CD133 and membrane microdomains: Old facets for future hypotheses

Christine A Fargeas, Jana Karbanová, József Jászai, Denis Corbeil

Abstract
Understanding all facets of membrane microdomains in normal and cancerous cells within the digestive tract is highly important, not only from a clinical point of view, but also in terms of our basic knowledge of cellular transformation. By studying the normal and cancer stem cell-associated molecule CD133 (prominin-1), novel aspects of the organization and dynamics of polarized epithelial cells have been revealed during the last decade. Its association with particular membrane microdomains is highly relevant in these contexts and might also offer new avenues in diagnosis and/or targeting of cancer stem cells.

Key words: AC133; Cancer; CD133; Membrane microdomains; Membrane vesicles; Prominin-1; Stem cell

Peer reviewer: Zoran Krivokapic, Professor, Dr., MD, FRCS, Institute for Digestive Disease, First Surgical Clinic, Clinical Center of Serbia, 6, Dr Koste Todorovica, Belgrade 11000, Serbia

TO THE EDITOR

We read with great interest a recent Editorial entitled “Multifaceted nature of membrane microdomains in colorectal cancer” by Jahn et al[1] published in issue 17 of the World Journal of Gastroenterology 2011 which proposes to describe the pioneering and recent studies on membrane microdomains (the so-called lipid rafts) and their potential roles in cancers. An important section dealing with prominin-1 (alias CD133), a cholesterol-binding glycoprotein often described as a stem and cancer stem cell marker, is unfortunately entirely based on a single publication released in 2009[2], thus leaving out valuable biochemical and morphological information concerning CD133 and membrane microdomains from earlier works. We fear that as such, it might lead to underestimation of the importance and complexity of such a molecular association and contribute to certain confusion, particularly with regard to the debated AC133 epitope of CD133 and its association with cancer. We propose to expose here earlier overlooked data regarding its expression in epithelial cells and summarize the current knowledge on its cell biology and association with distinctive membrane microdomains. We hope that this might enlighten current issues regarding the implication of CD133 in colorectal cancer, whether it is in metastases, or as a prognostic marker or as a cancer stem cell marker. Actually, the demonstration of the presence of CD133 in Caco-2 cells and its association with membrane microdomains is much less recent than 2009, since it was more than a decade ago that we reported its presence.
in this widely used human colon carcinoma-derived cell line\cite{3}. The detection of CD133 by immunolabeling was originally documented by its particular epitope AC133 that appeared to be restricted to stem/progenitor cell populations but was also thought to be dependent on conformation and/or sensitive to changes in glycosylation\cite{4}. This antigen was attractive in the context of stem/progenitor and cancer stem cells and has often been used to define them in numerous organ systems including the digestive tract, but at the same time controversy was generated on the implication of CD133 as a specific marker\cite{5-10}.

We have previously demonstrated in a key publication of 2000 using the Caco-2 cells as a model of enterocyte epithelial differentiation\cite{11} together with a later study\cite{12}, that the AC133 epitope, but neither the CD133 transcript nor the CD133 protein, is down-regulated upon differentiation, with the result that only a minute sub-fraction of CD133 molecules will carry it\cite{13}. We have therefore stressed several times in the literature that it is important to consider that AC133 antibody detects only a subpopulation of human prominin-1/CD133 glycoproteins carrying the AC133 epitope, and that consequently, AC133 antigen is not fully synonymous with CD133\cite{11-13}. The importance of CD133 glycosylation states for the definition of cancer stem cells has been analyzed by Bindlmaier and colleagues\cite{14}. In the meantime, the prominin-1 (PROM1) gene was shown to be transcriptionally active all along the gastrointestinal tract as CD133 mRNA is detectable by Northern blot\cite{15}, and several studies have demonstrated that in humans, as in mice, its protein is physiologically expressed in several differentiated epithelia\cite{16-20}. Thus, the AC133 epitope might be simply down- or up-regulated during the process of differentiation or transformation, respectively\cite{11-13}. The alteration of the general glycosylation pattern of intestinal cells might explain such a phenomenon\cite{21}. Importantly, the lack of AC133 detection might additionally reflect its instability\cite{22} or its differential accessibility\cite{23} (see below). Of note, the proportion of CD133 molecules carrying (or not) the AC133 epitope in a given differentiated cell remains, however, unknown.

As proposed earlier\cite{19} and pointed out in the Editorial of Jahn and colleagues, the molecular environment surrounding CD133 within the plasma membrane might influence the detection of certain epitopes (e.g., AC133 or those within putative ganglioside-binding sites\cite{24}). To fully appreciate the importance of CD133, one should bear in mind that, at the subcellular level, CD133 selectively marks plasma membrane protrusions, e.g., microvilli and primary cilia, that are located in the apical domain of polarized epithelial cells including Caco-2 cells, and was therefore originally named prominin (from Latin, *prominen*, meaning to project). Within these protrusions, CD133 binds directly to plasma membrane cholesterol\cite{25,26} and is incorporated into membrane microdomains that differ from those found in non-protruding areas of the plasma membrane, as demonstrated biochemically using mild detergents\cite{27}, and morphologically by co-localization with the ganglioside GM1\cite{28}. Such protein-lipid interactions appear essential to maintain the proper localization of CD133 in microvilli\cite{29}, and potentially its physiological function which yet remains elusive\cite{29,30}. Thus, the direct binding of certain gangliosides to CD133\cite{26,27} within the densely packed lipid microdomain might mask some CD133 epitope(s), particularly those in the vicinity of the membrane. Technically, they might be revealed, at least in part, using sensitive methods including harsh conditions for antigen retrieval as in the case of native tissues\cite{8,9,30,31}, upon cell-detachment as in the case of cell lines (e.g., Caco-2 cells)\cite{32}, or by chemical interference with membrane microdomain integrity\cite{33}.

Although tightly associated with plasma membrane, CD133 is nonetheless released into numerous physiological body fluids including urine, saliva, seminal fluids and cerebrospinal fluids in association with small membrane vesicles\cite{34}. It is important to point out that such vesicles are budding from the tip of a microvillus or primary cilium by a molecular mechanism involving cholesterol-dependent membrane microdomains\cite{35,36}. In other words, their release might be modulated by the cholesterol level (and possibly that of other lipids) within the plasma membrane. Interestingly, such release occurs solely during and after the differentiation of Caco-2 cells or, *in vivo*, of neural progenitor cells\cite{37}. Based on the latter observation and the expression of CD133 (AC133 epitope in the case of humans) by numerous somatic stem cells, the concept of “stem cell-specific membrane microdomains” was postulated\cite{38}. Given that membrane microdomains are implicated in several signaling cascades by allowing the formation of active transduction complexes\cite{39}, CD133-containing membrane microdomains might carry and/or functionally organize molecular determinants essential to maintain the stem cell and undifferentiated cell properties and their loss or disposal, e.g., via membrane vesicles, and could modify the status or even the fate of the cells\cite{33,39}. Yet, these microdomains, given their dependence on cholesterol, seem to differ from those defined by Hakomori and co-workers in the glycosynapse concept, and which have been implicated in several biological phenomena related to tumorigenesis\cite{35,38}. However, the coalescence of small CD133-lipid entities into the largest platform within the microvillus membranes might be dragged by carbohydrate moieties, as proposed earlier\cite{33,35,36}. Thus, a certain interdependence of lipid rafts and glycosynapses *per se* might exist. Whether CD133 molecules carrying AC133 epitope are preferentially released upon differentiation remains to be determined. Collectively, numerous physiological and technical parameters might interfere with immuno-detection of certain CD133 epitopes, and importantly, the lack of their detection needs to be evaluated with some caution, and maybe alternative methods such as *in situ* hybridization should complement the investigation\cite{18,30}.

Clinically, in addition to its potential value as a biomarker in tissue diagnosis, the association of CD133/
lipid complexes with extracellular membrane vesicles might offer an alternative screening method for the detection of cancers associated with the digestive tract as demonstrated for central nervous system diseases. Moreover, CD133 expression by cancer stem cells might contribute to outlining new prospects for more effective cancer therapy by targeting tumor-initiating cells.

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S- Editor Sun H  L- Editor Logan S  E- Editor Zhang DN