High glucose induces epithelial-mesenchymal transition and results in the migration and invasion of colorectal cancer cells

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Abstract. Diabetes mellitus (DM) is associated with an increased risk of colorectal cancer (CRC). Hyperglycemia, a chronic abnormality in diabetes, is an independent predictor of cancer-associated mortality in CRC. However, the underlying biological mechanism of hyperglycemia in CRC cells is largely unknown. In the present study, HCT-116 and HT-29 cell proliferation, apoptosis, migration and invasion were assessed. In addition, the expression of epithelial (E)-cadherin, vimentin and high-mobility group A protein 2 (HMGA2) were assessed using western blotting. The results demonstrated that high glucose (HG; 30 mmol/l) caused CRC cells to lose their epithelial morphology, with a decrease in E-cadherin and an increase in vimentin, suggesting epithelial-mesenchymal transition (EMT). Furthermore, HG significantly enhanced the cell migration and invasion of CRC cells and the expression of HMGA2. Transfection with HMGA2 small interfering RNA reversed the HG-induced changes to CRC cells. In addition, HG promoted CRC cell proliferation and suppressed apoptosis. The results of the present study suggest that hyperglycemia promotes EMT, proliferation, migration and invasion in CRC cells and may provide novel insights into the link between HG and CRC.

Introduction

Colorectal cancer (CRC) is one of the most common malignant tumors and the leading cause of cancer-associated mortality in humans (1). CRC is the third most commonly diagnosed cancer in males and the second in females, with an estimated 1.4 million cases and 693,900 deaths occurring worldwide in 2012 (2). In the USA, CRC is the third leading cause of cancer-associated mortality (3), while tumor invasion and metastasis are the leading causes of patient mortality (4). Many CRCs are metastatic at the time of diagnosis (5). Diabetes mellitus (DM) is a metabolic disorder characterized by increased blood glucose levels (6) and is considered to be one of the most important health problems worldwide (7). It has been demonstrated that DM is associated with an elevated risk of CRC in both men and women (8). A meta-analysis of 8 studies identified a positive correlation between type 2 (T2)DM with a 1.21-fold increased risk of CRC (9). Patients with colorectal cancer and DM have an increased risk of cancer-specific mortality and have worse disease-free survival than those who do not have DM (10,11). DM has also been reported to be a risk factor for CRC, although this remains controversial (11-14).

Epithelial-mesenchymal transition (EMT) is the morphological transformation of epithelial-like cancer cells to an elongated mesenchymal phenotype (15). During EMT, cancer cells stop expressing adhesion proteins, including epithelial (E)-cadherin and claudin-1, and increase the expression of mesenchymal phenotype markers, including vimentin, neural (N)-cadherin and Snail (16). EMT serves an important role in the invasion and metastasis of CRC (17) and is able to induce circulating tumor cell properties in transformed colorectal epithelial cells (18). Furthermore, EMT is highly prognostic for colon cancer recurrence (19). High glucose (HG) induces EMT in breast cancer cells (20) and human peritoneal mesothelial cells (21); however, this effect has not been studied in CRC.

The aim of the present study was to investigate the association between HG and the migration, invasion and apoptosis of colorectal cancer cells. The expression of EMT-associated proteins was detected and the underlying mechanisms were investigated.
Materials and methods

Cell culture and transfection. The human CRC cell lines HCT-116 and HT-29 were obtained from American Type Culture Collection (Manassas, VA, USA). Both cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM; Genom Biotech Pvt., Ltd., Bhandup, Mumbai) containing 10% fetal bovine serum (FBS; Atlanta Biologicals, Flowery Branch, GA, USA), 100 unit/ml penicillin, 100 µg/ml streptomycin with normal glucose (NG; 5.5 mmol/l) or HG (30 mmol/l). Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO₂.

Human samples. A total of 6 CRCs with or without T2DM in this study were histologically and clinically diagnosed at Ningbo Urology and Nephrology Hospital between October 2015 to March 2016 and the tissues were collected immediately following surgical resection for diagnosis. The inclusion criteria was as follows: i) Patients had to be diagnosed with CRC by preoperative pathological biopsy using a colonoscope; ii) aged between 18 and 75 years; iii) exhibit no distant metastasis; and iv) with or without diabetes. Patients were excluded if they: i) Received radiotherapy and chemotherapy prior to surgery; ii) exhibited acute infection; or iii) had a history of abdominal surgery or other malignant tumors. The specimens were then stored at -80°C. The present study was approved by Ningbo Yinzhou Ethics Committee and signed informed consent was obtained from the patients or their family. Patient data is summarized in Table I.

Immunofluorescence. CRC tissues were fixed in 4% formaldehyde solution for 2 h at 25°C and then sectioned into 5-µM-thick frozen sections. The sections were washed in cold PBS 3 times and subsequently blocked with 2% bovine serum albumin V at 25°C (BSA-V; Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) for 1 h. Samples were incubated with primary antibodies against E-cadherin (1:20; sc-8426; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) or goat anti-rabbit secondary antibody (1:1,000; sc-362281; Santa Cruz Biotechnology, Inc.) at room temperature or their family. Patient data is summarized in Table I.

HMGA2 knockdown. The RNA interference technique was used to downregulate HMGA2 in HCT-116 and HT-29 cells (22). HMGA2 small interfering 400 ng (si)RNAs (siHMGA2-1, 5'-GAA AGC AGA GAC CAU UGG ATT-3'; siHMGA2-2, 5'-GAA AGC AGAGCCAUUGGATT-3'; Shanghai Genechem Co., Ltd., Shanghai, China) were synthesized and transfected into cells using RNAiMAX (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol. Following 48 h transfection, HMGA2 expression was confirmed by western blotting. Western blotting was then performed as aforementioned.

MTT assay. HCT-116 and HT-29 proliferation was measured using an MTT assay. Cells were incubated with 0.35 mg/ml MTT solution at 37°C for 4 h. The medium was removed, 100 µl dimethylsulfoxide (DMSO) was added and the mixture was vortexed at 112 x g for 10 min at 25°C. The optical density was read at 490 nm and all experiments were performed 3 times.

Ki-67 expression and apoptosis analysis. Cells were seeded in 6-well plates and treated with NG or HG, respectively, for 4 days. Cells were digested using 1 ml trypsin (#C0201; Beyotime Institute of Biotechnology, Beijing, China), washed twice with PBS and incubated in 100 µl fixation buffer (Biolegend, Inc., San Diego, CA, USA) at room temperature
for 15 min. Cells were then washed with 100 µl permeabilisation buffer (Biolegend, Inc.). Following centrifugation at 1,500 x g for 3 min at 25°C, the cells were resuspended in 100 µl permeabilisation wash buffer containing Alexa Fluor 647 mouse anti-Ki-67 antibody (1:100; 561126; BD Biosciences) and incubated at room temperature in the dark for 30 min. A total of 400 µl permeabilisation wash buffer was added to resuspend the cells for flow cytometric analysis using a FACS flow cytometer (BD Biosciences).

Cell apoptosis was assayed using the Annexin V-phycoerythrin (PE) Apoptosis Detection kit (BD Biosciences). Cells were washed twice with cold PBS and resuspended in Annexin V Binding buffer at a concentration of 1.0x10^5 cells/ml. Specifically, this suspension (100 µl) comprised 1 µl Annexin V-PE, 1 µl 7-aminoactinomycin D and 98 µl Binding buffer. The cells were vortexed gently and incubated for 15 min at room temperature in the dark. To each tube, 400 µl of Binding buffer added and cells were analyzed using a FACS flow cytometer (BD Biosciences) and FlowJo 7.6 software (FlowJo LLC, Ashland, OR, USA).

**Statistical analysis.** Data are expressed as the mean ± standard deviation. One-way analysis of variance was used to test the Homogeneity of variance, then a Mann-Whitney U was used to compare differences between groups. All statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA) and SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

**Results**

**HG induces EMT in CRC tissues and cells.** E-cadherin protein expression was measured in tumor tissues from 3 patients with CRC and DM and 3 patients with CRC without DM using immunofluorescence and western blotting. The area of tumor cells with positive E-cadherin staining was increased in patients without DM compared with those with DM (Fig. 1A). Furthermore, the results of western blotting confirmed that the expression of E-cadherin protein was lower in patients with DM compared with patients without DM (Fig. 1B); however, the expression of vimentin protein was significantly higher in patients with DM compared with those without DM (Fig. 1B). HCT-116 and HT-29 cells were exposed to HG for 4 days and it was demonstrated that HG reduced the expression of E-cadherin protein, whereas the expression of vimentin protein was increased (Fig. 1C). These results suggest that HG serves an important role in the EMT of CRC cells.

**HG promotes the migration and invasion of CRC cells.** EMT is characterized by a loss of cell-to-cell adhesion and increased cell migration and invasion (23). As such, the effect of HG on the metastatic capability of CRC cells was investigated. Scratch assays revealed that wound healing was faster in HCT-116 and HT-29 cells grown in HG conditions compared with those grown in NG (Fig. 2A and B). Furthermore, HG accelerated the cells ability to invade and migrate compared with NG (Fig. 2C and D). These results suggest that HG is able to promote the invasion and migration of CRC cells.

**HG promotes EMT by increasing the level of HMGA2 protein.** HMGA2 is known to control the expression of a diverse set of transcription factors associated with the regulation of E-cadherin transcription (24,25). HMGA2 has been reported to regulate EMT in gastric cancer (26,27), tongue squamous cell carcinoma (28) and prostate cancer cells (29). As such, it was hypothesized that HMGA2 may regulate HG-induced EMT and the expression of HMGA2 in CRC cells exposed to HG or NG for 4 days was assessed. HMGA2 was significantly upregulated in HG-stimulated cells compared with those treated with NG (Fig. 3A and B). HMGA2 expression was knocked down in HCT-116 and HT-29 cells and confirmed used western blotting (Fig. 3C and D). The results revealed that HMGA2 knockdown significantly increased E-cadherin protein expression and decreased vimentin protein expression in HG-stimulated cell compared with those treated with NG (Fig. 3E and F).

**HG enhances cell viability and suppresses apoptosis in CRC cells.** To characterize the functional roles of HG in cell proliferation, MTT assays were performed and Ki-67 was measured. The results revealed that HG enhances the viability of HCT-116 and HT-29 cells in a time-dependent manner (Fig. 4A and B). Ki-67 is a nuclear antigen present only in proliferating cells and is one of the most widely used proliferation-associated markers in cancer cells (30). Ki-67 staining demonstrated that HG enhances the expression of Ki-67 and therefore the proliferation of HCT-116 and HT-29 cells compared with NG (Fig. 4C and D). The role of HG on apoptosis in HCT-116 and HT-29 cells was also assessed and it was revealed that HG significantly decreased apoptosis compared with HG (Fig. 4E and F).

**Discussion**

Impaired metabolism and unlimited growth are two hallmarks of cancer and serve an important role in cancer progression (31) and DM promotes the growth and metastasis of tumor cells (32). The results of the present study demonstrate that HG increases HMGA2 expression and induces EMT in CRC cells.

The invasive and migratory capabilities of CRC cells were significantly enhanced by HG, while transfection with

### Table I. Patient data.

| Patients       | Number of patients | Age   | Sex ratio (F:M) | Comorbidities     |
|----------------|--------------------|-------|-----------------|-------------------|
| With diabetes  | 3                  | 56-64 | 2:1             | No comorbidities  |
| Without diabetes | 3              | 60-65 | 2:1             | One with hypertension |
HMGA2 siRNA suppressed HG-induced EMT in HCT-116 and HT-29 cells. In addition, HG enhanced the proliferation and reduced the apoptosis of CRC cells. These results suggest that DM causes EMT and promotes metastasis in CRC cells. As such, DM may induce CRC tumor growth.

DM has been reported to have pro-migratory and pro-invasive effects in both normal (33) and cancer cells (34-38). Epidemiological studies have previously established an association between inflammation and DM (39-41). The chronic inflammatory response may contribute to DM development by causing insulin resistance, which in turn intensifies hyperglycemia to promote long-term complications of diabetes (42). Furthermore, inflammation induces EMT in CRC (43,44). Previous research has verified that HG induces EMT in pancreatic and breast cancers (45). Similarly, the results of the present study demonstrate that DM is associated with the downregulation of E-cadherin and upregulation of vimentin in patients with CRC. Meanwhile, HG induces EMT in CRC.

Figure 1. HG promotes epithelial-mesenchymal transition in patients with CRC and CRC cells. (A) Immunofluorescence for E-cad in tumors from patients with CRC with or without DM (magnification, x40). Western blot analyses of E-cad and VIM in (B) CRC tissues and (C) HCT-116 and HT-29 cells. **P<0.01 vs. Ctrl. HG, high glucose (30 mmol/l); CRC, colorectal cancer; E-cad, epithelial cadherin; DM, diabetes mellitus; VIM, vimentin; Ctrl, control.
cells *in vitro*. Downregulated E-cadherin expression is associated with lymph node metastases, poor tumor differentiation and worse prognosis in patients with CRC (46,47). Conversely, increased vimentin expression is significantly associated with lymph node metastasis and poor prognosis in CRC (48). The results of the present study demonstrated that the invasion and migration capabilities of CRC cells were enhanced by the occurrence of EMT. HMGA2 is a chromatin remodeling factor that is able to alter chromatin architecture to activate transcriptional enhancers (49). High expression of HMGA2 is associated with cell proliferation and increased metastasis in a number of cancers (50). The results of the present study are consistent with a number of previous studies in which it was reported that HMGA2 activates EMT in cancer cells (51,52). At least 11 EMT-associated molecular pathways have been reported in the literature about CRC cells, including β-catenin-associated EMT, transforming growth factor-β and Wnt pathway-associated EMT and aberrant NOTCH-1 signaling associated EMT (53) Future studies should aim to elucidate whether there any other signaling pathways are associated with HG-induced EMT.

HG in patients with DM may alter the expression of genes that promote cell proliferation in the colon (32-37,54). The rate of proliferating cell nuclear antigen-positive cells is higher in patients with CRC and DM compared with patients with CRC alone (55). HG conditions enhance cell proliferation via decreasing the population of cells arrested in the G0/G1 phase (56). In accordance with the results of the present study, HG has previously been reported to increase the proliferation of CRC cells (57).

The present study is not without limitations. The effect of HG, which is the main feature of DM, was studied in isolation. T2DM is typically accompanied by other metabolic abnormalities, including hyperlipidemia and hyperinsulinemia (58,59). These abnormalities should be considered in future studies.

In summary, the results of the present study indicate that hyperglycemia is associated with a reduction in epithelial markers and an increase mesothelial markers in CRC. The HG-induced enhanced migratory and invasive abilities of CRC cells may be attributed to EMT via the upregulation of HMGA2. The results of the present study may provide novel insights into the association between DM and CRC.

Figure 2. HG promotes the migration and invasion of HCT-116 and HT-29 cells. The migratory abilities of (A) HCT-116 and (B) HT-29 cells were determined using a scratch assay following HG stimulation (magnification, x40). A Transwell assay was also performed to assess the migration and invasion abilities of (C) HCT-116 and (D) HT-29 cells (magnification, x40). HG, high glucose; Ctrl, control.
Figure 3. HG promotes EMT by increasing the level of HMGA2 protein. Western blotting was performed to assess the expression of HMGA2 protein in (A) HCT-116 and (B) HT-29 cells. HMGA2 knockdown in (C) HCT-116 and (D) HT-29 cells was confirmed using western blotting. Western blotting analysis revealed that HMGA2 knockdown reversed EMT in (E) HCT-116 and (F) HT-29 cells. *P<0.05 and **P<0.01 vs. Ctrl. HG, high glucose; EMT, epithelial-mesenchymal transition; HMGA2, high-mobility group A protein 2; Ctrl, control; siRNA, small interfering RNA.
Figure 4. HG promotes cell viability and suppresses apoptosis in colorectal cancer cells. Cell viability was assessed in (A) HCT-116 and (B) HT-29 cells using an MTT assay. Cell viability was also assessed in (C) HCT-116 and (D) HT-29 using flow cytometry. An Annexin V-PE/7AAD assay was performed to measure the number of (E) HCT-116 and (F) HT-29 cells in early apoptosis (lower-right quadrant) and late apoptosis/necrosis (upper-right quadrant) cells. *P<0.01 vs. Ctrl. HG, high glucose; PE, phycoerythrin; 7AAD, 7 aminoactinomycin D; Ctrl, control.
EMT is the dominant program in human colon cancer.

References

1. McGuire S: World cancer report 2014. Geneva, Switzerland: World health organization, international agency for research on cancer, WHO Press, 2015. Adv Nutr 7: 418-419, 2016.
2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. CA Cancer J Clin 65: 87-108, 2015.
3. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2015. CA Cancer J Clin 65: 5-29, 2015.
4. Khan N and Mukhtar H: Cancer and metastasis: Prevention and treatment by green tea. Cancer Metastasis Rev 29: 435-445, 2010.
5. Yoon SS and Tanabe KK: Surgical treatment and other regional treatments for colorectal cancer liver metastases. Oncologist 4: 197-208, 1999.
6. Puuvila G, Chanprasertyotin S and Sriprapradang A: Diagnostic criteria for diabetes mellitus and other categories of glucose intolerance: 1997 criteria by the expert committee on the diagnosis and classification of diabetes mellitus (ADA), 1998 WHO consultation criteria, and 1985 WHO criteria, world health organization. Diabetes Res Clin Pract 44: 21-26, 1999.

7. Shaw JE, Sicree RA and Zimmet PZ: Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 87: 4-14, 2010.
8. Larsson SC, Orsini N and Wolk A: Diabetes mellitus and risk of colorectal cancer: A meta-analysis. J Natl Cancer Inst 97: 1679-1687, 2005.
9. Gabriel B, Pinsard N, Gerard R and Louchet E: Association of myocardopathy and spino-cerebellar degeneration (Friedreich's disease) - Apopos of a case. Pediatr 29: 367-377, 1974 (In French).
10. Mills KT, Bellows CF, Hoffman AE, Kelly TN and Gaglardi G: Diabetes mellitus and colorectal cancer prognosis: A meta-analysis. Dis Colon Rectum 56: 1304-1310, 2013.
11. Stein KB, Snyder CF, Barone BB, Yeh HC, Pears KS, Derr RL, Wolff AC and Brancati FL: Colorectal cancer outcomes, recurrence, and complications in persons with and without diabetes mellitus: A systematic review and meta-analysis. Dis Colon Rectum 55: 1839-1851, 2010.
12. Flood A, Mai V, Pfeiffer R, Kahle L, Remaley AT, Lanza E and Schatzkin A: Elevated serum concentrations of insulin and glucose increase risk of recurrent colorectal adenomas. Gastroenterology 133: 1423-1429, 2007.
13. Jullumstro E, Kollind M, Lydersen S and Edna TH: Diabetes mellitus and outcomes of colorectal cancer. Acta Oncol 48: 361-367, 2009.
14. Noh GY, Hwang DY, Choi YH and Lee YY: Effect of diabetes mellitus on outcomes of colorectal cancer. J Korean Soc Coloproctol 26: 424-428, 2010.
15. Kalluri R: EMT: When epithelial cells decide to become mesenchymal-like cells. J Clin Invest 119: 1417-1419, 2009.
16. Lamouille S, Xu J and Derynck R: Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 15: 178-196, 2014.
17. Natalwala A, Spychal R and Tselepis C: Epithelial-mesenchymal transition mediated tumourigenesis in the gastrointestinal tract. World J Gastroenterol 14: 3792-3797, 2008.
18. Calangiu CM, Simionescu CE, Stepan AE, Cernea D, Zavoii RE and Margaritescu C: The expression of CK19, vimentin and E-cadherin in differentiated thyroid carcinomas. Rom J Morphol Embryol 55: 919-925, 2014.
19. Loboda A, Nebzhyn MV, Watters JW, Buser CA, Shaw PM, Huang PS, Varit Veer L, Tollenara RA, Jackson DB, Agrawal D, et al: EMT is the dominant program in human colon cancer. BMC Med Genomics 4: 9, 2011.
20. Flores-Lopez LA, Martinez-Hernandez MG, Viedma-Rodriguez R, Diaz-Flores M and Baiza-Gutman LA: High glucose and insulin enhance uPA expression, ROS formation and invasiveness in breast cancer-derived cells. Cell Oncol (Dordr) 39: 365-378, 2016.
21. He L, Lou W, Ji L, Liang W, Zhou M, Xu G, Zhao L, Huang C, Li R, Wang H, et al: Serum response factor accelerates the high glucose-induced Epithelial-to-Mesenchymal Transition (EMT) via Smad signaling in human peritoneal mesothelial cells. PLoS One 9: e108593, 2014.
22. Gou W, Zhou X, Liu Z, Wang L, Shen J, Xu X, Li Z, Zhai X, Zuo D and Wu Y: CD74-ROSI G2032R mutation transcriptionally up-regulates Twist1 in non-small cell lung cancer cells leading to increased migration, invasion, and resistance to erlotinib. Cancer Lett 422: 19-28, 2018.
23. Tam L and Weinberg A: The Epigenetics of epithelial-mesenchymal plasticity in cancer. Nat Med 19: 1438-1449, 2013.
24. Thauault S, Tan EJ, Peinado H, Cano A, Heldin CH and Moustakas A: HMGA2 and Smads co-regulate SNAIL1 expression during induction of epithelial-to-mesenchymal transition. J Biol Chem 283: 33437-33446, 2008.
25. Tan EJ, Thauault S, Caja L, Carletti T, Heldin CH and Moustakas A: Regulation of transcription factor Twist expression by the DNA architectural protein high mobility group A2 during epithelial-to-mesenchymal transition. J Biol Chem 287: 7134-7145, 2012.
26. Dong J, Wang R, Ren G, Li X, Wang J, Sun Y, Liang J, Nie Y, Wu K, Feng B, et al: HMGA2-FOX1L2 axis regulates metastases and epithelial-to-mesenchymal transition of chemoresistant gastric cancer. Clin Cancer Res 23: 3461-3473, 2017.
27. Li W, Wang Z, Zha L, Kong D, Liao G and Li H: HMGA2 regulates epithelial-mesenchymal transition and the acquisition of tumor stem cell properties through TWIST1 in gastric cancer. Oncotarget 8: 185-192, 2017.
28. Grossarth-Maticek R and Eysenck HF: Length of survival and lymphocyte percentage in women with mammary cancer as a function of psychotherapy. Psychol Rep 63: 315-321, 1998.
29. Shi Z, Wu D, Tang R, Li X, Chen R, Xue S, Zhang C and Sun X: Silencing of HMGA2 promotes apoptosis and inhibits migration and invasion of prostate cancer cells. J Biochem 41: 229-236, 2016.

30. Alco G, Bozdogan A, Selamoglu D, Pilanci KN, Tuzlali S, Ordu C, Igdem Ş, Okkan S, Dincer M, Demir G and Ozmen V: Clinical and histopathological factors associated with Ki-67 expression in breast cancer patients. Oncol Lett 9: 1046-1054, 2015.

31. Hanahan D and Weinberg RA: Hallmarks of cancer: The next generation. Cell 144: 646-674, 2011.

32. Ryu TY, Park J and Scherer PE: Hyperglycemia as a risk factor for cancer progression. Diabetes Metab J 38: 330-336, 2014.

33. Abhijit S, Bhaskaran R, Narayanasamy A, Chakroverty A, Manickam N, Dixit M, Mohan V and Balasubramaniam M: Hyperinsulinemia-induced vascular smooth muscle cell (VSMC) migration and proliferation is mediated by converging mechanisms of mitochondrial dysfunction and oxidative stress. Mol Cell Biochem 373: 95-105, 2013.

34. Beckner ME, Stracce ML, Liotta LA and Schiffmann E: Glycolysis as primary energy source in tumor cell chemotaxis. J Natl Cancer Inst 82: 1836-1840, 1990.

35. Rose DP and Vona-Davis L: The cellular and molecular mechanisms by which insulin influences breast cancer risk and progression. Endocr Relat Cancer 19: R225-R241, 2012.

36. Yoshi S, Liu M and Turner N: Diabetes and its link with cancer: Providing the fuel and spark to launch an aggressive growth regime. Biomed Res Int 2015: 930863, 2015.

37. Masur K, Vetter C, Hinz A, Tomas N, Henrich H, Niggemann B and Zanker KS: Diabetogenic glucose and insulin concentrations modulate transcriptome and protein levels involved in tumour cell migration, adhesion and proliferation. Br J Cancer 104: 345-352, 2011.

38. Kang X, Kong F, Wu X, Ren Y, Wu S, Wu K, Jiang Z and Zhang W: High glucose promotes tumor invasion and increases metastasis-associated protein expression in human lung epithelial cells by upregulating heme oxygenase-1 via reactive oxygen species or the TGF-β/PI3K/Akt signaling pathway. Cell Physiol Biochem 35: 1008-1022, 2015.

39. Lontchi-Yimagou E, Sobngwi E, Matsha TE and Kgengne AP: Diabetes mellitus and inflammation. Curr Diab Rep 13: 435-444, 2013.

40. Hu B, Meigs B, Li Y, Rifai N and Manson E: Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes 53: 693-700, 2004.

41. Han TS, Sattar N, Williams K, Gonzalez-Villalpando C, Alco G, Bozdogan A, Selamoglu D, Pilanci KN, Tuzlali S, Oord C, Igdem Ş, Okkan S, Dincer M, Demir G and Ozmen V: Clinical and histopathological factors associated with Ki-67 expression in breast cancer patients. Oncol Lett 9: 1046-1054, 2015.

42. Cheng XS, Li YF, Tan J, Sun B, Xiao YC, Fang XB, Zhang XF, Li Q, Dong JH, Li M, et al: CCL20 and CXCL8 synergize to promote progression and poor survival outcome in patients with colorectal cancer by collaborative induction of the epithelial-mesenchymal transition. Cancer Lett 348: 77-87, 2014.

43. Li W, Zhang L, Chen X, Jiang Z, Zong L and Ma Q: Hyperglycemia promotes the epithelial-mesenchymal transition of pancreatic cancer via hydrogen peroxide. Oxid Med Cell Longev 2016: 5190314, 2016.

44. Pena C, Garcia JM, Silva J, Garcia V, Rodriguez R, Alonso I, Millan I, Salas C, de Herreros AG, Munoz A and Bonilla F: E-cadherin and vitamin D receptor regulation by SNAIL and ZEB1 in colon cancer: Clinico-pathological correlations. Hum Mol Genet 14: 3361-3370, 2005.

45. He X, Chen Z, Jia M and Zhao X: Downregulated E-cadherin expression indicates worse prognosis in Asian patients with colorectal cancer: Evidence from meta-analysis. PLoS One 8: e70858, 2013.

46. Toyotama Y, Yasuda H, Saigusa S, Tanaka K, Inoue Y, Goei A and Kusunoki M: Increased expression of Slug and Vimentin as novel predictive biomarkers for lymph node metastasis and poor prognosis in colorectal cancer. Carcinogenesis 34: 2548-2557, 2013.

47. Boo LM, Lin HH, Chung V, Zhou B, Louie SG, O’Reilly MA, Yen Y and Ann DK: High mobility group A2 potentiates genotoxic stress in part through the modulation of basal and DNA damage-dependent phosphoryllysinol-5-kinase activation. Cancer Res 65: 6622-6630, 2005.

48. Fusco A and Fedele M: Roles of HMGA proteins in cancer. Nat Rev Cancer 7: 899-910, 2007.

49. Wang Y, Le Y, Xue JY, Zheng ZJ and Xue YM: Let-7d miRNA prevents TGF-beta1-induced EMT and renal fibrogenesis through regulation of HMGA2 expression. Biochem Biophys Res Commun 479: 676-682, 2016.

50. Zhao XP, Zhang H, Jiao JY, Tang DX, Wu YL and Pan CB: Overexpression of HMGA2 promotes tongue cancer metastasis through EMT pathway. J Transl Med 14: 26, 2016.

51. Matejka M, Finck J and Kraklickova M: Epithelial-mesenchymal transition in tumor tissue and its metastatic spread of cancer. Klin Onkol Winter 30: 20-27, 2017 (In Czech).

52. Tomas NM, Masur K, Pichca JC, Niggemann B and Zanker KS: Akt and phospholipase C gamma are involved in the regulation of growth and migration of MDA-MB-468 breast cancer and SW480 colon cancer cells when cultured with diabetogenic levels of glucose and insulin. BMC Res Notes 5: 214, 2012.

53. Yang B, Huang CZ, Yu T, Zhou SN, Liu Q, Liu GJ, Chen S and Han FH: Metformin depresses overactivated Notch1/Hes1 signaling in colorectal cancer patients with type 2 diabetes mellitus. Anticancer Drugs 28: 531-539, 2017.

54. Yang IP, Tsai HL, Huang CW, Lu CY, Miao ZF, Chang SF, Juo SH and Wang JY: High blood sugar levels significantly impact the prognosis of colorectal cancer patients through down-regulation of microRNA-16 by targeting Myb and VEGFR2. Oncotarget 7: 18837-18850, 2016.

55. Lee SK, Moon JW, Lee YW, Lee JO, Kim SJ, Kim N, Kim J, Kim HS and Park SH: The effect of high glucose levels on the hypermethylation of protein phosphatase 1 regulatory subunit 3C (PPP1R3C) gene in colorectal cancer. J Genet 94: 75-85, 2015.

56. Haffner M: Diabetes, hyperlipidemia, and coronary artery disease. Am J Cardiol 83: 17F-21F, 1999.

57. O’Dea K, Lion RJ, Lee A, Traianedes K, Hopper JL and Rae C: Diabetes, hyperinsulinism, hyperlipidemia in small aboriginal community in northern Australia. Diabetes Care 13: 830-835, 1990.

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