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The Effect of Ro48-8071 an Oxidosqualene Cyclase Inhibitor on the Proliferation of Gastric Cancer Cells

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ABSTRACT

Gastric carcinoma arises from uncontrolled cell growth in the stomach, ranking as the fifth most common malignancy. The primary risk factor, *Helicobacter pylori* infection, initiates gastric mucosal changes that can progress to cancer. Current treatment strategies predominantly rely on surgery although perioperative and adjuvant chemotherapy may be employed to improve outcomes, particularly in cases with extensive lymph node involvement. However, many patients experience relapse or metastasis post-surgery, limiting long-term survival rates. Cholesterol metabolism is critical in cancer progression, influencing cell membrane integrity, steroid hormone production, and signal transduction pathways within the tumor microenvironment (TME). The de novo synthesis of cholesterol involves a complex cascade of enzymatic reactions primarily occurring in the cytosol and endoplasmic reticulum. When cholesterol levels decrease, the expression of low-density lipoprotein (LDL) receptors (LDLR) increases. The increase and decrease of LDLR play a crucial role in maintaining cholesterol homeostasis and ensuring that cells obtain the cholesterol they need. Notably, Ro48-8071 acts as an inhibitor of oxidosqualene cyclase (OSC), disrupting the conversion of oxidosqualene to lanosterol, a pivotal step in cholesterol biosynthesis. This inhibition potentially impedes cancer cell proliferation and promotes apoptosis in cholesterol-dependent cells. Given the significance of cholesterol metabolism in carcinogenesis, this study aims to investigate the impact of Ro48-8071 on gastric cancer cells HGC-27 and MKN-45. Findings with the effect of Ro48-8071, an OSC inhibitor, indicate that the proliferation of MKN-45 cells shows a higher degree of dependence on cholesterol metabolism compared to the HGC-27 cell line. Consequently, LDLR gene expression is found at lower levels, inversely related to cholesterol production. The findings suggest that inhibiting cholesterol synthesis via Ro48-8071 could selectively affect the proliferation of MKN-45 gastric cancer cells, which are more dependent on cholesterol. This implies that targeting cholesterol metabolism might offer a novel approach to treating specific subtypes of gastric cancer, potentially improving outcomes in patients with aggressive cancer types.

Keywords: Ro48-8071, gastric cancer, LDLR, cholesterol metabolism

ÖZ

Ro48-8071 Oksidoskualen İnhibitörünün Mide Kanseri Hücrelerinin Çoğalması Üzerindeki Etkisi

Mide kanseri, mide içinde kontrolsüz hücre büyümesinden kaynaklanan ve beşinci en yaygın kanser türü olarak sıralanan bir hastalıktır. Başlıca risk faktörü olan *Helicobacter pylori* enfeksiyonu, mide mukozal değişikliklerini başlatır ve kansere ilerleyebilir. Mevcut tedavi stratejileri genellikle cerrahiye dayanmakta olup, perioperatif ve adjuvan kemoterapi, özellikle yaygın lenf düğümü tutulumu olan vakalarda sonuçları iyileştirmek için kullanılabilir. Ancak birçok hasta ameliyat sonrası nüks veya metastaz yaşayarak uzun vadeli sağkalım oranlarını sınırlar. Kolesterol metabolizması, kanser ilerlemesinde hücre zarı bütünlüğü, steroid hormon üretimi ve tümör mikroçevresinde sinyal iletim yolları üzerinde kritik bir rol oynar. Kolesterolün de novo sentezi, temel olarak sitozol ve endoplazmik retikulumda gerçekleşen karmaşık bir enzimatik reaksiyon kaskadı ile ilişkilidir. Kolesterol seviyelerinin azalması durumunda, düşük yoğunluklu lipoprotein (LDL) reseptörlerinin (LDLR) ifadesi artar. Düşük yoğunluklu lipoprotein reseptörlerinin artış ve azalışı, kolesterol homeostazının korunmasında ve hücrelerin ihtiyaç duyduğu kolesterolün

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temin edilmesinde büyük önem taşır. Bu bağlamda oksidoskualen siklaz (OSC) inhibitörü olarak görev yapan Ro48-8071, oksidoskualenin lanosterole dönüşümünü engeller, bu da kolesterol biyosentezinin önemli bir adımıdır. Bu inhibisyon, kolesterol bağımlı hücrelerde kanser hücrelerinin proliferasyonunu engelleyebilir ve apoptozisini arttırabilir. Kolesterol metabolizmasının kanser oluşumundaki önemi göz önüne alındığında, bu çalışma Ro48-8071'in mide kanseri hücreleri HGC-27 ve MKN-45 üzerindeki etkilerini araştırmayı amaçlamaktadır. Ro48-8071 OSC inhibitörünün etkisiyle bulgular, MKN-45 hücrelerinin proliferasyonu HGC-27 hücre hattına kıyasla daha yüksek derecede kolesterol metabolizmasına bağlılık gösterdiğini ve buna bağlı olarak LDLR gen ifadesinin kolesterol üretimiyle ters orantılı olarak düşük ekspresyon seviyesinde bulunduğunu göstermektedir. Bulgular, Ro48-8071 ile kolesterol sentezinin inhibe edilmesinin, kolesterole daha bağımlı olan MKN-45 mide kanseri hücrelerinin proliferasyonunu seçici olarak etkileyebileceğini göstermektedir. Bu durum, kolesterol metabolizmasının hedeflenmesinin, belirli mide kanseri alt tiplerini tedavi etmek için yeni bir yaklaşım sunabileceğini ve agresif kanser türlerine sahip hastalarda sonuçları potansiyel olarak iyileştirebileceğini düşündürmektedir.

Anahtar kelimeler: Ro48-8071, mide kanseri, LDLR, kolesterol metabolizması

INTRODUCTION

Gastric carcinoma is raised from the uncontrolled growth of the cells within the stomach, the fifth most common type of malignancy worldwide according to the World Health Organization (968.784 new cases per year in 2020) (1). Worldwide, gastric cancer (GC) is the leading cause of cancer-related mortality, with most deaths occurring within the first year after diagnosis (2). Gastric cancer occurs at twice the rate in men compared to women (3). *Helicobacter pylori* infection stands out as the foremost risk factor for non-cardia GC. The persistent presence of *H. pylori* in the stomach lining initiates a sequence of events that includes atrophic gastritis and progresses to intestinal metaplasia, establishing it as a significant risk factor for GC (4). The most commonly followed strategy against GC is surgery which remains the sole potentially curative treatment. Perioperative and adjuvant chemotherapy, along with chemoradiation, can enhance outcomes for resectable cases with extensive lymph node removal. However, more than half of the patients who undergo radical surgery experience local relapse, distant metastases, or are diagnosed with advanced-stage disease, resulting in a median survival of rarely more than 12 months and a five-year survival rate below 10%. In advanced cases (stage IV) where patients are suitable for chemotherapy, cisplatin and fluoropyrimidine-based regimens, often supplemented with trastuzumab for HER2-positive patients, are commonly used (5).

One of the hallmarks of cancer cells is the disruption of cholesterol homeostasis. Tumor microenvironment (TME) supports the survival and growth of tumor cells, and the interaction between cholesterol metabolism and TME plays a role in tumor development and progression (6).

De novo cholesterol synthesis involves more than 20 enzymes in the cytosol and endoplasmic reticulum (ER). The initial step is catalyzed by acetylacetyl-CoA thiolase, which condenses two acetyl-CoA molecules to form acetylacetyl-CoA (7). Next, 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCS) adds a third acetyl-CoA molecule to create 3-hydroxy-3-methylglutaryl-CoA, which is then reduced to mevalonate by 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) in the first rate-limiting step of cholesterol biosynthesis. Mevalonate undergoes two phosphorylations by mevalonate kinase (MVK) and phosphomevalonate kinase (PMVK) and an ATP-dependent decarboxylation to produce isopentyl pyrophosphate (IPP). Isopentyl pyrophosphate is converted to its isomer dimethylallyl pyrophosphate (DMAPP), and its condensation forms geranyl pyrophosphate (GPP). Geranyl pyrophosphate combines with another IPP molecule to yield farnesyl pyrophosphate (FPP). The mevalonate pathway can then lead to either non-sterol isoprenoids or sterols. Squalene synthase mediates the head-to-head condensation of two FPP molecules to produce squalene (8). Squalene is converted to 2.3-epoxysqualene by squalene epoxidase (SQLE) and then cyclized to lanosterol by lanosterol synthase. The final phase of cholesterol biosynthesis involves multiple oxygen-based reactions, transforming lanosterol through the Bloch branch or the Kandutsch-Russell pathway into desmosterol and 7-dehydrocholesterol, the direct precursors of cholesterol (9). A third hybrid pathway for converting lanosterol into cholesterol has also been suggested. Targeting cholesterol metabolism in carcinogenesis has shown substantial antitumor effects in both preclinical and clinical studies (6). The development of GC is linked to the activation of the mevalonate pathway or the intake of cholesterol through the low-density lipoprotein receptor (LDLR) (8). When cellular cholesterol levels drop, the expression of LDLR rises. The extracellular domain of LDLR can bind to circulating LDL, facilitating its uptake via endocytosis. Once inside the cell, LDL is transported to the lysosome, where lipases break it down, releasing free cholesterol for cellular use (9).

Ro48-8071 is a small molecule primarily studied for its role as an inhibitor of oxidosqualene cyclase (OSC), an enzyme crucial in the cholesterol biosynthesis pathway (10). Ro48-8071 inhibits OSC, which is responsible for converting oxidosqualene to lanosterol, a key step in the cholesterol biosynthesis pathway. By inhibiting OSC, Ro48-8071 reduces

the production of cholesterol within cells (11). Since cholesterol is essential for maintaining cell membrane integrity and producing steroid hormones, the inhibition of its synthesis by Ro48-8071 can have wide-ranging effects. This reduction in cholesterol synthesis can lead to decreased cell proliferation and increased apoptosis (programmed cell death) in cells that rely heavily on cholesterol (12).

In light of the literature, this study aims to examine the effect of the OSC inhibitor Ro48-8071 on GC cells HGC-27 and MKN-45 using real-time PCR (qRT-PCR) and cell viability assay.

MATERIALS AND METHOD

Cell Culture

HGC-27 human gastric adenocarcinoma and MKN-45 human GC cell line were utilized. HGC-27 cells were grown in High Glucose DMEM medium (Gibco, Thermo Fisher, USA), and MKN-45 cells were cultured in RPMI-1640 medium (Gibco, Thermo Fisher, USA). Culture media were supplemented with 10% fetal bovine serum (FBS) and 1% pen/strep. The cells were grown at 37 °C with a 5% CO_2 incubator. Ro61-8048 (Sigma, USA) was used as OSC inhibitor was used in cell culture experiments.

RNA Isolation and cDNA Synthesis

RNA extraction from gastric cell lines was performed using the RNeasy Mini kit (Qiagen, USA) according to the manufacturer's protocol, and the concentrations were measured. One microgram of RNA template was used for reverse transcription using high-capacity RNA-to-cDNA synthesis kit (Applied Biosystems, USA) according to the manufacturer's protocol.

qRT-PCR

qRT-PCR was performed to investigate LDLR expression in mRNA level. qPCR was conducted using the QIAGEN RotorGene Q system with SYBR Green (Applied Biosystems) and the following primers: LDLR forward: 5'-ACA TCT ACT GGA CCG ACT CT-3' and LDLR reverse: TGT TTT CAG TCA CCA GCG AG.

Cycle threshold (ct), results were standardized using the RPLP0 housekeeping gene for normalization. Primer sequence for RLP0 were as follows: forward: 5'-AGC ATC TAC AAC CCT GAA GTG-3' and reverse: 5'-AGC AAG TGG GAA GGT GTA ATC-3'. Relative mRNA fold changes were determined using the ΔΔCt method.

Cell Viability Assay

Cell viability assay was performed via CellTiter-Glo® Assay (Promega). HGC-27 and MKN-45 cells were initially seeded into 96 well-plate and incubated for 24 h for cells to be attached to the wells. Afterwards, between 20-100 µM, Ro48-8071 inhibitor was introduced to the cells for 96 h. After 96 h of treatment, Cell Titer Glo reagent (Promega) was introduced to each well according to the manufacturer's protocol.

RESULTS

Ro48-8071 inhibitor was applied at the indicated doses (100, 75, 50, 25, 20, 0 µM) to cell lines MKN-45 and HGC-27, and cell viability rates were calculated. Ro48-8071 inhibitor caused a strong dose-dependent inhibition of cell growth of MKN-45 cells; however, Ro48-8071 treatment of HGC-27 cells showed a moderate dose-dependent decrease in cell number as compared to MKN45 cells (Figure 1). Data presented as mean ± SD (**p< 0.001).

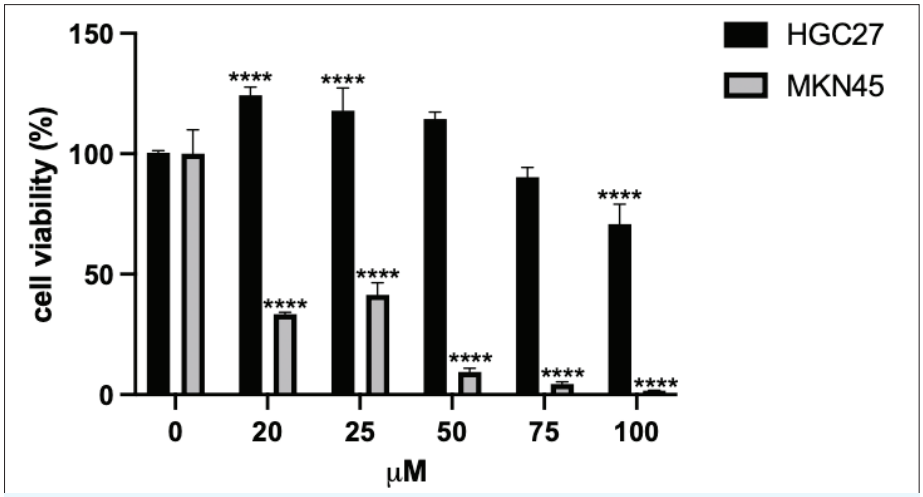

Figure 1. Ro48-8071 inhibitor reduces the growth in gastric carcinoma. MKN45 and HGC27 cells were plated and treated with 20, 25, 50, 75, 100 µM Ro48-8071 inhibitor. Data presented as mean ± SD.

*p< 0.05, **p< 0.01, ***p< 0.001, p< 0.0001.

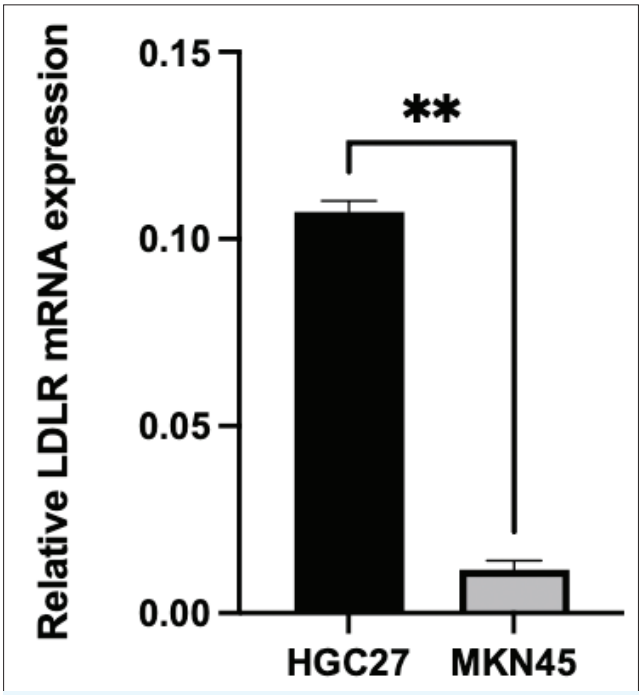

Figure 2. LDLR mRNA levels in MKN-45 and HGC-27 cell lines after 25 μM Ro48-8071 inhibitor treatment. Data presented as mean ± SD.

*p< 0.05, **p< 0.01, ***p<0.001, p< 0.0001.

Based on the cell viability profiles of MKN45 and HGC27 cells after treatment with the Ro48-8071 inhibitor, LDLR mRNA expression was investigated using a selected dose of 25 μM Ro48-8071. The MKN-45 (ΔΔCt 0.01164244) cell line had lower LDLR mRNA levels than the HGC-27 (ΔΔCt 0.107224125) cell line. In the MKN45 cell line, LDLR mRNA fold change was detected 10 times higher than HGC-27 (Figure 2). Data is presented as mean ± SD (****p< 0.0001).

DISCUSSION

Gastric cancer is among the most prevalent cancers globally, characterized by extensive metastasis and high mortality rates. Chemotherapy is a primary treatment for metastatic GC, but its effectiveness is often hindered by drug resistance, leading to treatment failure. The mechanisms behind chemotherapy resistance in GC are complex and multifactorial, with lipid metabolism playing a crucial role (13). Cholesterol [cholest-5-en-3-ol (3-beta) cholesterol], along with its metabolites and esters, is a major component of the plasma membrane and various other cellular organelles in animals. Cholesterol plays an important role in cellular homeostasis by maintaining the rigidity of cell membranes, providing a medium for signaling transduction, and being converted into other vital macromolecules, such as sterol hormones and bile acids (14). Chemically, cholesterol is distinctly different from two other lipids, triacylglycerols and phospholipids. It is primarily transported through the blood by LDL. Maintaining especially LDL cholesterol homeostasis is crucial for regulating cellular functions (15). Altered cholesterol metabolism is crucial in oncogenic signals and tumor development, invasion and metastasis. Epidemiological studies have shown the correlation between cholesterol content and cancer incidence worldwide (14). Accumulating evidence suggests that elevated cholesterol biosynthesis is a common characteristic of cancer and is linked to the neoplastic transformation normal tissue (16). Based on the existing data, cholesterol homeostasis is identified to be a new key player in cancer pathogenesis.

In our study, our objective was to investigate the reliance of GC cell growth on cholesterol metabolism. To achieve this, we utilized the Ro48-8071 inhibitor to block cholesterol synthesis, thereby inhibiting cholesterol-dependent cell growth. Our findings indicated a significant decrease in viability of MKN-45 cells following treatment with the Ro48-8071 inhibitor, underscoring their dependence on cholesterol for proliferation. Similar to our findings, one study has also revealed that the MKN-45 cells have lower LDLR expression level than other GC cell lines and thus the highest cellular cholesterol level (17). Tumor cells overexpress LDLR to meet their increased demand for cholesterol, which is crucial for rapid cell proliferation and the synthesis of new cell membranes. Our findings reveal that MKN-45 cells have lower levels of LDLR, suggesting higher cellular cholesterol levels than HGC-27 cells. This indicates that altered cholesterol levels in MKN-45 contribute to rapid cell proliferation. Excess cytoplasmic cholesterol typically accumulates in the lysosomes or mitochondria, activating oncogenic signaling pathways that enhance cell proliferation and survival while inhibiting apoptosis (18). The Ro48-8071 inhibitor inhibits cholesterol de novo synthesis, resulting in reduced cell proliferation. In contrast, HGC-27 cells exhibit higher LDLR expression at the gene level, leading to lower cellular cholesterol levels. Consequently, the viability of HGC-27 cells was not as significantly affected by the Ro48-8071 inhibitor compared to MKN-45 cells. This study underscores the critical role of cellular cholesterol levels in the proliferation of gastric cell lines. Various studies have suggested that cholesterol de novo synthesis and its carriers play a significant role in the development and progression of GC (19,20). Cholesterol de novo synthesis does not only influence the growth of cancer cells but also affects their metastatic potential and ability to migrate. Recent evidence indicates that inhibiting de novo cholesterol synthesis can also reduce the adhesion and migration of cancer cells (16). Therefore, when evaluating our results, it can be inferred

that blocking cholesterol synthesis in GC cells decreases their adhesion and migratory capabilities, highlighting the critical role of cholesterol in both breast and GC development and metastasis. In conclusion, based on the literature and our results, targeting cholesterol de novo synthesis may offer a new therapeutic potential for cancer treatment.

CONCLUSION

Cholesterol, a major component of cell membranes and essential for cellular homeostasis, plays a crucial role in cancer cell growth, proliferation, and survival. Our study aimed to investigate the effect of Ro48-8071 cholesterol biosynthesis inhibitor on GC cell growth on cholesterol metabolism. By utilizing the Ro48-8071 inhibitor to block cholesterol synthesis, we observed a significant decrease in the viability of MKN-45 cells, highlighting their reliance on cholesterol for proliferation. MKN-45 cells, which have lower LDLR expression levels, exhibit higher cellular cholesterol levels than HGC-27 cells. This suggests that the altered cholesterol levels in MKN-45 cells contribute to their rapid proliferation. Conversely, HGC-27 cells, with higher LDLR expression, maintained lower cellular cholesterol levels and showed less impact on viability when treated with the Ro48-8071 inhibitor. This study emphasizes the critical role of cellular cholesterol levels in the proliferation of GC cell lines. Given the critical role of cholesterol metabolism in GC, as a prospect, the studies can focus on developing specific inhibitors like Ro48-8071 to effectively target cholesterol synthesis in cancer cells. Combining these inhibitors with existing chemotherapy agents could enhance treatment efficacy and overcome drug resistance. Furthermore, identifying biomarkers related to cholesterol metabolism could enable personalized treatment strategies.

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