

# RESEARCH PROGRESS ON THE ANTITUMOR MECHANISM OF PENFLURIDOL

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## Abstract

Penfluridol, originally an antipsychotic drug that can easily penetrate the blood-brain barrier and is mainly used to treat severe mental disorders such as schizophrenia, has been re-evaluated due to its safety and pharmacological properties. Potential use in cancer treatment. This article first elaborates on the pharmacology, toxicology, drug resistance and combined drug analysis of penfluridol. In recent years, penfluridol has shown significant anti-tumor activity in tumor treatment research, and the anti-tumor mechanism of penfluridol has been deeply explored, including cell cycle regulation, activation of pro-apoptotic signaling pathways, anti-angiogenic effects, Interference in cell metabolism, inhibition of tumor metastasis and invasion by penfluridol, regulation of immune response in the tumor microenvironment, potential impact on cell replication, enhancement of the efficiency of growth inhibitory factors, impact on tumor-related inflammation, and regulation of tumor cell energy metabolism effect. The molecular mechanisms of penfluridol in a variety of different tumors were analyzed. At the same time, the future research and application prospects of penfluridol are elaborated. These studies not only enhance the potential value of penfluridol as an anti-cancer drug, but also provide new perspectives and strategies for the anti-tumor repositioning of other drugs.

**Keywords:** Penfluridol; Antitumor Effect; Cell Cycle Arrest; Apoptosis; Antiangiogenesis; Drug Repositioning

## 1. Introduction

In recent years, with the application of drug repurposing strategies in the field of oncology, the anti-tumor potential of penfluridol has gradually surfaced. First, as a drug that has been marketed, the safety and pharmacokinetic properties of penfluridol have been relatively well studied. Secondly, the effects of penfluridol on the metabolic pathways, signal transduction pathways, and tumor microenvironment of tumor cells provide new strategies for the comprehensive treatment of cancer. It has been confirmed that penfluridol mainly exerts its antipsychotic effect by acting on dopamine receptor D2 (DRD2) (Kline et al., 2018; Shintomi & Yamamura, 1975). In the field of cancer treatment, scientists have noticed that DRD2 is also expressed in many types of tumor cells and is associated with tumor growth, invasion, and metastasis. Therefore, research on the anti-tumor effect of penfluridol has also emerged. This article aims to deeply explore the latest research progress of penfluridol as a drug repositioning anti-tumor drug and analyze its molecular mechanism of action in detail.

## 2. Overviews of Penfluridol

### 2.1 Pharmacology

Chemical properties of penfluridol: The chemical name of penfluridol is 1-[4,4-bis(4-fluorophenyl) butyl]-4-[4-chloro-3-(trifluoromethyl) phenyl]-4-piperidinol, with a molecular formula of  $C_{28}H_{27}ClF_5NO$  and a molecular weight of 523.97.

It is a white or off-white crystalline powder, odorless and tasteless, easily soluble in methanol, ethanol, acetone or chloroform, and almost insoluble in water (Migdalof et al., 1979a). Dosage and administration: The therapeutic dose range is 20-120mg, once a week. Usually starts with 10-20 mg per week, and gradually increases by 10-20 mg every week or two to reduce extrapyramidal reactions. The usual therapeutic dose is 30-60 mg per week (Wang et al., 1982). Penfluridol not only blocks dopamine receptors (DA receptors) in the brain, but also blocks  $\alpha$ -adrenaline receptors in the nervous system. This dual blocking mechanism makes penfluridol have a strong and lasting antipsychotic effect (Cooper et al., 1975). In addition, penfluridol also has an antiemetic effect, but its sedative effect is weak and its effect on cardiovascular function is also mild (Migdalof et al., 1979b). Penfluridol is slowly absorbed orally, reaching its peak blood concentration within 24 to 72 hours, and can still be detected in the blood after 7 days. After absorption, the drug is stored in adipose tissue, slowly released, and gradually penetrates into the brain tissue. It is mainly excreted in the feces in its original form, and a small amount is excreted in the urine (Balant-Gorgia & Balant, 1987).

### 2.2 toxicology

Penfluridol is widely distributed in the body and accumulates mainly in tissues such as the liver, kidneys, lungs, and gastrointestinal tract. The half-life of penfluridol is about 10 hours (Janssen et al.,

1970). Acute toxicity: As a long-acting oral antipsychotic drug, penfluridol's acute toxicity is mainly manifested as extrapyramidal reactions, such as akathisia, acute dystonia, and Parkinson's disease. Patients who take too much at one time or have poor tolerance may experience acute dystonia, such as torticollis, oculomotor crisis, or torsion spasm, the next day after taking the drug(Andrade, 2022). Cardiotoxicity: Penfluridol may affect the heart. The main toxic reactions are myocardial damage and interference with intracardiac conduction, resulting in severe arrhythmia, chest tightness, shortness of breath, etc(Storror et al., 2014). Hepatotoxicity: Penfluridol may cause a transient increase in transaminase, so it should be used with caution in patients with impaired liver function. Effects on the endocrine system: Penfluridol may affect the endocrine system, and some patients may experience menstrual disorders, galactorrhea and other reactions. Allergic reactions: Allergic rashes are occasionally seen with penfluridol, so it is contraindicated for those who are allergic to this product. Effects on the blood system: Penfluridol may cause granulocytopenia, so the white blood cell count should be checked regularly. Effects on the central nervous system: Penfluridol may cause drowsiness, fatigue, anxiety or depression, etc. Reproductive toxicity: Pregnant women should use penfluridol with caution, and lactating women should stop breastfeeding while using this product (Chen et al., 2019).

### 2.3 Drug resistance analysis

The molecular mechanism of drug resistance to penfluridol is crucial to overcome drug resistance. The following are the molecular mechanism pathways of drug resistance:

**Upregulation of anti-apoptotic proteins:** Drug-resistant tumor cells may upregulate anti-apoptotic proteins to escape drug-induced cell death. Penfluridol inhibits tumor growth by inducing endoplasmic reticulum stress and autophagy, and drug-resistant tumor cells may resist this effect by upregulating proteins with anti-apoptotic functions, like members of the Bcl-2 family(Tung et al., 2022a).

**Defective function of tumor-infiltrating immune cells:** Impairments in the functionality of immune cells within the tumor microenvironment could result in resistance to therapeutics. Penfluridol has demonstrated the ability to impede the processing of N-linked glycoproteins and to boost T cell-driven anti-tumor immunity. Tumors that develop resistance might circumvent immune detection by modulating the activity of immune cells that have infiltrated the tumor, including dampening the efficacy of T cells(Xu et al., 2024).

**Loss of tumor neoantigens:** The absence of neoantigens in tumors can hinder the immune system's capacity to recognize and target cancer cells, potentially causing resistance to treatment. Penfluridol curbs tumor proliferation by influencing the properties of cancer stem cells, whereas resistant tumors might escape immune detection by

diminishing the presence of neoantigens(Roudko et al., 2021).

**Abnormal DNA damage repair pathway:** Drug-resistant tumors may resist the cytotoxic effects of penfluridol by activating the DNA damage repair pathway. Penfluridol can induce cell apoptosis, and drug-resistant tumors may repair drug-induced DNA damage by upregulating the expression of DNA repair-related genes, thereby developing drug resistance(Brinkman et al., 2021).

**Alterations in metabolic pathways:** Alterations in the metabolic pathways of cancer cells could contribute to their resistance to penfluridol. Penfluridol impedes tumor expansion by targeting glycolysis. In response, drug-resistant tumors might adjust their metabolic strategies, possibly by increasing oxidative phosphorylation, to fulfill their energy requirements and counteract the drug's impact(Martin-Bernabe et al., 2021).

## 2.4 Combination therapy

**Concurrent Administration with Chemotherapeutic Agents:** As per the ASCO 2016 research, the synergistic use of 5-FU (5-fluorouracil) and cisplatin exhibited synergistic pharmacokinetic and pharmacogenetic benefits in individuals with advanced nasopharyngeal carcinoma. This combined approach can enhance therapeutic outcomes and mitigate adverse effects. The AUC (Area Under the Curve) of 5-FU is significantly correlated with both the objective response rate and the incidence of toxicity, suggesting that treatment

efficacy can be optimized by adjusting drug dosage and monitoring AUC levels. Consequently, the integration of penfluridol with 5-FU and cisplatin could represent a potent therapeutic strategy(Sun et al., 2016).

**Combined application with immune checkpoint inhibitors:** Research by Professor Zhang Li's team shows that camrelizumab (a PD-1 inhibitor) combined with chemotherapy (GP regimen) has achieved significant results in the first-line treatment of recurrent or metastatic nasopharyngeal carcinoma. Effective efficacy, objective response rate reaches 91%(Yang et al., 2021). Penfluridol, known for its ability to boost T cell-mediated tumor immunity, may improve anti-tumor efficacy when used in conjunction with immune checkpoint inhibitors by stimulating the T cell immune response.

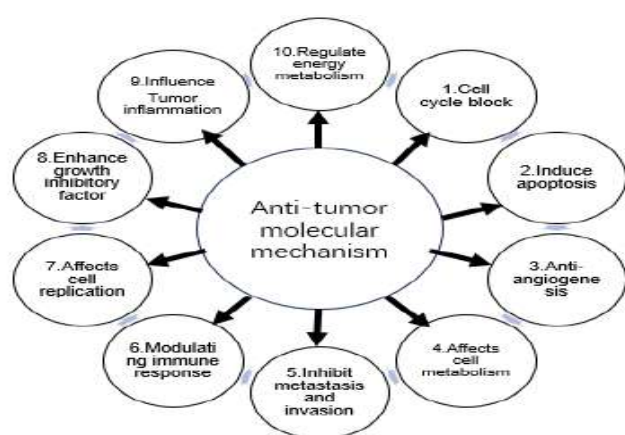
**Combination with other anti-tumor drugs:** Research indicates that penfluridol specifically targets PFKL, thereby suppressing glycolysis and curbing the development of esophageal cancer in a pathway that is dependent on AMPK/FOXO3a/BIM. This mode of action implies that combining penfluridol with other anti-neoplastic agents that target metabolic pathways might lead to a synergistic therapeutic impact(Zheng et al., 2022a).

**Combined application with targeted drugs:** Given that penfluridol can inhibit the stemness of tumor stem cells, its combined application with targeted drugs such as gemcitabine may enhance the therapeutic effect by inhibiting the self-renewal

ability of tumor stem cells(Gandhi et al., 2015).

### 3. Anti-tumor mechanism of penfluridol

Penfluridol's anti-neoplastic efficacy is mediated through a multitude of intricate molecular pathways, as illustrated in the accompanying figure. Below is an in-depth examination of these particular mechanisms of action.



**Figure 1:** Anti-tumor mechanisms

#### 3.1 Cell cycle arrest

Penfluridol has the ability to induce cell cycle arrest in different types of tumor cells. Research has demonstrated that penfluridol can reduce the viability of various lung cancer cell lines and induce G0/G1 phase cell cycle arrest by modulating the expression of relevant proteins. In addition, penfluridol can also reduce the mitochondrial membrane potential of lung cancer cells and increase the level of reactive oxygen species, which may induce mitochondrial-mediated endogenous apoptosis. penfluridol can also inhibit the migration and invasion of lung cancer cells by downregulating

the FAK-MMP signaling pathway(Xue et al., 2020).

#### 3.2 Induction of cell apoptosis

Research indicates that penfluridol suppresses the proliferation of pancreatic cancer cell lines, including Panc-1, BxPC-3, and AsPC-1, and triggers apoptosis through the activation of pro-apoptotic signaling pathways. Furthermore, penfluridol is capable of inducing endoplasmic reticulum (ER) stress and autophagic responses. The assessment of cellular apoptosis and autophagy was conducted using Annexin/FITC and acridine orange staining techniques. Western blotting data revealed alterations in the levels of autophagy-related proteins LC3B and p62. These outcomes establish a theoretical foundation for considering penfluridol as a potential anticancer agent. Ongoing research is elucidating the impact of ER stress and autophagy on pancreatic cancer cell proliferation and their association with the inhibition of cell growth. These insights offer novel avenues for the therapeutic application of penfluridol in oncology(Ranjan & Srivastava, 2014). In vitro studies have demonstrated penfluridol's inhibitory impact on B16 melanoma, LL/2 lung cancer, CT26 colon cancer, and 4T1 breast cancer cell lines. Additionally, penfluridol has been shown to significantly suppress the growth of LL/2 lung tumors in vivo, thereby extending the survival period of mice bearing these tumors. In penfluridol-treated cancer cells, an accumulation of nonesterified cholesterol was detected, alongside a dose-dependent reduction in total cholesterol levels

within the tumor tissues. These observations imply that penfluridol might achieve its antitumor activity by disrupting cholesterol balance(Wu et al., 2014).

### 3.3 Anti-angiogenic effect

The findings revealed that while low doses of penfluridol did not exhibit toxicity to endothelial cells, they effectively hindered angiogenesis both in vitro and in vivo. Specifically, penfluridol impeded VEGF-triggered migration and tube formation in primary endothelial cells in vitro, and it also suppressed VEGF- and FGF-driven angiogenesis in the matrigel plug assay in vivo. Moreover, when cancer cells were exposed to these low doses of penfluridol, there was a slight reduction in the activation levels of basal protein kinase (Src), which impacted their migratory capabilities and the rate of wound closure. Collectively, the data suggest that low levels of penfluridol, akin to those utilized clinically for psychiatric conditions, can inhibit angiogenesis within the tumor microenvironment. These insights offer valuable directions for further exploration of penfluridol's potential role in cancer treatment(Srivastava et al., 2020a).

### 3.4 Impact on Cell Metabolism

Penfluridol curbs the proliferation of lung cancer cells by causing a deficiency in ATP energy, diminishing the quantity and integrity of mitochondria, and elevating the levels of glycolytic proteins. The suppression of glycolysis or the application of 2-deoxy-D-glucose (2DG) can potentiate the inhibitory impact of penfluridol.

Penfluridol might deplete energy by targeting the SIRT1/PGC-1 $\alpha$  pathway. Clinical samples of lung cancer have demonstrated a positive correlation between the expression levels of PGC-1 $\alpha$  and SIRT1. The concurrent administration of penfluridol and 2DG can amplify its anti-neoplastic effects, offering a novel therapeutic perspective for penfluridol in lung cancer management(Lai et al., 2022a). The study found that penfluridol directly acts on liver phosphofructokinase (PFKL), a key enzyme in glycolysis, to inhibit glucose consumption, lactate and ATP production, leading to nuclear translocation of FOXO3a (forkhead box O-type transcription factor 3a) and subsequent transcriptional activation of BIM (Bcl-2-interacting death-promoting protein) in an AMPK (AMP-activated protein kinase)-dependent manner. Therefore, PFKL could serve as a candidate prognostic indicator and a therapeutic target for esophageal squamous cell carcinoma (ESCC), with penfluridol potentially emerging as a novel treatment strategy for combating this life-threatening condition(Zheng et al., 2022b).

### 3.5 Inhibition of tumor metastasis and invasion

In breast cancer-related studies, it was found that the expression of HER2 (receptor tyrosine kinase bound to the cell membrane surface) and  $\beta$ -catenin (intracellular glycoprotein) signaling pathways increased in resistant breast cancer cell lines, suggesting that they may mediate resistance to paclitaxel (paclitaxel). Furthermore, it has been observed that penfluridol is capable of effectively

suppressing these two signaling pathways, decreasing the viability of drug-resistant cells, and potentiating the anti-tumor efficacy of paclitaxel in animal models(Gupta et al., 2019a). Glioblastoma is the most common and deadly brain tumor, and its treatment remains challenging. Studies have found that the antipsychotic drug penfluridol has significant anti-tumor effects and can effectively inhibit the growth of glioblastoma. Penfluridol has been shown to markedly suppress myeloid-derived suppressor cells (MDSCs) and regulatory T cells, and to enhance the presence of M1 macrophages, consequently diminishing the inflammatory response within tumors(Ranjan & Wright et al., 2017a).

### **3.6 Modulating the immune response of the tumor microenvironment**

In one study, it was determined that penfluridol administered in a metronomic chemotherapy regimen exerted a substantial influence on the immunological milieu of gastric cancer. Experimental evidence indicated that this dosing strategy could decrease the quantity of circulating endothelial progenitor cells within the peripheral bloodstream of athymic mice, modulate the concentrations of vascular endothelial growth factor, and influence the polarization state of tumor-associated macrophages. These findings indicate that penfluridol not only inhibits tumor growth but also exerts anti-tumor effects by regulating immune responses in the tumor microenvironment. This provides a new perspective and possible treatment

strategy for the treatment of gastric cancer(Yuan et al., 2015). In another study, it was observed that penfluridol markedly curbed the proliferation of glioblastoma and enhanced the immune response within the tumor microenvironment. This was achieved by suppressing myeloid-derived suppressor cells (MDSCs) and regulatory T cells, and by promoting M1 macrophages, which contributed to its anti-tumor efficacy(Ranjan & Wright et al., 2017a).

### **3.7 Potential effects on cell replication**

Renal cell carcinoma (RCC) is a lethal tumor that is resistant to multiple therapeutic approaches, and its treatment remains challenging. Recent studies have found that the antipsychotic drug penfluridol, which targets dopamine receptor D2 (DRD2), has antitumor effects on multiple cancer types. Experimental results demonstrated that penfluridol was effective in suppressing the proliferation of various renal cell carcinoma (RCC) cell lines in vitro and in restraining tumorigenesis in vivo, with a particular enhancement in the growth suppression of clear cell (cc) RCC lines when combined with Sutent (sunitinib). Mechanistic investigations revealed that the induction of the unfolded protein response (UPR) due to endoplasmic reticulum (ER) stress and the suppression of the GLI1/OCT4/Nanog signaling axis play crucial roles in penfluridol-induced autophagy-mediated apoptosis and the inhibition of stem-like properties. In clinical specimens, a positive correlation was observed between DRD2 and the expression levels

of GLI1, OCT4, and Nanog, which also correlated with unfavorable prognosis. Consequently, DRD2 antagonists, including penfluridol, are anticipated to be repurposed as potential therapeutic agents for the management of ccRCC(Tung et al., 2022b).

### 3.8 Enhancing the efficiency of growth inhibitors

Data from multiple studies have shown that penfluridol can directly activate the enzymatic activity of protein phosphatase 2A (PP2A), thereby triggering a series of downstream anti-cancer cascade reactions. In human pancreatic cancer cell lines, penfluridol treatment increased PP2A activity by 136% after 24 hours(Chien et al., 2015). Researchers have found that the antipsychotic medication penfluridol exhibits substantial anti-neoplastic effects against non-small cell lung cancer (NSCLC). Penfluridol triggers non-apoptotic cell demise by impeding autophagic flux, resulting in the accumulation of light chain 3 (LC3) B-II, a protein associated with autophagosomes. Furthermore, penfluridol is capable of initiating endoplasmic reticulum (ER) stress and the activation of p38 mitogen-activated protein kinase (MAPK), which in turn leads to unfolded protein response (UPR)-induced non-apoptotic cell death. These discoveries indicate the potential therapeutic utility of penfluridol for NSCLC(Hung et al., 2019).

### 3.9 Effects on Tumor-Related Inflammation

Chronic inflammation is closely related to the occurrence and development of tumors and has become a new target for anti-cancer treatment in the

future. Penfluridol, an antipsychotic drug, was found to significantly inhibit the growth of glioblastoma, the study found. This effect is primarily realized by inhibiting myeloid-derived suppressor cells (MDSCs), which are recognized for elevating the population of regulatory T cells (Tregs) with immunosuppressive functions, and for dampening M1 macrophages, which possess tumor-suppressive characteristics. Penfluridol treatment also resulted in suppression of regulatory T cells and an increase in M1 macrophages by 58% and 57%, respectively. Additionally, reductions in CCL4 and IFN $\gamma$  were observed with penfluridol treatment, indicating a reduction in overall tumor inflammation. These findings suggest that penfluridol may have therapeutic potential in glioblastoma, particularly in modulating tumor-associated inflammation(Ranjan & Wright et al., 2017a). These two factors play a key driving role in tumor progression(Gorgun et al., 2013).

### 3.10 Role in regulating energy metabolism in tumor cells

More and more research results reveal that penfluridol can regulate the energy metabolism process of tumor cells through unique multiple pathways, thereby exerting anti-cancer activity. First of all, penfluridol can significantly inhibit the activity of the cholesterol synthesis pathway, reducing it by 92%(Wu et al., 2014). This disrupts the cholesterol balance inside tumor cells. This unique new anti-cancer mechanism is relatively rare in existing anti-cancer drugs. Secondly, penfluridol



can also induce the accumulation of a large number of autophagosomes in tumor cells, leading to the depletion of ATP reserves and a 72% drop in energy supply levels, which in turn activates the unfolded protein response and ultimately triggers non-apoptotic programmed cell death. The mortality rate observed in lung cancer cell lines is as high as 83%(Hung et al., 2019).

#### **4. The mechanism of action of penfluridol in various tumors**

##### **4.1 Glioblastoma**

Glioblastoma stem cells (GSCs) are widely acknowledged for their role in fostering drug resistance within glioblastoma, and it is noteworthy that antipsychotic medications can readily pass through the blood-brain barrier (Srivastava et al., 2020b). Penfluridol demonstrated its ability to inhibit cell proliferation in a manner that was both time- and concentration-dependent. At the IC<sub>50</sub> level, there was a notable reduction in both the size of spheroids and the development of subsequent spheroid generations. Expression levels of stemness-associated genes SOX2 and OCT4 were diminished. The treatment with penfluridol also curtailed the migration and invasive capabilities of cancer cells by lowering the levels of integrin  $\alpha$ 6 and uPAR, and by suppressing the expression of EMT-related proteins vimentin and Zeb1(Kim et al., 2019). Data revealed that the antipsychotic agent penfluridol markedly decreased viability and triggered apoptosis in a selection of ten

glioblastoma cell lines from both adults and children. Treatment with penfluridol curbed the phosphorylation of Akt at the Ser473 residue and diminished the levels of GLI1, OCT4, Nanog, and Sox2 across various glioblastoma cell lines in a dose-dependent fashion. By targeting Akt and GLI1, penfluridol's suppressive impact on cellular proliferation was augmented. Nevertheless, elevated GLI1 expression considerably mitigated penfluridol's efficacy. Further, it was shown that penfluridol curtailed U87MG tumor growth in both subcutaneous and intracranial glioblastoma xenograft models by 65% and 72%, correspondingly. Analysis via immunohistochemistry and Western blotting of the tumors indicated a reduction in pAkt (Ser 473), GLI1, and OCT4 levels, while there was an increase in caspase-3 activation and TUNEL positivity. Collectively, these findings suggest that penfluridol exerts a significant impact on glioblastoma, with its comprehensive suppression of tumor growth being linked to the Akt-mediated inhibition of GLI1(Ranjan & Srivastava, 2017). Another study showed that penfluridol treatment reduced regulatory T cells and increased M1 macrophages by 58% and 57%, respectively. Penfluridol treatment also observed a decrease in CCL4 and IFN $\gamma$ , indicating a decrease in overall tumor inflammation, demonstrating that penfluridol has an inhibitory effect on glioblastoma tumor growth(Ranjan & Wright et al., 2017b).

##### **4.2 Breast cancer**

Penfluridol has a pronounced anti-proliferative

impact on diverse tumor cell lines. For instance, in the case of breast cancer cell lines MCF-7 and 4T1, penfluridol markedly suppressed cellular proliferation. Employing MTT and clonogenic assays, it was demonstrated that penfluridol curtailed cell viability in a concentration-dependent pattern. At a dosage of 10  $\mu$ M, the viability of MCF-7 cells dropped to below 30%, contrasting with the untreated control group where the survival rate was nearly 100%(Hedrick et al., 2017). Research have shown that penfluridol is capable of reversing paclitaxel resistance by suppressing the HER2/ $\beta$ -catenin signaling pathway. Moreover, in mice treated with both paclitaxel and penfluridol, there was a notable reduction in the expression levels of HER2 and  $\beta$ -catenin in tumors, accompanied by an increase in apoptotic activity(Gupta et al., 2019b). In addition, another study demonstrated that penfluridol administration markedly decreased the levels of integrin  $\alpha$ 6, integrin  $\beta$ 4, Fak, paxillin, Rac1/2/3, and ROCK1 in vitro, and that penfluridol's efficacy was mediated through the inhibition of integrin signaling. This treatment was effective in curbing the proliferation of primary triple-negative breast cancer (TNBC) tumors, with particular efficacy against brain metastases(Ranjan et al., 2016).

### 4.3 Lung cancer

In a study, benign concentrations of penfluridol were found to diminish the migratory, invasive, and adhesive capabilities of lung adenocarcinoma (LADC) cells. Matrix metalloproteinase-12 (MMP-

12) appears to be a likely target for penfluridol's action. Penfluridol downregulates MMP-12 expression by impeding the urokinase-type plasminogen activator (uPA)/uPA receptor/transforming growth factor- $\beta$ /Akt signaling pathway. Examination of clinical LADC specimens indicated a positive correlation between MMP12 and the levels of expression of genes associated with mesenchymal properties. There is a lower survival rate observed in patients characterized by high SNA11/MMP12 expression compared to those with low SNA11/MMP12 expression(Hung et al., 2021). Another study used the BALB/c nude mouse model to investigate the anti-neoplastic effects of Penfluridol. Findings indicated that Penfluridol markedly decreased tumor dimensions in mice. Further validation through Western blot and immunohistochemical analysis confirmed that Penfluridol curbed the proliferation and metastasis of lung cancer by modulating the AKT and MMP signaling pathways. Throughout the treatment period, no adverse effects were detected, indicating that the mice tolerated Penfluridol treatment well(Xue et al., 2020). Mechanistic studies have shown that penfluridol's reduction of cellular energy is attributed to the suppression of the principal modulator of mitochondrial biogenesis, specifically the sirtuin 1 (SIRT1)/peroxisome proliferator-activated receptor coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) pathway. The activation of the SIRT1/PGC-1 $\alpha$  pathway counteracted the inhibitory impacts of penfluridol on mitochondrial biogenesis and cellular viability. Consequently, the concurrent use of penfluridol with glycolytic

inhibitors may represent an effective therapeutic approach to potentiate the anti-neoplastic effects of penfluridol in lung cancer(Lai et al., 2022b).

#### 4.4 Pancreatic cancer

Autophagy has become an important target for cancer. Some people believe that triggering autophagy could serve as a therapeutic approach for pancreatic cancer treatment(Mukubou et al., 2010). Pancreatic cancer cells have high levels of basal autophagy(Kang & Tang, 2012). Animal model studies indicate that penfluridol administration prompts the apoptosis of pancreatic cancer cells and curbs their proliferation. The IC50 concentration after a 24-hour period is recorded at 6-7  $\mu$ M. Penfluridol elicited considerable autophagic responses. The blockade of autophagy hindered penfluridol-induced apoptosis, suggesting that autophagy is a precursor to apoptosis. Penfluridol's suppressive effect on BxPC-3 and AsPC-1 tumor xenografts was less pronounced when combined with chloroquine. TUNEL staining and caspase-3 activation indicated reduced apoptosis in tumors of mice treated with chloroquine. Penfluridol was found to inhibit the growth of orthotopically implanted Panc-1 tumors by 80% through the induction of autophagy-mediated apoptosis. Collectively, these findings underscore that penfluridol restrains the growth of pancreatic tumors via autophagy-mediated apoptosis(Ranjan & Srivastava, 2016). The research established that endoplasmic reticulum (ER) stress indicators, including binding protein (BIP), C/EBP

homologous protein (CHOP), and inositol requiring 1 $\alpha$  (IRE1 $\alpha$ ), exhibited increased expression in a dose-dependent fashion following penfluridol administration. The suppression of ER stress through pre-treatment with agents like sodium phenylbutyrate and mithramycin, or by employing CHOP small interfering RNA to silence CHOP, effectively hindered penfluridol-induced autophagy. These findings unequivocally demonstrate that ER stress triggered by penfluridol precipitates autophagy(Ranjan & German et al., 2017a).

#### 4.5 Colon cancer

Modified aerobic glycolysis serves as a pivotal mechanism for sustaining the survival and proliferation of cancer cells. This process is crucial not only for maintaining cellular energy metabolism but also significantly contributes to tumor growth and metastasis(Littleflower et al., 2024). Tumor cells are able to adapt their metabolic pathways to meet the energy and biosynthetic precursor requirements for rapid proliferation(Liberti & Locasale, 2016). Malignant cells tend to produce energy predominantly via increased glycolysis instead of relying on oxidative phosphorylation(Feng et al., 2018). According to the Warburg effect, the glycolysis rate of rapidly proliferating tumor cells can be as high as 200 times that of normal cells(Pistritto et al., 2016). Therefore, blocking the glycolysis process and limiting the energy supply of cancer cells may become an effective cancer treatment strategy(Ranjan & German et al., 2017b). This strategy can not only

limit the energy production of cancer cells, but also affect their biosynthetic capacity, thereby inhibiting tumor growth and spread. Related experimental reports show that Penfluridol can effectively reduce the survival rate of CRC cells and promote cell apoptosis through the mitochondrial-mediated intrinsic pathway. In addition, the administration of Penfluridol has been shown to suppress the glycolytic activity in HCT-116 and HT-29 cells, evidenced by reduced glucose uptake, lactate output, and intracellular ATP concentrations. Subsequent mechanistic investigations disclosed that Penfluridol influences apoptosis and glycolysis in colorectal cancer (CRC) cells by reducing the expression of hexokinase-2 (HK-2). Overexpression of HK-2 can efficiently counteract the pro-apoptotic and glycolytic inhibitory effects induced by Penfluridol(Wang et al., 2021).

#### 4.6 Acute leukemia

A study on acute leukemia observed that penfluridol suppressed the activity of AML cell lines with FLT3-WT (represented by HL60 and U937) and FLT3-ITD (MV4–11) in a concentration-dependent manner. The study revealed that penfluridol not only prompted apoptotic changes, including enhanced nuclear disintegration, an increase in the sub-G1 cell fraction, PARP cleavage, and caspase-3 activation, but also initiated autophagic reactions, marked by the conversion of light chain 3 (LC3) and the formation of acidic vesicular organelles (AVOs). Moreover, the elevation of intracellular ROS levels by penfluridol was identified as a key factor in the

autophagic response it elicited(Wu et al., 2019). The use of pharmacological inhibitors 3-methyladenine and chloroquine to impede autophagy substantially amplified the apoptotic effects induced by penfluridol, indicating that autophagy serves a protective function in AML cells treated with penfluridol(Zheng et al., 2015). At the molecular level, penfluridol triggers apoptosis by stimulating the activity of protein phosphatase 2A (PP2A), which in turn suppresses the activities of Akt and mitogen-activated protein kinase (MAPK)(Peris et al., 2023).

#### 4.7 Inflammatory autoimmune diseases

Related studies have delved into the therapeutic potential of Penfluridol, an FDA-approved small molecule, in the context of TNF $\alpha$ -driven inflammatory autoimmune conditions, with a particular focus on inflammatory arthritis. The study employed various in vitro assays to ascertain Penfluridol's inhibitory impact on TNF $\alpha$ -induced NF- $\kappa$ B activity and to assess its therapeutic efficacy across several disease models. Findings indicated that Penfluridol potently curbed TNF $\alpha$ -induced NF- $\kappa$ B activity and mitigated the symptoms of arthritis and colitis(Chen et al., 2022). Mechanistic research discloses that Penfluridol interacts with acid sphingomyelinase, thereby suppressing its enzymatic function (Zhao et al., 2021). In addition, Penfluridol curbed the differentiation of splenic naive CD4<sup>+</sup> T cells into TH1 and TH17 subsets and dampened the polarization of M1 macrophages(Ye et al., 2023). Therefore, this research establishes a

basis for considering penfluridol as a newly discovered small molecule therapeutic for the management of TNF $\alpha$ -mediated conditions, including inflammatory arthritis and colitis.

#### 4.8 Bladder cancer

According to a study report, cationic amphiphilic drugs (CADs) can reduce the viability of human UCB cell lines in vitro and induce lysosomal puncta formation (van der Horst, van de Merbel, Ruigrok, van der Mark, Ploeg, Appelman, Tvingsholm, & Jaatela et al., 2020). In mouse models, the intravesical administration of penfluridol as part of a chemotherapy drug (CAD) regimen markedly inhibited tumor expansion and metastatic spread. Additionally, the treatment of cultured human urothelial carcinoma of the bladder (UCB) tissues derived from patients elicited notable antitumor effects in the majority of engrafted tumor tissues, suggesting that penfluridol is a potential therapeutic candidate for bladder cancer treatment (van der Horst, van de Merbel, Ruigrok, van der Mark, Ploeg, Appelman, Tvingsholm, & Jäätelä et al., 2020).

### 5. Challenges and future development directions

Although penfluridol has shown significant potential in tumor treatment research, it still faces many challenges in its translation into clinical applications. This section discusses these challenges in depth and explores solution strategies and future research directions.

#### 5.1 Pharmacokinetics and pharmacodynamic

#### issues

The pharmacokinetics of penfluridol, including its absorption, distribution, metabolism, and excretion in the body, are crucial for its clinical use. Studies have shown that penfluridol has a low bioavailability, which may limit its ability to reach effective antitumor concentrations in the body. In addition, penfluridol's high metabolism in the liver may lead to a rapid reduction in its active form (Turner et al., 2011). Therefore, improving its pharmacokinetics, such as increasing its solubility and stability through nanotechnology, is an important direction for future research (Ahmed et al., 2012).

#### 5.2 Individual Differences and Patient Screening

There are significant differences in the response of different patients to penfluridol, which may be related to the individual's genetic background, tumor type and microenvironment. For example, some tumor cells may show resistance to penfluridol due to specific genetic mutations. Identifying patients who are more likely to benefit from penfluridol treatment in clinical trials is key to achieving precision medicine (Gao et al., 2013). Using genomic and proteomic approaches to predict patient response to penfluridol will provide more personalized decision support for treatment.

#### 5.3 Long-term safety and side effects

Although penfluridol has a good safety record in the treatment of mental illness, its long-term safety in

cancer treatment still needs further study. In particular, in high-dose or long-term treatment, possible side effects include QT interval prolongation, metabolic abnormalities and other cardiovascular problems (Smith et al., 2018). Therefore, long-term clinical observation and follow-up studies are essential to evaluate its safety and tolerability.

#### 5.4 Drug Design and Optimization

Given the multiple anti-tumor mechanisms of penfluridol, it is possible to further improve its efficacy and selectivity through drug design and optimization. For example, increasing its affinity for specific cancer markers through structural modification, or developing derivatives that can specifically target the tumor microenvironment, may enhance its potential for application in tumor treatment (Tuan & Lee, 2019).

#### 5.5 Interdisciplinary cooperation and application of innovative technologies

Future research should strengthen interdisciplinary collaboration and integrate knowledge and technology from the fields of medicinal chemistry, molecular biology, and clinical medicine to address the challenges encountered in the research and development of penfluridol. At the same time, the use of the latest biotechnologies, such as CRISPR gene editing technology and AI-driven drug discovery platforms, can accelerate the development of penfluridol and its derivatives (Zhouravleva et al., 2022).

### 6. Conclusion

As a potential anti-tumor drug, penfluridol has a diversified mechanism of action and effectiveness demonstrated in preclinical models, laying a solid foundation for its application in the field of tumor treatment. Studies have revealed that penfluridol can effectively inhibit the growth of various types of tumor cells, including glioblastoma, breast cancer, pancreatic cancer, lung cancer cells, bladder cancer, colon cancer, leukemia, and certain autoimmune diseases. Penfluridol exerts its anti-tumor effect through cell cycle regulation, activation of pro-apoptotic signaling pathways, anti-angiogenic effects, interference with cell metabolism, inhibition of tumor metastasis and invasion by penfluridol, regulation of tumor microenvironment immune response, potential effects on cell replication, enhancement of the efficiency of growth inhibitory factors, effects on tumor-related inflammation, and regulation of tumor cell energy metabolism. Future research needs to strengthen preclinical and clinical research while deeply exploring its mechanism of action to promote the application of penfluridol in clinical treatment.

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