IDENTIFICATION OF SB216763 AS A PROMISING TARGETED THERAPY FOR POOR-PROGNOSIS CHOLANGIOCARCINOMA PATIENTS

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ABSTRACT
Cholangiocarcinoma (CCA) is an aggressive malignancy arising from the bile ducts. Its incidence is the highest and represents a major public health burden in Thailand. CCA patients generally exhibit poor prognosis due to late diagnosis and the lack of effective therapeutic options. Our previous bioinformatic analysis stratified CCA patients into two groups with distinct survival outcomes and classified CCA cell lines into two groups representing the distinct patient groups. To identify a promising therapeutic strategy for CCA patients, particularly those with poor prognosis, we integrated bioinformatic and experimental approaches. In this study, SB216763, a GSK3β inhibitor, was identified as a promising targeted therapeutic candidate for CCA patients with poorer prognosis. RT-qPCR and Western blot analyses revealed that GSK3β was upregulated in RMCCA-1 cells, representing CCA with poor-prognosis group, compared to CCLP-1 cells, representing CCA with better-prognosis group. SB216763 treatment activated β-catenin, confirming GSK3β inhibition. MTT assays demonstrated that RMCCA-1 cells were more sensitive to SB216763 treatment than CCLP-1 cells. Microscopic observations also supported this differential response. Collectively, our study highlights GSK3β inhibition by SB216763 as a potential precision medicine strategy for CCA patients with poor prognosis, underscoring the importance of patient group stratification in guiding personalized therapeutic approaches.
Keywords: Cholangiocarcinoma, SB216763, GSK3β

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INTRODUCTION

Cholangiocarcinoma (CCA) is a cancer that arises from the epithelial cells of bile ducts. In the early stages, CCA patients show no symptoms. Diagnosis often occurs at an advanced stage when the tumor has progressed and metastasized. Consequently, CCA is associated with poor prognosis and high mortality rates (Banales et al., 2020). Surgical resection remains the most effective and potentially curative treatment option for CCA. However, most patients are diagnosed at late state, surgery alone is often insufficient. Therefore, chemotherapy and radiotherapy are commonly used as adjuvant or palliative treatments. Nevertheless, treatment outcomes remain unsatisfactory, as these therapies destroy both normal and cancerous cells, resulting in limited therapeutic benefit and significant side effects (Elvevi et al., 2022; Strijker et al., 2019).

Additionally, the complexity of CCA among patients makes it difficult to achieve universally effective outcomes. Although several targeted therapies have been developed in recent years, their clinical efficacy remains limited due to the absence of well-defined and specific targets in CCA (Elvevi et al., 2022). Therefore, developing targeted and personalized treatment strategies that can improve therapeutic responses, particularly for patients with poor prognosis. Given the heterogeneous nature of CCA, molecular stratification of patients into distinct groups based on gene expression profiles has emerged as a promising approach to enable precision medicine. Our previous study used transcriptomic data from public databases to develop a gene signature for stratifying CCA patients into two groups: a low-risk (better-prognosis) group and a high-risk (poorer-prognosis) group (Sae-Fung et al., 2022). Moreover, we applied the same gene signature to classify CCA cell lines into two representative groups corresponding to these patient categories for further investigation. This classification provides a valuable platform for investigating potential therapeutic agents to each group of CCA patients.

To improve treatment outcomes for patients with poor prognosis, the identification of small molecules that specifically target vulnerabilities in high-risk CCA is essential. Through bioinformatic analysis of drug response and gene expression profiles, we identified SB216763, a GSK3β inhibitor, as a promising candidate compound with potential selective efficacy in the poor-prognosis CCA group. This study aims to validate the role of GSK3β inhibition by SB216763 as a precision therapeutic strategy for CCA patients with poor survival outcomes.

LITERATURE REVIEWS

Cholangiocarcinoma and current treatment

Cholangiocarcinoma (CCA) is a highly heterogeneous group of cancers and is the second most common type of primary liver cancer, accounting for approximately 15% of all liver tumors and 3% of all gastrointestinal cancers (Banales et al., 2016). In Thailand, the highest incidence of CCA is found in the northeastern region, with approximately 85 cases per 100,000 people. The main case is liver fluke infection as known *Opisthorchis viverrine* (Banales et al., 2020; Strijker et al., 2019). In the early stages of the disease is usually asymptomatic, leading to diagnoses often occurring at an advanced stage when symptoms such as chronic fatigue, abdominal pain, loss of appetite, anorexia and nausea (Banales et al., 2020; Elvevi et al., 2022). The standard treatment for CCA is surgery, involving the removal of both hepatic and biliary structures to eliminate the tumor while preserving the function of the remaining liver tissue. Surgery is considered highly effective; however, the lack of early diagnosis reduces the effectiveness of treatment. Non-surgical treatments for CCA include chemotherapy, radiotherapy and targeted therapy. For chemotherapy, the first-line drugs are gemcitabine combined with cisplatin. Although chemotherapy and radiotherapy have been employed, their efficacy remains limited due to the heterogeneity and variability of CCA patient; these approaches less universally effective and damage to both cancerous and normal cells. Targeted therapy depends on specific targets and the drugs used (Elvevi et al., 2022). Nevertheless,

developing effective targeted therapy is crucial because of the diverse nature of CCA and the lack of specific therapeutic targets.

Glycogen synthase kinase 3 beta (GSK3β)

Glycogen synthase kinase 3 beta (GSK3β) is a serine/threonine protein kinase. One of the main mechanisms of GSK3β is the regulation of the WNT/β-catenin pathway, in which GSK3β phosphorylates β-catenin, leading to its degradation via the proteasomal system, thereby inhibiting the transcription of genes involved in cell proliferation (Fu et al., 2011). However, the role of GSK3β in cancer can act as either a tumor suppressor or tumor promoter, depending on the type of cancer (Duda et al., 2020). Moreover, the role of GSK3β in CCA remains unclear. From the literature review, the conceptual framework can be drawn as shown in Figure 1.

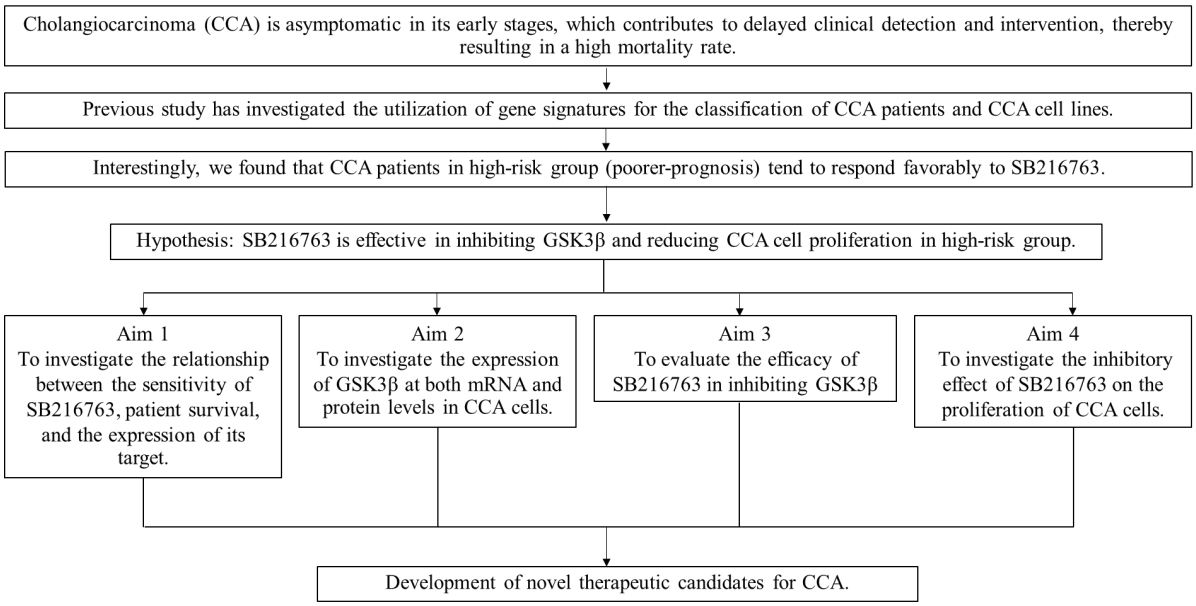


Figure 1 Conceptual Framework

RESEARCH METHODOLOGY

This study employed an integrative approach, beginning with bioinformatic analysis followed by *in vitro* validation, to identify potential therapeutic candidates for cholangiocarcinoma (CCA). Bioinformatic analysis was performed using the Gene Expression Omnibus (GEO) database, specifically dataset GSE89749, which includes data from 111 CCA patients with an overall survival of more than 30 days. Drug sensitivity among CCA patient groups was analyzed using the pRRophetic algorithm to estimate the half-maximal inhibitory concentration (IC_{50}) for each patient. Kaplan-Meier survival curves and the log-rank test were applied to evaluate survival differences between CCA patient groups. Moreover, we utilized data from The Cancer Genome Atlas (TCGA) via the GEPIA platform to investigate *GSK3B* expression in normal and CCA tissues. Subsequently, *in vitro* experiments were performed using two CCA cell lines, CCLP-1 and RMCCA-1, which have previously been established as representative models of distinct CCA patient subgroups. The expression of GSK3β was examined at the mRNA level by RT-qPCR and at the protein level by Western blot. The inhibition of GSK3β following treatment with SB216763 was evaluated by detecting β-catenin levels through Western blot analysis. To assess the effect of SB216763 treatment, cell viability was measured using the MTT assay at 24, 48 hours. SB216763 sensitivity between CCA cell lines was visualized and analyzed using "IC50 Calculator" (https://www.aatbio.com/tools/ic50-calculator). All experimental results are presented as mean ± SD from at least independent experiments. Statistical significance between two groups was considered at p-value < 0.05 (*), < 0.01 (**) and < 0.001 (***)

RESEARCH RESULTS

SB216763 is promising drug for poor prognosis CCA patients

Our previous study demonstrated an association between poor survival outcomes and the high-risk group of CCA patients identified from the GSE89749 dataset (Sae-Fung et al., 2022). In the present study, we used the GSE89749 dataset to estimate the half-maximal inhibitory concentration (IC_{50}) of various drugs using the R "pRRophetic" package in order to predict drug sensitivity between the low-risk and high-risk groups and to identify potential effective compounds for the high-risk group. Among the tested compounds, SB216763, a GSK3β inhibitor, showed a significantly lower estimated IC_{50} in the high-risk group compared with the low-risk group, indicating more sensitivity in high-risk patients (Fig. 2A). Furthermore, when CCA patients were classified based on their predicted sensitivity to SB216763, those who were more sensitive to SB216763 were found to have shorter overall survival (Fig. 2B). This finding suggests that SB216763 may serve as an effective therapeutic agent for CCA patients with poor prognosis. To further support this observation, we analyzed the expression of GSK3β, the target of SB216763 in CCA patients, using data from The Cancer Genome Atlas (TCGA) via the GEPIA platform. GSK3β expression was significantly upregulated in CCA tissues compared to normal bile duct tissues (Fig. 2C). These results suggest that targeting GSK3β with SB216763 may represent a promising therapeutic approach for CCA patients, particularly the poor-prognosis CCA patient group.

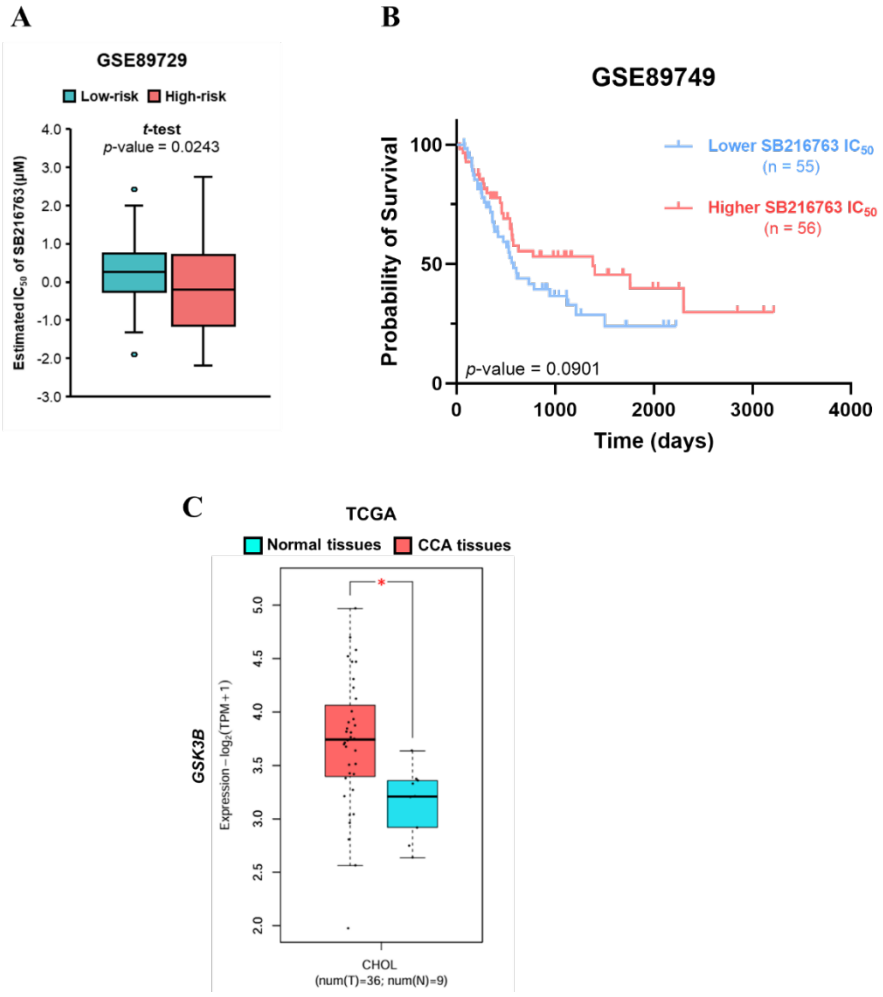


Figure 2 Bioinformatic analysis of CCA patients. (A) Estimated IC_{50} of SB216763 between CCA patients with low and high-risk group. (B) Kaplan-Meier overall survival curves in CCA patients with low and high sensitivity to SB216763. (C) Comparison of GSK3B expression in CCA and normal bile duct tissues.

GSK3β expression in CCA cell lines

According to previous research, CCA cell lines have been classified into two groups based on gene signature: a low-risk group was represented better-prognosis patients and a high-risk group was represented poorer-prognosis patients (Sae-Fung et al., 2022). Furthermore, bioinformatics analysis showed that high-risk group was likely to SB216763 susceptible and CCA tissues were high GSK3β expression. To investigate GSK3β expression using CCA cell lines, this study employed two representative cell lines that are CCLP-1 as a model for low-risk group, and RMCCA-1 as a model for high-risk group. The results demonstrated that RMCCA-1 exhibited significantly higher GSK3β expression compared to CCLP-1, both at the mRNA and protein levels (Fig. 3A-B).

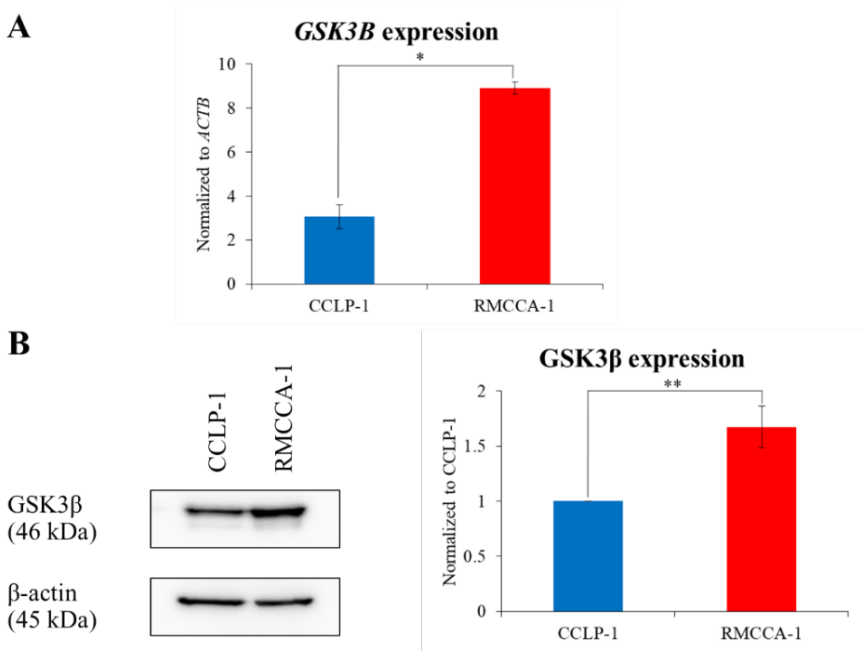


Figure 3 GSK3β expression in two CCA cell lines, CCLP-1 and RMCCA-1. (A) mRNA level. (B) protein level.

Validation of the inhibitory effect of SB216763 on GSK3β in CCA cell lines

SB216763 functions as both a GSK3β inhibitor and an activator of WNT/β-catenin signaling pathway, it is known that under normal conditions, GSK3β phosphorylates β-catenin and promotes β-catenin degradation. Inhibition of GSK3β results in the accumulation of β-catenin within the cell. To determine whether SB216763 can effectively inhibit GSK3β activity, CCA cells were treated with SB216763 and examined protein expression of β-catenin. The results showed an increase β-catenin levels following treatment with SB216763 in CCLP-1 and RMCCA-1 (Fig. 4A-B). These findings indicate that SB216763 effectively inhibits GSK3β function in CCA cells.

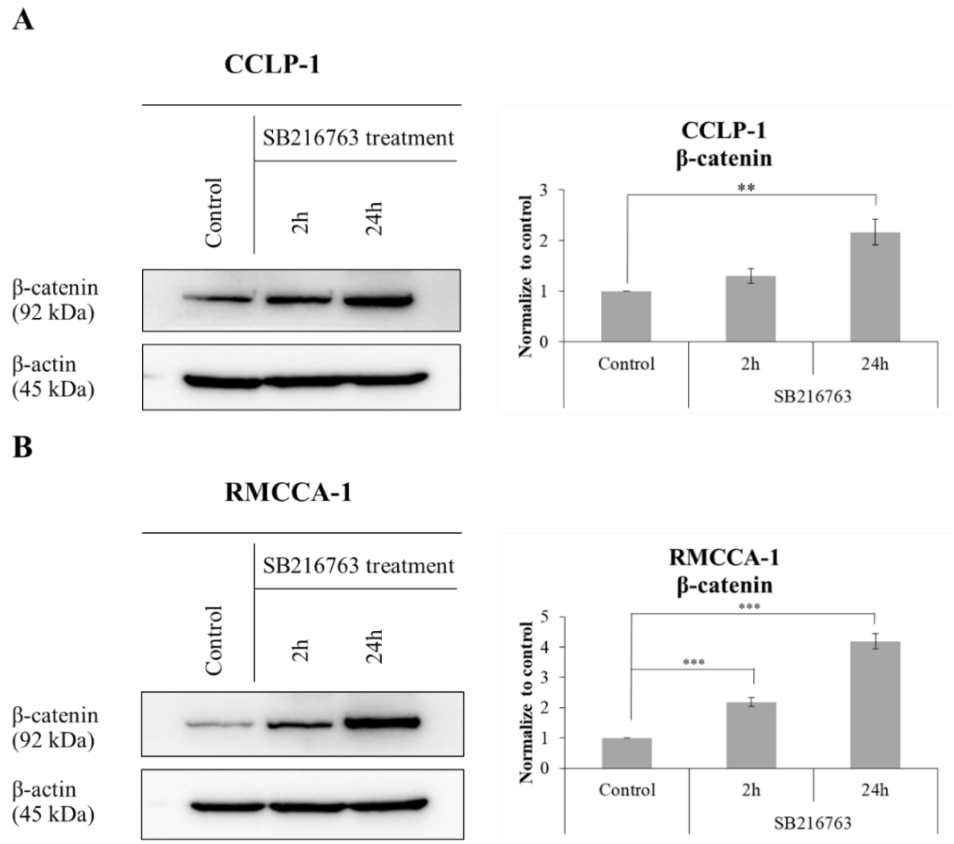
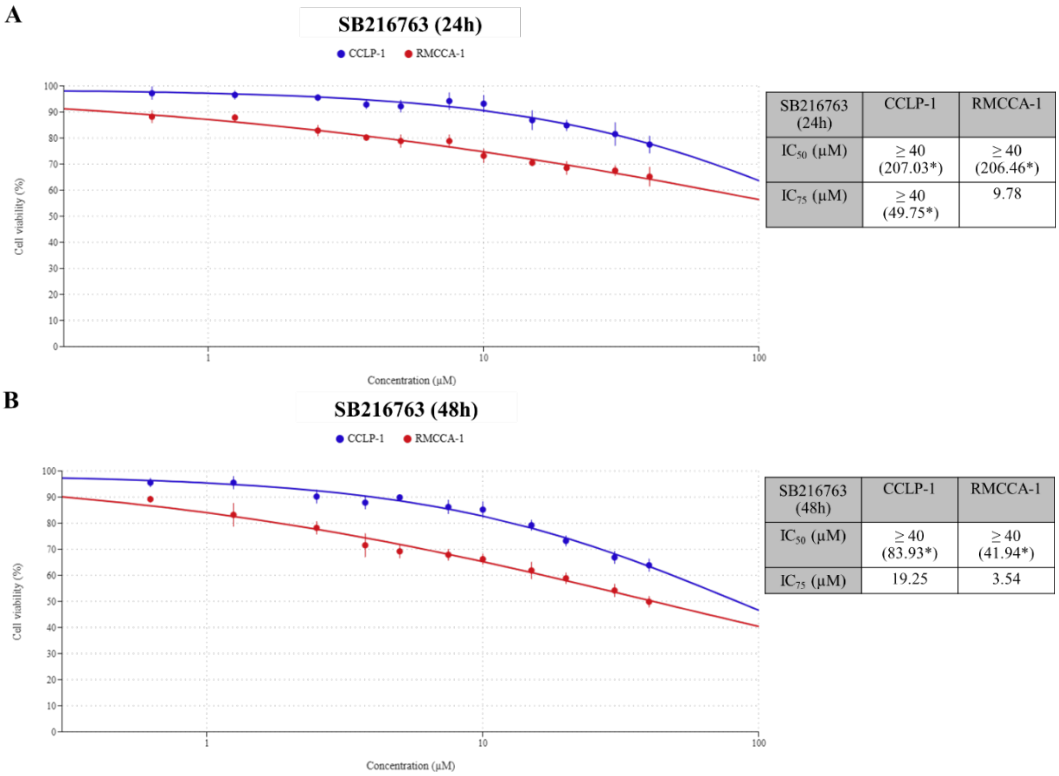


Figure 4 The inhibitory effect of SB216763 on GSKβ in CCA cell lines. (A) CCLP-1 and (B) RMCCA-1.

Evaluation of the effect of SB216763 on cell viability in CCA cell lines

Bioinformatic analyses in this study indicated that high-risk group patients, who exhibit poorer-prognosis, tend to respond better to SB216763 compared to low-risk group patients. To investigate the cellular response to SB216763, CCA cell lines were treated with SB216763 for 24, 48 hours and cell viability was assessed using MTT assay. The results showed that RMCCA-1, representing patients in a high-risk group, exhibited greater sensitivity to SB216763 than CCLP-1, as indicated by the IC_{75} values (Fig. 5A-B). This observation suggests that even at higher concentrations, two groups of CCA cell lines did not reach the level of response corresponding to the IC_{50} concentration. Nevertheless, when the estimated IC_{50} values were calculated, the results were consistent, supporting that RMCCA-1 is more responsive to SB216763 than CCLP-1. Furthermore, SB216763 treatment inhibited cell proliferation in RMCCA-1 at lower concentrations than in CCLP-1 (Fig. 6). These findings suggest that RMCCA-1, a high-risk group cell line, responded more to SB216763, and SB216763 suppressed cell proliferation.



SB216763 (24h)	CCLP-1	RMCCA-1
IC_{50} (μM)	≥ 40 (207.03*)	≥ 40 (206.46*)
IC_{75} (μM)	≥ 40 (49.75*)	9.78

SB216763 (48h)	CCLP-1	RMCCA-1
IC_{50} (μM)	≥ 40 (83.93*)	≥ 40 (41.94*)
IC_{75} (μM)	19.25	3.54

Figure 5 Dose response curve of CCA cell lines treated with SB216763 for (A) 24 hours, (B) 48 hours.

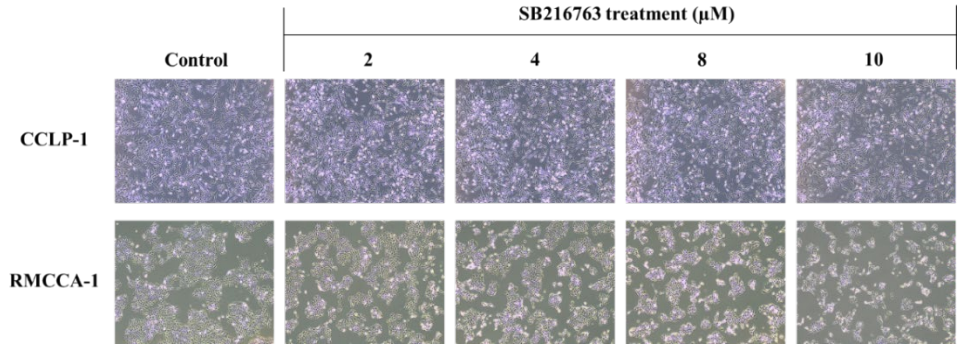


Figure 6 Treatment of CCA cell lines with SB216763 reduces cell proliferation.

DISCUSSION & CONCLUSION

This study provides new insights into the potential of GSK3β inhibition as a targeted therapeutic strategy for cholangiocarcinoma (CCA), particularly for patients with poor prognosis. Using an integrated bioinformatic and experimental approach, we identified SB216763 as a compound with selective efficacy in high-risk CCA, highlighting the importance of patient stratification in the development of precision medicine.

GSK3β has been implicated in multiple oncogenic pathways, including WNT/β-catenin signaling, cell survival, and drug resistance in various cancer types (Duda et al., 2020). For instance, in ovarian cancer, GSKβ expression has been shown to promote cell proliferation, and treatment with GSKβ inhibitor can suppress tumor growth both *in vitro* and *in vivo* (Cao et al., 2006). Similarly, in hepatocellular carcinoma, high GSKβ expression has been associated with poor prognosis, and its inhibition led to reduce cell proliferation and invasion (Wang et al., 2024). In contrast, studies in skin cancer have shown that downregulation of GSKβ can induce malignant transformation of normal skin cells (Ma et al., 2007). These findings indicate that GSKβ may act either as a tumor promoter or a tumor suppressor, depending on the cancer

context. In this study, its elevated expression in CCA tissues suggests a potential role in maintaining tumor aggressiveness. Inhibition of GSK3β by SB216763 led to β-catenin accumulation, confirming pathway modulation and validating its mechanism of action in CCA cells. Moreover, SB216763 exhibited preferential cytotoxicity in RMCCA-1 cells, which have higher GSK3β expression and represent the poor-prognosis group, suggesting that tumors dependent on GSK3β signaling may be particularly vulnerable to its inhibition.

These findings underscore the potential of targeting GSK3β-driven signaling as a therapeutic vulnerability in aggressive CCA. Importantly, integrating transcriptomic data to guide drug selection provides a rational framework for identifying promising compounds to defined patient subgroups. This precision approach may help overcome the limitations of conventional therapies that fail to account for CCA heterogeneity.

In conclusion, SB216763 emerges as a promising precision therapeutic candidate for poor-prognosis CCA. Future studies should validate its antitumor efficacy *in vivo* and explore its combination with standard or immune-based therapies to enhance treatment outcomes in this challenging malignancy.

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Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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