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COMMENTARY

Comments on the "Prognostic Impact and Clinicopathological Correlation of CD133 and ALDH1 Expression in Invasive Breast Cancer" and the "Commentary by Antonio Ieni and Giovanni Tuccari"

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To the Editor,

The recent publication by Sung Jeep Kim et al. [1] entitled "Prognostic impact and clinicopathological correlation of CD133 and ALDH1 expression in invasive breast cancer" and the Commentary by Ieni and Tuccari [2] along with the Author's reply prompted us to share several considerations on the immunodetection of CD133 (Prominin-1) and data regarding its expression in mammary epithelial cells. When assessing the predictive role of CD133 in breast cancer, it is important to consider these data. The authors of both publications fairly discuss the importance of the scoring methods used and the origin of the surgical samples in the observed differences of CD133 immunopositivity rates, and the association of CD133 with breast cancer subtypes or other predictive parameters in several recent studies (Table 1).

However, differences may also be attributed to the antibodies used (Table 1). The immunodetection of the pentaspan membrane glycoprotein CD133 has generated discrepancies on numerous occasions. For example, Hermansen et al. [3] have reported inconsistent immunohistochemical patterns on replicates of glioblastoma samples using different anti-CD133 antibodies. Moreover, beyond the fact that variability in the reactivity of polyclonal antibodies is inherent to their production, a given company may market (or may have) several rabbit polyclonal anti-CD133 with distinct specificities. Unfortunately, the description of the antibodies used in these studies is sometimes minimal or incomplete, creating ambiguity about their nature or making it difficult to track information about their precise specificity and to compare the different results [4-6]. In addition, although most studies are very recent, several anti-CD133 antibodies are not commercially available any longer, impeding any further investigation. In some cases, the antibody simply cannot be found with the sellers [7] or is actually specific for a different molecule, E-cadherin [8] (Table 1). Surprisingly, this latter publication by Aomatsu et al. [8] is repeatedly quoted by newer publications, including Kim et al. [1] and Ieni and Tuccari [2], in support of the prognostic role of CD133 in breast cancer [5,9,10].

The specificity of the anti-CD133 antibody is essential for the interpretation of data since the targeted portions of the molecule are in certain cases absent from some splicing variant isoforms [11]. A notable example is the cytoplasmic C-terminal domain of CD133, which constitutes a splicing cassette where alternative and facultative exons are expressed [12]. Therefore, caveats apply to observations made with rabbit antisera against the last 18 amino acids of CD133 as long as the nature of the CD133 isoforms examined is not ascertained (Table 1). Moreover, the glycosylation status of CD133 is known to interfere with the accessibility of certain epitopes, particularly CD133/1, which is recognized by the AC133 monoclonal antibody (Table 1) [13,14]. Therefore, depending on the antibody, one might or not be looking at different entities with potentially different biological and/or pathological roles [15].

When analyzing the expression of CD133 in healthy and pathological samples, two other aspects need to be considered. First, the expression of CD133 is not limited to stem and cancer stem cells. Providing adequate antibodies and immunological techniques (antigen retrieval) are applied, CD133

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Table 1. Nonexhaustive list of immunohistochemistry studies of the expression of the pentaspan membrane glycoprotein CD133 in breast cancers

Study (year)	Ref.	Breast cancer subtype/ specimen	CD133 immune-labeling*	Antibody (name/clone†) applied	Host/clonality	Specificity‡
Kim et al. (2015)	[1]	Invasive breast cancer/tumor	Membrane and cytoplasm	Unspecified[1]	Rabbit/p	C-term[§,‖]
Han et al. (2015)	[5]	Infiltrating ductal carcinoma/tumor	Membrane and cytoplasm	Unspecified[2]	Mouse/m	?
Collina et al. (2015)	[29]	Triple-negative carcinoma/tumor	Membrane and cytoplasm	AC133[3]	Mouse/m	2nd extracellular loop
Kapucuoğlu et al. (2015)	[30]	Invasive ductal carcinoma/tumor	Cytoplasm	66141[2]	Rabbit/p	C-term[§]
Mansour and Atwa (2015)	[7]	Invasive ductal carcinoma/tumor	Membrane and cytoplasm	Unknown[4]	Mouse/m	?
Aomatsu et al. (2012)	[8]	Breast cancer/tumor	-	NCH-38[5]	Mouse/m	E-cadherin
Zhao et al. (2011)	[6]	Triple-negative carcinoma/tumor	Membrane (cytoplasm)	Unspecified[2]	Rabbit/p	?
Ieni et al. (2011)	[4]	Node-negative invasive breast carcinoma/lymph nodes	-	Unspecified[6]	Rabbit/p	?

Question marks indicate the impossibility to track down the immunogenic region of CD133 since the antibody used was not specified and/or are not available any longer. Note that the studies listed were determined by the Commentary by Ieni and Tuccari and the Author's reply.
p = polyclonal antibody; m = monoclonal antibody.
*Base for the scoring; in parentheses, staining reported but not considered for scoring; †As stated by the supplier ([1]Abnova; [2]Abcam; [3]Miltenyi Biotec; [4]Santa Cruz Biotechnology; [5]DAKO; [6]Abgent); ‡As stated by the supplier; §Antiserum directed against the last 18 amino acids of CD133 splice variant s1 and s2; ‖Although not specified, only anti-C-term seems to be proposed by Abnova.

can be detected in various differentiated epithelia found in kidney, prostate, liver, and pancreas [15-17], and in mature glial cells [18]. In glandular epithelia, $CD133^+$ cells might constitute facultative stem cells acting during regeneration [19]. In good concordance with the strong expression of *PROM1* mRNA in the mammary glands, CD133 was detected by immunohistochemistry at the apical side of the lactiferous ducts in normal human mammary glands using rabbit antiserum αhE2 directed against its first extracellular loop (residues 240–388 of splice variant s2) but not its AC133 (CD133/1) epitope [13]. Such linear staining along the luminal surface in benign mammary lobules or ductules has been used as a positive control for CD133 detection in a recent study by Lin et al. [10] showing (with an unspecified antibody) a differential expression of CD133 between benign and malignant papillary lesions, with strong expression in benign papillomas and most atypical intraductal papillomas and loss of expression in malignant papillary carcinoma. Moreover, apical/endoluminal membrane staining with AC133 monoclonal antibody and antigen retrieval was reported in normal mammary epithelia and carcinoma [17]. In the hypothesis that breast cancer subtypes are linked to an epithelial differentiation hierarchy, the cellular origin of $CD133^+$ cancer cells and its impact on cancer progression and metastases would be particularly relevant [20]. It is also important to distinguish CD133 expression in hematopoietic progenitor cells within lymph nodes from that in epithelial cells or tumor masses to evaluate the meaning of CD133 in breast cancer [4].

Second, because CD133 is associated with plasma membrane protrusions (e.g., microvilli, primary cilium) and extracellular vesicles budding thereof [21,22], differing subcellular localization of the CD133 staining is likely to reflect modified cellular activity. Nevertheless, intracellular localization of CD133 has been observed under normal conditions, with accumulation in multivesicular bodies that are released as $CD133^+$ exosomes upon fusion with the plasma membrane [16,23]. The presence of CD133 in various physiological body fluids or glandular lumina in cancer samples might be considered to be a biomarker [22,24], and the characterization of exosomes in plasma serum might be instructive regarding cancer origin [25]. A cytoplasmic localization of CD133 might reflect deficient cell polarization related to epithelial/mesenchymal transition [26], although the latter issue remains to be demonstrated. In addition, it seems important, in the presence of cytoplasmic expression of CD133, to consider that some anti-CD133 monoclonal antibodies have shown cross-reactivity with cytokeratin 18 [27].

In conclusion, to reach a standard in scoring systems for CD133 it is highly important to ascertain the identity of the antigenic molecule being detected and to use clearly defined and characterized reagents. However, an exhaustive expression profile of this biomarker in both normal breast and cancer tissues still needs to be completed. CD133, which was proposed to promote mammary gland branching in a mouse model [28], clearly appears to be of significance both for development and for cancer progression and prognosis.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

1. Kim SJ, Kim YS, Jang ED, Seo KJ, Kim JS. Prognostic impact and clini-

copathological correlation of CD133 and ALDH1 expression in invasive breast cancer. J Breast Cancer 2015;18:347-55.
2. Ieni A, Tuccari G. Comments on the "Prognostic Impact and Clinicopathological Correlation of CD133 and ALDH1 Expression in Invasive Breast Cancer". J Breast Cancer 2016;19:96-8.
3. Hermansen SK, Christensen KG, Jensen SS, Kristensen BW. Inconsistent immunohistochemical expression patterns of four different CD133 antibody clones in glioblastoma. J Histochem Cytochem 2011;59:391-407.
4. Ieni A, Giuffrè G, Adamo V, Tuccari G. Prognostic impact of CD133 immunoexpression in node-negative invasive breast carcinomas. Anticancer Res 2011;31:1315-20.
5. Han Z, Chen Z, Zheng R, Cheng Z, Gong X, Wang D. Clinicopathological significance of CD133 and CD44 expression in infiltrating ductal carcinoma and their relationship to angiogenesis. World J Surg Oncol 2015;13:56.
6. Zhao P, Lu Y, Jiang X, Li X. Clinicopathological significance and prognostic value of CD133 expression in triple-negative breast carcinoma. Cancer Sci 2011;102:1107-11.
7. Mansour SF, Atwa MM. Clinicopathological significance of CD133 and ALDH1 cancer stem cell marker expression in invasive ductal breast carcinoma. Asian Pac J Cancer Prev 2015;16:7491-6.
8. Aomatsu N, Yashiro M, Kashiwagi S, Takashima T, Ishikawa T, Ohsawa M, et al. CD133 is a useful surrogate marker for predicting chemosensitivity to neoadjuvant chemotherapy in breast cancer. PLoS One 2012;7:e45865.
9. Bock C, Kuhn C, Ditsch N, Krebold R, Heublein S, Mayr D, et al. Strong correlation between N-cadherin and CD133 in breast cancer: role of both markers in metastatic events. J Cancer Res Clin Oncol 2014;140:1873-81.
10. Lin CH, Liu CH, Wen CH, Ko PL, Chai CY. Differential CD133 expression distinguishes malignant from benign papillary lesions of the breast. Virchows Arch 2015;466:177-84.
11. Fargeas CA, Huttner WB, Corbeil D. Nomenclature of prominin-1 (CD133) splice variants: an update. Tissue Antigens 2007;69:602-6.
12. Corbeil D, Karbanová J, Fargeas CA, Jászai J. Prominin-1 (CD133): molecular and cellular features across species. Adv Exp Med Biol 2013;777:3-24.
13. Florek M, Haase M, Marzesco AM, Freund D, Ehninger G, Huttner WB, et al. Prominin-1/CD133, a neural and hematopoietic stem cell marker, is expressed in adult human differentiated cells and certain types of kidney cancer. Cell Tissue Res 2005;319:15-26.
14. Kemper K, Sprick MR, de Bree M, Scopelliti A, Vermeulen L, Hoek M, et al. The AC133 epitope, but not the CD133 protein, is lost upon cancer stem cell differentiation. Cancer Res 2010;70:719-29.
15. Karbanová J, Missol-Kolka E, Fonseca AV, Lorra C, Janich P, Hollerová H, et al. The stem cell marker CD133 (Prominin-1) is expressed in various human glandular epithelia. J Histochem Cytochem 2008;56:977-93.
16. Missol-Kolka E, Karbanová J, Janich P, Haase M, Fargeas CA, Huttner WB, et al. Prominin-1 (CD133) is not restricted to stem cells located in the basal compartment of murine and human prostate. Prostate 2011;71:254-67.
17. Immervoll H, Hoem D, Sakariassen PØ, Steffensen OJ, Molven A. Expression of the "stem cell marker" CD133 in pancreas and pancreatic ductal adenocarcinomas. BMC Cancer 2008;8:48.
18. Corbeil D. Prominin-1 (CD133): New Insights on Stem & Cancer Stem Cell Biology. New York: Springer-Verlag; 2013.
19. Corbeil D, Fargeas CA, Jászai J. CD133 might be a pan marker of epithelial cells with dedifferentiation capacity. Proc Natl Acad Sci U S A 2014;111:E1451-2.
20. Prat A, Perou CM. Mammary development meets cancer genomics. Nat Med 2009;15:842-4.
21. Weigmann A, Corbeil D, Hellwig A, Huttner WB. Prominin, a novel microvilli-specific polytopic membrane protein of the apical surface of epithelial cells, is targeted to plasmalemmal protrusions of non-epithelial cells. Proc Natl Acad Sci U S A 1997;94:12425-30.
22. Marzesco AM. Prominin-1-containing membrane vesicles: origins, formation, and utility. Adv Exp Med Biol 2013;777:41-54.
23. Bauer N, Wilsch-Bräuninger M, Karbanová J, Fonseca AV, Strauss D, Freund D, et al. Haematopoietic stem cell differentiation promotes the release of prominin-1/CD133-containing membrane vesicles: a role of the endocytic-exocytic pathway. EMBO Mol Med 2011;3:398-409.
24. Karbanová J, Laco J, Marzesco AM, Janich P, Voborníková M, Mokrý J, et al. Human prominin-1 (CD133) is detected in both neoplastic and non-neoplastic salivary gland diseases and released into saliva in a ubiquitinated form. PLoS One 2014;9:e98927.
25. O'Brien K, Rani S, Corcoran C, Wallace R, Hughes L, Friel AM, et al. Exosomes from triple-negative breast cancer cells can transfer phenotypic traits representing their cells of origin to secondary cells. Eur J Cancer 2013;49:1845-59.
26. Latorre E, Carelli S, Raimondi I, D'Agostino V, Castiglioni I, Zucal C, et al. The ribonucleic complex HuR-MALAT1 represses CD133 expression and suppresses epithelial-mesenchymal transition in breast cancer. Cancer Res 2016;76:2626-36.
27. Pötgens AJ, Schmitz U, Kaufmann P, Frank HG. Monoclonal antibody CD133-2 (AC141) against hematopoietic stem cell antigen CD133 shows crossreactivity with cytokeratin 18. J Histochem Cytochem 2002;50:1131-4.
28. Anderson LH, Boulanger CA, Smith GH, Carmeliet P, Watson CJ. Stem cell marker prominin-1 regulates branching morphogenesis, but not regenerative capacity, in the mammary gland. Dev Dyn 2011;240:674-81.
29. Collina F, Di Bonito M, Li Bergolis V, De Laurentiis M, Vitagliano C, Cerrone M, et al. Prognostic value of cancer stem cells markers in triple-negative breast cancer. Biomed Res Int 2015;2015:158682.
30. Kapucuoğlu N, Bozkurt KK, Başpınar Ş, Koçer M, Eroğlu HE, Akdeniz R, et al. The clinicopathological and prognostic significance of CD24, CD44, CD133, ALDH1 expressions in invasive ductal carcinoma of the breast: CD44/CD24 expression in breast cancer. Pathol Res Pract 2015;211:740-7.

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