

## Effect of omentin-1 on cancer stem cell surface markers and tumour-suppressive miRNA expression in a high-glucose environment associated with colorectal cancer

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### Abstract

**Objective:** To investigate how omentin-1 impacts colorectal cancer stem cell surface markers and the expression levels of tumour-suppressive micro ribonucleic acid in a colorectal cancer-associated high-glucose environment.

**Methods:** The study was conducted in the First Affiliated Hospital of Anhui Medical University, Anhui, China, from April 2018 to January 2019 and comprised cluster of differentiation133 and colorectal cancer stem cells from the SW480 cell line (the human colon adenocarcinoma cell line) obtained through immunomagnetic beads-based cell isolation. The colon cancer stem cells were divided into 6 groups: Z0 (control), Z1 (1ug/mL omentin-1), Z2 (2ug/mL omentin-1), G0 (5.0g/mL glucose), G1 (1ug/mL omentin-1 and 5.0g/mL glucose), and G2 (2ug/mL omentin-1 and 5.0 g/mL glucose). After 24 hours of intervention, quantitative polymerase chain reaction and western blot test were used for the detection of messenger ribonucleic acid and protein levels of stem cell surface markers. The colorectal cancer stem cells were divided into three groups: the control group, omentin group 1 (1ug/mL omentin-1) and omentin group 2 (2ug/mL omentin-1). After 24 hours of intervention, the expression of tumour suppressor micro ribonucleic acid was measured using quantitative polymerase chain reaction. Data was analysed using SPSS 23.

**Results:** Compared to the Z0 group, cluster of differentiation133 messenger ribonucleic acid expression reduced sharply in Z1 group ( $p<0.05$ ), while Z2 group saw a marked increase in the expression ( $p<0.05$ ). With respect to tumour-suppressive micro ribonucleic acid expression, micro ribonucleic acid 126, 145, 34a and 342-5P in omentin group 2 exhibited an expression level significantly higher than those in the control group and the omentin group 1 ( $p<0.05$ ).

**Conclusions:** High glucose levels were found to upregulate the expression of colorectal cancer stem cell surface markers cluster of differentiation133 messenger ribonucleic acid and protein. Also, omentin-1 was found to be associated with the downregulation of cluster of differentiation133 messenger ribonucleic acid and protein expression and the upregulation of cluster of differentiation 44 messenger ribonucleic acid expression in a high-glucose environment. Finally, omentin-1 was found to have the ability to promote the expression of relevant tumour-suppressive micro ribonucleic acids 126, 14, 34a and 342-5P.

**Keywords:** Omentin-1, Colorectal, Cancer, Cancer stem cell, miRNA, Cell surface marker. (JPMA 72: 430; 2022)

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### Introduction

Colorectal cancer (CRC) is one of the most common malignancies worldwide and the third most commonly diagnosed cancer in China, followed by lung cancer and gastric cancer.<sup>1</sup> The International Diabetes Federation (IDF)<sup>2</sup> estimated in 2019 that about 463 million people had diabetes worldwide. Considering the close association between diabetes and cancer, individuals with diabetes are exposed to a higher risk of cancer, including CRC and breast cancer.<sup>3-5</sup>

Omentin-1 is a novel adipokine composed of 313 amino

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acids. Human omentin-1 is shown to have a close relationship with the incidence of type 2 diabetes mellitus (T2DM) in different ethnic populations, making it a candidate gene for T2DM susceptibility in humans; plasma omentin-1 levels are remarkably reduced in patients with diabetes, obesity and metabolic syndrome.<sup>6-8</sup> Also, omentin-1 is known to be linked to several malignant tumours, such as gastric cancer, malignant pleural mesothelioma and kidney cancer.<sup>9-11</sup>

Cancer stem cells (CSCs) are a rare subset of cancer cells within a tumour that have the ability to self-renew infinitely, differentiate into multiple types of cancer cells and induce uncontrolled growth of malignancies. They are closely associated with the development, progression and prognosis of malignant tumours, and are difficult to be completely eliminated by regular chemo- and radiotherapy. Evidence shows that CSCs lie in the root of tumour recurrence.<sup>12</sup> With precise therapy targeting CSCs being

actively discussed, specific cell surface markers for CSCs have attracted closer attention. Although a range of colorectal cancer stem cell (CRCSC) surface markers have already been identified, cluster of differentiation (CD)133 is still the most frequently used cell surface molecule in CRC screening. Besides, CD133+ expression is proved to be strongly associated with the metastasis and prognosis of CRC. In our preliminary study,<sup>13</sup> it was noted that adipokine omentin-1 could promote apoptosis of CRCSCs and inhibit CRCSC growth. The current follow-up study was planned to focus on the expression profile of CD133+ in CRCSCs, and to investigate how omentin-1 impacts CRCSC surface markers and the expression levels of tumour-suppressive micro ribonucleic acid (miRNA) in a CRC-associated high-glucose environment.

## Materials and Methods

The study was conducted in the First Affiliated Hospital of Anhui Medical University, Anhui, China, from April 2018 to January 2019 and comprised CD133+CRCSCs from the SW480 cell line (the human colon adenocarcinoma cell line) obtained through immunomagnetic beads-based cell isolation in the previous study.<sup>13</sup> The CD133+ CRCSCs were subjected to culture, subculture, cold storage and resuscitation.

After culture and subculture, the CRCSCs were frozen and kept under refrigeration so that the cells could be resuscitated before any experiment.

When CRCSCs gathered into spheres and grew well, 900µl serum-free medium (SFM) and 100µl dimethyl sulfoxide (DMSO) was added for long-term preservation in liquid nitrogen. They were resuscitated when needed.

Immunohistochemical (IHC) staining was performed to visualise CD133+ CRCSCs.

Quantitative polymerase chain reaction (qPCR) was used to evaluate the effect of omentin-1 at different concentrations on the messenger RNA (mRNA) and miRNA expression levels of such CRCSC surface markers as CD133, CD44 and Aldehyde dehydrogenase 1 (ALDH1) in a high-glucose environment.

To assess the impact of omentin-1 and glucose levels on the expression of CRCSC surface markers, the CRCSCs were divided into 6 groups: Z0 (control), Z1 (1µg/mL omentin-1), Z2 (2µg/mL omentin-1), G0 (5.0g/mL glucose), G1 (1µg/mL omentin-1 and 5.0g/mL glucose), and G2 (2µg/mL omentin-1 and 5.0 g/mL glucose). After 24 hours of intervention, qPCR and western blot test (WBT) were used for the detection of mRNA and protein levels of the surface markers. To evaluate the effect of omentin-1 levels on

tumour-suppressive miRNA expression, the CRCSCs were divided into three groups: the control group, omentin group 1 (1µg/mL omentin-1) and omentin group 2 (2µg/mL omentin-1).

During the exponential phase of growth, CD133+ CRCSCs were used for total RNA extraction. Then, RNA concentration and purity were determined to calculate the total RNA for reverse transcription (RT).

After RT and complementary deoxyribonucleic acid (cDNA) synthesis, RT-qPCR was performed. Reaction conditions were set as 95°C 35s (initial denaturation), 95°C 10s, and 65°C 35s for 42 cycles.

### RT-qPCR

Primer sequences of indicators used were:

Human CD133: amplicon size 73bp.

Forward primer: 5'-AGAGGCGTTGGAGAACATGA-3';

reverse primer: 5'-CAGACTGCTGCTAAGCTGTG-3';

Human CD44: amplicon size 110bp. Forward primer:

5'-ACTAAATCAGGGCTGGGCTT-3'; reverse primer:

5'-GAGAGGGTAGACAGGGAGGA-3';

Human ALDH1: amplicon size 79bp. Forward primer:

5'-CCAGCCCACAGTGTCTCTA-3'; reverse primer:

5'-GATTTGCTGCTACTGGTCCAA-3'; Human β-actin:

amplicon size 180bp. Forward primer:

5'-GGGAAATCGTGCGTGACATTAAGG-3'; reverse primer:

5'-CAGGAAGGAAGGCTGGAAGAGTG-3'.

The miRNA primer sequences were: miR-126:

5'-TCGTACCGTGAGTAATAATGCG-3'; miR-34a:

5'-TGCCAGTGTCTTAGCTGGTTGT-3'; miR-143:

5'-TGAGATGAAGCACTGTAGCTC-3'; miR-145:

5'-GTCCAGTTTTCCAGGAATCCCT-3'; miR-342-5P:

5'-AGGGGTGCTATCTGTGATTGA-3'; and miR-342-3p: 5'-

TCTCACACAGAAATCGCACCCGT-3'.

Reaction conditions were set as 95°C for 15 min (initial denaturation), 95°C 15s, 60°C 25s, and 72°C 15s for 42 cycles.

WBT was performed to measure the effect of omentin-1 at varying concentrations on the expression levels of CRCSC surface markers CD133, CD44, ALDH1 in a high-glucose environment.

For total protein extraction all groups of CRCSCs were treated respectively and placed in a 5% carbon dioxide (CO<sub>2</sub>) incubator to culture at 37°C for 24h. Then the CRCSCs in each group were subjected to centrifugation, and the supernatant was removed. Following that, the bicinchoninic acid (BCA) protein assay was performed for

the determination of protein concentration. The solutions were stored for future use.

Another WBT was carried out to measure the expression levels of relevant proteins.

Data was analysed using SPSS 23. The had a normal distribution and was expressed as mean±standard deviation (SD). Intergroup comparisons were done using analysis of variance (ANOVA) for three or more groups, while least significant difference (LSD) test was used in case of two groups.  $P < 0.05$  was considered statistically significant.

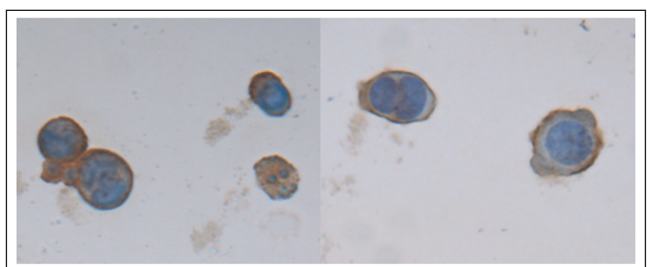
**Results**

IHC-stained images provided evidence for the presence of CD133 surface expression (Figure-1).

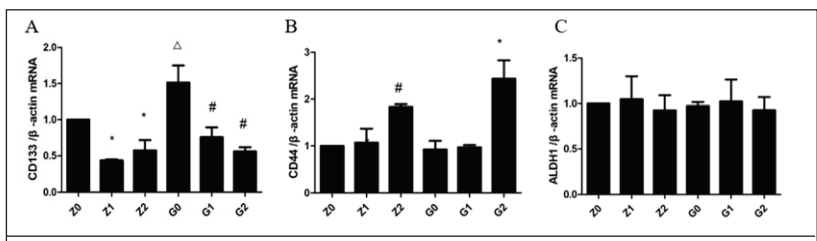
After omentin-1 treatment for 24h, the expression levels of CD133 mRNA in groups Z1 (0.435±0.025) and Z2 (0.573±0.203) decreased in comparison with control group Z0 (control<0.05)). In contrast, CD133 mRNA expression in group G0 (1.511±0.335) increased significantly ( $p < 0.05$ ), while there was no significant difference between Z1 and Z2 regarding CD133 mRNA expression ( $p > 0.05$ ). Compared to G0, CD133 mRNA expression reduced significantly in G1 and G2 ( $p < 0.05$ ), but the difference was not significant between G1 (0.758±0.191) and G2 (0.560±0.083) ( $p > 0.05$ ).

ALDH1 mRNA expression after omentin-1 treatment for 24h was not significantly different between group Z0 and the other five groups ( $p > 0.05$ ).

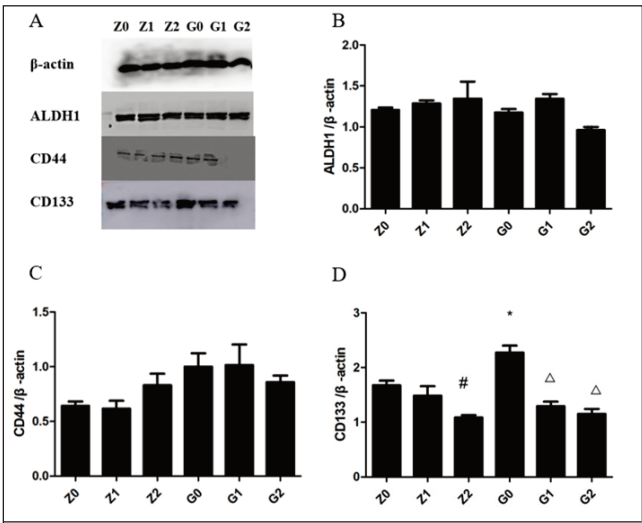
CD44 mRNA expression after omentin-1 treatment for 24h in Z2 (1.841±0.074) and G2 (2.438±0.551) increased



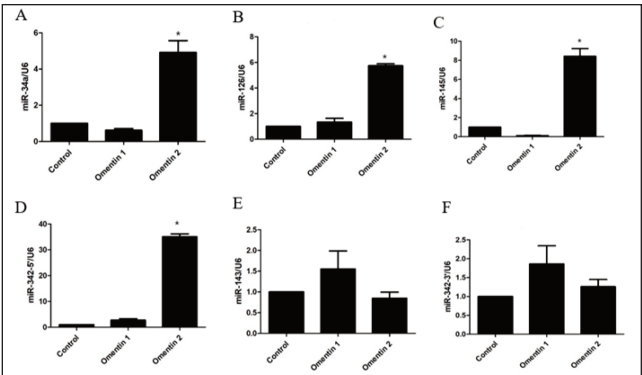
**Figure-1:** Expression of cluster of differentiation (CD)133 on the cell membrane of colon cancer stem cells (100×).



**Figure-2:** Messenger ribonucleic acid (mRNA) expression of colorectal cancer stem cell (CRCSC) surface markers.



**Figure-3:** Protein expression of colorectal cancer stem cell (CRCSC) surface markers.



**Figure-4:** Effect of omentin-1 at different concentrations on tumour-suppressive micro ribonucleic acid (miRNA) expression (miRNA-126, miRNA-34a, miRNA-143, miRNA-145, miRNA-342) in colorectal cancer stem cells (CRCSCs).

significantly compared to Z0 ( $p < 0.05$ ), while Z1 (1.071±0.4187), G0 (0.925±0.259) and G1 (0.970±0.067) had no significant differences in this regard ( $p > 0.05$ ) (Figure-2).

ALDH1 protein expression after omentin-1 treatment for 24h was not significantly different between Z0 and the other five groups ( $p > 0.05$ ).

CD44 protein expression after omentin-1 treatment for 24h was not significantly different between group Z0 (relative grayscale: 1.675±0.120) and Z1 (0.616±0.1016), Z2 (0.830±0.148), G0 (1.001±0.173), G1 (1.016±0.264) and G2 (0.858±0.083) ( $p > 0.05$ ).

CD133 protein expression after omentin-1 treatment for 24h in Z1 (relative grayscale: 1.485±0.242) was not significantly different from Z0 (1.675±0.120) ( $p > 0.05$ ), while Z2 (1.086±0.062) had a substantially reduced level of CD133 protein expression compared to Z0

( $p < 0.05$ ), and CD133 protein expression in G0 was markedly increased ( $p < 0.05$ ). Compared to G0, CD133 protein expression decreased significantly in G1 and G2 ( $p < 0.05$ ), but the differences between G1 and G2 lacked statistical significance ( $p > 0.05$ ) (Figure 3).

Compared to the control group, miR-126, miR-34a, miR-145 and miR-342-5P expression remarkably increased in omentin group 2 ( $p < 0.05$ ), while there was no significant difference between the control group and omentin group 1 with respect to miR-126, miR-34a, miR-145, miR-342-5P, and miR-342-3P expression ( $p > 0.05$ ).

Differences between the control group and omentin groups 1 and 2 with regard to miR-143, miR-342-3P expression were not statistically significant ( $p > 0.05$ ) (Figure-4).

## Discussion

CRC accounts for >9% of the world's cancer incidence, with the vast majority, approximately 90%, having adenocarcinomas (AdCs) in their colonic and rectal epithelial cells.<sup>14-16</sup> Liver metastases are found in >70% CRC-related deaths. Although surgical intervention may produce favourable outcomes, no more than 25% CRC patients are eligible for surgery and the recurrence rate is up to 70%. For inoperable, recurrent or metastatic cases, palliative chemotherapy is deemed as the first-line treatment.<sup>17</sup> Since CSCs are closely associated with cancer development and progression, elimination of CSCs is an important treatment for the radical cure of cancer. CRCSCs share many key biological properties of the stem cells of other solid tumours,<sup>18</sup> Omentin-1 inhibited the proliferation and promoted apoptosis of colon CSCs in a dose and time-dependent manner, which could be strengthened by the PI3K/Akt inhibitor (Akt/PKB is a member of the PI3K signaling pathway, which is a core signaling pathway of stimulating growth factors. PI3K stimulates the intracellular signaling pathways of malignant tumour cells by activating Akt to inhibit tumour cell apoptosis and promote proliferation. In contrast, inhibition of the Akt signaling pathway may induce apoptosis in some malignant tumour cells).<sup>19</sup> CD133 is a transmembrane glycoprotein expressed in haematopoietic cells, endothelial cells and neuroepithelial cells.<sup>20</sup> CD133 is considered a specific biomarker for the identification of primary CRCSCs. CD133 expression is possibly linked to CRC cell differentiation and the recurrence, survival and size of colorectal carcinomas.<sup>21</sup> CD133+ in CRC is radio- and chemo-resistant.<sup>22,23</sup>

During the previous experiment,<sup>13</sup> the research group obtained CD133-rich cell populations based on indirect immunomagnetic separation (IMS); and prior to the

experiment, the researchers determined CD133 content up to 80.3% in the cell populations using flow cytometry. A study<sup>24</sup> found that the percentage of a CSC surface molecular marker, such as CD133 and CD44, might be associated with the stage and the microenvironment of cancer. The current study investigated three different CRCSC surface markers: CD133, CD44 and ALDH1. This was done by creating high-glucose environments to simulate the blood sugar levels of patients with diabetes.

Adipokines are metabolism-related active molecules secreted by adipose tissue or interstitial tissue and blood vessels around adipose tissue and sharing functional and structural features of cytokines. Known adipokines include adiponectin, leptin, resistin, omentum, and visfatin.

During the preliminary study,<sup>13</sup> the researchers noted that omentin-1 levels were positively correlated with high-density lipoprotein cholesterol (HDL-C) levels and negatively correlated with triglycerides (TG), fasting insulin, and waist-to-hip ratio (WHR) levels. As obese individuals have a greater risk of developing CRC, omentin-1 levels may have associations with obesity and CRC. A study<sup>25</sup> proposed that a reduced level of omentin-1 appeared to be associated with the poor prognosis of patients with progressive CRC. In the preliminary study,<sup>13</sup> the researchers found that metformin<sup>26</sup> could inhibit the proliferation of CRCSCs and CD133 surface expression. Also, evidence showed that omentin-1 could accelerate CRCSC apoptosis and inhibit CRCSC growth.<sup>13</sup> Following the preliminary study,<sup>13</sup> the researchers treated CRCSCs with omentin-1 at varying concentrations in a high-glucose environment, and found that CD133 expression in the high-glucose environment exceeded that in the control group. After omentin-1 treatment, the mRNA and protein expression levels of CD133 were markedly decreased, indicating the inhibiting role of omentin-1 in CD133 expression. In another study,<sup>27</sup> leptin was shown to promote CSC enrichment. Resistin<sup>28</sup> was found to prevent apoptosis of breast cancer cells. In the preliminary study,<sup>13</sup> adiponectin was found to accelerate the apoptosis of CRC cells<sup>29</sup> and Lgr5GFP+ stem cells (the leucine-rich-repeat-containing G-protein-coupled receptor 5(Lgr5)).<sup>30</sup>

Based on the findings above, we assumed that adipokines might have varying impacts on different CSCs as they did on different cancers. In the current study, omentin-1 inhibited CD133 expression and increased the expression level of CD44 mRNA even though no clear association was noted between omentin-1 and CD44 protein and ALDH1. Like leptin, resistin and adiponectin, omentin-1 also appeared to have varying impacts on different surface markers. Notably, the same CSC surface marker from different sources might even develop contradictory trends

after omentin-1 treatment.

The miRNAs<sup>31</sup> are a class of endogenous small non-coding RNA molecules acting by binding to complementary sites on target mRNAs to regulate the expression levels of specific proteins. They play an important role in embryonic life and pre-implantation embryo development, and regulate cell differentiation, proliferation and apoptosis as well as cancer development and progression. As oncogene and anti-oncogene inhibitors, miRNAs play an essential role in cancer development. As cancer cells share characteristics with normal stem cells, dysregulation of miRNAs may lead to loss or quiescence of cell lineages, which increases the risk of cancer development.

Ample evidence shows that some miRNAs are CRC inhibitors, and their expression levels are down-regulated in CRC. A study<sup>32</sup> measured miR-126 expression in CRC cells using a miRNA microarray scanner, and found that miR-126 was basically missing in CRC cells. Similar findings were presented by another study<sup>33</sup> which pointed out that miR-126-induced elements can regulate the activity of CRC cells via autophagy and apoptosis, and suggested a new mechanism of interaction between miR-126 and mechanistic target of rapamycin (mTOR) in CRC pathogenesis. A study<sup>34</sup> found that CRC tissue had an miR-34a expression level significantly lower than the normal adjacent tissue; miR-34a was closely associated with the depth of tumour infiltration and tumour-nodes-metastases (TNM) staging. This indicates that miR-34a is possibly involved in the development of CRC.

Although the molecular malleability of normal cells and CSCs is supported by growing evidence, the community still has a very limited understanding of the underlying mechanism. A range of scientific studies have proved that when CRC occurs,<sup>35,36</sup> individual miRNAs or miRNA clusters interrupt relevant key cellular pathways, such as Wnt, RAS, TGF- $\beta$ <sup>37</sup> and inflammation signalling pathways, which triggers the complicated regulation network.

Numerous studies have demonstrated that dysregulated miR-34a is closely related to the proliferation, differentiation, migration and invasion of tumour cells, as well as the diagnosis, prognosis, treatment and chemo-resistance of tumours.<sup>36</sup> A study<sup>38</sup> suggested that increased miR-34a expression was associated with the effectiveness of treating CRC with regorafenib where radio-resistance and initiator CRC cell phenotypes were reversed through down-regulation of WNT / $\beta$ -catenin. In the present study, miR-34a expression was elevated after treatment with 2 $\mu$ g/mL omentin-1; as to CD44, the mRNA expression level increased, while the protein expression level had no significant difference compared to the control group;

CD133 expression was decreased to a lower level. The findings were consistent with the above mentioned studies.<sup>36,38</sup>

The current study found that omentin-1 could up-regulate miR-145 expression and down-regulate CD133 mRNA and protein expression, which agrees with the findings of the previous studies.<sup>39,40</sup> A study<sup>41</sup> showed that PU-PEI (polyurethane-short branch-polyethylenimine) -mediated miR-145 could reduce the features of lung cancer stem cells, undermine chemo- and radio-resistance and inhibit tumour growth and metastasis in vivo. Similar findings were presented in a study concerning CRCSCs.<sup>42</sup>

## Conclusion

It was found that omentin-1 could up-regulate the expression levels of miR-126, miR-34a, miR-145 and miR-342-5P. These miRNAs are inhibitors against CSCs and relevant surface markers. The findings indicated that CD133+expression was reduced on mRNA and protein levels, and the preliminary study had also suggested that omentin-1 could inhibit CRCSC growth and promote CRCSC apoptosis. Therefore, omentin-1 can be said to be a promising CSC regulator as it may modulate inflammation and regulate adipose cell differentiation through alteration of miRNA expression levels in cancer cells.

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**Conflicts of Interest:** None.

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