Promoter CpG Hypermethylation and Downregulation of Caveolin Expression in Human Colon Cancers

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Background/Aims: Abnormal reduction of caveolins has been found in many human cancers while its overexpression also correlates with increased metastatic progression of some tumors. To elucidate the possible implication of caveolin abnormality in human colon tumorigenesis, the expression and mutational status of caveolins was explored. Methods: We investigated 11 human colon cancer cell lines, 49 primary carcinoma tissues, and its matched normal colonic tissues. Both mRNA and protein levels of caveolins (cav-1, cav-2) were evaluated by quantitative RT-PCR and immunoblotting. Effect of cav-1 expression on tumor growth was tested using cell counting and colony formation assay. Cav-1 expression was restored in nonexpressing cells, whereas cav-1 expression was inhibited by siRNA-mediated knockdown in expressing cells. Methylation status of 38 CpG sites was evaluated by bisulfite DNA sequencing. Results: Low expression of cav-1 transcript was found in 54.5% of cancer cell lines, whereas 45.5% of those showed strong expression. Expression level of cav-1 protein was very low in majority of cancer cell lines except two cell lines. Approximately 47% and 10% of primary carcinomas exhibited significant reduction and elevation in cav-1 expression, respectively. Cav-2 expression also showed down- and up-regulation in 28% and 3% of primary tumors, respectively. Cav-1 transcript was re-expressed in nonexpressing cells by 5-aza-dC treatment. Restoration of cav-1 inhibited growth of cav-1-negative cells and reduced phospho-Erk level, whereas ectopic overexpression of cav-1 further stimulated cav-1-expressing cells and activated p53 and p21. Conclusions: Caveolin undergoes epigenetic silencing in a considerable proportion of human colon cancers by aberrant promoter CpG hypermethylation. Also, cav-1 acts two opposite functions as a growth suppressor or growth stimulator in colon cancers. (Intest Res 2007;5:60-72)

Key words: Caveolins; Colonic neoplasms; Hypermethylation

INTRODUCTION

Caveolae are small invaginations of the plasma membrane, and have been proposed to have roles in transcytosis of macromolecules. Caveolin-1 (7q31.1), caveolin-2 (7q31.1) and caveolin-3 (3p25) were identified as major components of caveolae. Of the three caveolin types, caveolin-1 (cav-1) and caveolin-2 (cav-2) are found in endothelial cells, smooth muscle cells, adipocytes and fibroblasts, while caveolin-3 is found specifically in muscle cells. They function through the caveolin-scaffolding domain of the caveolin homo-oligomer formed by cav-1 only or the heterooligomer formed by cav-1 and cav-2. It has been known that cav-1 takes lead mainly to make caveolae structure rather than cav-2, but recent report showed that the phosphorylation of cav-2 also modulates cav-1-dependent caveolae formation.2,6

Loss or reduction of caveolae or cav-1 expression was
first described in NIH3T3 cells transformed by activated oncogenes\(^7\) and in breast and lung cancer cell lines. In addition, low expression of *cav-1* was identified in colon,\(^8\) ovarian\(^9\) and follicular thyroid carcinoma.\(^{10}\) Recombinant expression of *cav-1* in transformed NIH3T3 cell or breast cancer cell lines suppressed their anchorage-independent growth. Moreover, targeted down-regulation of *cav-1* using an antisense cDNA vector promotes anchorage-independent cell growth, derives tumorigenesis in nude mice, and hyperactivates the p42/44 MAP kinase cascade in NIH3T3 cells.\(^{11}\)

In contrast, elevated expression of *cav-1* has been detected in metastatic prostate cancers and identified as an independent prognostic marker for prostate cancer progression in lymph node-negative patients who have biochemical recurrence of disease after radical prostatectomy. Additionally, a significant association of increased *cav-1* in prostate cancer was observed in Africa-American men versus white-American men.\(^{12}\) Overexpression of *cav-1* was also observed in pancreatic cancer,\(^{13}\) and esophageal squamous cell carcinoma.\(^{14}\) Interestingly, although many cell types express *cav-1*, secretion of *cav-1* has been reported for normal pancreatic exocrine cells and pituitary cells in addition to prostate cancer cells.\(^{15}\)

*Cav-1* has antiapoptotic properties under variety of clinically relevant circumstances, including androgen and growth factor deprivation and oncogene over-expression.\(^{16,17}\) Recently, it was documented that prostate cancer cells secrete *cav-1*, and secreted *cav-1* can stimulate viability and clonal growth of prostate cancer cells that do not express *cav-1*. The concept of a secreted autocrine or paracrine factor that directly contributes to androgen resistance in prostate cancer is novel and represents an efficient mechanism for maximizing resistance to various proapoptotic stimuli that metastatic cells often encounter during the highly inefficient process of metastasis.\(^{18,19}\)

However, it has been existed several controversial reports about expression status of *cav-1* in breast and prostate cancers.\(^{20-22}\) In opposition to above mentions, increased *cav-1* staining was detected in human breast cancer specimens.

The gene encoding *cav-1* and *cav-2* is located on human chromosome 7q31.1.2-4. Loss of heterozygosity on 7q31.1 has been detected in carcinomas of the ovary, prostate, stomach and kidney. However, deletions or mutations within the *cav-1* gene were not detected.\(^{5}\) Recently, in only invasive scirrhous breast carcinoma, a mutation in *cav-1* at codon 132 (P132L) has been identified in 16% of cases.\(^{23,27}\)

Taking the previously observed aspects of *cav-1* into account, it is conceivable that *cav-1* has two opposite functions as tumor suppressor gene and oncogene. As tumor suppressor gene, expression of *cav-1* can inhibit anchorage-independent tumor growth and mitogenic signal pathway.\(^{11,22,24}\) In a viewpoint of oncogenic function, expression of *caveolin-1* in tumor progression elicits several advantages, such as *c-myc* oncogene-induced apoptosis,\(^{17}\) suppression to anoikis,\(^{24}\) multidrug resistance,\(^{25}\) androgen-independency in prostate cancer\(^{16}\) and elevated metastasis potential.\(^{18,28}\)

In the present study, expression and mutation status of *cav-1* was investigated using colon cancer cell lines and primary carcinoma tissues in order to elucidate the possible implication of *cav-1* alteration in the development of human colon cancer. In addition, effect of *cav-1* on the growth of tumor cells was analyzed to understand its biological significance in colon tumorigenesis.

**MATERIALS AND METHODS**

1. Human Colon Cancer Cell Line

Eleven human colon cancer cell lines (Caco-2, COLO205, HCT116, HT-29, KM12C, KM12SM, Lovo, RKO, SNU-C1, SW620 and WiDr) were obtained from Korea Cell Line Bank (Seoul National University, Seoul, Korea). Cell lines were maintained at 37°C in RPMI 1640 or DMEM medium supplemented with 10% fetal bovine serum (GIBCO BRL, Gaithersburg, MD).

2. Tissue Specimens

Forty-nine primary carcinoma tissues and matched normal colonic tissues were obtained from the same cancer patients in Kyung Hee University Medical Center (Seoul, Korea). Tissue specimens were snap-frozen in liquid N\(_2\) and stored at -70°C until used.