

RESEARCH ARTICLE

THERAPEUTIC TARGETING OF HYPOXIA-INDUCIBLE FACTOR SIGNALING PATHWAYS- A PROMISING APPROACH IN CANCER TREATMENT

Anjum Mohammad Shaik¹, Valli Harisomayajula¹, Saranya M.L¹, Phani Greeshma Veeramachaneni¹, Samhitha Reddy Gaddam¹ and Suryanarayana Veeravilli²

1. Department of Biotechnology, KoneruLakshmaiah Education Foundation, Andhra Pradesh, India.

2. Department of Humanities and Basic Sciences, Aditya Engineering College, Surampalem, Andhra Pradesh, India.

.....

Manuscript Info

Manuscript History Received: 25 July 2020 Final Accepted: 28 August 2020 Published: September 2020

Key words: -

HIF, Angiogenesis, Tumor Microenvironment, Hypoxia, Therapeutic, Cancer

Abstract

..... Oxygen and nutrients are delivered to the cells with the help of the vascular networking system, which makes availability of oxygen as primary regulator for many processes. Low oxygen availability condition activates the Hypoxia Inducible Factors (HIF), which are transcription regulators helping in the expression of genes related to cell cycle regulation and angiogenesis. HIF is hence regarded as the master regulator of angiogenesis. The oxygen deprival is due to the increased consumption of oxygen in the tumor microenvironment and in turn leads to hypoxia. A thorough understanding of how hypoxia influences angiogenesis mediated by several pathways has become essential for identifying novel strategies targeting HIF thereby blocking angiogenesis. In this review we would discuss about the HIF signaling pathways and altered functions of immune cells due to hypoxia by considering that reducing or targeting hypoxia may in turn prevent the suppression of anti-tumor immune response.

Copy Right, IJAR, 2020,. All rights reserved.

Introduction:-

Tumour contains certain regions which are deficient of oxygen supply, necrosis areas. Cells have well exposure to oxygen if they are nearer to blood vessels. An important mechanism as adaptive response to reduced oxygen availability is regulated by HIF pathways [1]. HIF can show their effects on hypoxia responsive genes thereby promoting cancer cell progression. Most recently many potential drugs were identified that inhibit HIF and were also validated as anti-cancer drugs [2]. HIF-1 is the key regulator that helps the tumor cells to adapt in hypoxic microenvironment. Later HIF mediated pathways were discovered which helped in tracing potential anti-cancer drugs [3]. In addition, the noteworthiness of focused HIF treatment is as not completely known in a clinical setting, and huge numbers of the preclinical examinations have discovered undesirable outcomes, demonstrating that the HIF and its pathways are yet not totally understood. It is appropriate that future examination is required around the impacts of focusing on hypoxia when combined with various types of immunotherapy [4]. While there is potential for diminishing hypoxia-interceded protection from immunotherapy, a few treatments might be hampered in oxygenated tumors, bringing about decreased helpful adequacy [5].

.....

Corresponding Author:- Suryanarayana Veeravilli Address:- Department of Humanities and Basic Sciences, Aditya Engineering College, Surampalem, Andhra Pradesh, India.

HIF signaling pathways in tumor microenvironment:

HIF'S are the key mediators of the hypoxic signalling pathway belong to the transcription factors family and PAS superfamily. These are heterodimeric factors which have two subunits α which is cytoplasmic, oxygen dependent and β which is constitutively nuclear [6]. The HIF α is known to take part in gene regulatory responses and iron chelation while HIF- β in known to take part in transcriptional responses to xenobiotic agents [7]. These two are the master regulators of the signalling pathway which form a complex. HIF- α Is stabilized by a group of Fe and oxygen dependent enzymes known as HIF- prolyl hydroxylase domain (PHD 1-3) enzymes [8]. As these enzymes are oxygen dependent, PHDs hydroxylate two proline residues of HIF α by factor inhibiting HIF (FIH) at an asparagine residueand allows binding of the Von Hippel-Lindau tumor-suppressor protein under normoxic conditions. This leads to subsequent ubiquitation and proteasomal degradation of the α subunit [9]. In contradiction to this, under hypoxic conditions, the PHD'S are very less active, PHD substrate O₂ and cofactor 2-oxoglutarate become limiting, attenuating HIF- α hydroxylation and resulting in HIF- α accumulation, binds to Arnt and forms a transcriptional complex with p300 and CBP through interactions with the HIF-a N- and C-terminal transactivation domain (N-, C-TAD) in the nucleus [10]. Then this complex binds to the hypoxia recessive elements and triggers the transcription of a selection of genes in nucleus which are involved in different processes like angiogenesis and erythropoiesis. HIF- α has three subunits HIF α 1, HIF α 2, HIF α 3 among which HIF-1 α is ubiquitously expressed whereas HIF-2 α is expressed in a more limited fashion primarily in adult lungs [11]. These two subunits are non-redundant and share 48% of their amino acid sequence and similar protein structure. HIF3 α , on the other hand, seems to act as a dominant negative regulator of HIF1 induced gene expression [12].

Metabolic Changes in TME due to Hypoxia induced pathways:

Hypoxic tumor microenvironment is well characterized by the elevated levels of lactic acid. It can be well explained by Warburg effect which is a metabolic move happening in profoundly multiplying cells that can convert glucose to lactate indeed the presence of oxygen [13]. In this process mainly cancer cells obtain their requirement by depending upon glycolysis rather than TCA cycle [14]. It is well known fact that in glycolysis phenomenon 2ATP of energy is produced per glucose molecule but in TCA cycle for the same molecule 36 ATP are generated. Anyhow studies state that glycolysis has faster kinetics compared to TCA cycle which significantly makes cancerous cell choose glycolysis over TCA cycle [15]. This is a potential hallmark of tumor cells. Many evidences showed that cMyc and Ras and p53 are also involved in regulation of the Warburg effect. To meet the demands of the tumor, HIF-1 regulates aerobic glycolysis and HIF-1 α activate glucose transporter gene expressions (Glut1 and 3) under hypoxia environment which helps in glucose uptake by cancer cells [16]. The influx of glucose to meet the requirement of glycolysis is ensured by the activated expression of hexokinases and phosphoglycerate kinase-1. These mechanisms are also regulated by HIF-1a. In few experiments it was demonstrated that PKM2 pyruvate kinase M2 is one of the target gene for HIF-1 α and thus its expression helps in uptake of glucose in cancer cells [17]. The lactate production finally leads to acidosis of tumourmicroenvironment which in turn increases the cancer cell invasion [18]. MtoR is mammalian target of rapamycin (protein kinase) has role in regulating cellular processes, but hypoxia mediated mechanisms do not allow expression of this protein kinase resulting in activity of tumor environment [19]. Hence, hypoxia mediated mTOR signaling mechanism may regulate cell behavior and gene expression [20].



Figure 1:- Uptake of glucose by Glut 1 and Glu3 receptors, pathway of HIF- 1α in activating hexokinases and phosphoglycerate kinase-1.

Immune cell functions in hypoxic tumourmicroenvironment:

Hypoxia is known to have great impact on immune cell functions. Function and differentiation of dendritic cells is altered under hypoxic conditions. Dendritic cell which are known for their antigen presenting function loose the capacity to present antigens in oxygen deprived conditions [21]. Hypoxic mature dendritic cells have profound change in their chemokine expression. Hypoxia plays a major role in regulation of tumour-associated macrophages (TAMS's). TAM's are attracted to hypoxic regions in TME [22].

Myeloid cells comprise granulocytes and monocytes collectively. They arise due to differentiation of progenitors of hematopoietic stem cells in the bone marrow [23]. Hypoxia-inducible factor 1α , induced by hypoxia in the tumour microenvironment is shown to regulate the function and differentiation of Myeloid-derived suppressor cells (MDSC) in the tumour microenvironment [24]. MDSC are characterized by common myeloid origin. Immature myeloid cells comprising the same phenotype as that of the MDSC are frequently generated in the bone marrow of healthy individuals whereas, in cancer cells, a regular myeloid cell instead of differentiating into a macrophage, a Dendritic cell or polymorphonuclear neutrophils (PMN), differentiates to a pathological MDSC, an immunosuppressive Tumour-associated macrophage (TAM) [25]. For this reason, MDSC is critical in regulating the immune responses in cancer. Hypoxia-Inducible Factors HIF is known to inhibit functions of tumours-infiltrating lymphocytes. Research has shown that in vitro cultures of hypoxic T cells lead to a suppressed proliferation, essentially because of an increase in iNOS and CD69, which impacts T-cell responses detrimentally [26]. The activation of HIF 1 α down-regulates T cell reception signal transduction, whereas the absence of HIF 1 α leads to an increase in pro-inflammatory cytokines, due to an increase in NF- κ B activation [27].

Inhibition of HIF- A promising target for Cancer therapy:

Exploring HIF pathways and selecting it as an inhibitory target for cancer was well established [28]. As of now there are few inhibitors against HIF molecular components at phase 1 and 2 clinical trials. Studies have demonstrated that hypoxia environment showed resistance to the immunotherapy and helps in the activation of few immunosuppressive cells like MDSCs (Myeloid derived suppressor cells) [29]. There by targeting hypoxia and its pathways can prevent the suppression of immune responses against tumor. Most of the therapies related to the HIF pathways help in inhibiting angiogenesis and affecting cancer cell metabolism. The potential outcome may be observed when combination of immunotherapy and HIF inhibition therapies are used [30].

One such study has been explored in murine prostate cancer where TH-302 reduces the hypoxia. In another study, ENTPD2 ectonucleoside triphosphate diphosphohydrolase 2 inhibitors and immune checkpoint inhibitors which increased the infiltrating capability of T-cells into cancer environment [31]. Increased anti-tumor function of T-cell and tumor clearance was observed in B16 and MC38 murine tumor models when Metformin, a type-2 diabetes drug was used along with PD-1 blockade [32]. By targeting Mtor or AKT pathways inhibition of HIF can be achieved. Tuberous sclerosis complex 2 (TSC2) is regulator of mTOR and TSC2 knockouts have shown accumulation of HIF-1 α and further activation of genes related to expression of VEGF [33]. Another potential drug named Rapamycin, has significantly reduced the levels of HIF-1 α in TSC2 knockouts. PD-L1 is an important regulator for immune escape of cancer cells. The blockade of such regulator can enhance the immune activity [34]. It has been explored that AKT and mTOR pathways regulate PD-L1 in lung cancer, colorectal cancer and pancreatic cancer. Targeting hypoxia related pathway with immunotherapy together is a considerable approach. But sometimes immunotherapies within the tumor microenvironment become beneficial on exposure to hypoxic environment. Oncolytic HSV-1 are already used for melanomas as first line treatment [35]. Recent studies also showed that these viruses replicate more efficiently in hypoxic conditions finally which resulted in lower progression rates of tumor. But it depends on the virus because Oncolytic adeno virus replication gets hampered under hypoxic conditions.

To date, several HIF inhibitors have been synthesized and identified, to inhibit the expression and function of HIF subunits through direct or indirect mechanisms [36]. Although the classification of HIF inhibitors may not be accurate, they are tentatively classified based on the reputed mechanisms of action. These are protein synthesis, agents modulating the expression, protein accumulation and degradation, dimerization, DNA binding, and transcriptional activity of HIFs [37]. While no potential agents have been developed to selectively inhibit HIF α protein synthesis, few natural compounds such as Chrysin inhibits synthesis of HIF-1 α protein by AKT signaling [38]. Whereas glyceollin, isolated from soybeans, by restricting the PI3K / AKT / mTOR pathway, significantly reduced HIF-1 α synthesis. In addition, 2-methoxyestradiol (2ME2) which is naturally produced by catechol-O-methyltransferase-mediated O-methylation of 2-hydroxyestradiol has been found to inhibit the synthesis of HIF-1 α

and HIF-2 α proteins, and suppress their nuclear translocation and transcriptional activity. KC7F2, which inhibits HIF-1 α protein synthesis, is also a lead compound [39].

A compound Indenopyrazole 21 was found to strongly inhibit HIF-1 alpha transcriptional activity without affecting the accumulation of HIF-1 α protein and HIF-1 heterodimerization [40]. While, YC-1, an anti-platelet aggregation agent, oppresses HIF transcriptional activity and may also affect HIF-1 α protein accumulation. Chetomin, a metabolite, was found to inhibit HIF-1 alpha association with p300 by disrupting the p300 protein's tertiary structure of the CH1 domain [41]. FM19G11, a novel chemical entity, effectively represses p300, a histone acetyltransferase necessary by inhibiting histone acetylation which is a co-factor for HIF-transcription activation [42].

Conclusion:-

Studies have proven that hypoxia/HIF mechanisms are master regulators in promoting angiogenesis in TME. This review represents that HIF related mechanisms are potential targets incapability to regulate the process of angiogenesis by manipulation of HIF pathways for treating cancers. Cancer cells can evade single anti-angiogenic procedures by promoting other pro-angiogenic pathways. As HIF regulate pro-angiogenic pathways there may be increased therapeutic outcome on targeting HIF mechanisms.

Conflict of interest:

The authors declare that there is no conflict of interests exist among them regarding the publication of this paper.

References:-

- 1. Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: role of the HIF system. Nature medicine. 2003 Jun;9(6):677-84.
- 2. Xia Y, Choi HK, Lee K. Recent advances in hypoxia-inducible factor (HIF)-1 inhibitors. European journal of medicinal chemistry. 2012 Mar 1;49:24-40.
- 3. Semenza GL. Tumor metabolism: cancer cells give and take lactate. The Journal of clinical investigation. 2008 Dec 1;118(12):3835-7.
- 4. Kumar VP, Prasanthi S, Lakshmi VR, Santosh MS. Cancer vaccines: a promising role in cancer therapy. Acad J Cancer Res. 2010;3(2):16-21.
- 5. Sitkovsky M, Ohta A. Targeting the hypoxia-adenosinergic signaling pathway to improve the adoptive immunotherapy of cancer. Journal of molecular medicine. 2013 Feb 1;91(2):147-55.
- 6. Cummins EP, Taylor CT. Hypoxia-responsive transcription factors. PflügersArchiv. 2005 Sep 1;450(6):363-71.
- 7. Kaluz S, Kaluzová M, Stanbridge EJ. Regulation of gene expression by hypoxia: integration of the HIFtransduced hypoxic signal at the hypoxia-responsive element. ClinicaChimica Acta. 2008 Sep 1;395(1-2):6-13.
- 8. Pugh CW, Ratcliffe PJ. The von Hippel–Lindau tumor suppressor, hypoxia-inducible factor-1 (HIF-1) degradation, and cancer pathogenesis. InSeminars in cancer biology 2003 Feb 1 (Vol. 13, No. 1, pp. 83-89). Academic Press.
- 9. Tanimoto K, Makino Y, Pereira T, Poellinger L. Mechanism of regulation of the hypoxia-inducible factor-1α by the von Hippel-Lindau tumor suppressor protein. The EMBO journal. 2000 Aug 15;19(16):4298-309.
- 10. Jokilehto T, Jaakkola PM. The role of HIF prolyl hydroxylases in tumour growth. Journal of cellular and molecular medicine. 2010 Apr;14(4):758-70.
- 11. Rajatapiti P, van der Horst IW, de Rooij JD, Tran MG, Maxwell PH, Tibboel D, Rottier R, de Krijger RR. Expression of hypoxia-inducible factors in normal human lung development. Pediatric and Developmental Pathology. 2008 May;11(3):193-9.
- 12. Duan C. Hypoxia-inducible factor 3 biology: complexities and emerging themes. American Journal of Physiology-Cell Physiology. 2016 Feb 15;310(4):C260-9.
- 13. Fadaka A, Ajiboye B, Ojo O, Adewale O, Olayide I, Emuowhochere R. Biology of glucose metabolization in cancer cells. Journal of Oncological Sciences. 2017 Jul 1;3(2):45-51.
- 14. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. science. 2009 May 22;324(5930):1029-33.
- 15. Wu W, Zhao S. Metabolic changes in cancer: beyond the Warburg effect. Acta biochimica et biophysicaSinica. 2013 Jan 1;45(1):18-26.
- 16. Kumar VP, Chakrapani R, Prasad MK. Cancer Immune System: A focus on clinical trials of Cancer Vaccines. Journal of Pure and Applied Microbiology. 2011 Apr 1;5(1):441-4.

- 17. Soga T. Cancer metabolism: key players in metabolic reprogramming. Cancer science. 2013 Mar;104(3):275-81.
- 18. Chiche J, Brahimi-Horn MC, Pouysségur J. Tumour hypoxia induces a metabolic shift causing acidosis: a common feature in cancer. Journal of cellular and molecular medicine. 2010 Apr;14(4):771-94.
- 19. Kimball SR. Interaction between the AMP-activated protein kinase and mTOR signaling pathways. Medicine and science in sports and exercise. 2006 Nov 1;38(11):1958.
- 20. van den Beucken T, Koritzinsky M, Wouters BG. Translational control of gene expression during hypoxia. Cancer biology & therapy. 2006 Jul 1;5(7):749-55.
- 21. Liu YJ. Dendritic cell subsets and lineages, and their functions in innate and adaptive immunity. Cell. 2001 Aug 10;106(3):259-62.
- Bingle L, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland. 2002 Mar;196(3):254-65.
- 23. Bassler K, Schulte-Schrepping J, Warnat-Herresthal S, Aschenbrenner AC, Schultze JL. The myeloid cell compartment—cell by cell. Annual Review of Immunology. 2019 Apr 26;37:269-93.
- Corzo CA, Condamine T, Lu L, Cotter MJ, Youn JI, Cheng P, Cho HI, Celis E, Quiceno DG, Padhya T, McCaffrey TV. HIF-1α regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. Journal of Experimental Medicine. 2010 Oct 25;207(11):2439-53.
- 25. Wynn TA. Myeloid-cell differentiation redefined in cancer. Nature immunology. 2013 Mar;14(3):197-9.
- Khan O, Headley M, Gerard A, Wei W, Liu L, Krummel MF. Regulation of T cell priming by lymphoid stroma. PloS one. 2011 Nov 14;6(11):e26138.
- Won C, Kim BH, Yi EH, Choi KJ, Kim EK, Jeong JM, Lee JH, Jang JJ, Yoon JH, Jeong WI, Park IC. Signal transducer and activator of transcription 3-mediated CD133 up-regulation contributes to promotion of hepatocellular carcinoma. Hepatology. 2015 Oct;62(4):1160-73.
- Kumar VP, Mounica MS, Swetha TN, Lakshmi GV, Anusri T, Amulya PL, Sirisha A. Cytokine therapy: clinical and progressive status in cancer. International Journal of Immunological Studies. 2011 Jan 1;1(3):264-71.
- 29. Najjar YG, Menk AV, Sander C, Rao U, Karunamurthy A, Bhatia R, Zhai S, Kirkwood JM, Delgoffe GM. Tumor cell oxidative metabolism as a barrier to PD-1 blockade immunotherapy in melanoma. JCI insight. 2019 Mar 7;4(5).
- 30. Tartour E, Pere H, Maillere B, Terme M, Merillon N, Taieb J, Sandoval F, Quintin-Colonna F, Lacerda K, Karadimou A, Badoual C. Angiogenesis and immunity: a bidirectional link potentially relevant for the monitoring of antiangiogenic therapy and the development of novel therapeutic combination with immunotherapy. Cancer and Metastasis Reviews. 2011 Mar 1;30(1):83-95.
- Chiu DK, Tse AP, Xu IM, Di Cui J, Lai RK, Li LL, Koh HY, Tsang FH, Wei LL, Wong CM, Ng IO. Hypoxia inducible factor HIF-1 promotes myeloid-derived suppressor cells accumulation through ENTPD2/CD39L1 in hepatocellular carcinoma. Nature communications. 2017 Sep 11;8(1):1-2.
- Scharping NE, Menk AV, Whetstone RD, Zeng X, Delgoffe GM. Efficacy of PD-1 blockade is potentiated by metformin-induced reduction of tumor hypoxia. Cancer immunology research. 2017 Jan 1;5(1):9-16.
- 33. Martin DE, Hall MN. The expanding TOR signaling network. Current opinion in cell biology. 2005 Apr 1;17(2):158-66.
- 34. Vemuri PK, Challagulla R, Kunta A. Plant Allergens and Immune System. December 2019, ISBN: 9781794803077, Penguin House.
- 35. Fukuhara H, Ino Y, Todo T. Oncolytic virus therapy: a new era of cancer treatment at dawn. Cancer science. 2016 Oct;107(10):1373-9.
- Onnis B, Rapisarda A, Melillo G. Development of HIF-1 inhibitors for cancer therapy. Journal of cellular and molecular medicine. 2009 Sep;13(9a):2780-6.
- 37. Yeo EJ, Chun YS, Park JW. New anticancer strategies targeting HIF-1. Biochemical pharmacology. 2004 Sep 15;68(6):1061-9.
- Fu B, Xue J, Li Z, Shi X, Jiang BH, Fang J. Chrysin inhibits expression of hypoxia-inducible factor-1α through reducing hypoxia-inducible factor-1α stability and inhibiting its protein synthesis. Molecular Cancer Therapeutics. 2007 Jan 1;6(1):220-6.
- Ouyang S, Yao YH, Zhang ZM, Liu JS, Xiang H. Curcumin inhibits hypoxia inducible factor-1α-induced inflammation and apoptosis in macrophages through an ERK dependent pathway. European Review for Medical and Pharmacological Sciences. 2019 Feb 1;23(4):1816-25.

- 40. Singleton DC, Harris AL. Targeting the ATF4 pathway in cancer therapy. Expert opinion on therapeutic targets. 2012 Dec 1;16(12):1189-202.
- Newcomb EW, Lukyanov Y, Schnee T, Ali MA, Lan L, Zagzag D. Noscapine inhibits hypoxia-mediated HIF-1α expression andangiogenesis in vitro: a novel function for an old drug. International journal of oncology. 2006 May 1;28(5):1121-30.
- 42. Moreno-Manzano V, Rodríguez-Jiménez FJ, Aceña-Bonilla JL, Fustero-Lardíes S, Erceg S, Dopazo J, Montaner D, Stojkovic M, Sánchez-Puelles JM. FM19G11, a new hypoxia-inducible factor (HIF) modulator, affects stem cell differentiation status. Journal of Biological Chemistry. 2010 Jan 8;285(2):1333-42.