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CD44 in Colon Cancer

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Alternative splicing of ten different variant exons (v1–v10) is responsible for the creation of a large number of different CD44 surface proteins. Some of these proteins play decisive roles in the metastatic spread of rat tumours. Also in human cancers, CD44 splice variants are frequently expressed in advanced states of tumorigenesis. In breast cancer and in non-Hodgkin's lymphomas expression of exon v6 is correlated with poor prognosis of patient survival. In colorectal carcinogenesis, expression of exon v5 is an early tumour marker since it is already detectable on small dysplastic polyps (but not on normal colon epithelium). In contrast, exon v6 expression occurred with increased frequency with tumour progression, and its expression on colorectal tumours indicated reduced survival probability. Most likely, tumours carrying the CD44 v6 epitope acquire selective advantage during tumour progression and metastasis formation. This could be a proliferative advantage since mice transgenic for the CD44 isoform CD44v4–v7 on T lymphocytes show an accelerated T-dependent immune response as compared with non-transgenic siblings.

Key words: alternative splicing, epitopes, survival probability, prognosis, transgenic mice
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INTRODUCTION

CD44 WAS RECOGNISED as a leucocyte antigen in 1982 [1]. With the detection of extensive alternative splicing [2–10] and various types of post-translational modification [11–15], the definition of its molecular functions has become complex. Current evidence suggests that CD44 proteins participate in a relatively large number of related molecular processes, which involve specific adhesions (e.g. to hyaluronate, collagen, fibronectin, reviewed in [16], signal transduction [17] and cell migration [18].

Several alternatively spliced variants of CD44 have been found on the surface of pancreatic and mammary carcinoma cell lines of the rat [3] (Figure 1). Their expression correlated with the metastatic potential of the cells. These CD44 variants (CD44v) differ from the most ubiquitously expressed CD44 standard form (CD44s) by the usage of ten variant exons in various combinations. Variant exon sequences are excised from transcripts giving rise to CD44s (Figure 1). These variant isoforms could confer metastatic properties to a non-metastatic tumour cell line [3]. Also, in human cancer, CD44 splice variants are frequently expressed in advanced states of tumorigenesis. In breast cancer, epitopes encoded by exons v5 and v6 indicate poor prognosis [19]. Their presence correlates inversely with patient survival [20, 21]. Also, the aggressive behaviour of non-Hodgkin's lymphomas depends on CD44 molecules carrying the v6 epitope [22, 23]. Not only the larger splice variants, but also the smallest CD44 protein, the leucocyte protein CD44s, appear to play a role in tumorigenesis: Namalwa cells transfected with an expression clone of CD44s grow and metastasise better in SCID mice than the non-transfected control cells [24].

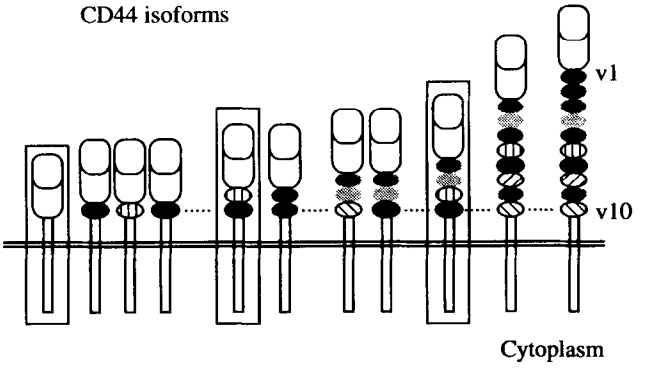

Figure 1. The family of CD44 proteins. Several isoforms of the CD44 protein family are presented. They have a common transmembrane and cytoplasmic region, and the amino terminal extracellular portion. They differ in the extracellular part by omission or insertion of sequences coded by ten different variant exons (v1–v10). The dots indicate that only a subset of the possible combinations are shown. The boxed isoforms indicate the smallest, most ubiquitously expressed form (CD44s) and the two variant isoforms that are expressed in a metastatic pancreatic carcinoma and have been shown to promote metastatic capability. The relative portions of the protein parts are not drawn to scale.

We will briefly review here the data concerning the expression of CD44 in colorectal cancer, and attempt to argue from experimental data the basis of the advantage of expressing CD44 variants. We will also briefly discuss the possible applications of CD44 reagents in diagnosis and therapy.

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CD44 EXPRESSION IN COLORECTAL CANCER

CD44 expression is controlled at several levels. Promoter activity appears to be regulated by growth stimuli [25]. One may, therefore, expect that proliferating cells of colorectal epithelium (at the base of the crypts) show some promoter activity. The second level of control is exerted during splicing. An as yet poorly understood regulatory factor (or factors) influences the splice machinery and determines which exon-encoded sequences will appear in the mature RNA and protein products. The CD44 proteins are then possibly recruited for specific functions by post-translational modifications. All these biological regulations will exhibit some leakiness and the transitions may well be gradual. In carcinogenesis, selective advantage may enforce the expansion of clones with defined expression patterns.

With all this in mind, the methodology of analysis of clinical samples needs to be chosen with caution. Reverse transcriptase-polymerase chain reaction (RT-PCR) and antibody-based techniques are available. For determining quantitative differences in RNA abundance, RT-PCR is helpful only if linear conditions of measurement are maintained. Most studies have, however, used exhaustive PCR conditions. Nevertheless, RT-PCR revealed the synthesis of larger splice variants in colorectal tumour progression [26–28]. Similar problems of quantitation arise with antibodies of different antigen affinities. Using a polyclonal serum raised against a peptide comprising exon sequences v3–v10, normal colorectal mucosa in frozen sections showed almost no staining [29]. Traces could be seen only in cells at the base of crypts. With a monoclonal antibody directed against the N-terminus common to all CD44 proteins, massive staining occurred in non-epithelial cells below the basement membrane while colorectal mucosa was negative. Monoclonal antibodies to individual epitopes encoded by exon v6 generally did not detect antigen in normal mucosa. Only one of these, the high affinity VFF18, gave some background staining.

Samples from various stages of colorectal carcinogenesis revealed interesting changes of variant exon expression [28, 29]. Small dysplastic polyps already carried on their cell surfaces elevated levels of CD44 proteins carrying an epitope encoded by exon v5. The expression of exon v5 persisted throughout carcinogenesis as polyps and samples of cancer tissue were almost invariably positive for the v5 epitope. An epitope encoded by exon v6, however, occurred with increasing frequency in tumour progression and was present in 9, 45, and 68% of the early and advanced polyps and invasive carcinomas, respectively [28].

Patients with v6 positive carcinomas had poorer prognosis than those with negative tumours [25, 30]. Also, the expression level appeared to be related to prognosis, with highest positive cell counts indicating the greatest reduced survival probability [30]. As the most straightforward explanation, it appears that tumours carrying CD44 with the v6 exon epitope acquire selective advantage during tumour progression and metastasis formation.

EVIDENCE FOR A PROLIFERATION-PROMOTING EFFECT OF CD44v4–v7

One of the molecules found in metastatic cancer, a splice variant carrying exon sequences v4–v7, was introduced into the germ-line of mice by oocyte injection of a Thy-1 promoter-driven cDNA construct (J. Moll and associates, unpublished data and [31]). Mice carrying this transgene express CD44v4–v7 on the surface of T cells. The mice are viable and show an interesting phenotype: upon antigenic or mitogenic stimulation their T cells enter S phase with increased efficiency. *In vivo* or *in vitro*, more cells respond to stimulation and they reach maximal [^{3}H]Tdr incorporation of effector functions (T_H, CTL) 24 h earlier than cells from sib controls. The presence of CD44v4–v7 thus either lowers a threshold for the antigen/mitogen response or potentiates relevant signalling during activation of lymphocytes. Indeed, the transgenic T cells respond to a 5-fold lower concanavalin A concentration than nontransgenic control cells (J. Moll, unpublished data). Interestingly, the repopulation kinetics of lethally irradiated mice are also improved using bone marrow from transgenic as compared to bone marrow from nontransgenic mice (unpublished data). Cancer cells may adopt this function and profit from the proliferative advantage mediated by the CD44 splice variant.

EXPLOITING CD44 EXPRESSION FOR DIAGNOSIS AND TREATMENTS

Except for the retrospective study cited above, the diagnostic and therapeutic possibilities of CD44 have not yet been explored. We consider some applications realistic. Since epitopes encoded by exon v5 appear early in colorectal carcinogenesis, e.g. most polyps carry the epitopes, they could serve as early indicators of colorectal epithelium transformation if detected in cellular debris of stool. The presence of epitopes encoded by exon v6 would then indicate the existence of a more aggressive tumour in the patient. As deduced from the Kaplan–Meier evaluations of survival probability in relation to the occurrence on tumour cells of the v6 epitopes, surface expression of such epitopes predicts poor prognosis independent of Dukes' status. Antibodies directed against v6 epitopes could be administered after surgery aimed at inhibiting or eliminating circulating residual tumour cells. An attempt of this sort has been successful previously [32]. In this study an antibody directed against another adhesion molecule improved survival from colorectal cancer.

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