COMMENTARY

Sweet beginning for cancer stem cells

Linda G. Baum[a,1]

The concept of "oncofetal antigens" (i.e., biomarkers that are expressed both on normal developing fetal tissue and on human cancer cells) was proposed in the 1960s, before we knew very much about cancer cell heterogeneity or had even conceptualized the cancer stem cell. It is now clear that tumors are indeed composed of very different, genetically distinct subpopulations of cancer cells and that a tiny fraction of those cells, the cancer stem cells, are endowed with the ability to self-renew and to persist and thus can establish new tumors. In PNAS, Cheung et al. (1) identify a specific glycolipid antigen, stage-specific embryonic antigen-3 (SSEA-3), that is preferentially expressed on a small fraction of cultured breast cancer cells that fulfill the definition of cancer stem cells. Importantly, this paper also identifies a single gene product, the β-1,3-galactosyltransferase 5 (β3GalT5) enzyme, that creates a critical domain of this glycolipid antigen (see figure S3 in ref. 1 for the glycolipid structures and the enzymes that create them). Thus, this work demonstrates that this oncofetal antigen is not merely a marker of breast cancer stem cells (BCSCs) but is also critical for survival of these cells.

Glycoantigens on Cancer Stem Cells

Why do we want to identify unique antigens expressed on cancer stem cells? Three reasons come to mind. First, having a set of antigens or markers that specifically define a cancer stem cell can facilitate isolation of relatively pure populations of these cells from heterogeneous tumors, so that the properties of the cancer stem cells can be studied. Cheung et al. (1) used two assays, cell colonies and mammosphere formation, to assay the relative fraction of BCSCs from heterogeneous cultures of two different human breast cancer cell lines. The group defined a repertoire of antigens that yielded a significantly enriched population of BCSCs from both human cell lines. Although Wong and coworkers (2, 3) have shown that BCSCs express a variety of glycolipid antigens, including SSEA-3, SSEA-4, and Globo-H, using SSEA-4 or Globo-H as isolation markers did not increase the yield of cells capable of forming cell colonies or mammospheres. However, using SSEA-3 as a marker yielded a population that formed a high percentage of cell colonies and mammospheres from both breast cancer cell lines. Critically, using SSEA-3 as one of the repertoire of markers to identify BCSCs yielded a subpopulation of cells that was very efficient at forming tumors in vivo in mice—as few as 10 cells expressing a repertoire of markers including SSEA-3 was sufficient to form tumors in mice, and these tumors grew to a larger size than those derived from cells not expressing SSEA-3. These results demonstrate the utility of the SSEA-3 antigen in defining BCSCs, so that these cells can be isolated and studied.

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These markers could also be used during histologic diagnosis, to label the fraction of cells in a primary tumor that meet the phenotypic definition of BCSCs. Although not currently used for prognostication, just as identifying BCSCs in vitro can be used to understand cancer stem cell biology, identification of BCSCs in primary tumors may be useful in the future to predict the aggressiveness or metastatic potential of a primary tumor (4).

Second, these unique antigens may serve as vaccine candidates for immunotherapy of tumors. The subset of oncofetal antigens that contain glycan epitopes have also been referred to as tumor-associated carbohydrate antigens (TACA) and several of these, including SSEA-3 and Globo H, have been the targets of immunotherapy strategies targeting both conventional T-cell recognition of antigens and T-inde-pendent antibody responses (5, 6). Again, identifying such markers in primary tumors could potentially inform the tumor immunotherapy approach that would be crafted for individual patients in the "personalized

[a]Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA 90095
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[1]Email: lbaum@mednet.ucla.edu.

medicine" era; being able to immunohistologically identify a full repertoire of oncofetal or TACA markers on primary biopsies may be increasingly important for therapy as well as for diagnosis and prognosis (4).

Third, these unique antigens may be critical for cancer stem cell behavior. It is highly likely that the consistent and stereotypic expression of these structures on cancer stem cells confers some type of biologic advantage to the tumor cells—it is not likely that tumors express such structures merely to make it easier for biologists, oncologists, and pathologists to find the stem cells. It is worth noting that the development of antibody markers for cell surface antigens has yielded an explosion of phenotypic information about these antigens without necessarily revealing what the function of these antigens might be. An exciting aspect of the work of Cheung et al. (1) is the identification of a functional requirement for the specific glycosyltransferase enzyme that creates the SSEA-3 antigen. The enzyme β3GalT5 is essential to create the SSEA-3 glycolipid; in Cheung et al. (1), overexpression of the β3GalT5 enzyme increased SSEA-3 expression on the two cancer cell lines. Conversely, siRNA knockdown of β3GalT5, and thus reduction in cell-surface SSEA-3, resulted in reduced cell proliferation and increased apoptosis specifically in breast cancer cell lines, but not in nontransformed cultured breast cells. This result clearly shows that this antigen has a unique role in the biology of neoplastic, but not nonneoplastic, cells. Moreover, the function of this antigen directly relates to the biology of the BCSCs, namely the ability of these cells to proliferate and survive. Thus, this glycolipid antigen may be a useful therapeutic target in breast cancer, not just as an oncofetal antigen used for a vaccine against tumor cells, but as the target of small molecules that could block the contribution of SSEA-3 structures on the cell surface to tumor cell proliferation and survival.

Beyond Flow Cytometry

An interesting aspect of this work is the meticulous characterization of glycolipid antigens on tumor cells by mass spectrometry, as well as by flow cytometry using fluorescently tagged antibodies. Given the ease of flow cytometry and the heterogeneity of tumor cell populations, rigorous structural analysis of glycolipid epitopes is not typically part of a study examining the biological properties of cancer stem cells. However, the work by Wong and coworkers (1) demonstrates that detection of an epitope by flow cytometry (i.e., the relative fluorescence of the number of tagged antibodies bound to the cell surface) may not directly reflect the abundance of the structures being interrogated. This lack of correlation may reflect the accessibility of the epitope to the antibody on the cell surface, the distribution of the epitopes on the cell surface, or the cross-reactivity of the antibody with related epitopes. An advantage of flow cytometry, especially using a panel of antibodies, is that a small population of unique cells can be identified and purified, but if the antibodies lack sensitivity or specificity for a particular type of tumor cells this approach will be problematic. An advantage of mass spectrometry is that the structures that are identified are clearly defined; however, because isolation of the pool of cells to be analyzed by mass spectrometry may require antibody-mediated isolation, there will continue to be a trade-off between the utility of rapid screening techniques such as flow cytometry and the more definitive characterization offered by mass spectrometry. Cheung et al. (1) stress that the development of better reagents to detect these glycolipid antigens will benefit the field.

It is not uncommon that phenotypic characterization and functional assessment of cell surface molecules on tumor cells is somewhat disconnected; unlike the broad scope of the work by Cheung et al. (1), a cancer biology group studying BCSCs may not have the resources of a group that performs glycan and glycolipid analysis. Again, sometimes it is very useful to simply have a marker that identifies a cancer stem cell to isolate the cell and possibly develop a vaccine against the cell. However, also revealing the functional properties of an oncofetal antigen opens up many additional possibilities for therapeutic innovation, to target the relatively small fraction of cells in a tumor that do the most damage. Of course, a number of questions remain. For example, from an evolutionary point of view, why would a tumor cell expend the energy to further modify the SSEA-3 backbone to make SSEA-4 and Globo H if these antigens do not seem to contribute to stem cell proliferation and survival? Additionally, how can we enhance human immunoreactivity to these antigens that were present during fetal life? Finally, what will be the optimal method of phenotyping a patient's primary tumor, to ensure that we focus on developing the most appropriate mix of therapies for each patient? The rapid evolution of new technologies and tools to make analysis of structure and function of glycans, glycolipids, and glycoproteins more accessible to all scientists, especially the recent efforts supported by the National Institutes of Health Common Fund (7), will hopefully accelerate progress in understanding the role of unique glycan antigens in cancer stem cell biology.

1 Cheung SKC, et al. (2016) Stage-specific embryonic antigen-3 (SSEA-3) and β3GalT5 are cancer specific and significant markers for breast cancer stem cells. *Proc Natl Acad Sci USA* 113:960–965.

2 Chang WW, et al. (2008) Expression of Globo H and SSEA3 in breast cancer stem cells and the involvement of fucosyl transferases 1 and 2 in Globo H synthesis. *Proc Natl Acad Sci USA* 105(33):11667–11672.

3 Lou YW, et al. (2014) Stage-specific embryonic antigen-4 as a potential therapeutic target in glioblastoma multiforme and other cancers. *Proc Natl Acad Sci USA* 111(7):2482–2487.

4 Novak A, et al. (2013) Cholesterol masks membrane glycosphingolipid tumor-associated antigens to reduce their immunodetection in human cancer biopsies. *Glycobiology* 23(11):1230–1239.

5 Heimburg-Molinaro J, et al. (2011) Cancer vaccines and carbohydrate epitopes. *Vaccine* 29(48):8802–8826.

6 Huang YL, et al. (2013) Carbohydrate-based vaccines with a glycolipid adjuvant for breast cancer. *Proc Natl Acad Sci USA* 110(7):2517–2522.

7 National Institutes of Health (2015) Office of Strategic Coordination – The Common Fund Glycoscience Program. Available at https://commonfund.nih.gov/glycoscience.