

## Original Article

# OSU-CG5, a novel energy restriction mimetic agent, targets human colorectal cancer cells *in vitro*

El-shaimaa A ARAFA<sup>1,3</sup>, Ahmed H ABDELAZEEM<sup>2,4</sup>, Hany H ARAB<sup>1,5</sup>, Hany A OMAR<sup>1,3,\*</sup>

<sup>1</sup>Department of Pharmacology and <sup>2</sup>Department of Pharmaceutical Chemistry, College of Pharmacy, Taif University, Taif 21974, Saudi Arabia; <sup>3</sup>Department of Pharmacology and <sup>4</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt; <sup>5</sup>Department of Biochemistry, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt

**Aim:** Energy-restriction mimetic agents (ERMAs) are small-molecule agents that target various aspects of energy metabolism, which has emerged as a promising approach in cancer therapy. In the current study, we tested the ability of OSU-CG5, a novel ERMA, to target human colorectal cancer (CRC) *in vitro*.

**Methods:** Two human CRC cell lines (HCT-116 and Caco-2) were tested. Cell viability was assessed using MTT assay. Caspase-3/7 activities were measured using Caspase-Glo 3/7 assay kit. Western blot analysis was used to measure the expression of relevant proteins in the cells. Glucose consumption of the cells was detected using glucose uptake cell-based assay kit.

**Results:** OSU-CG5 dose-dependently inhibited HCT-116 and Caco-2 cell proliferation with the IC<sub>50</sub> values of 3.9 and 4.6 μmol/L, respectively, which were 20–25-fold lower than those of resveratrol, a reference ERMA. Both OSU-CG5 (5, 10, and 20 μmol/L) and resveratrol (50, 100, and 200 μmol/L) dose-dependently increased caspase-3/7 activity and PARP level in the cells. Furthermore, both OSU-CG5 and resveratrol induced dose-dependent energy restriction in the cells: they suppressed glucose uptake and Akt phosphorylation, decreased the levels of p-mTOR and p-p70S6K, increased the levels of ER stress response proteins GRP78 and GADD153, and increased the level of β-TrCP, which led to the downregulation of cyclin D1 and Sp1.

**Conclusion:** OSU-CG5 exhibits promising anti-cancer activity against human CRC cells *in vitro*, which was, at least in part, due to energy restriction and the consequent induction of ER stress and apoptosis.

**Keywords:** colorectal cancer; energy restriction mimetic agent; OSU-CG5; resveratrol; apoptosis; glucose uptake; Akt; mTOR; ER stress; β-TrCP

Acta Pharmacologica Sinica (2014) 35: 394–400; doi: 10.1038/aps.2013.183; published online 27 Jan 2014

## Introduction

The growing interest in utilizing energy restriction without malnutrition as an approach for cancer therapy and prevention is based on the promising results from animal and human trials<sup>[1,2]</sup>. The principle of targeting energy metabolism as an anti-tumor strategy relies on the differences in the ways that normal and transformed cells generate energy<sup>[3]</sup>. Unlike non-neoplastic cells, transformed cells exhibit a high demand for glucose with very limited flexibility for modifying their means of ATP generation in response to changing environmental conditions and energy source availability<sup>[3,4]</sup>. Therefore, cancer cells are exquisitely vulnerable to energy restriction. However, it is extremely difficult to implement chronic energy restriction through actual dietary caloric restriction as an anti-

tumor strategy in humans.

Energy-restriction mimetic agents (ERMAs) are small-molecule agents that target various aspects of energy metabolism<sup>[5]</sup>. Energy restriction suppresses the clonal expansion of transformed cells by limiting the survival factors required for the regulation of cell proliferation, cell cycle control and angiogenesis<sup>[6]</sup>. While very effective in pre-clinical studies, the United States Food and Drug Administration has terminated the clinical trial investigating the use of ERMAs, such as 2-deoxyglucose (2-DG), in prostate cancer (NCT00633087) due to serious side effects<sup>[7]</sup>. Therefore, new ERMAs with higher efficacy and improved safety are currently needed.

Ciglitazone, a thiazolidinedione (TZD), has been used as a scaffold to develop novel ERMAs, such as OSU-CG5, to mimic glucose starvation. These compounds were able to induce multiple components of the starvation-associated responses, which offer advantages over an ERMA that targets only one component of this response<sup>[8]</sup>. In addition, they exhibited high

\* To whom correspondence should be addressed.

E-mail omar.22@buckeyemail.osu.edu

Received 2013-09-29 Accepted 2013-11-27

antiproliferative activity in human prostate cancer LNCaP and human breast cancer MCF-7 cell lines<sup>[9]</sup>.

In the current study, we employed OSU-CG5, a novel ERMA, as a new therapeutic option for the treatment of colorectal cancer (CRC). A major challenge in the systemic treatment of CRC is the cellular resistance to conventional cytotoxic agents, which may be attributed to the heterogeneity of genetic abnormalities that are acquired during the course of carcinogenesis<sup>[10]</sup>. The direct link between obesity and CRC was used as the basis for selecting ERMA as a novel treatment option for CRC<sup>[11-13]</sup>. We hypothesized that ERMA will provide considerable advantages over traditional chemotherapeutic agents and will foster potentially successful strategies for the treatment of CRC. In the present study, we evaluated the *in vitro* antitumor effect of OSU-CG5 and its ability to target CRC through energy restriction compared to the reference ERMA, resveratrol.

## Materials and methods

### Reagents and antibodies

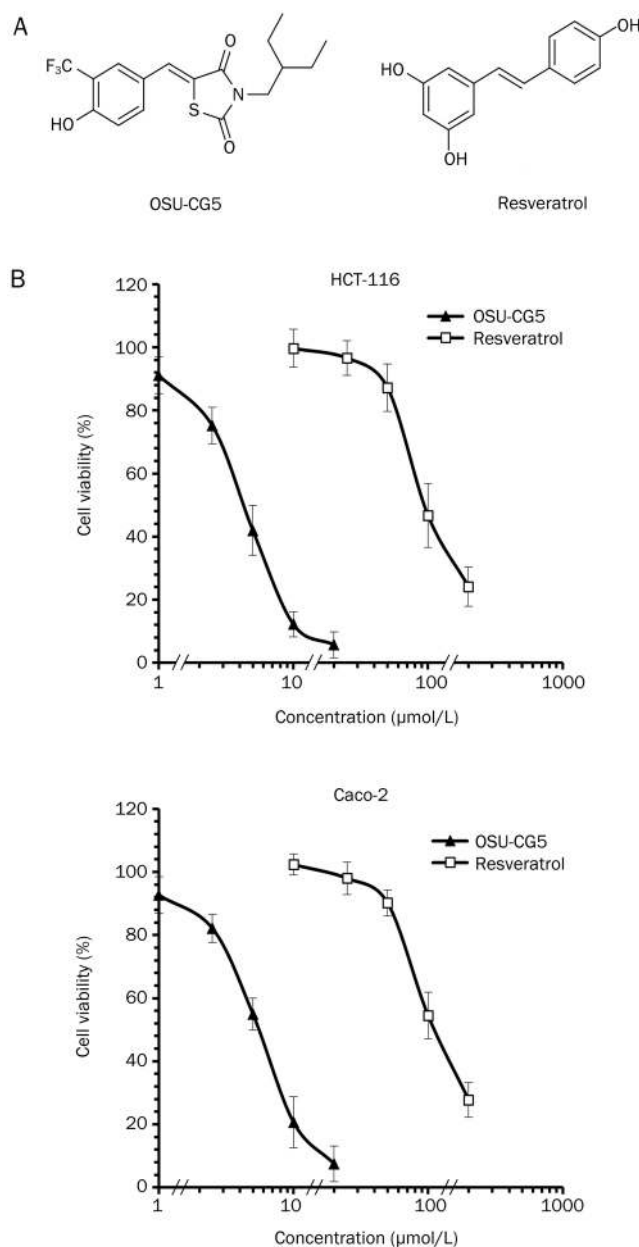
OSU-CG5 (Figure 1A) was obtained from Dr CHEN's laboratory at The Ohio State University. OSU-CG5 was dissolved in dimethyl sulfoxide (DMSO) at a final concentration of 0.1% or less and was then added to the cells. Resveratrol (Figure 1A) was obtained from Sigma-Aldrich Chemical Co (St Louis, MI, USA). Antibodies against various biomarkers were obtained from the following sources: 78 kDa glucose-regulated protein (GRP78), Akt, p-<sup>473</sup>Ser Akt, p-<sup>172</sup>Thr AMPK, AMPK, p-<sup>389</sup>Thr p70S6K, p70S6K, p-<sup>2448</sup>Ser mammalian target of rapamycin (mTOR) and mTOR from Cell Signaling Technologies (Danvers, MA, USA); Sp1, growth arrest and DNA damage-inducible gene (GADD)153 and cyclin D1 from Santa Cruz Biotechnology (Santa Cruz, CA, USA);  $\beta$ -transducin repeat containing protein ( $\beta$ -TrCP) from Invitrogen; and  $\beta$ -actin from Sigma-Aldrich (St Louis, MO, USA). Mouse anti-poly (ADP-ribose) polymerase (PARP) monoclonal antibody was obtained from BD Pharmingen (San Diego, CA, USA). The enhanced chemiluminescence system that was used to detect the immunoblotted proteins was purchased from GE Healthcare (Little Chalfont, Buckinghamshire, UK). Other chemicals and reagents were obtained from Sigma-Aldrich, unless otherwise mentioned.

### Cell culture

The human colorectal carcinoma cell lines HCT-116 and Caco-2 were obtained from the American Type Culture Collection (Manassas, VA, USA). The cell lines were cultured in RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), 1.5 g/L sodium bicarbonate, 2 mmol/L L-glutamine and 1% penicillin/streptomycin. The cells were cultured at 37°C in a humidified incubator containing 5% CO<sub>2</sub>.

### Cell viability analysis

Cell viability was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as previously



**Figure 1.** The antiproliferative activity of OSU-CG5 on CRC cells. (A) The structures of OSU-CG5 and resveratrol. (B) Dose-dependent effects of OSU-CG5 and resveratrol on the viability of the CRC HCT-116 and Caco-2 cell lines after 48 h treatment. Mean $\pm$ SD.  $n=6$ .

described<sup>[14, 15]</sup>; 6 replicates were performed for each sample. Briefly, CRC cells were seeded at a density of  $5 \times 10^3$  cells/well in 96-well flat-bottomed plates; then, 24 h later, the cells were treated with OSU-CG5 or resveratrol at the indicated concentrations. After 48 h, the medium was aspirated and replaced with 200  $\mu$ L of RPMI-1640 medium containing 0.5 mg/mL MTT; the cells were incubated for 2 h. Supernatants were aspirated, and the reduced MTT dye was dissolved in 200  $\mu$ L of DMSO per well. The absorbance at 570 nm was determined using a plate reader. The results were calculated by subtracting the blank readings, which did not contain cells, from the

sample readings.  $IC_{50}$  values were determined using CalcuSyn software (Biosoft, Cambridge, UK).

### Caspase assays

The caspase-3/7 activities in HCT-116 cells treated with OSU-CG5 or resveratrol were measured using the Caspase-Glo 3/7 assay kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Briefly, the cells were seeded into an opaque polystyrene 96-well microtiter plate in six replicates. Following treatment, the prepared reagents were added (100  $\mu$ L/well) and incubated in the dark for 30 min at room temperature. Following incubation, the luminescence was measured using a GloMax-96 Microplate Luminometer (Promega, Madison, WI, USA) at 560 nm, and the data are expressed in relative light units (RLU).

### Immunoblotting

Western blot analysis was performed as previously reported<sup>[16]</sup>. Briefly, the treated cells were washed with PBS, resuspended in sodium dodecyl sulfate (SDS) sample buffer, sonicated, and boiled for 5 min. The cell lysates were centrifuged, and equivalent amounts of proteins were subjected to 10% SDS-polyacrylamide gel electrophoresis. Blots were transferred onto a nitrocellulose membrane using a semi-dry transfer cell. The membranes were washed three times with TBS containing 0.05% Tween 20 (TBST) and were then blocked with TBST containing 5% nonfat milk for 120 min. The membranes were then incubated with the appropriate primary antibodies in TBST containing 5% low fat milk at 4°C overnight and were then washed three times with TBST. The membranes were probed with goat anti-mouse or anti-rabbit IgG-horseradish peroxidase conjugated secondary antibodies for 90 min at room temperature and then washed three times with TBST. The immunoblots were detected using enhanced chemiluminescence. Western blot analysis was conducted on the HCT-116 and Caco-2 cell lines in a parallel fashion. The protein expression/phosphorylation profile was similar for both cell lines; therefore, the results from the HCT116 cells were used as a representative to avoid any redundancy.

### Glucose uptake assay

The ability of the test compounds to inhibit glucose uptake was measured using the Glucose Uptake Cell-Based Assay Kit (Cayman, Ann Arbor, Michigan, USA) according to the manufacturer's instructions. Briefly, the cells were seeded at a density of  $5 \times 10^3$  cells/well in 96-well flat-bottomed plates and were incubated overnight. The next day, the cells were treated for 48 h with OSU-CG5, resveratrol or vehicle in 100  $\mu$ L of glucose- and serum-free media containing 150  $\mu$ g/mL of the fluorescent glucose analog 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose (2-NBDG). At the end of the treatment, the plates were centrifuged for 5 min at  $400 \times g$  at room temperature, and the supernatant was aspirated. Cell-Based Assay Buffer was added to each well (100  $\mu$ L/well). The reading of each well was immediately measured using a fluorescent microscope (excitation/emission=485 nm/

535 nm).

### Statistical analysis

Statistical analyses were conducted using SPSS (Statistical Package for the Social Sciences) 9.0 software (SPSS Inc, Chicago, IL, USA). All results are presented as the mean  $\pm$  standard deviation (SD). Significant differences between the mean values were evaluated using a one-way analysis of variance (ANOVA), followed by the Neuman-Keuls test for multiple comparisons. Differences were considered significant at  $P < 0.05$ .

### Results

#### OSU-CG5 causes greater cytotoxicity than resveratrol in the CRC cell lines

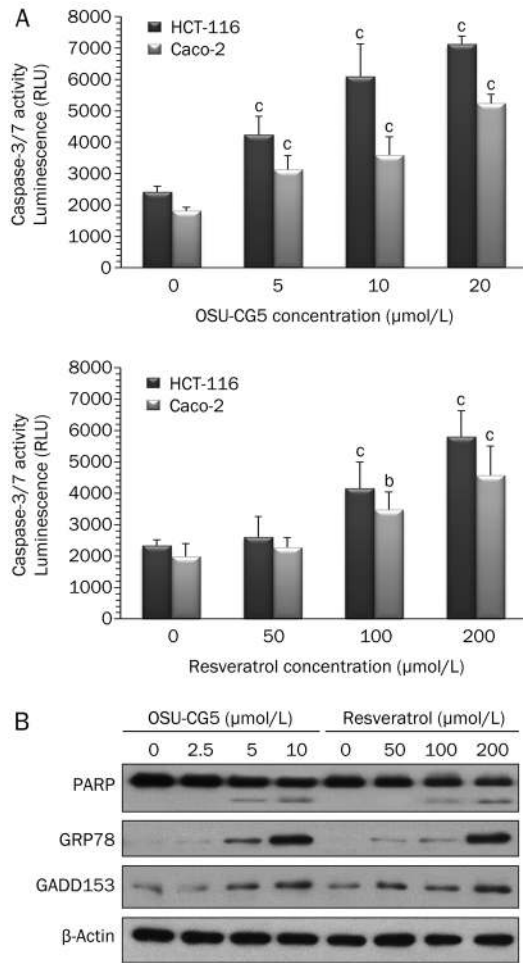
The dose-dependent cytotoxicity of OSU-CG5 was assessed in two CRC cell lines, HCT-116 and Caco-2, using MTT assays and was compared with that of resveratrol (Figure 1B). OSU-CG5 and resveratrol exhibited a differential, suppressive effect on the viability of these two cell lines, with the HCT-116 cell line being more susceptible than the Caco-2 cell line. The respective  $IC_{50}$  values of OSU-CG5 were 3.9 and 4.6  $\mu$ mol/L in the HCT-116 and Caco-2 cells, respectively, while the  $IC_{50}$  values for resveratrol were 94 and 116  $\mu$ mol/L in the HCT-116 and Caco-2 cells, respectively. The antiproliferative activities of OSU-CG5 against these two cell lines were 20–25-fold higher than that of resveratrol.

#### OSU-CG5 induces endoplasmic reticulum (ER) stress and apoptotic cell death in CRC cells

The caspase-3/7 assay indicated that treatment of the HCT-116 and Caco-2 cells with OSU-CG5 or resveratrol led to a dose-dependent increase in the proportion of activated caspase-3/7 (Figure 2A), suggesting that the OSU-CG5- or resveratrol-induced cell death was, at least in part, due to apoptosis. The effect of the test compounds on apoptosis induction was further confirmed by Western blot analysis of HCT-116 cells (Figure 2B). Both OSU-CG5 and resveratrol induced dose-dependent increases in the proteolytic cleavage of PARP, a hallmark of apoptosis. In addition, treating HCT-116 cells with OSU-CG5 or resveratrol induced ER stress, as indicated by the dose-dependent upregulation of ER stress response proteins, such as GRP78 and GADD153.

#### OSU-CG5 inhibits glucose uptake in the CRC cell lines

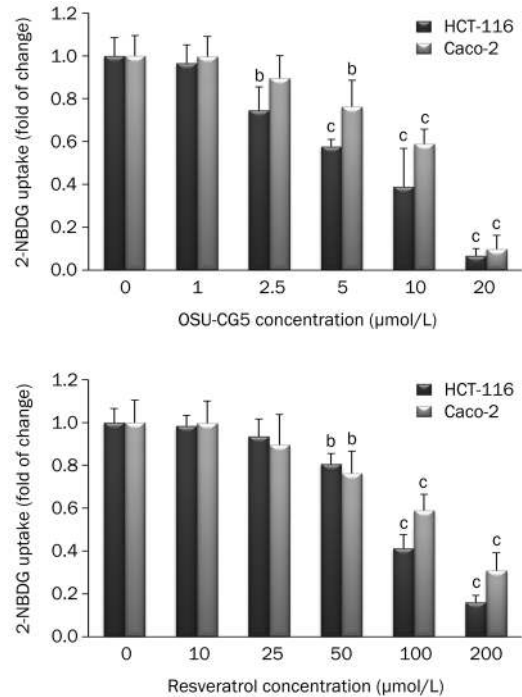
To investigate the hypothesis that OSU-CG5 targets glucose utilization in CRC cells, the suppressive effects of OSU-CG5 on the uptake of the fluorescent glucose analog, 2-NBDG were examined in comparison to resveratrol (Figure 3). Both agents inhibited glucose uptake in the CRC cell lines in a dose-dependent manner. Furthermore, the glucose uptake assay indicated that the ability of OSU-CG5 or resveratrol to suppress the glucose uptake of the CRC cell lines paralleled their respective inhibitory activities on cell viability (Figure 1B), suggesting a potential causal link between these two cellular events.



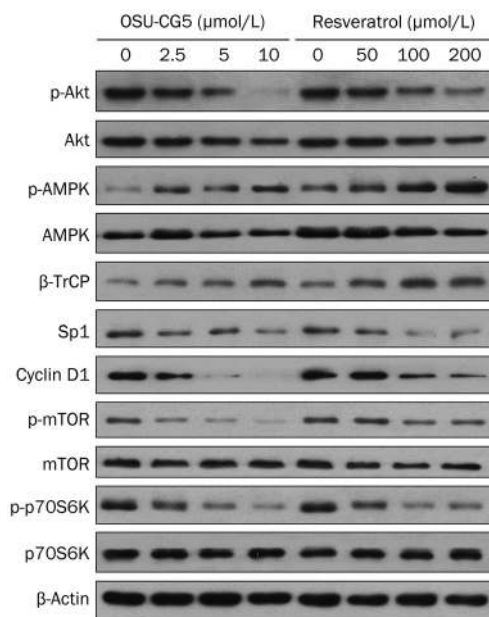
**Figure 2.** OSU-CG5 inhibits CRC cell growth by the induction of ER stress and apoptosis. (A) Caspase-3/7 activities were measured using the Caspase-Glo Assay Kit. Mean±SD.  $n=6$ . <sup>b</sup> $P<0.05$ , <sup>c</sup> $P<0.01$  compared to vehicle (DMSO)-treated cells. (B) Western blot analysis of the expression levels of PARP, GRP78, and GADD153 in HCT-116 cells after the indicated treatment for 48 h in RPMI-1640 medium supplemented with 10% FBS; 30 μg of total protein was loaded in each well.

### The antiproliferative activity of OSU-CG5 is associated with energy restriction cellular responses

Western blot analysis indicated that both OSU-CG5 and resveratrol shared the reported activities of glucose starvation in eliciting energy restriction cellular responses in CRC cell lines. These responses include β-TrCP-facilitated protein degradation, ER stress and adenosine monophosphate-activated protein kinase (AMPK) activation<sup>[9, 17]</sup>. OSU-CG5 and resveratrol treatment led to a dose-dependent increase in β-TrCP expression, which led to the downregulation of its substrates, cyclin D1 and Sp1 (Figure 4). Furthermore, because AMPK inhibits mTOR-p70S6K signaling<sup>[18]</sup>, the increase in AMPK phosphorylation caused by OSU-CG5 and resveratrol was accompanied by a decrease in the levels of p-mTOR and p-p70S6K. The induction of ER stress by OSU-CG5 and resveratrol was dem-



**Figure 3.** OSU-CG5 inhibits glucose uptake in CRC cells. Glucose uptake was measured using the Glucose Uptake Cell-Based Assay Kit after the indicated treatments for 48 h. Mean±SD.  $n=6$ . <sup>b</sup> $P<0.05$ , <sup>c</sup> $P<0.01$  compared to vehicle (DMSO)-treated cells.



**Figure 4.** The antiproliferative activity of OSU-CG5 in CRC cells is associated with energy restriction cellular responses. Western blot analysis of the expression levels of p-Akt, p-AMPK, β-TrCP, Sp1, Cyclin D1, p-mTOR, and p-p70S6K in HCT-116 cells after the indicated treatment for 48 h in RPMI-1640 medium supplemented with 10% FBS; 30 μg of total protein was loaded in each well.

onstrated by the upregulation of two ER stress biomarkers, DNA damage- and growth arrest-inducible gene (GADD)153 and glucose-regulated protein (GRP)78 (Figure 2B). Moreover, OSU-CG5 inhibited Akt phosphorylation, which is known to trigger aerobic glycolysis through different mechanisms<sup>[19, 20]</sup>. Akt inactivation might explain the enhanced inhibitory activity of OSU-CG5 on glucose utilization.

## Discussion

In the current study, we demonstrated the antitumor effects of OSU-CG5 in CRC cells through energy restriction. Compatible with its reported mode of action in prostate cancer cells<sup>[21]</sup>, OSU-CG5 mediates apoptosis in CRC cells through energy restriction and the subsequent induction of ER stress and apoptosis. Increasing knowledge of the pathogenesis of CRC has facilitated the identification of pathways that can be targeted by effective preventive and therapeutic strategies<sup>[22–24]</sup>. Among them, energy restriction represents a promising strategy due to the direct link between obesity and CRC<sup>[25, 26]</sup>. Additionally, the efficacy of energy restriction as a promising chemotherapeutic strategy was supported by the finding that the inhibition of glycolysis by dietary caloric restriction or ERMA, such as 2-DG, suppresses xenograft tumor growth in many animal models<sup>[7, 21]</sup>.

Previous studies have suggested the importance of energy restriction employment in CRC<sup>[25, 26]</sup>. Resveratrol is one of the compounds that have successfully changed the clinical course of patients with CRC through various mechanisms, including energy restriction<sup>[27–29]</sup>. On the other hand, OSU-CG5, by eliciting starvation-associated cellular responses through glucose uptake inhibition, has demonstrated a therapeutic value in targeting prostate cancer cells<sup>[21]</sup>.

Examination of the dose-dependent suppressive effects of OSU-CG5 compared to resveratrol on the viability of the CRC cell lines revealed differential antiproliferative potencies that paralleled the drug's inhibitory activities in glucose uptake (Figure 1B and 3). This OSU-CG5-induced cell death was, at least in part, attributable to apoptosis, as demonstrated by a dose-dependent increase in PARP cleavage. Both OSU-CG5 and resveratrol induced ER stress, and subsequently, apoptosis by eliciting starvation-associated cellular responses, such as

the inhibition of Akt and the activation of AMPK; these results are in agreement with our previous finding that the ERMA OSU-CG12 suppressed prostate cancer and breast cancer cells through the induction of energy restriction<sup>[9]</sup>. ER stress, as indicated by the upregulation of GRP78 and GADD153, as well as the activation of AMPK, has been reported as a target for selective cancer cell killing during calorie restriction<sup>[7, 30]</sup>. Additionally, the inhibition of the PI3K/Akt signaling pathway by OSU-CG5 was confirmed by its suppressive effect on Akt phosphorylation and its downstream targets, such as mTOR and p70S6K. This finding is in agreement with the reported inhibition of Akt phosphorylation by caloric restriction in Hi-Myc transgenic mice<sup>[31]</sup>.

Moreover, our results showed that OSU-CG5 can facilitate  $\beta$ -TrCP-mediated proteolysis, the hallmark of energy restriction execution in cells, similar to TZDs<sup>[9]</sup>. The activation of  $\beta$ -TrCP-mediated proteolysis of target proteins, including cyclin D1 and Sp1, leads to cell cycle arrest and the transcriptional repression of a series of genes involved in the oncogenic transformation in CRC<sup>[32–34]</sup>.

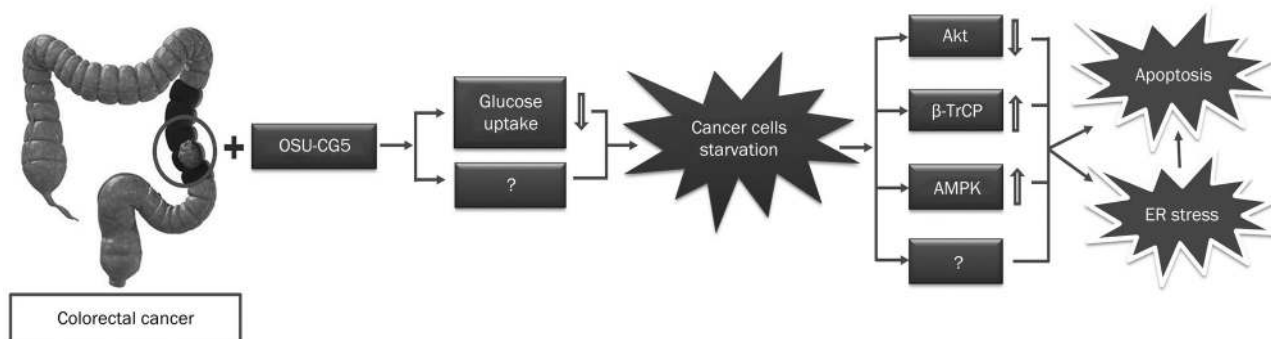
These data suggest that OSU-CG5 offers several advantages over resveratrol. First, it has a lower IC<sub>50</sub> in suppressing the growth of CRC cells. Second, OSU-CG5 treatment in mice was not associated with any evidence of systemic toxicity<sup>[21]</sup>. In conclusion, OSU-CG5 has promising anti-tumor activities against CRC cells. This ERMA induces ER stress and apoptosis in CRC cells through energy restriction mimetic effects (Figure 5). These findings support further investigation to clinically validate the antitumor effects of OSU-CG5.

## Acknowledgements

The current work was supported by grant No 1-434-2863 from the Deanship of Scientific Research, Taif University, Saudi Arabia. The authors would like to thank Dr Ching-shih CHEN at the Ohio State University for his insightful discussion and suggestions on the manuscript.

## Author contribution

El-shaimaa A ARAFA and Hany A OMAR designed the research; El-shaimaa A ARAFA, Ahmed H ABDELAZEEM, Hany H ARAB, and Hany A OMAR performed the research;



**Figure 5.** Graphical abstract depicting the proposed anticancer mechanism of OSU-CG5 in colorectal cancer.

El-Shaimaa A ARAFA, Hany H ARAB, and Hany A OMAR analyzed the data; El-shaimaa A ARAFA, Hany H ARAB, and Hany A OMAR wrote the paper.

### Abbreviations

2-DG, 2-deoxyglucose; CRC, colorectal cancer; DMSO, dimethyl sulfoxide; ERMs, energy restriction mimetic agents; GADD153, growth arrest and DNA damage-inducible gene 153; GRP78, 78 kDa glucose-regulated protein; mTOR, mammalian target of rapamycin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PARP, poly (ADP-ribose) polymerase; TZDs, thiazolidinedione;  $\beta$ -TrCP,  $\beta$ -transducin repeat containing protein.

### References

- Omar HA, Berman-Booty L, Weng JR. Energy restriction: Stepping stones towards cancer therapy. *Future Oncol* 2012; 8: 1503–6.
- Hursting S, Dunlap S, Ford N, Hursting M, Lashinger L. Calorie restriction and cancer prevention: a mechanistic perspective. *Cancer Metab* 2013; 1: 1–11.
- Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nat Rev Cancer* 2011; 11: 85–95.
- DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab* 2008; 7: 11–20.
- Omar HA, Berman-Booty L, Kulp SK, Chen CS. Energy restriction as an antitumor target. *Future Oncol* 2010; 6: 1675–9.
- Thompson HJ, Zhu Z, Jiang W. Dietary energy restriction in breast cancer prevention. *J Mammary Gland Biol Neoplasia* 2003; 8: 133–42.
- Jiang W, Zhu Z, Thompson HJ. Dietary energy restriction modulates the activity of AMP-activated protein kinase, Akt, and mammalian target of rapamycin in mammary carcinomas, mammary gland, and liver. *Cancer Res* 2008; 68: 5492–9.
- Omar HA, Salama SA, Arafa el SA, Weng JR. Antitumor effects of energy restriction-mimetic agents: thiazolidinediones. *Biol Chem* 2013; 394: 865–70.
- Wei S, Kulp SK, Chen CS. Energy restriction as an antitumor target of thiazolidinediones. *J Biol Chem* 2010; 285: 9780–91.
- Pancione M, Remo A, Colantuoni V. Genetic and epigenetic events generate multiple pathways in colorectal cancer progression. *Pathol Res Int* 2012; 2012: 509348.
- Dirx MJ, van den Brandt PA, Goldbohm RA, Lumey LH. Energy restriction early in life and colon carcinoma risk: results of The Netherlands Cohort Study after 7.3 years of follow-up. *Cancer* 2003; 97: 46–55.
- Hughes LA, van den Brandt PA, Goldbohm RA, de Goeij AF, de Bruine AP, van Engeland M, et al. Childhood and adolescent energy restriction and subsequent colorectal cancer risk: results from the Netherlands Cohort Study. *Int J Epidemiol* 2010; 39: 1333–44.
- Thompson HJ, Zhu Z, Jiang W. Protection against cancer by energy restriction: all experimental approaches are not equal. *J Nutr* 2002; 132: 1047–9.
- Omar HA, Sargeant AM, Weng JR, Wang D, Kulp SK, Patel T, et al. Targeting of the Akt-nuclear factor-kappa B signaling network by [1-(4-chloro-3-nitrobenzenesulfonyl)-1H-indol-3-yl]-methanol (OSU-A9), a novel indole-3-carbinol derivative, in a mouse model of hepatocellular carcinoma. *Mol Pharmacol* 2009; 76: 957–68.
- Omar HA, Arafa el-SA, Salama SA, Arab HH, Wu CH, Weng JR. OSU-A9 inhibits angiogenesis in human umbilical vein endothelial cells via disrupting Akt–NF- $\kappa$ B and MAPK signaling pathways. *Toxicol Appl Pharmacol* 2013; 272: 616–24.
- Omar HA, Chou CC, Berman-Booty LD, Ma Y, Hung JH, Wang D, et al. Antitumor effects of OSU-2S, a nonimmunosuppressive analogue of FTY720, in hepatocellular carcinoma. *Hepatology* 2011; 53: 1943–58.
- Chen CH, Huang PH, Chu PC, Chen MC, Chou CC, Wang D, et al. Energy restriction-mimetic agents induce apoptosis in prostate cancer cells in part through epigenetic activation of KLF6 tumor suppressor gene expression. *J Biol Chem* 2011; 286: 9968–76.
- Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* 2003; 115: 577–90.
- Rathmell JC, Fox CJ, Plas DR, Hammerman PS, Cinalli RM, Thompson CB. Akt-directed glucose metabolism can prevent Bax conformation change and promote growth factor-independent survival. *Mol Cell Biol* 2003; 23: 7315–28.
- Elstrom RL, Bauer DE, Buzzai M, Karnauskas R, Harris MH, Plas DR, et al. Akt stimulates aerobic glycolysis in cancer cells. *Cancer Res* 2004; 64: 3892–9.
- Berman-Booty LD, Chu PC, Thomas-Ahner JM, Bolon B, Wang D, Yang T, et al. Suppression of prostate epithelial proliferation and intraprostatic progrowth signaling in transgenic mice by a new energy restriction-mimetic agent. *Cancer Prev Res (Phila)* 2013; 6: 232–41.
- Bialkowska AB, Yang VW. High-throughput screening strategies for targeted identification of therapeutic compounds in colorectal cancer. *Future Oncol* 2012; 8: 259–72.
- Solier S, Zhang YW, Ballestrero A, Pommier Y, Zoppoli G. DNA damage response pathways and cell cycle checkpoints in colorectal cancer: current concepts and future perspectives for targeted treatment. *Curr Cancer Drug Targets* 2012; 12: 356–71.
- Raina K, Agarwal C, Wadhwa R, Serkova NJ, Agarwal R. Energy deprivation by silibinin in colorectal cancer cells: a double-edged sword targeting both apoptotic and autophagic machineries. *Autophagy* 2013; 9: 697–713.
- Kritchevsky D. Colorectal cancer: the role of dietary fat and caloric restriction. *Mutat Res* 1993; 290: 63–70.
- Macrae FA. Fat and calories in colon and breast cancer: from animal studies to controlled clinical trials. *Prev Med* 1993; 22: 750–66.
- Del Follo-Martinez A, Banerjee N, Li X, Safe S, Mertens-Talcott S. Resveratrol and quercetin in combination have anticancer activity in colon cancer cells and repress oncogenic microRNA-27a. *Nutr Cancer* 2013; 65: 494–504.
- Park S, Park O. Modulation of cancer cell proliferation by cell survival signal Akt and tumor suppressive energy sensor AMP-activated protein kinase in colon cancer cells treated with resveratrol. *Food Sci Biotechnol* 2010; 19: 1537–41.
- Fouad M, Agha A, Merzabani MA, Shouman S. Resveratrol inhibits proliferation, angiogenesis and induces apoptosis in colon cancer cells: Calorie restriction is the force to the cytotoxicity. *Hum Exp Toxicol* 2013; 32: 1067–80.
- Saito S, Furuno A, Sakurai J, Sakamoto A, Park HR, Shin-Ya K, et al. Chemical genomics identifies the unfolded protein response as a target for selective cancer cell killing during glucose deprivation. *Cancer Res* 2009; 69: 4225–34.
- Blando J, Moore T, Hursting S, Jiang G, Saha A, Beltran L, et al. Dietary energy balance modulates prostate cancer progression in Hi-Myc mice. *Cancer Prev Res (Phila)* 2011; 4: 2002–14.
- Wei S, Chuang HC, Tsai WC, Yang HC, Ho SR, Paterson AJ, et al. Thiazolidinediones mimic glucose starvation in facilitating Sp1

- degradation through the up-regulation of beta-transducin repeat-containing protein. *Mol Pharmacol* 2009; 76: 47–57.
- 33 Choi HJ, Lim do Y, Park JH. Induction of G<sub>1</sub> and G<sub>2</sub>/M cell cycle arrests by the dietary compound 3,3'-diindolylmethane in HT-29 human colon cancer cells. *BMC Gastroenterol* 2009; 9: 39–50.
- 34 Zhao Y, Zhang W, Guo Z, Ma F, Wu Y, Bai Y, et al. Inhibition of the transcription factor Sp1 suppresses colon cancer stem cell growth and induces apoptosis *in vitro* and in nude mouse xenografts. *Oncol Rep* 2013; 30: 1782–92.