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Original Research Paper

Colon cancer development and histomorphological appearance correlated to immune markers

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Abstract: Colon cancer is one of the most common cancers worldwide, ranking third in incidence and second in cancer-related deaths. This study aimed to evaluate the histological and molecular changes associated with colon cancer, focusing on molecular markers related to chemotherapy resistance. **Methods:** The study included 60 paraffin-embedded tissue samples from patients diagnosed with colon adenocarcinoma, along with 10 normal tissue samples as a control. All samples underwent routine histological examination using hematoxylin and eosin (H&E) staining to assess overall histological structure, and Giemsa staining was used to examine nuclear changes in cancer cells. Immunohistochemical assays using CK7 were also performed. **Results:** The results showed clear histological changes in the affected colon and rectum tissues, characterized by glandular deformation, increased cellular irregularity, and nuclear clumping in cancer cells compared to the control sample. **Conclusion:** The study also showed a strong negative result for the immunohistochemical marker CK7, which is within the normal expression range for colorectal cancer.

Keywords: colon cancer, histological appearance, IHC, CK7

1. Introduction

Cancer is a disease of uncontrolled proliferation by transformed cells subject to evolution by natural selection. We believe this definition captures the essence of the majority of previous and current definitions. To the simplest definition of cancer as a disease of uncontrolled proliferation of cells, our definition adds in the adjective “transformed” to capture the many tumorigenic processes that cancer cells adopt to metastasize [1]. Cancer cells evade immune detection and destruction through various strategies, including altering antigen presentation, creating an immunosuppressive environment, and inhibiting immune cell function [2].

Colon cancer, also known as colorectal cancer, is a malignant tumor that begins in the epithelial cells lining the colon or rectum. Its development involves a multistep process driven by genetic mutations, epigenetic changes, and environmental factors [25]. Lifestyle choices, such as a diet high in red and processed meats, a sedentary lifestyle, and chronic inflammatory conditions such as inflammatory bowel disease, also play an important role. Colon cancer is widespread worldwide, with high incidence rates,

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particularly in developed countries. It is the third most common cancer worldwide and the second leading cause of cancer-related deaths. Early detection through screening methods, such as colonoscopy, significantly improves outcomes [22].

Colorectal cancer is the third most common cancer in men and the second most common in women, accounting for 10% of all cancers worldwide. The incidence rate is 25% higher in males and varies considerably between countries. With an estimated 600,000 deaths each year, colorectal cancer is the fourth leading cause of cancer-related deaths globally. Among colorectal cancers, there are 1,096,000 new cases of colon cancer (CC) annually, with distinct differences from cancers of the rectum [14]. Tumor histology often reflects tumor aggressiveness, patient prognosis, and curability, and has therefore been used as a de facto diagnostic tool and for clinical decision-making. However, it remains challenging to define how the diverse histological subtypes arise and translate into pleiotropic biological phenotypes [6]. Since Dr. Allen Gawn first described the diagnostic utility of differential expression of keratin-7 (keratin-7; CK7) and cytokeratin-20 (keratin-20; CK20) in 1995, CK7 and CK20 have been among the most frequently used markers in pathology for predicting tumor site of origin or classifying tumors. Numerous algorithmic approaches have been proposed using immunohistochemical (IHC) profiling for cancers of unknown primary origin, and these almost always incorporate the coordinate expression of CK7 and CK20 [24]. The immunophenotype CK7−/CK20+ has been proven to be a distinctive feature of colorectal cancer 5. It is considered a good marker for primary lung cancer and metastatic colorectal cancer spread to the lungs, but not all colorectal cancers lack CK7 expression. Various studies have shown that the positivity rate of CK7 can range from 0 to 22% [9]. Chemotherapy is a cornerstone of cancer treatment. In addition to shrinking the malignant mass by killing tumor cells, chemotherapy also promotes tumor control through immune stimulation. However, chemotherapy resistance and tumor relapse remain significant problems, indicating that the immune stimulation of chemotherapy is not usually effective, does not last long, or is easily overcome by immune evasion [21].

2. Methodology

A total of 60 paraffin embedded blocks containing fine needle aspiration tissues from patients suspected clinically confirmed colorectal adenocarcinoma as per the histopathological report between the periods of October and November 2025 from histopathology laboratories in Al-Najaf Al-Ashraf, Iraq. Tissues were sectioned by rotary microtome (5µm thick sections) and sections were stained with hematoxylin and eosin.

Immunohistochemistry of CK7

Ck7 antibodies were purchased from (BIO SB) was carried out using manufacturer protocol. Paraffin blocks tissues were sectioned and processed accordingly for H and E and immunohistochemistry. Charged Slides were marked with hydrophilic pen and Antibodies were applied at a dilution of 1:50 and incubated for 2 hours at room temperature. Slides were then washed with PBS 3 times for 2 minutes. Reagent 1 Bio SB (polymer helper) was applied to the slides and incubated for 20 minutes at room temperature and then washed with PBS 3 times for 2 minutes. Reagent 2 Bio SB (polyperioxidase-anti-mouse/ rabbit IgG) is then added and slides were incubated at room temperature for 20 minutes and slides were washed with PBS 3 times for 2 minutes. Slide colour development was carried out using DAB (3,3 diaminobenzidine) and slides were washed with deionized water. Slides were counterstained using haematoxylin stain. Mounting medium was applied before a cover slip was attached to each slide.

Immunohistochemical score

Positive stained cells were counted using image j, image processing software to identify and count the number of positively stained cells. Positive cells were counted and intensity of staining recorded. Each immunohistochemical slide was examined for 10 fields at a magnification of 100 X and mean data was recorded. Immuno reactive score (IRS) was worked out according to the following table and represented by plus system according to (20).

Statistical analysis

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Each histological slide was photographed 10 times to assess the IHC intensity. Data were analyzed by comparing differences between values using a one -way analysis of variance (ANOVA) parametric test to determine the statistical significance. Value was considered significant at $p \leq 0.05$. All those statistical analyses were performed using SPSS version 21.0 (IBM Corp., Armonk, NY, USA).

3. Results and discussion

Tissue staining of colon and rectal cancer using H&E and Giemsa stains.

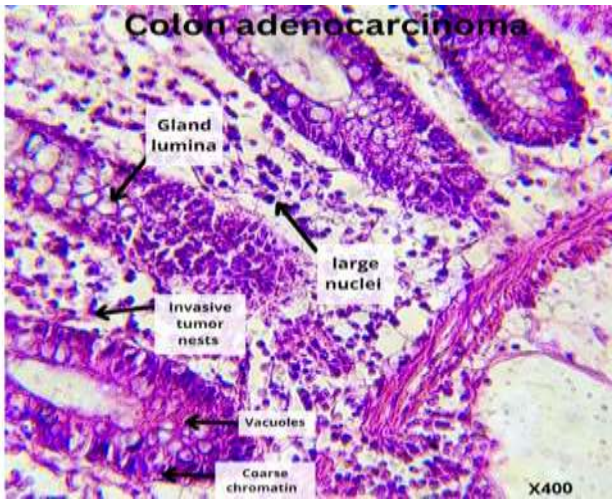

Fig 1: Colon adenocarcinoma tissue showing glandular lumina, large hyperchromatic nuclei, invasive tumor nests, cytoplasmic vacuoles, and coarse chromatin at ×400 magnification.

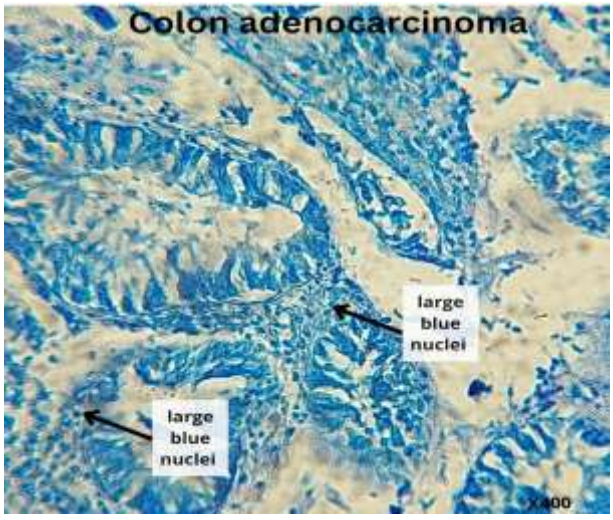

Fig 2: Giemsa stains of colon adenocarcinoma showing irregular glandular arrangements with large, deeply stained blue nuclei at 400x.

The results of this study showed histological section of well-differentiated adenocarcinoma of the colorectal characterized tissue by microscopic evidence Figure (1) shows sections stained with hematoxylin and eosin, magnified 400 times, showing a clear disruption in glandular organization, which characterizes colon adenocarcinoma. Irregularly shaped glands were observed, consisting of transformed epithelial cells with large, hyperchromatic nuclei, coarse chromatin, and prominent nucleoli. Cytoplasmic vacuoles were also found in some cells, reflecting the secretion of mucin. Gaseous tumor foci penetrating the surrounding connective tissue were also observed, confirming the malignant nature of the lesion. As Figure (2) shows in the sections stained with James' staining at a magnification of 400 times, the appearance of tumor cells in a deep blue color in the nuclei reflects an increase in DNA density and high nuclear activity. The large, irregular-shaped nuclei also indicate a high degree of malignancy in thyroid cancer. The glands appeared irregular with a decrease in cavity formation and clear nuclear congestion, indicating a loss of polarity and differentiation, which are two distinctive features of malignant transformation.

Colorectal cancer (CRC) is one of the most common cancers and accounts for a significant proportion of cancer-related deaths. The prognosis for colon cancer depends primarily on the stage. The 5-year relative survival rate for early, localized colon cancer can reach 91%; however, it drops to 72% for regional disease and 13% for patients with distant metastases [3]. Pathological histological analysis is used with tissue biopsy slides stained with hematoxylin and eosin (H&E). This staining technique allows the pathologist to identify tumor patterns, as the cells show abnormal shapes and textures [17]. As a new diagnostic biological marker based on hematoxylin and eosin (H&E) in patients with colon or stomach cancer. SARIFA positivity is defined as the direct contact between tumor cells and fat cells at the invasion front without intervening stroma (for example, without the so-called sticky stroma reaction) or intervening inflammatory infiltration, i.e., absence of lymphocytes, plasma cells, and granulocytes [19].

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Most cases of adenocarcinoma consist of branched and intricate glandular structures with uneven cavities, mainly composed of columnar cells, and sometimes caliciform cells located on a basal membrane. The tumor glands showed an empty cavity or a cavity occupied by necrotic cells, cytoplasmic fragments, nuclear debris, and fibrous material, referred to as "dirty necrosis." The tumor epithelium often appeared columnar or pseudostratified, with cytoplasm varying in abundance and heterogeneity, with large hypochromatic nuclei, vesicular nuclei, or one or more small nucleoli [11]. It is the cornerstone in the diagnosis of malignant hematologic tumors where the quality of staining plays a major role. Hypercellularity of the smear interferes with the quality of staining. We compared the newly modified Giemsa stain (MGS) as a routine method for staining hypercellular bone marrow smears with the traditional Leishman method and the May Grunwald Giemsa (MGG) method in a completely randomized study [7].

Giemsa stain can also help visualize chromosomal abnormalities through "Banding-Based Giemsa," or by observing alternating dark and light nucleotide regions on chromosomes during division [8].

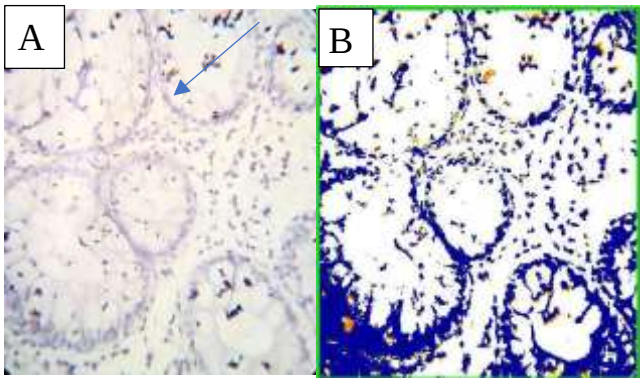

Fig 3: A histological section of well-differentiated of colorectal adenocarcinoma stained with immunome marker CK7 using IHC at 400X magnification. In image (A), the arrowhead (→) points to immunostaining are clearly negative for CK7 in the cytoplasm of the cancer cells, while image (B) shows the digital analysis of the same section to illustrate the staining intensity and positive areas.

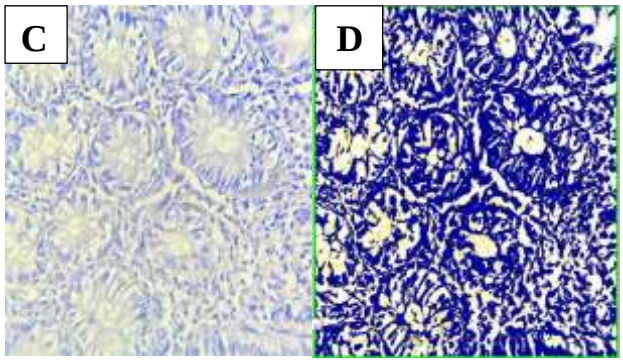

Fig 4: A tissue section of a control sample from the colorectal tissue immune stained with CK7 using IHC at 400X magnification. In image (C), the immune staining is clearly negative for CK7 in the cytoplasm of colon cells, while image (D) shows the digital analysis of the same section to illustrate the staining intensity and positive areas.

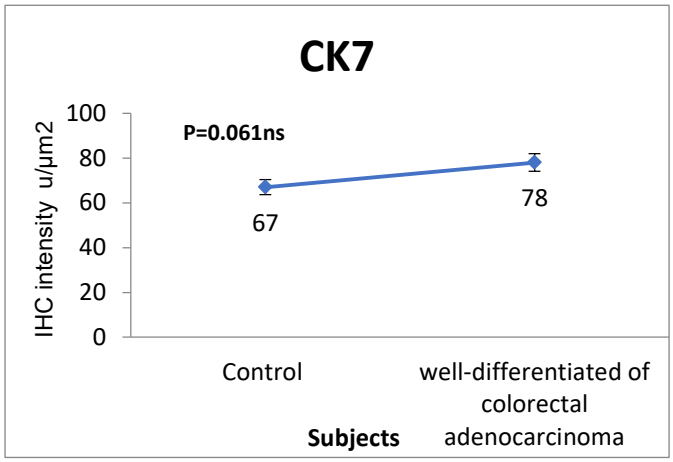

Fig 5: Ck7 staining intensity average IHC staining, control 67, well-differentiated of colorectal adenocarcinoma 78, P-value =0.061ns.

Figure (3) shows the histological section of well-differentiated of colorectal adenocarcinoma stained with an immunohistochemical (IHC) marker and examined under 400X magnification. Image (A) the arrowhead (→) points to clearly shows negativity and no association of the immunohistochemical marker CK7 in the tumor cells, as no distinctive brown staining appears, only light scattered staining with nonspecific colorations. Image (B) results from processing the same section through an image analysis program to highlighting the coloring areas where the blue regions indicate negative results. These results correspond to the immunophenotype of colorectal cancer, supporting its colonic origin and excluding positive patterns for this

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marker. While the figure (4) shows the histological section of a control sample from the colorectal stained with an immunohistochemical marker (IHC) and examined under 400X magnification. In image (C) the arrowhead (→) indicates, a clear negativity and lack of association of the immunohistochemical marker CK7 in the colonic cells is observed, as no distinctive brown staining appears, only nonspecific staining. Image (D) results from processing the same section through an image analysis program to highlight the negative blue staining areas and more precisely determine their intensity. These negative results are consistent with the immunophenotype of colorectal cells, supporting its colonic origin and excluding positive patterns for this marker.And the figure (5) shows a graph displaying the expression intensity of the immune protein CK7 in tumor cells well-differentiated of colorectal adenocarcinoma. The graph indicates that despite a slight increase in tumor cells reaching 78 compared to the control sample which reached 67, the difference was not statistically significant (P=0.061ns). This result indicates that CK7 does not signify a positive marker for colorectal cancer, which is consistent with the known immunophenotype of these tumors characterized by negativity or weak expression of CK7.

Cytokeratins (CKs) are intermediate filament proteins containing keratin and are characteristically expressed in epithelial tissues. The expression profile of CK subtypes varies according to the type and differentiation state of the epithelium. CK7 is commonly expressed in the epithelium of the stomach as well as in the biliary and pancreatic ductal epithelium [16].

Immunohistochemical analysis is also useful in determining the origin of cancer. Classically, a combination of cytokine keratin 7/20 expression has been used to evaluate cancers with an uncertain primary site. Most colon cancers are CK20-positive and CK7-negative, while most breast cancers are CK20-negative and CK7-positive. Interestingly, small bowel cancers frequently express CK7 and lack CK20, despite their intestinal morphology. Although this atypical immunophenotype may help predict the origin of intestinal adenocarcinoma, atypical CK7-positive and/or CK20-negative patterns have also been observed in approximately one-quarter of colon cancers with mismatch repair deficiency [15]. Non-keratinized epithelium expresses CK7, a type II keratin with a molecular weight of 54 kDa. Since CK20 is expressed in intestinal and gastric epithelial cells, while CK7 is found in breast, lung, ovarian, and urinary epithelium, a positive CK20 test and a negative CK7 test strongly suggest an intestinal origin for the cancer cells [20].

Cytoplasmic and/or membranous positivity for CK20 was observed in 66.2% of colorectal cancers. Positive immunostaining for CK7 was observed in 7% of cases. Regarding the co-expression of CK20 and CK7, the CK20+/CK7− immunophenotype was the most common, representing 46 out of 71 colorectal cancers, followed by CK20−/CK7−, then CK20−/CK7+ and CK20+/CK7+. Regarding CDX2, the majority of colorectal cancers (87.3%) showed positive staining. A statistically significant association was found between CDX2 expression and histologic grade and tumor invasion depth. Loss of CK20 positivity was associated with higher histologic grade. No association was found between CK7 expression and pathological histologic features [12]. Although seal cell carcinoma of the colon is rare, it is essential to differentiate between primary colon cancer and metastatic colonic gastro-cancer. Tumor cells with the immunohistochemical staining pattern CK7(−)/CK20(+) are indicative of primary colon cancer, while the staining pattern CK7(+)/CK20(−) is indicative of primary gastro-cancer. Therefore, based on histological features and immunohistochemical analysis results, metastatic colonic gastro-cancer was diagnosed [23].

Some recent studies have adopted a positivity threshold of 10% for considering a tumor CK7 positive. According to study by, Fei et al, (2019) [4]. CK7 expression can be considered a hallmark of retrograde differentiation or dedifferentiation related to the reacquisition of an embryonic phenotype, which is associated with mesenchymal epithelial transformation that confers metastasis to the tumor, described the absence of glandular differentiation and a primitive, duct-like morphology in CK7-expressing gastric mucosa. All these findings characterize CK7 expression in colorectal cancer as a marker of loss of differentiation associated with more aggressive behavior [10]. The study by, Kalita et al (2022) reported Immunohistochemistry was

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performed on ten cases. Of these, nine (90%) were CK20-positive and CK7-negative, and only one (10%) was CK20-positive and focally CK7-positive. Chu PG et al. reported that the most common immunohistochemical pattern for colorectal cancer is CK20-positive and CK7-negative, a staining pattern relatively specific to colorectal origin. However, up to 20% of tumors may exhibit a CK7-positive/CK20-negative or CK7-negative/CK20-negative staining pattern. The study by, Fleming M et al (2012) reported similar data indicating that colorectal cancer is CK20-positive and CK7-negative. Thus, the immunohistochemical results in this study were consistent with other studies [13].

Immunohistochemical staining is crucial for the accurate diagnosis of primary tumors and rare metastatic sites. Typically, the immunophenotype of adenocarcinomas of the colon is CK20-positive, CEA-positive, and CK7-negative. CK7 expression is observed in ductal and glandular epithelium, such as that of the salivary glands, pancreas, ovary, and endometrium. However, a small subset of colorectal cancers may be locally or weakly CK7-positive, as in our patient. Increased CK7 expression is associated with a poorer prognosis in colorectal cancer patients [18].

Conclusion

Colorectal cancer exhibits a typical immunohistochemical pattern characterized by CK7 negativity, with the possibility of abnormal or focal expression of CK7 in a limited number of cases. This abnormal expression is an indicator of loss of cellular differentiation and is associated with more aggressive tumor behavior and a worse prognosis.

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none

Author's Contribution

All authors had equal contribution to this manuscript

Ethics

This study was conducted under approval by the medical ethics committee at University of Kufa (2025).

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