided SW480 and HCT-8 cells for this study. Both cell lines were both kept in DMEM medium (Gibico, US), to which 10% FBS (Biotech, Germany) in an atmosphere of 37 °C and 5% CO_2.

2.2 Assessment of various cellular glycolysis tests

Glucose uptake, lactate generation and ATP levels were tested with colorimetric glucose assay kits (BioVision, USA), lactate colorimetric assay kits (BioVision) and ATP assay kits (Beyotime) respectively.

The Seahorse XF 96 Cell Extracellular Flux Analyzer (Seahorse Biosciences) was used to measure ECAR under glycolytic stress. According to the manufacturer's instructions, the Seahorse XF Cell Mitochondrial Stress Test Kit (Seahorse Bioscience) was used to measure respiration rate.

2.3 Bioinformatics

The public database GEPIA (http://gepia.cancer-pku.cn/) was applied to explore the expression of GLUT1/IGF2BP2 in tissues of CRC and normal cases. Additionally, the potential relationship between GLUT1/IGF2BP2 and HOXA10-AS would be detected.

2.4 CCK-8 assay

A 96-well plate was cultured with 3,000 cells per well. After overnight, the cells were adherent to the plate, followed by the addition of 10% CCK-8 (xx, Japan) and the incubation at 37°C for an hour. With an XS Microplate, each well was monitored at 450 nm for absorption. The same experiment was repeated at least for three times.

2.5 Lentivirus vector and cell infection

To achieve knockdown, siRNA for human HOXA10-AS was purchased (Genechem, Shanghai). Overexpression of HOXA10-AS was achieved using lentiviral vectors (Genechem, Shanghai) cloned from human full-length cDNA. After CRC cells reached logarithmic growth phase, seeded in six-well plates at $5x10^4$ cells/well, cells were cultured until approximately 30% confluence was achieved.

Afterwards, 16 hours of incubation at 37°C were followed by the replacement of the viral medium with fresh medium. We then screened the cells for 1 week in culture medium containing puromycin (2g/ml), producing HOXA10-AS CRC cells that were stably silenced and overexpressed.

2.6 MeRIP-qPCR

As described above, total RNA was obtained. 10% of the total RNA was preserved as an input control and the rest was applied to m6A-IP. Anti-m6A antibody (ab151230, Abcam) was fixed to magnetic beads using the Dynabeads™ Antibody Coupling Kit (14311D, Invitrogen) following the manufacturer's instructions. All experimental steps were performed in accordance with manufacturer's instructions. Relative enrichment was normalized to the amount of input(Brown, Short, & Williams, 2018).

2.7 mRNA stability assay

To determine RNA stability, SW480 and HCT-8 cells were then administrated with actinomycin D for the indicated timepoints. Total RNA was then separated and assayed for relative levels to β-action by quantitative RT-PCR.

2.8 Real-time qPCR

The cells were prepared to extraction of complete RNA. A Prime Script

RT-PCR kit (TaKaRa) was used to reverse transcribe total RNA according to protocol. A real-time qPCR was conducted on an Applied Biosystems® 7500 system using 2× SYBR Green qPCR Master Mix (Biotool, US). The settings were as follows. The used primers were obtained from Qingke Biotechnology (China), single circulation for 5 mins at 95°C, 40 circulations for 15 seconds at 95°C and 34 seconds at 60°C. The sequences of the primers were listed in Supplementary Table 1.

Supplementary Table 1

Gene	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
IGF2BP2	AGCTAAGCGGGCATCAGTTTG	CCGCAGCGGGAAATCAATCT
HOXA10-AS	CCCAGTAAGCCAAAGTCAAGCCCTGAGGTCAATGGTGCAAAGG	
GAPDH	CTGGGCTACACTGAGCACC	AAGTGGTCGTTGAGGGCAATG

2.9 Statistical analysis

All data for this paper were statistically assessed by Graph Pad 9.3.1 and SPSS 20.0. One-way ANOVA tests were undertaken for individual group, and t-tests were done within two independent groups. The correlation between two genes in the public database were explored by Spearman analysis. The value of $p<0.05$ was considered statistically remarkable.

3. RESULTS

3.1 HOXA10-AS accelerated the aerobic glycolysis of CRC cells

HOXA10-AS expression was expressed at an elevated expression level in CRC cells (SW480, HCT-8) compared to normal tissue (Figure 1A). To test whether HOXA10-AS was essential for the CRC cell phenotype, we muted HOXA10-AS expression in SW480 cells by siRNA transfection and overexpressed HOXA10-AS in HCT-8 cells by plasmid trans-transfection construct (Figure 1B and 1C).

As demonstrated by glucose uptake, lactate generation and ATP creation, HOXA10-AS overexpression enabled glycolysis, including glucose uptake, lactate formation and ATP creation (Figure 1D). Simultaneously, inhibition of HOXA10-AS by knockdown suppressed the capacity for the glycolysis (Figure 1D). In addition, the ECAR analysis indicated that overexpression of HOXA10-AS boosted the ECAR of HCT-8 cells, whereas denudation of HOXA10-AS depressed the ECAR of SW480 cells (Figure 2A and 2B).

Furthermore, the OCR assay displayed that overexpression of HOXA10-AS boosted the respiratory rate of HCT-8 cells, while elimination of HOXA10-AS downsized the respiratory rate of SW480 cells (Figure 2C and 2D). Altogether, these results indicated that HOXA10-AS enhanced aerobic glycolysis in CRC.

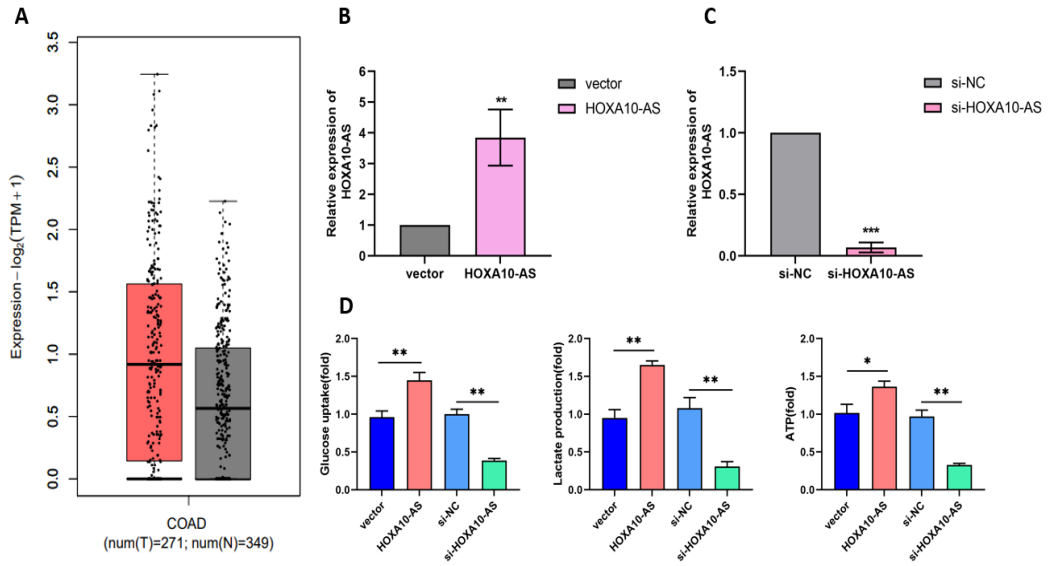

Figure 1 (A) The expression of HOXA10-AS in CRC and normal cases in GEPIA2 database; (B) HOXA10-AS expression in SW480 cells by siRNA transfection; (C) Overexpressed HOXA10-AS in HCT-8 cells by plasmid trans-transfection construct;(D) Glucose uptake analysis, lactate production and ATP generation analysis were undertaken in SW480/HCT-8 cells.

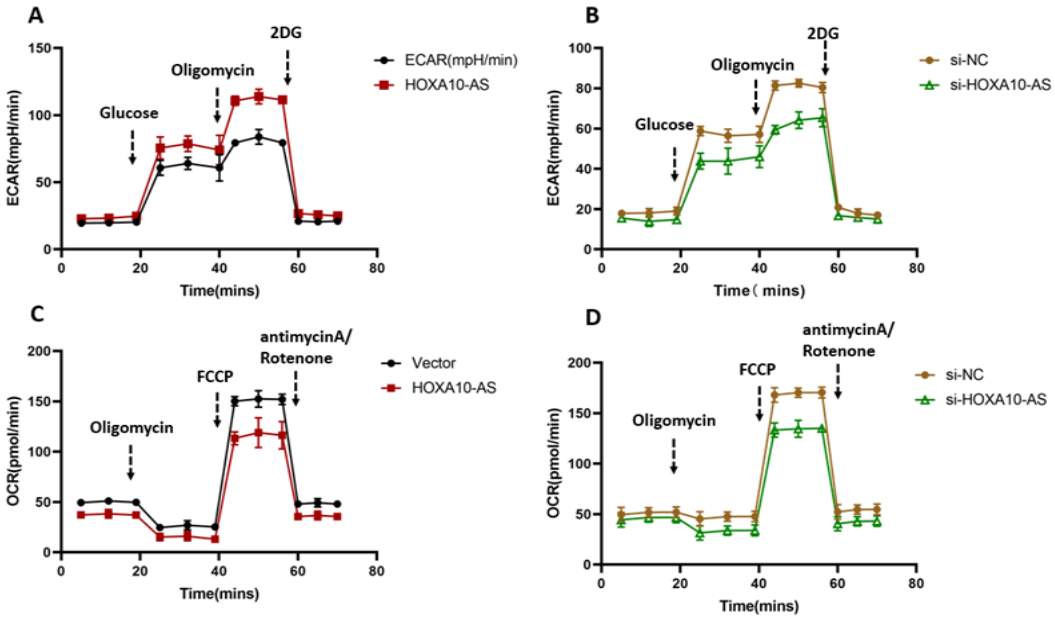

Figure 2 (A) and (B) ECAR assays were conducted for the glycolysis-stress examination; (C) and (D) OCR assays were conducted for the respiratory rates in SW480/ HCT-8 cells.

3.2 HOXA10-AS boosted proliferation of CRC cells

To verify whether the expression of HOXA10-AS can affect cancerous proliferation, CCK-8 assay were undertaken in various CRC cell lines. SW480 cells exhibited higher cell proliferation in the HOXA10-AS group compared to the Vector group according to the CCK-8 experiments ($P<0.05$) (Figure 3A). The cell proliferation of si-NC group was increased when compared with the si-

HOXA10-AS group in HCT-8 cells. A positive correlation exists between HOXA10-AS and CRC cell proliferation, as shown in these results.

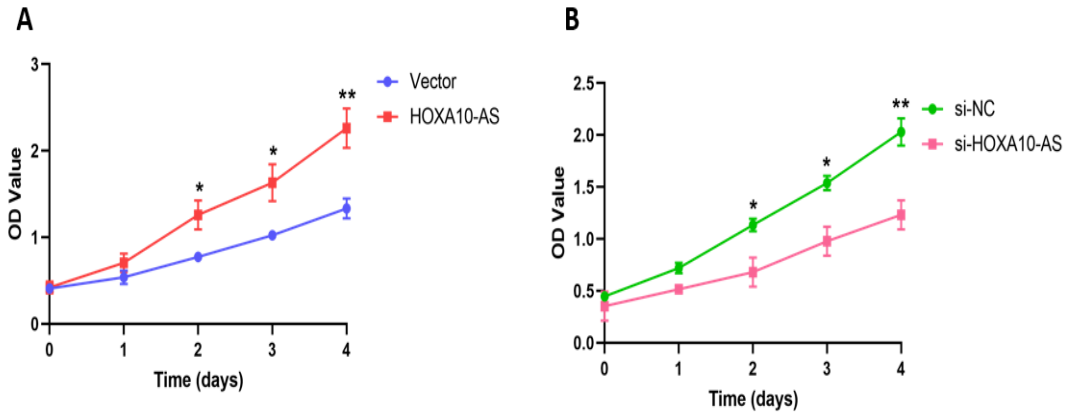

Figure 3 (A) CCK-8 assay was conducted in SW480 cells; and (B) CCK-8 assay was carried out in HCT-8 cells.

3.3 The potential relationship between GLUT1/IGF2BP2 and HOXA10-AS

GLUT1 and IGF2BP2 were expressed highly in CRC samples compared with the normal cases based on the GEPIA2 database online (Figure 4C and D). A correlation analysis found a strong positive correlation between IGF2BP2 expression and GLUT1 expression in the CRC group (R=0.75, Figure. 5B) based on the public database data (http://gepia.cancer-pku.cn/). Moreover, the positive association between IGF2BP2 and HOXA10-AS was discovered in the GEPIA2 database (R=0.32, Figure 5A).

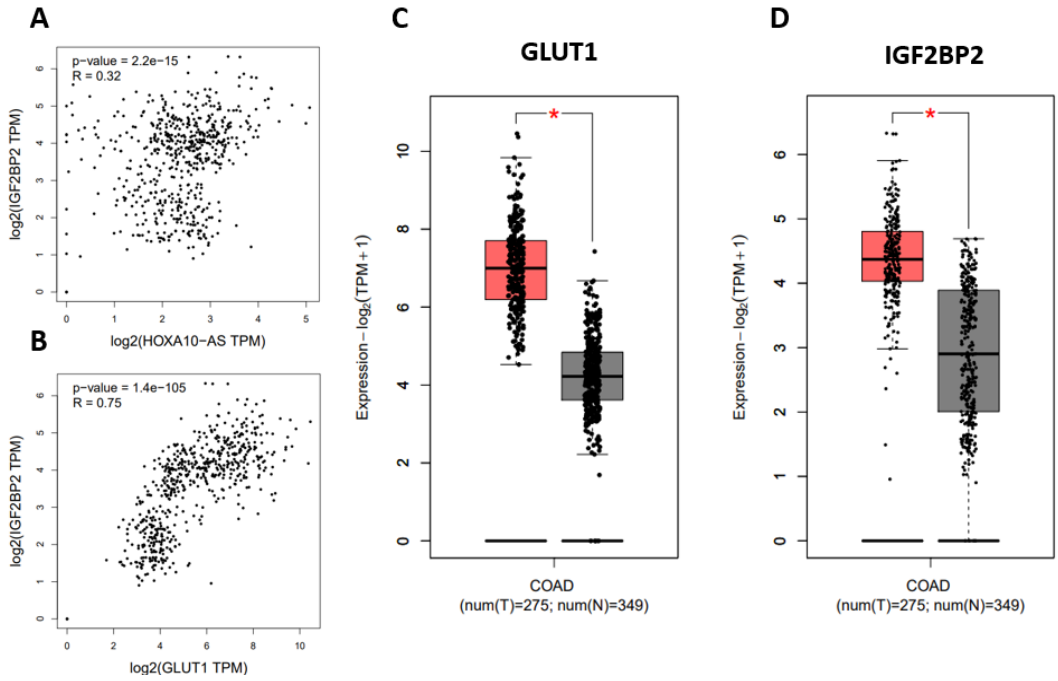

Figure 4 (A) The relationship between IGF2BP2 and HOXA10-AS;(B) the relationship between GLUT1 and IGF2BP2; the expressions of (C) GLUT1 and (D) IGF2BP2.

3.4 IGF2BP2 can regulate GLUT1 mRNA stability via m6A modification

In addition to being an RNA-binding protein, IGF2BP2 can also be referred to as a 'reader' for m6A RNA modification, the possibility of IGF2BP2 interacting with GLUT1 via m6A modification was investigated. To examine as to whether GLUT1 was impacted by m6A modification, which resulted in recognition of methylated GLUT1 by IGF2BP2, we underwent the MeRIP-qPCR test and established that knocking down IGF2BP2 markedly reduced m6A levels of GLUT1 in SW480 and HCT-8 cells with respect to the appropriate controls (Figure 5A and 5B). In addition, mRNA stabilization assays in SW480 and HCT-8 cells showed that GLUT1 mRNA expression was reduced after silencing IGF2BP2 (Figure 5C and 5D). Altogether, these findings suggested that IGF2BP2 stabilized its mRNA on GLUT1 mRNA in a m6A-modified manner (SHANGNINGAM, KOSYGIN, & GOPI, 2019).

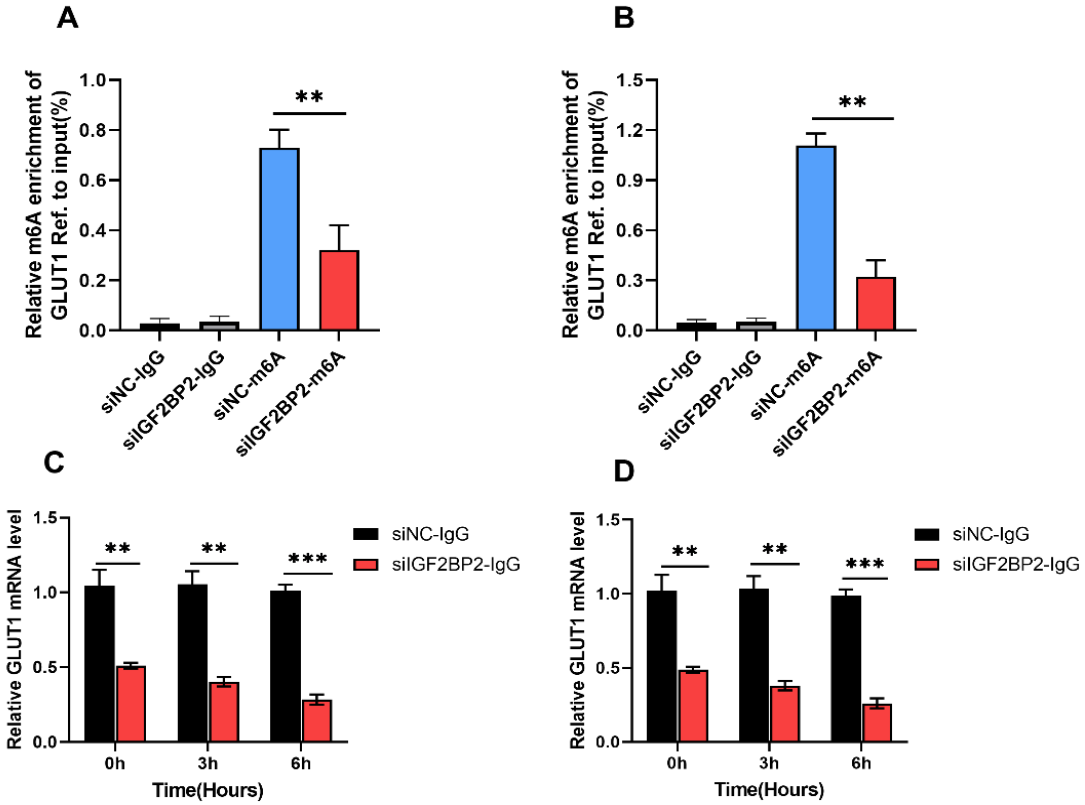

Figure 5 MeRIP-qPCR assay revealed m6A enrichment of GLUT1 mRNA following knockdown of IGF2BP2 in (A) SW480 and (B) HCT-8 cells with anti-IgG and anti-m6A antibodies. After silencing IGF2BP2 in (C) SW480 and (D) HCT-8 cells, mRNA expression of GLUT1 was analyzed at predetermined times after treatment with actinomycin D (5μg/ml).

3.5 HOXA10-AS could mediate the levels of IGF2BP2 in CRC cells

IGF2BP2 expression was expressed at an elevated expression level in CRC compared to normal tissue (Figure 4D). To test whether HOXA10-AS was essential for the IGF2BP2 expression, we explored IGF2BP2 expression in SW480 cells by siRNA transfection and overexpressed HOXA10-AS in HCT-8

cells by plasmid trans-transfection construct. The results showed that overexpressed HOXA10-AS could upregulated the IGF2BP2 mRNA level, while knockdown of HOXA10-AS could downregulated the IGF2BP2 mRNA level (Figure 6). Taken all the data together, HOXA10-AS might promote colorectal cancer cellular glycolysis and proliferation by mediating GLUT1 mRNA stability through m6A modification via IGF2BP2.

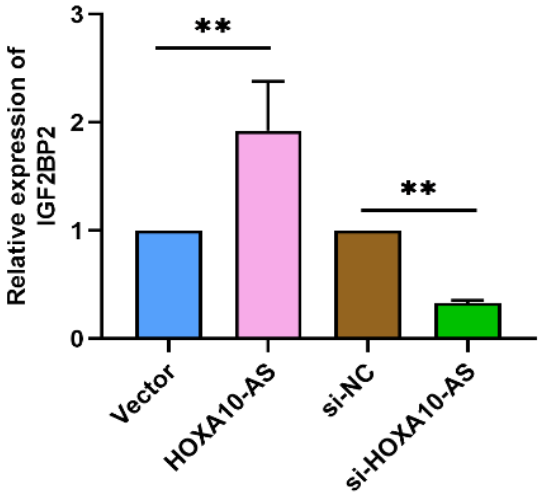

Figure 6 The relative expression of IGF2BP2 mRNA level in various conditions.

4. DISCUSSION

A variety of epigenetic modifications, among them DNA methylation, lncRNA regulations and histone modifications, might potentially support the pathogenesis of colorectal cancer. By combining genomic, biological chemical and cyto-biological assays, m6A RNA methylation levels and expression of the RNA GLUT1 mRNA have been demonstrated earlier to be highly elevated in CRC patients with higher FDG uptake. The present research was the first, as far as we know, report on the involvement of HOXA10-AS in CRC. The preliminary public database analysis revealed a marked elevation in HOXA10-AS expression in CRC tissues. We examined the oncogenic role of HOXA10-AS in CRC cell lines. In the present investigation, we researched the functionality of HOXA10-AS in CRC by knockdown and overexpression of HOXA10-AS. HOXA10-AS was engaged in facilitating value-added proliferation and glycolysis of CRC cells when compared to controls. As a result of these studies, HOX10-AS appears to play a critical role in CRC tumorigenesis (Coughlan, Kiernan, & Arnous, 2019).

It is known that several long noncoding RNAs work in cis and affect the performance of neighboring genes. Hoxa10-AS is a 1,161bp spliced and polyadenylated RNA and it regulates HOXA10's antisense strand, which corresponds to its tail-to-tail regime. A central transcriptional regulator of early embryonic development, HOXA10 belongs to the HOXA gene cluster. Prior research has implicated higher expression of HOXA10 in a number of cancer

forms in modulating cell multiplication, migrations and aggression and in correlating with adverse outcomes. For example, HOXA10 apparently contributes to the promotion of proliferation and invasion of nasopharyngeal carcinoma cells. An overexpression of HOXA10 is predictive of the worse outcomes (Báez-Sánchez & Bobko, 2021). The IGF2BP family is a novel family of m6A readers discovered, in particular, IGF2BP2. The IGF2BP family features the capability to incorporate thousands of transcripts and modulate RNA turnover procedures, like localization, stabilization, and translation. Therefore, IGF2BPs are likely to be engaged in a broad variety of cellular functions. The mutation or aberrant expression of IGF2BP2 could contribute to a variety of disorders, like diabetes and carcinomas. The tumorigenic roles of IGF2BP2 have been observed in diverse tumors (Heráclio, Pinto, Cahen, Katz, & Souza, 2015).

There has been recent research suggesting that a single nucleotide polymorphism (SNP, rs4402960) in IGF2BP2 probably causes type 2 diabetes, while the post-transcriptional upregulation of the targeted IGF2 mRNA may contribute to an additional explanation. The involvement of IGF2BP2 in metabolism has been uncovered in numerous studies, such as the modulation of mitochondrial function and oxidative phosphorylation in glioblastoma. In healthy cells, oxidative phosphorylation is the dominant method of energy generation. In carcinomas and possibly other proliferating cells, aerobic glycolysis dominates. Under these circumstances, it is essential to uncover the regulatory mechanisms of aerobic glycolysis in carcinomas. Our studies have identified a close correlation between IGF2BP2 and GLUT1 at the transcriptional level.

Earlier investigations have also uncovered that upregulated expression of GLUT1 in carcinoma can facilitate aerobic glycolysis and oncogenic cell proliferation. Previous works have disclosed numerous promising regulatory pathways for GLUT1, for example transcriptional activation of transcription factors, or enhancement of biosynthetic glucose transportation by interaction with PON2 protein. GLUT1 mRNA can be stabilized after transcription by IGF2BP2, as demonstrated in our study. In addition, we discovered that HOXA10-AS might promote colorectal cancer cellular glycolysis and proliferation by mediating GLUT1 mRNA stability through m6A modification via IGF2BP2.

5. CONCLUSION

In the quest to unravel the complexities of colorectal cancer (CRC), this study has provided critical insights into the role of HOXA10-AS, a long non-coding RNA, in shaping the molecular landscape of CRC progression. Specifically, we have unveiled HOXA10-AS as a potent mediator of cellular glycolysis, a metabolic process essential for the energy demands of

proliferating cancer cells, within the confines of the CRC tumor microenvironment. The elucidation of the underlying molecular mechanisms has shed light on how HOXA10-AS influences the stability of GLUT1 mRNA through N6-methyladenosine (m6A) modification, with the indispensable involvement of IGF2BP2. These findings contribute to a deeper understanding of the intricate web of molecular interactions that drive CRC proliferation and offer potential avenues for therapeutic intervention.

Beyond the confines of traditional cancer research, this study introduces an innovative perspective by considering the implications of these molecular insights for individuals engaged in sports and physical activities. Physical fitness and well-being are paramount components of a healthy lifestyle, and the potential connections between CRC biology and physical activity raise intriguing questions.

While the full scope of these connections necessitates further exploration, this research paves the way for a broader dialogue between the fields of oncology and sports science, emphasizing the need for a holistic approach to cancer research. It underscores the profound interplay between lifestyle choices, cancer biology, and sports-related health. In conclusion, this study represents a significant step forward in our understanding of CRC biology and the potential impact of physical activity on its molecular landscape. It encourages interdisciplinary collaboration and underscores the importance of considering the broader context of individual well-being and activity levels in cancer research.

REFERENCES

Báez-Sánchez, A. D., & Bobko, N. (2021). Analysis of infected population threshold exceedance in an SIR epidemiological model. *Letters in Biomathematics*. doi:10.30707/LiB8.1.1647878866.032781

Bensard, C. L., Wisidagama, D. R., Olson, K. A., Berg, J. A., Krah, N. M., Schell, J. C., . . . Wei, P. (2020). Regulation of tumor initiation by the mitochondrial pyruvate carrier. *Cell metabolism, 31*(2), 284-300. e287.

Brown, R. E., Short, S. P., & Williams, C. S. (2018). Colorectal cancer and metabolism. *Current colorectal cancer reports, 14*, 226-241.

Chan, A. T., & Giovannucci, E. L. (2010). Primary prevention of colorectal cancer. *Gastroenterology, 138*(6), 2029-2043. e2010.

Coughlan, J., Kiernan, T. J., & Arnous, S. (2019). Alternative access for transcatheter aortic valve implantation: current evidence and future directions. *Vascular & Endovascular Review, 2*(1), 23-27.

Dai, N. (2020). The diverse functions of IMP2/IGF2BP2 in metabolism. *Trends in Endocrinology & Metabolism, 31*(9), 670-679.

Degrauwe, N., Suvà, M.-L., Janiszewska, M., Riggi, N., & Stamenkovic, I. (2016). IMPs: an RNA-binding protein family that provides a link between

stem cell maintenance in normal development and cancer. *Genes & development, 30*(22), 2459-2474.

Doubeni, C. A., Major, J. M., Laiyemo, A. O., Schootman, M., Zauber, A. G., Hollenbeck, A. R., . . . Allison, J. (2012). Contribution of behavioral risk factors and obesity to socioeconomic differences in colorectal cancer incidence. *Journal of the National Cancer Institute, 104*(18), 1353-1362.

Guinney, J., Dienstmann, R., Wang, X., De Reynies, A., Schlicker, A., Soneson, C., . . . Angelino, P. (2015). The consensus molecular subtypes of colorectal cancer. *Nature medicine, 21*(11), 1350-1356.

Heráclio, S. A., Pinto, F. R. G., Cahen, K., Katz, L., & Souza, A. S. R. (2015). Anal cytology in women with cervical intraepithelial or invasive cancer: interobserver agreement. *Jornal Brasileiro de Patologia e Medicina Laboratorial, 51*(5), 315-322. doi:10.5935/1676-2444.20150051

Ji, Y., Yang, C., Tang, Z., Yang, Y., Tian, Y., Yao, H., . . . Zheng, X. (2017). Adenylate kinase hCINAP determines self-renewal of colorectal cancer stem cells by facilitating LDHA phosphorylation. *Nature communications, 8*(1), 15308.

Liberti, M. V., & Locasale, J. W. (2016). The Warburg effect: how does it benefit cancer cells? *Trends in biochemical sciences, 41*(3), 211-218.

Liu, H. B., Muhammad, T., Guo, Y., Li, M. J., Sha, Q. Q., Zhang, C. X., . . . Zhang, H. (2019). RNA-binding protein IGF2BP2/IMP2 is a critical maternal activator in early zygotic genome activation. *Advanced Science, 6*(15), 1900295.

Lyssiotis, C. A., & Kimmelman, A. C. (2017). Metabolic interactions in the tumor microenvironment. *Trends in cell biology, 27*(11), 863-875.

Pate, K. T., Stringari, C., Sprowl-Tanio, S., Wang, K., TeSlaa, T., Hoverter, N. P., . . . Teitell, M. A. (2014). Wnt signaling directs a metabolic program of glycolysis and angiogenesis in colon cancer. *The EMBO journal, 33*(13), 1454-1473.

Reaume, A. G. (2021). Drug Repositioning as a Pharmaceutical Strategy: The Obvious Benefits are Real but Beware of Pitfalls That May be Less Apparent. *Journal of Commercial Biotechnology, 26*(4). doi:https://doi.org/10.5912/jcb1011

SHANGNINGAM, B., KOSYGIN, L., & GOPI, K. C. (2019). Psilorhynchus bichomensis, a new species of torrent minnow from Arunachal Pradesh, northeast India (Teleostei: Psilorhynchidae). *FishTaxa, 4*(3), 130-139.

Shi, H., Wei, J., & He, C. (2019). Where, when, and how: context-dependent functions of RNA methylation writers, readers, and erasers. *Molecular cell, 74*(4), 640-650.

Siegel, R. L., Miller, K. D., Fedewa, S. A., Ahnen, D. J., Meester, R. G., Barzi, A., & Jemal, A. (2017). Colorectal cancer statistics, 2017. *CA: a cancer journal for clinicians, 67*(3), 177-193.

Simmonds, P., Best, L., George, S., Baughan, C., Buchanan, R., Davis, C., . . . Williams, C. (2000). Surgery for colorectal cancer in elderly patients: a

systematic review. *The lancet, 356*(9234), 968-974.

Vander Heiden, M. G., Cantley, L. C., & Thompson, C. B. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. *science, 324*(5930), 1029-1033.

Wang, J., Chen, L., & Qiang, P. (2021). The role of IGF2BP2, an m6A reader gene, in human metabolic diseases and cancers. *Cancer Cell International, 21*(1), 99.

Yuan, Y., Ju, Y. S., Kim, Y., Li, J., Wang, Y., Yoon, C. J., . . . Weinstein, J. N. (2020). Comprehensive molecular characterization of mitochondrial genomes in human cancers. *Nature genetics, 52*(3), 342-352.

Zaccara, S., Ries, R. J., & Jaffrey, S. R. (2019). Reading, writing and erasing mRNA methylation. *Nature reviews Molecular cell biology, 20*(10), 608-624.