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HIF-1α inhibitor PX-478 for cancer therapy and other fields

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Abstract. Hypoxia-inducible factor 1-alpha (HIF-1α) is a crucial transcription factor for tumor growth and progression. PX-478, a small molecule HIF-1α inhibitor, has been extensively studied for its potential as an anti-cancer agent in preclinical studies. This paper provides an overview of the current status of PX-478 in cancer treatment, with a focus on its application in treating pancreatic ductal adenocarcinoma (PDAC), lung cancer, breast cancer, glioma, and other kinds of cancer. This article discusses the mechanisms of action, pharmacokinetics, and pharmacodynamics of PX-478, as well as the findings of preclinical and clinical studies conducted to date. The evidence suggests that PX-478 has promising potential as a therapeutic agent for various cancer types. However, further research is needed to fully comprehend the compound's potential benefits and limitations and its optimal clinical application.

Keywords: HIF-1α, PX-478, cancer therapy, combination treatment.

1. Introduction

It is widely known that cancer disease is the second leading cause of death and morbidity all over the world. It has been reported and estimated that cancer kills nearly 10 million people annually and this number is increasing year by year [1]. Due to the unique tumor microenvironment, the effect of many anti-cancer drugs and therapy in cancer treatment and prognosis is very poor and limited, especially in the PDAC, where the five-year survival rate is less than 10% [2]. Additionally, some tumors can evolve during the treatment and secrete some chemicals to alter their microenvironment, reducing their sensitivity to the therapies and promoting tumor aggravation.

Hypoxia, a characteristic feature in the tumor microenvironment, has been found in most solid tumors [2]. Under hypoxia, HIF-1α will translocate to the nucleus and then bind with HIF-1β to regulate the expression of various downstream genes, which play a non-substitutable role in the construction of the tumor microenvironment, such as angiogenesis, extracellular matrix remodeling, and cancer-associated fibroblasts formation [3]. These contribute to tumor progression, metabolic, metastasis, and even evocation from the immune system, which has been tightly associated with poor therapeutic effects and prognosis [2, 3].

PX-478 has shown great power in the treatment of various cancers by inhibiting the HIF-1α at multiple levels, including decreasing mRNA, reducing translation, and promoting degradation [4]. These inhibitions occurred in both hypoxia and normoxia and are independent from pVHL and p53 [4]. In previous studies, it has shown promising achievements in the alone treatment or in combination with other therapies for some cancers with high HIF-1α expression, such as pancreatic, prostate, lung

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cancer et al [4]. Recently, scientists have found its great potential in the treatment of Atherosclerosis and diabetes mellitus. Therefore, this paper will review the application of PX-478 in cancer treatment and discuss its prospects in other emerging fields.

2. Cancer therapy

Hypoxia is always associated with therapy-resistant and poor prognosis in cancer treatment by promoting cancer cell proliferation and metastasis [3]. Recent studies have reported that it contributes to tumor resistance to radiotherapy, chemotherapy, and immunotherapy in various cancer, especially in PDAC, and breast cancer [3]. In previous research, PX-478 has been demonstrated significant efficacy in cancer treatment by inhibiting HIF-1α at multiple levels [5]. Current studies are thus focused on the combination treatment of PX-478 with other therapies, which has been shown to significantly improve treatment outcomes in various types of cancer. This section aims to summarize the powerful effects of PX-478 in enhancing the anti-tumor activity of other therapies and briefly explain the underlying mechanisms.

2.1. Alone treatment

PX-478 has shown great power in treating cancer, especially solid tumors with high level of hypoxia, such as PDAC, ESSC, breast cancer, and lung cancer. It has been reported that can inhibit tumor growth and induce cell apoptosis by inhibiting G2/M transition, EMT, and angiogenesis for cancer treatment in vivo and in vitro [6]. In a study, the Administration of 20 mg/kg PX-478 resulted in a remarkable reduction of the median primary volume of NSCLC and SCLC by 87% and 99% respectively, and delayed their metastases [7]. Additionally, PX-478 single treatment appears to have the ability to induce and enhance immune response. Inflammatory factor COX-2 and immunosuppressive factor PD-L1, directly targeted by HIF-1α, were overexpressed in ESSC and associated with poor survival rate and prognosis [5]. Administration of PX-478 alone significantly suppresses the expression of PD-L1 and COX-2 and inhibits the tumor growth of ESSC in vivo and in vitro [6].

2.2. Combination treatment

Hypoxia and HIF-1α are involved in slow-proliferation stem-cell-like phenotype cell transformation, angiogenesis promotion, microenvironment remodeling, and metastasis augmentation, which all further induce therapy resistance [3]. Therefore, targeting HIF-1α to reduce tumor resistance to chemotherapy, radiotherapy is a promising strategy and has been extensively researched in recent studies [3]. Additionally, with immune therapy emerging, PX-478 also has been shown that can enhance the immune response when combined with immunotherapy.

2.2.1. Combination with radiotherapy. Hypoxia is highly associated with resistance of radiotherapy. On the one hand, cancer cells exposed to radiation in low-oxygen environments tend to resist cell death because there are fewer DNA radicals produced as a result of the reduced formation of ROS and DNA damage [3]. On the other hand, it can activate HIF-1α and downstream signaling pathways to promote angiogenesis and stromal recovery [5].

PX-478 was discovered that can enhance the sensitivity of prostate cancer cells to radiotherapy under hypoxia and normixa by prolonging the phosphorylation of H2AX histone which is positive for DNA damage [9]. Additionally, PX-478 has been demonstrated that can improve the radiosensitivity in Panc-1, C6 glioma, UMSCCa10, and HN5 squamous cell lines by blocking HIF-1α dependent microenvironment remodeling and stomal vascular support [8]. Treatment with PX-478 in hypoxia one day before radiation provided a consistent radiation sensitization enhancement of 1.42 [8]. In pancreatic xenografts of Panc-1, SU.86.86, and CF-PAC-1, PX-478 enhanced the antitumor effects of fractionated radiation, whether combined with gemcitabine or not [10]. Additionally, 5 days of GY radiation combined with PX-478 treatment made 56.6% and 80.9% maximum tumor regression respectively in Panc-1 pancreatic cancer xenografts [10].

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2.2.2. Combination with chemotherapy. Combination therapy of many first-line chemotherapeutic drugs with PX-478 for cancer treatment has been widely reported in recent studies and shows great efficiency in many types of cancer, especially in PDAC, breast cancer, and lung cancer.

Gemcitabine (Gem) is one of the most widely used chemotherapeutic drugs for advanced pancreatic cancer. However, due to the high degree of drug resistance in PDAC which is the most hypoxia solid tumor, gem is often ineffective In PDAC treatment [2]. PX-478 has been reported that can significantly enhance the gem effect of inhibition of tumor growth and promotion of Immunogenic cell death (ICD) [2]. During ICD, dying cancer cells express a variety of signals, known as damage-associated molecule patterns to activate immune cells such as dendritic cells, macrophages, and T cells, which can recognize and attack other cancer cells [2]. In recent research, PX-478 has been demonstrated that can increase the antitumor effect of gem via inducing ICD in PDAC by upregulating P-elF2a [2]. In the xenograft model, immune-competent mice were vaccinated with Panc02 cells which were treated with Gem and PX-478 before [2]. The mice were then injected with Panc02 cells on the other flank for 1 week. The survival rate of the immunized group has significantly increased (5/8 alive) compared with the non-immunized or Gem-alone immunized group. Additionally, in spleen and tumor tissues, CD3+ and CD8+ cytotoxic T cells have been detected significantly increased [2]. In vitro, expression of CRT, HMGBI, and ATP, three of the hallmarks of ICD were highly increased in the combination treatment group. Combination treatments also have been shown to promote maturation and increase the phagocytosis activity of dendritic T cells [2].

Reactive oxygen species (ROS) play a complex role in cellular signaling and are implicated in the etiology of cancer. A moderate quantity of ROS aids in the development and growth of tumors, whereas excessive level of ROS can destroy cancer cell DNA, leading to cell death [11]. Arsenic trioxide (As2O3, ATO) is a ROS inducer that is frequently applied to treat a variety of malignancies, including lymphoma, and leukemia [11]. However, the ATO alone treatment in PDAC has been demonstrated that only had a minimal anti-tumor effect. HIF-1α has been reported that can partially inhibit ROS production under hypoxia [11]. Lang et al. recently have shown that PX-478 can significantly enhance the antitumor effect of arsenic trioxide by promoting apoptosis of cancer cells induced by excessive ROS [11]. Additionally, they found HIF-1α lessoned ROS independent of the mitochondrial pathway but through FOXO1/SESN3 pathway in PDAC (Panc-1 and BxPC-3 cells) [11]. Similarly, Dichloroacetic acid, a potential drug in oncology, and PX-478 have been shown to have synergistic effects in various cancer cell lines, including lung, colorectal, cervical, breast, brain, and liver cancers [12]. Additionally, ROS generation and apoptosis have been proven that plays important roles in this synergism [12].

Recent research has reported that miRNA also played a key role in the development of drug resistance in tumors [13]. Li et al found that Survivin, a protein inhibiting apoptosis, is significantly upregulated in TNBC by downregulating miRNA-494 in TNBC under hypoxia, which is associated with drug resistance [13]. Docetaxel (DTX), inhibiting the growth and division of cancer cells by interfering with the microtubes, is commonly used to treat various types of cancer, including breast cancer [13]. PX-478 has been shown that significantly decrease the resistance of TNBC to DTX through HIF-1α/miR-494/Survivin signaling pathway on both MB-231 and MB-468 cell lines [13]. Additionally, in the previous study, hypoxia was shown that can reduce the apoptosis of colorectal carcinoma (CRC) cells induced by oxaliplatin (OXA) [14]. Xu et al. recently revealed that HIF-1α/miR-338-5p/IL-6 feedback loop appears to be responsible for this. MiR-338-5p is significantly downregulated in HCT116 and HCT8 colorectal cancer cell lines. PX-478 administration can suppress this loop and enhance the cytotoxic effects of OXA on CRC [14].

Glioma is the most common primary malignant brain tumor [15]. Hypoxia and HIFs influence glioma development and survival by regulating angiogenesis, glycolytic metabolism, and treatment-resistant [15]. Therefore, targeting HIF-1α in glioma treatment appears to be a possible therapy. In a statical survey, ferroptosis was found to be associated with malignancy progression and drug resistance in 1750 glioma cases [15]. Sulfasalazine (SAS), which is commonly used to treat rheumatoid arthritis, has recently been shown to have anticancer properties in a variety of

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malignancies, including gliomas, via triggering ferroptosis and inhibiting SLC7ALL [15]. Sun et al. revealed that hypoxia enhances the resistance of glioma cells to SAS-induced ferroptosis via the P13K/AKT/HIF-1α/SLC7ALL pathway [15]. In this research, PX-478 and SAS have been proven to have a synergistic effect on anticancer activities both in vivo and in vitro [15].

In addition to the cancer xenograft model that was directly cultured in the lab, patient-derived models were employed to investigate the potential of PX-478 combined with other drugs. Ryu et al. have reported that PX-478 single treatment or its combination with neratinib (20 mg/kg) significantly suppresses tumor growth in cases of trastuzumab-exposed HR-/HER2+ patient-derived breast cancer xenograft models and VEGF was downregulated [16].

2.2.3. Combination with immunotherapy. Recent advances in immunotherapy have shown significant progress in treating various types of cancer. However, hypoxia remains a major challenge for immunotherapy, as it triggers the activation of HIF-1α and its downstream pathway, which is crucial for the construction of the immunosuppressive microenvironment. Therefore, targeting HIF-1 for cancer treatment seems to be a promising strategy to dismantle the immunosuppressive network of the microenvironment.

Immune checkpoint inhibitors (ICI) are a popular therapy by blocking certain checkpoints on immune cells, allowing them to attack cancer cells more effectively [5]. However, the effectiveness of ICI treatment with NSCLC is not significant which may associate with low T-cell infiltration [5]. From previous research, it has become more and more clear that epithelial-mesenchymal transition (EMT) reduces TILs in the tumor microenvironment to inhibit anti-tumor immunity. Luo et al. has recently revealed that HIF-1α is crucial in inducing EMT with LOXL2 as an important bridge molecule and appears to decrease the quantity of TILS via promoting hypoxia-induced EMT [5]. In this research, ICI anti-PD-I synergized with PX-478 and got a remarkable result in NSCLC treatment [5]. Compared with signal treatment, combination treatment significantly increased tumor growth regression. Large increases in CD4+ and CD8+ T cells, IFN-γ production, and granzyme B were observed [5]. Additionally, Kheshtchin et al. have reported that in the 4T1 breast cancer model, PX-478 significantly enhances the anti-tumor effect of cell-based vaccination [13]. Co-administration of PX-478 with antigen-based DC vaccination resulted in total tumor regression in 50% of mice, as well as a significant increase in survival [13]. These findings show that inhibiting HIF-1α with PX-478 is a promising therapeutic for boosting anti-tumor immunity.

3. Other fields

In addition to its inhibitory effect on tumors, PX-478 has shown great potential in other diseases in recent research.

Hypoxia commonly occurs in adipose tissue due to the tissue's underdeveloped vascular system and the excess expansion of adipocytes to store fat [17]. Instead of promoting angiogenesis, ECM will accumulate abnormally under hypoxia in adipose tissue, causing extensive tissue fibrosis and resulting in dysfunction [17]. According to research by Sun et al., PX-478 treatment successfully inhibits the high-fat diet (HFD)-induced activation of HIF-1α in adipose tissue but did not affect weight gain for mice with a chow diet [17]. The treatment strengthens energy expenditure and increases resistance to metabolic parameter deterioration caused by HFD [17]. Furthermore, a reduction of fibrosis and inflammatory infiltrates were observed in the adipose tissue of mice treated with PX-478 [17].

Atherosclerosis is also significantly influenced by HIF-1α. Increased hypoxia is known to occur in atherosclerotic lesions, which cause inflammation, plaque development, and vascular remodeling [18]. Villa-Roel et al has demonstrated that PX-478 inhibits HIF-1α and its target genes in ECs that are associated with lipid and fatty acid catabolic pathways and reduces atherosclerosis in chronic mouse model injected with AAV-PCSK911 [17]. Additionally, the reduction of plasma cholesterol levels by 69% and 30% respectively, and prevention of diet-induced weight gain are observed in C57BL/6 and ApoE-/- mice, showing that PX-478 may be a promising drug in atherogenic treatment [18].

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In type 2 diabetes development, pancreatic β cells undergo expansion to maintain normoglycemia, but prolonged metabolic overload leads to β cell dysfunction [19]. According to IIegems et al., metabolic overstimulation in mice pancreatic islets led to a hypoxic phenotype [19]. In mouse islet organoid PX-478 was shown to be able to restore normal insulin production in response to glucose [18]. Administration of PX-478 to db/db and STZ-induced diabetic mice caused a reduction in blood glucose levels and an improvement in β cell function, which suggests the potential therapeutic effects of PX-478 in treating diabetes [19].

4. Conclusion

PX-478 has demonstrated significant efficacy in the treatment of various types of cancer with high hypoxia, especially in PDAC, breast cancer, and lung cancer. In addition to its alone treatment, its use in combination with other therapies has yielded promising results in vitro and in vivo. It can enhance the activity of radiotherapy in several xenograft models. In terms of chemotherapy, it can enhance the anti-tumor effect of several first-line chemotherapeutic drugs, such as gem, ATO, DTX, and neratinib in various cancer treatments by inducing ICD, ROS, and ferroptosis. Additionally, when combined with immunotherapy, it can enhance the immune response, increasing the expression of cytotoxic T cells, cytokine, and other immune factors. However, despite its remarkable potential, there is still a need for further investigation to fully comprehend the underlying mechanisms of PX-478. Additionally, more clinical trials, animal studies, and in vivo experiments are necessary to determine the optimal utilization of PX-478 and explore its broader range of applications. Beyond its impact on cancer treatment, recent studies have found its great potential in the field of diabetes and atherosclerosis. However, the mechanism behind these remains limited, which also needs to be further researched.

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