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# Distribution of complement regulatory proteins, decay-accelerating factor, CD59/homologous restriction factor 20 and membrane cofactor protein in human colorectal adenoma and cancer.

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**Abstract**

To clarify the events related to complement-mediated immune responses in human colorectal cancers, we immunohistochemically examined the distribution of decay-accelerating factor (DAF), CD59/homologous restriction factor 20 (HRF20), membrane cofactor protein (MCP) and terminal complement complex (TCC) in human colorectal adenomas and cancers, and then compared the findings with their distribution in normal colonic mucosa. In the normal mucosa, TCC was not present on epithelial cells. Whereas DAF and CD59/HRF20 were present only occasionally on the apical surfaces of normal epithelial cells, MCP was diffusely distributed on the basolateral surfaces of most epithelial cells of the colon. These findings suggest that MCP has a primary role in the regulation of complement activation on these cells. In adenoma cells, the expression of both DAF and CD59/HRF20 was enhanced. In cancer cells, the expression of CD59/HRF20 and MCP was diminished, whereas DAF expression was markedly enhanced. Since DAF was frequently stained in the lumen of the cancer glands, it was suggested that DAF was released into the colonic lumen in patients with colorectal cancer.

**KEYWORDS:** complement regulatory protein, decayaccelerating factor, membrane cofactor protein, homologous restriction factor 20, colorectal cancer

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# Distribution of Complement Regulatory Proteins, Decay-Accelerating Factor, CD59/Homologous Restriction Factor 20 and Membrane Cofactor Protein in Human Colorectal Adenoma and Cancer

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To clarify the events related to complement-mediated immune responses in human colorectal cancers, we immunohistochemically examined the distribution of decay-accelerating factor (DAF), CD59/homologous restriction factor 20 (HRF20), membrane cofactor protein (MCP) and terminal complement complex (TCC) in human colorectal adenomas and cancers, and then compared the findings with their distribution in normal colonic mucosa. In the normal mucosa, TCC was not present on epithelial cells. Whereas DAF and CD59/HRF20 were present only occasionally on the apical surfaces of normal epithelial cells, MCP was diffusely distributed on the basolateral surfaces of most epithelial cells of the colon. These findings suggest that MCP has a primary role in the regulation of complement activation on these cells. In adenoma cells, the expression of both DAF and CD59/HRF20 was enhanced. In cancer cells, the expression of CD59/HRF20 and MCP was diminished, whereas DAF expression was markedly enhanced. Since DAF was frequently stained in the lumen of the cancer glands, it was suggested that DAF was released into the colonic lumen in patients with colorectal cancer.

**Key words:** complement regulatory protein, decay-accelerating factor, membrane cofactor protein, homologous restriction factor 20, colorectal cancer

The development of malignant tumors elicits cell-mediated and humoral factor-mediated immune responses against tumor cells as part of several host defense mechanisms, and various therapeutic modalities utilizing these responses have been studied (1). Among the humoral factor-mediated responses, the complement system is a major effector pathway. The complement system has classically been implicated as a defense mechanism against infection by microorganisms and parasites, but the possibility that it participates in host immune responses to cancers also should be considered (2).

Recently, several membrane-bound molecules that regulate complement activation have been identified: Decay-accelerating factor (DAF, CD55) inhibits the formation of C3/ C5 convertases and promotes their catabolism (3, 4). Membrane cofactor protein (MCP, CD46) is a cofactor for factor I-mediated cleavage of C3b and C4b (5). CD59/homologous restriction factor 20 (HRF20) inhibits the formation of terminal complement complex (TCC) by preventing the binding of C9 to C5b-8 (6, 7). Knowledge of the distribution of these complement regulatory proteins is important to understand the complement-related immune responses to human cancers. Although the expression of these molecules in human malignancies, especially hematological malignancies has been reported (8-11); reports on colorectal cancers are limited (12, 13). In this study, to help elucidate the events related to the complement-mediated immune responses in human colorectal cancers, we immunohistochemically examined the distribution of DAF, CD59/HRF20, MCP and TCC in human colorectal adenomas and cancers.

## Materials and Methods

***Tissues.*** Tissue specimens of normal colonic mucosa and the tumor of the colon were obtained by endoscopic biopsy, polypectomy or surgical resection

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from 12 patients (8 men and 4 women; mean age, 61 years) with colorectal adenomas and 13 patients (7 men and 6 women; mean age, 61 years) with colorectal cancer, respectively. Informed consent was obtained from each patient. Samples were collected from the tumor lesion and from the unaffected mucosa at least 5 cm distant. Fourteen specimens of histologically normal mucosa were examined as controls. Of the 12 adenomas, 9 were tubular and 3 were tubulovillous. The diameter of the adenomas ranged from 3 to 22 mm. Five adenomas were located in the right, and 7 in the left side of the colon. Of the 13 cancers, 4 were well differentiated and 9 were moderately differentiated adenocarcinomas. Two cancers were located in the right, and 11 in the left side of the colon. The stage of the tumors, according to the modified Dukes' classification of the Gastrointestinal Tumor Study Group of the National Cancer Institute (14), was: A (n = 2), B (n = 4), and C (n = 7). None of the patients had liver metastasis.

***Immunohistochemistry.*** The tissue specimens were fixed in periodate-lysine-paraformaldehyde fixative (15), and cryostat sections were stained using an indirect peroxidase-labeled antibody method. The following mouse monoclonals were used as primary antibodies: 1C6 antibody to DAF (IgG1 isotype, Wako Pure Chemical Industries, Osaka, Japan, and a gift from Prof. Teizo Fujita, Fukushima Medical College, Fukushima, Japan) (4); 1F5 antibody to CD59/HRF20 (IgG1 isotype, a gift from Prof. Hidechika Okada, Nagoya City University, School of Medicine, Nagoya, Japan) (7); J4-48 antibody to MCP (IgG1 isotype, IMMUNOTECH S.A., Marseilles, France) (16); and aE11 antibody to a C9 neoepitope of TCC (IgG2a isotype, Dako, Glosrup, Denmark) (17). After incubation with the primary antibodies, the sections were incubated with horseradish peroxidase-labeled Fab' fragments of rabbit anti-mouse immunoglobulins, prepared as described previously (18), and then with diaminobenzidine containing hydrogen peroxide. The stained sections were counterstained with methyl green, dehydrated and mounted.

The expression of the antigens in the normal and neoplastic epithelia was evaluated semiquantitatively: Antigen expression was scored (2+) when specific staining was detectable on more than half the normal or neoplastic epithelia in the section, (1+) when specific staining was detectable but in less than half, and (−) when faint or no antigen was detectable. For statistical analysis, the chi-squared method was used.

## Results

***DAF.*** In 9 of 14 non-neoplastic normal mucosae, DAF staining was observed occasionally in small foci of the apical surface of colonic epithelial cells (Fig. 1A); and it was negative in the remaining 5. In contrast, in 6 of 12 adenomas and 10 of 13 colorectal cancers, DAF staining was enhanced on the luminal surface of the neoplastic glands (Fig. 1B, C). This was more prominent in the cancer than in the adenoma (Fig. 1B, C, Table 1, $P < 0.01$). DAF was often stained not only on the luminal surface but in the lumen of the cancer glands (Fig. 1C).

***CD59/HRF20.*** CD59/HRF20 was weakly stained in 12 of 14 normal mucosae along the luminal surface of the epithelial cells in the upper part of most crypts (Fig. 2A). It was also present in stromal cells of the mucosa. In 7 of 12 adenomas, the expression of this molecule was enhanced at the luminal surface of the adenoma gland (Fig. 2B). Of 13 cancers, CD59/HRF20 was stained intensely in 3 along the apical surface of the cancer cells (Fig. 2C) and weakly in 7 as seen in the normal epithelia. In the remaining 3, the cancer cells were

**Table 1** Expression of the complement regulatory proteins and deposition of terminal complement complex (TCC) in normal and neoplastic colorectal epithelia

| | | No. of specimens | | | |
|---|---|---|---|---|---|
| | | − | + | 2+ | Total |
| DAF | Normal | 5 | 9 | 0* | 14 |
| | Adenoma | 0 | 6 | 6 | 12 |
| | Cancer | 0 | 3 | 10 | 13 |
| CD59/HRF20 | Normal | 0 | 12 | 2** | 14 |
| | Adenoma | 0 | 5 | 7 | 12 |
| | Cancer | 3 | 7 | 3 | 13 |
| MCP | Normal | 0 | 0 | 14* | 14 |
| | Adenoma | 0 | 4 | 8 | 12 |
| | Cancer | 0 | 8 | 5 | 13 |
| TCC | Normal | 14 | 0 | 0 | 14 |
| | Adenoma | 11 | 1 | 0 | 12 |
| | Cancer | 11 | 2 | 0 | 13 |

2+: specific staining on more than half of normal or neoplastic epithelia. 1+: specific staining on less than half. −: faint or no specific staining. DAF: decay-accelerating factor; MCP: membrane cofactor protein; TCC: terminal complement complex. *$p < 0.01$, **$p < 0.02$.

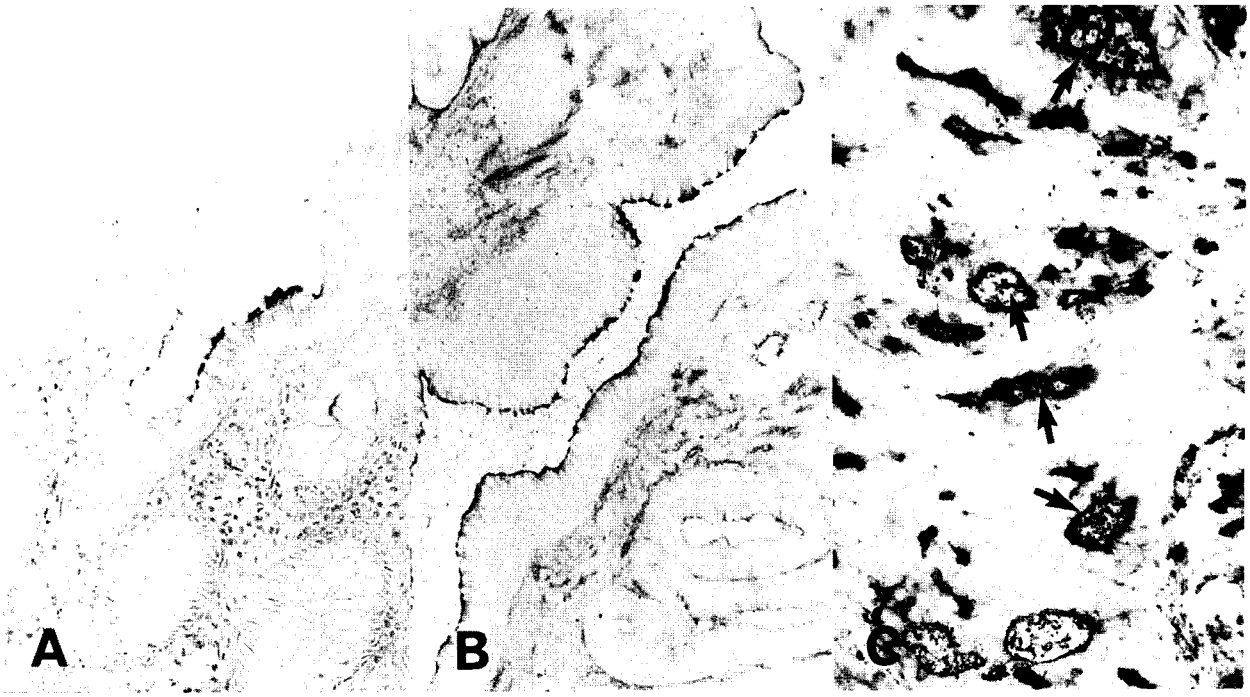


**Fig. 1** Immunohistochemical localization of decay-accelerating factor (DAF). In normal colonic mucosa (**A**), DAF is present in small foci of the apical surface of colonic epithelial cells. In adenoma (**B**), DAF staining is enhanced along the luminal surface of the adenoma cells. In cancer (**C**), intense DAF staining is present on the luminal surface of cancer cells and in the lumen of the cancer glands (arrows).

not stained, but the surrounding connective tissues were positive for CD59/HRF20 (Fig. 2D, Table 1, $P < 0.02$).

***MCP.*** MCP was observed on all normal mucosae. In contrast to the apical and occasional staining of DAF and CD59/HRF20, MCP was diffusely present on the basolateral surface of the epithelial cells throughout the crypts (Fig. 3A). In 8 of 12 adenomas and 5 of 13 cancers, MCP was stained with similar pattern and intensity to the normal mucosa (Fig. 3B); in the remaining 4 adenomas and 8 cancers, MCP was weakly stained (Fig. 3C, Table 1, $P < 0.01$).

***TCC.*** TCC was not stained in all normal epithelia (Fig. 4A). In 1 of 12 adenomas and 2 of 13 cancers, TCC was deposited along the basement membranes of the tumor gland (Fig. 4B, Table 1).

## Discussion

In this study, we immunohistochemically examined the distribution of the complement regulatory proteins (DAF, CD59/HRF20 and MCP) and TCC in normal and neoplastic human colonic tissues. In the normal mucosa, the expression of DAF was sporadic, and CD59/HRF20 was weakly stained, and expression of both molecules was limited to the apical surface of the epithelial cells. In contrast, MCP was diffusely distributed on the basolateral surfaces of normal epithelial cells throughout the crypt. These findings suggest that MCP has a primary role in the regulation of complement activation on the colonic epithelial cells.

We found that TCC was rarely stained on the neoplastic epithelia, suggesting that the colorectal cancer cells may not induce significant activation of autologous complement. MCP, which was expressed in neoplastic epithelia although at a somewhat decreased level, may protect the neoplastic cells from TCC deposition. Our findings are important in complement-mediated immune therapy of colorectal cancer (2).

Both DAF and CD59/HRF20 are glycosyl-

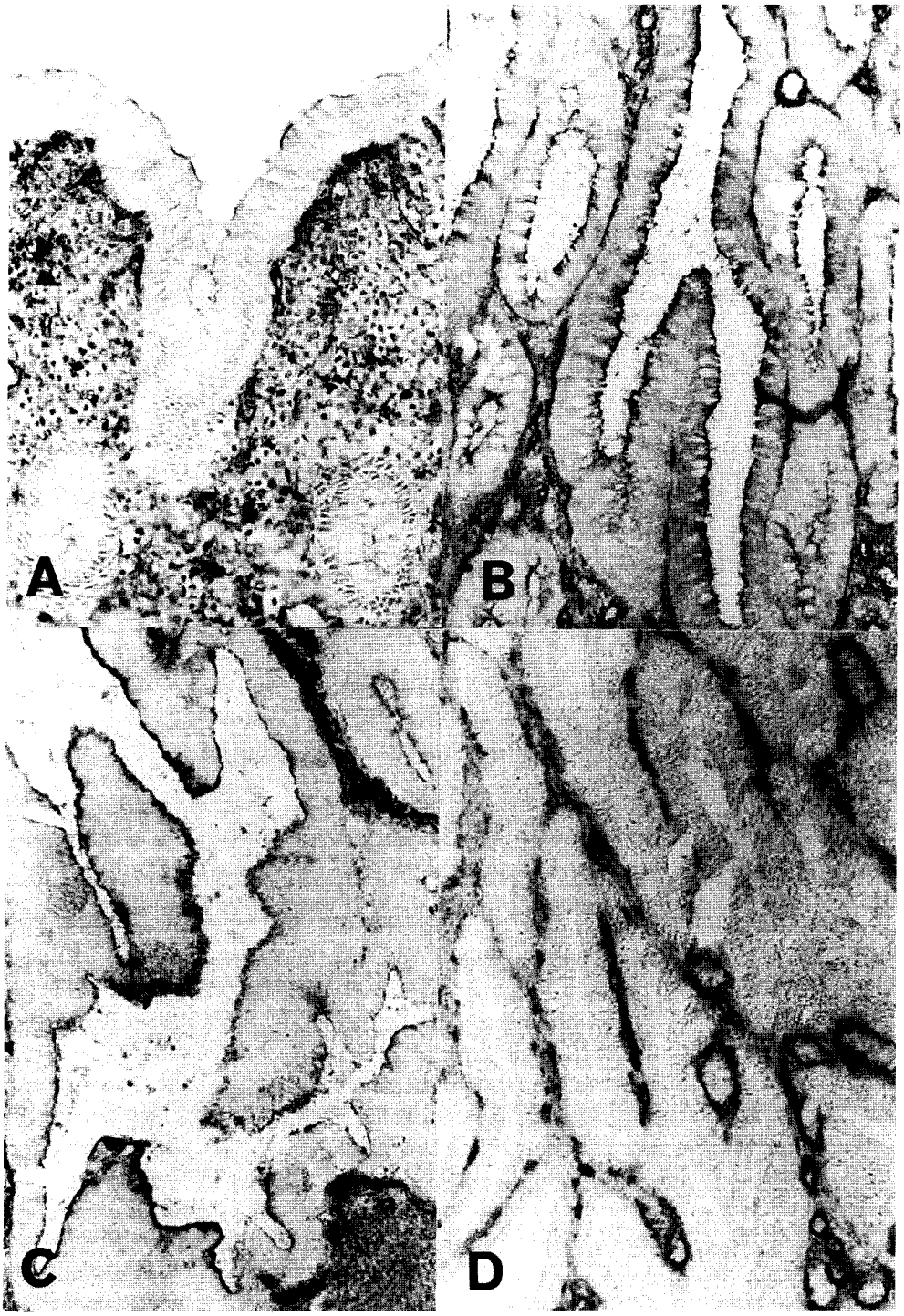


**Fig. 2** Immunohistochemical localization of CD59/HRF20. In normal colonic mucosa (**A**), weak linear staining of CD59/HRF20 is visible along the luminal surface of the normal epithelium in the upper part of the crypt. It is also present in stromal cells of the mucosa. In adenoma (**B**), the expression of CD59/HRF20 is enhanced at the luminal surface of the adenoma glands. Intense staining is seen along the apical surface of cancer cells (**C**). In another sample (**D**), connective tissues surrounding cancer cells are stained for CD59/HRF20, whereas the cancer cells are not.

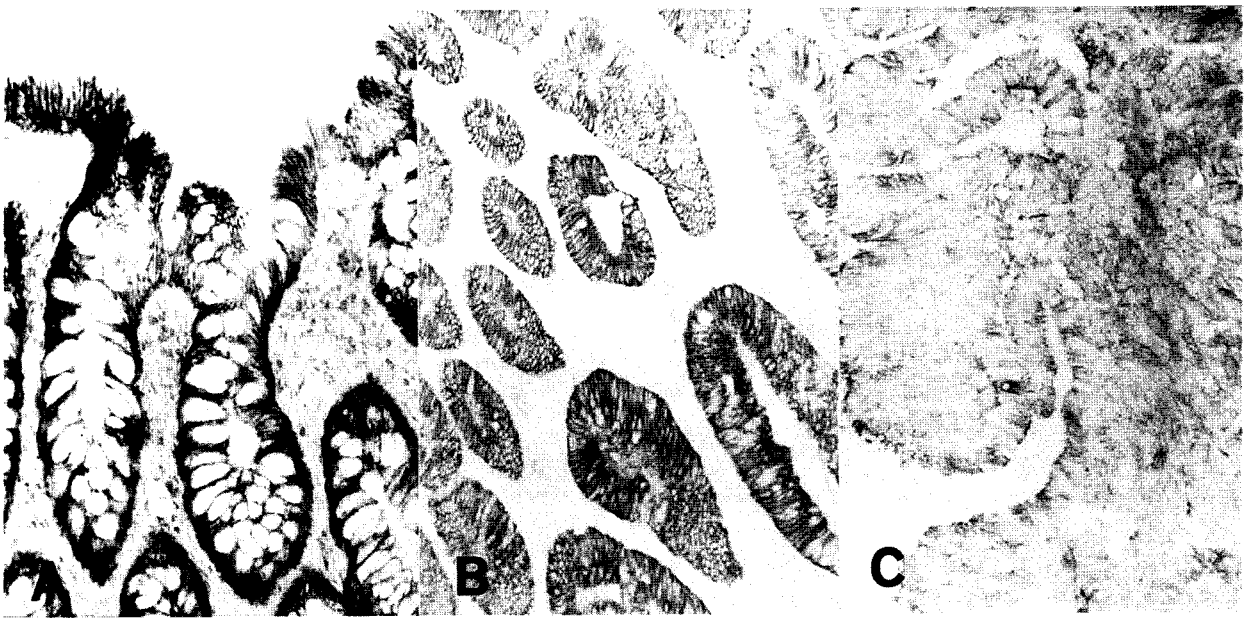


**Fig. 3** Immunohistochemical localization of menbrane cofactor protein (MCP). In normal colonic mucosa (**A**), MCP is present on the basolateral surface of the epithelial cells throughout the crypt. In adenoma and cancer, MCP is stained in a manner and intensity corresponding to that of the normal mucosa (**B**; an adenoma), or MCP staining on the neoplastic cells is decreased (**C**; a cancer).

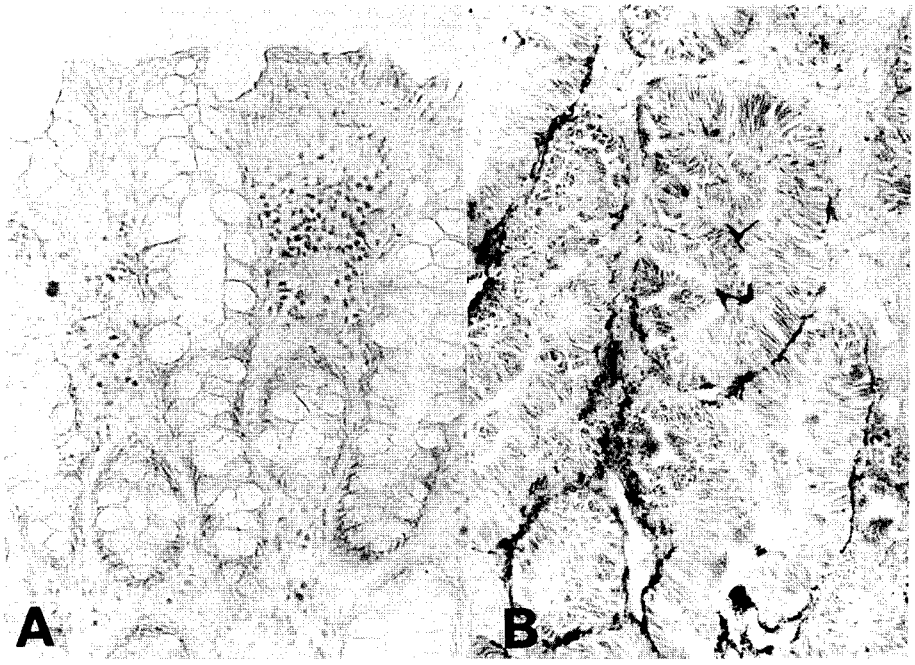


**Fig. 4** Immunohistochemical localization of terminal complement complex (TCC). In normal mucosa (**A**), there is no deposition of TCC. In colon cancer (**B**), TCC is deposited along the basement membranes of the cancer cells.

phosphatidylinositol (GPI)-anchored membrane proteins (7, 19, 20), whereas MCP is a transmembrane protein (5). As to the sorting mechanism for plasma membrane proteins in polarized epithelial cells, a GPI-membrane anchor has been suggested as a targeting signal for the apical surface (21), and our observations are compatible with this notion.

We found that the expression of both DAF and CD59/HRF20 was enhanced in adenoma. In cancer, DAF expression was markedly enhanced, whereas the expression of CD59/HRF20 was diminished. Shichishima *et al.* reported a similar profile of DAF and CD59/HRF20 expression on leukemic cells and suggested that DAF could compete more effectively than CD59/HRF20 for the limited number of anchor molecules available on proliferating leukemic cells (10). This could also be true of the colorectal cancer cells in this study. Although the mechanisms involved in the altered expression of these molecules in colorectal cancer are unknown, they are almost certainly multifactorial due to the complex nature of host responses.

The present results suggest that the expression of complement regulatory proteins was changed during neoplastic transformation in colorectal epithelia, but these results differ from those reported by Koretz *et al.* (12, 13) in some respects. We found that DAF expression was weaker in the normal epithelia, and more remarkable and more frequently enhanced in the neoplastic epithelia. The inconsistency in these studies could be due to the different antibodies used or the immunohistochemical procedures employed, but the differences are more likely due to the antibodies since discordant expression of DAF epitopes recognized by different antibodies has been shown in lung cancer cells (22). We further found that DAF was intensely and frequently stained in the lumen of the cancer glands, suggesting the release of DAF into the colonic lumen. This finding raises the possibility that the stools of patients with colorectal cancer contain increased amounts of DAF. We have developed an immunoassay for DAF and are currently examining whether measurement of stool DAF could be useful in the detection of colorectal cancer.

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## References

1. Parmiani G, Anichini A and Fossati G: Cellular immune response against autologous human malignant melanoma: Are *in vitro* studies providing a famework for a more effective immunotherapy. J Natl Cancer Inst (1990) **82**, 361-370.
2. Seya T, Hara T, Matsumoto M, Sugita Y and Akedo H: Complement-mediated tumor cell damage induced by antibodies against membrane cofactor protein (MCP, CD46). J Exp Med (1990) **172**, 1673-1680.
3. Nicholson-Weller A, Burge J, Fearon DT, Weller PF and Austen KF: Isolation of a human erythrocyte membrane glycoprotein with decay-accelerating activity for C3 convertases of the complement system. J Immunol (1982) **129**, 184-189.
4. Fujita T, Inoue T, Ogawa K, Iida K and Tamura N: The mechanism of action of decay-accelerating factor (DAF): DAF inhibits the assembly of C3 convertases by dissociating C2a and Bb. J Exp Med (1987) **166**, 1221-1228.
5. Seya T, Turner JR and Atkinson J: Purification and characterization of a membrane protein (gp45-70) that is a cofactor for cleavage of C3b and C4b. J Exp Med (1986) **163**, 837-855.
6. Sugita Y, Nakano Y and Tomita M: Isolation from human erythrocytes of a new membrane protein which inhibits the formation of complement transmembrane channels. J Biochem (1988) **104**, 633-638.
7. Okada N, Harada R, Fujita T and Okada H: A novel membrane glycoprotein capable of inhibiting membrane attack by homologous complement. Int Immunol (1989) **1**, 205-208.
8. Seya T, Hara T, Matsumoto M and Akeda H: Quantitative analysis of membrane cofactor protein (MCP) of complement: High expression of MCP on human leukemia cell lines, which is down-regulated during cell-differentiation. J Immunol (1990) **145**, 238-245.
9. Fukuda H, Seya T, Hara T, Matsumoto M, Kinosita T and Masaoka T: Deficiency of decay-accelerating factor (DAF, CD55) in Non-Hodgkin's lymphoma. Immunol Lett (1991) **29**, 205-210.
10. Shichishima T, Terasawa T, Hashimoto C, Ohto H, Takahashi M, Shibata A and Maruyama Y: Discordant and heterogeneous expression of GPI-anchored membrane proteins on leukemic cells in a patient with paroxysmal nocturnal hemoglobinuria. Blood (1993) **81**, 1855-1862.
11. Hara T, Kojima A, Fukuda H and Matsumoto M: Levels of complement regulatory proteins, CD35 (CR1), CD46 (MCP), CD55 (DAF) in human haematological malignancies. Br J Hematol (1992) **82**, 368-373.
12. Koretz K, Brüderlein S, Henne C and Möller P: Decay-accelerating factor (DAF, CD55) in normal colorectal mucosa, adenomas and carcinomas. Br J Cancer (1992) **66**, 810-814.
13. Koretz K, Brüderlein S, Henne C, and Möller P: Expression of CD59, a complement regulator protein and a second ligand of the CD2 molecule, and CD46 in normal and neoplastic colorectal epithelium. Br J Cancer (1993) **68**, 926-931.
14. Enker WE, Laffer UT and Block GE: Enhanced survival of patients with colon and rectal cancer is based upon wide anatomic resection. Ann Surg (1979) **190**, 350-360.
15. McLean IW and Nakane PK: Periodate-lysine-paraformaldehyde fixative: A new fixative for immunoelectron microscopy. J Histochem Cytochem (1974) **22**, 1077-1083.
16. Pesando JM, Hoffman P and Abed M: Antibody-induced antigenic modulation is antigen dependent: Characterization of 22 proteins on a malignant human B cell line. J Immunol (1986) **137**, 3689-3695.
17. Mollnes TE, Lea T, Harboe M and Tschopp J: Monoclonal antibodies recognizing a neoantigen of poly (C9) detect the human terminal complement complex in tissue and plasma. Scand J Immunol (1985)

**22**, 183-195.

18. Nakane PK and Kawaoi A: Peroxidase-labeled antibody: A new method of conjugation. J Histochem Cytochem (1974) **22**, 1084-1091.
19. Davitz MA, Low MG and Nussenzweig V: Release of decay-accelerating factor (DAF) from the cell membrane by phosphatidylinositol-specific phospholipase C (PIPLC). J Exp Med (1986) **163**, 1150-1161.
20. Medof ME, Walter EI, Roberts WL, Haas R and Rosenberry TL: Decay-accelerating factor of complement is anchored to cells by a C-terminal glycolipid. Biochemistry (1986) **25**, 6740-6747.
21. Lisanti MP, Caras IW, Davitz MA and Rodriguez-Boulan E: A glycophospholipid membrane anchor acts as an apical targeting signal in polarized epithelial cells. J Cell Biol (1989) **109**, 2145-2156.
22. Sakuma T, Kodama K, Hara T, Eshita Y, Shibata N, Matsumoto M, Seya T and Mori Y: Levels of complement regulatory molecules in lung cancer: Disappearance of the DI7 epitope of CD55 in small-cell carcinoma. Jpn J Cancer Res (1993) **84**, 753-759.

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