Targeting breast cancer stem cells: fishing season open!

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Centre de Recherche en Cancérologie de Marseille, Laboratoire d'Oncologie Moléculaire, UMR891 Inserm/Institut Paoli-Calmettes, Marseille, France

Abstract

Studies describing tumor as a hierarchically-organized cell population have changed the classical oncogenesis view and proposes new therapeutic strategies. Cancer stem cells (CSC) are thought to sustain tumor initiation/maintenance, therapy resistance, and systemic metastases. Targeting this tumor cell population is crucial to achieve a true cure. A large research effort is now aiming atdeveloping drugs targeting CSCs based either on *a priori* understanding of key pathways regulating CSC biology or high-throughput screening to identify novel targets and compounds.

Background

In recent years, tumor-initiating cells, so-called cancer stem cells (CSC), have been characterized in multiple cancers including breast cancer [1]. This component of cancer cells retains key stem cell properties, including self-renewal, which initiates and drives tumorigenesis, and differentiation albeit aberrant, which contributes to cellular heterogeneity. Moreover, CSCs are thought to be the seed for distant metastasis responsible for poor clinical outcome [2,3]. The discovery of CSCs provides an explanation as to why cancer may be so difficult to cure, and suggests new therapeutic strategies. Several studies demonstrate that breast CSCs are resistant to conventional therapeutic strategies such as radiotherapy or chemotherapy [2]. Thus, neoadjuvant chemotherapy leads to an increase in breast $CD24^-/CD44^+$ or $ALDH^{high}$ CSCs and tumorsphere-initiating-cells [4,5]. If these cells are the tumor root they are the cells to be killed.

Two approaches have been developed to design the best therapeutic strategies targeting CSCs. The first approach is based on targeting key pathways regulating CSC survival, differentiation and self-renewal. Several master pathways (Hedgehog, NOTCH, and AKT/WNT/βcatenin signaling) commonly involved in self-renewal of embryonic and adult stem cells are known to be deregulated in CSCs and induce an expansion of this population [6]. A number of agents targeting these pathways are currently tested preclinically and some have entered clinical trials. Meanwhile, studies of CSC-enriched populations using Omics technologies are rapidly defining additional regulatory pathways and networks regulating CSC biology. We have recently established a gene expression signature that allowed the identification of CXCR1/IL8 signaling as a key regulator pathway of breast CSC biology [7]. Utilizing a small molecule inhibitor of CXCR1, repertaxin, we were able to specifically target the CSC population in human breast cancer xenografts, retarding tumor growth and reducing metastasis [8].

The article

To identify novel drugs that target specifically CSCs researchers from Ciliberto's group have privileged the second approach based on unbiased high-throughput screening (HTS) of small molecules libraries on CSC-enriched populations [9]. Because tumor cell population could contain very few CSCs, HTS needs be redesigned to specifically measure gene inhibition or drug effect on the CSC population. In the MCF7 breast cancer cell line the authors described a cell population staining pale toluidine blue (Light Cells-LCs) enriched in CSCs. LCs had an increase in tumorsphere forming efficiency and were enriched for $ALDH^{bright}$ cells, described to exclusively contain the CSC population [7]. When transplanted in immunodeficient mice, LCs cells were highly tumorigenic compared to bulk MCF7 cells. Utilizing this experimental system; Cioce et al. have done a drug screen assay. A total of 26 compounds were screened for their ability to kill specifically the LCs at a greater rate than the bulk MCF7 cells. The screen identified four such compounds, which all turned up to interfere with NFκB signaling [9].

The viewpoint

This unbiased drug screen strategy on CSC-enriched population was initially developed by the laboratories of R. Weinberg and E. Lander. The model used was experimentally-transformed HMLER breast cancer cells modified by shRNA-mediated inhibition of the human E-cadherin gene. Inhibition of E-cadherin expression induced an epithelial-mesenchymal transition (EMT) resulting in an increase in $CD44^{high}/CD24^{low}$ cancer cells. A total of 32 compounds in a library of 16,000 chemicals had selective toxicity for these artificially-enriched breast CSCs. Among these, salinomycin was the most potent. The use of this potassium ionophore inhibitor as a potential cancer drug is novel and was validated *in vivo* using breast cancer cell line xenografts, with a decrease in tumor growth and metastasis formation [10]. A similar approach has been developed for human brain tumors with the establishment of several glioma neural stem cell lines stably enriched in CSCs.

Utilizing cell imaging-based chemical screen (450 FDA-approved drugs) P. Dirks' group identified both differential sensitivities of CSCs and a common susceptibility to perturbation of serotonin signaling [11]. This suggests that CSCs might be highly susceptible to metabolic changes and open new therapeutic possibilities.

Other than test selective drug toxicity on an enriched-CSC population compared to bulk cancer cells, HTS can be designed to directly measure drug effect on CSC function. Exploiting the relationship between neural stem cells self-renewal and neurosphere proliferation, a screen of more than 1,200 compounds identified several neuromodulators as key regulators of stem cell biology [12]. A similar approach may be envisaged with tumorsphere from breast cancer cell lines.

Instead of chemicals, RNA interference libraries can be screened to identify factors that control CSC tumorigenicity and to stimulate the development of novel anti-CSC therapies. A recent kinome-wide RNA interference screen identified factors that control the balance between maintenance and differentiation of glioblastoma CSCs. For example, silencing of TRRAP was described to increase differentiation of glioblastoma CSCs *in vitro* and also suppressed tumor formation *in vivo* [13].

In conclusion, HTS assays of CSCs provide opportunities to identify multiple compounds that could represent new revolutionary therapies. Because these novel compounds will be selected *in vitro*, it will be crucial to extensively validate *in vivo* the selective toxicity of these drugs toward CSCs , utilizing primary tumor xenografts as a preclinical step. Moreover, serial transplantation of the residual cells isolated from treated-tumors will be needed to prove the complete eradication of the tumor-initiating cell population. If the fishing season is officially open, the question remains how to choose the best bait. Hence, developing therapeutic strategies to target CSCs will need a thorough and rigorous effort as many challenges remain to overcome such as the evaluation of drug efficiency in the patients [14].

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