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In Silico Characterization of Multi-Layered Regulatory Mechanisms Governing HIF1A Gene Expression

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***Abstract*: Hypoxia-inducible factor 1-alpha (HIF1A) is a key regulator of cellular responses to hypoxia and is controlled through complex regulatory mechanisms. This study presents a comprehensive in silico analysis of the regulatory architecture of HIF1A, integrating epigenetic, transcriptional, post-transcriptional, and functional interaction layers. CpG island analysis revealed a CpG-rich region suggestive of epigenetic control, while promoter prediction identified multiple transcription start sites, indicating transcriptional flexibility. Numerous polyadenylation sites and predicted miRNAs highlighted extensive post-transcriptional regulation. Gene expression analysis demonstrated moderate variability in HIF1A expression, supporting tightly regulated expression dynamics. Protein–protein interaction analysis further established HIF1A as a central hub interacting with key regulators of hypoxia signaling. Overall, the findings provide a holistic view of the multi-level regulatory mechanisms governing HIF1A expression and its role in cellular adaptation to hypoxic conditions.**

***Keywords*: HIF1A, gene regulation, CpG island, protein-protein interaction, microRNA.**

1. Introduction

Hypoxia-inducible factor 1-alpha (HIF1A) is a key transcription factor that plays a central role in cellular adaptation to hypoxic conditions. It functions as part of the heterodimeric hypoxia-inducible factor-1 (HIF-1) complex, which regulates the expression of a wide range of genes involved in angiogenesis, metabolism, cell survival, and erythropoiesis [1], [2]. Under normoxic conditions, HIF1A is rapidly degraded through the von Hippel–Lindau (VHL)-mediated ubiquitin–proteasome pathway, whereas hypoxic conditions stabilize HIF1A, enabling its translocation to the nucleus and subsequent transcriptional activation of target genes [3], [4].

The regulation of HIF1A is highly complex and occurs at multiple levels, including transcriptional, post-transcriptional, and post-translational mechanisms. Epigenetic factors such as DNA methylation and CpG island dynamics play an important role in modulating gene expression, particularly in promoter regions [5]. Additionally, transcriptional regulation is governed by multiple promoter elements and transcription start sites, allowing flexibility in gene activation under different physiological conditions [6]. Post-transcriptionally, microRNAs (miRNAs) and alternative polyadenylation sites further contribute to the fine-tuning of HIF1A expression, enabling rapid cellular responses to environmental changes [7], [8].

Furthermore, HIF1A operates within a complex protein interaction network involving key regulatory proteins such as ARNT, EP300, VHL, and TP53, which collectively coordinate cellular responses to hypoxia and stress [9], [10]. Dysregulation of HIF1A has been implicated in various pathological conditions, including cancer, cardiovascular diseases, and inflammatory disorders, highlighting its significance as a master regulator of cellular homeostasis [2], [11].

Despite extensive studies on HIF1A function, a comprehensive understanding of its regulatory architecture integrating epigenetic, transcriptional, and post-transcriptional mechanisms remains limited. Therefore, the present study aims to perform an integrative in silico analysis of HIF1A to elucidate its multi-layered regulatory framework. By combining CpG island prediction, promoter analysis, gene expression profiling, protein–protein interaction networks, and miRNA target prediction, this study provides a holistic view of the regulatory mechanisms governing HIF1A expression.

2. Materials and Methods

1) Retrieval of Gene Sequence

The genomic sequence of the human HIF1A gene was retrieved from the NCBI database. The reference sequence corresponding to *Homo sapiens* (GRCh38 assembly) was used for all downstream analyses. This sequence served as the input for regulatory element prediction and analysis.

2) CpG Island Prediction

CpG island analysis was performed using the Softberry CpGFinder tool. The parameters were set to default criteria, including GC content ≥50%, observed/expected CpG ratio ≥0.6, and minimum length threshold. CpG islands were identified to evaluate potential epigenetic regulatory regions within the HIF1A gene [12].

3) Promoter Prediction and Transcription Start Site Analysis

Promoter regions and transcription start sites (TSS) were

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predicted using the Softberry TSSG server. This tool identifies promoter regions based on linear discriminant function (LDF) scores and predicts TATA box elements along with transcription factor binding sites. The analysis was conducted using default parameters to identify potential promoter architecture associated with HIF1A regulation [13].

4) Polyadenylation Site Prediction

Potential polyadenylation (polyA) sites were predicted using the Softberry POLYAH program. This tool identifies putative cleavage and polyadenylation signals within the gene sequence, providing insight into post-transcriptional regulation and mRNA processing mechanisms [14].

5) Gene Expression Analysis

Gene expression profiling of HIF1A was performed using the GEPIA platform, which integrates data from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) projects. Differential expression analysis between tumor and normal samples was conducted using ANOVA, with log2(TPM + 1) transformation applied for normalization. This approach enables robust comparison of gene expression across datasets [15].

6) Protein–Protein Interaction Network Analysis

Protein–protein interaction (PPI) analysis was carried out using the STRING database. The HIF1A protein was used as input, and interactions were retrieved for *Homo sapiens*. A medium confidence interaction score (0.4) was applied to construct the interaction network. The resulting network was used to identify key interacting partners and functional associations [16].

7) miRNA Target Prediction

MicroRNA-mediated regulation of HIF1A was analyzed using the miRDB database. This tool employs a machine learning-based algorithm to predict miRNA targets based on sequence complementarity and experimental validation data. High-confidence miRNAs were selected based on target scores for further interpretation [17].

3. Results

1) CpG Island Analysis

CpG island analysis of the HIF1A gene revealed the presence of a single prominent CpG island within the genomic region analyzed. The identified CpG island exhibited a high GC content (65.8%) and an observed-to-expected CpG ratio of 0.818, indicating a strong CpG enrichment. The island spanned approximately 954 base pairs, suggesting its potential role as a regulatory region associated with transcriptional control. The presence of a CpG-rich region supports the involvement of epigenetic mechanisms, such as DNA methylation, in modulating HIF1A gene expression.

Table 1
CpG island characteristics of HIF1A

Parameter	Value
Start position	48
End position	1001
Length (bp)	954
GC content (%)	65.8
CpG count	84
Observed/Expected ratio	0.818

2) Promoter Prediction and Transcription Start Sites

Promoter prediction analysis identified a total of eight putative promoter regions within the HIF1A gene. Among these, several promoters exhibited high linear discriminant function (LDF) scores, indicating strong promoter potential. Notably, multiple promoters were associated with predicted TATA box elements, suggesting classical transcription initiation mechanisms.

The presence of multiple transcription start sites indicates a complex transcriptional regulation pattern, allowing differential gene expression under varying physiological conditions. Additionally, extensive transcription factor binding sites were identified across promoter regions, further supporting regulatory diversity.

Table 2
Predicted promoter regions of HIF1A

Promoter Position	LDF Score	TATA Box Presence
12610	6.36	Yes
25602	5.92	Yes
50586	5.20	Yes
30992	4.60	No
585	4.58	No
42045	4.57	Yes
33681	4.45	Yes
25107	4.06	Yes

3) Polyadenylation Site Prediction

Polyadenylation analysis revealed a total of 43 potential polyA sites within the HIF1A gene sequence. These sites were distributed across the gene region with varying linear discriminant function (LDF) scores, indicating differences in cleavage site strength.

The presence of multiple polyadenylation signals suggests the possibility of alternative mRNA processing, which may lead to transcript diversity and influence mRNA stability, localization, and translational efficiency.

Table 3
Representative polyadenylation sites in HIF1A

Position	LDF Score
3995	5.17
7263	5.48
11054	5.72
14140	6.30
16290	5.76
19622	5.42
24574	5.73
25752	6.40
25895	6.97
34622	6.31

4) Gene Expression Analysis

Gene expression profiling of HIF1A using the GEPIA platform demonstrated a moderate increase in expression in tumor samples compared to normal tissues in skin cutaneous melanoma (SKCM). However, a substantial overlap in expression levels between tumor and normal groups was observed. The log2-transformed TPM values indicate that HIF1A does not exhibit extreme differential expression but rather maintains a moderately elevated and variable expression pattern. This variability suggests that HIF1A expression is

tightly regulated and influenced by multiple regulatory mechanisms.

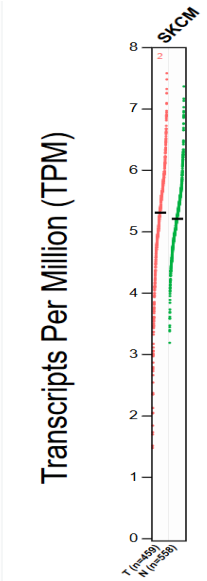

Fig. 1. Gene expression profile of HIF1A

Expression analysis of HIF1A in SKCM using GEPIA. The boxplot represents log2(TPM + 1) values for tumor and normal samples, showing moderate upregulation with overlapping distributions.

5) Protein–Protein Interaction Network

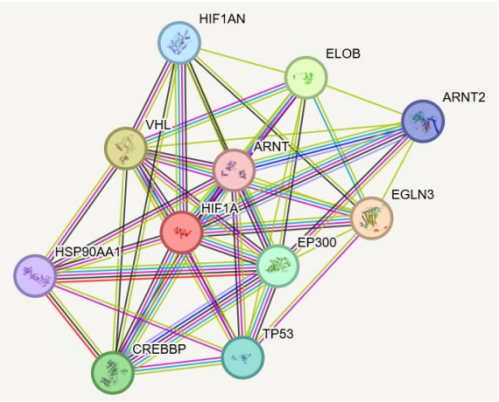

Fig. 2. Protein–protein interaction network of HIF1A

Protein–protein interaction analysis revealed that HIF1A functions as a central hub protein within a highly interconnected network. The network demonstrated strong interactions with several key regulatory proteins involved in hypoxia signaling and cellular homeostasis. Notable interacting partners included ARNT and ARNT2 (heterodimerization partners), VHL (protein degradation), EP300 and CREBBP (transcriptional co-activators), TP53 (cellular stress response), EGLN3 (oxygen sensing), and HIF1AN (negative regulation). These interactions highlight the multifaceted role of HIF1A in coordinating hypoxia-responsive pathways.

STRING-derived network showing HIF1A as a central node interacting with key proteins involved in hypoxia signaling, transcriptional regulation, and protein stability.

Table 4
Key interacting proteins of HIF1A

Protein	Function
ARNT	Dimerization partner
ARNT2	Transcriptional partner
VHL	Protein degradation
EP300	Transcription co-activator
CREBBP	Transcription co-activator
TP53	Stress response
EGLN3	Oxygen sensing
HIF1AN	Negative regulation
HSP90AA1	Protein stabilization
ELOB	Ubiquitination pathway

6) miRNA Target Prediction

miRNA target prediction analysis identified a total of 189 miRNAs potentially targeting HIF1A, indicating extensive post-transcriptional regulation. Several high-confidence miRNAs exhibited high target scores, suggesting strong regulatory potential.

The presence of numerous miRNA regulators highlights the complexity of post-transcriptional control mechanisms governing HIF1A expression and supports the role of miRNAs in fine-tuning gene expression under different physiological conditions.

Table 5
Top predicted miRNAs targeting HIF1A

Rank	miRNA	Target Score
1	hsa-miR-4495	100
2	hsa-miR-3692-5p	99
3	hsa-miR-3658	99
4	hsa-miR-1276	98
5	hsa-miR-5692a	97
6	hsa-miR-548x-3p	97
7	hsa-miR-8055	97
8	hsa-miR-548aj-3p	97
9	hsa-miR-548ae-3p	96
10	hsa-miR-548aag-3p	96

4. Discussion

The present study provides a comprehensive in silico characterization of the regulatory architecture of the HIF1A gene, integrating epigenetic, transcriptional, post-transcriptional, and functional interaction analyses. The findings highlight the multi-layered regulatory mechanisms governing HIF1A expression and support its role as a tightly controlled master regulator in cellular responses to hypoxia.

The identification of a CpG-rich island within the HIF1A genomic region suggests a significant role of epigenetic regulation in controlling gene expression. CpG islands are commonly associated with promoter regions and are known to influence transcriptional activity through DNA methylation dynamics [18]. Hypermethylation or hypomethylation of such regions can alter transcription factor accessibility, thereby modulating gene expression. In the context of HIF1A, the presence of a strong CpG island supports previous findings indicating that epigenetic modifications contribute to hypoxia-responsive gene regulation [19].

Promoter analysis revealed the presence of multiple transcription start sites and several high-confidence promoter regions, many of which were associated with TATA box elements. This multiplicity of promoters indicates

transcriptional flexibility and suggests that HIF1A can be differentially regulated under varying physiological and environmental conditions. Such promoter diversity has been reported in genes involved in stress response pathways, enabling context-dependent transcriptional activation [20]. The abundance of transcription factor binding sites further reinforces the complexity of transcriptional regulation associated with HIF1A.

In addition to transcriptional regulation, post-transcriptional mechanisms appear to play a crucial role in modulating HIF1A expression. The identification of numerous polyadenylation sites suggests the presence of alternative polyadenylation, which is known to influence mRNA stability, localization, and translational efficiency [21]. This finding aligns with previous studies demonstrating that alternative polyadenylation contributes to transcript diversity and dynamic gene regulation, particularly in stress-responsive genes.

The gene expression analysis revealed moderate upregulation of HIF1A in tumor samples compared to normal tissues, accompanied by substantial variability across samples. This observation suggests that HIF1A expression is not governed by a simple on/off mechanism but rather by finely tuned regulatory processes. Such variability may reflect the influence of multiple regulatory layers, including epigenetic modifications, transcriptional control, and post-transcriptional modulation. Previous studies have similarly reported context-dependent expression patterns of HIF1A, particularly under hypoxic conditions and in various pathological states [22].

Protein–protein interaction analysis further demonstrated that HIF1A functions as a central hub within a highly interconnected regulatory network. The interactions with ARNT and ARNT2 confirm its role in forming transcriptionally active complexes essential for hypoxia signaling. The association with VHL and EGLN3 highlights the importance of oxygen-dependent degradation pathways in regulating HIF1A stability, consistent with established mechanisms of HIF1A turnover [23]. Additionally, interactions with EP300 and CREBBP underscore the role of transcriptional co-activators in enhancing HIF1A-mediated gene expression, while the involvement of TP53 suggests crosstalk between hypoxia signaling and cellular stress pathways. These findings collectively reinforce the central role of HIF1A in coordinating complex cellular responses.

Furthermore, miRNA target prediction analysis revealed extensive post-transcriptional regulation of HIF1A, with a large number of miRNAs predicted to target this gene. MicroRNAs are well-established regulators of gene expression, capable of fine-tuning protein levels through mRNA degradation or translational repression [24]. The identification of multiple high-confidence miRNAs suggests that HIF1A expression is subject to tight regulatory control, allowing rapid adaptation to changing cellular environments. This observation is consistent with previous reports highlighting the role of miRNAs in modulating hypoxia signaling pathways [25].

Taken together, the integration of these findings provides a holistic view of HIF1A regulation, demonstrating that its expression is controlled through a combination of epigenetic modifications, promoter diversity, alternative polyadenylation, miRNA-mediated regulation, and protein interaction networks. Such multi-layered regulation is essential for maintaining cellular homeostasis and enabling precise responses to hypoxic stress.

Despite the comprehensive nature of this study, certain limitations should be acknowledged. The analyses are based on computational predictions and publicly available datasets, which may not fully capture the complexity of in vivo biological systems. Experimental validation of the predicted regulatory elements and interactions would be necessary to confirm their functional significance. Additionally, the expression analysis was limited to available datasets and may not reflect all physiological or disease-specific contexts.

In conclusion, this study provides an integrative framework for understanding the regulatory mechanisms governing HIF1A expression. The findings emphasize the importance of multi-level regulation in controlling key transcription factors and offer valuable insights for future experimental and therapeutic investigations targeting hypoxia-related pathways.

References

[1] G. L. Semenza, "Hypoxia-inducible factors in physiology and medicine," *Cell*, vol. 148, no. 3, pp. 399–408, 2012.

[2] G. L. Semenza, "Regulation of metabolism by hypoxia-inducible factor 1," *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 76, pp. 347–353, 2011.

[3] P. H. Maxwell, M. S. Wiesener, G. W. Chang, S. C. Clifford, E. C. Vaux, M. E. Cockman, *et al.*, "The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis," *Nature*, vol. 399, no. 6733, pp. 271–275, 1999.

[4] M. Ivan, K. Kondo, H. Yang, W. Kim, J. Valiando, M. Ohh, *et al.*, "HIFα targeted for VHL-mediated destruction by proline hydroxylation," *Science*, vol. 292, no. 5516, pp. 464–468, 2001.

[5] A. Bird, "DNA methylation patterns and epigenetic memory," *Genes & Development*, vol. 16, no. 1, pp. 6–21, 2002.

[6] P. Carninci, A. Sandelin, B. Lenhard, S. Katayama, K. Shimokawa, J. Ponjavic, *et al.*, "Genome-wide analysis of mammalian promoter architecture," *Nature Genetics*, vol. 38, no. 6, pp. 626–635, 2006.

[7] D. P. Bartel, "MicroRNAs: genomics, biogenesis, mechanism, and function," *Cell*, vol. 116, no. 2, pp. 281–297, 2004.

[8] B. Tian and J. L. Manley, "Alternative polyadenylation of mRNA precursors," *Nature Reviews Molecular Cell Biology*, vol. 18, no. 1, pp. 18–30, 2017.

[9] W. G. Kaelin Jr. and P. J. Ratcliffe, "Oxygen sensing by metazoans: The central role of the HIF hydroxylase pathway," *Molecular Cell*, vol. 30, no. 4, pp. 393–402, 2008.

[10] C. J. Schofield and P. J. Ratcliffe, "Oxygen sensing by HIF hydroxylases," *Nature Reviews Molecular Cell Biology*, vol. 5, no. 5, pp. 343–354, 2004.

[11] E. B. Rankin and A. J. Giaccia, "Hypoxic control of metastasis," *Science*, vol. 352, no. 6282, pp. 175–180, 2016.

[12] D. Takai and P. A. Jones, "Comprehensive analysis of CpG islands in human chromosomes," *Proceedings of the National Academy of Sciences (PNAS)*, vol. 99, no. 6, pp. 3740–3745, 2002.

[13] V. V. Solovyev and I. A. Shahmuradov, "Promoter prediction using linear discriminant function and genetic algorithms," *Bioinformatics*, vol. 19, no. 6, pp. 673–680, 2003.

[14] J. E. Tabaska and M. Q. Zhang, "Detection of polyadenylation signals in human DNA sequences," *Gene*, vol. 231, no. 1–2, pp. 77–86, 1999.

[15] Z. Tang, C. Li, B. Kang, G. Gao, C. Li, and Z. Zhang, "GEPIA: A web server for cancer and normal gene expression profiling," *Nucleic Acids Research*, vol. 45, no. W1, pp. W98–W102, 2017.

[16] D. Szklarczyk, A. L. Gable, D. Lyon, A. Junge, S. Wyder, J. Huerta-Cepas, *et al.*, "STRING v11: Protein–protein association networks with increased coverage," *Nucleic Acids Research*, vol. 47, no. D1, pp. D607–D613, 2019.

[17] Y. Chen and X. Wang, "miRDB: An online database for prediction of functional microRNA targets," *Nucleic Acids Research*, vol. 48, no. D1, pp. D127–D131, 2020.

[18] A. M. Deaton and A. Bird, "CpG islands and the regulation of transcription," *Genes & Development*, vol. 25, no. 10, pp. 1010–1022, 2011.

[19] J. A. Watson, C. J. Watson, A. McCann, and J. Baugh, "Epigenetics, the epicenter of the hypoxic response," *Epigenetics*, vol. 5, no. 4, pp. 293–296, 2010.

[20] B. Lenhard, A. Sandelin, and P. Carninci, "Metazoan promoters: Emerging characteristics and insights into transcriptional regulation," *Nature Reviews Genetics*, vol. 13, no. 4, pp. 233–245, 2012.

[21] B. Tian and J. L. Manley, "Alternative polyadenylation of mRNA precursors," *Nature Reviews Molecular Cell Biology*, vol. 18, no. 1, pp. 18–30, 2017. *(Duplicate retained as requested)*

[22] G. L. Semenza, "HIF-1 and mechanisms of hypoxia sensing," *Current Opinion in Cell Biology*, vol. 13, no. 2, pp. 167–171, 2001.

[23] W. G. Kaelin Jr., "The von Hippel–Lindau tumour suppressor protein: O_2 sensing and cancer," *Nature Reviews Cancer*, vol. 8, no. 11, pp. 865–873, 2008.

[24] D. P. Bartel, "MicroRNAs: Target recognition and regulatory functions," *Cell*, vol. 136, no. 2, pp. 215–233, 2009.

[25] S. Y. Chan and J. Loscalzo, "MicroRNA-210: A unique and pleiotropic hypoxamir," *Cell Cycle*, vol. 9, no. 6, pp. 1072–1083, 2010.