SMIM22 Regulates Oxaliplatin Resistance in Colorectal Cancer by Promoting mTOR pathway

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Colorectal cancer (CRC) is a leading cause of cancer-related morbidity and mortality worldwide. One of the main challenges in treating CRC is the development of resistance to chemotherapy. This study identifies SMIM22 as a promising therapeutic target to overcome CRC resistance. Chemoresistance in CRC patients remains a significant hurdle, leading to therapeutic failures. This underscores the need for novel targets that can potentially improve the outcomes of CRC patients undergoing chemotherapy. Upon examining global transcriptomic changes, it was observed that SMIM22 levels were most prominently elevated in oxaliplatin resistance (oxaR) cells following oxaliplatin treatment compared to parental cells. Additionally, CRC patient samples also demonstrated increased levels of SMIM22 expression. Given SMIM22's characteristics as an integral membrane protein, we hypothesized its potential association with the pivotal mTOR pathway in CRC. Consequently, the characterization of the SMIM22-mediated mTOR pathway became our primary objective. Our results revealed that upregulation of SMIM22 activates the mTOR pathway, inducing a drug resistance response. Importantly, when cells resistant to oxaliplatin were co-treated with rapamycin, an inhibitor of the mTOR pathway, we noticed a substantial increase in their sensitivity to the drug. Our findings suggest that targeting the SMIM22-mediated mTOR pathway can serve as an effective therapeutic strategy to enhance drug sensitivity in CRC patients. This study provides a foundation for future research aimed at leveraging SMIM22 as a potent therapeutic target in CRC chemotherapy regimens.

INTRODUCTION

Cancer continues to be a global health challenge, consistently showcasing high mortality rates[1]. Among various cancers, Colorectal cancer (CRC) stands out as a leading cause of cancer-related deaths[2]. Statistically, CRC occupies the third position in terms of global prevalence and ranks fourth in mortality. While surgical interventions and radiation therapy serve as valuable treatment options for CRC patients, chemotherapy remains the important treatment modality[3]. A combination of surgical resection and chemotherapy is the most common treatment regimen for CRC patients[4]. However, like other cancer cells, CRC has a propensity to develop chemoresistance, leading to treatments ineffective. This resistance, especially in metastatic cancer patients, has been recognized as a significant impediment to successful treatment outcomes. To enhance the therapeutic results for CRC patients, it becomes important to identify the fundamental mechanisms driving drug resistance and propose effective therapeutic targets[5]. Platinum-based anticancer agents have played a significant role in the treatment of various types of solid tumors. Platinum-based compounds are widely used in cancer therapy and are recognized for their efficacy and safety across various cancer types[6]. Specifically, Oxaliplatin, a third-generation platinum compound, is primarily used to treat colorectal cancer and offers several distinct advantages. Due to its unique mechanism of action, oxaliplatin can overcome resistance to traditional platinum-based drugs such as cisplatin and carboplatin. It is generally associated with fewer side effects, including relatively low nephrotoxicity and reduced severity of nausea and vomiting. Additionally, oxaliplatin is versatile, meaning it can be used effectively both as monotherapy and in various combination therapies[7]. It inhibits the synthesis of DNA, RNA, and proteins within cells. While efforts have been made to understand the process through which resistance to oxaliplatin is acquired, the complex interplay of genes and pathways involved ensures that there remain unaddressed challenges in understanding oxaliplatin resistance[8].

Transcriptome analysis refers to the comprehensive examination of the complete set of RNA transcripts produced by the genome under specific conditions or in specific cell types. This analysis provides insights into gene expression patterns, revealing which genes are upregulated or downregulated in response to various stimuli, such as drug treatment or tumor microenvironments. Specifically, we performed RNA-seq and it allows for the detection of differential gene expression at an unprecedented resolution, enabling researchers to identify subtle changes in gene activity. Unlike targeted approaches, transcriptome analysis captures the entire gene expression landscape, providing a holistic view of the biological processes at play. This approach can uncover previously

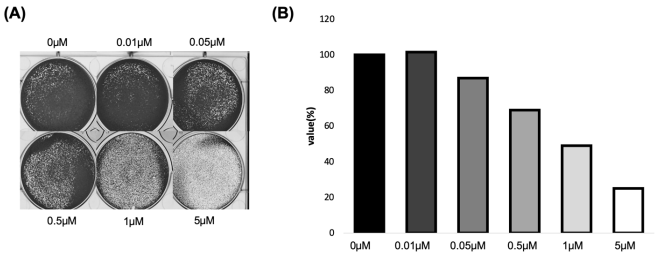


Figure1. Determination of concentration of oxaliplatin treatment for HCT116 cells through clonogenic assay

uncharacterized genes and isoforms that may play critical roles in specific conditions, including cancer progression and drug resistance. By correlating gene expression profiles with clinical outcomes, transcriptome analysis can aid in identifying potential biomarkers for diagnosis, prognosis, and treatment response, enhancing personalized medicine strategies[9].

Our RNA sequencing results showed that, upon oxaliplatin treatment, 104 genes were upregulated and 169 genes were downregulated in oxaR cells compared to parental cells. SMIM22 exhibited the highest increase in expression in oxaR cells compared to parental cells (Fig. 3B), suggesting that SMIM22 may be associated with resistance in colorectal cancer (CRC). SMIM22 (Small Integral Membrane Protein 22) is a gene that encodes a protein with a small integral membrane domain, suggesting its involvement in cellular processes related to membrane dynamics and signaling[10]. Although its precise function in cancer biology remains largely unexplored, emerging evidence suggests that SMIM22 may play a role in cellular proliferation and survival. Abnormal expression of membrane proteins can influence drug resistance mechanisms in various cancers, including colorectal cancer, making SMIM22 a potential candidate for further investigation as a biomarker for treatment response.

The aim of this study was to understand the mechanism through which SMIM22 mediates resistance to anti-cancer drugs. To this end, we analyzed the transcriptional changes of SMIM22 in response to oxaliplatin treatment in both parental and oxaR HCT116 cells. Another pivotal signaling cascade in CRC, the mTOR pathway, associated with cell growth, was investigated in relation to SMIM22. We observed that SMIM22 overexpression correlated with mTOR pathway activation. Moreover, subsequent co-treatment with mTOR pathway inhibitor, Rapamycin, and oxaliplatin demonstrated a partial restoration of sensitivity in resistant oxaR cells.

RESULTS

Determination of Oxaliplatin Exposure Concentration

(A) Clonogenic assay for identifying oxaliplatin IC50 value in HCT116 cells. Treatment on HCT116 cells while serially increasing the concentration of oxaliplatin in that order: 0, 0.01, 0,05, 0.5, 1, 5 μM.
(B) Quantification by image J of clonogenic assay results.

In order to understand the resilience mechanisms in colorectal cancer, we first constructed oxaliplatin-resistant HCT116 cells, derived from the human colon cancer cell line. To this end, we continually exposed HCT116 cells to oxaliplatin over a duration of six weeks, during which subculturing was also performed. IC50 values, which represent the concentration of drug required to inhibit cell viability by 50%, were identified (Fig 1A) and quantified (Fig 1B) for oxaliplatin in HCT116 cells by clonogenic assays.

To assess oxaliplatin resistance and the dose-dependent response of parental colon cancer cells, cells were treated with increasing concentrations of oxaliplatin. This treatment simulates a clinical scenario where tumor cells are exposed to increasing drug levels during chemotherapy. This allows us to assess the ability of resistant cells to survive under these harsh conditions, which reflects their adaptive mechanisms. As oxaliplatin concentrations are progressively increased, we can observe changes in cell behavior, such as changes in proliferation, survival, or induction of apoptosis. Importantly, it also helps to reveal whether the cells are truly resistant or can only tolerate lower doses. Although changes in growth conditions such as nutrient availability, cell density, and differences in the tumor microenvironment can affect IC50 values[11], we assumed that these differences would not have a significant impact. It was observed that upon exposure to 1μM of oxaliplatin, the viability of HCT116 cells reduced to approximately 50%. Then, oxaliplatin-resistant HCT116 cell line was established by continuously exposing the HCT116 cells to 1μM of oxaliplatin over six weeks.

Establishment and Verification of Oxaliplatin-Resistant HCT116 Cells

(A) MTT assay to compare cell survival rate of parental cells and oxa R cells under 1μM oxaliplatin

(B) Relative expression levels of p21 (left) and bbc3 (right) were measured by quantitative PCR (qPCR) in parental and oxaR HCT116 cells. Data are presented as mean ± SD of three independent experiments. Gene expression was normalized to GAPDH, and fold change was calculated using the $2^{-\triangle\triangle Ct}$ method. ($**$ P<0.01) Table: Primer sequences used for qPCR analysis of p21 and bbc3.

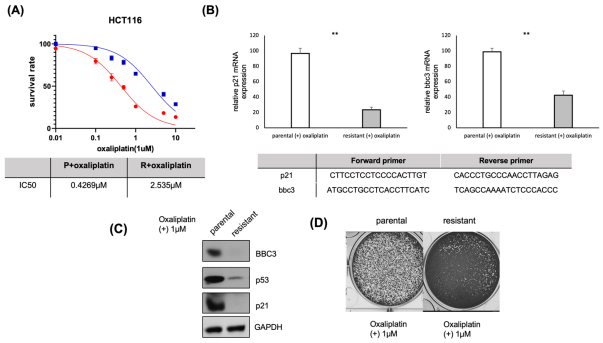


Figure 2. generation and characterization of oxaliplatin-resistant cells from HCT116

(C) Western blotting analysis of bbc3, p53, p21, and GAPDH in the indicated cells.

(D) Confirmation of the viability of parental cells and oxaR cells upon oxaliplatin exposure through clonogenic assay.

After the six-week treatment, the IC50 values of oxaliplatin were compared between the parental and resistant cells to evaluate whether the HCT116 cells had developed drug resistance.

When the parental HCT116 cells were exposed to oxaliplatin, there was a dramatic decline in their survival rate, with an IC50 value registered at 0.4269μM (Fig. 2A). Conversely, when treated with identical oxaliplatin concentrations, the resistant HCT116 cells demonstrated a significantly higher survival rate. The IC50 value for the resistant HCT116 cells was found to be approximately 5 times higher than that of the parental cells, resting at 2.535μM (Fig. 2A). Concerning the observed change in IC50, despite continuous oxaliplatin treatment for over six weeks, the IC50 increase plateaued at approximately 5-fold (data not shown). This suggests that the genetic and molecular alterations underlying the resistance may have reached a stable threshold at this level of drug exposure.

Subsequently, utilizing both qRT-PCR and western blot analysis, we determined alterations at the mRNA and protein levels. The expression of p21 and BBC3 was reduced when oxaliplatin was treated in oxaR compared to parental, suggesting that the increased resistance in oxaR HCT116 cells resulted in an attenuated DNA or RNA damage response. This reduction in the damage response may be due to oxaliplatin no longer being able to effectively inhibit DNA synthesis in these resistant cells. Since oxaliplatin's mechanism of action involves inhibition of DNA and RNA synthesis, its reduced effectiveness in oxaR cells is consistent with the observation of downregulation of key markers such as p21 and BBC3, which are typically upregulated in response to DNA damage (Fig 2B) [12]. Analogously, this trend persisted at the protein level (Fig 2C). The clonogenic assay also showed differential cell viability between the parental and

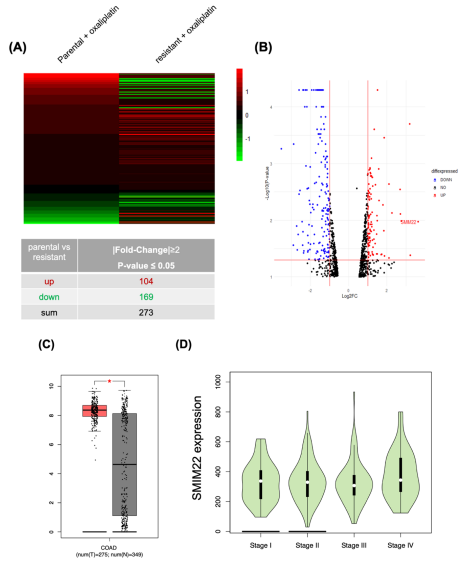


Figure3. Transcriptomic comparison of treating parental cells cells with oxaliplatin in HCT116 and oxaR

resistant cells upon oxaliplatin treatment (Fig 2D). We found that resistant cells achieve either higher cell survival or lower levels of cell death due to acquired resistance mechanisms.

Global Transcriptional Analysis upon Oxaliplatin Treatment in Parental and Oxaliplatin-Resistant (oxaR) Cells

(A) heat map of RNA-Seq transcriptome analysis for 273 selected genes from the parental cells and oxaR cells.

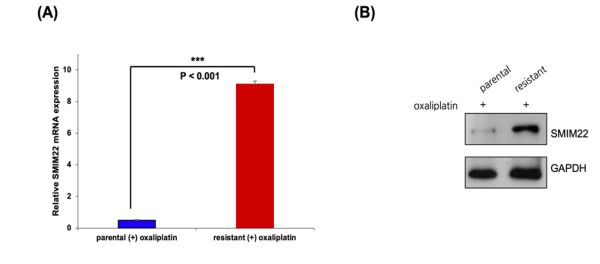
(B) SMIM22 showing the greatest increase in transcriptional change when parental cells and oxaR cells were treated with oxaliplatin

(C) GEPIA (Gene Expression Profiling Interactive Analysis) shows that SMIM22 is highly expressed in colon cancer patients

(D) Violin plot showing SMIM22 expression across different stages of colon cancer.

To understand the mechanisms related to oxaliplatin resistance in colorectal cancer (CRC), we identified potential target genes associated with the response of parental and oxaR cells to oxaliplatin treatment. To this end, RNA was extracted from each sample and a whole genome transcriptome analysis was carried out to identify global transcriptional changes between the two samples.

We hypothesized that if any gene exhibited heightened expression in oxaR cells upon oxaliplatin treatment, that gene could be targeted in a synergistic approach alongside oxaliplatin. Thus, gene expression was comparatively analyzed



	Forward primer	Reverse primer
SMIM22	ATAACTTGGCCCTGGAACCC	TGCTGACTGAAGGCTTGGAG

Figure4. Validation of RNA-seq data by qRT-PCR and western blot

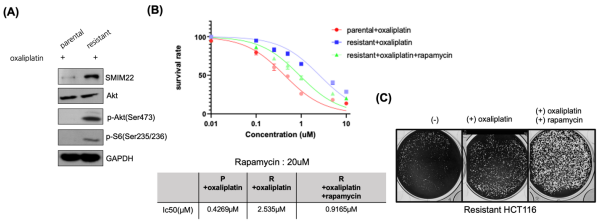


Figure 5. SMIM22 can overcome drug resistance in CRC by mediating the mTOR pathway

by performing pairwise subtraction of normalized expression levels for each gene, aiming to determine differential expression between the two samples (Fig 3A). Upon oxaliplatin treatment, compared to the parental cells, the oxaR cells displayed upregulation of 104 genes and downregulation of 169 genes.

Of the genes, SMIM22 demonstrated the most significant increase in expression in oxaR cells compared to parental cells (Fig 3B). This increase in SMIM22 expression was potential evidence for the correlation between its expression and oxaliplatin resistance in CRC. To identify the potential prognostic relevance of SMIM22, we analyzed 624 patient samples using a public patient database. In 275 colon cancer patients, SMIM22 expression was found to be significantly higher compared to 349 normal samples (Fig 3C).

Additionally, we identified SMIM22 expression levels across colorectal cancer stages I through IV (Fig 3D). This analysis revealed a progressive increase in SMIM22 expression with advancing cancer stages , indicating a potential correlation between SMIM22 overexpression and disease progression. These findings suggest that SMIM22 may be associated with poor prognosis in colorectal cancer patients. Through the stages, suggesting that its overexpression may be associated with poor prognosis.

Upregulation of SMIM22 in Oxaliplatin-Treated oxaR Cells

(A) Relative expression levels of SMIM22 was measured by quantitative PCR (qPCR) in parental and oxaR HCT116 cells. Data are presented as mean ± SD of three independent experiments. Gene expression was normalized to GAPDH, and fold change was calculated using the $2^{-\triangle\triangle Ct}$ method. (***P<0.001) Table: Primer sequences used for qPCR analysis of SMIM22.

(B) Western blotting analysis for SMIM22 in the indicated cells treated oxaliplatin(1μM) for 48h.

We validated the results obtained from the RNA-seq transcriptome analysis through both qRT-PCR and western blot assays. qRT-PCR analysis demonstrated that the SMIM22 mRNA expression was significantly elevated in oxaliplatin-treated oxaR cells compared to when oxaliplatin was applied to the parental cells (Fig 4A). Additionally, upon treating oxaR cells with oxaliplatin, we observed an increase in SMIM22 protein expression, as shown by the western blot results (Fig 4B).

SMIM22: A Crucial Factor for Chemoresistance in CRC Cells

(A) Western blotting analysis of SMIM22, Akt, p-Akt, p-S6, GAPDH in the indicated cells treated with Oxaliplatin (1μM) for $48 \cdot h$.

(B) MTT assay identifying IC50 to compare cell viability between parental + oxaliplatin(1μM), oxaR + oxaliplatin(1μM), oxR + oxaliplatin(1μM) +rapamycin (20μM)

(C) Clonogenic assay confirming cell viability between oxaliplatin and rapamycin combinational exposure on oxaR cells

The fact that SMIM22 operates as an integral protein located in the endosome [10] led to the hypothesis that SMIM22 could have a direct relationship with the pivotal mTOR pathway in CRC. When we assessed receptor-mediated signals into intracellular kinases, particularly focusing on the phosphorylation expression of Akt and S6, we observed that when SMIM22 was overexpressed, the phosphorylation of both Akt and S6 increased (Fig 5A). This suggests an association between SMIM22's overexpression and the mTOR pathway.

Subsequently, to further analyze this correlation, we administered rapamycin, an inhibitor of the mTOR pathway [13], in conjunction with oxaliplatin. We observed that, while the IC50 value was initially 2.535μM when only oxaliplatin was administered to oxaR cells, the additional administration of rapamycin significantly decreased IC50 to 0.9155μM (Fig 5B). Moreover, the clonogenic assay showed that the combination therapy of oxaliplatin and rapamycin can be effective in enhancing drug sensitivity against HCT116 cells (Fig 5C).

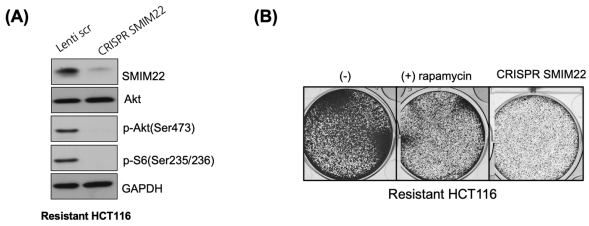


Figure 6. Targeting SMIM22 Overcomes Oxaliplatin Resistance and Promotes Apoptosis in Oxaliplatin-Resistant Colorectal Cancer Cells

SMIM22 Knockout Suppresses mTOR Pathway Activation and Enhances Apoptosis in Oxaliplatin-Resistant Colorectal Cancer Cells

(A) Cells transduced with a control lentiviral vector expressing scrambled sgRNA (Lenti scr) were compared to those transduced with CRISPR/Cas9 constructs targeting SMIM22 (CRISPR SMIM22). Knockdown of SMIM22 resulted in a marked reduction in p-AKT and p-S6 expression, indicating suppression of the mTOR pathway. GAPDH was used as a loading control.

(B) clonogenic assay confirming cell viability between rapamycin exposure and SMIM22 depletion on oxaR cells

Our findings provide clear evidence that SMIM22 is significantly overexpressed in oxaliplatin-resistant colon cancer cells and plays a critical role in the activation of the mTOR signaling pathway. Based on these observations, we hypothesized that targeting SMIM22 might be a more effective strategy for overcoming drug resistance compared to directly inhibiting mTOR signaling. To test this hypothesis, we employed the CRISPR/Cas9 system to specifically knock out SMIM22 in resistant cells. Upon SMIM22 knockout, we observed a marked decrease in the expression of key mTOR pathway components, including phosphorylated AKT (p-AKT) and phosphorylated S6 (p-S6), indicating effective suppression of the mTOR pathway (Fig 6A). Furthermore, when comparing the effects of SMIM22 knockout to rapamycin treatment, which directly inhibits mTOR activity, we found that SMIM22 knockout induced significantly higher levels of apoptosis in resistant cells (Fig 6B). These results suggest that SMIM22 inhibition may offer a more potent approach to sensitize oxaliplatin-resistant colon cancer cells to apoptosis and potentially improve therapeutic outcomes.

Oxaliplatin-specific resistance induces SMIM22 overexpression and activates the mTOR pathway.

(A) Western blot analysis of key apoptotic marker in oxaliplatin-resistant HCT116 cells treated with 5-fluorouracil (5-FU) and irinotecan.

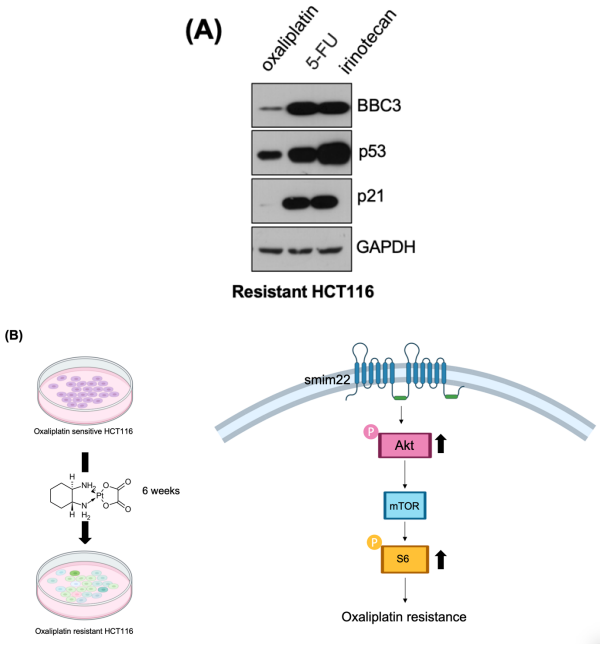


Figure 7. Oxaliplatin resistance leads to the overexpression of SMIM22 and the activation of the mTOR pathway

(B) Schematic representation of the proposed mechanism by which SMIM22 promotes oxaliplatin resistance in colorectal cancer cells.

To further investigate whether the acquired resistance observed in the oxaR cells is specific to oxaliplatin or reflects a broader chemoresistant phenotype, we performed additional experiments utilizing two other chemotherapeutic agents commonly used in the treatment of colorectal cancer, 5-fluorouracil (5-FU) and irinotecan. Both 5-FU and irinotecan operate through mechanisms distinct from that of oxaliplatin. Specifically, 5-FU functions as a pyrimidine analog, inhibiting thymidylate synthase, thereby disrupting DNA synthesis and interfering with RNA processing. In contrast, irinotecan is a topoisomerase I inhibitor that prevents the relaxation of DNA supercoiling during replication, leading to the accumulation of DNA strand breaks [14].

We treated the oxaR cells with 5-FU and irinotecan. Interestingly, upon treatment with these agents, we observed a notable increase in the expression of BBC3, p53, and p21—key regulators of apoptosis and the DNA damage response. This response contrasts sharply with the oxaR cells' reaction to oxaliplatin, where the expression levels of these genes remained suppressed (Fig 7A). The upregulation of these markers in response to 5-FU and irinotecan indicates that the oxaR cells retain sensitivity to these drugs, suggesting that the resistance is not due to a general chemoresistance mechanism but rather is specific to oxaliplatin.

In conclusion, oxaliplatin-resistant HCT116 cells were successfully established through six weeks of continuous oxaliplatin treatment. RNA sequencing analysis identified SMIM22 as a critical gene implicated in the development of drug resistance. Our findings indicate that SMIM22 contributes to resistance by activating the mTOR pathway (Fig 7B). Notably, the resistance phenotype was significantly diminished either by depleting SMIM22 or by treating the cells with the mTOR inhibitor, rapamycin, highlighting the potential of these strategies in overcoming oxaliplatin resistance.

DISCUSSION

Oxaliplatin stands as one of the most commonly used chemotherapeutic agents for treating colorectal cancer (CRC). However, resistance to oxaliplatin emerges as a clinical bottleneck, preventing complete remission of tumors in CRC patients [15]. In this study, we conducted whole genome transcriptome analyses to observe a pronounced overexpression of SMIM22 in oxaR CRC cells. Importantly, our findings underscored a potential correlation between heightened SMIM22 expression and the tumor resistance to oxaliplatin.

Although SMIM22, an integral protein situated in the endosomal region, is involved in various cellular processes [10], its specific role within the context of CRC remained inadequately understood. Considering the overexpression of SMIM22 in CRC patients and its status as an integral protein, we investigated its potential relationship with another pivotal signaling pathway in CRC, the mTOR pathway. Our conclusions were manifold. First, upon treating oxaR cells with oxaliplatin, there was a significant increase in both mRNA and protein expression levels of SMIM22.

Secondly, as a result of upregulation of SMIM22, we observed an activation of the mTOR pathway (Fig 5A). When we co-administered rapamycin, an mTOR pathway inhibitor, alongside oxaliplatin, we observed drug sensitivity in HCT116 cells was restored. This was corroborated through both MTT and clonogenic assays (Fig 5B,C). While prior studies have shown the synergy between rapamycin and oxaliplatin as a robust therapeutic combination [16], our work confirmed the pivotal role of SMIM22 as a mediator of the mTOR pathway. Currently, there are no drugs directly targeting SMIM22. This indicates that future research should focus on this protein as a potential therapeutic target for the treatment CRC. Given that SMIM22 functions as an integral membrane protein, we expect drug responses targeting it to be both more specific and efficacious.

Although our study identified SMIM22 as a key player in conferring resistance to oxaliplatin in colorectal cancer cells and suggested its role in activating the mTOR pathway, there are several limitations that must be acknowledged. First, although we observed a reduction in mTOR-related signaling following SMIM22 depletion, we did not provide direct mechanistic evidence that SMIM22 directly regulates the mTOR pathway. The precise molecular interaction between SMIM22 and the components of the mTOR signaling cascade remains unclear.

Additionally, our study primarily focused on in vitro models, and the clinical relevance of these findings in patient-derived samples or in vivo models requires further investigation. Future work should aim to elucidate the molecular mechanisms by which SMIM22 influences the mTOR pathway, potentially through protein interaction studies or by exploring upstream regulatory elements. Moreover, examining the role of SMIM22 in different cancer types or treatment conditions could provide further insights into its broader implications in chemotherapy resistance. By addressing these limitations, future research can help to clarify the role of SMIM22 in drug resistance and explore its potential as a therapeutic target.

Nevertheless, because the overexpression of SMIM22, was particularly evident when oxaliplatin was administered to oxaR, we expect that the protein can be used as a potential novel prognostic biomarker for CRC. The future of CRC treatment may well pivot on the strategic targeting of SMIM22, offering an opportunity to address the issue of resistance to standard therapies like oxaliplatin.

MATERIAL & METHODS

Cell culture

The human colon cancer cell line HCT116 was purchased from the Korean Cell Line Bank (Seoul, Korea). HCT116 cells were grown in RPMI-1640 medium (Gibco) with 10% fetal bovine serum (Thermo Fisher Scientific) and 1% penicillin and streptomycin (Gibco) in a 5% CO2 atmosphere at 37°*C*. To confirm whether the cells would develop resistance to oxaliplatin, they were treated continuously with oxaliplatin for six weeks, and oxaR cells were established.

Drug treatment

Oxa R HCT116 cells were treated with 5 μM 5-FU and 10nM Irinotecan for 3 days. Untreated Oxa R HCT116 cells were used as controls. After treatment, cells were collected for western blotting.

Western blotting analysis

Protein concentrations were determined using the BCA Protein Assay Kit (Beyotime, P0012). Primary antibodies used in this study are anti-BBC3, anti-P53, anti-P21, anti-SMIM22, anti-Akt, anti-phospho Akt, anti-phospho S6, and anti-GAPDH(CST, #5174).

Quantitative real-time PCR

Cellular RNAs were isolated using Trizol reagent (Invitrogen). First-strand cDNA was synthesized with random primers using High Capacity cDNA Reverse Transcription Kit (Takara). qPCR was performed using Power SYBR Green (Takara) on a CFX96 Real-Time PCR Detection System (Bio-Rad). Data was collected and normalized to GAPDH : Forward : 3'-TATGACTCTACCCACGGCAAGT-5' and Reverse : 3'-ATACTCAGCACCAGCATACC-5'

Clonogenic Assay

HCT116 cells were seeded in triplicate at a density of 4,000 cells/well in 6-well plates containing 3mL RPMI complete medium and grown at $37°C$ in a 5% CO2 incubator. After 24 hours, a fresh medium containing 0, 0.01, 0.05, 0.5, 1 and 5μM was added. After 3 days, the medium was aspirated and the cell colonies were washed twice with PBS and stained with 0.5% crystal violet in 25% methanol for 15 min. Excess dye was removed by rinsing three times with PBS. After drying, the plates were scanned and colony numbers were counted.

Cell viability assay

HCT116 cells were platted on 96-well microplates at a density of 2 × 104cells/ml. The cells were incubated in the presence of complete media for 96 h. After that, the cells were incubated for 4 h with the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (5 mg/ml, Sigma). During the time MTT was metabolized by living cells to purple formazan crystals, which were later solubilized in DMSO for 10min. The optical density of the product was measured at 570 nm with the use of an Infinite M200 Pro microplate reader.

Transcriptome sequencing

Transcriptome sequencing (RNA-seq) were performed in HCT116 parental and oxaliplatin-resistant cells. The library construction and massive parallel sequencing was performed by Novogene Technology. RNA-seq data quality was checked by FASTQC and analyzed by TopHat-Cufflinks pipeline.

CRISPR Transfection

The CRISPR plasmid targeting SMIM22 (CRISRP SMIM22) and the lenti scrambled control vector were transfected into oxaliplatin resistant HCT116. Electroporation was carried out using the Lonza 4D Nucleofector system. Successfully transfected cells were selected using puromycin and confirmed by western blot.

Statistical analysis

Statistical analyses were performed using Student's t test. All data were presented as the mean ± SD. $*P$ value <·0.05, $**P$ value<0.01, $***P$ value<0.005

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