Poised with purpose
Cell plasticity enhances tumorigenicity

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Individual carcinomas are composed of multiple, distinct subpopulations of neoplastic cells that exhibit distinct biological properties. The cancer stem cell (CSC) model has been invoked to explain how these diverse cancer cell types are organized within a given tumor. According to this model, minority subpopulations of CSCs are endowed with the potential to drive cancer progression, holding the majority, if not all, of a carcinoma’s tumor-initiating and metastatic potential, while the majority of neoplastic cells within such tumors, the non-CSCs, lack these traits.1,2 Accordingly, the CSC model offers the prospect of gaining a deeper understanding of the biological mechanisms that underlie tumor aggressiveness. For example, tumors that contain a high CSC content have been shown to be more aggressive and to carry poor clinical prognosis. Moreover, relative to the non-CSCs within individual tumors, the CSCs are known to display heightened resistance to currently used radio- and chemotherapies.3 This latter observation explains why an understanding of CSC biology is essential for the future development of novel therapies that succeed in eliciting curable patient responses in the oncology clinic.

One important clue to the organization and physiology of CSCs comes from a number of observations indicating that the stem cell (SC) program of these cells closely resembles that operating within the antecedent normal tissue-of-origin of a tumor, e.g., the CSC program of mammary carcinomas resembles in outline the corresponding program in the normal mammary gland. While it is not yet understood precisely how CSC biology is orchestrated, it is known that CSCs can undergo symmetric division to replenish the CSC pool, or asymmetric division to generate non-CSC progeny (which are considered poorly tumorigenic and poorly metastatic cancer cells). As such, CSCs, like their normal SC counterparts, are thought to reside at the apex of a cellular hierarchy and to differentiate in a unidirectional manner into their non-CSC counterparts. Implicit in this depiction is the idea that non-CSCs are unable to ascend the cellular hierarchy and re-enter the CSC state.

Recently, we and others established that not all cancers adhere to a unidirectional CSC model.4-6 In the setting of breast cancer (BrCa) cells, we identified subpopulations of non-CSCs that could readily switch from the non-CSC to CSC state. These initial findings indicated that many of the aggressive CSCs within individual tumors could be newly derived from their non-CSC counterparts, and that this process of dedifferentiation may occur continually during the development of the tumor. Of note, we also identified subpopulations of basal cells in the normal human mammary gland that could dedifferentiate into stem-like cells.5 Together these observations suggest that the current doctrine regarding unidirectional normal and neoplastic stem cell hierarchies should be revised.

In our recent publication,7 we set out to determine how broadly applicable non-CSC-to-CSC plasticity is in the context of BrCa. To do so, we analyzed a series of human BrCa cell lines encompassing both luminal- and basal-type BrCa cells. To summarize, we found that non-CSC-to-CSC plasticity is readily observed in basal-type BrCa cells, but not in luminal-type BrCa cells. We went on to show that this type of plasticity is enabled and potentially driven by the transcription factor ZEB1. Importantly, we also demonstrate that microenvironmental stimuli, such as TGFβ, by upregulating ZEB1 expression within BrCa cells, can enhance the rate of basal non-CSC cell conversion to the CSC state. Importantly, a corresponding set of luminal sub-type BrCa cells failed to undergo this conversion in response to TGF-β treatment. Together, these findings suggest that basal non-CSCs are inherently able to create CSCs, and that the tumor microenvironment may play an important role in determining the rate with which this type of conversion occurs. Moreover, the ability of non-CSCs to generate CSCs may contribute to the more aggressive clinical outcome of basal-type compared with luminal-type BrCa. Stated differently, the intrinsic aggressiveness of BrCa cells may be determined by their responsiveness to contextual signals that they receive from the nearby stromal microenvironment, with the less aggressive luminal BrCa cells showing little if any responsiveness to these signals and the basal cells being more responsive. We also note that coupled to this activation of ZEB1 and entrance into the CSC state is the acquisition of a number of mesenchymal features that are associated with the passage of carcinoma cells through an epithelial-to-mesenchymal transition (EMT).8

We were curious to understand why basal, but not luminal BrCa cells could readily activate ZEB1 expression.
Cancer stem cells (CSCs) were thought to adhere to a unidirectional hierarchical model where CSCs reside at the apex undergoing symmetric division to replenish the CSC pool or asymmetric division to generate non-CSC progeny. New evidence suggests that the CSC model is not unidirectional, rather non-CSCs can ascend the cellular hierarchy and re-enter the CSC state. Here we show that among breast cancer cells, basal-type non-CSCs are particularly proficient at generating CSCs. Switching from the non-CSC to CSC state is dependent on activation of the transcription factor ZEB1, and can be enhanced by the addition of factors such as TGFβ. Basal non-CSCs are readily able to activate ZEB1 due to the maintenance of its promoter in a bivalent chromatin configuration. In this formation, the ZEB1 promoter is marked simultaneously by repressive (H3K27me3) and activating (H3K4me3) histone modifications. In response to the appropriate signals, H3K27me3 is removed from the ZEB1 promoter, enabling the active H3K4me3 mark to persist and thereafter, presumably, foster active transcription of this gene. Accordingly, we chose to look at the chromatin at the ZEB1 promoter, which serves as a key determinant of the transcription of this gene. These experiments showed marked differences: whereas the ZEB1 promoter in luminal non-CSCs was marked exclusively by repressive histone modifications, in basal non-CSCs coexisting repressive and active histone modifications were found to be present. This unique chromatin configuration, termed bivalency, is thought to place a gene in a configuration that is poised for ready activation. Hence, the bivalent configuration of the ZEB1 gene in the basal subtype of BrCa cells suggested that the non-CSCs in these tumors carry a ZEB1 promoter that can readily switch from a poised to active state, resulting in the efficient induction of ZEB1. We went on to demonstrate that TGFβ, which enhances non-CSC-to-CSC plasticity, facilitates this process by markedly reducing the presence of the H3K27me3 repressive histone modification at the bivalent ZEB1 promoter, enabling the active H3K4me3 mark to persist and thereafter, presumably, foster active transcription of this gene.

The applicability of the CSC model to multiple cancer types has demonstrated unequivocally that the differentiation states of cancer cells directly impacts tumor growth, recurrence, and progression to metastatic disease. Adding to this complexity, we have now shown that select cancer cells can, in response to appropriate stimuli, readily switch between benign and aggressive cell states. Implied here is not only the notion that non-CSCs are plastic cell populations, but that non-CSCs are also highly adaptable cell populations. This in turn suggests that tumors as a whole need to be viewed as adaptable and evolving tissues. Consequently, where future therapies aimed at eradicating CSCs may offer some therapeutic benefit to patients, our data suggest that, under those circumstances, a tumor’s non-CSC component may replenish its pool of CSCs. In light of current findings, therapies targeting non-CSC-to-CSC plasticity should offer improved clinical outcome for cancer patients. (Fig. 1)

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