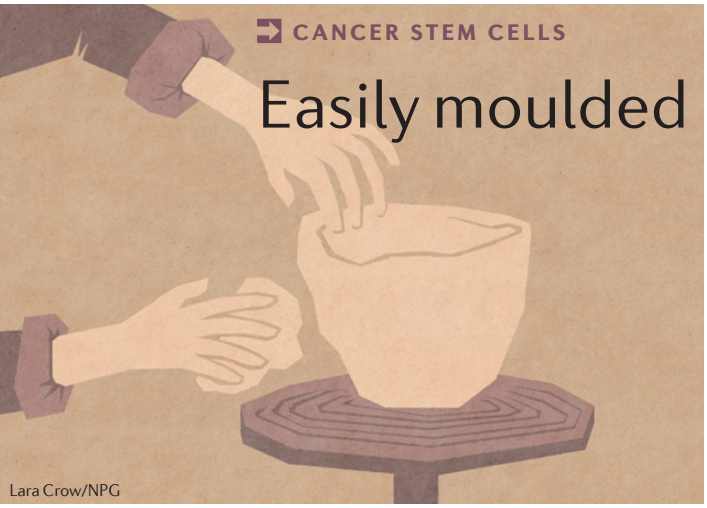
Nature Reviews Cancer | AOP, published online 18 July 2013; doi:10.1038/nrc3573



Previous studies have indicated that some cells that lack cancer stem cell (CSC) properties can transition to CSC-like cells. However, it is unclear how common these types of transitions are or how they are controlled. Chaffer, Marjanovic, Weinberg and colleagues have investigated this further in breast cancer cell lines.

CSCs in breast cancers have been shown to exist exclusively in the $CD44^{hi}$ compartment. The authors found that $CD44^{hi}$ and $CD44^{low}$ populations of cells coexist in several cell lines derived from basal breast cancers, but that cell lines from less aggressive luminal cancers are mostly composed of $CD44^{low}$ cell populations. Purified $CD44^{low}$ cells (>99.7% purity) from both basal and luminal cancer cell lines were able to seed tumours in the mammary fat pads of immunocompromised mice. Although tumours formed more frequently from $CD44^{hi}$ cells, these data suggested that populations that lack $CD44^{hi}$ cells (and that therefore presumably lack CSCs) can generate tumours. An investigation of the tumours arising from $CD44^{low}$ cells showed that they contained a small fraction of $CD44^{hi}$ cells (2–22% of the tumour cells), which indicates that some cells had converted from a $CD44^{low}$ to a $CD44^{hi}$ state.

> “populations that … presumably lack CSCs … can generate tumours”

How might these cells convert from a $CD44^{low}$ to a $CD44^{hi}$ state? Previous data have shown that stem-like cells have more mesenchymal properties than non-stem cells, and have linked this to the epithelial-to-mesenchymal transition (EMT) programme. Analysis of EMT-controlling transcription factors in HME-flopc cells (non-transformed mammary epithelial cells that frequently undergo spontaneous $CD44^{low}$-to-$CD44^{hi}$ transitions) indicated that zinc finger E-box binding homeobox 1 (*ZEB1*) was highly expressed in $CD44^{hi}$ cells compared with $CD44^{low}$ cells. Doxycycline-inducible short hairpin RNA (shRNA)-mediated knockdown of ZEB1 substantially reduced the ability of $CD44^{low}$ cells to convert to $CD44^{hi}$ cells, and this was reversed when doxycycline was withdrawn.

The miR-200 microRNA family and ZEB1 antagonize each other, and the authors showed that the inhibition of miR-200 increased *ZEB1* mRNA levels and the $CD44^{low}$-to-$CD44^{hi}$ transition in HME-flopc cells; this was abrogated when ZEB1 was also knocked down, which indicates that *ZEB1* is a key miR-200 target in this process. Similar results were observed in transformed HME-flopc cells. Furthermore, ZEB1 and miR-200 expression were inversely correlated in breast cancer cell lines, with higher expression of ZEB1 in cells from basal tumours (which have higher levels of CD44 expression) than in cells from luminal tumours.

The induction of *ZEB1*-targeted shRNAs in basal breast cancer cell lines decreased the ability of the $CD44^{low}$ population to give rise to tumours in immunocompromised mice, which shows the importance of ZEB1 expression for tumour initiation. Intriguingly, although ZEB1 was required for the $CD44^{low}$ to $CD44^{hi}$ conversion and continuous ZEB1 expression was necessary for the maintenance of stem-like properties *in vitro*, continuous ZEB1 expression was not required for the maintenance of high cell surface expression of CD44, which indicates that stemness and CD44 expression can be uncoupled.

The authors hypothesized that *ZEB1* might be rapidly induced as a result of epigenetic regulation; specifically, that *ZEB1* might exist in a bivalent state in which the gene promoter contains both permissive and repressive histone H3 modifications so that the gene is repressed but is also ready for rapid activation. This is common in genes that control the cell state in embryonic stem cells. Analysis of histone methylation patterns in $CD44^{low}$ HME-flopc cells and basal breast cancer cells (which are associated with more aggressive clinical behaviour) showed that the *ZEB1* promoter was indeed in a bivalent state in these cells, whereas the *ZEB1* promoter in $CD44^{hi}$ cells had mostly activating marks. By contrast, the more benign $CD44^{low}$ luminal breast cancer cells carried mostly repressive marks on the *ZEB1* promoter, which indicates that these cells are unable to rapidly upregulate *ZEB1* expression. Furthermore, transforming growth factor-β (TGFβ), a common inducer of EMT that upregulates *ZEB1* expression, was shown to enhance $CD44^{low}$-to-$CD44^{hi}$ transitions in basal but not in luminal breast cancer cells. Therefore, the differing clinical behaviours of these two subtypes of breast cancer cells could partly be explained by the differences in the configuration of the *ZEB1* promoter.

These data raise several interesting questions, including whether the plasticity of CSC-like states is present in primary human breast tumours, whether it differs depending on the subtype and the aggressiveness of the tumour, and whether it exists in other tumour types.

Sarah Seton-Rogers

ORIGINAL RESEARCH PAPER Chaffer, C. L. *et al.* Poised chromatin at the ZEB1 promoter enables breast cancer cell plasticity and enhances tumorigenicity. *Cell* **154**, 61–74 (2013)