doi.org/10.36721/PJPS.2025.38.5.REG.13016.1

Propofol suppresses migration and vascular mimicry of breast cancer MCF-7 cells by down regulating interleukin-6

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Abstract: Propofol is widely used in anesthesia, but its role in breast cancer progression remains controversial. This study investigated the molecular mechanisms of propofol in breast cancer, focusing on IL-6 and tumor microenvironment modulation. Bioinformatics analysis identified IL-6 as a potential target of propofol. MCF-7 cells were treated with varying propofol concentrations (0–100 μg/mL), and cell viability was assessed via CCK-8 assay. Propofol at 50 μg/mL significantly reduced viability, while 25 μg/mL (a non-cytotoxic dose) was selected for further experiments. Western blot confirmed propofol down regulated IL-6 expression. Functional assays demonstrated that propofol suppressed migration, invasion, and angiogenesis in MCF-7 cells; and effects that were reversed by recombinant human IL-6 (rhIL-6). Molecular docking analysis further supported the interaction between propofol and IL-6. Additionally, IL-6 and VEGF-C were found to be co-expressed, suggesting a possible link between propofol and vascular mimicry inhibition. These findings indicate that propofol may exert anti-tumor effects in breast cancer by targeting IL-6, thereby inhibiting key oncogenic processes. This study provides new insights into the potential therapeutic benefits of propofol in breast cancer management.

Keywords: Breast neoplasms; propofol; neoangiogenesis; cell migration; interleukin-6

Submitted on 24-06-2024 – Revised on 18-11-2024– Accepted on 31-12-2024

INTRODUCTION

Breast cancer is a common malignancy that is becoming increasingly common among females year by year and the rate of breast cancer is likely to stay “high for long”. Patients are trending younger and breast cancer has become the most dominant cause of death from malignant tumours in young women (Chen *et al.*, 2016), which means that this disease not only brings physical pain, but also causes severe psychological problems in patients. Radiotherapy, chemotherapy, targeted cancer therapy and endocrine therapy are very common treatments in patients, while surgery is a significant procedure (Lim *et al.*, 2019; Mudgway *et al.*, 2020; Riedel *et al.*, 2020; Villasco and D'Alonzo, 2020). Despite the different therapeutic methods that are available, tumour metastasis remains a very difficult problem for both doctors and patients (Parsons and Francavilla, 2019). Complex phenomen a are found during tumour cell metastasis, including cell migration, invasion, angiogenesis and so on (Stuelten *et al.*, 2018). Angiogenesis is characterized by the development of a preexisting vascular network (Huang *et al.*, 2004), which can be considered a conduit to provide oxygen and nutrients for tumorigenesis (Andonegui-Elguera *et al.*, 2020). Thus, at the beginning and during progression and metastasis of cancer, tumour angiogenesis is a key factor. Tumour angiogenesis is connected to systemic circulation, which offers a perfect opportunity to enable haematogenous metastatic spread (Aalders *et al.*, 2017). In addition to supplying nutrients to the tumor, this is particularly concerning as angiogenesis is closely linked to tumor migration and invasion. (Hulin *et al.*, 2019). Evidence is growing the VEGF family, including VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PIGF), plays a key role in regulating angiogenesis during metastasis (Jain, 2003; Lara *et al.*, 2018).

Interleukin-6 (IL-6) has garnered significant attention due to its important physiological functions and its production by various cell types, including immune cells and certain tumor cells (Yao *et al.*, 2014). Many inflammatory diseases and malignant tumours are associated with IL-6 metabolic disorders (Su *et al.*, 2017). There is abundant evidence indicating that IL-6 has an influence on not only proliferation, angiogenesis, migration and invasion of tumour cells, but also the ability of cancer cells to respond to anticancer therapies (Dmitrieva *et al.*, 2016). Tawara *et al* found a correlation between the coexpression of VEGF and IL-6 in HER-2 negative breast cancer (Tawara *et al.*, 2019). The literature shows that IL-6 and VEGF are associated with invasion, migration and angiogenesis in tumour cells (Huang *et al.*, 2004; Andonegui-Elguera *et al.*, 2020).

Propofol is a general anesthetic commonly used in intravenous anesthesia for inducing and maintaining anesthesia, as well as for ambulatory surgery and sedation in the ICU (intensive care unit) (Ye *et al.*, 2013). In addition to its use in anaesthesia, its nonaenesthetic effects, such as antioxidant, anti-inflammatory and immunomodulatory effects, have recently received considerable attention (Irwin *et al.*, 2020; Tian *et al.*, 2020). It has been reported that propofol, a common drug, has anti-tumour effects in

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many cancers, such as lung cancer (Liu and Liu, 2018), osteosarcoma (Ye *et al.*, 2013) and gastric cancer (Liu *et al.*, 2020). Propofol is also reported to have inhibitory effect in breast cancer (Zhang and Li, 2019; Tian *et al.*, 2020) and Meng believes that a certain dose of propofol is able to promote the proliferation of breast cancer cells (Meng *et al.*, 2017). Our study hypothesized that when MCF-7 cells are treated with propofol, cellular fuctions can be altered and, which may be implicate IL-6. In this study, we investigated the effects of propofol on cell migration and biological angiogenesis in human breast cancer MCF-7 cells in vitro, examining its underlying molecular mechanisms in detail. The results of our research have predictive power and provide insights for cancer therapies.

MATERIALS AND METHODS

Prediction of potential targets of propofol

BATMAN-TCM (http://bionet.ncpsb.org/batman-tcm/) is an integrative database that stores both established and predicted connections between ingredients and target proteins. We conducted a search in the BATMAN-TCM database for the anesthetic drug propofol (InChI=1S/C12H18O/c1-8(2)10-6-5-7-11(9(3)4)12(10)13/h5-9,13H,1-4H3), ensuring a smoother integration into the study. Potential targets of propofol were predicted with a threshold score cut-off of ≥20 and $p \leq 0.05$. We used Cytoscape, an online bioinformatics tool, to generate visual molecular interaction networks and construct network diagrams for propofol and its corresponding targets.

Gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) analyses of targets

Leveraging the comprehensive capabilities of the Metascape database(http://metascape.org), we conducted an exhaustive functional annotation of the genes or proteins linked to our study(Zhou *et al.*, 2019). GO analysis serves as a bioinformatics tool to classify and describe the functions of genes and their products across various organisms (Ashburner *et al.*, 2000). KEGG analysis is another bioinformatics tool that helps elucidate the high-level functions and roles of biological systems, including cells, organisms and ecosystems. This analysis relies on molecular-level data, particularly large-scale datasets produced by genome sequencing and various high-throughput experimental techniques(Kanehisa, 2002). This analysis illuminated the most significantly enriched biological annotations across four domains: biological processes, molecular functions, cellular components and KEGG Pathways. $P<0.05$ was considered statistically significant. By focusing on the first 20 items within each category, we gained insights into the underlying biological significance of our target genes, presented through visual aids for easier comprehension.

Construction and analysis of the protein-protein interaction (PPI) network

Our study extended into the intricate realm of protein-protein interactions, utilizing STRING (http://string-db.org), an online platform for bioinformatics analysis. We began by searching for potential targets related to propofol in the BATMAN-TCM database. The resulting data were subsequently imported into the STRING database, where we set a combined score threshold of greater than 0.9 and specified the species as "Homo sapiens". Leveraging the predictive capabilities of STRING, we mapped out the PPI network of potential targets, which was further refined using Cytoscape software to enhance the visualization of these complex interactions. The Molecular Complex Detection (MCODE) plugin was instrumental in pinpointing densely interconnected regions within the network, aiding in the formation of key gene modules essential for interpreting the biological significance of our results.

cBioPortal analysis for gene co-expression

Through the innovative use of cBioPortal (http://www.cbioportal.org) (Gao *et al.*, 2013), we undertook a comprehensive analysis to explore gene co-expression patterns. An analysis of 1,904 patient samples from the cBioPortal database revealed genomic alterations in the IL-6 gene across a subset of breast cancer samples. This involved the meticulous examination of clinical samples, focusing on the expression of the IL-6 gene and its co-expression relationships with the VEGF family. By setting a threshold for moderate co-expression (Pearson and Spearman scores >0.3), we delineated the intricate genetic networks that underpin these relationships.

Cell culture techniques and treatment protocols

MCF-7 cells were cryopreserved in liquid nitrogen at the Central Laboratory of Yongchuan Hospital of Chongqing Medical University. They were cultured in RPMI 1640 medium (Gibco, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS, Gibco, Thermo Fisher Scientific) and 1% penicillin-streptomycin (Beyotime Biotechnology) at 37˚C in a 5% CO2 incubator. For 48 hours, cells were treated with propofol (25 μg/mL, Aladdin) and recombinant human IL-6 cytokine (50 ng/mL, Peprotech). Propofol was dissolved in dimethyl sulfoxide (DMSO), maintaining a DMSO concentration of less than 0.1%, while IL-6 was prepared in RPMI 1640 medium.

Cell proliferation

After reaching the logarithmic phase, the cells were seeded in a 96-well plate at a density of 1×10^4 cells per well and incubated at 37°C with 5% CO_2 for 12 hours. We added varying concentrations of propofol [0 (as control), 12.5, 25, 50 and 100 μg/mL] or propofol and IL-6 cytokine and continued culturing for 48 hours. Following treatment, cell viability was measured using the Cell Counting Kit-8 (CCK-8, Dojindo, Japan, 10 μL/well), with absorbance recorded at 450 nm.

Western blot assay

Trypsin (0.25%) was used to digest cells from culture

dishes and samples were collected in PBS after treatment. RIPA buffer (Boster Biological Technology Co., Ltd, Wuhan, China) was used to isolate the total protein in each group. Proteins were separated by 15% SDS-PAGE and transferred to PVDF membranes (Millipore, USA), which were then blocked with 5% nonfat dry milk for 1 hour.

The membranes were incubated at 4ºC in the presence of IL-6 (cat. no.ab233706; 1:1,000, Abcam, Cambridge, MA, UK) and GAPDH (cat. no.AF7021; 1:1,000, Affinity Biosciences, OH, USA) overnight. After washing with TBST, the membranes were incubated with secondary antibodies (Boster Biological Technology) for 1 hour. Protein visualization was performed using an ECL kit (Millipore, USA) and GAPDH was used as a loading control. Grayscale values of the protein bands were analyzed using ImageJ software.

Cell migration assay

Cells from each group were diluted to a concentration of 2.5×10^5 cells/mL in serum-free medium. A 200 µL cell suspension was added to the upper chambers, while RPMI 1640 containing 20% FBS was regarded as a chemokine in the lower chamber. After 24 hours, chambers were gently rinsed with aseptic PBS. Migrated cells in the lower layer were fixed with methanol for 10 minutes, followed by staining with crystal violet (Beyotime Biotechnology) for 10 minutes. Finally, all chambers were rinsed with single-distilled water. When observing cell morphology under a light microscope (×100) and taking photographs, select 5 fields of view from each chamber to assess the cell migration ability.

Cell tubule formation assay

After Matrigel (BD, Bedford, San Jose, CA, America) for melting at 4°C overnight, RPMI 1640 medium and Matrigel were mixed(1:1 dilution). The Matrigel was laid in a 48-well plate (160 uL/well) and then solidified at 37ºC in the incubator for 1 h. Approximately 4×10^5 cells per well were seeded onto the Matrigel-coated plates. The assay was photographed with a light microscope (×40) after 6 h. The number of masses was analysed by ImageJ.

Molecular docking

The three-dimensional (3D) molecular structure of IL-6 (PDB ID: 1ALU) was obtained from the Protein Data Bank (PDB; http://www.rcsb.org/pdb/)(Burley *et al.*, 2019), the three-dimensional (3D) molecular structure file of the IL-6 was retrieved with PDB ID 1ALU. The propofol structural file, which is a ligand moecule, was downloaded from the ZINC database (http://zinc15.docking.org/, ZINC ID: ZINC968303) (Sterling and Irwin, 2015).

Prior to molecular docking, IL-6 protein preparation involved removing water, adding hydrogen atoms, merging nonpolar bonds and calculating Gasteiger charges using AutoDock Software (http://mgltools.scripps.edu/). Ligand preparation was performed in Openbabel GUI (O'Boyle *et al.*, 2011) ithin the PyRx interface by adding hydrogen, minimizing energy and converting the file to pdbqt format. Docking was conducted using AutoDock Vina in PyRx, targeting the selected ligand database (Tha *et al.*, 2020). The docking results were visualized using PyMOL and potential binding interactions between propofol and IL-6 were analyzed.

STATISTICAL ANALYSIS

Data analysis was performed using GraphPad Prism 5.0 (GraphPad Software, Inc.). A Student's t-test was applied to compare differences between the two groups, while Pearson's correlation coefficients were used to evaluate the relationship between IL-6 mRNA expression and the VEGF family. Results are presented as mean ± SD, with $p<0.05$ regarded as statistically significant. All experiments were conducted in triplicate in this study

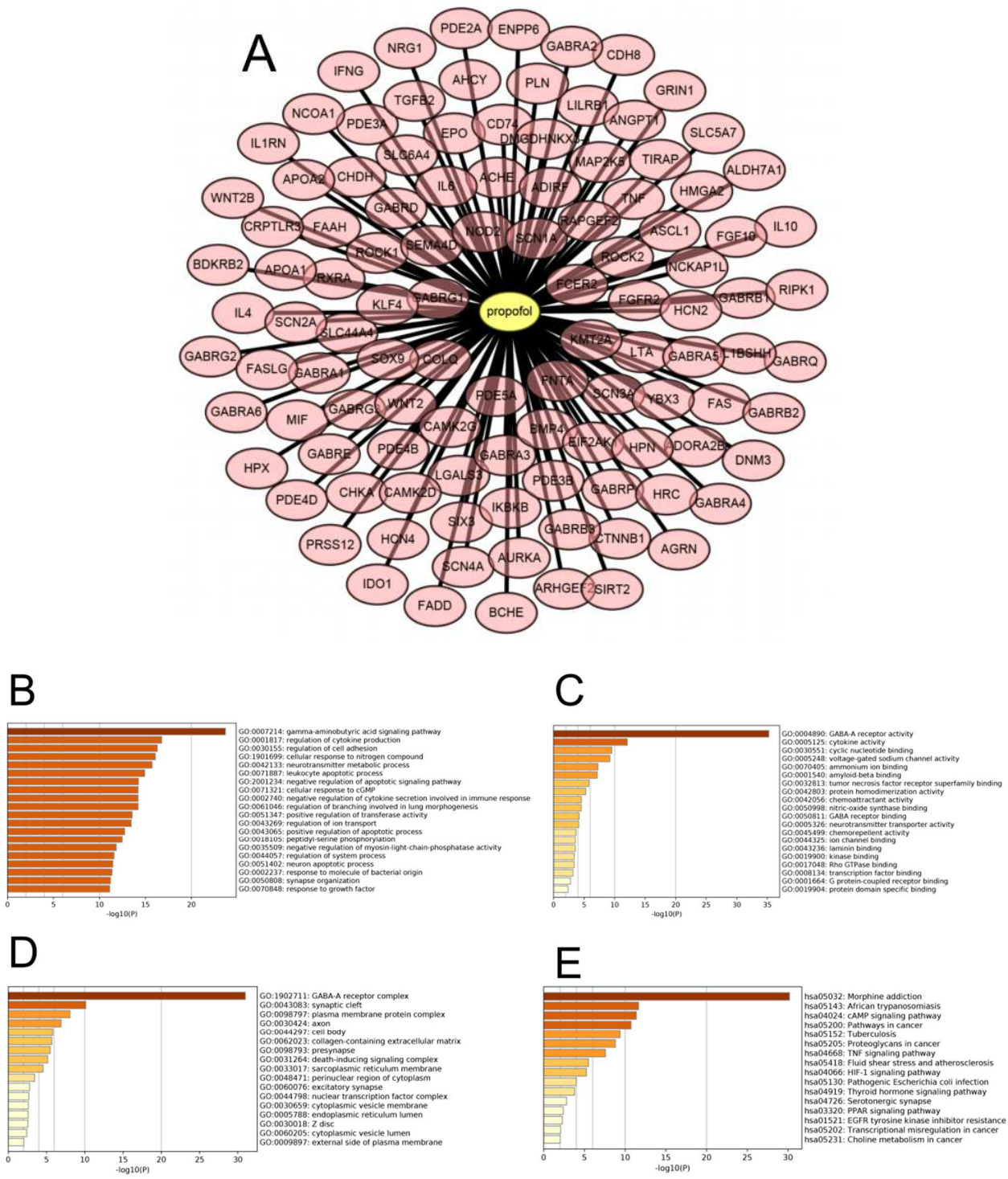
RESULTS

Prediction of targets based on the Batman-TCM platform and KEGG/GO enrichment analyses.

Utilizing the BATMAN-TCM database, we identified 109 potential targets for propofol, including GABRA2, GABRD, GABRB1, GABRG3, GABRE, SCN2A, GABRA3, GABRG1 and IL-6 (fig. 1A). Gene Ontology (GO) analysis highlighted significant enrichments in biological processes (BP) such as gamma-aminobutyric acid signaling pathway, cytokine production regulation, cell adhesion regulation, cellular response to nitrogen compounds and neurotransmitter metabolic processes (fig. 1B). Molecular function (MF) enrichments were observed in GABA-A receptor activity, cytokine activity, cyclic nucleotide binding, voltage-gated sodium channel activity and ammonium ion binding (fig. 1C). Changes in cell component (CC) were predominantly enriched in the GABA-A receptor complex, synaptic cleft, plasma membrane protein complex, axon and cell body **(**fig. 1D). Concurrently, KEGG pathway analysis indicated enrichment in pathways related to morphine addiction, African trypanosomiasis, cAMP signaling, cancer pathways and Tuberculosis (fig. 1E). The enriched GO terms and KEGG pathways provide a deeper understanding of the molecular mechanisms through which propofol may exert its therapeutic and non-therapeutic effects, paving the way for further experimental validation and exploration of its clinical applications.

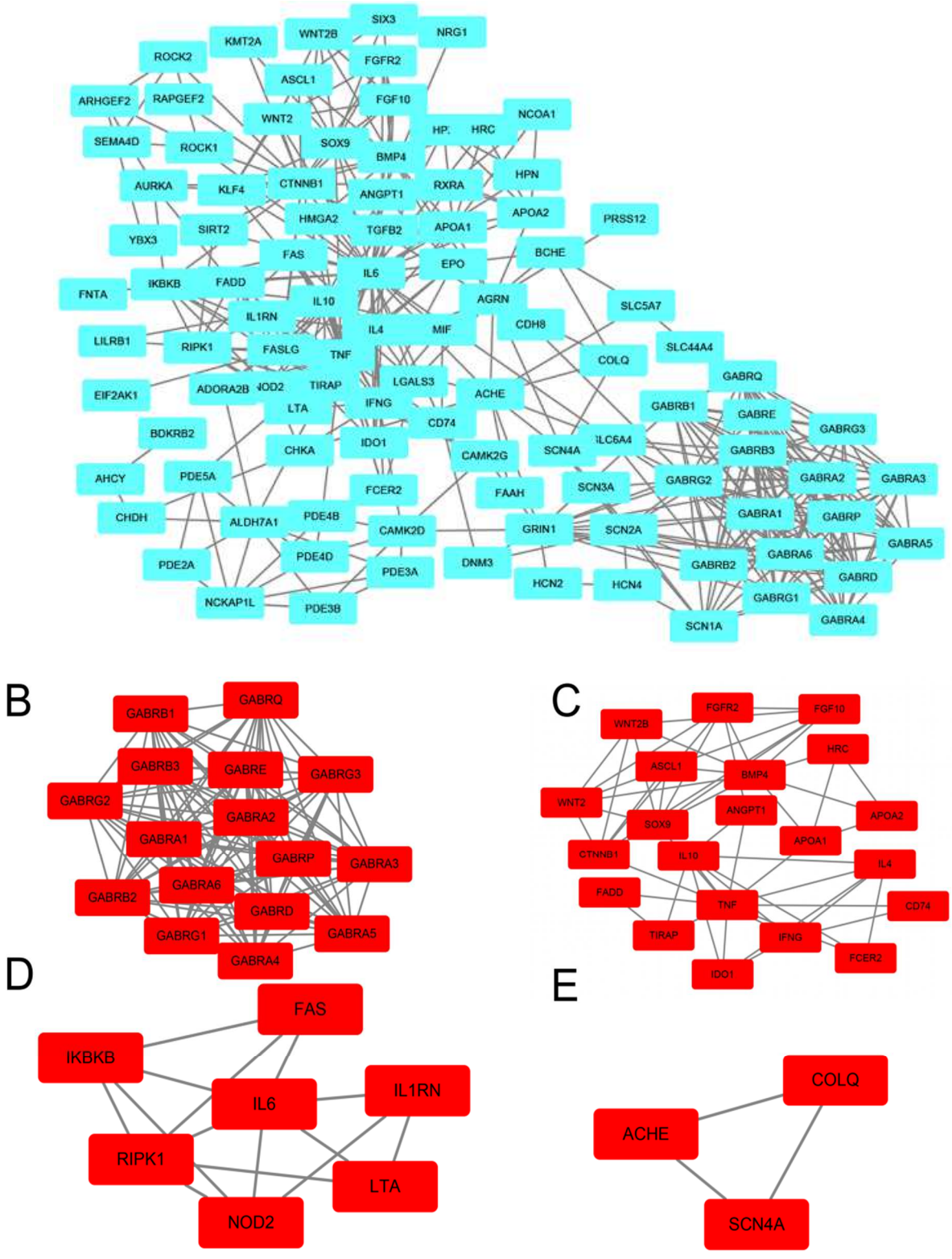
PPI network construction and module analysis

The PPI network illustrates the intricate relationships between predicted target genes of propofol (fig. 2A). The network shows densely connected regions, indicating key gene modules associated with propofol. These dense regions are crucial for understanding the complex interactions and potential mechanisms of action of propofol in the biological system.



(A) The network of propofol and its potential targets. The yellow circle represents propofol, and the pale red circles represent the predicted targets. (B) Gene Ontology (GO) analysis for biological processes (BP) indicates significant enrichments in pathways such as cytokine production regulation and neurotransmitter metabolic processes. (C) GO analysis for molecular functions (MF) reveals enrichments in GABA-A receptor activity and cytokine activity. (D)GO analysis for cellular components (CC) highlights enrichments in the GABA-A receptor complex and synaptic cleft. (E)KEGG pathway analysis demonstrates significant pathways related to morphine addiction and cancer pathways. ($p<0.01$)

Fig. 1: Potential Targets for Propofol and GO/KEGG Pathway Enrichment Analysis.



(A) The constructed PPI (protein-protein interaction) network of predicted target genes shows dense regions indicating key gene modules associated with propofol. (B, C, D, E) Significant gene modules identified using the MCODE algorithm in Cytoscape, showing the connectivity and significance of these gene modules.

Fig. 2: Key Gene Modules Related to Propofol in the PPI Network.

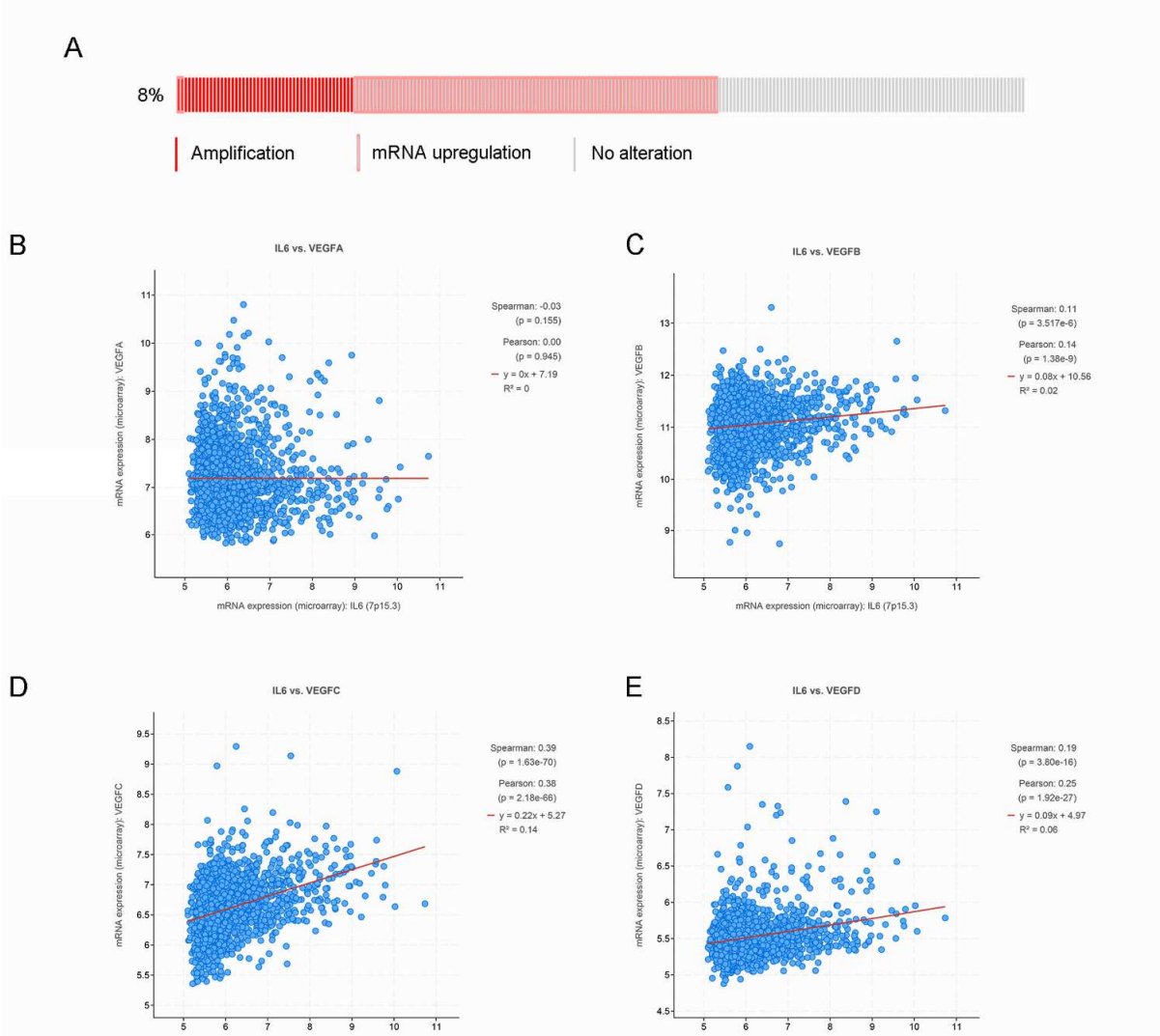


Fig. 3: The genomic alterations of the IL-6 gene and its co-expression relationship with the VEGF family in breast cancer patients. (A) The OncoPrint tab from the cBioPortal database shows genomic alterations in the IL-6 gene across breast cancer samples, with significant mRNA upregulation. Deep red bars represent gene amplifications, light red bars represent mRNA upregulation and grey squares indicate no alteration. (B, C, D, E) Co-expression analysis reveals significant correlations between IL-6 and VEGF family members, particularly VEGF-C, among breast cancer samples.

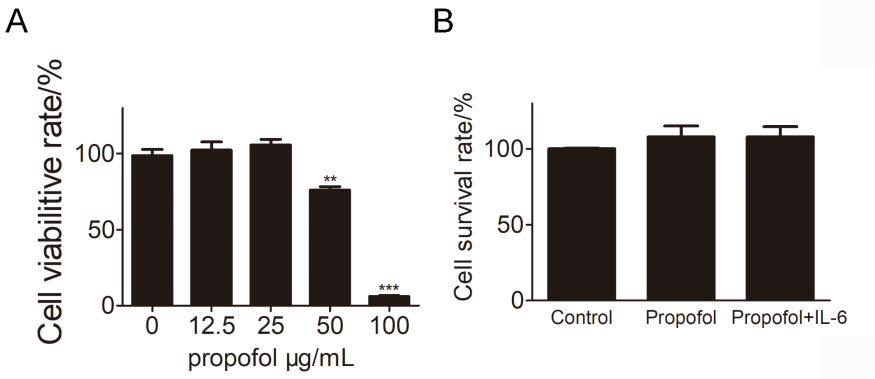


Fig. 4: The Survival rates of MCF-7 cells under different treatments were detected by CCK-8 assay.
(A) The cell viability of MCF-7 cells stimulated with 0–100 μg/mL propofol for 48 h. (B) The effect of propofol and rhIL-6 treatment on the survival rate of MCF-7 cells, showing the role of IL-6 in propofol. **p <0.01, ***p <0.001, *vs* 0 μg/mL propofol treatment group (as the control group); n=3.

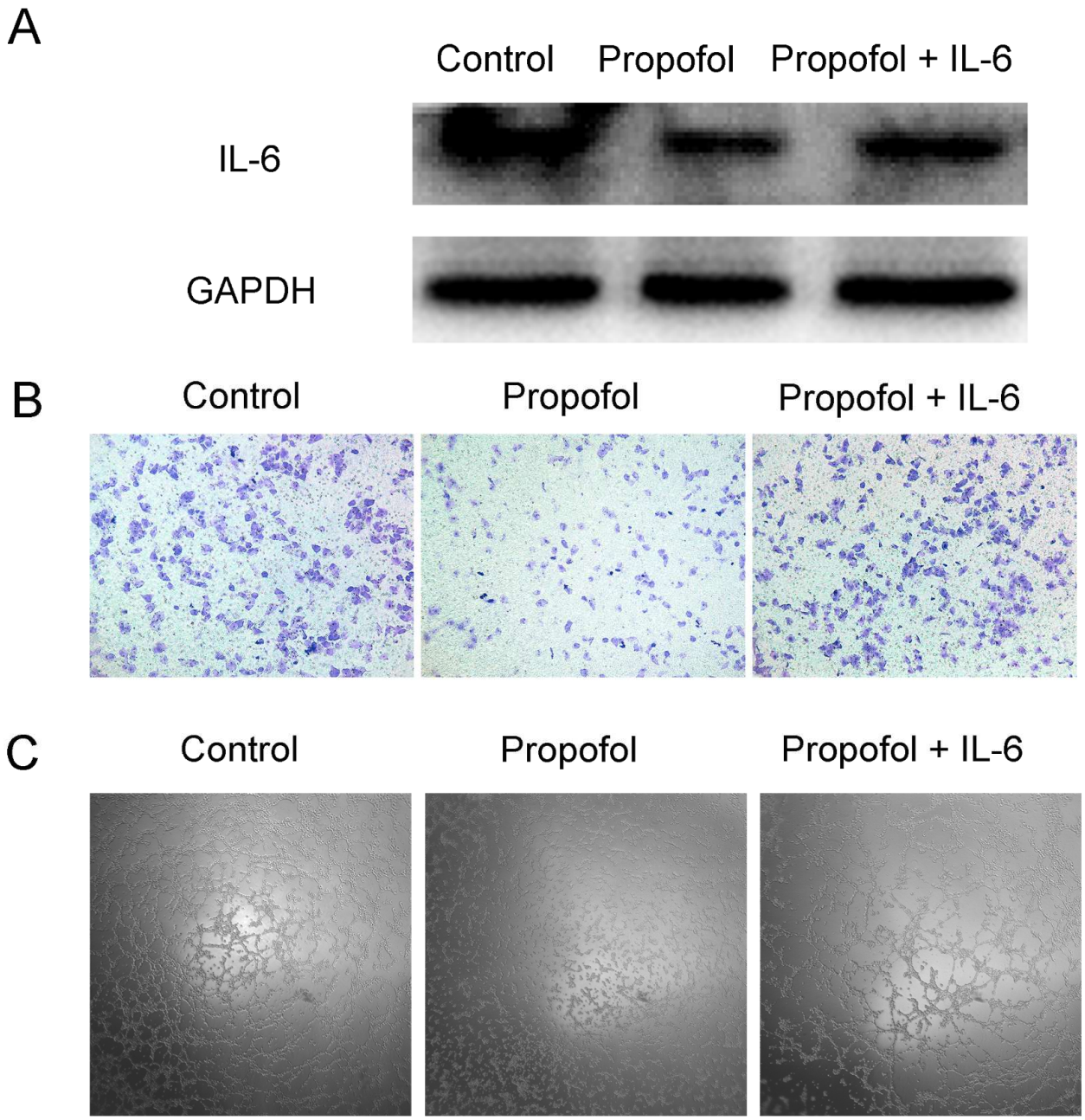


Fig. 5: Effects of propofol mediated tumor suppression via IL-6.
(A) Western blot assay confirmed that propofol and IL-6 cytokine treatment reversed the expression of IL-6 in the group which treated propofol alone. (B) Cell migration was examined using a Transwell chamber. Propofol inhibited cells from passing through the chamber while cotreated with the IL-6 cytokine partly abolished propofol-mediated inhibition (crystal violet staining, ×100, Scale bar = 50 μm). (C) The tube formation assay illustrated that the IL-6 cytokine rescued the suppressive effect of propofol-mediated capillary-like structure formation (×40, Scale bar = 100 μm).

Utilizing the MCODE algorithm in Cytoscape, we identified four significant gene modules within the PPI network, encompassing 21, 16, 7 and 3 genes, respectively (fig. 2B, 2C, 2D, 2E). These 47 genes represent key elements linked to propofol of mechanism of action. In fig. 2D, the PPI network analysis identifies IL-6 as a critical node within one of the significant gene modules.

Genomic alterations and co-expression analysis of il-6 in breast cancer samples

An analysis of 1,904 patient samples from the cBioPortal database revealed genomic alterations in the IL-6 gene across a subset of breast cancer samples, with alterations observed in 150 (8%) of the cases, predominantly manifesting as high mRNA expression (fig. 3A).

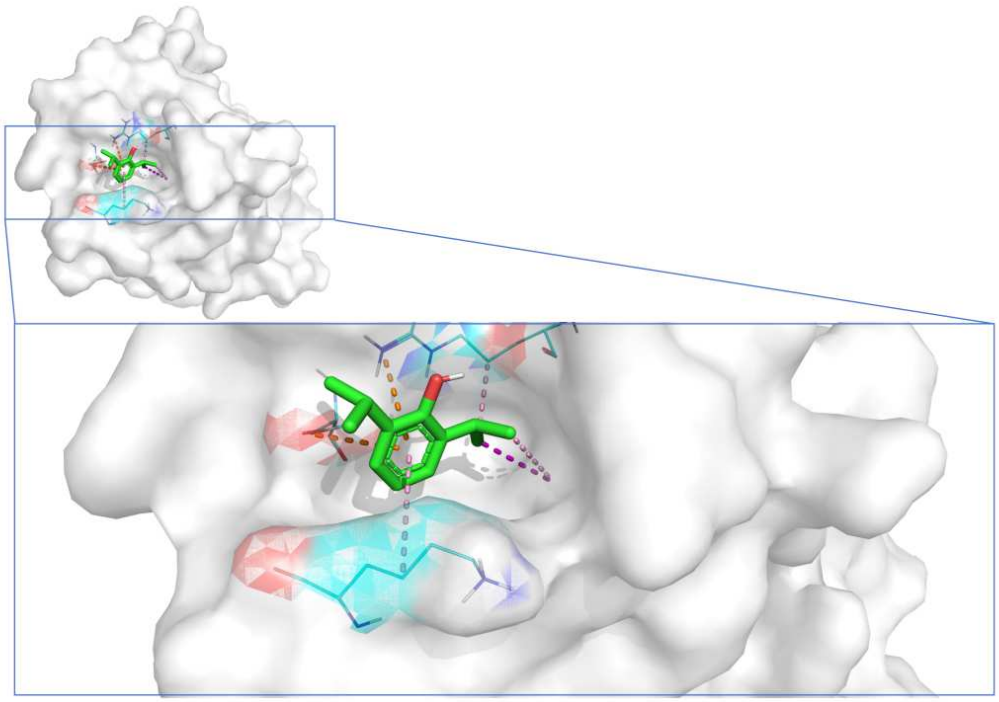


Fig. 6: The molecular docking simulation and binding dynamics of propofol to IL-6.
The 3D conformation shows propofol binding to the IL-6 receptor binding pocket, generating specific interaction forces with amino acid residues at the active site.

Our investigation suggested a potential association between IL-6 and the VEGF family (fig. 3B, 3C, 3D, 3E). Co-expression analysis revealed a significant correlation between IL-6 and VEGFC (Pearson correlation coefficient = 0.38, Spearman correlation coefficient = 0.39) among 1,904 breast cancer samples. No significant co-expression relationships were found between IL-6 and other members of the VEGF family (VEGFA, VEGFB, VEGFD).

Cytotoxicity of propofol on MCF-7 cells

Cell viability assays indicated no significant differences in survival rates between the 12.5 µg/mL and 25 µg/mL propofol treatment groups ($p=0.619$, $p= 0.280$). However, cell viability significantly decreased at 50 µg/mL ($p= 0.008$), with further reductions observed at 100 µg/mL ($p<0.001$) (fig. 4A). Based on these findings, a propofol concentration of 25 µg/mL was selected for subsequent experiments. No significant changes were observed in the viability of MCF-7 cells treated with propofol alone or in combination with IL-6 compared to the control group (fig. 4B). This suggests no direct correlation between propofol and IL-6 cytokine-induced biological changes in cell survival at the tested concentrations.

Propofol suppresses cell function by downregulating IL-6

IL-6, a key pro-inflammatory cytokine, is critical in tumor development. Our bioinformatics analysis identified IL-6 as a potential drug target for propofol, with increased expression levels found in breast cancer. We employed a western blot assay to analyze the effects of propofol on MCF-7 cells. Compared to the control cells, propofol significantly inhibited the protein expression of IL-6 ($p=0.0206$). However, co-treatment with IL-6 restored the protein expression levels ($p=0.0325$, fig. 5A). The results of the migration experiment (fig. 5B) showed that the number of migrated cells in the propofol group (62.20±9.107 cells, N=5) was strikingly lower than that in the control group (244.8±23.69 cells, N=5, $p<0.001$). Further investigation into the role of IL-6 in propofol-induced cell migration revealed that the number of migrated cells increased in the propofol + IL-6 group compared to the propofol group alone (193.8±10.77 cells, N=5, $p<0.001$). Additionally, propofol significantly suppressed the tube-forming capacity of MCF-7 cells (fig. 5C), with the propofol group showing fewer formations than the control group (68.33±9.171 vs. 173.0±9.713, N=3, $p=0.0014$). When MCF-7 cells were treated with both propofol and rhIL-6, the mass formation increased (117.0±13.50, N=3, $p=0.0407$) compared to the propofol group alone. These findings suggest that IL-6 is integral to propofol's ability to suppress tumor migration and angiogenesis in MCF-7 cells. Overall, the data supports the potential use of propofol to inhibit migration and angiogenesis in MCF-7 cells through IL-6 downregulation.

Molecular simulation of the binding of propofol to IL-6

As shown in fig. 6, the molecular docking of propofol with IL-6 revealed a three-dimensional conformation. The

molecular docking simulation highlights the specific binding dynamics of propofol to the IL-6 protein, demonstrating how propofol inserts into the IL-6 receptor binding pocket and interacts with several key amino acid residues. The results indicate that propofol inserts into the IL-6 receptor binding pocket, generating specific interaction forces with the amino acid residues at its active site. The active site residues include ARG-104, ASP-160, LYS-46, PHE-105, THR-163, GLN-156, GLU-106, THR-43 and SER-47. The benzene ring of propofol forms pi-cation or pi-anion interactions with the ARG-104 and ASP-160 residues of IL-6 and engages in alkyl or pi-alkyl interactions with LYS-46. Additionally, PHE-105 can form a pi-sigma interaction with the -CH_3 group on propofol. Propofol also forms van der Waals forces with THR-163, GLN-156, GLU-106, THR-43 and SER-47. This molecular docking simulation illustrates the binding dynamics of propofol to IL-6.

DISCUSSION

The incidence of breast cancer continues to rise year after year (Ahmad, 2019; Iacoviello *et al.*, 2020), establishing breast neoplasms as a significant health concern that impacts both the quality of life and psychological well-being of patients(Ataollahi *et al.*, 2015). Presently, surgery is considered the primary treatment for the majority of breast cancer patients (Maughan *et al.*, 2010). Research in onco-anaesthesiology indicates that perioperative anaesthetic might have an influence on long-term oncologic outcomes because of non-anesthetic effects (Rahmani *et al.*, 2023). Emerging research in onco-anesthesiology suggests that perioperative anesthetics may influence long-term oncologic outcomes due to their non-anesthetic effects. Propofol, a commonly used anesthetic in surgeries for decades, has recently garnered significant attention due to its potential impact on tumor metastasis during surgical procedures (Sun *et al.*, 2022). Its biological role in cellular functions makes it particularly interesting for further study.

Metastasis remains a critical and challenging step in tumor progression and is one of the most daunting issues in the treatment of breast cancer. Moreover, propofol, a widely utilized intravenous anesthetic agent, has captured significant interest in exploring the relationship between anesthetic practices and disease outcomes (Meng *et al.*, 2017; Liu *et al.*, 2020). Surgical tissue trauma is known to induce an inflammatory response, leading to elevated cytokine levels (Franzén *et al.*, 2023). IL-6, a pleiotropic cytokine, plays a pivotal role as one of the most significant mediators in the systemic response to surgery. Research has demonstrated that propofol reduces the expression of inflammatory mediators, including IL-1β, IL-6 and TNF-α(Zhou *et al.*, 2021). There is extensive evidence indicating that propofol administration not only attenuated reperfusion-induced kidney damage but also reduced the expression of renal TNF-α, IL-6 and CXCL-10. (Liu *et al.*, 2021). In oncological surgery, current findings suggest that propofol is linked to reduced metastasis and lower postoperative IL-6 levels compared to inhaled anesthetics (Li *et al.*, 2023).

The identification of protein or gene targets is essential for elucidating the molecular mechanisms underlying the direct influence of propofol on tumor cells, especially concerning the metastasis of breast cancer. Bioinformatics technology allows researchers to explore genetic alterations in diseases, proving to be a highly effective approach for identifying new biomarkers in tumor diseases. Our research employed bioinformatics methods to predict potential targets of propofol and validated these predictions through *in vitro* cell experiments.

Initially, our study pinpointed potential target genes of propofol in the BAT-MAN database. GO and KEGG enrichment analyses demonstrated that these targets were enriched in the regulation of cytokine production, cytokine activity and cancer pathways, potentially impacting tumor cell regulation. Notably, the IL-6 cytokine, which plays diverse roles in inflammation and oncogenesis, was identified as one of the most crucial gene modules for propofol.(Kishimoto, 2010). The PPI network showed that IL-6 directly interacts with FAS, IKBKB, IL1RN, RIPK1, NOD2 and LTA, emphasizing the key role of IL-6.

Further analysis of 1904 samples from the TCGA database revealed that 8% of the IL-6 gene exhibited changes, with the most significant being the upregulation of mRNA expression. Consequently, the role of propofol via IL-6 in breast cancer requires additional investigation. The study of cancer microenvironments, including anesthetic influences, is gaining traction as it may help elucidate novel anticancer mechanisms.

Previous research, such as that by NANAKO ANDO *et al*., found that reducing IL-6 and VEGF can suppress angiogenesis in colon cancer(Ando *et al.*, 2019). IL-6 has been demonstrated to regulate VEGF levels in various tissues (Borg *et al.*, 2005), capable of inducing VEGF secretion from breast cancer cells(Tawara *et al.*, 2019). Therefore, a drug that decreases IL-6 expression might also reduce VEGF levels in breast cancer. VEGF-C, a member of the VEGF family, has been shown to guide angiogenesis. (Jain, 2003; Liu *et al.*, 2020). Our study indicated that the mRNA level of VEGF-C was significantly associated with IL-6 in breast cancer, as analyzed using cBioPortal.

Considering its potential role in oncologic outcomes, our findings revealed that propofol inhibited biological functions by decreasing the expression of IL-6 in MCF-7 cells. We then verified the function of propofol in MCF-7 cell proliferation. The CCK8 assay showed that high concentrations of propofol strongly inhibited the viability of MCF-7 cells. For these experiments, a concentration of 25 μg/mL was selected. To determine the mechanisms of

propofol-mediated tumor suppression, a Western blot assay was performed, showing a significant decrease in IL-6 expression in the propofol group compared to control cells. However, this trend was reversed in the propofol+IL-6 group, highlighting the regulatory effects of propofol on IL-6 in MCF-7 cells. Tumor cell migration and angiogenesis are crucial pathological links in tumor progression, where migration provides a new growth environment for tumor cells and neovascularization supplies the necessary oxygen and nutrients for tumor cell proliferation. Further cell experiments revealed that propofol inhibited the migration and formation of new vascular meshes in MCF-7 cells. Compared with the group treated with propofol only, the inhibitory effect of propofol on MCF-7 cells was reversed after treatment with both propofol and recombinant human IL-6, indicating the potential involvement of propofol in regulating cell migration and neoangiogenesis via IL-6.

Additionally, virtual screening is an important tool for drug discovery in disease contexts. (Shan *et al.*, 2020). Molecular docking was performed to simulate the binding of propofol to IL-6, primarily interacting with the DNA-binding domain of the IL-6 protein, including key amino acid residues such as ARG-104, ASP-160 and LYS-46. This predicted interaction of propofol with IL-6 provides further insight into the mechanisms of action of propofol in MCF-7 cells. Research has demonstrated that propofol reduces the expression of inflammatory mediators, including IL-1β, IL-6 and TNF-α (Zhou *et al.*, 2021). Furthermore, substantial evidence suggests that administering propofol mitigates renal injury induced by reperfusion and decreases the levels of TNF-α, IL-6 and CXCL-10(Liu *et al.*, 2021). Animal experiments have also highlighted the anti-tumor effects of propofol, particularly in decreasing IL-6 levels, as demonstrated in a rat model of cisplatin-induced neuropathy, where the propofol-treated group exhibited significantly lower IL-6 levels(Gonullu *et al.*, 2023).

Propofol is widely used for anesthesia and sedation, demonstrating excellent safety and tolerability in clinical practice (Miller *et al.*, 2019). This study indicates that propofol significantly inhibits breast cancer cell activity, providing experimental evidence for its prioritized use in perioperative sedation for breast cancer patients. Furthermore, for patients with advanced breast cancer who also suffer from sleep disturbances (Soltanipur *et al.*, 2024), propofol is recommended under the supervision of specialists to improve sleep quality and enhance overall quality of life(McClintock *et al.*, 2024). However, the potential long-term effects of propofol on cancer progression, particularly its anesthetic properties, warrant further investigation. Clinical trials should focus on the long-term efficacy and safety of propofol, including its potential immunosuppressive effects and influence on the tumor microenvironment. These findings underscore the need for robust experimental and clinical data to support the broader application of propofol in oncology. Future research should aim to provide additional clarification, such as determining the precise effects of propofol on breast cancer in animal models or clinical settings, which is crucial for advancing our understanding and treatment of this disease.

CONCLUSION

This study was designed to ascertain whether propofol inhibits cell migration and tubule formation via IL-6 in MCF-7 cells. The more detailed mechanisms by which propofol influences IL-6 expression still require further exploration.

Funding

The research was supported by the Innovation Fund for Yongchuan Hospital of Chongqing Medical University (No.YJSCX201902); Hospital Fund of Yongchuan Hospital of Chongqing Medical University (No. YJJL2024023).

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to Professor Juan Liao for her technical assistance in the Central Laboratory at Yongchuan Hospital of Chongqing Medical University. We would like to express my special thanks to my husband, Mr. Pan Wenqiang, for his valuable statistical advice, which greatly contributed to the successful completion of this paper.

Conflict of interest

Authors will include statement about no conflict of interest.

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