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Breast cancer stem cells: initiating a new sort of thinking

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Cancer stem cells (CSCs) are currently a provocative and somewhat controversial topic among cancer researchers. CSCs, also referred to as tumor-initiating cells (TICs), are cells within a tumor that are capable of self-renewal and of producing a new tumor with the heterogeneity that is characteristic of the parental tumor. Although CSCs are named for their stem cell-like properties, they need not arise from normal stem cells. They may be derived from the transformation of tissue progenitors or even from differentiated cells gaining the ability of self-renewal. Although the CSC hypothesis is not new, current technologies, namely fluorescence activated cell sorting (FACS) and the ability to reconstitute cancers in mice, facilitated the functional experiments to formally prove their existence. The first experiments to validate the hypothesis that some cancer cells in the hematopoietic system have stem cell properties were presented in a seminal study by John Dick and colleagues. They enriched for human acute myeloid leukemia (AML) cells expressing a specific set of cell surface markers (Bonnett and Dick, 1997). The cells that they isolated initiated AML upon transplantation into non-obese diabetic mice with severe combined immunodeficiency disease (NOD/SCID mice). This proved that the isolated cells had the potential for self-renewal and differentiation, which is expected of a bona fide leukemia stem cell.

In 2003, Al-Hajj et al. published what may become a classic paper in the emerging field of CSCs (Al-Hajj et al., 2003). The authors borrowed the techniques previously applied to the hematopoietic system and applied them to a solid tumor type: breast cancers. They discovered that the cell surface immunophenotype $CD44^{+}CD24^{-/low}lineage^{-}$ characterizes a population of cells with an increased propensity to seed tumors in NOD/SCID mice. Consistently, 1000 cells with these markers were able to form tumors, representing a 10–50-fold enrichment over the tumor-initiating ability of unfractionated tumor cells. When this cell population was further selected for another cell-surface marker, epithelial specific antigen (ESA), only 200 of these cells were needed to induce tumors: a greater than 50-fold enrichment for tumor-initiating ability compared with unfractionated tumor cells. Moreover, this tumorigenic population generated tumors that were similar to the original donor cells, as assessed by FACS analysis for CD24 and CD44 or ESA. These cells re-established the phenotypic heterogeneity of the parental tumors, which helped fulfill the definition of CSCs. The $ESA^{+}CD44^{+}CD24^{-/low}$ cells formed tumors on serial transplantation, supporting the idea that these CSCs have the ability to self-renew. This study was the first to use cell surface markers to isolate functional CSCs in solid tumors and accordingly generated considerable excitement.

The take rate for establishing xenografts from primary tumors, and especially the ability to serially transplant tumors, has been generally reported to be very low. Some tumors and tumor types, including many estrogen receptor (ER)-positive breast cancers, may not grow as xenotransplants, making it currently impossible to assay for tumor-initiating ability. Rather than examining random patients presenting with breast cancer, Al-Hajj et al. examined only certain patients, eight out of nine of whom presented with metastatic disease. They isolated tumorigenic cells mainly from pleural effusions, which are excess fluids that accumulate in the pleural cavity. Many of these cells were pre-passaged through the mouse, which may have selected for highly aggressive and metastatic cells. Thus, these cells may have been more aggressive or hardy as they exhibited the ability to undergo several of the steps of metastasis. The study may not have been possible if the authors had examined the most prevalent, but usually less aggressive, type of ER-positive, luminal A-type breast cancers. The results of this study have yet to be independently replicated or extended to larger numbers and subtypes of primary breast cancers. This illustrates the technically challenging nature of these experiments with solid tumors rather than the validity of the results.

Although the tumors in this study represented different histological types, the small number of tumors analyzed did not represent all types of breast cancer. Gene expression profiling studies have identified at least five distinct subtypes of breast cancer. The question remains whether these same surface markers used by Al-Hajj et al. can identify CSCs across all breast cancer subtypes (Nakshatri et al., 2009). If not, methods for isolating tumor stem cells from each of the molecular subtypes will need to be developed. One tumor in the Al-Hajj study did not follow the same pattern as the others, illustrating this as a distinct possibility. Another method for analyzing breast cancer cells, which has allowed the isolation of cells with increased tumorigenic potential, uses an enzymatic assay to measure the activity of aldehyde dehydrogenase 1 (ALDH1). In a study by Ginestier et al., it was shown that the ALDH1-positive population exhibited only a small overlap with the $CD44^{+}CD24^{-/low}$ immunotype (Ginestier et al., 2007). However, in one example, the combination of these two methods did yield a population that was highly enriched for tumor-initiating ability.

Recently, the validity of studies employing methods similar to those employed by Al-Hajj et al. were challenged. These experiments rely on the xenotransplantation of human cancer cells into mice that are immunocompromised to prevent rejection. Some argue that these model systems may not support a physiologically relevant microenvironment for evaluating tumor-initiating ability. Using transgenic lymphoma and leukemia mouse models, Strasser and colleagues show that at least one in ten of the tumor cells transplanted to another histocompatible mouse seeded tumor growth, and that this event did not require a rare population of cells (Kelly et al., 2007). Similarly, Morrison and colleagues used highly immunocompromised NOD/SCID interleukin-2 receptor gamma chain null mice to enhance the detection of human tumorigenic melanoma cells and found that around 25% of unselected

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melanoma cells could induce tumors (Quintana et al., 2008).

Studies using syngeneic mouse tumor models reaffirm the existence of CSCs, showing that they are not unique to xenotransplanted human tumors. In two of these studies employing mouse mammary tumor models (p53 null and Wnt-1), the authors were able to use cell surface markers to identify tumor-initiating subpopulations of cells within these models, which were validated by limiting dilution transplantation assays that involved seeding tumors in syngeneic mice (Cho et al., 2008; Zhang et al., 2008). Despite the existence of rare subpopulations in many tumors, it should also be stressed that CSCs need not be a rare subpopulation and that aggressive cancers may have high frequencies of CSCs.

The work of Al-Hajj and colleagues has led to a surge in the identification of cell surface markers that characterize CSCs in various solid cancers, including glioblastoma, colon and pancreatic cancers (Visvader and Lindeman, 2008). Most importantly, these studies have influenced the entire field of cancer biology. Instead of broadly looking at whole tumors or cell lines, it now seems that identifying specific subpopulations within the tumor that may promote tumor formation may be more relevant for predicting therapeutic response. By using isolated CSCs, we now can elucidate the pathways that regulate the growth and survival of this unique population of cells.

It has been suggested that CSCs are intrinsically resistant to therapeutics and that they serve as a reservoir for cancer recurrence and metastasis. Jenny Chang and colleagues recently showed that, following chemotherapy, cells expressing the markers identified by Al-Hajj, $CD44^{+}CD24^{-/low}lineage^{-}$, were enriched in tumors compared with other cell populations (Li et al., 2008). The recent ability to generate or expand cells with these cell surface markers and stem cell-like properties through the induction of epithelial-to-mesenchymal transition has facilitated a screen to discover compounds that can selectively target CSCs (Gupta et al., 2009; Mani et al., 2008).

Al-Hajj et al. identified markers that characterize tumor-initiating cancer cells. These markers and others are now being used to monitor CSCs during preclinical and clinical studies. They should help to determine the best treatment protocol for a patient and enable screening to assess the efficacy of different treatment approaches. Ultimately, the most effective therapy combinations will not only need to shrink the overall tumor mass, but to specifically target the CSCs as well.

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