RESEARCH ARTICLE

Immuno Histochemical Expression of CD133 as Cancer Stem Cells Marker in a Sample of Iraqi Patients with Colorectal Carcinoma

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ABSTRACT

Colorectal cancer is one of the most frequent malignancies in the world, and it is also one of the most deadly. Distant metastases and recurrence frequently cause patient death. Cancer stem cells (CSCs) play a crucial role in colorectal cancer spread and relapse. CSCs are a type of cancer cell that can self-renew, divide indefinitely, and differentiate in multiple directions. The CD44, CD133, CD24, EpCAM, LGR5, and ALDH are some cell surface markers used to identify colorectal CSCs. They are highly tumorigenic, chemoresistant, and radioresistant, making them important in colorectal cancer metastasis and recurrence as well as disease-free survival. The current study included 60 Iraqi male patients. They were assigned into three groups. Group I consisted of 20 newly diagnosed colorectal cancer patients, group II consisted of 20 relapsed patients who were treated with chemotherapy and were cured, but the tumor relapsed, and group III consisted of 20 patients who demonstrated resistance to chemotherapy treatment. All clinicopathological markers were analyzed, and they demonstrated a higher tumor grade in group II compared to the other groups and a difference in age between groups. In comparison to group I, CD133 expression was higher in groups II and III, with the observation that some IHC slides were clear of typical colorectal cancer cells but positive for CD133, indicating the presence of cancer stem cells. In the resistance group, similar effects were reported. The results could be explained by the fact that the highest expression of CD133 suggests a significant number of cancer stem cells, which play a key role in tumor relapse and treatment resistance. As a result of these findings, CD133 may be employed as a biomarker for cancer stem cells, allowing for their detection and aiding in the process of tailoring therapy for these cells within the tumor mass, preventing tumor relapse and resistance to chemotherapy.

Keywords: CD133, Cancer stem cells, Colorectal carcinoma, Tumor relapsing.

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INTRODUCTION

Colorectal cancer (CRC), the third-deadliest cancer in the United States, is a disease that arises from epithelial cells lining the colon or rectum of the gastrointestinal tract, most commonly as a result of Wnt signaling pathway alterations that increase signaling activity. The mutations can be inherited or acquired, and they are most likely to arise in the stem cells of the intestinal crypt.[1] The adenomatous polyposis coli (APC) gene, which encodes the APC protein, is the most often altered in all colorectal cancers. The APC protein stops the catenin protein from accumulating. Without APC, catenin accumulates in high quantities and translocates into the nucleus, where it binds to DNA and stimulates proto-oncogene transcription. These genes are generally involved in stem cell renewal and differentiation, but when they are overexpressed, they can lead to cancer.[2] CRC is one of the most common cancers globally, with a 5-year relative survival rate of roughly 10% for both metastatic rectal and colon cancer. Although early detection, prevention, and customized medicine have increased the response rate to traditional treatments, therapy resistance and metastasis remain the leading reasons for CRC death.[3]

Evidence has accumulated over the last 15 years that suggests malignancies, including CRC, are stem-cell diseases. The key to a better prognosis is early discovery and treatment. Early-stage CRC patients have a five-year survival rate of more than 90%, compared to only 11% for those with locally progressed or metastatic illness.[4] Furthermore, despite many therapeutic options, including surgical resection, chemoradiation, monoclonal antibodies to tumor growth factors, and liver-directed therapy for metastatic disease, patients with metastatic CRC have a median survival of only two years.[5] Unfortunately, only a small percentage of

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metastases respond to these treatments, and even fewer are cured, underlining our lack of understanding of the molecular mechanisms underlying this most lethal stage of CRC.[6]

The transmembrane glycoprotein CD133 (also known as AC133 or prominin-1) is found in hematopoietic cells, endothelial cells, and neuroepithelial cells.[7] CD133+ colorectal cancer cells (Isolated from colorectal cancer patients and detected in the Go and G1 phases) have stem cell properties such as self-renewal and multi-directional differentiation ability. CD133 expression is linked to colorectal cancer cell development and tumor growth, and it is regarded as a unique marker of primary colorectal CSCs.[8] Colorectal cancer cells that are CD133+ are also resistant to radiotherapy and chemotherapy.[9] However, there have been some mixed results, with some suggesting that CD133- cells are more aggressive.[10,11] Using immunohistochemical analysis for CD44, CD166, and CD133 as cancer stem cell markers, this study attempted to find cancer stem cells inside the tumor tissue clinical samples of Iraqi patients with colorectal carcinoma.

MATERIALS AND METHODS

Samples Collection

The study included 60 male patients ranging in age from 30 to 60 years old. The samples were gathered in partnership with Dr. Majed Al-Dari's clinic from the histology unit of the medical city hospital, the Department of Education Laboratories, and the Ministry of Health and Environment. Sections of samples were taken from the Raji Al-hadithy laboratories. Group I contained 20 samples from patients newly diagnosed with colorectal cancer, Group II included samples from relapsed patients who were subjected to chemotherapy treatment, and the tumor relapsed, and Group III included 20 samples from patients who had developed resistance to chemotherapy.

Immunohistochemistry Protocol

- The tissue sections were determined by drawing a circle around them with a pap pen.
- Enough drops of hydrogen peroxide block were added to cover the sections, incubated for 5 to 10 minutes, and washed three times in TBS washing.
- Protein block was applied and incubated for 5 to 10 minutes at room temperature to block nonspecific background staining and washed three times in TBS washing.
- Diluted primary antibody at a ratio (1/100 for CD44, CD166, and CD133) was added and incubated for 30 minutes.
- Sections were washed three times in TBS and incubated for 30 minutes in a humidified chamber at room temperature.
- Sections were washed three times in TBS, secondary antibody conjugated with HRP was applied and incubated for 30 min in a humidified chamber at room temperature.
- Chromogen substrate (DAP) was added and incubated for 5 minutes at room temperature. Sections were washed with distilled water and TBS wash.
- Hematoxylin was added for 1 to 3 minutes at room temperature and washed in distilled water and Tap water wash.
- Dehydration: Sections were dehydrated by immersing the slide sequentially in
- 70% ethanol for 2 minutes.
- 80% ethanol for 2 minutes.
- 100% ethanol for 2 minutes.
- 100%ethanol for 5 minutes.
- Xylene for 3–5 minutes.

1. The slides were dehydrated before being mounted with DPX and covered with a coverslip. The slides were examined under a light microscope at 10X, 20X, and 40X magnifications. The results were compared to the positive control, which was defined by the kit's brochure. The intensity and proportion of staining were used to determine positivity in a semi-quantitative manner. When the cell membrane was dyed with brown color, a scale was calculated for CD166, CD133, and CD44.
2. Score 0 (negative): (none of the cells revealed positivity for the marker)
3. Score 1 (weak positive (1+): number of positive cells represent 10% or less of total (few scatter ≤10%)
4. Score 2 (moderate positive (2+): the positive cells 11≤30%.
5. Score 3 (strong positive (3+): the positive cells 31≤50 %.
6. Score 4 (very strong (4+): the positive cells more than 50%.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) program version 24 was used to analyze the data, and the results were expressed using simple statistical metrics like mean and standard deviation. Analysis of variance (ANOVA) was used to determine to mean differences, which were then tested using either the LSD or Duncan test. $p \leq 0.05$ was deemed to be an acceptable threshold of significance.

RESULTS AND DISCUSSION

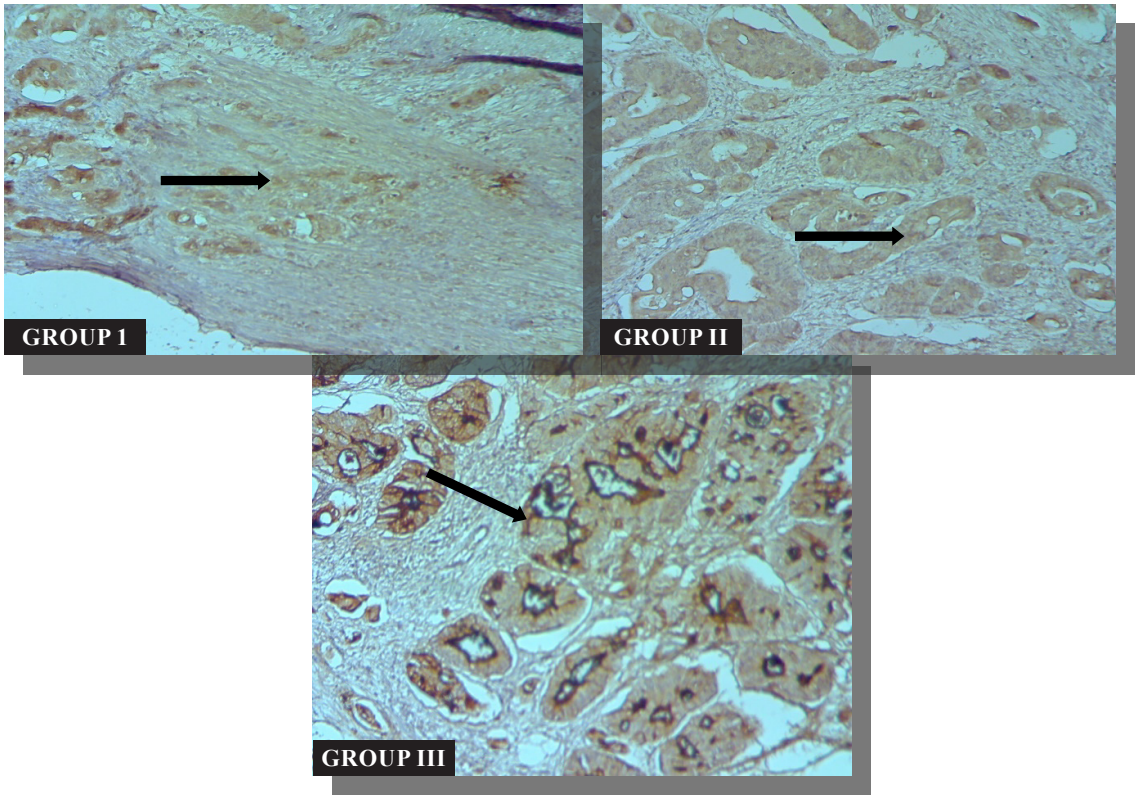
The results of the clinicopathological analysis are showed in Table 1. The results show a variety of age and grade and tumor location as compared between groups with the highest tumor grade in group I. The percentage of CD133 expression is higher in the resistant group as compared with the other groups indicating the highest number of cancer stem cells, which is explained by the ability of these cells (CSCs) to resist the chemotherapy using different mechanisms,[14] including their having to specific receptors on their surface showing their ability to introduce the chemotherapy inside the cells and degrade it via specific enzymes.[15]

In this study, all the slides obtained from the groups of patients were subjected to immunohistochemical (IHC) staining and the CD133 marker expression was measured and compared for each of the three groups of the study, and then analyzed microscopically. The percentage of CD marker was measured for five fields for each slide. The positive tumor cells stained with DAB stain reveal a brownish color indicating the positivity of CD133 marker expression, while the deep or light brownish color is the way of measuring the approximate intensity or the percentage of the marker expression within the IHC stained tumor mass slides. The accurately expressed

Table 1: Clinicopathological parameters of patients with colorectal carcinoma.

Clinical pathological parameters	*Group I (No. 20 newly diagnosed)*	*Group II (No. 20 relapsed)*	*Group III (No. 20 resistant)*
Age	44.4 ± 13.9	55.7±12.3	38.7±12.7
Grade	High	High	Low
Tumor location	30 % rectum 70 % colon	23% rectum 77% colon	15% rectum 85% colon
Type	Adenocarcinoma 70% Mucoidcarcinoma 30%	Adenocarcinoma 88% Mucoidcarcinoma 12%	Adenocarcinoma 65% Mucoidcarcinoma 35%
CD133 expression	15%	33%	44%

Note: Data are Mean±SD.

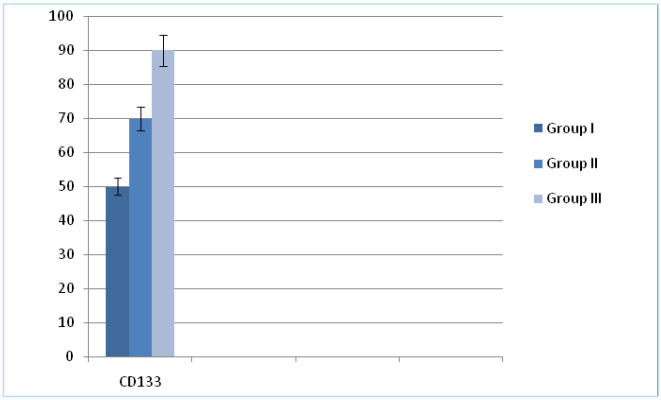


Figures 1: Immunohistochemical expression of CSCs in each group of the study, 40X.

cancer stem cells within the tumor mass were evaluated for CD marker through calculating the positive CD133 marker cells and then comparing their numbers to the total tumor cells in the field. The American Society Accredits the Method for Clinical Pathology (ASCP) by dividing the number of positive cells for the selected marker in five selective microscopic fields to the total number of the positive and the negative cells the result is shown in (Figure 1).[16,17]

The results of CD133 marker are shown in Figure 1. The positive CD133 cells within the tumor mass of group I appear as a brownish colored indicating their positivity to the CD133 marker and calculated as cancer stem cells within the tumor mass while the deep blue to violet-colored cells indicate the negative cells towards their reactivity to each measured CD marker.[18,19]

The percentages of positivity for each CD within each group are shown in Figure 2. The results show significant



Figures 2: Percentage of immunohistochemical expression of CD133 for the groups I (newly diagnosed patients), II (relapsed patients), and III (resistant patients) showing significant differences between groups, (data are mean±SD).

differences between groups. Group I (newly diagnosed patients) demonstrates a moderate expression to CD133 marker as compared to other groups, while the most highly expressed CD markers is shown in the resistant group compared to the other groups. Group II (relapsed patients) reveals a higher expression of CD marker than the newly diagnosed group, with a significant difference among groups. These results are in agreement with.[19-21] The possible explanation is that the resistant group possesses a high concentration of cancer stem cells within the tumor mass. As a result, the patients of these samples showed a history of resistance to the chemotherapy without any responses through and at the end of the recurrent therapy sessions courses.

CONCLUSION

Cancer stem cells can be the major cause of colorectal cancer resistance to treatment and tumor relapse. CD133 marker can be targeted as a surface marker for cancer stem cells. The immunohistochemical expression of cancer stem cells marker CD133 was the highest in the resistant patient group, reaching 90%. Immunohistochemical analysis can be efficiently used for the detection of cancer stem cells in colorectal cancer. The detection of cancer stem cells in colorectal cancer clinical samples is very important and can be used to prevent the relapsing of tumors in patients after treatment and in resistance of tumors to treatments. Tumors targeting therapy strategies could be easily and readily achieved via the early diagnosis of cancer stem cells within the tumors since these types of cells possess different mechanisms for resistance to chemotherapy.

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