Expression of CD44 Variant Proteins in Human Colorectal Cancer Is Related to Tumor Progression

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Introduction

During tumor progression, a subset of cells acquires metastatic properties, presumably through a series of genetic alterations. As a result, cells detach from the primary tumor, penetrate the basement membrane into the connective tissue, and invade adjacent structures including lymph and blood vessels. The tumor cells are subsequently transported to sites of metastatic outgrowth via lymph and/or blood. Loss of adhesive functions and gain of new adhesive functions are thought to play a crucial role in this metastatic cascade (1). Since tumor metastasis is the principle cause of death for cancer patients, there is consensus that a search for tools that allow effective assessment of the metastatic potential of tumors is a prime goal for cancer research. Recently, we have shown that CD44 variant glycoproteins containing sequences encoded by exon v6 confer full metastatic potential to rat carcinoma and sarcoma cell lines. Coinjection of variant-containing sequences encoded by exon v6 confer full metastatic potential to rat carcinoma and sarcoma cell lines. The pattern of CD44 expression in colorectal tumors differs dramatically from that in normal epithelium. Pan-CD44 mAb shows an overall increase of CD44 on the surface of tumor cells. Similarly, expression of CD44 variants encoding exons v7–v10 was greatly diminished in tumor cells.
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Fig. 1. A, schematic representation of the CD44 gene. □, exons that are spliced in the “standard” type of CD44. TM, transmembrane region. B, schematic representation of the CD44 protein with location of the epitopes which are recognized by the monoclonal antibodies NKI-P1, VFF4, VFF7, VFF8, VFF11, VFF14, VFF16, and VFF17 and the polyclonal antiserum CD44v. Anti-variant antibodies were raised against a bacterially expressed fusion protein encoded by pGEX CD44v HPKII (v3-v10). □, standard CD44; v1-v10, domains encoded by variant exons.

Fig. 2. Expression of CD44 variant proteins in colorectal tumor progression. Dark gray columns, percentage of positive tumors. Light gray columns, specimens with focal staining only. *, weak staining at base of crypts.

Moreover, new epitopes not found on normal epithelium were detected on the tumor cells. Overexpression of CD44 and appearance of a new epitope was already observed at a very early stage of colorectal tumor progression, i.e., in early adenomas. In these adenomas almost all cells expressed an exon v5 epitope. From the fact that not all v7-v10 positive cells carried also the v5 epitope and from PCR data (Fig. 3), we conclude that there is progressive change of CD44 splice variant production and that, compared to normal colon mucosa, the expression pattern of CD44 variants in tumor cells is much more complex (Fig. 3). At the more advanced stages of colorectal tumor progression, i.e., in advanced polyps and invasive carcinomas, the level of expression of v5 containing CD44 isoforms increased. Interestingly, tumor progression was strongly related to overexpression of CD44 isoforms carrying epitope(s) of exon v6, which in the rat has been shown to be involved in metastasis formation (Fig. 2). Expression of this exon was detectable in none of the normal colon specimens but in 9, 45, and 68% of the early and advanced polyps and invasive carcinomas, respectively (χ² trend 1 d.f. = 13.1; P = 0.0003). Moreover, in carcinomas, the expression of exon v6 was correlated to Dukes stage. The percentage of positive cases in the nonmetastatic Dukes A and B tumors was 55%, whereas in the metastatic Dukes C/D group 83% of the cases were positive (χ² 1 d.f. = 4.4; P = 0.03). Importantly, the overexpression of v6 containing CD44 isoforms during tumor progression was reflected not only by increasing percentages of positive cases but also by increasing numbers of positive cells within the tumors as well as by higher average expression levels apparent from the more intense staining (not shown). Focal expression of v6 in adenomas was correlated to another parameter of tumor progression, i.e., to the histological tumor grade. Expression was detectable in 1 of 17 low-grade but in 4 of 5 high-grade adenomas.

Our present finding that expression of CD44 splice variants, particularly those containing v6, in colorectal cancer is strongly related to tumor progression supports the concept that these CD44 variants play a role in human colorectal tumor metastasis similar to that in the rat model (2, 3). Interestingly, CD44 variants containing v6 are also up-regulated on activated lymphocytes (4, 12) and are involved in the normal immune response (12). Activated lymphocytes and metastasizing tumor cells share many properties, e.g., invasive behavior, migration involving reversible adhesive contacts, accumulation and expansion in draining lymphoid tissue, release into the circulation, and extravasation. CD44 splice variants appear to play a decisive role in one (or several) of these steps (13).

Multistep carcinogenesis, exemplified in the colorectal adenoma-carcinoma sequence, is believed to involve mutation and clonal selection (8). From normal mucosa through various adenoma stages to invasive and metastatic carcinoma, morphological phenotypes have been associated with progressive accumulation of genetic changes involving oncogenes and tumor suppressor genes like ras, APC, DCC, and p53, which appear to cause growth advantage. Our finding of progressive overexpression of CD44 variants at the successive stages of colorectal tumor progression suggests that expression of those variants also confers a selection advantage to the tumor cells. Altered adhesion mediated via the CD44 variants and growth signals associ-
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Fig. 3. Southern blot analysis of reverse transcription-PCR amplification products from specimens of normal colorectal mucosa and colorectal adenocarcinomas. In A, the PCR products obtained with CD44 specific primers 5' and 3' of the variant part were resolved on 1.2% agarose, stained with ethidium bromide, and visualized under UV light. The bright 350-base pair (bp) band present in both normal and tumor samples corresponds to the expected standard CD44 amplification product. Compared with normal colon mucosa, colorectal carcinomas grossly overexpression several larger splice variants. In B-E, after transfer to a Hybond N+ membrane, the same filters was hybridized consecutively to (B) exon v5, (C) exon v6 (D), exon v9, and (E) standard CD44 specific probes. The results show that, compared with normal colon mucosa, the expression pattern of CD44 variants in colorectal carcinomas is much more complex. The relative strong bands obtained with normal tissue in B-D result from long exposure times needed to allow adequate qualitative analysis of the variants. Hence, it should be stressed that a quantitative comparison between expression of variants in normal tissues and tumors in B-D is not possible. Lane 7, negative control.

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References


Research.
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