Abstract. Hypoxia-inducible factor (HIF)-1 is a central regulator in the adaptation process of cell response to hypoxia (low oxygen). Emerging evidence has demonstrated that HIF-1 plays an important role in the development and progression of many types of human diseases, including pathogen-associated cancers. In the present review, we summarize the recent understandings of how human pathogenic agents including viruses, bacteria and parasites deregulate cellular HIF-1 signaling pathway in their associated cancer cells, and highlight the common molecular mechanisms of HIF-1 signaling activated by these pathogenic infection, which could act as potential diagnostic markers and new therapeutic strategies against human infectious cancers.

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1. Introduction
To date approximately one-sixth of global cancers are attributable to an infectious agent (1). The fraction of cancers linked to pathogen infection varies greatly due to geographical location and socioeconomic factors: approximately 8% in developed countries, up to 23% in developing countries, and above one-third in Sub-Saharan Africa (1). Carcinogenesis associated with infections is a complex process, often mediated by chronic inflammatory conditions that is a progressive component in the tumor microenvironment and represents a key hallmark of cancer.

Hypoxia (low oxygen) is a phenotype of hostile microenvironment usually formed by cancer cell rapid growth (2). It has been demonstrated that hypoxia occur in many types of human malignancies caused by pathogen infections and tightly associated with chronic inflammation (3). Hypoxia-inducible factor (HIF) is the master regulator molecule in response to hypoxic stress. HIF, that belongs to basic-helix-loop-helix-PAS family, is a heterodimer transcriptional factor composed of an inducible α subunit (HIF-1α, oxygen-dependent) and a constitutively expressed β subunit (HIF-1β) (4). Three HIF isoforms (HIF-1, HIF-2 and HIF-3) have been identified. Since the majority of studies have reported on HIF-1, here we focus on HIF-1 not HIF-2 or HIF-3. The half-life of HIF-1α protein is very short, and the stability of HIF-1α will determine its effect. The specific proline residue of HIF-1α is hydroxylated by prolyl hydroxylases (PHDs) in the presence of oxygen. Upon hydroxylation, HIF-1α is targeted by the VHL tumor suppressor for ubiquitylation and proteasome-mediated degradation; while in hypoxia condition, the activity of PHD is blocked due to the absence of oxygen, and in turn HIF-1α is stabilized. The stabilized HIF-1α translocates into the nucleus and form a heterodimer with HIF-1β, where they function as a transcription factor to regulate many downstream genes that are critical for various cellular processes including inflammation and cell survival in hypoxia (5). In addition, substantial evidence has shown that transcription and translation of HIF-1 is regulated by many post-translational modifications including phosphorylation, acetylation and SUMOylation, as well as complex formation with other molecules which are important for HIF-1 accumulation and transactivation under hypoxic conditions (Fig. 1). Generally, the overexpression of HIF-1 in cancers was
primarily attributed to induction by environmental hypoxia or genetic mutations in the HIF-1-degradation pathways; however, it has now become clear that many human oncogenic pathogens directly enhance HIF-1 stability and activity through various mechanisms. Thus, elevated expression of HIF-1 represents a common outcome of comprehensive regulation by human oncogenic pathogens.

Here we will summarize the current works in understanding the mechanisms of HIF-1 stability and activity promoted by oncogenic pathogens including viruses, bacteria and parasites (Table I), to further address the role of HIF-1 in different oncogenic pathogen-associated cancers, and highlight the potential diagnostic markers and therapeutic targets.

2. HIF-1 activity is directly enhanced by oncogenic viruses at transcriptional or translational level

In the past decades, substantial evidence from both epidemiology and experimental study have accumulated pointing out seven different human viruses, namely EBV, HPV, HBV, HTLV, HCV, KSHV and MCV, as causal agents of various human cancers. Inspiringly, six of them, excluding newly discovered MCV, all have been clearly indicated involving in the deregulation of cellular HIF-1 signaling pathway. *EBV* Epstein-Barr virus (EBV) is a ubiquitous human γ-herpesvirus that is associated with lymphoproliferative disease in immunosuppressed patients as well as several types of malignancies, such as Burkitt’s lymphoma, lymphoproliferative disorders, T-cell lymphomas, Hodgkin’s disease and some gastric carcinomas (6). Previous studies from different research groups successively proposed distinct mechanisms that EBV infection results in accumulation and activation of HIF-1. In particular, the latent-membrane protein 1 (LMP-1), a principal EBV-encoded oncoprotein, has emerged as one of the most important viral proteins associated with HIF-1α. LMP1 expression in the EBV positive cell lines KR-4 and KR-1 was found to lead to increasing expression of HIF-1α, which is through to be activation of p42/p44 MAPK activity and oxidative stress signaling. LMP1 could promote HIF-1α DNA binding activity and increase activation of JNK/c-Jun signaling which in turn enhanced HIF-1α downstream gene expression (i.e. VEGF, Glut...) (7,8). In addition, LMP1 was also shown to stabilize HIF-1α in nasopharyngeal epithelial cells through upregulating the level of Siah1 E3 ubiquitin ligase, which induces proteasomal degradation of proline-hydroxylases PHD1 and PHD3 and ultimately inhibits HIF-1 degradation (9). Likewise, EBNA1, as the key antigen during EBV latency, has also been found to enhance transcription...
of HIF-1α through modulating the AP-1 transcriptional factor (10). At the post-translational level, more recent studies revealed that HIF-1α is also stabilized by EBV-encoded EBNA-5 and EBNA-3 in the EBV-transformed lymphoblastoid cells through binding to PHD1 and PHD2, two of which participate in the degradation of HIF-1α (11). Interestingly, it has also been shown that hypoxia stress is able to induce EBV lytic replication (12).

HPV. HPV, as a typical human oncogenic non-enveloped DNA virus, is characterized by definite causal role in a subset of squamous cell carcinoma, mainly cervical cancer. HPV-encoded E6 and E7 oncoproteins are shown to continuously express in cervical cancer lesions, which reveal the critical role of these two oncoproteins in the development of cervical tumors. Tang and colleagues (13) have demonstrated an increased expression of HIF-1α and HIF-1α-dependent VEGF in cervical cancer cells with the overexpression of E6 and E7. Further studies identified that E7 protein enhances HIF-1 activity by blocking the association of HIF-1 with histone deacetylases HDAC1, HDAC4 and HDAC7 (14). Notably, HPV-encoded E6 was found to display a distinct mechanism in regulation of hypoxia signal pathway. For example, E6 protein can prolong hypoxia-induced NF-κB activation by inhibiting CYLD deubiquitinase, which is a negative regulator of the NF-κB pathway, and then promote HPV-associated malignance (15). These findings shed light on the mechanisms of how HPV contributes to the stabilization and activation of HIF-1.

HBV. Hepatitis B virus (HBV), a small DNA virus belongs to hepadnaviridae family, is a globally distributed human pathogen that can cause life-threatening diseases like liver cirrhosis and hepatocellular carcinoma (HCC) (16). The

Table I. Deregulation of pathogens-associated HIF signaling.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Associated cancers (28,61,67,68)</th>
<th>Pathogen molecules</th>
<th>Mechanisms of pathogenic HIF signaling</th>
<th>Refs.</th>
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</thead>
<tbody>
<tr>
<td>EBV</td>
<td>Burkitt’s lymphoma, Hodgkin’s B cell lymphoma, gastric and nasopharyngeal carcinoma</td>
<td>LMP1</td>
<td>Degradation of PHD by Siah1-mediated ubiquitylation; Phosphorylation of CBP by MAPK (p42/p44); Activation of JNK/c-Jun</td>
<td>(7-9)</td>
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<td></td>
<td></td>
<td>EBNA1, EBNA5, EBNA3</td>
<td>Stabilization of HIF-1α by blocking interaction with PHD1 and PHD2</td>
<td>(10)</td>
</tr>
<tr>
<td>KSHV</td>
<td>Kaposi’s sarcoma, primary effusion lymphoma, multicentric Castlman’s disease</td>
<td>vGPCR, LANA</td>
<td>Activation of MAPK (p42, p44), p38 by phosphorylation; Stabilization and relocation of HIF-1α; Degradation of VHL through EC5S ubiquitin complex</td>
<td>(41,42)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vIFR3</td>
<td>Stabilize HIF-1α</td>
<td></td>
</tr>
<tr>
<td>HPV</td>
<td>Cervix, anus, vulva, penis, oropharynx</td>
<td>E6, E7</td>
<td>NF-κB activation by inhibiting CYLD deubiquitinase; Enhanced activity of HIF-1 by blocking the association with HDAC1, HDAC4 and HDAC7</td>
<td>(15)</td>
</tr>
<tr>
<td></td>
<td>Liver cancer (hepatocellular carcinoma)</td>
<td>X</td>
<td>Deactylation by MTA1 and HDAC1; Phosphorylation of CBP by MAPK (p42/p44);</td>
<td>(18,19)</td>
</tr>
<tr>
<td>HVB</td>
<td>(hepatocellular carcinoma)</td>
<td>Tax</td>
<td>Phosphorylation by Akt/PI3K pathway</td>
<td>(24)</td>
</tr>
<tr>
<td>HLTV</td>
<td>T cell lymphoma</td>
<td>Core</td>
<td>Likely through phosphorylation of NF-κB, STAT3, PI3K/Akt, MAPK (p42/p44)</td>
<td>(29,31,32)</td>
</tr>
<tr>
<td>HCV</td>
<td>Liver cancer (hepatocellular carcinoma)</td>
<td>Core</td>
<td>Enhanced transcription of HIF-1 by ROS-induced APE1; Inhibition of HIF-1 by iNOS</td>
<td>(51,54)</td>
</tr>
<tr>
<td>H. pylori</td>
<td>Gastric adenocarcinoma</td>
<td>ND</td>
<td>Enhanced transcription of HIF-1 by ROS-induced APE1; Inhibition of HIF-1 by iNOS</td>
<td>(51,54)</td>
</tr>
<tr>
<td>Bartonella</td>
<td>Bacillary angiomatosis (BA)</td>
<td>BadA</td>
<td>ND</td>
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<tr>
<td>Helminth</td>
<td>Bladder carcinoma, cholangiocarcinoma</td>
<td>ND</td>
<td>Induction of a hypoxic microenvironment</td>
<td>(62)</td>
</tr>
<tr>
<td>Leishmania</td>
<td>Skin cancer</td>
<td>ND</td>
<td>Exhaustion cellular iron pool; Elevated expression of HIF-1 by infected macrophage</td>
<td>(66)</td>
</tr>
</tbody>
</table>

ND, not determined.
HBV-encoded X protein (HBx), as one of the four HBV overlapping open reading frames encoded proteins, were proven with accumulating evidence to play a primary function in angiogenesis during the malignant development of HCC (17). Considering the established role of HIF-1 in angiogenesis, HBx links to HIF-1 has been extensively explored. Among these, a research study from Yoo group depicts a positive association between HBx and the MTA1/HDAC complex in stabilizing HIF-1α. HBx induces expression of both MTA1 and HDAC1 to enhance deacetylation of HIF-1 within oxygen-dependent degradation domain (18). Consistently, Holotnakova et al (19) further found that HBx could also increase the transcriptional activity of HIF-1, which in turn enhances a hypoxia-responsive gene CA9 promoter activity and contributes to the development of HCC.

**HTLV.** Among human retrovirus, human T-cell leukemia virus type 1 (HTLV-I), is the only identified retrovirus that directly linked to certain types of human cancer, such as ATL (adult T-cell leukemia). Tax protein has been recognized as one of the most important oncogenic proteins encoded by HTLV-1 (20). Tax has been shown to interact with several transcription factors and involve in activation of several oncogenic pathways, including CREB/ATF, API, NF-κB and the PI3K/Akt signaling pathway (21-23). In hypoxia signaling pathway, Tomita et al (24) showed that phosphorylation of PI3K/Akt induced by Tax leads to activation of HIF-1 in HTLV-infected cell lines, and proposed a PI3K/Akt-dependent mechanism that is responsible for HIF-1 protein accumulation and DNA-binding activity.

**HCV.** Hepatitis C virus (HCV) is a positive-strand RNA virus (25). Since the success of identification of HCV in 1989 by Houghton and colleagues, similar to HBV, the relationship between HCV chronic infection and the development of HCC has been established (26,27). In contrast to the mechanism by which HBV causes HCC, no HCV-derived viral protein has been found to function as oncoprotein. However, chronic inflammation and sustained liver damage caused by HCV infection could likely account for HCV-related hepatocellular carcinoma (28). Also, increasing evidence have shown that a state of oxidative stress is a characteristic manifestation induced by HCV infection (28). Stabilization of HIF-1α induced by HCV is potentially attributable to oxidative stress (29,30). For example, Nasimuzzaman et al (29) showed that the activation of NF-κB, STAT-3, PI3K/Akt and p42/44 mitogen-activated protein kinase under oxidative stress strongly link to the HIF-1α stabilization and VEGF synthesis. In contrast, Ripoli et al (30) provided another explanation that HCV infection causes severe impairment of mitochondrial oxidative phosphorylation, which results in HIF-1α stabilization under normoxic condition. Increased HIF-1α further stimulates the expression of HIF-controlled genes including glycolytic enzymes. More recent studies carried by different research groups disclose that HCV core protein plays a role in upregulation of HIF-1α both in transcription and protein level (31,32). However, the related mechanism remains to be further determined. Taken together, all these findings provide new insights into the role of HIF-1 signaling on the carcinogenesis of HCV-related HCC.

**KSHV.** Kaposi’s sarcoma-associated herpesvirus (KSHV), as a member of the γ-herpesviruses, also referred to as human herpesvirus 8 (HHV-8). It is well known that KSHV is tightly associated with Kaposi’s sarcoma (KS), primary effusion lymphoma (PEL) and multicentric Castleman’s disease (MCD) (33,34). Notably, Kaposi’s sarcoma, commonly occurred in untreated AIDS, is an angioproliferative and endothelial cell-derived tumor (35). Given the close link between active HIF-1 and angiogenesis, extensive studies indicate that KSHV has developed multiple distinct mechanisms to regulate HIF-1 signaling in KSHV-infected cells. The latency-associated nuclear antigen (LANA), as a key antigen in KSHV latency state, plays a crucial role not only in KSHV episomal persistence, but also in modulating viral and cellular gene expression (36,37). Research from our group revealed the role of LANA in regulating HIF-1 signaling. LANA is capable of stabilizing HIF-1α by targeting its suppressor von Hippel-Lindau (VHL) protein and p53 for degradation. This process depends on its suppressor of cytokine signaling (SOCS)-box motif that can recruit the (Elongin BC-Cullin 5-SOCS) EC55 ubiquitin complex (38). In addition, a potential α-helical amino-terminal domain of LANA was found responsible for inducing nuclear accumulation of HIF-1α in normoxic condition (35). In the transcriptional level, LANA is also shown to augment HIF-1α mRNA level (37). Remarkably, LANA also presents to directly interact with HIF-1α, and binds to the hypoxia-responsive element (HRE) motifs of the viral replication transcription activator (RTA) promoter, which partly explain the mechanism of hypoxia-induced KSHV lytic replication (37).

Although LANA represents a critical role in the deregulation of hypoxia signaling, several other viral proteins encoded by KSHV have also been implicated to associate with HIF-1α. For example, LANAl2 (also named vIRF3), which is reported exclusively expressed in KSHV-infected B cells (39), has been shown to stabilize and stimulate HIF-1α transcriptional activity. LANAl2 also interacts with HIF-1α via a double α-helix motif, which could inhibit HIF-1α degradation under normoxia (40). G protein-coupled receptor (vGPCR), which is encoded by KSHV with the nature of potent transformation and proangiogenesis in KS development (28). Earlier studies have documented the effect of vGPCR in HIF-1α activity is involved by several intercellular pathways. Sodhi et al (41) elucidated that the phosphorylation of the inhibitory domain of HIF-1α induced by vGPCR is through the activation of p38 and MAPK signaling pathways, which leads to increased transcriptional activity of HIF-1α and VEGF protein levels. In addition, it is worth mentioning that cytokines secretion induced by vGPCR also activate several kinase pathways including AKT, p38 and IKKβ, which ultimately stimulate HIF activity and VEGF secretion in a mTOR-independent manner (42). Recently, lytic proteins PF-8 and gpK8.1 and vIL-6 encoded by KSHV are also involved in HIF pathways. In both chronic and acute hypoxia, lytic proteins PF-8 and gpK8.1 are strongly expressed in KSHV-infected primary effusion lymphoma (PEL) cells (43). vIL-6 as an important cytokine in the pathogenesis of KS, is also higher expressed in hypoxia than in normoxia. Collectively, this evidence imply a critical role of HIF-1α in promoting KSHV latency and lytic replication (43).
3. HIF-1 is indirectly activated by bacteria-induced oxidative stress

Although it is still debated whether bacterial infection can directly cause human cancer (44), two bacteria *Helicobacter pylori* (*H. pylori*) and *Bartonella* have been widely demonstrated to highly associate with different human cancer development, and their infection can indirectly increase the expression levels of HIF in host cells in innate immune response (45).

*H. pylori.* Since the success of isolating *Helicobacter pylori* in 1984, many studies have focused on the causal relationship between the pathogen and peptic ulcer disease as well as gastric cancer (46). *H. pylori* infection is believed to elevate the risk of gastric cancer development (47,48). Subsequently research work of Griffiths et al has shown an increased expression of hypoxia-inducible protein, like HIF-1α, HIF-2α and VEGF, in the Barrett's metaplasia-dysplasia-adenocarcinoma sequence (49). Also, they found that the expression of HIF-1α in gastric cancer development is increasing and is linked to a poor prognosis (48). The evidence together suggest that HIF-1α is associated with malignant progression of gastric cancer and may play a critical role in carcinogenesis. HIF-1α has been implicated in proliferation, apoptosis events and the inflammatory process that as a result of *H. pylori* infection, is believed to potentially transform *H. pylori*-induced chronic gastritis into intestinal-type carcinoma. The study of Park et al (50) revealed that HIF-1α protein is aberrantly expressed in gastric cancer cells under normoxia, and this phenomenon is correlated with endogenous ROS generation. Importantly, they found that *H. pylori*-stimulated gastric epithelial non-mitochondrial ROS can induce HIF-1α expression as well as activate HIF-1α-mediated transcription. Therefore, they proposed a novel mechanism of stabilization of HIF-1α, which is separate from genetic abnormalities, such as functional loss of VHL tumor suppressor. A recent study from Bhattacharyya et al (51) further explains the underlying mechanism of HIF-1α stabilization and accumulation in *H. pylori*-infected gastric epithelia under normoxic condition. ROS generation, due to neutrophil infiltration in response to *H. pylori* infection, induces the expression of APE1 in human gastric epithelial cells. Subsequently, APE1 not only augments HIF-1α expression, but also interacts with transcriptional co-activator p300 enhancing the transcriptional activity of HIF-1α.

In addition to ROS, exogenous reactive nitrogen species has been extensively indicated in gastric carcinogenesis as well as chronic inflammation relevant to gastric cancer (52). A previous study by Mannick et al (53) has shown an increased expression of iNOS and sustained formation of nitric oxide in gastric mucosa in response to *H. pylori* infection. To unravel the molecular mechanism of HIF-1α accumulation under normoxia by nitric oxide, another group has found that nitric oxide impairs the degradation of HIF-1α under normoxia by inhibiting S-nitrosoglutathione (GSNO)-mediated prolyl hydroxylases (54). Collectively, the evidence provides us with a novel approach that can be employed by *H. pylori* to regulate the accumulation and activation of HIF-1α under normoxic condition.

*Bartonella.* *Bartonella* species is a gram-negative, fastidious, facultative intracellular bacteria that can infect many different mammalian hosts, and is considered as the only known bacterial pathogen causing vasculoproliferative disorders in humans (55,56). Among of more than 20 species, *B. henselae* and *B. quintana* are the major causative agents of bacillary angiomatosis (BA) and bacillary peliosis (BP) in immunocompromised patients (57). Kempf et al (56) showed that in BA lesions and *B. henselae* infected host cells, the expression of HIF-1 is very high, and *B. henselae* infection could result in the activation of cellular genes targeted by hypoxia. Further studies showed that Bartonella adhesin A (BadA) could be crucial for this bacteria to induce angiogenic reprogramming of the host cells via activation of HIF-1 (56,58), however, the related mechanism remains to be further investigated.

4. HIF-1 is indirectly upregulated by parasite-mediated iron exhaustion

Although the association of parasite infection with human cancers is not very clear, some evidence has shown that infection of some parasites including *Helminth* and *Leishmania* also indirectly impair HIF-1α signaling in their associated cancers.

*Helminth.* The prevalence of *Helminth* infections has a high correlation with geographic region and sanitary condition. Current evidence from epidemiological investigation strongly support the link between a certain type of cancer and a specific parasite (59). Among these, the relationship between bladder cancer and schistosomiasis is relatively explicit, which is mainly based on the observation of superficial transition cell carcinomas in animals infected with *S. haematobium* as well as case-control study (59-61). In addition, the other two trematodes, *Opisthorchis viverrini* and *Clonorchis sinensis*, are also believed to be the etiology of cholangiocarcinoma (61). Generally, the development of helminth-related cancer is a chronic process, often require exposure to the infection for many years. Both granulomas formation and inflammatory cell infiltration that responsible for much of the symptom of schistosomiasis, are important inflammation forms in response to *S. mansoni* infection (62,63). Recently, a research group found that Schistosomal granulomas are hypoxic, accompanied by the expression of HIF-1α and VEGF. The findings for the first time reveal a strong positive correlation between hypoxic tissue microenvironment produced by *S. mansoni* infection and HIF-1α expression. HIF-1α expression, in turn, promotes the development and growth of the granulomas (62). Considering the role of chronic inflammation in the development of cancer, hypoxia microenvironment induced HIF-1α expression is possibly another mechanism employed by a parasite, *S. mansoni*, to involve in the transformation of helminth-infected tissues.

*Leishmania.* To date, the links between HIF-1α and parasites were rarely reported, wherein *Leishmania* in association with HIF-1α expand our knowledge on the molecular strategy used by the parasite to deregulate the HIF-1α signal pathway. Studies from the same group showed that the overexpression of HIF-1α was found in both *Leishmania amazonensis*-infected mono-
nuclear phagocytes and cutaneous lesions of BALB/c mice (64,65). However, the related mechanism of activation needs to be further discovered. Recently, Singh et al (66) showed there are two potential distinct mechanisms in activation of HIF-1α in Leishmania donovani (LD)-infected macrophages. One is that LD can exhaust host cellular iron pool and then affect prolyl hydroxylase activity, which contributes to the stabilization of HIF-1α protein. On the other hand, an elevated expression of HIF-1α may due to the transcriptional regulation in the LD-infected macrophage.

5. Conclusions

Distinct from other non-pathogen associated cancers, elucidation of the molecular events underlying the carcinogenesis of human tumors associated with pathogen will facilitate to develop a specific pathogen-targeted therapeutic strategy. In the view of the fact that HIF-1 acts as a central regulator in the adaptation process of cells and organisms to hypoxia, and plays an important role in human pathogen-associated inflammatory cancers, it is possible to prevent pathogenic infection by using HIF-1-specific inhibitors. Hence, we highlight that HIF-1 plays an important role in human pathogen-associated inflammation of the molecular events underlying the carcinogenesis of the virus-associated tumors. In the present review, we enumerate at least six oncopogenic viruses that have clearly been implicated in the infected host cells. In the present review, we enumerate at least six oncopogenic viruses that have clearly been implicated in the infected host cells. In the present review, we enumerate at least six oncopogenic viruses that have clearly been implicated in the infected host cells.

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