Sox2, a marker for stem-like tumor cells in skin squamous cell carcinoma and hedgehog subgroup medulloblastoma

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Heterogeneity within tumors is becoming increasingly recognized as an important cause of treatment failure in cancer. Two recent studies use fate-mapping and limiting dilution transplantation assays to identify SRY (sex determining region Y)-box 2 (Sox2) as cancer stem-cell marker and driver of cancer stemness.

See also: **RJ Vanner *et al*** (July 2014), **S Boumahdi *et al*** (July 2014) and **M Kool *et al*** (March 2014)

There are several sources of tumor heterogeneity which include clonal evolution, heterogeneity in the micro-environment, and the hierarchical organization of certain cancers whereby highly tumorigenic cancer stem cells differentiate into less tumorigenic progeny (Meacham & Morrison, 2013). Employing genetic fate-mapping and limited dilution transplantation analyses, two studies independently discovered Sox2 as a cancer stem-cell marker in medulloblastoma and squamous-cell carcinoma, respectively (Vanner *et al*, 2014; Boumahdi *et al*, 2014). In the first study, Peter Dirks and colleagues perform elegant lineage tracing experiments to demonstrate the role of rare, relatively quiescent, Sox2⁺ tumor cells in propagating a murine model of sonic hedgehog subgroup medulloblastoma (MB) in primary tumors and allografts. In the second study, Cedric Blanpain and colleagues unravel the role of Sox2 in both tumor initiation and tumor maintenance in murine models of skin squamous-cell carcinoma (SCC) and also provide evidence that Sox2 is a driver of stemness.

While both cancer models display a hierarchical organization reminiscent of the normal tissue from which they arise, there are some notable differences between the two hierarchies (Fig 1). The hierarchy presented by Dirks and colleagues is steep with a rare population of slow-cycling Sox2⁺ cancer stem cells (comprising 5% of tumor population) giving rise to rapidly proliferating doublecortin⁺ progenitor cells (comprising 60% of tumor population), which subsequently differentiate to short-lived postmitotic NeuN⁺ cells (comprising 30% of the tumor population). By contrast, the Sox2⁺ cancer stem cell population of skin SCC is larger accounting for approximately 25% of tumor epithelial cells. Furthermore, this population in skin SCC is not slow cycling as evident by the increasing percentage of Sox2⁺ cells with each round of transplantation (80% after the second transplantation). These results suggest that the hierarchical organization of each cancer resembles the normal tissue homeostasis from which it arises. Importantly, both hierarchies are preserved in limiting dilution transplantation assays.

A related concept to the hierarchical growth of some cancers is the reversible plasticity of cancer cells. The cancer stem cell hypothesis predicts that the conversion of highly tumorigenic cancer stem cell to non-stem cell progeny is irreversible. However, there is mounting evidence in several cancers such as colon cancer, glioblastoma, and melanoma that the conversion of cancer stem cells to differentiated progeny is reversible (Charles *et al*, 2010; Roesch *et al*, 2010; Schwitalla *et al*, 2013; Suva *et al*, 2014). This has been demonstrated in melanoma with JARID1B as a cancer stem cell marker, in the perivascular niche of gliomas with nitric oxide promoting stem cell character, and in colon cancer where inflammation triggers dedifferentiation of non-stem cells to stem cells. Blanpain and colleagues provide further evidence in support of reversible plasticity between cancer stem cells and their differentiated progeny by demonstrating that SOX2⁺GFP⁺ tumor epithelial cells can give rise to tumors that contain both SOX2⁺GFP⁺ and SOX2⁺GFP⁺ tumor epithelial cells. Thus, the conversion between cancer stem cells and their non-stem cell progeny appears reversible in skin SCC. Dirks and colleagues also observe that Sox2⁺GFP⁺ tumor cells can form tumors in transplantation assays. It would be interesting to know whether such tumors also contain Sox2⁺GFP⁺ cells. Nonetheless, lineage tracing from differentiated cell types will be required to definitively evaluate the plasticity between Sox2⁺ and Sox2⁺ tumor populations in the two tumor types and determine whether these tumor subtypes follow the cancer stem cell model.

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Figure 1. Tumor Hierarchy in Sonic Hedgehog Subgroup MB and skin SCC.

(A) In a murine model of sonic hedgehog subgroup medulloblastoma, rare, relatively quiescent Sox2⁺ cancer stem cells give rise to rapidly proliferating doublecortin⁺ progenitor cells, which subsequently give rise to postmitotic NeuN⁺ cells. Whether this hierarchy is reversible, that is, whether a DCX⁺ cell can become a Sox2⁺ cell, or whether a NeuN⁺ cell can revert to a DCX⁺ cell remains to be elucidated. (B) In a murine model of skin squamous cell carcinoma, rapidly proliferating Sox2⁺ cells, which account for 25% of the total tumor population, give rise to Sox2⁻ cells. This differentiation step appears inefficiently reversible. Furthermore, Sox2 is not only a cancer stem cell marker but also a driver of stemness in this tumor type.

epitopes such as CD15, CD133, and nestin. By contrast, Olig2, a bHLH transcriptional repressor has been demonstrated to be both a marker and a driver of cancer stemness in gliomas as Olig2 function is required for glioma formation (Ligon *et al*, 2007). Similarly, Blanpain and colleagues demonstrate that lineage ablation of Sox2-expressing cells and conditional deletion of Sox2 in pre-existing skin papilloma and SCC results in tumor regression. Thus, Sox2 is both a marker and a driver of stemness in skin SCC. Dirks and colleagues demonstrate that Sox2 is a marker of stemness by observing that Sox2⁺ cells are relatively quiescent, capable of self-renewal, differentiation, and have tumor propagating properties. Conditional deletion of Sox2 in pre-existing murine MBs will be required to evaluate whether Sox2 is also a driver of stemness in MB.

These two studies have several important therapeutic implications. First, Sox2 or its downstream targets are an important therapeutic target in skin SCC. Second, as Sox2⁺ cancer stem cells are enriched following antimitotic therapy and smoothed inhibition in the murine MB model,

novel agents that target the quiescent Sox2⁺ population in MB are desperately needed. The observation that the Sox2⁺ population is resistant to smoothed inhibition is unexpected as both the Sox2⁺ population and Sox2⁻ population demonstrate similar levels of hedgehog pathway activation. The mechanism for the relative resistance of the Sox2⁺ cells to smoothed inhibition is not clear and will require further study. One possibility is that the mechanism for this drug tolerant state is reversible and chromatin mediated (Sharma *et al*, 2010). This adds another level of complexity to targeted therapies as recent genomic analysis of human MB has identified a genotype-related response to smoothed inhibition (Kool *et al*, 2014).

In conclusion, both of these studies shed light on Sox2 as a marker for stem-like tumor cells. While both cancer models have hierarchical organization, fate mapping of the non-stem population to determine if the hierarchies are bidirectional are important future experiments with clear therapeutic implications. It is important not to generalize these results to other cancers as the

cell-of-origin, and/or genetic alterations, can all potentially impact tumor hierarchy and stem cell markers and/or drivers. While the heterogeneity of cancer has numerous causes, studies like these that illuminate the complexity of one source of heterogeneity—the hierarchical organization of cancers—move us one step closer toward curing these devastating diseases.

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