Expression of CD44 splice variants in human skin and epidermal tumours

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Abstract

Splice variants of the adhesion molecule CD44 (CD44v) are important in the lymphatic spread of rat carcinoma cells. In several human tumours expression of CD44v correlates with tumour progression. However, little is known about the physiological functions of distinct variant exons. Here we report on the immunohistochemical evaluation of CD44 expression in normal human skin and epidermal tumours which do not metastasise, or do so very rarely. Frozen tissues were stained with a panel of monoclonal antibodies, recognizing epitopes of the CD44 standard isoform, as well as of variant exons v5, v6, v7, v7-v8 and v10. Stratum basale and spinosum as well as the root shaft of hairs reacted strongly with the whole panel of anti-CD44 antibodies. Stratum corneum, acinar cells of sebaceous and eccrine sweat glands stained with anti-CD44v5, anti-CD44v6 and anti-CD44v7, but not with anti-CD44v10, the latter recognizing the "epithelial isoform" (CD44v8-v10) of CD44. Ductal cells of glands and apocrine glands did not express CD44v. Compared with its expression in normal human skin, CD44v expression was reduced in basal cell carcinoma and squamous cell carcinoma of the skin. This was particularly true of CD44v10. The expression of CD44v in normal skin and dermal appendages indicates that not all combinations of variant exons are involved in tumour progression. Since the epithelial isoform is particularly downregulated in basal cell carcinoma and squamous cell carcinoma of the skin, it is unlikely that exons v8-v10 play a role in tumour progression. Rather, they may be of functional importance in maintenance of the epidermal structure.

Key words
CD44 variant isoforms • Skin • Basal cell carcinoma • Spindle cell carcinoma

Introduction

CD44 comprises a family of glycoproteins which vary by glycosylation and protein structure [5, 9, 29, 35, 39, 41]. As demonstrated in Figure 1, the latter is mainly due to alternative splicing, whereby up to ten additional, so-called variant exons can be inserted into the membrane proximal extracellular domain of the standard or haematopoetic form of the molecule (CD44s) [45, 52]. These variant exons can be expressed individually or in a variety of combinations [16, 20, 25–28, 46, 52], and the same cell can express more than one combination of variant exons [2, 6, 10, 11, 14, 16, 42, 48]. Some of these combinations are found, in particular, in specific organs or tissues, like the epithelial form, which contains the exons v8–v10, and the keratinocyte form, which contains the exons v3–v10 [4, 8, 20, 34, 49, 54]. Both the exons of the standard part of the molecule and the variant exons are heavily glycosylated: N-linked and O-linked glycosylations as well as chondroitin sulphate side chains have been described [4, 5, 21, 30, 35]. Furthermore, the region of the variant exons is highly hydrophilic and may thus be involved in specific ligand interactions [15, 21]. Finally, since alternative splicing of CD44 is stringently regulated, it can be assumed that the different exons or combinations of exons exert defined and divergent functions [15, 22].

Special interest in CD44 variant isoforms (CD44v) arose when it was demonstrated in a rat pancreatic carcinoma model that the expression of splice variants, in par-
Fig. 1 Schematic representation of CD44 isoforms. Variations in the protein structure of CD44 are mainly due to the insertion of up to ten (mouse and rat) or nine (human) so-called variant exons between exon 5s and 6s of the haematopoietic form of the molecule. Exon 8s comprises the transmembrane, exon 9s or 10s the cytoplasmic region.

Materials and methods

The monoclonal antibodies SFF2 (anti-CD44s, IgG1), VFF8 (anti-CD44v5, IgG1), VFF7 (anti-CD44v4-v6, IgG1), VFF9 (anti-CD44v7, IgG1), VFF17 (anti-CD44v7-v8, IgG2b) and VFF16 (anti-CD44v10, IgG1) were used as first antibodies. SFF2, VFF7, VFF8, VFF9, VFF16 and VFF17 were derived from BALB/c mice immunized with GST-CD44v3-v10 fusion protein. The exon-specificity was identified by binding to GST-fusion proteins, which contained single CD44 variant exons. The recognized epitopes were defined by a competition enzyme-linked immunosorbent assay using sets of overlapping peptides as competitors. The antibodies were further selected for the recognition of CD44v on tumour lines, which were known to express CD44v to guarantee that the specific epitopes were not hidden by glycosylation. In addition, Scatchard plot analysis revealed that the antibodies exhibited similar binding constants (a manuscript describing these antibodies is in preparation). Culture supernatants were puriﬁed and antibody. First and second antibodies were diluted in phosphate buffered saline and titrated on positive and negative control cells. Final working concentrations for the first antibodies were in the range of 2.5–5 μg/ml; the second antibody was diluted 1:150.

Tissues were obtained from surgical specimens and biopsies. Fresh tissue was snap-frozen in liquid nitrogen. Serial sections of about 1 cm² were cut in 4–6 μm thick sections, mounted on gelatin coated slides and air dried overnight. They were fixed in acetone for 10 min at −20°C and stored at −20°C or immunostained immediately. Typing of epithelial cultures was carried out according to the guidelines of the WHO. The collection contained 37 basal cell carcinomas (5 solid, 8 solid multicentric, 8 sclerosing, 4 nodulo-ulcerative, 4 adenoid cystic, 4 pigmented and 4 superficial) and 24 epidermal squamous cell carcinomas. By differentiation, 6 squamous cell carcinoma were grade 1, 11 were grade 2, 4 were grade 3 and 3 were grade 4. None of the patients with squamous cell carcinoma had developed metastases at the time of excision of the primary tumour. Also, during the post-surgery observation periods of 12–36 months neither local recurrence nor metastatic settlement were observed.

For immunohistochemistry (7), fixed and dried sections were incubated for 1 h at room temperature with the first antibody. Slides were washed intensively and incubated at room temperature for 30 min with the second, biotinylated anti-mouse Ig antibody. After washing sections were incubated with a horseradish-peroxidase-conjugated streptavidin complex. 3-Amino-9-ethylcarbazole (Sigma) was used as substrate for the enzyme. The peroxidase reaction resulted in an intense red precipitate. The sections were faintly counterstained with Mayer’s haematoxylin, air dried and mounted.

For negative controls the staining of each sample was performed without applying the primary antibody or by using an antibody of irrelevant specificity. With the exception of the reaction of the granulocytes due to their endogenous peroxidase, which had not been blocked, no staining was observed. The results were evaluated according to the following score system: high intensity of staining, ++; distinct staining, +; weak staining, ±; heterogeneous staining, +/− (more stained cells), − (equal numbers of stained and unstained cells), −/− (more unstained cells).

Results

Using immunohistochemistry and a panel of monoclonal antibodies which recognize epitopes on CD44s, on exon v5, v6, v7, the transition of exon v7 or v8 and exon v10, we observed that in human skin predominantly CD44v are expressed (Table 1). With the exception of the stratum corneum all epidermal layers expressed at least the variant exons v5, v6, v7, v8 and v10. The intensity of staining for individual epitopes showed marked variations (Fig. 2). While the epitope on the standard domain stained very intensely, staining of variant exons was generally weaker. Furthermore, there was a grading of the staining (the intensity decreased from the basal towards the apical layers). Exon v7 in particular, was hardly detectable in the upper layers of the stratum spinosum. The external and internal root shaft (lower and upper part) of the hair stained essentially like the stratum basale and stratum spinosum. Expression of CD44v in the different glands was more restricted. In the sebaceous glands the basal region stained distinctly with anti-CD44v5 and anti-CD44v6, but only weakly with anti-CD44v7, anti-CD44v7-v8 and anti-CD44v10. Ductal and acinar cells stained weakly with anti-CD44v5, anti-CD44v7-v8 and anti-CD44v10. Acinar and ductal cells of the eccrine sweat glands stained with anti-CD44v5, anti-CD44v6