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The expression of CD44v6 in colon: from normal to malignant

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ABSTRACT

CD44v6, an integral transmembrane protein belonging to a family of adhesion molecule receptors, plays an important role in tumor growth, progression and metastasis. The purpose of this study was to evaluate the expression of CD44v6 in normal, hyperplastic, adenomatous, and malignant colonic epithelium and to determine its correlation with tumor pathologic stage and lymph node metastasis. We examined the immunohistochemical expression of CD44v6 in normal colonic tissue (n = 25), hyperplastic polyps (n = 45), tubular adenomas (n = 57), tubulovillous adenomas (n = 25), villous adenomas (n = 9), adenocarcinomas stage I (n = 26), adenocarcinomas stage III (n = 26), and lymph node metastasis (n = 26). The percentage of positive cells and the staining intensity were assessed and scored. Statistical analysis was performed using logistic regression and McNemar test. All normal colonic tissue and hyperplastic polyps showed CD44v6 staining confined to the base of the crypt. In tubular adenomas, the dysplastic surface adenomatous epithelium expressed CD44v6 in 49 (86%) cases. CD44v6 was expressed in the glandular areas of tubulovillous adenomas in 21 (84%) cases and in the villous portion in 18 (72%) cases. All villous adenomas expressed CD44v6. CD44v6 was expressed in 23 (88%) cases of stage I adenocarcinomas, in 24 (92%) cases of stage III adenocarcinomas, and in 9 (35%) cases of metastatic adenocarcinomas. We concluded that the gain of CD44v6 expression in premalignant and malignant colonic lesions suggests that CD44v6 may be functionally involved in the adenoma-to-carcinoma progression. CD44v6 did not correlate to tumor pathologic stage and is lost during the acquisition of migratory function by metastatic tumor cells.

1. Introduction

Colorectal cancer is the second leading cause of cancer-related deaths in the United States and the third most common cancer in men and women. The American Cancer Society estimates that, in the United States in 2015, about 132,700 cases will be diagnosed with colorectal cancer and about 49,700 people will die of the disease [1]. Colorectal cancers commonly develop from preexisting polyps. Other risk factors include long-standing inflammatory bowel disease, certain types of diets, physical inactivity, obesity, smoking, and heavy alcohol consumption. Histologically, colonic polyps are classified as hyperplastic (90%) and adenomatous (10%), with the latter encompassing tubular, tubulovillous, and villous adenomas. Tubular adenomas are encountered most frequently (80%-86%) followed by tubulovillous adenomas (8%-16%) and villous adenomas (5%). Adenoma-to-carcinoma progression is well established as evidenced by the presence of 1 or more synchronous adenomas in one-third of operative specimens containing colon cancer, the risk of colon cancer is increased with the number of adenomatous polyps, and adenomatous tissue is frequently found contiguous to carcinoma. Molecular genetic studies also support an adenoma-to-carcinoma sequence with the accumulation of a number of genetic and epigenetic mutation [2,3].

Adenoma-to-carcinoma progression, tumor cell invasion, and metastasis involve a series of complex interactions between malignant cells and peritumoral stroma. These interactions are accomplished through transmembrane receptors on tumor cells that interact with stromal extracellular matrix molecules. One of these receptors, CD44, binds to the extracellular matrix component hyaluronan [4]. CD44 is an 85- to 90-kDa integral transmembrane glycoprotein belonging to a distinct family of adhesion molecule receptors. Multiple variant isoforms exist (CD44v2-v10, including CD44v6) arising from alternate mRNA splicing between exons 5 and 16 [5]. The variation at the gene level translates to variations on the proximal segment of extracellular portion of the CD44 receptor. These variations alter the cell-to-cell and cell-to-matrix adhesion properties of the receptor. There has been recent intense interest in the association of variant CD44v6 expression and tumor progression and metastasis [6,7]. Although CD44v6 expression has been correlated with higher tumor stage and metastatic potential in many tumors, its expression in colorectal tumors has been inconsistent and confusing.

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The purpose of this study was to evaluate the expression and distribution of CD44v6 during the progression of colorectal neoplasia by analyzing normal, hyperplastic, adenomatous, and malignant colonic epithelium and to determine its correlation with tumor pathologic stage and lymph node metastasis.

2. Materials and methods

2.1. Case selection

This retrospective study was conducted following approval by the institutional review board. A total of 234 cases were identified via the center's laboratory information system. The study included normal colonic tissue (n = 25), hyperplastic polyps (HP, n = 45), tubular adenomas (TA, n = 57), tubulovillous adenomas (TV, n = 25), villous adenomas (VA, n = 9), adenocarcinomas stage I (n = 26), adenocarcinomas stage III (n = 26), and adenocarcinoma metastatic to lymph nodes (n = 26). Tumors were staged according to American Joint Committee on Cancer [8].

The clinical histories were reviewed, and only cases obtained from screening colonoscopy with no history of cancer, colonic polyp, or inflammatory bowel disease and with normal pathology were included in the study as examples of normal tissue. Hematoxylin and eosin–stained slides of each case were reviewed to confirm the diagnosis. A representative slide was selected from each case, and the corresponding formalin-fixed, paraffin-embedded tissue blocks were retrieved from the surgical pathology archives. To compare the CD44v6 expression in the primary tumor and the metastatic tumor, we retrieved 2 tissue blocks from every case of stage III adenocarcinoma: 1 block from the primary tumor (n = 26) and the second block from the lymph node harboring the metastatic adenocarcinoma (n = 26).

2.2. Immunohistochemical staining for CD44v6

Five-micrometer–thick sections were cut from paraffin-embedded tissue and placed onto coated slides, deparaffinized in xylene, and rehydrated in descending grades of ethanol (100%-70%). Sections were then microwaved at 97°C in 150 mL of 10 mmol/L citrate buffer (pH 6) for 15 minutes to induce epitope retrieval. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol (Vector, Burlingame, CA) for 10 minutes. Sections were incubated at room temperature for 1 hour with a monoclonal antibody against CD44v6, the monoclonal antibody VFF7 against the amino acid sequence encoded by exon CD44V6 (1:1500, Bender MedSystems, CA). The slides were incubated with a biotinylated secondary antibody at room temperature for 30 minutes and thereafter with horseradish peroxidase–conjugated avidin-biotin complex at room temperature for 1 hour. Immunostaining was visualized using 3,3′-diaminobenzidine tetrahydrochloride/H_2O_2 substrate and counterstained with hematoxylin. Sections from normal skin served as positive control for CD44v6. For negative controls, the primary antibody was substituted with an isotypic mouse immunoglobulin G.

2.3. Results interpretation

Tissue sections from all cases were evaluated independently by 2 pathologists for the expression of CD44v6. *Reactivity for CD44v6* was defined as uniform cytoplasmic membrane staining, and its intensity was graded as follows: 1, weak; 02, moderate; 3, strong. Weak stain was considered negative. The stain was considered positive if at least 10% of cells showed strong positive stain. The percentage of positive staining was classified as focal if foci of positive cells were separated by nonstaining areas and diffuse if more than 75% of the area of interest was positive. Reviewers agreed on the interpretation of all variables; in the event of a disagreement, a consensus interpretation was generated after simultaneous review of the slide. Histologically, the TV polyp consists of an adenomatous and villous component. Therefore, in each case of TV polyp, we evaluated the expression of CD44v6 in the adenomatous and villous component separately. The stains results were tabulated, and statistical analysis was performed.

2.4. Statistical analysis

The odds of positive (3+ or 2+) CD44v6 intensity staining, of any (3+, 2+, or 1+) CD44v6 staining between groups, or of staining distribution (diffuse vs focal staining) were compared between groups using logistic regression; Firth's [9] penalized likelihood method was used to address staining/group combinations with zero counts. Odds ratio, 95% confidence interval, and *P* value were tabulated and compared. The proportions of samples with positive CD44v6 staining were compared between stage III primary adenocarcinoma and matched lymph node metastasis samples using the McNemar test [10]. Analyses were conducted using SAS software for Windows, version 9.4 (SAS Institute, Cary, NC).

3. Results

3.1. Evaluation of histopathology

Reevaluation of the hematoxylin and eosin–stained tissue sections confirmed the original diagnosis on all cases. Therefore, we included all the 234 cases we retrieved from our files.

3.2. CD44v6 staining results

In all cases of normal colon, strong (3+) CD44v6 staining was noted only in rare cells located at the base of the crypt (Fig. 1A). The remaining epithelium of the crypts and the surface epithelium did not show any CD44v6 staining. In all 45 (100%) HP cases, the hyperplastic epithelium did not express CD44v6; however, rare positive (3+) epithelial cells were noted at the base of the crypt (Fig. 1B). Tubular adenomas expressed CD44v6 staining in 49 of 57 (86%) cases and were negative in 8 cases. The stain was diffuse in 39 cases and focal in 10 cases (3+ in 30 cases and 2+ in 19 cases). Analogous to normal colonic crypts and HP, TA showed rare CD44v6 positive (3+) epithelial cells at the base of the crypt, whereas the remaining epithelium of the crypt was negative (Figs. 2 and 3). In TV, we evaluated CD44v6 expression in the adenomatous (tubular) and villous component independently in each case. The adenomatous component of TV polyps expressed CD44v6 in 21 of 25 (84%) cases and was negative in 4 cases. The stain was diffuse in 15 cases and focal in 6 cases (3+ in 11 cases and 2+ in 10 cases). The villous component of the TV was positive for CD44v6 in 18 (72%) cases and negative in 7 cases. The stain was diffuse in 3 and focal in 15 (3+ in 3 and 2+ in 15 cases). All cases that showed positive CD44v6 staining in the villous component also exhibited positive staining in the adenomatous areas. Three cases were positive only in the adenomatous areas, and 4 cases were negative in both components (Fig. 3). Villous adenomas expressed CD44v6 in all cases (9/9; 100%). The stain was diffuse in 5 cases and focal in 4 cases (3+ in 8 cases and 2+ in 1 case).

CD44v6 was expressed in 23 (88%) cases of adenocarcinomas stage I (3+ in 17 cases and 2+ in 6 cases) and in 24 (92%) cases of adenocarcinomas stage III (3+ in 21 cases and 2+ in 3 cases) (Fig. 4). Metastatic foci in the lymph nodes expressed CD44v6 in 9 (35%) of cases (3+ in 8 cases and 2+ in 1 cases). Stromal cells in all cases did not express CD44v6. Scattered lymphocytes were noted to be positive.

4. Statistical analysis results

Tables 1, 2, and 3 summarize CD44v6 staining intensity, numbers of positive samples, and numbers of diffuse vs focal samples, respectively, by group. Table 4 shows numbers of positive samples in matched stage III adenocarcinoma and lymph node metastasis samples.