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COMMENTARY

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# Mechanical softness: a true stemness feature for cancer cells

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**ABSTRACT**

Developing a method that can effectively define and sort cancer stem cells (CSCs) is extremely desirable. Mechanical stiffness is of paramount importance for a cell to differentiate and can reflect the differentiation state of cells. In line with this notion, cell softness is identified to be a unique marker for highly tumorigenic CSCs.

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The concept of cancer stem cells (CSCs) may date back to 1937 when a single murine leukemia cell could initiate a tumor in mice.[1] Until 1994, this concept was initially accepted by the seminal discovery of AML stem cells with the $CD34^+CD38^-$ phenotype in a mouse model by John Dick and colleagues in 1994.[2] Thereafter, $CD44^+CD24^-$ breast CSCs[3] and $CD133^+$ brain CSCs[4] were identified, respectively, which greatly promoted the CSC field development. The CSC theory can be well used to explain the drug resistance and the tumor recurrence and metastasis. Notwithstanding these, conventional cell surface markers, which are commonly used to identify CSCs, are unreliable and highly variable among different cancers. Currently, rigorous methods are not available to isolate CSCs from a tumor, limiting the investigation of the properties of this crucial tumor cell subpopulation. Therefore, developing new method(s) that can effectively sort and define highly tumorigenic CSCs is extremely desirable.

Biomechanics is a fundamental feature of a cell. During the last decades, accumulating evidence has shown that forces are able to regulate gene expression, protein synthesis, and macromolecule conformation, thus profoundly influencing cell migration, proliferation, and apoptosis.[5] Applying a force to a cell results in stress on the cell via the contact area. This stress is described by the force per unit contact area, and the cell use deformation in response to the stress. To characterize the ability to resist the deformation, a term "stiffness" is commonly used, which is defined as the ratio of stress to deformation. Thus, stiffness is an intrinsic mechanical property of a cell, which is mainly stemmed from F-actin. Different cell types display various levels of stiffness, which match the stiffness of local tissues, allowing the cells to properly sense and respond to the surrounding mechanical microenvironments.[6] Cell stiffness can range from 0.01 kPa for egg cells, 0.5 kPa for embryonic stem cells, 1–5 kPa for differentiated tissue cells, and to 12 kPa for skeletal muscle cells.[7] Thus, cellular stiffness appears to be related to a differentiation state of cells, and a weak stiffness represents a less differentiation. In line with this concept, undifferentiated tumorigenic cells are much softer (soft is inverse of stiff) than differentiated counterparts in various cancer types. Based on these lines of information, we speculate that mechanical softness might represent an inherent feature for CSCs.

In our recent study,[8] taking advantage of the deformability of soft cells, we designed a unique microfluidic chip for sorting soft and stiff tumor cells, respectively. A less than 400 Pa stiffness is used to define soft tumor cells and a higher than 700 Pa stiffness is used to describe stiff tumor cells. Biologically, the injection of 100 soft tumor cells (4T1 or MCF-7) into the mammary fat pads of wild-type or NOD/SCID IL-2Rc-null (NSG) mice could form an orthotopic tumor (4T1) or a metastatic tumor in the lungs (MCF-7). However, the injection of 100 stiff tumor cells could not form a tumor. Soft 3D fibrin gels can select highly tumorigenic cells and allow them to proliferate within the gel without differentiation.[9] It is the soft tumor cells but not the stiff ones that form spheroid colonies. These data clearly display that the soft tumor cells are highly tumorigenic in their ability to form a tumor at both primary and metastatic sites.

To date, ideal methods are lack in sorting CSCs. Surface markers like CD133, an intracellular enzyme like Acetaldehyde dehydrogenase1 (ALDH1), and side population (SP) are widely used to isolate CSCs despite their unreliability. When we used these three methods to sort CSC and non-CSC two subpopulation cells, we found that among $ALDH1^+$ breast, $CD133^+$ melanoma, and $SP^+$ tumor cells, 20–40% cells are stiff; and among $ALDH1^-$ breast $CD133^-$ melanoma and $SP^-$ tumor cells, 10–20% tumor cells are soft. Intriguingly, stiff $ALDH1^+$, $CD133^+$ and $SP^+$ cells do not have the ability to form a tumor, but soft $ALDH1^-$, $CD133^-$ and $SP^-$ cells do have the ability to

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form a tumor. Thus, the intrinsic softness may be an ideal marker to label CSCs, which is better than the conventional markers.

To better understand the merit of softness as a marker for tumorigenic cells, we conducted RNA sequencing and the gene set enrichment analysis (GSEA) was performed, which showed the enrichment of stemness-related genes. Among them, B-cell CLL/lymphoma 9-like (BCL9L), a homolog of B-cell CLL/lymphoma 9 (BCL9) with functional redundancy, is especially interesting, because Wnt signaling is critical in the regulation of stem cell pluripotency and self-renewal, and BCL9/BCL9L act additional transcriptional co-activators and form part of the Wnt enhanceosome. Indeed, this Wnt signaling protein BCL9L is identified to be upregulated in soft tumor cells and regulate their stemness and tumorigenicity.

CSC theory implicates the use of CSC markers to predict patient prognosis. Unlike the variability of biochemical molecules, the physical trait of a cell is more stable. Indeed, chemical molecule-based CSC markers are equivocal to predict the prognosis of cancer patients. Such vagueness might be ascribed to that marker-based CSCs contain both soft and stiff tumor cells. Thus, cellular softness may act as a biomechanical marker for CSCs to better predict the prognosis of cancer patients. Besides, such softness can be a potential target in cancer immunotherapy. Cancer cells can use mechanical softness to prevent membrane pore formation by cytotoxic T cell-released perforin,[10] suggesting a mechanics-based approach for tumor immunotherapy. We term this approach as mechno-immunotherapy.

## Disclosure of potential conflicts of interest

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