LETTER TO THE EDITOR

CD44 glycoprotein in cancer: a molecular conundrum hampering clinical applications

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Abstract

CD44 is a heavily glycosylated membrane receptor playing a key role in cell adhesion, signal transduction and cytoskeleton remodelling. It is also one of the most studied glycoproteins in cancer, frequently explored for stem cell identification, and associated with chemoresistance and metastasis. However, CD44 is a general designation for a large family of splicing variants exhibiting different degrees of glycosylation and, potentially, functionally distinct roles. Moreover, structural diversity associated with ambiguous nomenclature has delayed clinical developments. Herein, we attempt to comprehensively address these aspects and systematize CD44 nomenclature, setting milestones for biomarker discovery. In addition, we support that CD44 may be an important source of cancer neoantigens, most likely resulting from altered splicing and/or glycosylation. The discovery of potentially targetable CD44 (glyco)isoforms will require the combination of glycomics with proteogenomics approaches, exploring customized protein sequence databases generated using genomics and transcriptomics. Nevertheless, the necessary high-throughput analytical and bioinformatics tools are now available to address CD44 role in health and disease.

Keywords: CD44, Glycosylation, Cancer biomarkers, Nomenclature, CD44 isoforms

The transmembrane glycoprotein receptor CD44 plays a key role in cell adhesion to the extracellular matrix and interacts with growth factors and several extracellular ligands, including hyaluronic acid, collagen, osteopontin and many metalloproteinases to drive signal transduction and cytoskeleton rearrangements [1]. By interacting with co-factors and adaptor proteins, CD44 has been further implicated in lymphocyte homing, haematopoiesis, cell migration and adhesion, tumour invasion and metastasis [1]. Several isoforms of CD44 can be generated through the insertion of alternative exons at the variable region in a process regulated at both tissue and cellular levels. While ubiquitously expressed in healthy adult and foetal tissues, the molecular plasticity of alternatively spliced CD44 accounts for diversified functional roles. However, the intricate correlation between CD44 isoforms and underlying biological functions is yet to be fully disclosed. It has been long described that malignant transformation and progression are accompanied by a deregulation of CD44 splicing mechanisms, comprehensively addressed in recent reviews [2, 3]. The events leading to CD44 isoform molecular remodelling have direct implications in several cancer hallmarks and appear to vary according to the type of lesion, supporting the existence of disease-specific molecular fingerprints and potentially targetable biomarkers [4]. Not surprisingly, CD44 has been a hot topic in cancer research, frequently associated with more aggressive phenotypes and widely explored for cancer stem-cell identification [5]. Particular focus has been set on narrowing CD44 screening to its cancer-associated isoforms envisaging the necessary sensitivity and specificity for clinical applications. However, the lack of protocols for its full isoform discrimination at the protein level, and the existence of many functionally distinct isoforms poses a major drawback. Currently, these hurdles can only be partially circumvented by targeting CD44 transcripts using variant-specific probes or...
by emerging RNAseq approaches. Analytical difficulties are aggravated by vast post-translational modifications, with emphasis on the very high glycosylation density of variable regions. Moreover, glycans often present a non-templated and context-dependent nature, with several glycoforms coexisting for the same protein on a given biological milieu, further increasing CD44 molecular and functional diversity (Table 1). This poses a major challenge for identification by conventional immunoassays as well as high-throughput proteomics, which has delayed the definition of CD44 isoforms in health and disease.

The absence of nomenclature standardization also emerges as a key issue, making inter-study comparisons and clinical translation almost impossible. Showcasing some examples, CD44H and CD44E terminologies arise from the first observations in hematopoietic and epithelial cells; gp116 and gp85 distinguish glycoforms by molecular weights (116 and 85 kDa, respectively); CD44 hematopoietic cell E-/L-selectin ligand (HCELL) refers to CD44 isoforms expressed in hematopoietic and cancer cells showing elevated siafofucosylated glycans content and high affinity for E-/L-selectin ligands [6, 7]. All these designations fail to provide clear insights on the molecular nature of the isoforms. Notwithstanding, some studies adopt a nomenclature based on commercial names of the used monoclonal antibodies, highlighting the targeted variable exon, being CD44v3, CD44v6, CD44v9 amongst the most associated with cancer, including chemoresistance and prognosis [8, 9]. However, only eight have been experimentally confirmed (detailed in Table 1 and Fig. 1), specifically six isoforms with variable extracellular domain extensions, one isoform with a truncated cytoplasmic tail, and one isoform truncated at the extracellular domain. Here we attempt to standardize the nomenclature for the above described isoforms through a logical terminology. Following pre-existing designations, we propose that human CD44 isoforms originated by alternative splicing of the variable region should highlight the included exons. Conversely,

| Proposed nomenclature | UniProt | NCBI | Predicted glycosylation sites |
|-----------------------|---------|------|-------------------------------|
|                       |         |      | N-glycosylation | O-glycosylation | Total  |
| CD44v2-10             | Isoform 1| Isoform 1| 8 | 146 | 154 |
| CD44v3-10             | Isoform 4| Isoform 2| 8 | 133 | 141 |
| CD44v8-10             | Isoform 10| Isoform 3| 7 | 79  | 86  |
| CD44v10               | Isoform 11| Isoform 6| 7 | 56  | 63  |
| CD44s                 | Isoform 12| Isoform 4| 6 | 32  | 38  |
| CD44st                | Isoform 15| Isoform 8| 6 | 32  | 38  |
| CD44s-exon 15         | Isoform 18| Isoform 7| 6 | 23  | 29  |
| CD44soluble           | Isoform 19| Isoform 5| 2 | 3   | 5   |

*a* N-glycosylation sites predicted using NetNGlyc server 1.0 (http://www.cbs.dtu.dk/services/NetNGlyc/)

*b* O-glycosylation sites predicted using NetOGlyc server 4.0 (http://www.cbs.dtu.dk/services/NetOGlyc/)
isoforms lacking the variable region should present a nominal designation. Figure 1 constitutes a schematic representation of the experimentally determined human CD44 isoforms, and the proposed nomenclature is described below:

- **CD44v2-10** The canonical CD44 isoform includes a peptide sequence encoded by exons 6–14, while splicing out exon 18. This isoform has a predicted molecular weight of 82 kDa but presents extensive glycosylation, thereby arising to approximately 250 kDa or higher.
- **CD44v3-10** Also known as epican, this isoform results from the retention of exons 7–14 (v3–v10) and splicing out of exon 18. Its unmodified form has 77 kDa, resulting in an up to 200 kDa glycoprotein after post-translational modifications.
- **CD44v8-10** Also known as CD44E or CD44R1, this isoform is originated through retention of exons 12–14 (v8–v10) and splicing out of exon 18. It has...
53 kDa in its unaltered form, while reaching 130 kDa in its glycosylated form.

- **CD44v10** Also known as gp116 or CD44R2, this isoform retains the variable exon 14 (v10), while splicing out exon 18. The unglycosylated form has approximately 47 kDa whereas the glycosylated forms have been observed at 120 kDa.

- **CD44s** Also known as CD44H or gp85, the standard form of CD44 splices out all variable exons and exon 18. Originally with 39 kDa, the subsequent post-translational addition of N-linked and O-linked oligosaccharides gives rise to a 85–90 kDa glycoprotein.

- **CD44st** Also known as short-tail or tail-less, is a 32 kDa isoform splicing out the variable region and exon 19, while retaining exon 18. Importantly, exon 18 contains a stop codon that originates a truncated cytoplasmic tail, consequently leading to the loss of intracellular protein domains and signalling motifs necessary for interaction with cytoskeletal components.

- **CD44s-exon15** A CD44s homolog of 37 kDa lacking the peptide sequence encoded by exon 15.

- **CD44sol** This CD44 soluble isoform only retains exons 1–4, while presenting truncated forms of the exons 3 and 4. The modification of the two later exons leads to a smaller extracellular domain as well as to the loss of transmembrane and cytoplasmic domains. This 16 kDa isoform is often shed to bodily fluids through matrix metalloprotease activity.

In summary, the wide array of structurally similar CD44 isoforms, associated with dense glycosylation and inadvertent lack of nomenclature consensus has posed a significant challenge for inferring on CD44 role in cancer. These aspects have biased many previous conclusions, provided several conflicting data, and significantly delayed clinical development. Most studies disregard CD44 glycosylated domains, which sometimes more than double the molecular weight of the isoforms, decisively modulating biophysical, biochemical and functional properties of the receptor (predicted glycosylation sites detailed in Table 1). Glycosylation also raises a tremendous challenge for CD44 mapping based on conventional proteomics, urging the introduction of glycan-targeted approaches. As such, more comprehensive strategies will certainly require the integration of glycomics/glycoproteomics with emerging proteogenomics, exploring customized protein sequence databases generated using genomics and transcriptomics. This approach is also expected to pinpoint relevant cancer neoantigens for driving targeted therapeutics and immunotherapy development. Nevertheless, we augment that the necessary technologies are now available for addressing CD44 molecular and functional diversity in health and disease, ultimately providing targetable biomarkers for oncology.

**Abbreviations**
CD44: cluster of differentiation 44; HCELL: hematopoietic cell E-/L-selectin ligand; RNAseq: RNA sequencing; mRNA: messenger ribonucleic acid.

**Authors’ contributions**
RA, CG and JAF: developed the concept; RA and AP: revised the literature and performed bioinformatics analysis; RA, CG, AP and JAF: wrote the manuscript; MRS, LL and LLS: revised the manuscript. All authors read and approved the final manuscript.

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