·Review·

Hypoxia-inducible factor-1α: a promising target for tumor therapy

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[Abstract] Hypoxia-inducible factor-1α (HIF-1α), a nuclear transcriptional factor, is constitutively expressed in mammalian cells under hypoxia, which contributes a lot to the regulation of internal O_2 homeostasis. Micro-environmental hypoxia is a common feature of solid tumors. Under the stress of hypoxia, HIF-1α is accumulated and activated, which leads to activation of a vast array of downstream genes that contribute to tumor O_2 homeostasis and energy metabolic equilibrium. HIF-1α weighs heavily in favor of tumor genesis and progression. So far, HIF-1α has became an attracting tumor research topic, which improves understanding on how HIF-1α functions in tumor progression and key signaling pathways that regulate HIF-1α, therefore, provides new scientific supports and ideas to look for novel target for tumor therapy.

Key words: hypoxia-inducible factor-1α, neoplasm, biological behavior, signaling pathway, therapeutic target

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p Oxygen homeostasis is necessary for cells to maintain their physiological function. Hypoxia is a common phenomenon occurs in the highly proliferative and metabolic tumor tissues, which results in deprivative supply of blood and oxygen in tumor. It can limit tumor growth rate and metastasis to a certain extent. In response to hypoxia, tumor will undergo a series of change to adapt to the hypoxic microenvironment, among which HIF-1 is one of the most important regulators. HIF-1 is a heterodimer protein constituted of α and β subunits and will exert its transcriptional function only when the two subunits bind together. HIF-1β expresses in all kinds of cells with a relatively stable amount in an oxygen-independent manner. HIF-1α is owned by HIF-1 specifically and acts as a regulatory subunit, which determines the activity of HIF-1. Considering its important role on tumor progress and its potential application for tumor diagnosis and therapy, HIF-1α attracts more and more attention. With further understanding of its structure, expression, regulatory activity and its role on tumor biological behavior, HIF-1α will probably become an important target for tumor therapy in the near future.

Structure of HIF-1α and its high expression in tumor tissue

HIF-1α gene locates between region q21and q24 of human fourteenth chromosome, encoding HIF-1α protein who is a member of basic helix-hoop-helix Per/Arnt/Sim (bHLH-PAS)

transcriptional factors superfamily. HIF-1α contains several important domains including :1) bHLH and PAS domains mediating the dimerization with HIF-1β and binding with DNA. 2) Nuclear location signals (NLS) of N-and C-terminals, mediating the translocation of HIF-1α into nuclear, 3) oxygen-dependent degradation domain(ODD), mediating HIF-1α degradation by proteasomes; 4) two transactivation domains (TAD); N-TAD near N-terminal and C-TAD near C terminal, mediating the transactivation of HIF-1α .

It has been proved that HIF-1α is highly expressed in various kinds of human tumor. Zhong et al. [1] analysed the expression of HIF-1α in 19 kinds of human tumor tissue by immunohistochemistry and discovered that HIF-1α was overexpressed in 13 kinds of solid tumor and the precancerous lesion of urogenital system and digestive system, whereas no expression was detected in in their surrounding normal tissues. The subsequent clinical researches reported that HIF-1α was overexpressed in a series of malignant tumors such as esophageal carcinoma,[2] prostate carcinoma,[3] and so on. Most studies suggested that high expression of HIF-1α was not only a useful indicator of hypoxia in tumor tissue, but also a marker of poor prognosis, with significantly positive correlation to tumor grade and negative correlation to therapeutic sensitivity and patient survival rate. There were also a minority of clinical researches showed that the positive correlation between HIF-1α overexpression in tumor tissue and patient survival rate was not, however, universal.[4]

High expressed HIF-1α in tumor tissue accumulates in cytoplasm and translocates into nuclear to combine with HIF-1β to form HIF-1 dimer with transcriptional activity. HIF-1 recognizes and interacts with hypoxia response element (HRE) on the promoters of downstream target genes, then activates the transcription of a series of downstream target genes via co-transactivators such as CBP/p300. These genes are associated with tumor energy and substance metabolism, cell immortalization, apoptosis, tumor invasion, metastasis, therapeutic resistance and so on.

Influence of HIF-1α on tumor biological behavior

The relatively hypoxic microenvironment around tumor forces it to have a series of adaptation change in its energy production and utilization. Glucose, a main energy source, obtains ATP mainly through glycolysis under hypoxic condition, whose ATP production is only 1/19 compared to that of oxidative phosphorylation under normoxic condition. During the process, cell will upregulate the expression of glucose transporters[5] and glycolytic kinase[6] by HIF-1α and enhance glucose uptake and glycolysis to increase ATP production, compensating the ATP insufficiency resulting from hypoxia. But even if the oxygen concentration can meet the need of oxidative phosphorylation, tumor cell will still maintain a relatively high glycolytic rate. Its mechanism may be associated with the inhibition of the translocation of glycolytic final product to mitochondria by HIF-1α . Pyruvate Dehydrogenase Kinase 1 (PDK1), downstream product of HIF-1α , inhibits the process of Pyruvate transforming into acetyl-CoA catalized by pyruvate dehydrogenase complex.[7] Some oncogenic products associated with tumor cell proliferation and survival, such as c-myc and Akt, co-regulate tumor cell glucose metabolism with HIF-1α .[7]

Besides glucose metabolism, protein metabolism is another important aspect of tumor substance metabolism. Hypoxia or nutritional deficiency can lead to abnormal regulation of mTOR (mammalian target of rapamycin) pathway who regulates protein synthesis and tumor cell growth. Growth factors, hormone and external cell complex accumulate on the TSC (tuberous sclerosis complex) 1/2 complex which is the upstream regulator of mTOR, then activate mTOR through Ras/ERK or PI3K/Akt pathway. mTOR is inhibited under hypoxic condition mainly by functional change of TSC1/2 complex, which is influenced by HIF-independent REDD1 protein.[8] TSC1/2 is also regulated by tumor suppressor PTEN (phosphatase and

tensin). PTEN is a phosphotase that negatively regulates PI3K-Akt-mTOR pathway, whose functional loss mutation will lead to the persistent activation of PI3K-Akt-mTOR pathway and accumulation of HIF-1α. Therefore, an auto-regulatory cycle that connects hypoxia and tumor protein metabolism may exsist in the stabilization of HIF-1α which is mediated by mTOR.[9] Hypoxia and nutritional deficiency inhibit the classical cap-dependent translation, but mRNA that contains internal ribosome entry sites (IRES) can still be translated. HIF-1α and its downstream target gene VEGF-A contain the sequence described above, so its ability to induce angiogenesis still remain under stress like hypoxia and nutritional deficiency. [10]

Similar to the phenomenon that some mRNA can be constantly translated by IRES, tumor cells can also proliferate continuously through certain mechanisms, which is leading to cell immortalization and tumorigenesis. The immortalization of tumor is transactivated by telemorase encoding by TERT gene, whose 5 terminal flanking regions contain HRE. HIF-1α will activate telemorase transcription through binding to HRE, resulting in cell immortalization.[11] Zhou et al.[5] have observed that HIF-1α expression in colorectal adenocarcinoma tissue was significantly positive correlated with cell proliferation reflecting by proliferating cell nuclear antigen (PCNA).

Immortalization and abnormal proliferation lead to the biological transformation of normal cell to tumor cell. On the other hand, cell abnormal apoptosis is another important reason of tumorigenesis, during which HIF-1α playsa key role.[12] Cell metabolic alterations in response to hypoxia and nutritional deficiency enable it to adapt to the environment and survive. However, with the progression of hypoxia and nutritional deficiency stress, cell will have to seek survival strategies such as autophagy.

Autophagy. , which is of significant importance to tumorigenesis, [13] undergoes the process of hydropic degradation of cytoplasmal protein and recruit of cell organ to lysosome/vacuoles, which is mediated by the bnip3, a downstream pro-apoptosis molecular of HIF-1α. [14] Cell apoptosis mediated by bnip3 occurs under the condition of growth factor deficiency, cell acidosis or glucose shortage. Therefore cell may experience the process of survival or apoptosis under different hypoxic stages. Wei et al.[15] proved that HIF-1α can promote the proliferation of cervix carcinoma cell under normoxic condition and at early stage of hypoxia as well. In contrast, inhibition of HIF-1α activity can decrease the proliferation of cervix carcinoma cell and promote its apoptosis at early stage of hypoxia.[16] With the progress of hypoxia, the protective role of HIF-1α attenuates gradually, and may even, at the late stage of hypoxia, exerts a cytotoxic effect. Therefore, under the condition of different stages of hypoxia, HIF-1α can determine tumor cell fate, either apoptosis or adapt to the hypoxic environment and survive, through the regulation of downstream pro-apoptosis and anti-apoptosis genes balance by HIF-1α, which may provide an explanation for the correlation between HIF-1α expression in tumor tissue and patient survival rate.

Many studies have suggested that HIF-1α activates the formation of many pro-angiogenic factors including VEGF.[6,17] VEGF can not only directly stimulate endothelial cell proliferation and neovascularization, but also provide tumor cells with oxygen, nutrition and pathway for metabolic products removement, enhance vasopermeability, and promote the extravasation and accumulation of fibrinogen, plasma protein, thus facilitates tumor invasion and metastasis. The inactivation of HIF-1α in tumor tissue leads to angiogenesis retardation and tumor growth arrest.[18]

Though HIF-1α can relieve the stress of insufficient oxygen provision and nutrition to a certain degree by regulating auto-metabolism and stimulating angiogenesis, the hostile hypoxic and nutrition deficiency microenvironment in tumor will ultimately force tumor metastatsis from primary site to less hypoxic area of body. Metastasis includes the detachment between cell and cell, cell and external matrix stroma, facilitating cell entering into blood circulation

and lymphatic system through basal membrane and interstitial tissue. Most protein that participate tumor metastasis are induced by HIF-1α, including vimentin, keratin, fibronectin, matrix metalloproteinase 2, cathepetic enzyme D, chemokine receptor CXCR4 and its specific ligand SDF-1.[19,20] Vimentin and keratin are key factors mediating cell and cell adhesion and epithelial to mesenchymal transformation, the latter was necessary for tumor invasion. Fibronectin assists the adhesion between tumor cell and basal membrane, as well as external matrix. MMP2 and cathepsin D degrade cell external matrix, enhancing the invasive ability of tumor cell. CXCR4 activation can induce the rearrangement of cytoskeleton, resulting in the accumulation of internal cell actin and parapodium, consequently enhance cell movement and migration ability. In addition, specific chemokine receptor expressed by different tumors may bind to its correspondering ligand expressed by some organs, assisting tumor cells migrate to these organs specifically.

Most tumors are treated by surgery, chemotherapy, radiotherapy and so on Hypoxia/HIF-1α also mediates tumor therapeutic resistance. ATP synthesis is decreased under hypoxic condition, leading to the drug accumulation inside the cells because of the failure of drug export by ATP-dependent drug transporter, thus increases cell sensitivity to drugs.[21] HIF-1α can reverse the this process by accelerating glycolysis. On the contrary, inactivation of HIF-1α inhibited P-gp expression, which is a drug transporter, increased the sensitivity of leukemia K562 cell line to homoharrigtonine.[6] Besides, HIF-1α can slow down tumor cell growth by regulation of cell cycle,[22] attenuate the efficacy of cell cycle specific chemical drug such as methotrexate. Radiotherapy can induce the activity of HIF-1α in tumor cells, increase the secretion of VEGF and FGF-2, both of which can activate downstream anti-apoptotic signal pathway by binding to their receptors and protect tumor cells from killing or damage by radiotherapy, leading to tumors resistance to radiotherapy.[23] The chemo- and radio-sensitivities are significantly enhanced in tumors with HIF-1α inactivation.[6, 24]

Regulation of HIF-1α by Signal pathway

Regulation of HIF-1α by signal pathway is a complex network including many regulators and regulatory machineries. It can mainly be categorized into two types, negative regulation and positive regulation.[25]

Negative regulation of HIF-1α. Regulation of HIF-1α is mainly on the protein level: the 402th and 564th proline residues on ODD domain are recognized and hydroxylated by prolyl hydroxylase (PHD) under normoxic condition, leading to the binding of protein von Hippel-Lindau (VHL) to all of these hydroxylated proline residues, in which ubiquitin protein ligase is recruited, resulting in the ubiquitination of HIF-1α subunit and its quickly degradation by ubiquitin-proteasomes pathway.[25] In addition to the 402th and 564th proline subunits, the 803th aspartate residue (Asn803) is also an important hydroxylated site. Through hydroxylating this residue, FIH-1 (factional inhibitor of HIF-1) can prevent C-TAD from interacting with Transco-activator p300, thus losing its transcriptional activity.[26] Besides, FIH-1 can also enhance the interaction between VHL and HIF-1α and accelerate the degradation of HIF-1α. VHL can inhibit the transcriptional activation of HIF-1α on its downstream genes by activating histone deacetylase (HDAC). Acetyltransferase ARD1 (arrest defective 1) can stabilize the interaction between HIF-1α and VHL to accelerate HIF-1α ubiquitin degradation and down-regulate its protein expression through the 532th acetylated lysine under either normoxic or hypoxic condition in a dose-dependent manner.[27] Some downstream factors regulated by Akt such as GSK3β and FOXO4 can also negatively regulate HIF-1α. It has been proved that dominant negative mutant of GSK3β can increase HIF-1 activity under hypoxic condition. Hhypoxia can activate Akt and upregulate HIF-1α through phosphorylated inhibiting

GSK3β. But with of the progression of hypoxia, Akt activity will decrease, this will inversely activate GSK3β, avoiding further accumulation of HIF-1α.[28] Therefore, the hypoxia stage determines the balance of HIF-1α between stabilization and degradation. FOXO4 is a member of transcriptional factors superfamily, negatively regulated by Akt. The constant overexpression of activated FOXO4 can downregulate HIF-1α protein level under hypoxia and inhibit the transcriptional activation of HIF-1α target genes.[29]

In addition to regulation at protein level, the regulation of HIF-1α protein function is another important aspect of HIF-1α regulation. Dominant negative HIF-1α (dn HIF-1α) is a spliced mutant constituted by domain loss of ODD, N-TAD, C-TAD, N-NLS and C-NLS. Its combination with HIF-1β will form a HIF-1 dimer which can not bind to and activate the transcription of downstream target genes. dn HIF-1α impedes the formation of HIF-1 with transcriptional activity by competing HIF-1β binding site with wide-type HIF-1α.[16] CITED2 is activated via a mechanism similar to that of HIF-1α (blocking the degradation of proteasomes) under hypoxia and regulate the expression and transcriptional activity in a negative feed back manner.[30] IPAS is a kind of bHLH-PAS protein induced by HIF-1α. Its structure is similar to HIF-1α but lack C-TAD domain. It has been discovered by co-immunopreciptation and DNA binding experiment that the combination of IPAS and HIF-1α would make it unable to bind to HIF-1β and DNA. Therefore the co-expression of IPAS and HIF-1α can significantly decrease HIF-1α transcriptional activity and constitute a negative feed back for HIF-1α signal pathway.[31] P53, a tumor suppressor, can also negatively regulate HIF-1α, that is hypoxia leads to p53 accumulation, who will bind to HIF-1α and exert negatively regulatory role by protein stabilization, DNA binding and transcription.[32] Both HIF-1α and p53 can combine with p300. Low level expression of p53 can cause HIF-1α degradation. It should be noticed that in the fibrosacroma cell line HT1080 with normal p53, inactivated HIF-1α will reverse cells resistance to chemotherapeutics cisplatin and lead to cell apoptosis, but the efficacy described above was not obvious in fibrosacroma cell line HT1080 with mutated p53.[33] Therefore, when regarding HIF-1α as a target for tumor inhibition, whether p53 is in a non-mutated state should be considered.

Positive regulation of HIF-1α. As it has been described above, the degradation of HIF-1α mediating by proteasomes under normoxia is the main pathway for HIF-1α regulation. But under hypoxia, the degradation pathway for HIF-1α subunit is blocked, resulting in HIF-1α accumulation in cytoplasm, activated, translocating into nuclear and binding to HIF-1β subunit to form a dimmer with transcriptional activity and activate the transcription of downstream target genes. Besides oxygen saturation, pH also affect the stability of HIF-1α. Acid environment can decrease the degradation of HIF-1α under normoxia by nuclear VHL inactivation. Though oxygen saturation is the main factor influencing HIF-1α functional activation, its stabilization and activation is not merely hypoxia dependent. Under normoxia, signal pathways constituted by various factors such as growth factors, PI3K, MAPK, oncogene, Akt downstream products HDM2 and mTOR, transcricptional coactivators p300, molecular chaperones HSP90, NO and ROS can stimulate the activation of HIF-1α. Similar to the mechanism of negative regulation of HIF-1α but in an opposite manner, these negative regulators can either increase the expression level of HIF-1α mRNA/protein (increase the synthesis and block the degradation of HIF-1α protein), or enhance the binding of HIF-1α and DNA, or promote the transcriptional activity of HIF-1α on its downstream target genes.[25]

Targeted HIF-1α for tumor therapy

More and more anti-tumor strategies exert tumor inhibition function by acting on HIF-1α signal pathway described above mainly by increasing negatively regulation and / or

decreasing positive regulation of HIF-1α .

Decrease HIF-1α protein level. Inhibiting the synthesis of HIF-1α Just as mentioned above, activity of mTOR in many tumor cells is a key factor for determining the synthesis of many tumor associated proteins including HIF-1α . The persistent activation of tyrosine kinase receptor and its downstream PI3K/Akt and RAS/MAPK signal transduction pathway can increase the activity of mTOR and HIF-1α and further activate the tumorigenic function of HIF-1α downstream products. Therefore inhibiting mTOR and interfering HIF-1α may become an important target for tumor therapy. In the transgenic mouse model for prostatic intraepithelial neoplasm, whose Akt was highly activated, RAD-001, a mTOR inhibitors, could inhibit tumor growth. It has been suggested by gene expression microarray analysis that mRNA of HIF-1α target gene products in the transgenic prostate tissue was significantly increased compared with that in non-transgenic prostate tissue and was significantly decreased compared with those without RAD-001 treatment in transgenic prostate tissue.[34] Similar to the discovery above, tumor cell was very sensitive to mTOR inhibitor CCI-779 in renal cell carcinoma..[35] CCI-779 was a kind of rapamycin derivatives associated with inhibition of HIF-1α mRNA translation. At present, its anti-tumor activity study is mainly focus on patients with PTEN mutation and persistent PI3K-Akt-mTOR pathway activation.

Small molecules designed for HIF-1α including antisense mRNA, small interfering RNA (si RNA) and short hairpin RNA, (sh RNA), which can specifically block HIF-1α transcription or translation by directly inhibiting HIF-1α and its downstream target gene production and exert various anti-tumor activity. Prostaglandin E2 (PGE2) is another biomacromolecule induced by HIF-1α besides tyrosine receptor kinase. The amount of COX-2, who catalyzes PGE2, was closely related to VEGF expression, angiogenesis and patient mortality in colon cancer patients. Non steroid anti-inflammatory drugs inhibiting COX-2 can decrease the risk of colorectal cancer. The VEGF mRNA expression induced by PGE2 in colon cancer can also be blocked by HIF-1 inhibiting through RNA interfering.[36] Li et al.[37] have introduced sh RNA designed for HIF-1α into human breast cancer cell line MCF-7. It increased tumor cell apoptosis, inhibited tumor proliferation and enhanced its sensitivity to methotrexate by inhibiting HIF-1α signal pathway and decreasing the downstream target genes expression including VEGF, Glut-1, PGK, P-gp. Application of sh RNA designed for HIF-1α for non solid tumor leukemia cell line K562 also got results similar to solid tumor, which was the increasing the sensitivity of K562 to chemotherapeutic drug homoharringtonine.[6] In common children tumor cell line including rhabdomyosarcoma cell A204 RMS and Ewing sarcoma cell A673 ES, siRNA could specifically block the expression of HIF-1α and its downstream gene Glut-1 induced by hypoxia, thus decreased cells resistance to apoptosis induced by physical or chemical hypoxia.[12] Though this kind of molecule exerts a certain degree of anti-tumor activity, its characteristic of high cost and relatively low efficacy makes it still stay at the stage of in vitro experiment at present. Furthermore, there are still no methods that can ensure the delivery of these small molecules into every tumor cell, which also limits its application to some extent.

Decrease the stabilization of HIF-1α . Connective tissue growth factor (CTGF) is a kind of external cell signal molecule participating in various physiological and pathological process and can inhibit tumor growth and metastasis of transplantation tumor model in nude mice for human lung cancer. Chang et al have discovered that this inhibition is through promoting degradation of HIF-1α protein and decreasing VEGF expression and its corresponding angiogenesis. The acetyltransferase ARD1 in tumor cells transfected with CTGF was increased by HIF-1α acetylation to accelerate its ubiquitin degradation. But the HIF-1α with 532th lysine mutation will not degrade even if CTGF was overexpressed.[38]

HSP90, thioredoxin (Trx) and HDAC inhibitors can also decrease the stabilization of

HIF-1α to exert tumor inhibition effect. HSP90 is a kind of molecule chaperone which can interact with HIF-1α and is necessary to maintain its transcriptional activity. HSP90 inhibitors geldanamycin and its analogue 17-AAG and 17-DMAG can still induce ubiquitination of HIF-1α and degradation of proteasomes even if VHL does not exist. Receptor for activated C kinase 1 (RACK1) can inhibit HIF-1α competing with HSP90. It contains a domain similar to the one that mediating the interaction between VHL and Elongin C, through which RACK1 can recruit ubiquitin ligase such as Elongin C and constitute a proteasome degradation pathway for HIF-1α similar to the VHL model but in a oxygen-independent manner.[39] HSP90 inhibiting by 17-AAG can lead to the strong binding of RACK1and HIF-1α and further ubiquitination and proteasome degradation. In addition to its role in promoting the stabilization of HIF-1α protein, HSP90 can also protect many activated or overexpressed oncoprotein including tyrosine receptor kinase and serine-threonine kinase from being degraded.[40] Therefore HSP90 inhibitors is a kind of promising anti-tumor drugs. And 17-AAG and 17-DMAG are entering into clinical trial at present.

Just as molecule chaperones like HSP90 to maintain its correctly folding, it also need Trx system to keep its cysteine subunit in a correct redox state and maintain its stabilization and function. The overexpression of Trx in tumor cells will upregulate the expression of HIF-1α protein, enhance its transcriptional activity and increase VEGF expression and angiogenesis. The inhibitor of Trx has the reverse efficacy.[41] Opposite to the role of acetyltransferase ARD1, which is accelerating degradation of HIF-1α by acetylating 532th lysine. HDAC can increase the stabilization of HIF-1α by deacetylating 532th lysine. HDAC inhibitors, trichostatin, can reverse the stabilization role of HIF-1α by downregulating HIF-1α and VEGF expression in osteosarcoma cell line MG-63 and significantly decrease its invasiveness.[42]

Decrease the transcriptional activity of HIF-1α . Just as mentioned above, some HIF-1α inhibitors do not affect its mRNA or protein level, but can impede the transcription of HIF-1α activated downstream target genes. Levomycin and DJ12 can inhibit transcription by blocking the binding of HIF-1α and DNA. Levomycin can bind to DNA sequences 5-ACGT-3 or 5-TCGT-3 in priority, thus can compete with HIF-1α whose binding site contains the central sequences 5-ACGTG-3 or 5-GCGTG-3.[43] DJ12 is a common HIF-1α inhibitor screening from 150, 000 compounds by Jones et al. It can exert anti-tumor efficacy by inhibiting the transcriptional activity of HIF-1α and downregulating tumor cell growth associated gene expression such as VEGF in several kinds of human tumors including breast cancer, melanoma, renal cell carcinoma. Its mechanism is similar to levomycins and is independent of cell type.[44] Shin et al.[26] have observed that bortezomib, proteasomes inhibitor, can inhibit tumor angiogenesis and its adaptation to hypoxia in diverse myeloma and hepatoma carcinoma cell lines. Further studies discovered that bortezomib s inhibition on tumor was through enhancing the interaction between C-TAD in HIF-1α and FIH, interfering the recruit of p300 by C-TAD, impeding the transcriptional activation of HIF-1α and inhibiting the expression of downstream target gene VEGF and EPO (erythropoietin). FIH inactivation or loss of Asn803 in C-TAD will lead to the inefficacy of Bortezomib on tumor.[26] We have discussed above that HDAC inhibitors can induce HIF-1α degradation under the condition that drug concentration should accumulate to a certain degree. HDAC inhibitors SAHA (suberoylanilide hydroxamic acid) and trichostatin A inhibit the function of C-TAD and its interaction with p300 through a aspartate subunit hydroxylation independent manner.[45] Homeodomain-interacting protein kinase 2 (HIPK2) is a newly discovered Transco-repressor protein who has pro-apoptotic role. It has dual roles of promoting the degradation of HIF-1α and inhibiting transcriptional activity of HIF-1α . The overexpression of HIPK2 cancer cell lines including colon cancer cell RKO, glioma cell T98G and lung adenocarcinoma cell

H1299 can downregulate the expression of HIF-1α and its downstream drug resistance related gene MDR1 and anti-apoptotic related gene Bcl-2, and increase the apoptosis sensitivity of chemoresistant tumor cells induced by chemotherapeutics adrimycin.[46]

Similar to the mechanisms of small molecule such as si RNAi, sh RNA specifically inhibits HIF-1α at the synthesis level. dn HIF-1α can also directly inhibit HIF-1α specifically. The only difference is that dn HIF-1α inhibits HIF-1α at the transcriptional level without direct influence on HIF-1α protein expression. Jensen et al. have transfected humor glioma cell with dn HIF-1α and discovered that inactivation of HIF-1α in tumor tissue can lead to the decreasing of VEGF synthesis, tumor angiogenesis disturbance and tumor growth inhibition.[18] Stoeltzing et al also got a similar result in the experiment of transfecting human gastric carcinoma cell TMK-1 with dn HIF-1α and made a further discovery that tumor angiogenesis decrease was closely related to the maturation arrest of tumor vascular endothelial cells by immunohistochemistry analysis.[47] Brown ea al. have detected the influence of dn HIF-1α on six kinds of human tumor cell lines and discovered that dn HIF-1α can reverse the resistance of tumor cells to chemotherapeutics etoposide.[48] We have also observed that dn HIF-1α can accelerate cell apoptosis by reversing VEGF expression induced by HIF-1α and reverse the protective role of wide-type HIF-1α on cervix carcinoma cell SiHa, exerting certain degrees of anti-tumor activity.[16]

Utilizing high expression of HIF-1α . Recent years, a novel tumor targeted therapeutic strategy has been developed by utilizing the recognization and combination of HRE by HIF-1α . It employs VEGF or EPO promoters containing HRE or with several HRE structures connects together to constitute a HIF-1α /HRE gene expressing system, enabling the targeted gene selectively expressed in tumor cell with hypoxia or HIF-1α overexpression. The more number of the stringed HRE structures, the more genes are expressed in tumor cells with hypoxia or HIF-1α overexpression, the better of the targeted ability of gene expression. Post et al. constituted a hypoxia/HIF-1α dependent adenovirus vector based on the idea described above. The replication of the adenovirus with cell lysis ability is dependent on the expression of E1A virus replicators. The upstream of E1A gene contains promoter sequence which can interact with HIF-1α (stringed HRE). Aftering transfecting tumor cells with hypoxia or HIF-1α overexpression by HYPR-Ad, HIF-1α will activate the expression of E1A gene and lead to large amount of virus replication and cell lysis. The normoxia cells wont be affected because they dont express E1A gene. No matter what origin or genetic alterations are of the tumor cells, so long as the cells have the characteristics of hypoxia or HIF-1α overexpression, they can be transfected by HYPR-Ad and lysed by virus undergoing constant replication, Therefore, this hypoxia/HIF-1α dependent adenovirus vector is a promising vector for tumor targeted gene therapy with a bright future.[49] Post et al have firstly applied HYPR-Ad system to human brain tumor cells in vitro including glioma cell LN229, U251MG and medulloblastoma cell Daoy and induced lysis of these tumor cell successfully.[49] They also applied the system to in vivo tumor model research and discovered that HYPR-Ad could highly replicate in the hypoxic region of tumor and could kill tumor cells specifically, as well as slowed down tumor growth. The regimen of HYPR-Ad and BCNU combination can significantly enhance the anti-tumor efficacy.[50] Recently they inserted a IL-4 sequence into HYPR-Ad vector and constituted HYPR-Ad-IL4. IL-4 is a cytokine mediating host s strong immunoactivity to tumor and has a potential anti-tumor activity. This newly HIF-1α dependent HYPR-Ad-IL4 has better tumor-inhibition effect than traditional HYPR-Ad does in both in vitro and in vivo studies.[51] In addition to that, it has been mentioned that radiotherapy can increase the releasing of VEGF and FGF-2 by HIF-1α induction and protect tumor cell from apoptosis caused by radiotherapy. But if the tumor is pretreated with angiogenesis inhibitors canstatin, the activation of HIF-1α induced by

radiotherapy will lead to tumor apoptosis instead of radioresistance.[52] All of the studies above have suggested that HIF-1α can act as a tumor killer under certain conditions by being inhibited or activated and the combination of several strategies can exert better anti-tumor efficacy.

Conclusion

The biggest challenge for effective human tumor therapy is the disease. heterologenity caused by individual differences. There was many genetic and epigenetic changes in tumor cells. Understanding the changes represented by key therapeutic targets in definite patients is the challenge at present. Owing to its high expression in various human tumors and its control on the genes expression that are of important influence on tumor process, HIF-1α may become an important target for tumor therapy. However, just as we have discussed above, HIF-1α affects tumor biological behavior in many aspects with multifaceted nature. For instance, in different tumor types or the same tumor type with different microenvironment (such as oxygen tension, concentration of metabolic products), the expression of the same HIF-1α downstream target gene and its protein may increase or stay the same level or even decrease. Correspondingly, the biological behavior associated with the gene may become stronger, weaker or even changes to the opposite direction. The balanced regulation of HIF-1α on tumor cell survival and apoptosis is a good example. Though many gene expression induced by HIF-1α can promote tumor survival, HIF-1α may upregulate the target gene expression associated with tumor growth arrest and tumor cell apoptosis under specific tumor type and environment. Therefore, when regarding the signal pathway as an anti-tumor target, the complex factors that influence the activity of HIF-1α and its downstream target gene production should be considered. A successful anti-tumor strategy should be carefully planned and best designed to obtain the efficacy we are looking for in target tissue. A series of inner cell process including HIF-1α mRNA transcription, protein translation, stabilization and degradation of protein, nuclear translocation, combining with HIF-1β to form a functional dimmer, binding to downstream DNA and activating its transcription at the end are affected by many regulatory factors. They recruit the regulatory signals to HIF-1α in an inversed pyramid manner by various mechanisms. Therefore the signal pathway centered by HIF-1α constituted a complex network. Any of the factors associated with any of the signal pathway may become a target to interfering HIF-1α s function. How to make a breakthrough among the complex network consisted of hundreds of signal regulators is still a big challenge. Further studies of HIF-1α signal pathway need to be performed, illuminate unknown molecular mechanisms and develop more antitumor therapy targeting HIF-1α with better efficacy. In order to transform these new strategies from basic research to clinical application successfully, a new suitable system should be constituted, including choosing appropriate patients and the combination of various therapies such as surgery, radiotherapy and chemotherapy, which is a new direction for future tumor therapy development.

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