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Small-molecule inhibitors of the PERK-mediated Unfolded Protein Response signaling pathway in targeted therapy for colorectal cancer

Authors' Contribution:
A – Study Design
B – Data Collection
C – Statistical Analysis
D – Data Interpretation
E – Manuscript Preparation
F – Literature Search
G – Funds Collection

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ABSTRACT:

Introduction: The newest data has reported that endoplasmic reticulum (ER) stress and PERK-dependent Unfolded Protein Response (UPR) signaling pathway may constitute a key factor in colorectal cancer (CRC) pathogenesis on the molecular level. Nowadays used anti-cancer treatment strategies are still insufficient, since patients suffer from various side effects that are directly evoked via therapeutic agents characterized by non-specific action in normal and cancer cells.

Aim: Thereby, the main aim of the presented research was to analyze the effectiveness of the small-molecule PERK inhibitor NCI 12487 in an *in vitro* cellular model of CRC.

Materials and methods: The study was performed on colorectal cancer HT-29 and normal human colon epithelial CCD 841 CoN cell lines. The cytotoxicity was measured by XTT assay, evaluation of apoptosis was performed by caspase-3 assay, whereas cell cycle analysis via the propidium iodide (PI) staining.

Results: Results obtained have demonstrated that the investigated compound is selective only for HT-29 cancer cells, since at 25 μM concentration it significantly decreased HT-29 cells viability in a dose- and time-dependent manner, evoked increased caspase-3 activity and arrest in the G2/M phase of the cell cycle. Moreover, NCI 12487 compound markedly decreased HT-29 cells viability, increased caspase-3 activity and percentage of cells in sub-G0/G1, thus promoted apoptosis of cancer HT-29 cells with induced ER stress conditions.

Conclusion: Thus, based on the results obtained in this study it may be concluded that small-molecule modulators of the PERK-dependent UPR signaling pathway may constitute an innovative, targeted treatment strategy against CRC.

KEYWORDS: apoptosis, cancer treatment, colorectal cancer, endoplasmic reticulum stress, PERK inhibitors, unfolded protein response

ABBREVIATIONS

ATF4 – activating transcription factor 4
ATF6 – activating transcription factor 6
BiP – binding immunoglobulin protein
CHOP – CCAAT/enhancer-binding protein
CRC – colorectal cancer
DMSO – dimethyl sulfoxide
eIF2α – eukaryotic initiation factor 2α
ER – endoplasmic reticulum
FBS – fetal bovine serum
IRE1 – inositol-requiring enzyme 1
NCI – National Cancer Institute
p-CA – p-Coumaric acid
PERK – protein kinase RNA-like ER kinase
PI – propidium iodide
RIPK1 – receptor-interacting serine/threonine-protein kinase 1
Th – thapsigargin
TNF – tumor necrosis factor
UPR – Unfolded Protein Response
XTT – 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide

INTRODUCTION

The newest data has demonstrated that colorectal cancer (CRC) constitutes the most common malignant neoplasm and it is characterized by a still increasing tendency in morbidity and deaths [1]. The global burden of CRC may increase by 60% to more than 2.2 million new cases and 1.1 million deaths by 2030. CRC constitutes the third most common cancer in men and the second most common cancer in women [2]. It is estimated that the rise in the global burden of CRC will persist until the year 2035 and likely beyond [3]. To the present day the molecular basis of CRC has not been fully characterized, but it is known that multiple risk factors may be implicated in the development of CRC. Besides an ageing population and dietary habits, obesity, lack of physical exercise, and increase in smoking constitute a key risk factor of CRC [4].

The mechanisms of CRC development and progression still remain poorly understood, whereas there are multiple research data confirming a prominent role of the protein kinase RNA-like ER kinase (PERK)-dependent Unfolded Protein Response (UPR) signaling pathway in CRC pathogenesis [5–7]. The endoplasmic reticulum (ER) constitutes a multifunctional organelle that is responsible

for major cellular processes such as nascent protein folding and modification, calcium storage, liquid biosynthesis and detoxification. However, multiple conditions including hypoxia, nutrient deprivation, drug-induced toxicity, acidic extracellular pH and genetic mutations may lead to the accumulation of unfolded and misfolded proteins within the ER lumen and subsequently evoke ER stress conditions and activation of the UPR signaling pathway to restore protein homeostasis [8]. ER stress activated within cancer cells may restore homeostasis, contribute to cancer cells survival and thereby to tumor expansion [9]. Upon tumorigenesis, the high proliferation rates of cancer cells are strictly correlated with increased activities of ER protein folding, assembly and transport, that may induce ER stress conditions [10]. The ER stress-mediated response is considered cytoprotective as well as it is directly involved in tumor growth and adaptation of cancer cells against unfavorable environment [11].

To decrease abrogated protein load in the ER three transmembrane ER receptors may be activated including PERK, inositol-requiring enzyme 1 (IRE1) and activating transcription factor 6 (ATF6) that play a key role in the activation of three major branches of the UPR signaling pathway. However, it has been reported that the PERK-mediated UPR signaling arm plays a crucial role in promoting adaptation and survival upon ER stress conditions induced by hypoxia and should be the focus of future anticancer therapeutic efforts [12]. Interestingly, there are multiple research data confirming that ER stress-related signaling pathways may constitute a potential target for developing novel treatment strategy that may reduce adaptation of cancer cells to hypoxia, inflammation and angiogenesis, thus overcome drug resistance. However, the effects of these drugs on nontumorigenic cells remain under investigation [13, 14]. Under ER stress conditions PERK undergoes oligomerization, by the dissociation of Grp78/binding immunoglobulin protein (BiP), and autophosphorylation, that subsequently evoke phosphorylation of the eukaryotic initiation factor 2α (eIF2α), which constitutes the main substrate of PERK. Subsequently, the mentioned phosphorylation of PERK and eIF2α directly leads to the attenuation of global protein translation and enhanced translation of only selective proteins including activating transcription factor 4 (ATF4). ATF4 possesses a dual role, since under mild to moderate ER stress conditions it is responsible for restoration of the cellular homeostasis, however under severe and chronic ER stress conditions ATF4 may activate CCAAT-enhancer-binding protein homologous protein (CHOP)-mediated apoptosis [15–18].

The understanding of CRC carcinogenesis at the molecular level is essential for the development of an innovative treatment strategy. Thus, based on the latest research data demonstrating that the occurrence of the ER stress conditions as well as activation of the PERK-dependent UPR signaling pathway may be strictly associated with CRC pathogenesis and its further progression at the molecular level, the main aim of the present study was to evaluate the effectiveness of the small-molecule PERK inhibitor NCI 12487 in an in vitro cellular model of CRC.

MATERIALS AND METHODS

The investigated small-molecule PERK inhibitor NCI 12487 was screened, characterized and provided for further analysis in collaboration with the Department of Biochemistry and Molecular Biology, Hollings Cancer Center, Medical University of South Carolina, Charleston, SC, USA. National Cancer Institute (NCI) compound library was used for the high-throughput assay (inhibitor screening).

Experiments were performed using commercially available, purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA), human colon adenocarcinoma cell line HT-29 (ATCC® HTB-38™) and the normal human colon epithelial cell line CCD 841 CoN (ATCC® CRL1790™). Cells were cultured under standard conditions (37°C; 5% CO_2; 95% humidity), according to the guidelines provided by the vendors. The HT-29 cell line was cultured in McCoy's 5A medium (Sigma-Aldrich Corp., St. Louis, MO, USA) supplemented with 10% (v/v) fetal bovine serum (FBS) (Sigma-Aldrich Corp., St. Louis, MO, USA), 100 units/mL penicillin and 100 µg/mL streptomycin (GIBCO-BRL, Life Technologies,. Ltd., Paisley, Scotland), whereas the CCD 841 CoN cell line was cultured in Eagle's Minimum Essential Medium (Sigma-Aldrich Corp., St. Louis, MO, USA), supplemented with 10% (v/v) FBS (Sigma-Aldrich Corp., St. Louis, MO, USA), 100 units/mL penicillin and 100 µg/mL streptomycin (GIBCO-BRL, Life Technologies,. Ltd., Paisley, Scotland).

The cytotoxicity analysis was performed using the 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide (XTT) colorimetric assay (Thermo Scientific, Waltham, MA, USA). All experiments were performed in triplicate with similar results. Cells were seeded in 96-well plates (5×10^3/well) and cultured in 100 µL of complete growth medium for 24 h. After adhesion, cells were treated with 100 µL of complete cell culture medium containing the tested PERK inhibitor in a wide concentration range (0.75 µM, 3 µM, 6 µM, 12 µM, 25 µM, 50 µM, 75 µM, 100 µM, 50 mM) or 0.1% DMSO (Sigma-Aldrich Corp., St. Louis, MO, USA), which was used as a solvent for the investigated compound. Untreated cells cultured in a complete medium were used as a negative control, whereas cells treated with 100% DMSO comprised a positive control. Additionally, to demonstrate the effect of the tested compound upon ER stress conditions, HT-29 and CCD 841 CoN cells were seeded in 96-well plates (5×10^3/well) and cultured in 100 µL of complete growth medium for 24 h. After adhesion, cells were pretreated for 1 h with 100 µL of complete cell culture medium containing PERK inhibitor NCI 12487 at the concentrations of 3 µM and 25 µM and then cells were treated with Th (500 nM), as an ER stress inducer. Cells were also treated with Th (500 nM) only. Untreated cells cultured in a complete growth medium were used as a negative control, whereas cells incubated with 100% DMSO comprised a positive control. All samples were incubated for 16, 24 and 48 h. Next, 25 µL of XTT/PMS mixture were added to each well. After a 2 h incubation at 37°C in a 5% CO2 incubator, absorbance was measured at a wavelength of 450 nm using Synergy HT (BioTek, Hong Kong, China) spectrophotometer.

Analysis of apoptosis was carried out using a colorimetric caspase-3 assay kit (Abcam, Cambridge, UK). All experiments were repeated three times with similar results. Cells were seeded in 6-well plates (5×10^5/well) and cultured in a complete growth medium for 24 h. After adhesion, cells were treated with the PERK inhibitor in a large concentration range (3 µM, 6 µM, 12 µM, 25 µM, 50 µM, 75 µM, 100 µM) or 0.1% DMSO (Sigma-Aldrich Corp., St. Louis, MO, USA), and incubated for 24 h. Cells treated with staurosporine (Sigma-Aldrich Corp., St. Louis, MO, USA) at a 1 µM concentration

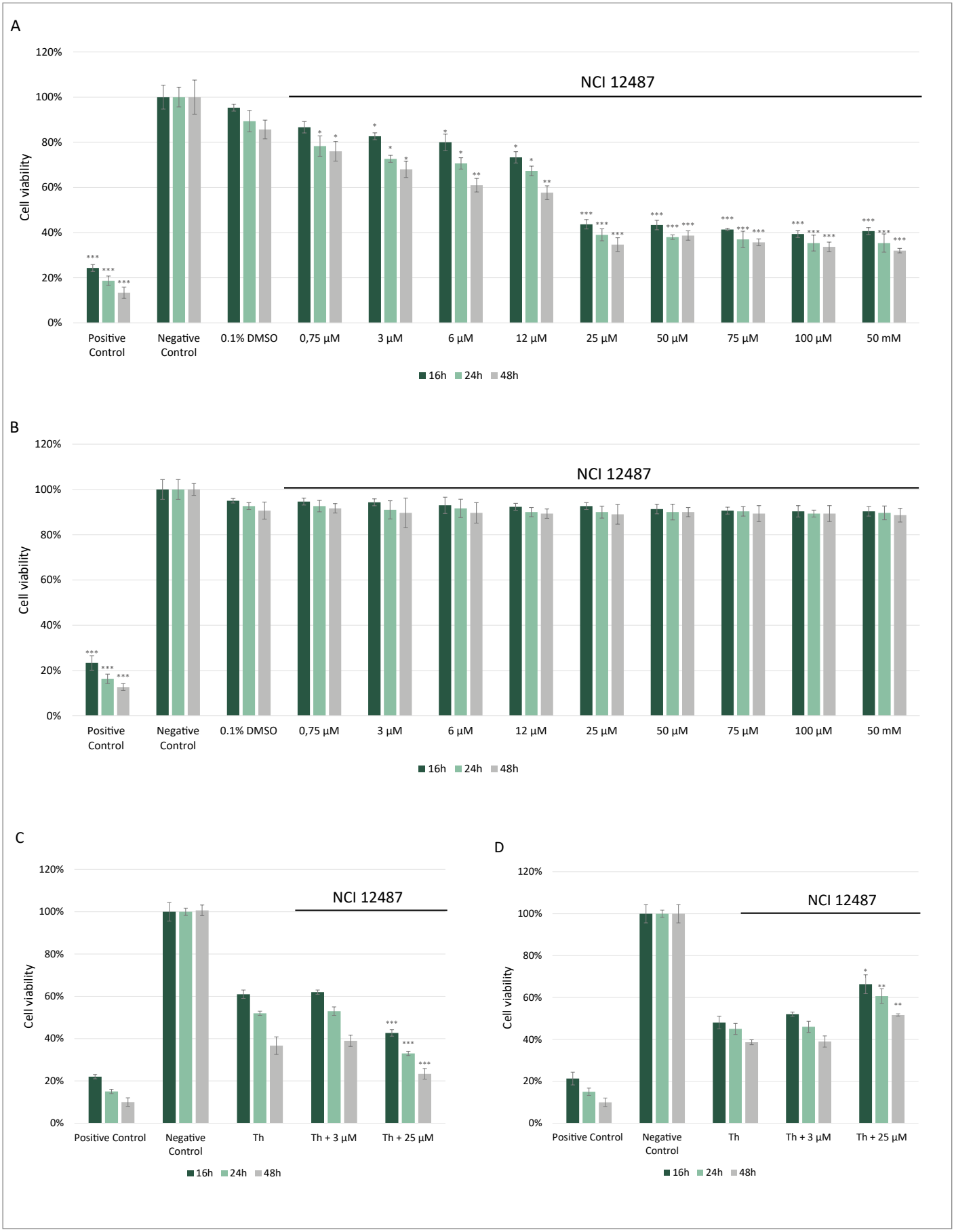

Fig. 1. Analysis of cytotoxicity with the XTT assay after treatment of HT-29 cells (A) and CCD 841 CoN cells (B) with PERK inhibitor NCI 12487 only and after treatment of HT-29 cells (C) and CCD 841 CoN (D) cells both with Th and PERK inhibitor NCI 12487. Each of the statistical analyses in individual experiments was based on the results of three independent tests. Data are expressed as mean ± SE (n = 3). On the graphs, the differences were statistically significant as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ *versus* the negative control (A, B) and *versus* Th (C, D). DMSO – dimethyl sulfoxide, Th – thapsigargin.

for 16 h constituted a positive control, whereas cells incubated only in a complete medium for 24 h served as a negative control. To investigate the effect of the analysed PERK inhibitor in HT-29 and CCD 841 CoN cells, with induced ER stress conditions, cells were seeded in 6-well plates (5×10^5/well) and cultured in a complete growth medium for 24 h. After adhesion, cells were pretreated for 1 h with the complete cell culture medium containing PERK inhibitor at the concentrations of 3 μM and 25 μM and then cells were treated with Th (500 nM) for 24 h. Cells were also treated only with Th (500 nM) for 24 h. Cells treated with staurosporine (Sigma-Aldrich Corp., St. Louis, MO, USA) at a 1 μM concentration for 16 h constituted a positive control, whereas cells incubated for 24 h only in a complete medium served as a negative control. Next, culture medium was removed, cells were washed once with 1 X DPBS (Sigma-Aldrich Corp., St. Louis, MO, USA) and detached using 1 X trypsin/EDTA solution (GIBCO-BRL, Life Technologies, Ltd., Paisley, Scotland). Cells' suspension was centrifuged (5 min, 1000 rpm at RT) and a pellet containing 1 × 106 cells was resuspended in 50 μL of cold Cell Lysis Buffer. Following 10 min of incubation on ice, a suspension of cells was centrifuged (10,000 × g, 1 min) and supernatants were transferred to fresh 2 mL tubes and the protein concentration was measured by performing a standard Bradford assay. BSA was used as a protein standard. Cell lysate containing 100 μg of protein was used for each assay. The 2 X Reaction Buffer containing 10 mM DTT and subsequently 4 mM DEVD-pNA substrate (200 μM final concentration) were added to each sample. After a 2h incubation at 37°C, the p-NA absorbance was measured at a wavelength of 405 nm using the Synergy HT (BioTek, Hong Kong, China) spectrophotometer.

Analysis of the cell cycle was performed by flow cytometry using the propidium iodide (PI) staining. All experiments were performed in triplicate with similar results. Cells were seeded in 6-well plates (5×10^5/well) and cultured in a complete growth medium for 24 h. After adhesion, cells were treated with the investigated PERK inhibitor in a wide concentration range (3 μM, 6 μM, 12 μM, 25 μM, 50 μM, 75 μM, 100 μM) or 0.1% DMSO (Sigma-Aldrich Corp., St. Louis, MO, USA). Cells treated with nocodazole (Sigma-Aldrich Corp., St. Louis, MO, USA) at a concentration of 1 μM for 16 h constituted a positive control, whereas cells cultured only in complete medium for 24 h were used as a negative control. To investigate the effect of the PERK inhibitor in HT-29 and CCD 841 CoN cells, with the induced ER stress conditions, cells were seeded in 6-well plates (5×10^5/well) and cultured in a complete growth medium for 24 h. After adhesion cells were pretreated for 1 h with the complete cell culture medium containing PERK inhibitor NCI 12487 of 3 μM and 25 μM concentrations and then cells were treated with Th (500 nM) and incubated for 24 h. Cells were also treated only with Th (500 nM) and incubated for 24 h. Cells treated with nocodazole (Sigma-Aldrich Corp., St. Louis, MO, USA) at a concentration of 1 μM for 16 h constituted a positive control, whereas cells cultured in complete medium for 24 h were used as a negative control. After treatment and incubation with the investigated compounds, cells were harvested and washed twice with cold 1 X DPBS (Sigma-Aldrich Corp., St. Louis, MO, USA). There were 1 × 106 cells/mL fixed with ice-cold 70% ethanol at –20°C for 20 min. Next, ethanol-suspended cells were centrifuged at 5000 rpm for 5 min. Pellets of cells were suspended in 250 μL of 1 X DPBS (Sigma-Aldrich Corp., St. Louis, MO, USA) and then cells were treated with RNase A, DNase & Protease-free (10 mg/mL) (Canvax Biotech, Córdoba, Spain) and incubated at 37 °C for 1 h before staining with PI solution (10 μg/mL) (Sigma-Aldrich Corp., St. Louis, MO, USA). After a 30-minute incubation at 4°C, samples were analyzed by flow cytometry using the Beckman Coulter CytoFLEX. The percentage of cells in each cell-cycle phase, based on DNA content, was determined using Kaluza analysis 1.5 A software (Beckman Coulter, Brea, CA, USA).

Statistical analysis was performed using the Sigma Plot (Systat Software, Inc., San Jose, CA, USA). The normality test was performed for each statistical analysis in all conducted studies using the Shapiro-Wilk test. All statistical analyses were normally distributed, therefore the statistical analysis between two groups was performed using the Student's t-test. Each of the statistical analyses in individual experiments was based on the results of three independent tests. On the graphs, the differences were statistically significant as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

RESULTS

Analysis of cytotoxicity, measured via the colorimetric XTT assay, demonstrated that the investigated compound NCI 12487 inhibited HT-29 cells viability in a dose- and time-dependent manner. The results obtained showed that the analyzed compound has the highest inhibitory potential at a concentration of 25 μM at all incubation times. A similar response was observed after treatment of HT-29 cells with the tested compound at 50 μM, 75 μM, 100 μM and 50 mM (Fig. 1A.). No significant cytotoxic effect was observed in normal CCD 841 CoN cells at any concentration of NCI 12487 and incubation times (Fig. 1B.). The 0.1% DMSO, used as a solvent for the tested PERK inhibitor, did not cause a significant cytotoxic effect toward HT-29 and CCD 841 CoN cells after 16, 24 and 48 h incubation (Fig. 1A., 1B.). Moreover, obtained results demonstrated that Th, an ER stress inducer, markedly decreased HT-29 and CCD 841 CoN cells viability at all incubation times in comparison with the negative control (Fig. 1C., 1D.). Treatment of HT-29 cells both with Th and tested compound NCI 12487 at 25 μM concentration evoked a significant decrease of cell viability at all incubation times in comparison with Th only treated cells (Fig. 1C.). Moreover, treatment of CCD 841 CoN cells, with induced ER stress conditions, with the tested compound NCI 12487 at 25 μM concentration evoked a significant inhibition of Th-induced ER stress (Fig. 1D.).

Analysis of apoptosis, performed by the colorimetric caspase-3 assay, showed that treatment of HT-29 and CCD 841 CoN cells with 1 μM staurosporine for 16 h significantly increased the level of caspase-3 activity in comparison with the negative control (Fig 2A., 2B.). The results obtained demonstrated that the investigated compound NCI 12487 evoked increased activity of caspase-3 in HT-29 cells in a dose-dependent manner. The highest increase in caspase-3 activity in HT-29 cells NCI 12487 triggered at concentration of 25 μM after 24 h incubation. A similar results were observed after 24 incubation of HT-29 cells with the tested compound at 50 μM, 75 μM and 100 μM (Fig. 2A.). Besides, a significant increase in the activity of caspase-3 was not observed in CCD 841 CoN cells after 24 h incubation with NCI 12487 at any used concentration (Fig. 2B.). The 0.1% DMSO, a solvent for the PERK inhibitor, did not result in a significant activation of caspase-3-dependent apoptosis in the investigated cell lines after 24 h incubation (Fig. 2A., 2B.). Following 24 h incubation of HT-29 cells both

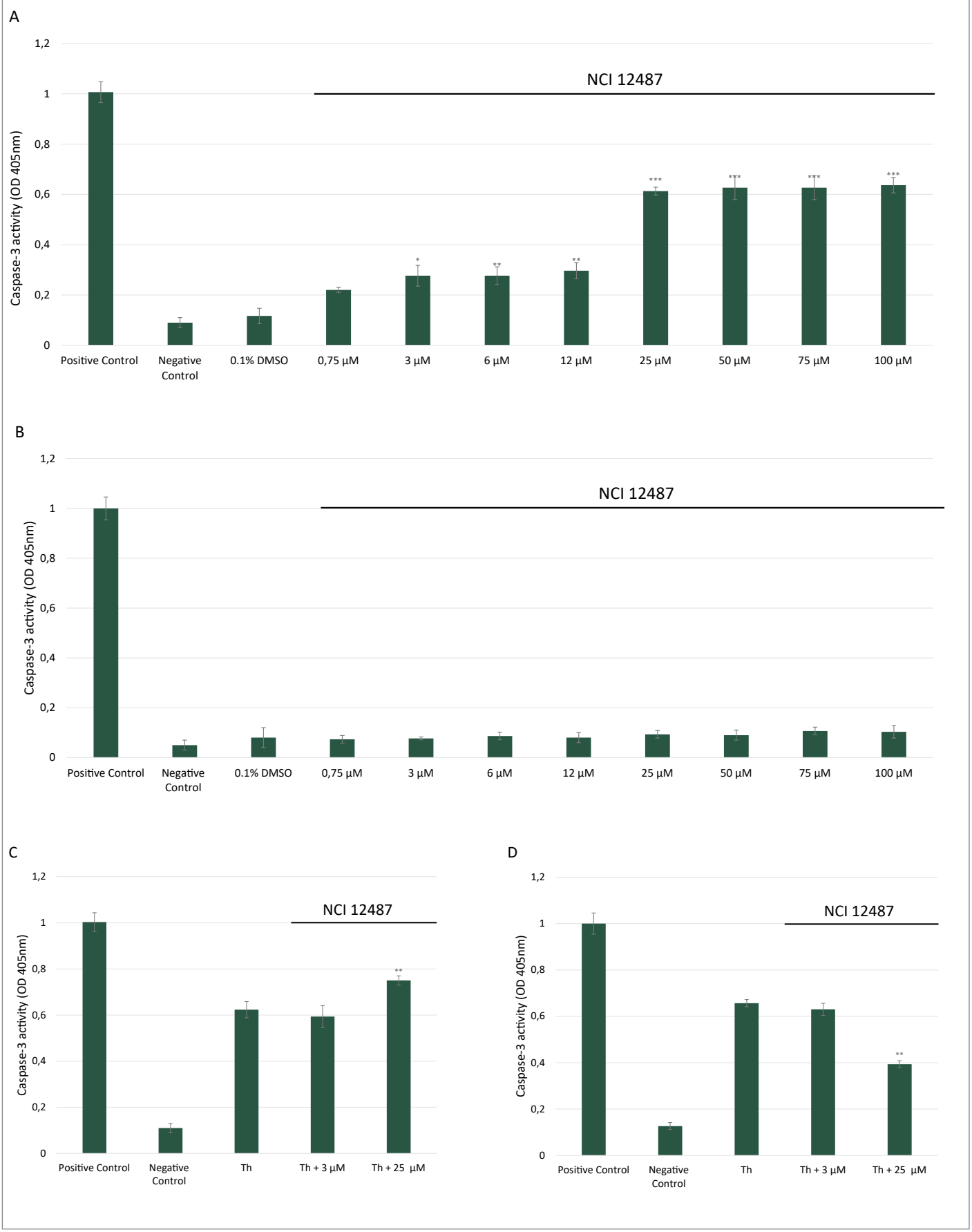

Fig. 2. Analysis of apoptosis with the caspase-3 activity assay after treatment of HT-29 cells (A) and CCD 841 CoN cells (B) with PERK inhibitor NCI 12487 only and after treatment of HT-29 cells (C) and CCD 841 CoN (D) cells both with Th and PERK inhibitor NCI 12487. Each of the statistical analyses in the individual experiments was based on the results of three independent tests. Data are expressed as mean ± SE (n = 3). On the graphs, the differences were statistically significant as follows: * P < 0.05, ** P < 0.01, *** P < 0.001 *versus* the negative control (A, B) and *versus* Th (C, D). DMSO – dimethyl sulfoxide, Th – thapsigargin.

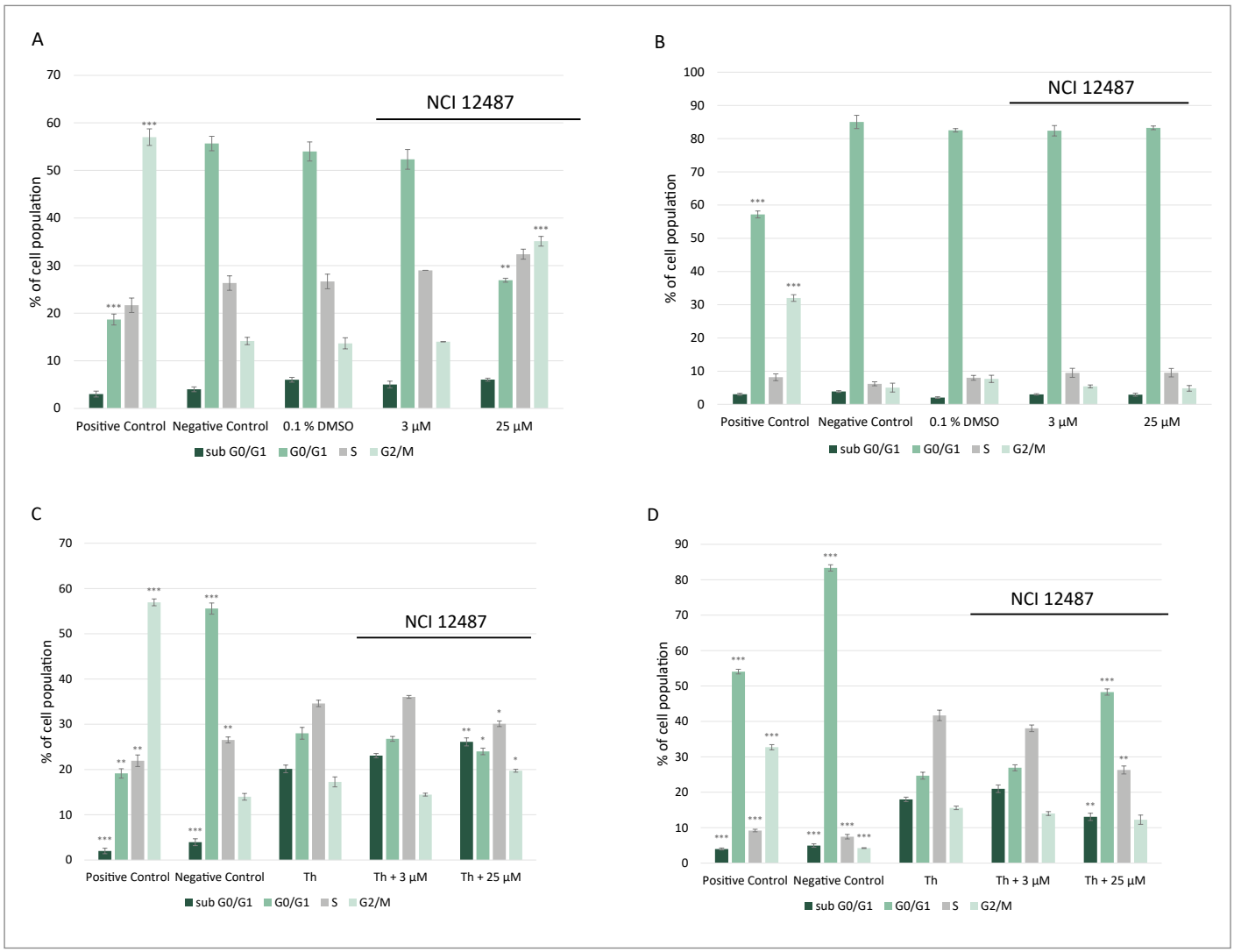

Fig. 3. Cell cycle analysis with flow cytometric propidium iodide (PI) staining after treatment of HT-29 cells (A) and CCD 841 CoN cells (B) with PERK inhibitor NCI 12487 only and after treatment of HT-29 cells (C) and CCD 841 CoN (D) cells both with Th and PERK inhibitor NCI 12487. Each of the statistical analyses in individual experiments was based on the results of three independent tests. Data are expressed as mean ± SE (n = 3). On the graphs, the differences were statistically significant as follows: * P < 0.05, ** P < 0.01, *** P < 0.001 *versus* the negative control (A, B) and *versus* Th (C, D). DMSO – dimethyl sulfoxide, Th – thapsigargin.

with Th and the investigated compound at 25 µM concentration the caspase-3 activity was significantly increased as compared to only-Th-treated HT-29 cells (Fig. 2C.), whereas 24 h incubation of CCD 841 CoN cells with Th and the investigated PERK inhibitor at 25 µM concentration evoked inhibition of Th-induced ER stress conditions, thus decreased caspase-3 activity in comparison with only-Th-treated CCD 841 CoN cells (Fig. 2D.).

Cell cycle analysis was performed by PI staining. It was demonstrated that treatment of both HT-29 and CCD 841 CoN cells with 1 µM nocodazole for 16 h evoked their cell cycle arrest in the G2/M phase. Besides, the results obtained showed no effect of 0.1% DMSO on the course of the cell cycle of HT-29 and CCD 841 CoN cells after 24 h incubation (Fig. 3A., 3B.). Interestingly, treatment of HT-29 cells with the investigated compound at a concentration of 25 µM for 24 h resulted in a significant arrest in the G2/M phase of the cell cycle (Fig. 3A.). However, there was no effect of the investigated compound on the course of the cell cycle of CCD 841 CoN cells (Fig. 3B.). Following treatment of HT-29 and CCD 841 CoN cells with Th only the percentage of cells in sub-G0/G1 was increased as compared to negative control, which proves Th-induced ER stress conditions and subsequent cell death (Fig. 3C., 3D.). It was also demonstrated that after treatment of HT-29 cells both with Th and the investigated PERK inhibitor NCI 12487 at 25 µM concentration the percentage of cells in sub-G0/G1 was significantly increased as compared to only-Th-treated HT-29 cells. However, following treatment of CCD 841 CoN cells both with Th and NCI 12487 compound at 25 µM concentration the percentage of cells in the sub-G0/G1 phase of the cell cycle was significantly decreased in comparison with only-Th-treated CCD 841 CoN cells, which confirms the inhibitory activity of NCI 12487 against ER stress and cell death induction (Fig. 3D.).

DISCUSSION

Currently available anti-cancer treatment strategies, including surgical intervention, chemotherapy and radiation therapy or a combination of these treatment strategies, are not effective enough to provide full protection from cancer. The major disadvantages of nowadays used anti-cancer treatment are its poor bioavailability, high-dose requirements, adverse effects, low therapeutic indices,

development of multiple drug resistance as well as non-specific targeting. Thereby, anti-cancer treatment strategies, which possess an ability to target only cancerous cells are needed to reduce adverse effects in patients and improve therapeutic efficacy [19, 20]. In this research we evaluated the effectiveness of the small-molecule inhibitor of the PERK-mediated UPR signaling pathway, previously selected from the NCI compound library, using the in vitro model of CRC. To demonstrate the specificity of the tested PERK inhibitor toward HT-29 cancer cells and except adverse side effects all analysis have also been conducted on a normal human colon epithelial CCD 841 CoN cell line. Results obtained in this study have confirmed that the PERK-dependent UPR signaling pathway plays a prominent role in CRC pathogenesis and its further progression as well as modulators of this pathway may constitute an innovative, targeted treatment strategy against CRC.

There are multiple research data confirming a significant role of PERK-mediated UPR signaling pathway activation in carcinogenesis. It has been suggested that PERK plays a pivotal role in cancer cell adaptation to hypoxic stress via regulation of the translation of angiogenic factors important for the development of functional microvessels, thus the PERK-dependent UPR signaling pathway may constitute an important target for the development of novel antitumor modalities [21]. Although there are numerous data confirming a significant role of the ER stress and UPR signaling pathway in cancer development, progression and its resistance to currently available therapies, it is necessary to gather detailed knowledge about the molecular mechanism responsible for the switch of the PERK-dependent UPR signaling pathway from the pro-adaptive to pro-apoptotic branch. Currently, many modulators of the PERK-mediated UPR signaling are being analyzed, whereas only a detailed characterization of the mechanism of activation of the pro-apoptotic arm of the UPR signaling pathway will allow to answer the question of whether to stimulate or inhibit PERK to increase cancer cells' sensitivity to apoptotic cell death. Nowadays, there are still no specific and clinically available positive or negative modulators of the ER stress-mediated UPR signaling pathway, whereas the specific pharmacological modulators of the survival/death switch may constitute a novel anti-cancer treatment strategy [22]. Currently numerous research data have demonstrated that both stimulation and inhibition of the PERK-dependent signaling pathway have paradoxical effects on different stages of carcinogenesis [23]. Thus, there are two opposite approaches to cancer treatment correlated with UPR signaling modulation: activation of more severe and prolonged ER stress conditions and subsequently apoptotic cell death or inhibition of the pro-adaptive UPR signaling pathways leading to increased sensitivity to anti-cancer treatment [24].

The role of the PERK-mediated UPR signaling pathway during oncogenesis, cancer growth, metastasis and chemoresistance has been confirmed by numerous research studies [25]. Interestingly, the level of p-eIF2α, the main PERK substrate, has been significantly elevated, in comparison to normal cells, within cancer cells in bronchioloalveolar carcinoma, Hodgkin's lymphoma, gastrointestinal carcinoma, breast cancer as well as benign and malignant melanocytic and colonic epithelial neoplasms. However, multiple research data have demonstrated that p-eIF2α may play a crucial role in cancer prevention [6]. Multiple research data have demonstrated that in CRC cell lines such as HT-29, SW1116, LoVo, CoCM-1 or SW620, the ER stress and PERK-mediated signaling are closely correlated with more aggressive and resistant phenotypes [18, 26]. Hypoxia is a common feature of tumors that determines both their malignancy and treatment resistance. It has been reported that PERK/eIF2α signaling constitutes a prominent contributor to the tolerance of therapy-resistant cells that arise as a consequence of transient changes in oxygenation in solid tumors [27]. Numerous research studies worldwide are focused on anti-cancer treatment therapies based on the inhibition of the PERK-mediated UPR signaling. GSK260414, the first generation of PERK inhibitors, evoked significant inhibition of PERK in vitro and inhibited the growth of human pancreatic tumor xenograft in mice, whereas triggered a high cytotoxic effect toward pancreatic cells [28–30]. GSK2656157 represented the second generation of PERK inhibitors, exhibited anti-cancer activity, since evoked in vivo dose-dependent inhibition of multiple human tumor xenografts. The potential mechanisms for the anti-cancer effect include altered amino acid metabolism, decreased blood vessel density as well as vascular perfusion. However, GSK2656157, like GSK260414, markedly inhibited pancreatic function and after its withdrawal anti-cancer activity was reversible [31]. Moreover, it has been demonstrated that both GSK2606414 and GSK2656157 may constitute, independently of PERK inactivation, inhibitors of tumor necrosis factor (TNF)-mediated receptor-interacting serine/threonine-protein kinase 1 (RIPK1)-mediated cell death [32]. It has also been reported that p-Coumaric acid (p-CA) may activate the CHOP-mediated pro-apoptotic branch of the UPR signaling pathway in CRC models [33], whereas another study has demonstrated that p-CA has no effect on cancer cell cycle distribution. Additionally, Linalool treatment results in the activation of the pro-apoptotic arm of the UPR signaling, whereas together with p-CA it may only enhance currently available anti-cancer treatment strategies [34]. Interestingly, it has been confirmed that ER stress constitutes a key mechanism for chemoresistance to CRC. The newest research data have demonstrated that PERK modulator CCT020312 markedly decreased CRC cell viability in a dose- and time-dependent manner. However, CCT020312 cannot constitute a single anti-neoplastic treatment. It has been demonstrated that CCT020312 significantly decreased cell viability of CRC cells that were drug-sensitive or -resistant to Taxol treatment. Besides, CCT020312 possesses synergistic effects with Taxol to in vitro induce apoptosis and cell cycle arrest in the G2/M phase. CCT020312, combined with Taxol, evoked increased ER stress conditions via enhanced phosphorylation of PERK, eIF2α and CHOP expression. An in vivo analysis demonstrated that CCT020312, combined with Taxol, reduced tumor growth in CRC xenografts. Thus, the mentioned research has suggested that PERK modulators may significantly improve chemosensitivity of CRC to treatment with other anti-cancer drugs [35]. Also, a study by Jianjun et al. showed that ciclopirox leads to disruption of cellular bioenergetics and activates PERK-mediated ER stress to evoke apoptosis and overcome drug resistance in CRC, whereas its mechanism of antitumor activity is still unexplained [36].

CONCLUSIONS

As is has been mentioned in this article the ER stress-related UPR signaling pathway is considered as a double-edged sword in cancer pathogenesis, since under ER stress conditions cancer cells may survive via activation of adaptation mechanisms or may undergo apoptotic cell death. Although the molecular mechanism that is

directly responsible for the shift of the UPR signaling pathway from pro-adaptive to pro-apoptotic branch of UPR still remains unclear, it has been demonstrated that both ER stress and UPR signaling pathway activation play a key role in cancer pathogenesis as well as in all stages of multistep oncogenic progression. Further research should be conducted to gather detailed knowledge about the PERK-dependent UPR signaling pathway mechanisms in cancer cells as well as to understand how the UPR signaling pathways interact with other signal transduction pathways and how other signaling pathways cooperate with UPR signaling pathways to determine cell fate. However, based on the literature data and results obtained in the presented study, targeting of the components of the UPR signaling pathways via small-molecule modulators of the PERK-dependent UPR signaling pathway may constitute a groundbreaking, targeted treatment strategy against CRC.

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REFERENCES

1. Sawicki T., Ruszkowska M., Danielewicz A. et al.: A Review of Colorectal Cancer in Terms of Epidemiology, Risk Factors, Development, Symptoms and Diagnosis. Cancers (Basel), 2021; 13(9): 2025.
2. Arnold M., Sierra M.S., Laversanne M. et al.: Global patterns and trends in colorectal cancer incidence and mortality. Gut, 2017; 66(4): 683–691.
3. Douaiher J., Ravipati A., Grams B. et al.: Colorectal cancer-global burden, trends, and geographical variations. J. Surg. Oncol., 2017; 115(5): 619–630.
4. Dekker E., Tanis P.J., Vleugels J.L.A., Kasi P.M., Wallace M.B.: Colorectal cancer. Lancet, 2019; 394(10207): 1467–1480.
5. Huang J., Pan H., Wang J. et al.: Unfolded protein response in colorectal cancer. Cell Biosci., 2021; 11(1): 26.
6. Siwecka N., Rozpedek W., Pytel D. et al.: Dual role of Endoplasmic Reticulum Stress-Mediated Unfolded Protein Response Signaling Pathway in Carcinogenesis. Int. J. Mol. Sci., 2019; 20(18): 4354.
7. Rozpedek W., Pytel D., Wawrzynkiewicz A. et al.: Use of Small-molecule Inhibitory Compound of PERK-dependent Signaling Pathway as a Promising Target-based Therapy for Colorectal Cancer. Curr. Cancer Drug Targets, 2020; 20(3): 223–238.
8. Zhao T., Du J., Zeng H.: Interplay between endoplasmic reticulum stress and non-coding RNAs in cancer. J. Hematol. Oncol., 2020; 13(1): 163.
9. Martinon F.: Targeting endoplasmic reticulum signaling pathways in cancer. Acta Oncol., 2012; 51(7): 822–830.
10. Lee A.S.: GRP78 induction in cancer: therapeutic and prognostic implications. Cancer Res., 2007; 67(8): 3496–3499.
11. Ma Y., Hendershot L.M.: The role of the unfolded protein response in tumour development: friend or foe? Nature reviews. Cancer, 2004; 4(12): 966–977.
12. Cojocari D., Vellanki R.N., Sit B. et al.: New small molecule inhibitors of UPR activation demonstrate that PERK, but not IRE1alpha signaling is essential for promoting adaptation and survival to hypoxia. Radiother. Oncol., 2013; 108(3): 541–547.
13. Kraskiewicz H., FitzGerald U.: InterfERing with endoplasmic reticulum stress. Trends Pharmacol. Sci., 2012; 33(2): 53–63.
14. Healy S.J., Gorman A.M., Mousavi-Shafaei P., Gupta S., Samali A.: Targeting the endoplasmic reticulum-stress response as an anticancer strategy. Eur. J. Pharmacol., 2009; 625(1–3): 234–246.
15. Liu Z., Lv Y., Zhao N., Guan G., Wang J.: Protein kinase R-like ER kinase and its role in endoplasmic reticulum stress-decided cell fate. Cell Death Dis., 2015; 6: e1822.
16. Yao Y., Lu Q., Hu Z. et al.: A non-canonical pathway regulates ER stress signaling and blocks ER stress-induced apoptosis and heart failure. Nat. Commun., 2017; 8(1): 133.
17. Szegezdi E., Logue S.E., Gorman A.M., Samali A.: Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Rep., 2006; 7(9): 880–885.
18. Rozpedek W., Markiewicz L., Diehl J.A., Pytel D., Majsterek I.: The role of the adaptive stress response in the pathogenesis of neurodegenerative diseases, cancer and diabetes mellitus type 2. Pol Merkur Lekarski, 2015; 39(234): 393–397.
19. Chakraborty S., Rahman T.: The difficulties in cancer treatment. Ecancermedicalscience, 2012; 6: ed16.
20. Senapati S., Mahanta A.K., Kumar S., and Maiti P.: Controlled drug delivery vehicles for cancer treatment and their performance. Signal transduction and targeted therapy, 2018; 3: 7.
21. Blais J.D., Addison C.L., Edge R. et al.: Perk-dependent translational regulation promotes tumor cell adaptation and angiogenesis in response to hypoxic stress. Mol. Cell. Biol., 2006; 26(24): 9517–9532.
22. Corazzari M., Gagliardi M., Fimia G.M., Piacentini M.: Endoplasmic Reticulum Stress, Unfolded Protein Response, and Cancer Cell Fate. Front Oncol., 2017; 7: 78.
23. Pytel D., Seyb K., Liu M. et al.: Enzymatic Characterization of ER Stress-Dependent Kinase, PERK, and Development of a High-Throughput Assay for Identification of PERK Inhibitors. J. Biomol. Screen., 2014; 19(7): 1024–1034.
24. Yadav R.K., Chae S.W., Kim H.R., Chae H.J.: Endoplasmic reticulum stress and cancer. JCP, 2014; 19(2): 75–88.
25. Madden E., Logue S.E., Healy S.J., Manie S., Samali A.: The role of the unfolded protein response in cancer progression: From oncogenesis to chemoresistance. Biol. Cell., 2019; 111(1): 1–17.
26. Limonta P., Moretti R.M., Marzagalli M. et al.: Role of Endoplasmic Reticulum Stress in the Anticancer Activity of Natural Compounds. Int J Mol Sci., 2019; 20(4): 961.
27. Rouschop K.M., Dubois L.J., Keulers T.G. et al.: PERK/eIF2alpha signaling protects therapy resistant hypoxic cells through induction of glutathione synthesis and protection against ROS. Proc Natl Acad Sci USA., 2013; 110(12): 4622–4627.
28. Axten J.M., Medina J.R., Feng Y. et al.: Discovery of 7-methyl-5-(1-{[3-(trifluoromethyl)phenyl]acetyl}-2,3-dihydro-1H-indol-5-yl)-7H-p yrrolo[2,3-d] pyrimidin-4-amine (GSK2606414), a potent and selective first-in-class inhibitor of protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK). J. Med. Chem., 2012; 55(16): 7193–7207.
29. Moreno J.A., Halliday M., Molloy C. et al.: Oral treatment targeting the unfolded protein response prevents neurodegeneration and clinical disease in prion-infected mice. Sci. Transl. Med., 2013; 5(206): 206ra138.
30. Mercado G., Castillo V., Soto P. et al.: Targeting PERK signaling with the small molecule GSK2606414 prevents neurodegeneration in a model of Parkinson's disease. Neurobiol. Dis, 2018; 112: 136–148.
31. Atkins C., Liu Q., Minthorn E. et al.: Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity. Cancer Res., 2013; 73(6): 1993–2002.
32. Rojas-Rivera D., Delvaeye T., Roelandt R. et al.: When PERK inhibitors turn out to be new potent RIPK1 inhibitors: critical issues on the specificity and use of GSK2606414 and GSK2656157. Cell Death Differ, 2017; 24(6): 1100–1110.
33. Sharma S.H., Rajamanickam V., Nagarajan S.: Antiproliferative effect of p-Coumaric acid targets UPR activation by downregulating Grp78 in colon cancer. Chem. Biol. Interact, 2018; 291: 16–28.
34. Chang M.Y., Shen Y.L.: Linalool exhibits cytotoxic effects by activating antitumor immunity. Molecules, 2014; 19(5): 6694–6706.
35. Lei Y., He L., Yan C., Wang Y., Lv G.: PERK activation by CCT020312 chemosensitizes colorectal cancer through inducing apoptosis regulated by ER stress. Biochem. Biophys. Res. Commun., 2021; 557: 316–322.
36. Qi J., Zhou N., Li L. et al.: Ciclopirox activates PERK-dependent endoplasmic reticulum stress to drive cell death in colorectal cancer. Cell Death Dis, 2020; 11(7): 582.

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