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Review

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The role of hypoxia-induced factor 1α in breast cancer

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Abstract

Breast cancer usually grows very quickly, becoming insensitive to blood flow in nearby veins; because of that, inside solid tumors it's possible to find a hypoxic environment, in other words, an environment where oxygen is less available. Another feature of cancer is its angiogenesis rate, because of the high energy demand, new blood vessels must be produced to take nutrients inside the solid tumor mass. Even with normal blood flow bringing the cancer oxygen and nutrients, its cells favor hypoxia, in an event known as Warburg Effect. According to the Warburg Effect, cells, even with normal oxygen rates, prefer to use fermentation instead of the citric acid cycle to produce ATP. For the cancer to operate normally in hypoxia, a transcription factor family is activated, known as hypoxia-induced factors (HIF), composed of a HIF-1β and a HIF-1α subunits. As HIF-1α is expressed during hypoxia, it is a great target for treatments and a breast cancer biomarker. Because of the role of HIF-1α in cancer and the high incidence of breast cancer worlwide, this review was performed in order to bring the most recent results concerning the role HIF-1α can exert in breast cancer development and progression.

Keywords: Breast neoplasms, hypoxia-induced factor 1, HIF-1α

INTRODUCTION

Cancer, an extremely aggressive heterogeneous disease, is the second leading cause of death in the world[1]. The hallmarks of cancer are evasion of apoptosis, self-sufficiency in growth signals, insensitivity to anti-

growth factors, sustained angiogenesis, limitless replication potential, and tissue invasion/metastasis[2]. To proliferate, tumors must form their own vascular network and blood supply, which is due to the influence of tumor angiogenesis factors. However, the new vascular network that is formed differs from that found in healthy tissues because it presents a series of structural and functional alterations. These alterations may interfere with the adequate supply of blood and oxygen, leading to hypoxia, a state in which the organism is functioning with reduced and/or no oxygen supply[3]. In order for the cancer to prevent its metabolism from being diminished or ceased in this situation, a family of transcription factors called hypoxia-induced factors (HIF) is activated.

HIF is known to act on hematopoiesis, angiogenesis, apoptosis, cell reproduction, cancer cell metabolism among other functions[2-5]. HIF-1α action is complemented by the transcriptional co-activator with PDZ-binding motif (TAZ), activating different genes that are involved in the same cascade. Both HIF-1α and TAZ play an important role in remodeling the extracellular matrix, one of the important steps for tissue invasion to occur; these genes act as co-activators of each other, potentiating the expression of HIF-1α target genes[6,7].

HIF-1α is especially important in the metabolism of cancers, being part of several metabolic pathways that cancers use, such as metabolism in hypoxia, angiogenesis, cell reproduction, *etc*. Thus, cancers with a high rate of metastasis and recurrence - as is the case with breast cancer - have their physiology strongly linked to HIF-1α. Based on that, this review sought to group the most recent experimental studies on the role that HIF-1α plays in the development and progression of breast cancer - one of the most incident and deadly cancers worldwide - resistance to conventional treatments, as well as proposals for new treatments based on its expression and/or inhibition.

HIF-1α MODULATION UNDER HYPOXIA

The genes in HIF family are composed of two subunits: HIF-1α (or one of its isoforms, HIF-2α or HIF-3α) and HIF-1β (which is expressed constitutively, acting on a range of transcriptional systems)[8]. The HIF-1α subunit, when in normoxia, is rapidly degraded via the 26S ubiquitin-proteasome pathway by the von Hippel-Lindau tumor suppressor protein; in situations of hypoxia, however, it has increased activity[2-4].

Under periods of normoxia, proline residues of HIF-1α are hydrolyzed by prolyl-4-hydroxylases. Hydroxylated HIF-1α is then recognized by VHL and ubiquitinated by the E3 ubiquitin-protein ligase complex. After, its proteasomal degradation occurs[9]. Breast cancer grows very rapidly and usually the tumor microenvironment has reduced oxygen distribution[10]. In situations of hypoxia, hydroxylation of HIF-1α does not occur, after which it is translocated and accumulates in the nucleus, where it binds to HIF-1β; the HIF-1α/HIF-1β complex binds to hypoxia-response element, adhering to hypoxia target genes such as vascular endothelial growth factor receptor (VEGF) and insulin-like growth factor 2 (IGF-2), stimulating angiogenesis and proliferation[9]. Inhibition of HIF-1α generated inhibition of signaling by VEGF/VEGFR2 factors, which in turn prevented angiogenesis[10].

A model of HIF-1α modulation in hypoxia involves receptor of activated protein C kinase-1 (RACK1) and heat shock protein-90 (HSP90)[11]. In it, human rhomboid family-1 (RHBDF1) controls the competition between RACK1 and HSP90 for binding to HIF-1α. The interaction between RACK1 and HIF-1α results in its degradation, however prior to that RHBDF1 binds to RACK1, giving way for HIF-1α to bind to HSP90, resulting in its stabilization. This model would explain the high levels of HIF-1α, Rack1, HSP90 and RHBDF1 in various cancer cells, including breast cancer. This facilitation of HIF-1α activity as a result of the action of RHBDF1 would also explain the relationship between the high protein level of RHBDF1 in distant metastases and lymph nodes[11].

The RAS protein activator 1 (RASAL1) protein is known to be involved in the progression of hypoxia resistance in breast cancer cells. The increase of intracellular Ca^{2+} ions stimulates RASAL1, which leads to

attenuation of RAS activation and mitogen-activated protein kinase (MAPK) activity and contributes to tumor progression through weakened anti-RAS activity[12]. In periods of hypoxia, reactive oxygen species accumulation activates PI3k/Akt (Protein-kinase B) and MAPK/ERK (extracellular signal-regulated kinase) pathways, essential for the maintenance of carcinogenesis, tumor angiogenesis and activation of HIF-1α.

PROGNOSIS VALUE OF HIF-1α EXPRESSION

Several genes are influenced by HIF-1α expression. In addition to the high expression of HIF-1α being directly associated with tumor growth rate, metastatic potential, and consequently to the poor prognosis of patients, it is also associated with tumor progression, since as the cancer stage advances, HIF-1α expression increases[13]. Thus, the possibility of using HIF-1α as a prognostic marker has already been discussed by several authors[14-16].

HIF-1α up-regulates Snail expression - a gene involved in induction of the epithelial-mesenchymal transition (EMT) - promoting increased migration and aggressiveness of breast cancer cells. HIF-1α together with Snail are responsible for the down-regulation of E-Cadherin expression, promoting EMT[17]. After inhibiting HIF-1α and Snail expression in triple negative breast cancer (TNBC) cells by adding small doses of farnesyltransferase inhibitors, the following mRNA levels of HIF-1α expression pathway genes were also decreased: Snail, glucose transporter 1 (GLUT1), pyruvate dehydrogenase kinase 1 and lactate dehydrogenase A, all involved in the metabolism of hypoxia.

There is also a correlation between HIF-1α protein expression and the erythropoietin receptor protein in aggressive breast cancer samples, which leads to the worsening of the cases, since both play an important role in angiogenesis[18]. γ-secretase is an intramembranous protease that cleaves transmembrane proteins[19]. HIF-1α containing the γ-secretase complex also contributes to the migration and invasiveness phenotype of breast cancer cells. Another gene that is influenced by HIF-1α is heregulin, a growth factor that binds to Erb-B2 receptor tyrosine kinase 3 (ErbB3) and ErbB4 receptors with a known role in cell proliferation, survival, motility and tumorigenesis[20]. According to the authors, stimulation of the ErbB3 receptor and the activation of the P-Rex1/Rac1 pathway via induction of C-X-C Motif Chemokine Receptor 4 are transcriptionally mediated by HIF-1α.

In breast neoplasms, SETD8, a methyltransferase that methylates H4K20, along with a transcription factor related to cancer progression, has the function of regulating the increase and of N-Cadherin expression and the decrease of E-Cadherin[21]. This modulation would be responsible for the epithelial-mesenchymal transition (EMT) phenotype, which increases the invasive potential of breast cancer cells. An interaction between STED8 and HIF-1α was identified, as well as co-localization in the human embryonic kidney 293T and MDA-MB-231 cell nuclei, indicating that they interact with each other. STED8 was seen to slightly regulate HIF-1α expression, and STED8 silencing increased the acetylation and hydroxylation of HIF-1α, demonstrating that STED8 plays a role in the stabilization of HIF-1α. It was also found that STED8 and HIF-1α expression in patients with TNBC and HER2+ breast cancer were higher than in the Luminal A and Luminal B molecular profile[21].

Interleukin-17 (IL-17) is a cytokine produced by T Helper lymphocytes associated with inflammatory diseases that has also been related to onset, development and metastasis of tumors[22]. IL-17 can modulate the expression of some genes in breast cancer cells by altering their adhesion properties through pathways of expression including that of HIF-1α. Tumor necrosis factor α (TNF-α), in turn, is a cytokine associated with cell survival, apoptosis, inflammation and immunity[22]. In addition, TNF-α regulates the expression of cell adhesion proteins, which aid in the determination of a metastatic phenotype in tumor cells. Increased levels of HIF-1α were also found in groups of cells treated with IL-17 and TNF-α in a dose-dependent manner[22].

HIF-1α expression in breast cancer cells influences AKT and ERK pathways, which promote angiogenesis, further worsening the patient's prognosis[10]. Another transcription pathway that was also seen to be more expressed along with the high expression of HIF-1α was the PI3K/AKT pathway, which is important in signaling proliferation, invasion, angiogenesis, *etc.*[23].

HIF-1α expression is directly related to the expression of VEGF[16,24] and Axl (Tyrosine-kinase receptor), which is described as being more expressed in a hypoxic environment and acts in much the same way as HIF-1α (angiogenesis, metastasis, invasion, *etc.*). It was also seen that both aided in the regulation of EMT, which in turn is an important step in the progression and metastasis of cancers[24].

When the increase of HIF-1α expression is regulated by lysophosphatidic acid receptor 2, it has been associated with increased proliferation, migration and cell invasion parameters[15,24]. An increase in the expression of the *EZH2* gene (enhancer of zeste homologue 2, known to act on the aggressive profile of tumors) is completely linked to that of HIF-1α[25]. Patients expressing high levels of HIF-1α and *EZH2* had worse prognoses, data showing that both can potentially lead the individual to death[25]. A direct relationship between the high HIF-1α expression and a greater p53 expression has also been described, which is an aggravating factor for the progression of breast cancer, since the loss of p53 function implies an increase in vascular endothelial growth factor (VEGF) expression mediated by HIF-1α, and consequently, increased angiogenesis[13].

An assessment of HIF-1α and nuclear factor-kappa B (NF-κB) levels as prognostic factors in breast cancer, related to the sub-type of cancer and overall survival (OS) was done by Rajkovic-Molek *et al.*[26]. Activation of HIF-1α and NF-κB (detected by nuclear staining) was found in 41% and 31% of tumors, respectively. In addition, HIF-1α was related to worse OS, making its expression an unfavorable prognostic factor[26].

HIF-1α IN DRUG RESISTENCE

The high expression of HIF-1α has been related to worse prognosis not only by the pathways affected that lead to more aggressive tumors, but also by its association with resistance to hormone, chemo - and radiotherpies in breast cancer[27,28].

Tamoxifen is a drug which acts as an estrogen receptor antagonist, widely used in the treatment of breast cancer, as well as in prevention in women who are prone to develop breast cancer[29]. Lactate generated through cell fermentation in breast cancer (mediated by HIF-1α and the Warburg effect) is related to high rates of resistance to Tamoxifen, and can therefore be a marker of resistance to treatment[29,30]. HIF-1α expression inhibits estrogen receptor α (ERα), which contributes to Tamoxifen resistance and a worse prognosis. Tumors expressing HIF-1α along with ERα were observed to have a much more aggressive profile[29]. Letrozole, in turn, is an aromatase inhibitor, a class of drugs used to treat ER positive breast neoplasms[31]. That the non-hypoxic expression of HIF-1α mediated the resistance to Letrozole effected by HER2 was also seen. HIF-1α protein synthesis increases when activation of phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin (mTOR) pathway by HER2 occurs in long term letrozole-treated cells. As a result, HIF-1α up-regulates the expression of breast cancer resistant protein, which contributes to resistance to Letrozole[31]. HIF-1α is also involved in AI resistance in acquired cases and/or in *de novo* cases, showing a potential therapeutic target for resistance to Letrozole and other AIs.

Trastuzumab is a monoclonal antibody against the HER2 domain, one of the most commonly used drugs for the treatment of HER2+ breast cancers. The induction of resistance to Trastuzumab in breast cancer cells through the activation of the signal transducer and activator of transcription protein (STAT3) pathway/HIF-1α/hairy and enhancer of split-1 (Hes-1) leading to a down-regulation of phosphatase and tensin homolog (PTEN)

was evaluated[32]. Knockdown of HIF-1α in Trastuzumab-resistant cells was observed to cause a decrease in the proliferation of HIF-1α, just as the up-expression of HIF-1α in cells caused resistance to the anti-proliferative effect of Trastuzumab, showing that there is a direct link between the levels of HIF-1αexpression and resistance to Trastuzumab. HIF-1α up-regulates HES-1 expression, a negative regulator of the PTEN promoter, implying that HIF-1α can modulate PTEN expression in Trastuzumab-resistant cells with the constitutive activation of the STAT3 protein[32].

4-Hydroxynonenal (4-HNE) is produced in the lipid peroxidation of cells and is present at high levels in invasive breast cancer cells. The role of 4-HNE in the stabilization of HIF-1α in breast cancer cells was assessed by Li *et al.*[32]. Sirtuin-1 (SIRT1) and Sirtuin-3 (SIRT3) are covalently modified by 4-HNE, leading to SIRT3 deacetylase activity. SIRT3 was also observed to destabilize the HIF-1α protein and inhibit its activity. Regulation of cell growth, invasion and expression of VEGF may be the result of the destabilization of the HIF-1α protein by the inhibition of SIRT3, induced by 4-HNE. These results led researchers to conclude that 4-HNE is involved in resistance to chemotherapeutic drugs via up-regulation of HIF-1α[33].

Zhong *et al.*[34] studied the role of HIF-1α in the regulation of radiation-induced autophagy in mammary neoplasia cells. Michigan Cancer Foundation-7 (MCF-7) in cultures mimicking hypoxia had greater survival of cells when exposed to ionizing radiation. Isogenic cells constitutively expressing HIF-1α small hairpin RNA decreased the expression of HIF-1α and also the formation of colonies. This fact suggested that the HIF-1α knockdown increased radiosensitivity.

Long non-coding RNAs (LncRNAs) are non-coding transcripts involved in the modulation of various signaling pathways, acting as oncogenes or tumor suppressors during tumorigenesis[35]. The urothelial carcinoma-associated 1 (UCA1) lncRNA, which induces adriamycin resistance in MCF-7 cells, was used to test for resistance to tamoxifen in breast cancer cells. Cells expressing UCA1 lncRNAs were transfected with an up-expressing HIF-1α vector, and UCA1 lncRNA was found to be regulated in ER+ cells in a HIF-1α-dependent manner[35]. UCA1 expression was also observed to be increased by an miR-18 - HIF-1α feedback loop.

HIF-1α INHIBITION AS THERAPEUTIC AGENT

As seen previously, the high expression of HIF-1a is associated with a worse prognosis and resistance to antineoplastic treatments. The low expression of HIF-1α, by its turn, is linked to several metabolic alterations, since it influences the regulation of other genes that are important in the process of metastasis of breast cancer[36,37]. The low expression of HIF-1α leads to decreased expression of the *Snail* and twist-related protein 1 (*TWIST1*) genes, signaled by transforming growth factor β1 (TGF-β1), a protein that stimulates cell differentiation and proliferation that is known to regulate the EMT process in tumor cells[36]. Both *Snail* and *TWIST1* are genes most commonly expressed in hypoxia. The silencing of HIF-1α led to the termination of TGF-β-regulated breast cancer cell invasion[36]. Reduced expression of HIF-1α increased apoptosis when on serum starvation. At the same time, Caspase 3 fragments were identified with enhanced activity, indicating that HIF-1α maintains cell growth and survival by inhibiting apoptosis[38]. It was also observed that reduced expression of HIF-1α inhibited TNBC migration and invasion ability[38]. Thus, given the importance of the role of HIF-1α in the development and progression of neoplasias, this gene has become the target of several studies that have been carried out to identify or create HIF-1α inhibitors that could be used as therapeutical agent, as can be seen in the studies below.

Brachyury is a transcription factor of the T-Box family characterized by a highly conserved DNA binding domain called T-Domain, which is directly related with the progression of tumor cells[39]. High Brachyury expression was related to a significant increase in HIF-1α expression in MCF-7 and T47D cells. Akt/PTEN

signaling is known to be a regulator of HIF-1α expression, and on that account the specific inhibitor LY290004, which inhibited Akt phosphorylation and consequently prevented Brachyury-induced HIF-1α expression, revealing that Brachyury is a transcription factor that can be targeted in breast cancer treatments along with HIF-1α[39].

A HIF-1α putative inhibitor is T2A or TSN[36,40-42]. Breast cancer cells treated with this drug showed a decrease in expression levels of HIF-1a and VEGF proportional to the concentration of T2A administered[36]. T2A was also used to inhibit EMT in breast cancer cells via HIF-1α[40]. Two cell lines with hypoxia-induced doxorubicin (DOX) resistance in relation to T2A, MCF-1 and HCC1937, were analyzed. T2A decreased the DOX resistance of both lines in hypoxia partially via HIF-1α[40,41]. It was also found that T2A reduced the viability and proliferation rate in both cell lines, indicating that T2A can target the cell cycle[40].

Dutasteride, a double-blocker of type 1 and type 2 isoforms of the steroid-5α-reductase (*SRD541*) gene is used in the treatment of benign prostatic hyperplasia. As *SRD541* is highly expressed in breast tumor cells, Dutasteride was tested in MDA-MB-231 and MDA-MB-468 cells *in vitro*[43]. MDA-MB-231 cells showed a decrease in VEGF expression, while the detection of HIF-1α was at the limit of detection of the technique employed. MDA-MB-468 cells had decreased VEGF and HIF-1α, showing the potential effect of Dutasteride treatment in response to chemotherapy of TNBCs via altered protein expression of HIF-1α and VEGF.

In order to verify the effects of pigallocatechin-3-gallate on the expression of HIF-1α and VEGF in breast cancer cells, MCF-7 cells were treated with different concentrations of this compound[44]. These cells had a dramatic decrease in growth and decreased expression of VEGF and HIF-1α in a dose-dependent manner. Also working with cells, Dewangan *et al.*[23] evaluated the inhibitory effects of centchroman, a non-steroidal oral contraceptive on HIF-1α. As was expected, an elevated expression of HIF-1α in cells prior to treatment was observed, which was inhibited with drug administration. After treatment, a decrease in VEGF/VEGFR2 expression was observed, with a consequent decrease in angiogenesis[23]. Sarkar *et al.*[10], on the other hand, demonstrated that the administration of phenethyl isothiocyanate, an organic sulfur of the Isotiosianate family found in cruciferous plants decreased HIF-1α expression in normoxia and hypoxia. In addition, administration of N-acetyl cysteine, a known antioxidant, which had been used as a positive control was also observed to inhibit HIF-1α expression. A correlation analysis was then performed to try to determine the association between the parameters involved in the low regulation of HIF-1α by phenethyl isothiocyanate. A positive correlation was found between HIF-1α and HSP90, and these two parameters were correlated with reactive oxygen species (ROS). According to the authors, ROS can be considered the main regulator of both HIF-1α and HSP90[10].

The drug M410 [(Z)-3,4',5-trimethoxylstilbene-3'-Ophosphate disodium, a CA4 analog], which is a potent tubulin polymerization inhibitor in bovine brain *in vitro*, had its inhibitory potential for HIF-1α evaluated by Yang *et al.*[45] Upon treatment, the expression of HIF-1β was found to be unaltered; however, HIF-1α expression was decreased. The amount of VEGF and glucose transporter 1 messenger RNA in the cells was also analyzed, which was very low in relative to normality, showing that M410 was directly involved in the transcriptional activity of HIF-1α[45].

DCQ (2-benzoyl-3-phenyl-6,7-dichloroquinoxaline 1,4-dioxide) contains an N-oxyde portion (present in hypoxia-activated drugs used in cancer treatments) similar to tirapazamine (TPZ) used in treatments against other types of cancer, but inefficient in breast cancer[46]. Similarly to the results with TPZ treatments found in the literature, DCQ induced an increase in p-Akt levels and a reduction in p-mTOR levels suggesting possible anti-translational activity in MCF-7 cells, which would decrease the rates of HIF-1α. DCQ was identified as a promising drug in the treatment of breast cancer because it exhibits pro-apoptotic and anti-metastatic features by reducing HIF-1α and the post-HIF-1α gene signaling cascade[46].

Extracellular matrix metalloproteinase inducer (EMMPRIN) glycoprotein, also known as cluster of differentiation 147 (CD147), is a densely glycosylated type I transmembrane glycoprotein, highly expressed in tumors[47]. This glycoprotein is known to induce a number of malignancy characteristics in breast cancer cells, such as invasiveness, angiogenesis, anchorage independent growth and chemioresistance. SUM102 and BT474 cells were treated with human recombinant EMMPRIN, in which the levels of STAT3 and HIF-1α were observed to be increased. To investigate the role of HIF-1α, the cells were infected with lentivirus carrying HIF-1α shRNA[47]. HIF-1α was observed to increase the percentage of CD24 surface markers (stem-like cells) and decreased the percentage of CD44 via STAT3. In addition, miR-106a/b targeted STAT3 and HIF-1α, and mammary neoplasms treated with miR-106a/b had decreased STAT3 and HIF-1α, and consequently a significant attenuation of stem-like characteristics[47]. Also working with miRNA, Pakravan *et al.*[48] evaluated whether the transfer of exosomes from mesenchymal stem cells (MSCs) containing miR-100 - a tumor suppressor - could have an effect on angiogenesis in breast cancer cells. The miR-100 present in MSCs exosomes was found to inhibit the expression of HIF-1α and mTOR in MDA-MB-231 and MCF-7 cells, which consequently inhibited VEGF expression, contributing to the control of angiogenesis[48].

To understand the functioning of pathways of acquisition of malignancy characteristics, notorious in breast cancer, Kuo *et al.*[49] treated several breast cancer cell lines with pharmacological doses of dimethyl-2-ketoglutarate (DKG). DKG induced HIF-1α in 6 cell lines, rapidly but transiently, and DKG treatment was shown to create a "pseudohypoxic" state in normoxia. An increase in succinate and fumarate rates was then seen, along with a decline in SDH and FH levels. This imbalance of metabolites may then impair PHD2 activity, stabilizing HIF-1α and reprogramming the surface of breast cancer cells[49]. The phenotype was maintained for up to 3 cell passages, and an increase of Carbonic anhydrase 9 (CAIX) (which is one of the genes downstream of the HIF-1α signaling pathway) was seen in DKG-treated breast cancer cells. Taking into account that CAIX is seen in high-grade cancers, this result suggests mitochondrial dysregulation may induce tumorigenicity[49]. Considering that, Tosatto *et al.*[50] evaluated mitochondrial calcium uniporter (MCU) in the progression of breast cancer cells. The MCU is a selective channel that absorbs Ca2+ ions in the mitochondria, which is correlated with infiltration in lymph nodes and tumor size. Silencing of the MCU drastically decreased mitochondrial ROS levels and down-regulated HIF-1α. When treated with Paraquat (a substance that generates superoxide production), there was an increase in the transcription of HIF-1α, which led the researchers to relate HIF-1α to an effector of MCU depletion[50].

A novel class of an antigenic strategy known as the decoy approach was used by Zhu *et al.*[51], who showed that cells treated with a HIF-1α decoy had the transcriptional activity of HIF-1α inhibited and entered apoptosis.

HIF-1α INHIBITION BY NATURAL COMPOUNDS

Still considering the inhibition of HIF-1α as antineoplastic therapy, we have listed here the most recent works dealing with natural compounds, be they extracted from animal plants or microbes.

Manassantin A (MA), a compound isolated from the organism *Saururus cernuus*, which was identified as a potent inhibitor of HIF-1α was used in an attempt to inhibit the function of HIF-1α in breast cancer cells[9]. Several MA derivatives were synthesized in the search for one presenting low toxicity and a good inhibitory effect on HIF-1α. LXY6090 was developed which initially inhibited HIF-1α activity well. The compound was used in T47D, MCF-7 and MX-1 lines, and a decrease in the protein levels of HIF-1α in the cells was observed in a dose-dependent manner. LXY6090 also inhibited the accumulation of HIF-1α mRNA by promoting proteasome degradation in a VHL-dependent way. In addition to these effects, LXY6090 was also observed to inhibit the hypoxia-induced protein expression of HIF-1α target genes such as VEGF and IGF-2, demonstrating a great potential as the target of further research for the treatment of breast cancer associated with an up-expression of HIF-1α.

Another compound that is known to modulate glycolysis in tumor cells is oroxylin A (OA), an active flavonoid found in the plant *Scutellaria baicalensis Georgi*, widely used in Chinese medicine. In breast cancer cells, OA was observed to decrease HIF-1α expression at the protein level. SIRT3 acts as a tumor suppressor by regulating the mitochondrial protein manganese superoxide dismutase, reducing ROS levels. In hypoxia, HIF-1α is stabilized by the inhibition of prolyl hydroxylases by ROS generation. The regulation of O^{2-} (Superoxide anion) by SIRT3 plays an important role in the activation of PHD induced by OA, destabilization of HIF-1α and suppression of glycolysis[52].

Ascofuranone is an antibiotic with antifungal properties that has been isolated from cultures of the phytopathogenic fungus *Ascochyta viciae*. In addition to its antibiotic and antiviral effects, it also has some physiological effects such as hypolipidemic activity, suppression of hypertension, immuno-modulation, and amelioration of type II diabetes[53]. Therefore, the effect of Ascofuranone on the expression of VEGF and HIF-1α was examined, in ord er to determine if the antibiotic would also act on angiogenesis. Ascofuranone decreased HIF-1α expression with a consequent decrease of VEGF expression in MDA-MB-231 cells in a dose-dependent manner. Ascorfuranone was subsequently shown to inhibit the phosphorylation of Akt, mTOR and p70S6K, inhibiting epidermal growth factor-induced protein synthesis[46]. Another compound that suppress HIF-1α by the Akt/mTOR/p70S6K and MAPK pathways in a dose-dependent manner is Ft1 (notoginsenoside Ft1) a saponin isolated from *Panax notoginseng*[54].

Andrographolide (Andro), a substance extracted from *Andrographis paniculata*, an Asian plant used for fever, diarrhea and infectious diseases, also has anti-tumor characteristics such as attenuation of cell invasion, inhibition of cell cycle progression, among others[55]. Thus, the authors evaluated the relationship between Andro and HIF-1α and its possible antitumor effect in breast cancer cells. In cultures of MDA-MB-231 and T47D cells with Andro, a decrease in HIF-1α levels was observed in a dose-dependent manner[55]. It was also found that Andro down-regulated HIF-1α-mediated VEGF expression, which would be an indicator of improvement in angiogenesis.

That some lactobacilli have a cytotoxic effect on neoplastic cells has already been described in the literature. Based on that, Esfandiary *et al.*[56] decided to use the culture supernatant of *Lactobacillus crispatus* (*L. crispatus*) (LCS - *L. crispatus* supernatant) and *Lactobacillus rhamnosus* (*L. rhamnosus*) (LRS - *L. rhamnosus* supernatant) in cultures of TNBC MDA-MB-231. It was found that LRS had a greater cytotoxic effect on MDA-MB-231 cells than LCS. Treatment with LRS led to a decrease in mRNA levels of HIF-1α, HSP90 and SCL2A1 (GLUT1), whereas LCS treatment down-regulated the expression of HSP90, SHARP1 (gene encoding proteins that act on the degradation of HIF-1α) and VHL.

Diallyl trisulfides is an organic sulfur compound present in garlic that modulates various biological processes and its effects were studied on the process of metastasis in breast cancer cells[57]. According to the authors, the compound at different concentrations inhibited not only the transcription but also the translation of HIF-1α[57].

Berberine is an alkaloid purified from a plant of the genus *Berberis*, widely used in Eastern medicine, and exhibits many pharmacological effects such as antihypertensive, antibacterial and antitumor effects. Berberine was observed to inhibit the expression of P-glycoprotein (P-gp), which when up-expressed is a marker of drug resistance. AMP-activated protein kinase (AMPK) is also seen in processes of resistance to antitumor treatments, since it is a P-gp modulator in human cancer cells in hypoxia[58]. Hypoxia was observed to induce AMPK activation, elevating the protein levels of AMPK, P-gp and HIF-1α in MCF-7 cells. HIF-1α was subsequently found to be a protein of the AMPK pathway and that regulates P-gp expression in MCF-7 cells, and that small doses of Berberine increased DOX chemosensitivity by inhibiting AMPK, subsequently downregulating the expression of HIF-1α and of P-gp. Yet high doses of Berberine down-regulated HIF-1α, which induced p53 activation, leading the mammary tumor cells to apoptosis *in vitro*[58].

CONCLUSION

In this review, several experimental studies the role of HIF-1α in breast cancer, whether in *in vitro* or *in vivo* models, have been included. HIF-1α has an immense potential both as a target for various breast cancer treatments and for the prevention and diagnosis of breast cancer. The importance of research with HIF-1α is undeniable. Because of its wide range and several signaling pathways, it has a huge potential for use in the treatment, follow-up and diagnosis of breast cancer. Many innovative treatments using chemicals, drugs and substances exogenous to the body have been employed to better understand the way HIF-1α works in the organism. Analysis of the different points in the HIF-1α expression cascade is of upmost importance in order to gain greater knowledge about how to use HIF-1α in favor of the treatment and prevention of breast cancer and other diseases in which this factor plays an important role.

However, that there is a dearth of studies using HIF-1α directly has been observed, since most of the articles found used HIF-1α as a complement to confirm the data obtained with other genes/proteins/signaling pathways, or only as one of the components of the work. It is hoped that in the future more research will be done using HIF-1α to better understand its function, and new ways of using the knowledge of the functioning of this transcription factor to our advantage.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Carlos HFP, Glaucia LV, Fernando F, Beatriz CAA

Performed data acquisition, as well as provided administrative, technical, and material support: Carlos HFP, Jessica AE, Matheus MP, Glauco SAA, Beatriz CAA

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

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Consent for publication

Not applicable.

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