Several studies have identified a subset of cancer cells, designated as tumor-initiating cells or cancer stem cells (CSCs), with the ability for self-renewal and differentiation into distinct cell lineages. In recent years, the hypothesis has emerged, and gained great momentum, that tumors are hierarchically arranged, with CSCs being the principal drivers of tumor growth for proliferation, resistance to chemotherapy, and metastasis (1–3). A combination of flow cytometry and xenotransplantation techniques led to the identification of leukemia-initiating cells (CD34+/CD38−) and breast cancer–initiating cells (CD44+/CD24−/low) and provided the scientific basis for the CSC hypothesis (3–5). Needless to say, if CSCs exist, their attributes need to be studied, and specific therapies directed against these cells will have to be developed. Although there are some excellent studies suggesting the presence of CSCs, several controversial issues remain.

The development of drug resistance and disease recurrence and metastasis could simply be consequences of tumor heterogeneity and plasticity. The latter characteristics also could result in cells having distinct characteristics, including sensitivity to therapy. What factors (i.e., clonal evolution, CSCs, or tumor-microenvironment niche) contribute to adverse outcomes remains an important question. The answer to this question is undoubtedly going to be complex and may vary in different tumors. One of the concerns with the CSC hypothesis is that the methods used to identify these cells are limited. The presence of CSCs is usually inferred on the basis of one of the following characteristics: (a) flow cytometry results indicating the presence of “specific” cell surface markers; (b) clonogenic assay findings such as mammosphere formation; and (c) ability of cells to give rise to metastases in xenotransplantation models (6).

Several cell surface markers have been associated with CSC activity. These tend to differ across different studies, with minor degrees of overlap. For example, in breast cancer, CSC activity was first associated with CD44+/CD24− cells and later with aldehyde dehydrogenase 1-positive (ALDH1+) cells; more recently, CSC activity has been associated with CD49F+ cells and with retention of a lipophilic fluorescent dye, PKH26 (6). Similar findings have accrued in colon, pancreatic, ovarian, and lung cancers and glioblastomas (3). The cells identified by each of these methods have features compatible with CSCs but the cells are distinct. Analyses of some commonly used breast cancer cell lines have revealed some cell lines to have almost 100% of cells with a CSC phenotype, whereas others are almost completely devoid of these cells (7). More specifically, CD44+/CD24− cells were found predominantly in basal/mesenchymal cell lines, whereas none of the estrogen receptor (ER)-positive cell lines were found to have important numbers of these cells. More recently, cells with a distinct phenotype have been ascribed to CSC activity in ER-positive and/or progesterone receptor–positive tumors. This finding raises questions about whether each breast cancer tumor subtype has its own CSCs. Recent genomic analyses seem to suggest that there are 10 types of breast cancer. One of these types (basal cell–like/triple-negative cancer) has been further divided into 6 additional subtypes. Would it be reasonable to anticipate that different CSCs will be found for each one of these types?

In mouse models of mammary cancer, different driver mutations are tumorigenic; for example, Wnt1-driven breast cancers are enriched with CD61 for CSC activity in mice, whereas mice with Neu/ErbB2-driven breast cancers do not possess stem cell activity (8). Mouse models of lung cancer have also exhibited different surface phenotypes with different mutations (9). Thus, different oncogenic mutations may give rise to cancers with different tumorigenic capacity. In addition, the spectrum of driver mutations and stem cell properties appears to change with age (3, 10). In some studies, it has been documented that cells with a non-CSC phenotype can “acquire” a CSC phenotype,
whereas in others this has not been possible. The CSC phenotype can be enriched by simple manipulations, such as exposure of the cells to hypoxia or upregulation of 1 or more genes (11, 12) that are associated with development of an epithelial-to-mesenchymal transition. Such cells can more efficiently form mammospheres in soft agar colonies and tumors in mice.

The ability to implant may be a function of better engraftment ability in immunocompromised mice rather than the functionality of CSCs. As few as 10 leukemic cells from mice genetically engineered to develop leukemia have been shown to cause leukemia when injected into genetically compatible healthy animals (13), raising questions about how many CSCs exist in leukemia. Furthermore, recent studies in melanoma showed that the frequency of cells with CSC characteristics increased dramatically from 1 per 1000 000 cells to 1 per 4 cells simply from a change in the level of immunodeficiency in recipient mice (14). The ability of mammary cancer cells and leukemic cells to implant in mice depends on the genetic background of the mice and their level of immunocompetence (14, 15). Study results such as these raise questions about the applicability of data derived from immunocompromised xenotransplantation models, because these mouse models do not recapitulate the environmental conditions present in human tumors.

One of the cardinal principles of the CSC hypothesis is that (only) CSCs have the capacity to undergo symmetric cell division (for self-renewal) as well as asymmetric cell division (to give rise to differentiated progeny). Asymmetric division in Drosophila is controlled by the Numb protein, whereas in Caenorhabditis elegans it is controlled by Par proteins. These mechanisms are conserved in vertebrates because they are necessary for simple processes such as determination of cellular polarity. Some of the polarity-related genes such as STK11 (serine/threonine kinase 11, also known as LKB1) are known tumor suppressors and have been implicated in several cancers. Deregulation of adhesion and polarity proteins has been shown to cause misoriented mitotic spindles that could lead to symmetric division in nonstem cells and increased self-renewal (16).

Evidence from clinical practice does not appear to be in line with the CSC hypothesis. Although a percentage of patients go on to develop metastases following surgery for early stage solid cancer, the vast majority do not. Despite the fact that CSCs are not known to be ER positive, breast cancers have been successfully treated with antiestrogenic therapy only. Similarly, targeted therapy against HER2 (human epidermal growth factor receptor 2), a differentiation marker, has been shown to be extremely effective, with complete remission of disease occurring in randomized clinical trials.

In our studies, the expression of ALDH1 in tumor cells was not associated with response or resistance to therapy; intriguingly, stromal expression was found to be prognostic in triple-negative tumors. Lastly, although it is believed that cells with a CSC phenotype are relatively rare in tumors (a few per million cells), such evidence makes it difficult to explain the utility of gene expression profiles from whole primary tumors for determining prognosis or predicting sensitivity to endocrine therapy or chemotherapy.

Four of the 6 hallmarks of cancer proposed by Hanahan and Weinberg (17)—enabling replicative immortality, resisting cell death, sustaining proliferative signaling, and evading growth suppressors—are likely to represent early events in cancer initiation. The other 2 hallmarks, angiogenesis and activating invasion and metastasis pathways, could be linked to cancer cells undergoing epithelial-to-mesenchymal transformation (and expression of a CSC phenotype). In addition, cancer cells undertake multiple cell modifications to evade the immune system; these include modifications to the MHC antigens and the production of immunoregulatory molecules. Additionally, there are indirect effects mediated via interactions with mesenchymal cells and tumor-associated lymphocytes, macrophages, and dendritic cells (2, 18). Examples of the latter include high expression of CD47 in tumor cells being associated with decreased phagocytosis by macrophages, and the expression of CD200 in tumors resulting in impaired tumor-specific effector T-cell responses. The contribution of mesenchymal cells to the development of resistance to common chemotherapeutic agents such as cis-platinum is well documented. Similarly, immune cells and macrophages contribute to alteration of the extracellular matrix, resulting in the release of stroma-associated growth factors.

In conclusion, cancer is a disease of genomic instability, evasion of immune cells, and adaptation of the tumor cells to the changing environment. Genetic heterogeneity caused by tumors and tumor microenvironmental factors (Fig. 1) forms the basis of aggressive behavior of some cancer cell populations. Given the right environment and unique mutational events, some of these cancer cells acquire plasticity, including stem cell–like characteristics, enabling them to act like “supervillains.” Their ever-mutating phenotype and extreme capacity to adapt to changes in the microenvironment, including the ability to recruit nontumor cells, enables them to flourish in adverse circumstances. Because the adaptive environments are likely to be context specific and different in every patient (personalized), it might be simplistic to believe there will be a single therapy that will be able to eradicate these cells in a given cancer type. More investigation clearly is needed, focused on understanding what fac-
tors affect tumor behavior and on developing therapeutics to inhibit mutations and adaptations of the microenvironment that favor tumor growth and to prevent the conversion of these ordinary tumors cells into supervillains.

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