Are All Cancer Stem Cells Created Equal?

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SUMMARY
Numerous solid malignancies have been reported to contain cancer stem cells (CSCs). Distinct functional characteristics have been attributed to CSCs, and thus it is widely believed that these unique cells may have genetic and phenotypic homogeneity. Recent exciting but limited evidence, however, contradicts this tenet and supports the intriguing concept of genetic and phenotypic diversity in the CSC population. We propose that CSC heterogeneity at the inter- and intrapatient levels may be due to the cell of origin, to environmental cues, and/or to human papillomavirus infection. Additional insight into CSC heterogeneity is needed to identify actionable targets for optimal eradication of the diverse CSC subpopulations within a tumor. STEM CELLS TRANSLATIONAL MEDICINE 2014;3:1111–1115

INTRODUCTION
The cancer stem cell (CSC) hypothesis posits that a unique population of cancer cells has the exclusive ability to self-renew and differentiate into multiple lineages of non-CSC cancer cells that constitute the bulk of the tumor. Based on this hypothesis, CSCs are believed to reside at the top of the cancer cell hierarchy and produce cell progenies responsible for tumor heterogeneity that exists in solid malignancies. Recent published work has challenged this notion and shown that non-CSCs can dedifferentiate and acquire CSC characteristics through activation of selected oncogenic pathways [1–3]. In addition, environmental stimuli, specifically secretion of growth factors into the tumor microenvironment, can promote the reprogramming of differentiated non-CSC cancer cells into CSCs [4]. These observations provide initial evidence that differentiated cells can be reprogrammed into CSCs under certain conditions and thus suggest that the cell of origin for CSCs may not be limited to stem cells. An intriguing report revealed plasticity in the CSC population such that CSCs can switch between two different states with distinct phenotypes; epithelial to mesenchymal transition-like (EMT-like) and non-EMT-like CSCs [5]. These findings add additional layers of complexity to the CSC hypothesis and argue against genetic and phenotypic homogeneity within the CSC population. In this paper, we discuss the concept and clinical implications of CSC diversity in solid malignancies.

GENETIC HETEROGENEITY OF CSCS
The cell of origin for CSCs is a fundamental question in the field that remains unanswered at this time. A common notion is that normal pluripotent stem and/or progenitor cells may be the cell of origin for CSCs because these two cell types possess similar hallmark functional characteristics, namely, self-renewal and differentiation. However, there is a lack of experimental evidence to provide insight into the molecular mechanisms required to reprogram normal pluripotent cells into CSCs. The most logical and straightforward hypothesis is that accumulation of genetic alterations may trigger the transformation of normal pluripotent cells into CSCs. It is unclear whether a distinct set and/or a threshold number of genetic alterations are needed to drive the transition of normal pluripotent cells into CSCs. If alteration in a distinct set of genes is the causative event, then genetic diversity of CSCs may be limited. Alternatively, if the threshold number of genetic alterations is the driver event to induce the reprogramming of normal pluripotent cells, then genetic heterogeneity in CSCs would be expected to increase considerably. Another possibility is that the combination of the two mechanisms mentioned may cooperate to produce CSCs and thus result in additional permutations in the genetic diversity of the CSC population.

Emerging literature is revealing bidirectional plasticity between the non-CSC and CSC populations to support non-CSCs as the cell of origin for CSCs. Several groups reported that non-CSCs can be induced to dedifferentiate into CSCs by co-opting oncogenic pathways and/or through interaction with the microenvironment. A recent study showed that hyperactive K-ras and Wnt signaling cooperate to dedifferentiate villus cells into intestinal CSCs in a nuclear factor-κB-dependent fashion [1]. Similarly, K-ras functioned in concert with another oncogene, c-Myc, to promote the conversion of differentiated mouse fibroblasts into CSCs [3]. Mammary non-CSC epithelial cells were shown to spontaneously dedifferentiate to CSCs in vitro and in vivo [6]. In addition, the conversion rate of non-CSCs to CSCs was more pronounced in vivo, suggesting that the tumor microenvironment may play a critical role in CSC expansion [6]. Subsequent work from the same group reported that tumor growth factor-β, a microenvironment stimulus, converts the ZEB1 promoter to an active chromatin configuration in non-CSCs, resulting in an increase in ZEB1 and subsequent transition to a CSC state [2]. Another study supported the importance of...
the tumor microenvironment in CSC expansion and demonstrated that myofibroblasts secrete hepatocyte growth factor to activate the Wnt signaling cascade in non-CSC tumors leading to a switch to a CSC state [4]. Taken together, these studies suggest that any tumor cell may have the capacity to be reprogrammed into CSCs under defined genetic and/or environmental conditions. This possibility argues that CSC diversity is not only a consequence of somatic mutations but also may be due to epigenetic modifications. It should be noted that a key limitation of these studies is the strict use of human cancer cell lines and murine models; therefore, it remains to be determined whether the bidirectional plasticity between non-CSCs and CSCs occurs in tumors from cancer patients.

**Phenotypic Heterogeneity of CSCs**

It is generally accepted that a key functional characteristic of CSCs is resistance to conventional chemotherapeutics and radiation. Several groups reported that CSCs are more refractory to standard chemotherapy and radiation than non-CSCs [7–12]. However, emerging work using different tumor model systems suggests that the chemoresistant and/or radioresistant phenotype of CSCs may not be a universal feature [13–15]. CSCs isolated from the primary tumors of a cohort of glioblastoma patients showed differential sensitivity to radiation [13]. In addition, CSCs isolated from a glioblastoma patient were more resistant to radiation compared with non-CSCs, whereas, in a different glioblastoma patient, CSCs and non-CSCs showed similar responses to radiation [13]. It is unclear if this finding is tissue-specific and limited to glioblastoma or translates to other solid malignancies. Another question that remains to be addressed is whether CSCs also exhibit differential response to chemotherapy at the interpatient level? In any event, these studies provide initial evidence that phenotypic heterogeneity of CSCs exists at the interpatient level.

There is literature to support the argument that phenotypic CSC diversity also may exist at the intrapatient level. CSCs were shown to have the capacity to transition between EMT-like CSCs with mesenchymal features or non-EMT-like CSCs with epithelial features [5]. The existence of non-EMT-like and EMT-like CSCs was confirmed in the primary tumors of oral squamous cell carcinoma patients (OSCC) [5]. Both EMT-like and non-EMT-like CSCs have similar in vivo tumor-initiating ability at the primary site; however, spontaneous metastatic disease was restricted to the EMT-like CSCs [5]. All of the non-EMT-like CSCs were able to produce a mixed population of cells consisting of non-EMT-like and EMT-like CSCs [5]. In contrast, the majority of the EMT-like CSCs were unpotent and generated only EMT-like CSCs [5]. These results indicate that the phenotype of CSCs is not static but rather dynamic depending on conditions that are yet to be fully elucidated. It will be interesting to determine whether EMT-like and non-EMT-like CSC states that exist in OSCC are present in other solid malignancies.

Another potential contributor to phenotypic heterogeneity of CSCs is the cell of origin. Overexpression of Ras in mammary stem-like cells formed poorly differentiated tumors with 100% penetrance in NOD/SCID mice. Whereas enforced Ras expression in differentiated mammary epithelial cells was less tumorigenic and formed well-differentiated tumors [6]. Another study reported that the in vivo tumor growth kinetics were faster when K-ras was overexpressed in Thy1/Sca1-negative less differentiated murine fibroblasts than in Thy1/Sca1-positive more differentiated murine fibroblasts [3]. These intriguing observations suggest that the differentiated state of the cell of origin of a CSC can modulate the behavior of the CSC and the resultant progenies.

**Viruses and CSC Heterogeneity**

Several viruses have been linked to the development of epithelial malignancies. Human papillomavirus (HPV), hepatitis B virus (HBV) or hepatitis C virus (HCV), and Epstein-Barr virus (EBV) account for the majority of virus-induced carcinomas worldwide. HPV, in particular HPV16, is associated with cervical carcinomas and oropharyngeal squamous cell carcinomas (OPSCCs). Infection with HBV or HCV is the predominant risk factor for the development of hepatocellular carcinomas (HCCs). EBV is an etiological factor for nasopharyngeal carcinomas and lymphomas. In general, these viruses are recognized as preferentially infecting a distinct population of host cells. High-risk HPV infection is limited to the basal cells (epidermal stem cells) of the stratified epithelium. A recent study demonstrated that HCV can replicate in human fetal hepatocytes, suggesting that HCV may be able to infect and replicate in hepatocytic stem and/or progenitor cells [16]. EBV is known to infect B-cells and epithelial cells; however, it is unclear at this time whether EBV has a proclivity to infect differentiated or undifferentiated epithelial cells. Because HPV, HBV/HCV, and EBV have cell and organ tropism, we speculate that virus-induced carcinomas are unique entities and may have genetic and phenotypic CSC heterogeneity due to differences in the cell of origin and the viral genome.

Recent work from our laboratory showed that HPV16 may promote interpatient CSC heterogeneity in OPSCCs. HPV16-positive OPSCC was found with a higher intrinsic CSC population than HPV16-negative OPSCC [17]. This finding was surprising because a randomized clinical trial showed that HPV16-positive OPSCC patients have better progression-free and overall survival than HPV-negative OPSCC patients in response to concurrent chemoradiation [18]. A plausible interpretation is that HPV16-positive CSCs are phenotypically distinct from HPV-negative CSCs and perhaps are more responsive to chemoradiation. Preliminary work from our laboratory supports this notion and showed that HPV16-positive OPSCC CSCs are more sensitive to cisplatin than HPV16-negative OPSCC CSCs (unpublished data). Based on our results, we speculate that CSC phenotype is more critical than CSC number as a biomarker for clinical response and outcome.

It can be argued that HPV16-positive OPSCC has a higher intrinsic CSC pool because HPV16 preferentially infects the basal cells (epidermal stem cells) of the reticulated epithelium of the tonsil. Our work cannot exclude this possibility; however, to our knowledge, no experimental evidence shows that HPV16 infection is sufficient to transform basal cells into CSCs. Alternatively, it is possible that HPV16-positive CSCs favor symmetric over asymmetric cell division to augment the CSC pool. Next-generation sequencing data showed that HPV16-positive OPSCCs contain numerous genetic alterations, although with less prevalence than in HPV-negative OPSCCs, providing evidence that HPV-negative and HPV16-positive OPSCCs have distinct etiologies [19, 20]. Moreover, the sequencing data suggest that additional genetic alterations may be necessary to transform HPV16-infected basal cells into CSCs. However, it is unclear at this time if the key
mutations necessary to promote a CSC phenotype occur more or less frequently in the HPV16-infected basal cells compared with the HPV-negative transformed epithelial cells.

**FUTURE RESEARCH DIRECTIONS AND CLINICAL IMPLICATIONS OF CSC HETEROGENEITY**

Sufficient evidence indicates that bidirectional non-CSC-CSC plasticity should be incorporated into the classic unidirectional hierarchy CSC hypothesis to fully model our current understanding of CSCs. We propose that four different types of cells—normal stem cells, normal progenitor cells, normal differentiated cells, and non-CSC tumor cells—have the potential to be the cell of origin for CSCs (Fig. 1). Because there is genetic and phenotypic heterogeneity between these cell types, a logical inference is that the cell of origin may be a critical determinant of CSC diversity at the genetic and phenotypic levels. This cell-of-origin thesis for CSC heterogeneity may not be limited within a particular tumor type but may extend to explain CSC heterogeneity between tumor types. Epithelial cells, differentiated and undifferentiated, from different organ sites are heterogeneous in nature; therefore, it is logical to propose that CSC diversity may exist at the level of intertumor type. In fact, numerous cell surface and functional markers have been used to prospectively isolate CSCs within a tumor site and across multiple tumor sites. The successful enrichment of functional CSCs using different approaches implies that CSC diversity exists at the intertumor-type and intratumor-type levels.

Research in the CSC field should be prioritized to enhance our understanding of genetic and phenotypic CSC diversity. Genetic CSC heterogeneity can be interrogated using different next-generation sequencing platforms. Whole-genome or exome sequencing at the single CSC level can be utilized to ascertain the extent of DNA heterogeneity of CSCs within an individual tumor and between tumors from different patients. In addition, RNA sequencing at the single-CSC level may provide insight into the diversity of gene expression due to epigenetic regulation in response to extracellular cues. Single-cell RNA-sequencing analysis of freshly dissociated primary glioblastomas revealed intrapatient gene expression diversity at the single-tumor-cell level [21]. Interestingly, individual tumor cells were distributed across the entire differentiated tumor cell-CSC expression-signature spectrum, suggesting that varying degrees of stemness exist among tumor cells within a particular tumor [21]. It remains to be determined whether the difference in the stemness expression profile will translate into a clinically relevant difference in CSC phenotype. A caveat of the current literature on phenotypic CSC heterogeneity is the extensive reliance on in vitro assays. There is limited evidence that CSC diversity at the phenotypic level exists in patient tumors or in animal models. Insight into phenotypic CSC diversity has been scarce because of several technical hurdles, such as the limited number of CSCs isolated from patient tumors and the lack of in vivo models available to study CSCs at a single-cell level. Patient-derived xenografts (PDXs) hold significant promise as a model system to facilitate the study of CSCs. PDXs were reported to retain a high degree of similarity in gene expression to the original patient tumor [22, 23] and thus may recapitulate the genetic and phenotypic CSC diversity of the original patient tumor. In addition, PDXs can be serially passaged and be a continual source for tumors from an individual patient. This model system should increase the number of CSCs available for experimental study and allow for comprehensive examination of CSC genotype and phenotype at an individualized level.

Intratumor heterogeneity at the genetic level has been demonstrated in solid malignancies and is associated with disease progression and treatment resistance [24–26]. The origin of intratumor heterogeneity is not well established; however, it is postulated that clonal evolution of tumor cells due to stochastic mutations may be the primary event responsible for genetic intratumor diversity. We propose an alternative hypothesis and speculate that intratumoral heterogeneity is a direct consequence of CSC diversity. A recent report demonstrated that HPV-negative OPSCC tumors were more diverse at a genetic level than HPV16-positive OPSCC tumors [26]. We proposed that high interpatient CSC heterogeneity exists in HPV-negative OPSCC tumors because the cell of origin for HPV-negative CSCs is not restricted to one cell type but may be from several cell types including normal stem cells, normal progenitor cells, and differentiated cells (Fig. 2A). HPV-negative OPSCC patients tend to be smokers and alcohol drinkers, two behaviors known to promote genetic instability and carcinogenesis. We speculate that these social behaviors may drive a continual cycle of reprogramming of HPV-negative non-CSC tumor cells to HPV-negative CSCs and thus promote dynamic genetic evolution of the CSC pool in HPV-negative OPSCC tumors. It is presumed that these newly evolved CSCs have additional genetic alterations beyond the original parental CSC and, consequently, increase intrapatient CSC diversity and tumor heterogeneity in HPV-negative OPSCC. In contrast, we speculate that HPV16-positive OPSCC tumors have low CSC diversity and tumor heterogeneity because HPV16 preferentially infects the basal stem cells, suggesting that the cell of origin for HPV16-positive CSCs is limited to one cell type (Fig. 2B). Our theory is consistent with the observation that HPV16-positive OPSCC tumors have a distinctive homogeneous nonkeratinizing basaloid morphology [27, 28]. In contrast to HPV-negative OPSCC patients, HPV16-positive OPSCC patients are predominantly nonsmokers and nondrinkers. Based on this observation, we further argue that HPV16-positive OPSCC tumors

![Figure 1](https://www.StemCellsTM.com)
may be more homogenous because the chance of DNA-damaging behaviors to alter the CSC or non-CSC genome will be less likely to occur.

The potential difference in prognosis and in CSC cell of origin between HPV-negative and HPV16-positive OPSCC raises several intriguing questions with implications that may be applicable to other solid malignancies. Is CSC diversity predictive of chemotherapy and/or radiation response? Is intratumor CSC heterogeneity altered following chemotherapy and/or radiation treatment? Is surgery the optimal treatment modality to negate the clinical consequence of CSC diversity? This notion is provocative, but we argue that surgical resection is the only treatment modality that would physically remove the pool of diverse CSCs from the patient. Further research to understand the role of CSC diversity in intratumor heterogeneity and treatment response in OPSCC and other solid malignancies is warranted. Additional insight into CSC diversity may reveal actionable targets and lead to the development of a class of designer molecules to eliminate the heterogeneous CSC pool.

Figure 2. CSC diversity and intratumor heterogeneity in HPV-negative and HPV16-positive OPSCC. (A): HPV-negative OPSCC. The cell of origin for HPV-negative OPSCC CSCs can include normal stem cells, normal progenitor cells, and differentiated cells leading to interpatient CSC and tumor heterogeneity. A continual cycle of reprogramming of HPV-negative non-CSC tumor cells to HPV-negative CSCs is postulated because of the accumulation of genetic alterations from smoking and/or alcohol exposure. These newly evolved CSCs will have additional genetic alterations beyond the original parental CSC, resulting in an increase in intrapatient CSC diversity and tumor heterogeneity in HPV-negative OPSCC. (B): HPV16-positive OPSCC. The cell of origin for HPV16-positive OPSCC CSCs is limited to the basal stem cells in the reticulated epithelium of the tonsillar crypt. An HPV16-positive OPSCC CSC is thought to undergo symmetric and asymmetric cell division to give rise to a genetically non-diverse population of CSCs and differentiated non-CSC tumor cells, resulting in a homogenous tumor at the genetic level. Abbreviations: CSC, cancer stem cell; HPV, human papillomavirus; OPSCC, oropharyngeal squamous cell carcinoma.
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AUTHOR CONTRIBUTIONS

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DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.