



Research Article

***In Vitro* Oncogenic Effects of Glycated Albumin in Human Colorectal Cancer Cell lines HT29 and SW620 Revealing EpCAM and Galectin-3 Upregulation in Type 2 Diabetic Colorectal Cancer Tissues as Potential Biomarkers**

Yahya Maashi^{1,2#}, Shaykhah Almutairi^{1,3#}, Maram Aldawood^{4,5}, Hamad Al-Eidi¹, Rehab AlRoshody⁵, Hind A Alghamdi^{5,6,7}, Sara Bahattab^{1,3}, Abdulmonem A Alsaleh⁵, Haitham Alkadi⁸, Saleh Alghamdi⁸, Bader Almuzzaini^{1*}, Sabine Matou-Nasri^{1,5,9,10*}

¹Medical Genomics Research Department, King Abdullah International Medical Research Center (KAIMRC), King Saud bin Abdulaziz University for Health Sciences (KSAU-HS), Ministry of National Guard-Health Affairs (MNG-HA), Riyadh 11481, Saudi Arabia

²Department of Pathology and Laboratory Medicine, Human leukocyte antigen Section, King Faisal Specialist Hospital & Research Centre, Jeddah 21499, Saudi Arabia

³Biochemistry Department, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

⁴Zoology Department, King Saud University, Riyadh 11451, Saudi Arabia

⁵Blood and Cancer Research Department, KAIMRC, KSAU-HS, MNG-HA, Riyadh 11481, Saudi Arabia

⁶Biochemistry Department, King Abdulaziz University, Jeddah 21589, Saudi Arabia

⁷Chemistry Department, Princess Nourah bint Abdulrahman University, Riyadh 11564, Saudi Arabia

⁸Research Center, King Fahad Medical City, Riyadh 11525, Saudi Arabia

⁹Biosciences Department, Faculty of the School of Systems Biology, George Mason University, Manassas, VA 20110, United States

¹⁰King Abdullah International Medical Research Center, Riyadh 11481, Saudi Arabia

[#]Contributed equally.

***Corresponding author:** Almuzzaini B & Matou-Nasri S, Medical Genomics Research Department, King Abdullah International Medical Research Center (KAIMRC), King Saud bin Abdulaziz University for Health Sciences (KSAU-HS), Ministry of National Guard-Health Affairs (MNG-HA), Riyadh 11481, Saudi Arabia.

Citation: Maashi Y, Almutairi S, Aldawood M, Al-Eidi H, AlRoshody R, et al. (2024) *In Vitro* Oncogenic Effects of Glycated Albumin in Human Colorectal Cancer Cell lines HT29 and SW620 Revealing EpCAM and Galectin-3 Upregulation in Type 2 Diabetic Colorectal Cancer Tissues as Potential Biomarkers. J Oncol Res Ther 9: 10227. DOI: 10.29011/2574-710X.10227.

Received Date: 12 June, 2024; **Accepted Date:** 20 June, 2024; **Published Date:** 24 June, 2024

Abstract

Glycated albumin (GA), an advanced glycation end product (AGE), is highly generated in patients with type 2 diabetes mellitus (T2DM), and associated with the increased development and progression of colorectal cancer (CRC). However, the biological effects of GA on colorectal adenocarcinoma and metastatic CRC pathogenesis remain unclear. Here, we investigated the impact of methylglyoxal-derived GA on colorectal adenocarcinoma HT29 and metastatic CRC (mCRC) SW620 cell lines, to explore key cellular events involved in oncogenesis. Using a cell viability assay and Transwell[®] inserts, GA stimulated CRC cell proliferation, migration and invasion, compared to the untreated cells. Western blot technology and protein arrays indicated that GA increased the phosphorylation levels of oncogenic extracellular signal-regulated kinase (ERK)1/2 and ribosomal protein kinase p70S6K1. Additionally, the gene and protein expression of epithelial cell adhesion molecule (EpCAM), glycan-binding protein Galectin-3 (Gal-3) and RAGE, the main receptor for AGEs, were upregulated by GA. Bioinformatics analysis confirmed the increased expression levels of EpCAM, Gal-3 and p70S6K1 genes in tissues of T2DM CRC patients compared to their non-diabetic counterparts. Using an anti-RAGE neutralizing antibody, the RAGE blockade prevented GA-induced ERK1/2 phosphorylation, EpCAM and Gal-3 overexpression in CRC cells. Throughout the study, the non-glycated albumin had no effect. Altogether, GA might contribute to the pathogenesis of colorectal adenocarcinoma and mCRC via GA-RAGE axis, leading to the upregulation of EpCAM and Gal-3, revealed as promising biomarkers for the early diagnosis of CRC development and progression in patients with T2DM.

Keywords: Glycated Albumin; Type 2 Diabetes Mellitus; Ig: immunoglobulin; Colorectal Cancer; Biomarkers; EpCAM; Galectin-3.

Abbreviations:

AGE: advanced glycation end product;

AP: activator protein;

BD: Becton Dickinson;

BSA: bovine serum albumin;

cDNA: complementary DNA;

CEL: cell intensity file,

CRC: colorectal cancer;

CTC: circulating tumor cell;

DMEM: Dulbecco's modified Eagle's medium;

EMT: epithelial-mesenchymal transition;

EpCAM: epithelial cell adhesion molecule;

ERK: extracellular signal-regulated kinase;

FBS: fetal bovine serum;

GA: glycated albumin;

Gal-3: galectin-3;

GAPDH: glyceraldehyde 3-phosphate dehydrogenase;

GEO: Gene Expression Omnibus;

GSE: GEO series;

IGF-1R: insulin growth factor-1 receptor;

JNK: c-jun kinase;

MAAPster: Microarray Analysis Pipeline;

MAPK: mitogen-activated protein kinase;

mCRC: metastatic colorectal cancer;

MG: methylglyoxal;

mRNA: messenger RNA;

mTOR: mammalian target of rapamycin;

NF-κB: nuclear factor-kappa B;

O.D.: optical density;

PBS: phosphate-buffered saline;

PI3K: phosphatidylinositol 3-kinase;

PL: phospholipase;

p: phospho;

p70S6K1: protein 70 S6 kinase beta-1;

PTEN: phosphatase and tensin homolog;

RAGE: receptor for AGEs;

RMA: robust multi-chip average;

RT-qPCR: reverse transcription-quantitative polymerase chain reaction;

SD: standard deviation;

SDS: sodium dodecyl sulfate;

SPM: serum-poor medium;

STAT: signal transducer and activator of transcription;

t: total;

T2DM: type 2 diabetes mellitus;

Introduction

Colorectal cancer (CRC) ranks second in cancer-related deaths worldwide and is the third most frequently diagnosed cancer [1]. With the highest incidence in developed countries, the number of CRC patients globally is predicted to increase to 2.5 million by 2035 as a result of hereditary, environmental factors, and unhealthy dietary habits [2, 3]. Mainly caused by insulin resistance and pancreatic β -cell dysfunction, high blood sugar (also known as hyperglycemia) and long-term complications of hyperglycemia can result in chronic consequences, such as type 2 diabetes mellitus (T2DM), diabetic retinopathy, neuropathy, nephropathy, impaired wound healing leading to foot injury, cardiovascular disease, and cancer, particularly CRC [4-6]. The onset, development, and progression of CRC stimulated substantial attention to the advanced glycation end products (AGEs), especially glycated albumin (GA), which are primarily produced under hyperglycemic conditions [7-9].

Food is the primary exogenous source of AGEs (also called dietary AGEs) which are generated inside the body during digestion, absorption, and metabolism, and contributing to the body's total AGEs pool [10]. Physiological endogenous AGEs represent a minor component; however, reprogramming cancer cell metabolism shifting to aerobic glycolysis (i.e., the Warburg effect) leads to increased production of methylglyoxal, a highly reactive α -dicarbonyl compound that enhances the production of AGEs [4, 11, 12]. Generated AGEs are toxic products formed during spontaneous non-enzymatic reactions between free amino groups of proteins, nucleotides, or lipids and reactive carbonyl groups of reducing sugars [13]. AGEs and their main cell surface receptor RAGE are implicated in inflammation and in the pathogenesis of several chronic diseases such as T2DM and cancer, including breast and colon cancer [14-18]. RAGE is weakly expressed in most of the tissues, including the small intestine and colon, but is strongly expressed in the developing embryo, adult brain, and lungs [19-21]. Excessive glucose, external stress, and AGEs can cause cancer cells to overexpress RAGE [22]. Numerous oncogenic signaling pathways are triggered by the AGEs-RAGE axis. These pathways include phosphatidylinositol 3-kinase (PI3K)/Akt and mitogen-activated protein kinase (MAPK), such as extracellular

signal-regulated kinase (ERK)1/2, p38 and c-jun kinase (JNK), which in turn initiates the activation of transcription factors like nuclear factor-kappa B (NF- κ B), activator protein (AP)1 and signal transducer and activator of transcription (STAT)-3 [18].

Colorectal cancer is a heterogeneous disease classified in clinical stratifications, biological and molecular subtypes, including adenocarcinoma and metastatic CRC [23, 24]. Epidemiological data and meta-analysis studies indicated that the colorectal adenoma incidence increases in patients diagnosed with T2DM compared to their non-diabetic counterparts [25, 26]. Clinical studies reported an increased detection of AGEs, including GA, in the blood collected from diabetic patients [27-29]. The clinical significance of the AGEs-RAGE axis in colorectal carcinogenesis was described after the detection of increased AGEs and RAGE expression levels in patient's CRC tissues compared to adjacent non-tumor tissues [30]. The main therapy for CRC patients with T2DM is hypoglycemic agents such as metformin, the first-line antidiabetic drug with anticancer effects, as well as insulin growth factor-1 receptor (IGF-1R) and RAGE inhibitors [2]. Due to the lack of obvious clinical symptoms affecting the early diagnosis of CRC, the primary cause of high morbidity [31], it is urgent to study the oncogenic effects of GA, the main circulating AGEs, and to identify new biomarkers, which could be used for the early diagnosis of CRC in T2DM patients.

During this study, the oncogenic effects of GA on colorectal adenocarcinoma HT29 and metastatic CRC (mCRC) SW620 cell lines were evaluated using cell-based assays. In addition, the biological impact of GA on the expression levels of signaling phospho-proteins and cancer-related proteins was investigated. Bioinformatics analysis was performed on the relevant cancer-related protein expressions induced by GA to confirm any upregulation of their gene expression levels in tissues of T2DM CRC patients compared to their non-diabetic counterparts, which could reveal potential biomarkers.

Materials and Methods

Generation of Glycated Albumin

Glycated albumin (GA), generated from bovine serum albumin (BSA) and highly reactive metabolite methylglyoxal (MG), as well as non-glycated albumin were prepared as previously described [14].

Cell Culture and Treatment

The human colorectal adenocarcinoma (advanced primary tumor) HT29 (#HTB-38) and lymph node metastasis-derived CRC SW620 (#CCL-277) cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA). The cells were cultured in complete medium composed of Dulbecco's modified

Eagle's medium (DMEM from Gibco® containing 4.5 g/l glucose, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS from Gibco®), 100 UI/ml penicillin, and 100 µg/ml streptomycin (Gibco®). The cultured HT29 and SW620 cells exhibited the characteristic cobblestone epithelial-like morphological features and ovoid shape with the formation of aggregates, respectively. The cells were maintained in an incubator at 37°C in a 5% CO₂ humidified atmosphere. When the cells reached 70-80% confluence, they were enzymatically harvested using Gibco® TrypLE™ Express Enzyme solution and then split in a 1:2 ratio. Between passages 3 and 7, the cells were used for downstream applications.

After cell seeding in complete medium, the next day, the medium was replaced with serum-poor medium (SPM), composed of DMEM containing low (1 g/l) glucose and supplemented with 2.5% FBS. After 24 h of incubation, the HT29 and SW620 cells were treated with various concentrations (25, 50, 100, and 200 mg/ml) of GA (reported from previous studies as MG-BSA-AGEs [14, 15]) and non-glycated albumin in SPM, for various incubation times. The untreated cells were the controls.

Cell Viability Assay

The HT29 and SW620 cells (1×10^5 /ml) were seeded in a 96-well plate (Nunc™, Thermo Fisher Scientific) in 100 µl of complete medium and conditioned as described. After 72 h of incubation in the presence or absence of various concentrations (25-200 mg/ml) of GA and non-glycated albumin, the cell viability was determined using the crystal violet assay method [32]. Briefly, the cells were gently washed with warm phosphate-buffered saline (PBS). After carefully removing the PBS solution, 0.2% crystal violet solution (containing 2% ethanol) was added to fix and stain the cells. After two washes in tap water, removing the non-viable cells, the staining of the viable adherent cells was solubilized after addition of 1% sodium dodecyl sulfate (SDS). The optical density (O.D.) of the solubilized solution was measured using SoftMax Pro 6.21 software on the SpectraMax Plus 384 spectrophotometer (Molecular devices, Wokingham, UK) with a reading at 570 nm. The percentage of cell viability was calculated as follows: $[\text{O.D. (treated cells)} - \text{O.D. (blank)}] / [\text{O.D. (untreated cells)} - \text{O.D. (blank)}] \times 100$.

Directional Cell Migration and Invasion Assays

The Boyden chamber system composed of Transwell® inserts in 24-well plates (Nunc™) with an uncoated porous membrane to assess directional cell migration, and a porous membrane coated with diluted Becton Dickinson (BD Biosciences, Franklin Lakes, NJ) growth factor-reduced Matrigel® matrix to assess cell invasion, as previously described [14]. The untreated and treated HT29 and SW620 cells (10^4 /100 µl) were seeded in SPM in the upper

chamber of the insert while the lower chamber (corresponding to the well) contained SPM in the presence or absence of various concentrations (50-200 mg/ml) of GA and non-glycated albumin (100 mg/ml, corresponding to the efficient GA concentration). Each condition was performed in duplicate and each experiment repeated independently four times. After 24 h of incubation, migrated and invaded cells were fixed, stained and counted as previously described [14].

Protein Extraction and Western Blotting Analysis

The HT29 and SW620 cells (0.5×10^5 /ml) were seeded in a 24-well plate (Nunc™, Fisher Scientific, Loughborough, UK). The untreated cells and cells treated with GA or non-glycated albumin were incubated for different incubation times (in minutes, to explore signaling pathways and in hours, to monitor cell surface molecule expression levels). The cells were lysed in cold NP40 lysis buffer (Invitrogen, Paisley, UK) for protein extraction and the protein extracts in the cell lysates were kept on ice. From the protein concentration determination, separation by 12% SDS-polyacrylamide gel electrophoresis to protein transfer onto polyvinylidene fluoride membranes, Western blot procedures were performed as previously described [33] for the detection of phospho-ERK (p-ERK)1/2 and total ERK1/2 (t-ERK1/2), RAGE, epithelial cell adhesion molecule (EpCAM) and galectin-3 (Gal-3) using mouse monoclonal anti-p-ERK1/2 (#sc-7976, 1:1,000 dilution, clone E4, Tyr204 of ERK1), polyclonal anti-t-ERK1/2 antibody (#sc-93, 1:1,000), rabbit monoclonal anti-RAGE antibody (#MA-29007, 1:250) from Invitrogen, and mouse monoclonal anti-Gal-3 antibody (#sc-32790, clone B2C10, 1:1,000) and anti-EpCAM antibody (#sc-66020, clone EBA-1, 1:1,000) were provided by Santa Cruz Biotechnology (Dallas, TX). Rabbit anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH, #ab9485, Abcam, 1:2,000) and anti-α-Tubulin (#ab18251, Abcam, 1:2,000) antibodies were used as loading controls for the detection of these housekeeping proteins. Infrared fluorescent IRDye® 680RD (red)-conjugated goat anti-rabbit or IRDye® 800RD (green)-conjugated goat anti-mouse secondary antibodies (LI-COR Biosciences, Lincoln, NE, USA) were used to detect primary antibodies. These secondary antibodies were diluted (1:5,000 dilution) in Odyssey® blocking buffer and continuously stirred for 1 h at room temperature. Following washing, the blots were scanned, viewed using a LI-COR Odyssey CLx Scanner (LI-COR Biosciences, Lincoln, NE, USA), and analyzed using ImageJ software (<http://rsbweb.nih.gov/ij/index.html>).

Phospho-kinase and oncology protein arrays

The detection of the phosphorylation of 43 human kinases was done using the Proteome Profiler Human phospho-kinase Array kit (#ARY003C, R&D systems, Minneapolis, MN, USA). After

30 min of incubation, the untreated HT29 and SW620 cells and the cells treated with 100 mg/ml (the most effective concentration) of GA and non-glycated albumin were centrifuged at $200 \times g$ for 5 min. The detection of cancer-related proteins in the untreated and treated cell lysates was performed using the Proteome Profiler Human XL Oncology Array kit (#ARY026, R&D systems) after 48 h of incubation. All the steps from the protein extraction to the protein visualization using the ECL Chemiluminescent Kit (Bio-Rad Laboratories) were performed according to the manufacturer's instructions. The proteins were detected as a black spot on a white background using the LI-COR C-DiGit® Blot Scanner and quantified through densitometry using ImageJ software.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis

From the untreated HT29 and SW620 cells and the cells treated with 100 mg/ml of GA and non-glycated albumin for 48 h of incubation, total RNA (1000 ng) was extracted using the RNeasy mini kit (#74104, Qiagen, Hilden, Germany). Due to the low stability of RNA, the RNA extract was converted to complementary DNA using the Superscript® First-Strand Synthesis System (#4368814, Invitrogen, Carlsbad, CA) and performed on a Tetrade thermal cycler (Bio-Rad Laboratories, Hercules, CA). Human messenger RNA (mRNA) was monitored for EpCAM, LGALS3, and GAPDH (internal control) gene expression using primer pairs and sequences (Integrated DNA Technologies, Inc., Coralville, IA) as follows: 5'-TGT GGT TGT GGT GAT AGC AGT T-3' (EpCAM forward); 5'-CCC ATC TCC TTT ATC TCA GCC TTC-3' (EpCAM reverse); 5'-GGC CAC TGA TTG TGC CTT AT-3' (LGALS3 forward); 5'-TCT TTC TTC CCT TCC CCA GT-3' (LGALS3 reverse); 5'-TGA TGA CAT CAA GAA GGT GGT GAA G-3' (GAPDH forward) and 5'-TCC TTG GAG GCC ATG TGG GCC AT-3' (GAPDH reverse). RT-qPCR was performed using the QuantiTect Reverse Transcription kit containing SYBR™ Green PCR Master Mix (#A25742, Applied Biosystem, Thermo Fisher) and each total reaction (10 ml) was prepared with cDNA (25 ng, 4.5 ml), SYBR™ Green PCR Master Mix (5 ml) and primer pair solution (0.5 ml, 10 mM). All reactions were run in triplicate on 7500 real-time PCR system (Applied Biosystem) using the following cyclin conditions: an initial step at 95°C for 10 min, followed by 40 cycles of amplification at 95°C for 15 sec and 60°C for 1 min. Each targeted gene expression was related to that of GAPDH. The relative fold change in gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method [34].

Bioinformatics analysis

Data repositories were searched for publicly available gene expression datasets containing T2DM and non-diabetic CRC patients. The raw signal intensity gene expression (cell intensity file, CEL) files were downloaded and pre-processed using the microarray analysis pipeline (MAAPster) with a robust multi-chip average (RMA) normalization method [35]. The normalized data were checked for quality and used for downstream expression analysis.

RAGE blockade

To demonstrate that GA acts via RAGE in the CRC cells, a RAGE blockade was performed using an anti-RAGE neutralizing monoclonal antibody (#MA5-29007, Invitrogen) along its isotype control immunoglobulin (Ig)G (#31235, Invitrogen) for a 2-h cell pretreatment followed by 30 min or 48 h of additional treatment, as previously described [15]. The protein extraction and Western blot analysis were also done.

Statistical Analysis

The results are expressed as mean $\pm$ standard deviation (SD). The data were obtained from at least three independent experiments. A one-way ANOVA followed by post-hoc Tukey's test was used for multiple comparison analysis. The mean expression level and two-sample independent student's t-test were used for the comparative analysis. A value of $p < 0.05$ was considered significant.

Results

GA modulates HT29 and SW620 Cell Proliferation in a Dose-Dependent Manner

As cell proliferation is the main cellular event contributing to tumor development, we tested the effects of various concentrations (25-200 mg/ml) of GA as well as non-glycated albumin on the human colorectal adenocarcinoma cell line HT29 and mCRC cell line SW620, after 72 h of incubation. The percentage of cell viability was determined after applying the crystal violet method. Compared to the untreated cells, defined as the control and corresponding to 100%, the addition of 50-100 mg/ml of AGEs resulted in the stimulation of HT29 (1.2-1.3-fold, $p < 0.01$, Figure 1A) and SW620 (1.4-1.5-fold, $p < 0.05$, Figure 1B) cell proliferation. Unlike the GA, under all the conditions tested, the non-glycated albumin did not modify the HT29 and SW620 cell proliferation, as compared to the untreated cells (Figure 1).

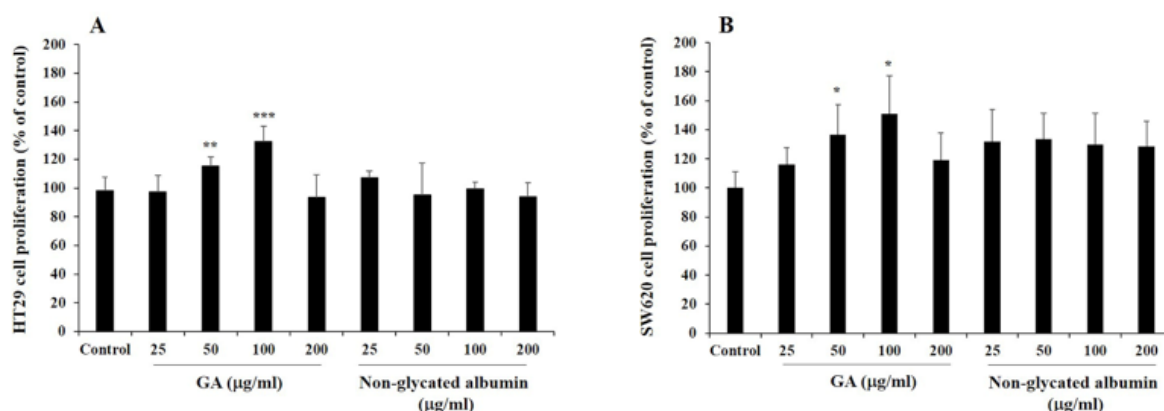


Figure 1: Effects of GA and non-glycated albumin on colorectal adenocarcinoma HT29 (A) and mCRC SW620 (B) cell viability. HT29 and SW620 cells were treated with various concentrations (25-200 µg/ml) of GA and non-glycated albumin for 72 h of incubation. Cell viability was determined using the crystal violet method and expressed as a percentage of the control, with untreated cell viability corresponding to 100%. Results are presented as mean ± SD of three independent experiments. (*), (**) and (***) signify a statistically significant difference ($p < 0.05$, $p < 0.01$ and $p < 0.001$), compared to the control.

GA stimulates HT29 and SW620 directional cell migration and cell invasion in a dose-dependent manner

Using the Boyden chamber systems, we explored the biological impact of GA on the directional migration of HT29 and SW620 cells and cell invasion through diluted growth factor-reduced Matrigel®, a reconstituted basement membrane. Compared to the control, the addition of 100 mg/ml of GA significantly increased (1.2-fold, $p = 0.004$, Figure 2A-B) the number of migrated HT29 cells, and when tested at 50 and 100 mg/ml, GA significantly augmented (1.4-fold, $p = 0.027$ at 50 mg/ml and 1.8-fold, $p = 0.00097$ at 100 mg/ml, Figure 2C-D) the number of migrated SW620 cells (Figure 2A-D). At the highest concentration (200

mg/ml), the GA had no effect on the directional migration of HT29 (Figures 2A-B) and SW620 (Figure 2C-D) cells, compared to the control (Figure 2C-D). During the cell invasion, the addition of 50-100 mg/ml of AGEs significantly increased the number of invaded HT29 cells (1.50-fold, $p = 0.034$ at 50 mg/ml and 1.9-fold, $p = 0.0013$ at 100 mg/ml, Figure 2E-F) and SW620 cells (1.39-fold, $p = 0.0052$ at 50 mg/ml and 1.59-fold, $p = 0.00074$ at 100 mg/ml, Figure 2G-H), compared to the control (Figure 2E-H). Once again, the GA had no effect on the HT29 and SW620 cell invasion when tested at 200 mg/ml, compared to the control (Figure 2). Tested at 100 mg/ml, the effective concentration of GA, the non-glycated albumin did not affect the HT29 and SW620 cell migration and invasion, compared to the control (Figure 2).

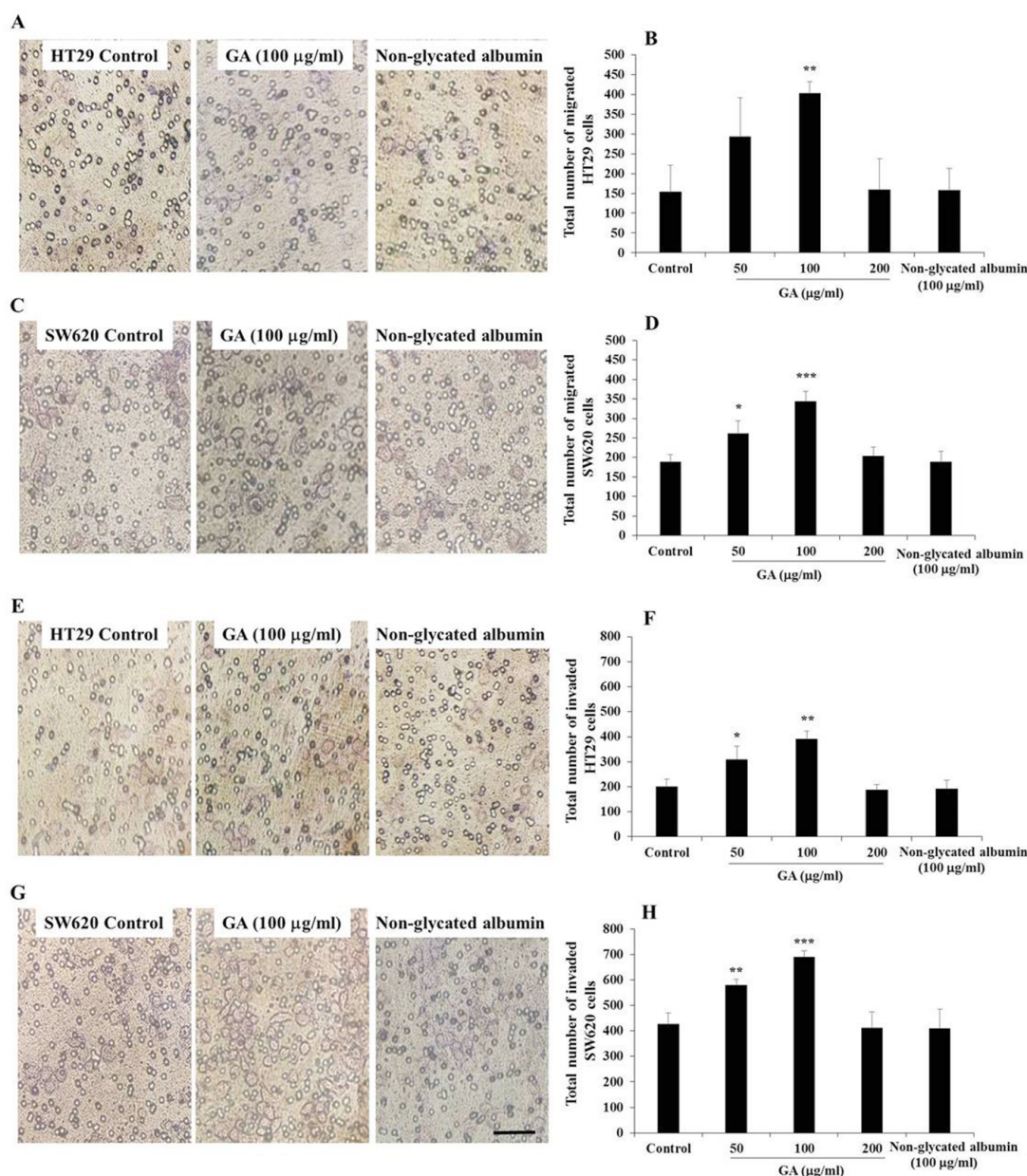
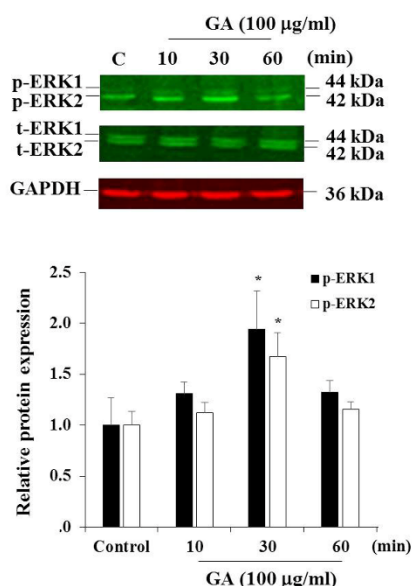


Figure 2: Effects of GA and non-glycated albumin on directional migration (A-D) and invasion (E-H) of colorectal adenocarcinoma HT29 (A-B, E-F) and mCRC SW620 (C-D, G-H) cells using the Boyden chamber. Representative photomicrographs showing HT29 (A) and SW620 (C) cells that migrated across a porous membrane after 24 h of incubation in the absence (Control) or the presence of GA and non-glycated albumin tested at 100 mg/ml. Scale bar = 100 µm. Representative photomicrographs showing HT29 (E) and SW620 (G) cells that invaded across a diluted Matrigel®-coated porous membrane after 24 h of incubation in the absence (Control) or the presence of GA and non-glycated albumin tested at 100 mg/ml. Scale bar = 100 µm. Quantification of the number of migrated HT29 (B) and SW620 (D) cells and the number of invaded HT29 (F) and SW620 (H) cells after treatment with 50-200 mg/ml of GA or 100 mg/ml of non-glycated albumin, compared to control, the untreated cells. Results are presented as the mean ± SD of four independent experiments. (*), (**) and (***) represent a statistically significant difference ($p < 0.05$, $p < 0.01$ and $p < 0.001$), compared to the control.

GA effect on ERK1/2 phosphorylation level in HT29 and SW620 cells

To investigate the signaling pathways involved in the stimulatory effects of GA, the phosphorylation level of the crucial oncogenic signaling protein ERK1/2 was monitored in HT29 and SW620 cells, at different incubation time periods (10, 30, and 60 min) in the presence of the most effective concentration (100 mg/ml) of GA. After 30 min of exposure to the AGEs, the highest degree of ERK1/2 phosphorylation was observed for both the HT29 and SW620 cells (Figure 3). At the shortest exposure time (10 min) and beyond 30 min of exposure (i.e., 60 min), the degree of ERK1/2 phosphorylation was significantly lower than that detected after 30 min of exposure in the SW620 cells and was non-significant in the HT29 cells (Figure 3).

HT29 cell lysates:



SW620 cell lysates:

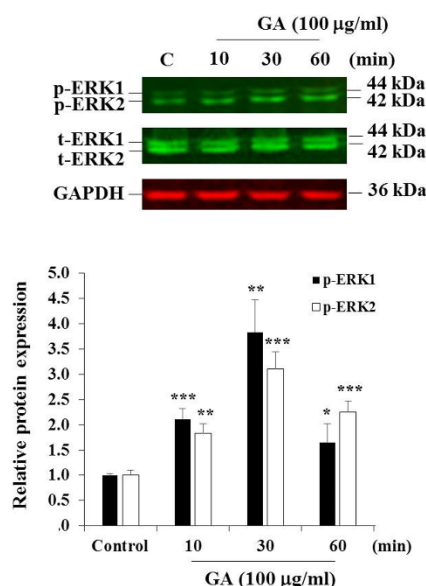


Figure 3: Time course of GA effect on phospho (p)-ERK1/2 expression levels monitored in HT29 and SW620 cells. Representative Western blots showing the variation in p-ERK1/2 expression levels in HT29 (left) and SW620 (right) cells stimulated by 100 µg/ml of GA after different incubation periods varying from 10 to 60 min, compared to untreated control cells. Bar graphs show the relative expression level of p-ERK1/2 in HT29 (left) and SW620 (right) cells, calculated as a ratio to total ERK1/2 (t-ERK1/2), the loading control. The results are presented as mean $\pm$ SD of three independent experiments. (*), (**) and (***) mean a statistically significant difference ($p < 0.05$, $p < 0.01$ and $p < 0.001$), compared to the control.

After defining the optimal exposure time corresponding to the highest degree of ERK1/2 phosphorylation induced by GA, the HT29 and SW620 cells were exposed to various concentrations (50-200 mg/ml) of the GA and non-glycated albumin. After 30 min of incubation, a progressive and statistically significant ($p < 0.05$) increase in ERK1/2 phosphorylation levels was observed in the HT29 and SW620 cells after the addition of 50 and 100 mg/ml of GA, compared to the low ERK1/2 phosphorylation level detected in the untreated cells (Figure 4). When tested at the highest concentration (200 mg/ml) on the HT29 and SW620 cells, no significant difference was obtained between the level of ERK1/2 phosphorylation detected in GA-treated cells and in untreated cells (Figure 4). In contrast to GA-induced ERK1/2 phosphorylation, no change in the ERK1/2 phosphorylation level was noticed in both the HT29 and SW620 cell lines at all the non-glycated albumin concentrations (Figure 4).

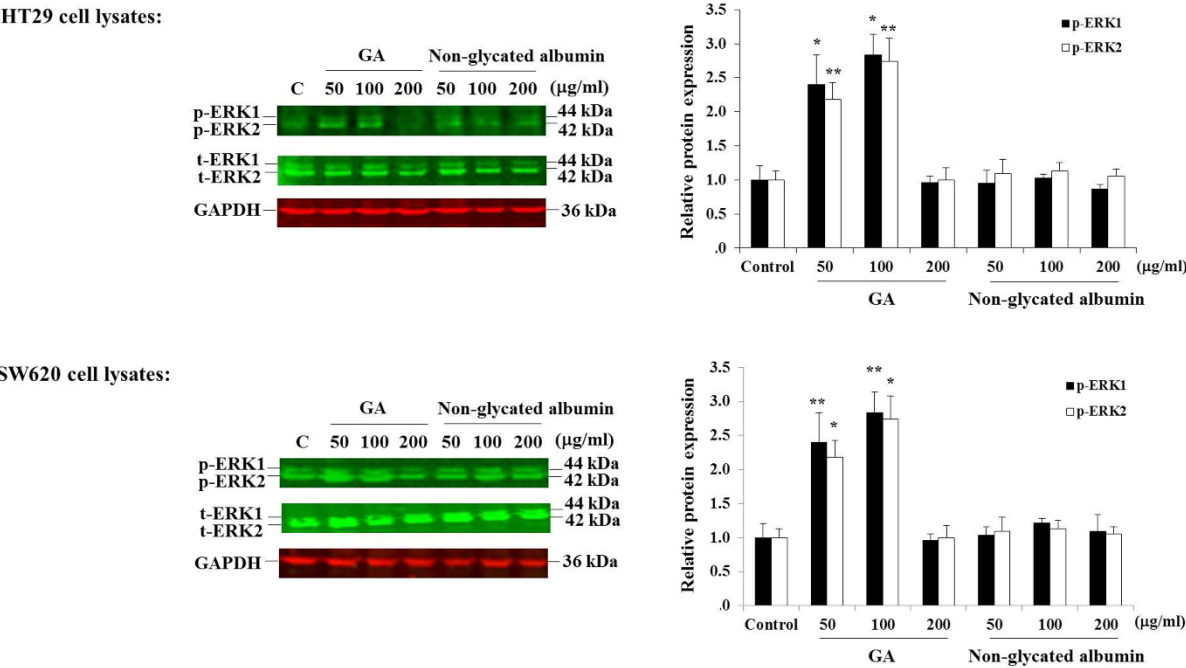


Figure 4: Dose-dependent modulatory effect of GA on p-ERK1/2 expression levels in HT29 and SW620 cells. Representative Western blots showing the effect of various concentrations (50, 100, and 200 mg/ml) of GA and non-glycated albumin on p-ERK1/2 expression levels detected in HT29 (top) and SW620 (bottom) cell lysates, compared to the untreated cells. Bar graphs show the relative expression levels of p-ERK1/2 detected in the HT29 (top) and SW620 (bottom) cell lysates, calculated as a ratio to total ERK1/2 (t-ERK1/2), the loading control. Results are presented as mean $\pm$ SD of three independent experiments. (*) and (**) represent a statistically significant difference ($p < 0.05$ and $p < 0.01$) compared to the control.

A wider investigation of the signaling phospho-proteins involved in the GA stimulatory effects was done using a phospho-kinase array after 30 min of cellular exposure to 100 mg/ml of GA. Compared to the basal expression level detected in the untreated cells (control), in the HT29 and SW620 cells, a strong increase in pro-tumorigenic phospho-proteins, including Akt1/2/3, phospholipase (PL)-Cg and p70S6K1 was observed while the tumor suppressor phospho-p53 was concomitantly overexpressed in the SW620 cells and, to a lesser extent, in the HT29 cells (Figure 5).

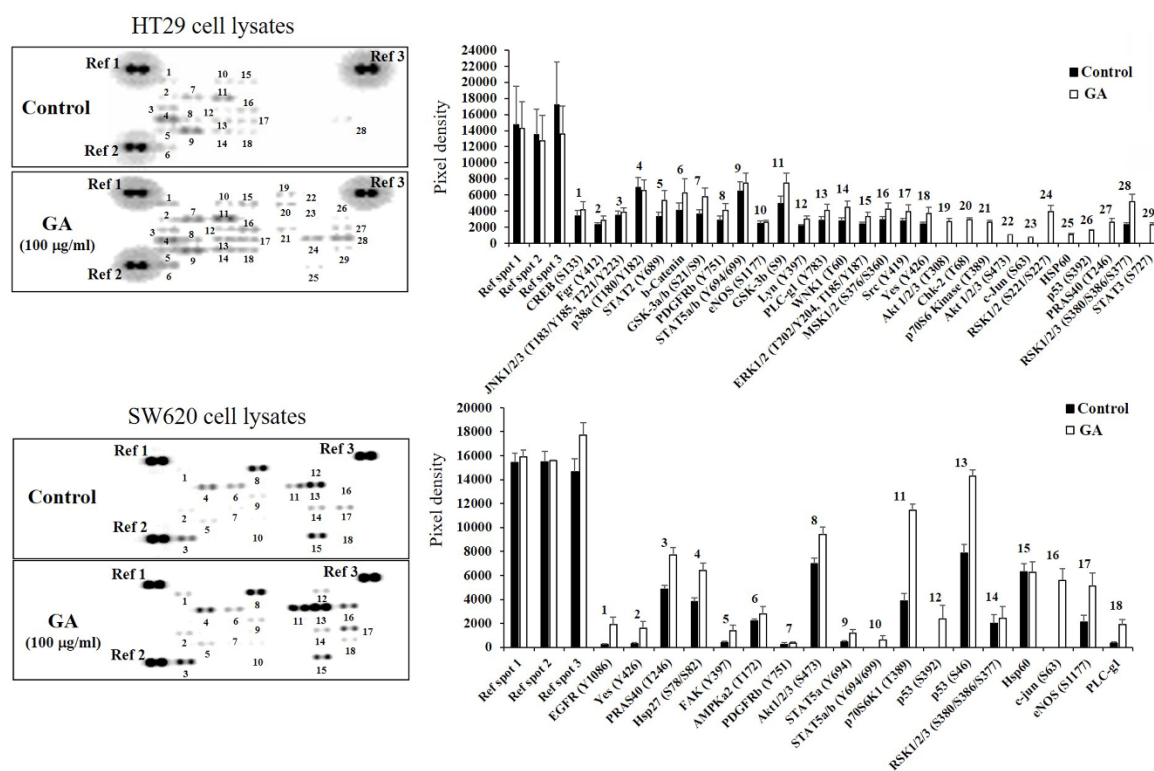


Figure 5: Effect of GA on phospho-protein expression levels in HT29 and SW620 cells. Multi-immunoblots of 39 phospho-proteins expressed in (100 mg/ml) GA-treated HT29 (upper panel) and SW620 (lower panel) cells, compared to the control (untreated cells). Bar graphs show the expression levels of the detected phospho-proteins expressed in pixels. Results are presented as mean $\pm$ SD of three independent experiments.

GA upregulates Galectin-3 and EpCAM expression levels in HT29 and SW620 cells

To pinpoint the cancer-related proteins driving the GA-induced colorectal adenocarcinoma and mCRC pathogenesis, human proteome profiler oncology arrays were performed after 48 h of (HT29 and SW620) cell incubation in the presence or absence of 100 mg/ml of GA and non-glycated albumin. Of the 84 proteins listed in the oncology array, only EpCAM and Gal-3 expressions were detected in both the HT29 and SW620 cell lysates (Figure 6A). The upregulation of the detected cancer-related EpCAM and Gal-3 proteins was also confirmed at the gene expression levels, compared to the basal expression level monitored in the untreated cells, the control (Figure 6B). Besides the EpCAM and Gal-3, the protein level of RAGE, the main receptor for AGEs, was also assessed in both the HT29 and SW620 cell lysates. Using Western blot technology, RAGE, EpCAM and Gal-3 protein expression levels were significantly increased in the GA-treated HT29 and SW620 cells, compared to the basal expression level detected in the untreated cells (Figure 6C).

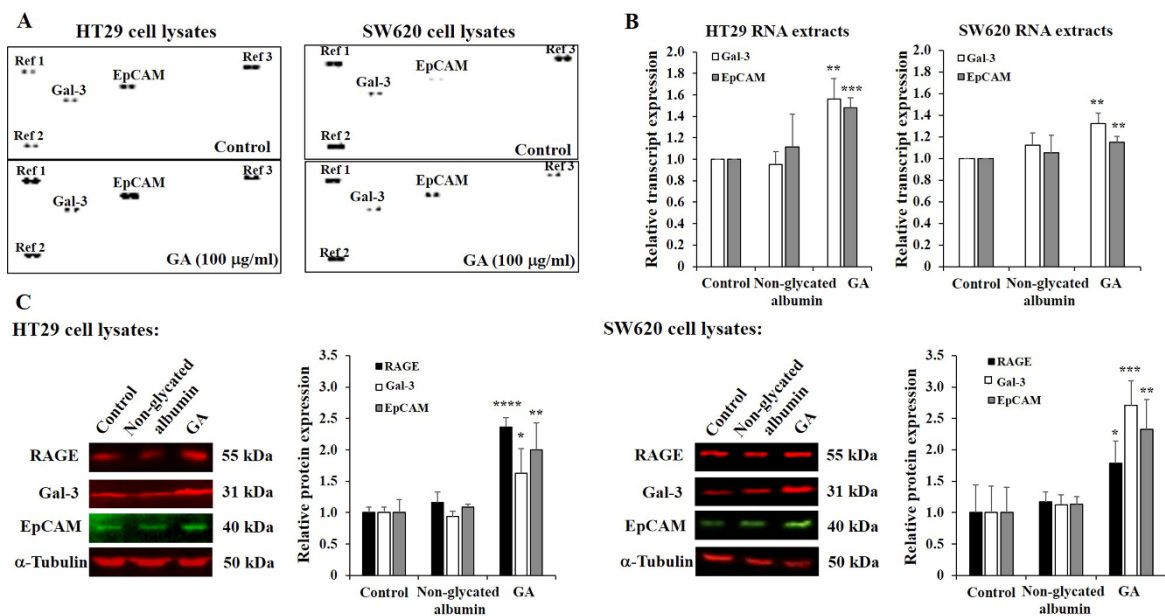


Figure 6: Effect of GA on cancer-related protein and gene expression levels in HT29 and SW620 cells. (A) Representative Oncology array membranes of 84 cancer-related proteins detected in the untreated HT29 (left panel) or SW620 (right panel) cell lysates and the cell lysates after treatment with 100 mg/ml of GA for 48 h of incubation, revealing the detection of Gal-3 and EpCAM. (B) RT-qPCR analyses showing the upregulation of Gal-3 and EpCAM transcripts in both the HT29 and SW620 RNA extracts after 48 h of treatment with 100 mg/ml of GA. (C) Representative Western blots showing GA-induced RAGE, Gal-3 and EpCAM upregulation detected in the HT29 (left) and SW620 (right) cell lysates, compared to the control. Bar graphs show the relative protein expression levels, calculated as a ratio to a-Tubulin. Results are presented as mean ± SD of three independent experiments. (*), (**), (***) and (****) represent a statistically significant difference ($p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.0001$), compared to the control.

Upregulation of Gal-3, EpCAM and p70S6K1 gene expression levels in the tissues of T2DM patients diagnosed with CRC

In the Gene Expression Omnibus (GEO) data repository for publicly available data related to CRC tissues and T2DM patients, a suitable dataset (GEO series, GSE115313) from Del Puerto-Nevedo and colleagues [36] was relevant for our current study. The dataset contains formalin-fixed, paraffin-embedded tissue samples collected from 23 (7 females and 16 males) T2DM and 19 (9 females and 10 males) non-diabetic CRC patients [36]. In silico analysis showed that there was no significant difference in the RAGE gene expression levels monitored in the tissues of T2DM CRC patients and their non-diabetic counterparts (Figure 7A). However, the gene expression levels of EPCAM and LGALS3 (i.e., Gal-3) were significantly upregulated in the tissues of T2DM CRC patients compared to their non-diabetic counterparts (Table 1, Figure 7).

Probe ID	Gene	Mean Expression (CRC with T2DM)	Mean Expression (CRC without T2DM)	Expression Difference	<i>p</i> value*
16879863	<i>EPCAM</i>	2.85	2.5	0.35	0.0038
16784381	<i>LGALS3</i>	2.87	1.97	0.89	0.0109
17026994		3.05	2.94	0.11	0.0272
17029594		3.05	2.96	0.09	0.0436
17034585		3.05	2.96	0.09	0.0436
17037086	<i>Galectin-3</i>	3.05	2.94	0.11	0.0272

17039794		3.05	2.96	0.09	0.0436
17042290		3.05	2.96	0.09	0.0436
*Two sample, independent Student's t-test. P-value < 0.05 is statistically significant.					

Table 1: A comparative analysis on the mean gene expression level for EPCAM and LGALS3 (i.e., Galectin-3) between CRC with T2DM and without T2D.

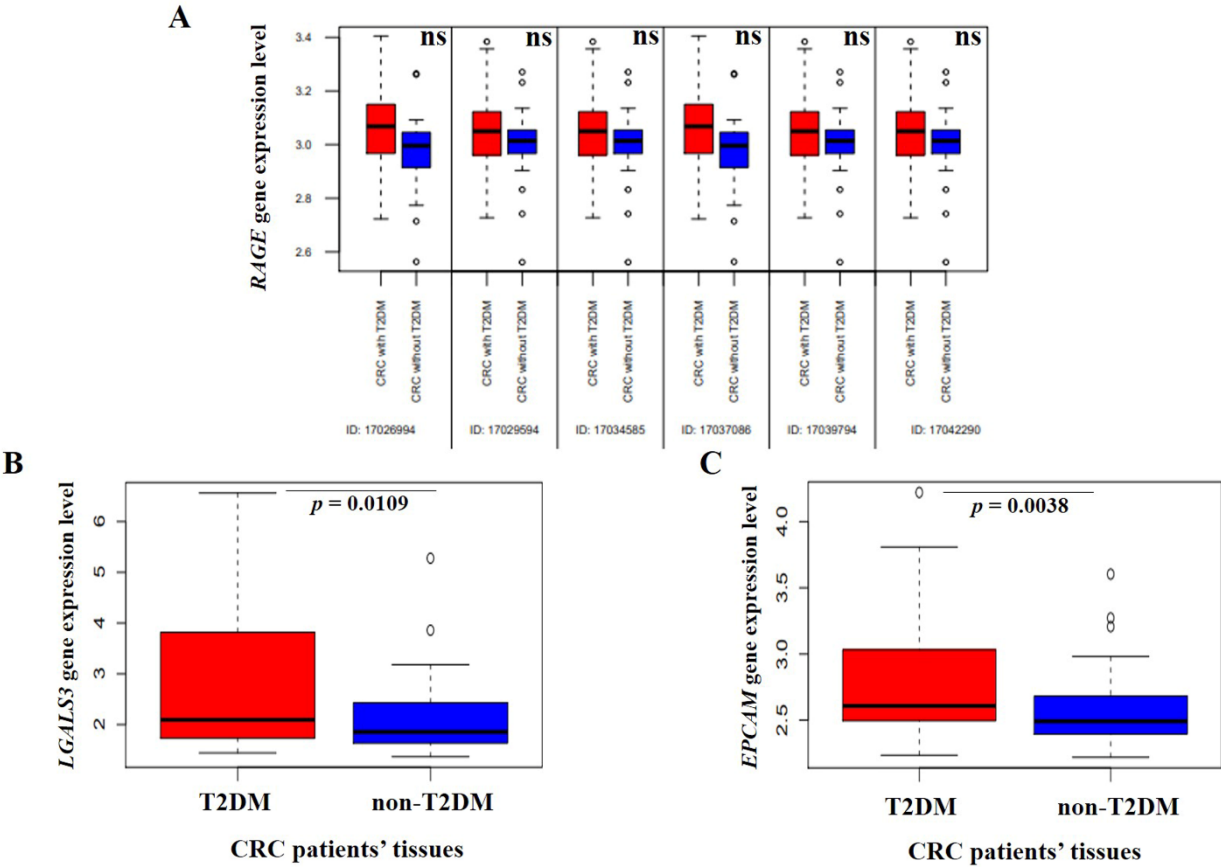


Figure 7: Boxplots showing the gene expression levels of RAGE (A), EPCAM (B) and LGALS3 (C) in tissues of T2DM CRC patients and their non-(T2DM) diabetic counterparts, retrieved from the dataset published by Del Puerto-Nevado et al [36].

Two enzymes of the phosphokinase pathway (i.e., p70S6K1 and phosphatidylinositol 3-kinase) showed significant upregulation of the gene expression in the tissues of T2DM CRC patients compared to their non-diabetic counterparts (upregulation of RPS6KB1 and PIK3C3 by 0.204 and 0.545-folds; $p = 0.0009$ and 0.0065 , respectively) (Figure 8). Of note, there was a significant downregulation of nitric oxide synthase NOS3 by 0.144-fold (p -value = 0.0179 , Figure 8).

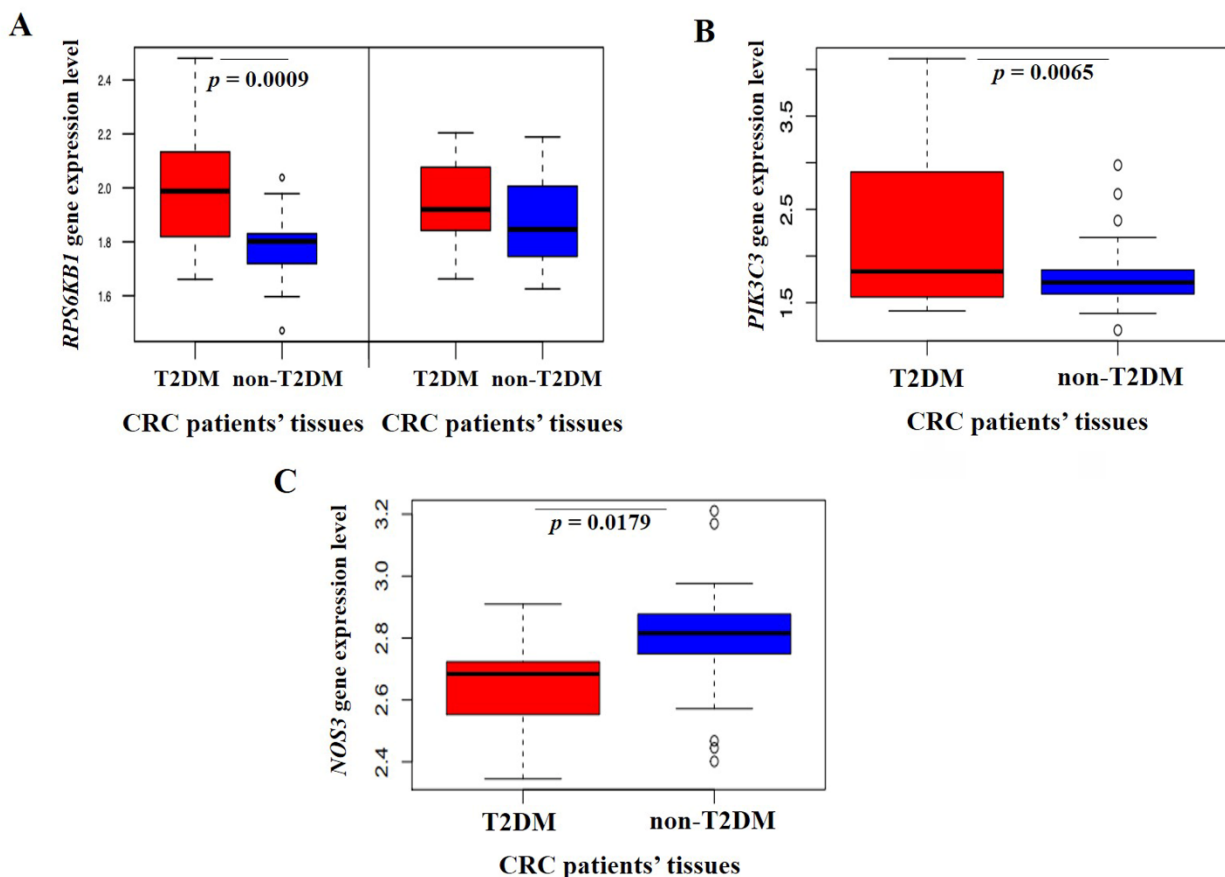


Figure 8: Boxplots showing the gene expression levels of RPS6KB1 (A; left, using probe: 16836626; right, using probe: 17121812), PIK3C3 (B), and NOS3 (C) genes monitored in the tissues of T2DM CRC patients and their non-(T2DM) diabetic counterparts. Data retrieved from dataset published by Del Puerto-Nevado et al [36].

Loss of GA-stimulated oncogenic ERK1/2 over-phosphorylation and EpCAM and Gal-3 upregulation after RAGE blockade

To verify the key role of the GA-RAGE axis in GA-induced oncogenic ERK1/2 over-phosphorylation and upregulation of EpCAM and Gal-3 in mCRC and colorectal adenocarcinoma in T2DM conditions, a blockade of the RAGE function was performed by pretreating the cells with a neutralizing anti-RAGE antibody along its isotype IgG, used as a negative control. In the IgG-pretreated HT29 and SW620 cells, the GA induced ERK1/2 over-phosphorylation and increased Gal-3 and EpCAM expression levels, compared to the basal levels detected in the untreated control cells (Figure 9). In the HT29 and SW620 cells pretreated with anti-RAGE antibodies, the addition of GA did not augment the expression levels of p-ERK1/2, Gal-3 and EpCAM (Figure 9). Thus, GA lost its stimulatory effect on the oncogenic ERK1/2 phosphorylation and upregulation of Gal-3 and EpCAM in colorectal adenocarcinoma HT29 and mCRC SW620 cells after the RAGE blockade.

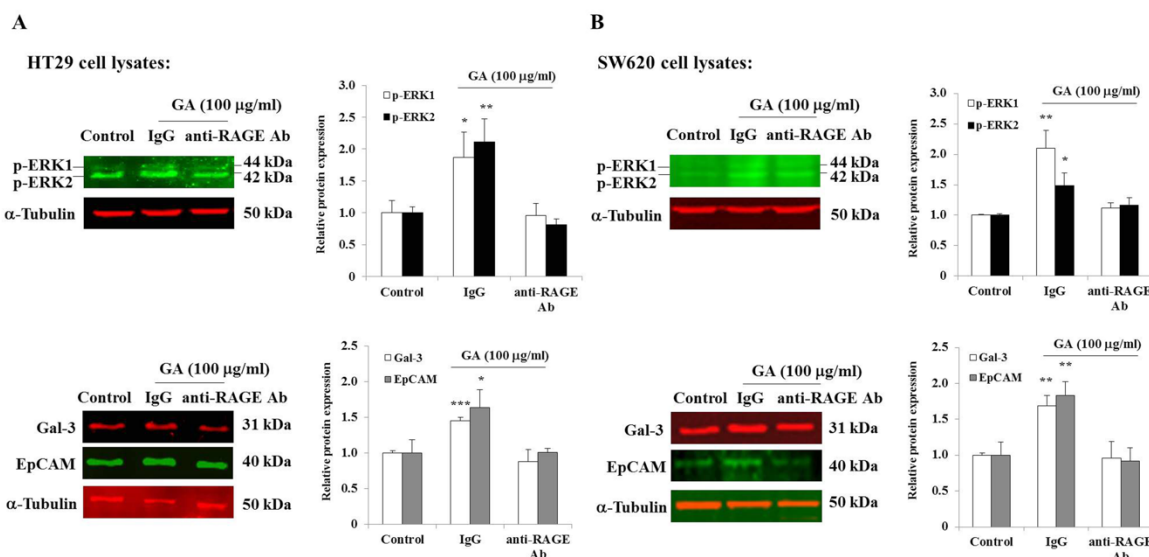


Figure 9: Loss of GA stimulatory effects on ERK1/2 phosphorylation, Gal-3 and EpCAM upregulation in HT29 (A) and SW620 (B) cells after RAGE blockade. Representative Western blot analysis showing ERK1/2 over-phosphorylation (upper panel) and the upregulation of Gal-3 and EpCAM (lower panel) in the IgG-pretreated cells exposed to GA while GA lost its stimulatory effect in the anti-RAGE antibody (Ab)-pretreated cells. Bar graphs show the relative expression of p-ERK1/2, Gal-3, and EpCAM, calculated as a ratio to α-Tubulin. The results are presented as mean ± SD of three independent experiments. (*), (**) and (***) mean a statistically significant difference ($p < 0.05$, $p < 0.01$ and $p < 0.001$), compared to the control.

Discussion

In this present study, we evaluated the biological impact of GA, a stable marker of glycemia, on the main cellular functions involved in the development and progression of CRC using the human colorectal adenocarcinoma cell line HT29 and the mCRC cell line SW620. For all the functional assays used, the CRC cell response to GA described a bell-shaped curve characteristic of the involvement of a dimeric receptor such as RAGE, a mediator of the increase in CRC cell proliferation, migration, invasion, oncogenic signaling pathways including ERK1/2 and p70S6K1 over-phosphorylation, and oncology-related protein expression such as EpCAM and Gal-3. Using a public repository data and bioinformatics analysis, the expression levels of EpCAM and Gal-3 genes were upregulated in CRC tissues of the T2DM patients, compared to their non-diabetic counterparts. The involvement of the AGEs-RAGE axis was confirmed by blocking the RAGE using an anti-RAGE neutralizing antibody, which resulted in the loss of the GA-induced ERK1/2 phosphorylation, EpCAM and Gal-3 upregulation, suggesting that the latter two oncology-related proteins are promising biomarkers for early diagnosis and to monitor the development and progression of CRC in T2DM patients.

Throughout the present study, the impact of T2DM on CRC pathogenesis was assessed by exposing the HT29 and SW620 cells to various concentrations of GA derived from methylglyoxal, a highly reactive glucose metabolite. An epidemiological study reported that a high plasma level of GA was statistically significantly associated with an increased risk of colon cancer [37]. Here, GA, tested at different concentrations (25-100 µg/ml), exerted a dose-dependent effect on all cellular events, including cell proliferation, migration, invasion, ERK1/2 over-phosphorylation, reaching peak stimulation when used at 100 µg/ml. This effective concentration of AGEs that stimulated all these major cellular events of oncogenesis was similar to that reported in our previous studies on breast cancer pathogenesis, including highly invasive non-hormone-dependent breast cancer and poorly invasive hormone-dependent breast cancer cell lines [14, 15].

After observing the pro-tumorigenic effects of GA in colorectal adenocarcinoma HT29 and mCRC SW620 cells, insight into the activated signaling phospho-proteins was revealed using a phospho-kinase protein array. The highest over-phosphorylation levels of the signaling proteins were detected in the SW620 cell lysates and corresponding to p70S6K1, a downstream effector of phosphatidylinositol 3-kinase/protein kinase B (Akt)/ mammalian

target of rapamycin (mTOR) pathway crucial for the main cellular events in CRC [38] and involved in glucose metabolism and protein biosynthesis [39]. In previous studies using the highly invasive triple-negative breast cancer cell line MDA-MB-231, p70S6K1 over-phosphorylation induced by GA was finally confirmed after strong detection of p-p70S6K1 in invasive ductal carcinoma tissues of patients with T2DM, while p-p70S6K1 was weakly expressed in the tissues of their non-diabetic counterparts [40]. Therefore, it will be of great interest to evaluate the expression levels of p-p70S6K1 in metastatic CRC tissues extracted from T2DM patients compared to non-diabetic patients. A high phosphorylation level of the tumor suppressor p53, which did not affect the oncogenic impact of over-phosphorylated c-Jun, was also observed in the SW620 cells compared to the HT29 cells after GA exposure. In agreement with our findings, hyperglycemia induces p53 phosphorylation, which regulates most of the metabolic reactions and contributes to insulin resistance [41]. This reported biological evidence validates our in vitro experimental conditions mimicking T2DM conditions.

To reveal the potential molecular mechanisms involved in the GA-stimulated CRC pathogenesis, the cancer-related protein expression was assessed in the untreated and GA-treated colorectal adenocarcinoma and mCRC cells. Of 84 cancer-related proteins that could be detected using an oncology protein array, only two proteins were visualized and corresponded to EpCAM and Gal-3, whose expression was induced by GA. EpCAM, a type I transmembrane glycoprotein, is a homophilic Ca^{2+} -independent epithelial cell-cell adhesion molecule. The EpCAM expression level varies depending on the cell types and organs. EpCAM is highly expressed in the epithelia of the colon, small intestine, pancreas, liver, and endometrium [42]. Although EpCAM was first discovered as a tumor-associated antigen in CRC, an inverse relationship between tumor stage and percentage of tissue staining in EpCAM was reported, suggesting EpCAM as a prognostic marker to predict the stage of colon adenocarcinoma [43]. Under normal conditions, at the cellular and functional level, EpCAM plays an important role not only in proliferation and cell cycle progression through the upregulation of c-myc, but also in differentiation, invasion, metastasis, cell signaling, formation and maintenance of organ morphology [44, 45]. Under abnormal conditions, EpCAM overexpression was reported to result in in vivo hyperplastic growth of primary human mammary epithelial cells using a chicken chorioallantoic membrane xenograft model [46]. In vitro and in vivo studies, using HT29 cells and nonobese diabetic/severe combined immunodeficiency mice for orthotopic colon cancer and metastasis models, demonstrated that EpCAM promotes colon cancer progression and metastasis via hepatocyte growth factor receptor regulation [47]. Previously, Wang et al [48] demonstrated that EpCAM regulates epithelial-mesenchymal transition (EMT), stemness and metastasis of nasopharyngeal

carcinoma cells through the phosphatase and tensin homolog (PTEN)/AKT/ mTOR pathway. This EpCAM-mediated increase in p70S6K1 is consistent with GA-induced p70S6K1 over-phosphorylation observed in our present study. Used as a diagnostic epithelial marker, EpCAM expressed on circulating tumor cells (CTCs) is commonly utilized for the capture of CTCs from the bloodstream of carcinoma patients [49]. CTCs are shed from the primary tumor site and provide a cellular source of drug resistance and relapse after tumor escape [50]. CTCs, as a surrogate of distant metastasis, may be potentially useful for cancer diagnosis and monitoring of therapeutic effects of malignancies, including CRC [51]. It will be interesting to compare EpCAM expression levels on CTCs captured in the bloodstream of T2DM CRC patients with those from their non-diabetic counterparts.

In the present study, besides the increased expression levels of EpCAM in GA-treated adenocarcinoma and mCRC cells, the expression levels of Gal-3 were also upregulated. Gal-3 is an emerging component involved in the pathogenesis of common diseases, including metabolic disorders, inflammation and cancer onset/progression [52, 53]. Gal-3, a b-galactoside-binding lectin, does not have a transmembrane anchor sequence or signal peptide suggesting that it acts through association with other AGEs-receptor components (i.e., OST48, 80 k-H or p60, p90). Unlike RAGE, a transmembrane protein, which acts independently to activate signaling pathways [18]. In physiological situations, Gal-3 is not expressed or weakly expressed but its expression is induced with aging, in diabetic conditions, and also in cancers including CRC [54]. A meta-analysis study reported that Gal-3 exerted oncogenic activities promoting CRC, suggesting that Gal-3 was a promising diagnostic marker for solid tumor progression [55]. To expand our knowledge on the cellular functions of Gal-3, down-regulation of overexpression of LGALS3 gene applied to cell-based assays mimicking GA-induced colorectal adenocarcinoma and mCRC cell proliferation, migration, invasion, and signaling pathways may reveal the key role of Gal-3 in GA oncogenic activities.

In conclusion, due to the high generation of circulating GA detected in T2DM patients diagnosed with CRC, in the present study, we showed that GA stimulated the main colonic cancer cellular events (i.e., proliferation, migration, invasion, and ERK1/2 over-phosphorylation) involved in colorectal adenocarcinoma and mCRC pathogenesis using the respective most studied in vitro models, HT29 and SW620 human cell lines. We also identified EpCAM and Gal-3, two oncogenic adhesion molecules overexpressed by GA, and, using bioinformatics analysis, upregulated in CRC tissues of T2DM patients, compared to their non-diabetic counterparts. In a therapeutic approach, by blocking RAGE with an anti-RAGE neutralizing antibody, we demonstrated that GA losses its stimulatory effects on ERK1/2 phosphorylation,

the key oncogenic signaling pathway, and on EpCAM and Gal-3 overexpression. For the first time, this present study reports the identification of GA-induced EpCAM and Gal-3 overexpression via RAGE, suggesting them as two potential biomarkers for the early diagnosis of CRC in T2DM patients. Further studies are warranted to verify whether these potential biomarkers are diagnostic biomarkers in a larger cohort and whether they play key roles in T2DM-induced colonic carcinogenesis and possibly therapeutic targets, investigated using in vivo models.

Acknowledgements

We are grateful to Ms Yara Almihmadi from KAIMRC for her technical assistance. We would also like to thank Dr Rawan Alnafisah, assistant professor in the Department of Pharmaceutical Sciences, College of Pharmacy, KSAU-HS, KAIMRC, Riyadh, Saudi Arabia, for producing the graphical abstract.

Funding: All the experiments were financially supported by King Abdullah International Medical Research Center (grant number RC13/249/R) and by King Fahad Medical City (IRF No. 022-020).

Availability of data and materials: The Data generated in the present study may be requested from the corresponding author.

Authors' Contributions: SMN conceived and conducted the study. YM, ShA, MA, HA, RA, HAA, and SB, carried out the cell-based assays, Western blot analysis, visualization, generation of raw data, and reviewed the manuscript. AAA and HA collected the data, performed the bioinformatics analysis and reviewed the manuscript. ShA, SA, BA and SMN interpreted the data, wrote, and reviewed the manuscript. SMN and BA confirm the authenticity of all the raw data. All the authors approved the final manuscript.

Ethics approval and consent to participate: Not applicable

Patient consent for publication: Not applicable

Competing interests: The authors declare no financial or commercial conflict of interest.

References

1. Xi Y and Xu P (2021) Global colorectal cancer burden in 2020 and projections to 2040. *Transl Oncol* 14:101174.
2. GHY, Li SF, Wei R, Jiang Z (2022) Diabetes and colorectal cancer risk: Clinical and therapeutic implications. *J Diabetes Res* 1747326.
3. Kim SH, Park DH, Lim YJ (2023) Impact of diet on colorectal cancer progression and prevention: From nutrients to neoplasms. *Korean J Gastroenterol* 82:73-83.
4. Al-Shehri FS (2019) Glycemic control and microvascular complications of type 2 diabetes among Saudis. *Journal of Diabetes Mellitus* 9:167-175.
5. Harding JL, Pavkov ME, Magliano DJ, Shaw JE, Gregg EW (2019) Global trends in diabetes complications: a review of current evidence. *Diabetologia* 62:3-16.
6. Lega IC and Lipscombe LL (2020) Review: Diabetes, obesity, and cancer – Pathophysiology and clinical implications. *Endocr Rev* 41:bnz014.
7. Chhipa AS, Borse SP, Bakshi R, Lalotra S, Nivsarkar M (2019) Targeting receptors of advanced glycation end products (RAGE): Preventing diabetes induced cancer and diabetic complications. *Pathol Res Pract* 215:152643.
8. Azizian-Farsani F, Abedpoor N, Sheikhha MH, Gure AO, Nasr-Esfahani MH, et al. (2020) Receptor for advanced glycation end products as a fuel to colorectal cancer development. *Front Oncol* 10:552283.
9. Rossi GR, Jensen A, Ng S, Yin Z, Li A, et al. (2023) Advanced glycation end product (AGE) targeting antibody SIWA318H is efficacious in preclinical models for pancreatic cancer. *Sci Rep* 13:16953.
10. Snelson M, Coughlan MT (2029) Dietary advanced glycation end products: Digestion, metabolism and modulation of gut microbial ecology. *Nutrients* 11:215.
11. Almajwal AM, Alam I, Abulmeaty M, Razak S, Pawelec G, et al. (2020) Intake of dietary advanced glycation end products influences inflammatory markers, immune phenotypes, and antiradical capacity of healthy elderly in a little-studied population. *Food Sci Nutr* 8:1046-1057.
12. Sruthi CR, Raghu KG (2022) Methylglyoxal induces ambience for cancer promotion in HepG2 cells via Warburg effect and promotes glycation. *J Cell Biochem* 123:1532-1543.
13. Twarda-Clapa A, Olczak A, Bialkowska AM, Koziolkiewicz M (2022) Advanced glycation end-products (AGEs): Formation, chemistry, classification, receptors, and disease related to AGEs. *Cells* 11:1312.
14. Sharaf H, Matou-Nasri S, Wang Q, Rabhan Z, Al-Eidi H, et al. (2015) Advanced glycation endproducts increase proliferation, migration and invasion of the breast cancer cell line MDA-MB-231. *Biochim Biophys Acta* 1852:429-41.
15. Matou-Nasri S, Sharaf H, Wang Q, Almobadel N, Rabhan Z, et al. (2017) Biological impact of advanced glycation endproducts on estrogen receptor-positive MCF-7 breast cancer cells. *Biochim Biophys Acta Mol Basis Dis* 1863:2808-2820.
16. Chakraborty A, Sami SA, Marma KKS (2021) A comprehensive review on RAGE-facilitated pathological pathways connecting Alzheimer's disease, diabetes mellitus, and cardiovascular diseases. *Egypt J Intern Med* 33: 47.
17. Faruqui T, Khan MS, Akhter Y, Khan S, Rafi Z, et al. (2022) RAGE inhibitors for targeted therapy of cancer: A comprehensive review. *Int J Mol Sci* 24:266.
18. Mollace A, Coluccio ML, Donato G, Mollace V, Malara N (2021) Cross-talks in colon cancer between RAGE/AGEs axis and inflammation/immunotherapy. *Oncotarget* 12:1281-1295.
19. Kim J, Waldvogel HJ, Faull RLM, Curtis MA, Nicholson LFB (2015) The RAGE receptor and its ligands are highly expressed in astrocytes in a grade-dependant manner in the stratum and subependymal layer in Huntington's disease. *J Neurochem* 134:927-42.
20. Lee H, Lee J, Hong SH, Rahman I, Yang SR (2018) Inhibition of RAGE attenuates cigarette smoke-induced lung epithelial cell damage via RAGE-mediated Nrf2/DAMP signaling. *Front Pharmacol* 9:684.
21. Schwertner K, Gelles K, Leitner J, Steinberger P, Gundacker C, et al. (2023) Human intestine and placenta exhibit tissue-specific expression of RAGE isoforms. *Heliyon* 9: e18247.
22. Palanissami G, Paul SFD (2018) RAGE and its ligands: Molecular interplay between glycation, inflammation, and hallmarks of cancer –

Citation: Maashi Y, Almutairi S, Aldawood M, Al-Eidi H, AlRoshody R, et al. (2024) In Vitro Oncogenic Effects of Glycated Albumin in Human Colorectal Cancer Cell lines HT29 and SW620 Revealing EpCAM and Galectin-3 Upregulation in Type 2 Diabetic Colorectal Cancer Tissues as Potential Biomarkers. *J Oncol Res Ther* 9: 10227. DOI: 10.29011/2574-710X.10227.

- A review. *Horm Cancer* 9:295-325.
23. Sagaert X, Vanstapel A, Verbeek S (2018) Tumor heterogeneity in colorectal cancer: What do we know so far. *Pathobiology* 85:72-84.
 24. Sirinukunwattana K, Domingo E, Richman SD, Redmond KL, Blake A, et al. (2021) Image-based consensus molecular subtype (imCMS) classification of colorectal cancer using deep learning. *Gut* 70:544-554.
 25. Yu F, Guo Y, Wang H, Feng J, Jin Z, et al. (2016) Type 2 diabetes mellitus and risk of colorectal adenoma: a meta-analysis of observational studies. *BMC Cancer* 16:642.
 26. Pang Y, Kartsonaki C, Guo Y, Chen Y, Yang L, et al. (2018) Diabetes, plasma glucose and incidence of colorectal cancer in Chinese adults: a prospective study of 0.5 million people. *J Epidemiol Community Health* 72:919-925.
 27. Júnior JPL, Brescansin CP, Santos-Weiss ICR, Welter M, Souza EMD, et al. (2017) Serum fluorescent advanced glycation end (F-AGE) products in gestational diabetes patients. *Arch Endocrinol Metab* 61:233-237.
 28. Indyk D, Bronowicka-Szydełko A, Gamian A, Kuzan A (2021) Advanced glycation end products and their receptors in serum of patients with type 2 diabetes. *Sci Rep* 11:13264.
 29. Xiong JY, Wang JM, Zhao XL, Yang C, Jiang XS, et al. (2021) Glycated albumin as a biomarker for diagnosis of diabetes mellitus: A systematic review and meta-analysis. *World J Clin Cases* 9:9520-9534.
 30. Sakellariou S, Fragkou P, Levidou G, Gargalionis A, Piperi C, et al. (2016) Clinical significance of AGE-RAGE axis in colorectal cancer: associations with glyoxalase-I, adiponectin receptor expression and prognosis. *BMC cancer* 16:174.
 31. Zhang Y, Wang Y, Zhang B, Li P, Zhao Y (2023) Methods and biomarkers for early detection, prediction, and diagnosis of colorectal cancer. *Biomed Pharmacother* 163:114786.
 32. Feoktistova M, Geserick P, Leverkus M (2016) Crystal violet assay for determining viability of cultured cells. *Cold Spring Harb Protoc* pdb. prot087379.
 33. El-Obeid A, Maashi Y, AlRoshody R, Alatar G, Aljudayi M, et al. (2023) Herbal melanin modulates PGE2 and IL-6 gastroprotective markers through COX-2 and TLR4 signaling in th gastric cancer cell line AGS. *BMC Complementary Medicine and Therapies* 23:305.
 34. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25:402-8.
 35. Valdez K, Kuhn S, felloumi (2020) CCBR/MicroArrayPipeline: Release v2.0.0 (v2.0.0).
 36. Del Puerto-Nevado L, Minguez P, Corton M, Solanes-Casado S, Prieto I, Mas S, et al. (2019) Molecular evidence of field cancerization initiated by diabetes in colon cancer patients. *Molecular Oncology* 13: 857-872.
 37. Hidaka A, Budhathoki S, Yamaji T, Sawada N, Tanaka-Mizuno S, et al. (2019) Plasma C-peptide and glycated albumin and subsequent risk of cancer: From a large prospective case-cohort study in Japan. *Int J Cancer* 144:718-729.
 38. Zhong J, Ding S, Zhang X, Di W, Wang X, et al. (2023) To investigate the occurrence and development of colorectal cancer based on the PI3K/AKT/mTOR signaling pathway. *Front. Biosci (Landmark Ed)* 28:37.
 39. Deng H, Chen Y, Li P, Hang Q, Zhang P, et al. (2022) PI3K/AKT/mTOR pathway, hypoxia, and glucose metabolism: Potential targets to overcome radioresistance in small cell lung cancer. *Cancer Pathogenesis and Therapy* 1: 56-66.
 40. Matou-Nasri S, Aldawood M, Alanazi F, Khan AL (2023) Updates on triple-negative breast cancer in type 2 diabetes mellitus patients: From risk factors to diagnosis, biomarkers and therapy. *Diagnostics (Basel)* 13:2390.
 41. Strycharz J, Drzewoski J, Szemraj J, Sliwinski A (2017) Is p53 involved in tissue-specific insulin resistance formation? *Oxid Med Cell Longev* 9270549.
 42. Keller L, Werner S, Pantel K (2019) Biology and clinical significance of EpCAM. *Cell Stress* 3:165-180.
 43. Mokhtari M, Zakerzade Z (2017) EPCAM expression in colon adenocarcinoma and its relationship with TNM staging. *Adv Biomed Res* 6:56.
 44. Huang L, Yang Y, Yang F, Liu S, Zhu Z, et al. (2018) Functions of EpCAM in physiological process and diseases (Review). *Int J Mol Med* 42:1771-1785.
 45. Münz M, Kieu C, Mack B, Schmitt B, Zeidler R, et al. (2004) The carcinoma-associated antigen EpCAM upregulates c-myc and induces cell proliferation. *Oncogene* 23:5748-58.
 46. Martowicz A, Rainer J, Lelong J, Spizzo G, Gastl G, et al. (2013) EpCAM overexpression prolongs proliferative capacity of primary human breast epithelial cells and supports hyperplastic growth. *Molecular Cancer* 12:56.
 47. Lee CC, Yu CJ, Panda SS, Chen KC, Liang KH, et al. (2023) Epithelial cell adhesion molecule (EpCAM) regulates HGR signaling to promote colon cancer progression and metastasis. *J Transl Med* 21:530.
 48. Wang MH, Sun R, Zhou HM, Zhang MY, Lu JB, et al. (2018) Epithelial cell adhesion molecule overexpression regulated epithelial-mesenchymal transition, stemness and metastasis of nasopharyngeal carcinoma cells via the PTEN/AKT/mTOR pathway. *Cell Death Dis* 9:2.
 49. Eslami-S Z, Cortés-Hernández LE, Alix-Panabières C (2020) Epithelial cell adhesion molecule: An anchor to isolate clinically relevant circulating tumor cells. *Cells* 9:1836.
 50. Pantel K, Alix-Panabières C (2022) Crucial roles of circulating tumor cells in the metastatic cascade and tumor immune escape: biology and clinical translation. *J Immunother Cancer* 10:e005615.
 51. Petrik J, Verbanac D, Fabijanec M, Hulina-Tomašković A, Čeri A, et al. (2022) Circulating tumor cells in colorectal cancer: Detection systems and clinical utility. *Int J Mol Sci* 23:13582.
 52. Li Y, Li T, Zhou Z, Xiao Y (2022) Emerging roles of galectin-3 in diabetes and diabetes complications: A snapshot. *Rev Endocr Metab Disord* 23:569-577.
 53. Aureli A, Cornò MD, Marziani B, Gessani S, Conti L (2022) Highlights on the role of galectin-3 in colorectal cancer and the preventive/therapeutic potential of food-derived inhibitors. *Cancers (Basel)* 15:52.
 54. Heusler I, Biron-Shental T, Farladansky-Gershnel S, Pasternak Y, Kidron D, et al. (2021) Enhances expression of Galectin-3 in gestational diabetes. *Nutr Metab Cardiovasc Dis* 31:1791-1797.
 55. Wang Y, Liu S, Tian Y, Wang Y, Zhang Q, et al. (2018) Prognostic role of galectin-3 expression in patients with solid tumors: a meta-analysis of 36 eligible studies. *Cancer Cell Int* 18:172.