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Comments and Critique

CD44 and its Role in Tumour Progression and Metastasis

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THE NATURE of the transmembrane glycoprotein molecule CD44 [1] and the role it plays in tumour development and progression are matters which are rapidly becoming of interest not only to basic researchers but also to cancer clinicians and pathologists. Initially identified and studied in many cell systems, CD44 was known by several synonyms, including phagocytic glycoprotein-1 (Pgp-1) [2], extracellular matrix receptor III (ECM-III) [3], and HUTCH-1 [4]. When these individual molecules were shown to be the same [4–6], the name CD44 was assigned at the Third International Workshop on Leukocyte Differentiation Antigens [7]. Resolution of the cDNA sequence [8] revealed considerable homology with the cartilage link proteins involved in adhesion between hyaluronate and other proteoglycans in the extracellular matrix [9]. Subsequently, CD44 was found to have at least two functions, as a receptor for the glycosaminoglycan hyaluronate [10] and as a lymphocyte homing receptor [11, 12]. It was this latter role in the normal recirculation of lymphocytes that led, initially by analogy, to interest in the possible role of CD44 in regulating tumour cell dissemination.

The standard or haemopoietic form of CD44 (CD44s), which is found predominantly on haemopoietic cells, is synthesised as a 37kD core protein which can be modified extensively by glycosylation and by addition of chondroitin sulphate [8, 13, 14]. Epithelial cells produce a larger core protein form of CD44, CD44E, which is generated by alternative RNA splicing [8]. At present the ligand for CD44E remains unknown though, in direct contrast to CD44s, it has been shown not to facilitate binding to hyaluronate [15, 16].

The discovery that alternative RNA splicing could lead to the generation of a larger epithelial isoform of CD44 was rapidly followed by the exciting findings of Gunthert *et al.* [17]. A molecule expressed only on metastatic rat pancreatic carcinoma cells, compared with their benign counterparts, was shown to be another variant of CD44, designated CD44v or pMeta-1. This variant was also generated by alternative splicing of the RNA, resulting in the addition of 162 amino acids into the same site as that used to generate CD44E [17].

Subsequent investigations have revealed that the extensive isoform heterogeneity of CD44 is in fact a consequence of splicing choice from 10 variant exons [18, 19]. Many of these variants have been shown to be expressed by a range of human tumour cell lines [20] suggesting that the association between isoform expression and metastatic behaviour, initially determined in rodent tumours [17, 19], may extend to neoplasia in man. Indeed, two recent reports, using the technique of reverse transcriptase-polymerase chain reaction (RT-PCR) with oligonucleotides which flank the variant-insertion site, have suggested a remarkable correlation between increased malignancy and splice-variant expression [21, 22]. Proof that such a correlation is a general phenomenon clearly would have very important implications in diagnosis and prognosis. Unfortunately, the situation is not yet this clear-cut. Confusion over the complicated exon boundaries, exacerbated by the different nomenclature adopted by various groups, has meant that the specific variant isoform identified in more malignant tumours need not necessarily be the generally accepted metastasis-associated isoform [22]. More fundamental still is the problem posed by the technology used to demonstrate the existence of specific variants. RT-PCR on clinical material [21, 22] is not as unequivocal in terms of cellular origin as when tissue culture lines are used as the starting material [20]. Infiltrating stromal cells can contribute to the isoform pattern detected; a particularly significant observation given that activated lymphocytes can express the so-called meta (metastasis-associated) variant of CD44 [23]. Though the unequivocal cellular source of specific isoforms need not be essential information, (as long as benign neoplasms are distinguishable from malignant, the technique would still be valid as a diagnostic/prognostic tool), the possible complicating presence of minor "contaminating" cell types has been cited as a reason for placing a greater dependence on immunohistochemistry as a more robust and dependable method of assessment [24]. An additional benefit to be gained from this latter approach would be the clear demonstration of a specific protein product; the existence of mRNA of different transcript sizes is no guarantee of the eventual presence of the appropriate protein. While such antibody-dependent analysis is still very much at an early stage it is clear that results obtained so far do not accord entirely with the straightforward correlation between variant expression and metastatic behaviour espoused above.

Wirth *et al.* [25], using the monoclonal antibody 1.1ASML [17], which recognises the rat variant CD44 molecule, investigated the tissue distribution of CD44v in newborn and adult rats. They found that the 1.1ASML epitope was expressed in adult rats in a range, albeit a restricted range, of normal tissues including the basal layer of the epidermis and the intestinal crypt cells; additional distribution of the CD44v protein was detected in the prenatal and neonatal stages of development in such tissues as the thyroid, submandibular gland and the ductal cells of the pancreas [25]. Analysis of tissue samples from normal human colon mucosa, colon adenocarcinoma and carcinoma metastases showed increased levels of expression of CD44v in the invasive tumour samples; however, this variant isoform was

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also found expressed in 17 out of 17 adenomatous polyps [26]. Clearly the metastasis-related variant is already expressed at an early stage in colorectal carcinogenesis and is not an invariant consequence of malignant progression. While masking of the CD44v epitope may lead to an underestimation of prevalance of this protein in this type of study, the clear unequivocal demonstration of its presence in at least some benign lesions and normal tissue highlights the likely complexity of the association of this variant glycoprotein with the metastatic process.

Against this background, Abassi *et al.* [27] have now shown that, using the antibody F10.44.2, which will not distinguish between the variant and standard forms, CD44 expression in human adenomatous polyps, colorectal adenocarcinomas (both primary and metastatic deposits) as well as adjacent normal tissue, is associated with cellular proliferation. Such a correlation has been suggested previously [28, 29] but the combination of staining for both expression of CD44 and the proliferation-associated nuclear antigen (Ki-67) clearly establishes this concordance [27]. Surely this association raises another possible mechanism whereby CD44 may facilitate secondary tumour development; that is, is it possible that this cell surface molecule is actually involved in stimulating cell growth? It has been shown in diverse biological systems that proteoglycans in the extracellular matrix can sequester growth factors and/or other cytokines [30, 31]. Could it be that the hyaluronate–cell interaction mediated via CD44 serves to bring the responding cancer cell within close proximity to these sequestered growth stimulating factors? Alternatively, the cell surface proteoglycans, such as CD44, could present growth stimulatory soluble factors to adjacent cells within the tissue/tumour mass. Indeed, Tanaka *et al.* [32] have shown that the cytokine, macrophage inflammatory protein-1β (MIP-1β) is capable of being immobilised via its glycosaminoglycan binding site to CD44 and that this immobilisation serves to enhance cytokine-induced modulation of T-cell adhesion.

Whether similar mechanisms are involved in regulating the proliferative response of CD44-expressing colorectal epithelial cells remains conjectural at this time. What is clear is that there will be considerable interest in the role(s) of CD44, both the splice variants and standard form, in tumour development and progression. Future years will see a clearer picture emerge of this involvement and such information may provide new approaches to diagnosis, prognostic assessment and therapy.

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