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# Systems Biology and Cancer Stem Cells

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## **Abstract**

The identification, purification, and characterization of cancer stem cells holds tremendous promise for improving the treatment of cancer. Mounting evidence is demonstrating that only certain tumor cells (i.e. the cancer stem cells) can give rise to tumors when injected and that these purified cell populations generate heterogeneous tumors. While the cell of origin is still not determined definitively, specific molecular markers for populations containing these cancer stem cells have been found for leukemia, brain cancer, and breast cancer, among others. Systems approaches, particularly molecular profiling, have proven to be of great utility for cancer diagnosis and characterization. These approaches also hold significant promise for identifying distinctive properties of the cancer stem cells, and progress is already being made.

**Keywords:** transcriptomics, proteomics, molecular signature, networks

## **Introduction**

Cancer is the second deadliest disease in the United States, accounting for one out of every four deaths. Despite tremendous efforts dedicated to conquering this disease, the age-adjusted mortality rate has remained almost unchanged for most cancers for the past five decades, with one compelling instance being a devastating brain tumor—glioblastoma—which carries a median survival rate of merely 9 to 12 months regardless of treatment. Among the conglomerate of cellular, molecular and genetic complications contributing to the persistence of cancer, the cellular origin provides a promising avenue that could lead to targeted elimination of cancer. Cancers often occur in tissues with constant proliferation. A paradigm learned from developmental biology suggests that only the clonogenic stem cells in the hierarchical tissue developmental processes are long-lived, whereas their differentiated progenies lose proliferating potential. Emerging evidence suggests that cancers may actually be a malignance derived from a small cell population dubbed cancer stem cells (CSCs), among the heterogeneous tumor cells, that demonstrate self-renewal and multiple lineage differentiation properties [1]. Discovered initially in leukemia [2], cancer stem cell populations have been isolated from several tissues, including breast, brain and colorectal cancers [3-5]. The identification of these cancer stem cell populations sets the stage for systems level analysis, and holds potentially tremendous ramifications in terms of our understanding of the molecular pathogenesis of cancer, patient stratification, and therapeutic intervention.

As with any complex biological system, cancer (including cancer stem cells) can be viewed and interrogated at the genome-scale using systems biology approaches. Systems approaches stress three concepts regarding biological information [6]. First, there are two fundamental types of biological information — the digital information of the genome and the environmental information that is outside our DNA. Second, the digital genome information encodes two types of biological networks — protein interactions and gene regulatory networks. Protein networks transmit and use biological information for development, physiology and metabolism. Gene regulatory networks — transcription factors and RNAs that regulate networks of other transcription factors and other RNAs —

receive information from, for example, signal-transduction networks, integrate and modulate it, and convey the processed information to networks of genes and proteins that execute developmental and physiological functions. In biological systems, these two types of networks are closely integrated. The organization of these networks can be inferred from various different types of measurements including, for example, global measurements of dynamically changing levels of mRNAs and proteins during developmental and physiological responses, as well as large-scale measurements of protein–protein and protein–DNA interactions. Third, there are many hierarchical levels of organization and information (for example, DNA, RNA and protein networks, cell signaling and metabolic networks, and organization and responses of organ systems). To understand biological systems, information must be gathered from as many information levels as possible and integrated into models that generate testable hypotheses about how biological systems function.

In this review article we delineate our view for investigating cancer stem cells using powerful integrated transcriptomic, proteomic and computational approaches. We will focus on gene expression profile signatures which will provide fundamental insights into the networks in the application of cancer diagnosis, patient stratification, and treatment management. We will also discuss emerging new experimental technologies and computational modeling approaches that empower systems strategies for tackling cancer stem cell challenges.

## **Molecular profiling for cancer classification**

Systems approaches have proven of great utility in the study of cancer, with increasing power expected to continue to emerge in the future. Despite notable and significant challenges that remain [7, 8], one area that has shown significant promise is in the mining of global gene expression data sets to identify molecular signatures that can be used for diagnosis and treatment selection [9].

These studies typically involve the collection of samples from two or more classes (e.g. cancer vs. normal, or responsive vs. non-responsive to treatment) and the use of a set of data on which to train the classifier and another set on which to test. In the absence of a true test set, resampling methods such as cross validation are generally used to estimate likely performance of the classifier on future data. Challenges often arise in these studies when different measurement platforms are used in training and test sets. The ability to generate an accurate classifier is a function of factors such as 1) the size of the training set relative to the number of features, 2) the computational method used, and 3) the inherent distinctness of the selected phenotypes. Typically, the number of samples is far less than the number of transcripts, leading to overfitting being a significant problem. This leads to the need for computational methods that aid in avoiding overfitting when selecting a classifier. A variety of methods have been applied to cancer diagnoses including approaches based on support vector machines [10, 11] and relative expression reversals [12-15], among many others.

Application of these methods has led to the discovery of molecular classifiers of varying degrees of accuracy to identify prognostic signatures for breast cancer [16-32], ovarian cancer [33-35], colon cancer [36, 37], prostate cancer [14, 38-43], and brain cancer [44, 45], among others. Given the success of such global approaches to identify signatures for cancer tumors, there is reason to suppose that such approaches will prove very useful in uncovering new biology, markers, and treatments in the emerging area of cancer stem cells. Indeed, progress in this direction is already being made.

## **Molecular profiling of cancer stem cells**

Following on the successes of molecular profiling in identifying prognostic signatures for many cancers, researchers have begun to perform profiling of cancer stem cells as well. We will discuss such efforts in the context of three cancers: leukemia, brain, and breast. In addition to profiling for signatures of specific cancer stem cells, interesting work has also been done to find general signatures for “stem-ness” in tumors. For example, an 11-gene signature for “stem-ness” in multiple cancer types has been identified [46] that predicts short interval to disease recurrence, distant metastasis and death from cancer. This signature reflects a BMI-1 oncogene-driven gene expression pathway, where the BMI-1 gene is essential for the self-renewal of hematopoietic and neural stem cells. Using Kaplan-Meier analysis, this signature for “stem-ness” was found to show predictive ability in 11 different cancers, including 5 epithelial cancers (prostate, breast, lung, ovairna, and bladder) and 6 nonepithelial (lymphoma, mesothelioma, medulloblastoma, glioma, and acute myeloid leukemia). Thus, there is evidence that the property of “stemness” (defined with this signature) is predictive of outcome in a wide variety of tumors. If validated, this observation could have a major impact on patient care [47]. Recent studies have also shown that cancer and normal stem cells share the same self-renewal mechanisms, such as the Bmi1 and Wnt canonical pathways [48, 49], further strengthening the link between stem cells and cancer stem cells.

### ***Historical perspective: Cancer stem cells in leukemia***

The fundamental concept of cancer stem cells came from early studies of blood cancer (leukemia) and the blood forming hematopoietic stem cells (HSC). Elegant work by Till and McCulloch in the early 1960's established the existence of bone marrow HSC capable of forming myelo-erythroid colonies in the spleen of lethally irradiated hosts [50]. These cells were later isolated by Weissman and colleagues and shown to be able to self-renewal and exhibit multipotent differentiation giving rise to all the blood lineages [51, 52]. Studies of human leukemia using *in vitro* and *in vivo* colony-formation assays demonstrated that only a small subset of leukemia cells possess extensive proliferative capability [53, 54] suggesting that leukemia may actually be derived from a small leukemic stem cell (LSC) population. This concept was further proved by the successful isolation of myeloid leukemia-initiating cells using cell surface phenotype CD34<sup>+</sup>CD38<sup>-</sup> and subsequent *in vivo* transplantation into severe combined immune-deficient (SCID)

mice [2]. One intriguing question that remained unanswered until very recently is whether the leukemic stem cells are derived from normal HSCs or from their downstream committed progenitor cells that regain the self-renewal property. Using mouse genetics and clinical studies, Weissman and colleagues demonstrated that both mechanisms exist in leukemia: while chronic myeloid leukemia can be derived from LSC residing at the HSC stage [55], the more differentiated progenitor—granulocyte-macrophage progenitor (GMP)—can also gain LSC property during the blast-crisis of myeloid leukemia [56]. One common characteristic between stem cells and cancer is the unlimited self-renewal capability. The hierarchical organization and the cellular heterogeneity in the hematopoietic systems, and lessons learned from leukemic stem cell have inspired later isolation of cancer stem cells from solid tumors [3], and will continue to provide theoretical framework for investigating tumorigenesis.

### ***Molecular profiling of brain cancer stem cells***

Brain cancer is a highly heterogeneous disease consisting of multiple tumor types affecting both children and adults [57]. The most malignant form of brain cancer, glioblastoma multiforme (GBM), is characterized by a diverse cellular phenotypic and genetic heterogeneity which has been a significant impediment to the development of effective targeted medical therapies. Traditional treatment for GBM consists of surgical resection to reduce the tumor mass followed by radiation and chemotherapy selective for highly-proliferating tumor cells. Despite continuous refinement over the past three decades, these treatments have not significantly impacted time to tumor recurrence or long-term survival in GBM patients. Given the low proliferate rate of normal brain tissue, it has long been hypothesized that brain cancer may arise from neural stem or progenitor cells which were thought to be more sensitive to oncogenic transformation [58]. Using neural stem cell culture techniques, two different types of malignant brain tumors were shown to possess subpopulations of tumor cells with characteristic stem cell-like properties such as self-renewal, potential for differentiation, and capacity to form neurospheres [59]. The cell (or cells) of origin for these putative brain cancer stem cells has not been determined definitively, but evidence on this subject is starting to accumulate. For example, a number of groups have confirmed that brain tumors contain a small fraction of cells that can be separated on the basis of the cell-surface marker CD133 and that have clonogenic potential as measured by the neurosphere assay. In a key *in vivo* experiment, a minority population of cells from brain tumors was shown to be tumorigenic by implantation into immuno-compromised mouse brain. In this model, injection of as few as 100 CD133<sup>+</sup> cells could generate tumors *in vivo*, whereas injecting of as many as 10<sup>5</sup> CD133<sup>-</sup> cells, which comprise the majority of the GBM tumor cells, did not lead to any viable tumor growth. Interestingly, CD133 had previously been found to be a marker of hematopoietic stem cells [60-62] and has now been implicated in the identification of a number of putative tumor stem cells from a variety of human cancers, including leukemia [63], prostate cancer [64], and colon cancer [4]. At this point, evidence supports that the subpopulation of tumorigenic brain cancer cells is a subset of CD133<sup>+</sup> cells as not all CD133<sup>+</sup> cells are tumorigenic [65]. Several labs are currently refining molecular markers to refine tumor stem cell identification and purification.

The paradigm that brain tumors possess a small fraction of cancer-initiating tumor stem cells which can reconstitute the tumor's cellular and functional hierarchy has important implications for treatment. To further explore the potential differences in subpopulations of GBM cells, Lee *et al.* used high-throughput genome-wide molecular profiling to characterize GBM-derived tumor stem cells cultured in serum-free conditions optimized to sustain neural stem cells [66]. Genome wide expression and genotyping studies revealed that these GBM-derived tumor stem cells had extensive similarities to normal neural stem cells and possessed the capacity to initiate brain tumors in a xenograft mouse model. Significantly, the genotype and phenotype of the mouse xenograft brain tumors resulting from implantation of tumor stem cells cultured in serum-free conditions matched that of the parent human GBM tumors from which they were derived. When GBM-derived tumor cell lines were generated using standard serum-based culture conditions not conducive to stem cell growth, the resulting cells lacked the ability to initiate or propagate tumors in the mouse xenograft model. Genome-wide transcription analysis and genotyping studies revealed significant differences between these serum-cultured cell lines and their corresponding parent GBM tumors and matched serum-free cultured tumor stem cell lines. Of note, the serum-cultured cell lines did manifest extensive similarities to the most commonly utilized immortalized cancer cell lines. These studies provided further evidence that GBM has a distinct subpopulation of tumor cells with stem cell-like properties, and that it is these cells that are biologically relevant in initiating and sustaining tumor growth. Unfortunately, most pre-clinical studies have historically been based on targeting therapies to features identified in serum-cultured immortalized cancer cell lines. This landmark large-scale molecular profiling study raises significant concerns about this approach and suggests the use of serum-free cultured tumor-stem cells as a more appropriate pre-clinical model [66].

There is great interest in identifying effective therapies that can target the tumor-initiating and tumor-maintaining subpopulation of tumor stem cells identified as CD 133<sup>+</sup>. Two recent studies used molecular profiling to characterize the differential response of CD133<sup>+</sup> cells to therapeutic intervention compared to CD 133<sup>-</sup> cells. After radiation treatment, GBM tumors exhibited an increase in the fraction of CD133<sup>+</sup> cells, more robust activation of DNA repair pathways in CD 133<sup>+</sup> than CD 133<sup>-</sup> cells, and more aggressive growth characteristics on serial transplantation [67]. Pre-treatment with an inhibitor of cell-cycle checkpoints CHK1/CHK2 sensitized CD 133<sup>+</sup> cells to radiation, suggesting a role for heightened DNA repair in the relative resistance of CD 133<sup>+</sup> tumor stem cells to radiation-induced DNA damage. CD133<sup>+</sup> cells also retain the ability to differentiate into more terminally-differentiated and less aggressive cells, a feature lacking in CD133<sup>-</sup> cells. Bone morphogenic protein (BMP), a potent differentiating molecule, led to significant differentiation of CD133<sup>+</sup> cells and decreased tumor growth in an *in vivo* mouse model [68]. This suggests that the use of differentiating agents may play an important role against tumor stem cells which, which unlike CD 133<sup>-</sup> cells, retain important differentiation capacity—presumably because different triggering leads to the loss of neoplastic potential. Another area of fertile discovery is the role of epigenetic regulation in the control of stem cell maintenance, self-renewal and differentiation. Transcriptional changes that result from the pharmacological reversal of epigenetic gene silencing, typically with either a histone deacetylase inhibitor or a demethylating agent,

allows for the identification of epigenetically-regulated genes on a genome-wide scale using molecular profiling techniques [69, 70]. Recent large scale genomic studies have confirmed the importance of epigenetic gene silencing in the biological behavior of GBM, including response to therapy [69, 71, 72]. Using a similar approach, several investigators have identified key genes which are epigenetically regulated during differentiation in neural stem cells and have been implicated in neoplasia [73-75]. Epigenetic modifications make attractive therapeutic targets as they are readily reversed and the underlying DNA sequence is not altered. Taken together, these studies highlight the importance of targeting therapies to the CD 133<sup>+</sup> subpopulation of cells which can exploit the tumor stem cell properties and vulnerabilities important in the effective therapeutic treatment of GBM.

The fraction of cells in a GBM tumor that express CD133 has been observed to be as little as 1% in low-grade tumors and as high as 30% for high-grade glioblastoma. However, in any particular grade, such as glioblastoma, there is high variability in this fraction [65]. Interestingly, time to progression and overall survival are not greatly affected by extent of resection in GBM patients. Despite the removal of the bulk tumor, consisting mostly of CD133<sup>-</sup> cells, the tumor recurs within one year on average. Thus, it is not yet clear whether the fraction of CD133<sup>+</sup> cells in the bulk tumor will prove to be useful as a prognostic marker. It could be that the important neoplastic-associated stem cells constitute only a small fraction of the total CD133<sup>+</sup> cells. Assuming that CD133<sup>+</sup> cells (or a subfraction) are the biologically active tumor stem cells responsible for tumor recurrence, identifying and targeting the location of these cells *in vivo*, within the tumor or in the surrounding brain after tumor resection, will be of great importance. A recent study indicates that tumor stem cells exist in the "perivascular niche", and that endothelial cells potentiate the tumor stem cells capacity to initiate and maintain tumor growth [76]. Application of inhibitors of endothelial cells significantly depleted tumor blood vessels resulting in a dramatic reduction of tumor stem cells but not CD 133<sup>-</sup> cells in the bulk tumor. This suggests that targeting the unique vulnerabilities of tumor stem cell populations should include further characterization of the micro-environment that support and sustains tumor stem cell self-renewal and differentiation. Large-scale molecular profiling of purified subpopulations of tumor cells from GBM will play a critical role in further defining these molecular targets [77].

### ***Molecular profiling of breast cancer stem cells***

Cancer stem cells have also been identified and characterized for breast tumors, a much more common malignancy than brain tumors. Breast cancer tumors contain a subpopulation of highly tumorigenic cells characterized by CD44 expression and no or low CD24 expression (CD44<sup>+</sup>CD24<sup>-/low</sup>), as demonstrated by their distinguishing capability to generate tumors in immunodeficient mice [3]. These CD44<sup>+</sup>CD24<sup>-/low</sup> cells have also been demonstrated to have the capacity to give rise to the various cell types characteristic of the bulk tumor. As with CD133<sup>+</sup> cells in brain tumors, the CD44<sup>+</sup>CD24<sup>-/low</sup> cells in breast tumors contain the population of cancer stem cells, but may not uniquely identify them. Indeed, additional cell-surface markers may further distinguish the tumorigenic breast cell population. For example, it has been shown that among

CD44<sup>+</sup>CD24<sup>-/low</sup> cells, those that were also ESA<sup>+</sup> had enriched tumor generating potential compared with those that were ESA<sup>-</sup>. Thus, one hypothesis would be that ESA<sup>+</sup>CD44<sup>+</sup>CD24<sup>-/low</sup> cells may represent a purer population of a subset of the cancer stem cells [3]. This interesting question for all different tumor stem cells is the extent to which neoplastic stem cell population may be purified by additional cell-surface markers. Also of interest is that CD44<sup>+</sup>CD24<sup>-/low</sup> cells generate additional CD44<sup>+</sup>CD24<sup>-/low</sup> cells as well as phenotypically distinct cells, providing evidence that breast cancer stem cells have the hallmark stem cell characteristics of self-renewal and the capacity to generate heterogeneous populations.

It has been shown that these tumorigenic breast cancer cells can propagate in culture and maintain properties of normal human mammary gland stem/progenitor cells that have the distinct ability to grow in selective culture conditions as non-adherent spherical clusters of cells [78, 79]. Most importantly, after *in vitro* culturing these cells were found to remain CD44<sup>+</sup>CD24<sup>-/low</sup> and maintain their ability to generate tumors in immunodeficient mice with as few as 1000 injected cells [78, 79]. Thus, there is strong evidence that breast cancer stem cells have indeed been isolated and can even be propagated in culture while maintaining their cancer stem cell characteristics.

Machine-learning from global gene expression data has been used to identify a gene signature from tumorigenic breast-cancer cells that is associated with both overall and metastasis-free survival in patients with breast cancer [24]. This signature was found through comparing the gene-expression profiles of the CD44<sup>+</sup>CD24<sup>-/low</sup> breast cancer stem cells, which demonstrate enriched invasiveness [80], with normal breast epithelium. The differentially expressed genes between these two groups were then used to generate a 186-gene signature for “invasiveness” that showed a significant association with overall and metastasis-free survival of patients with breast cancer [24]. This invasiveness signature was then also shown to be associated with prognosis in other cancers, including medulloblastoma, lung cancer, and prostate cancer, demonstrating the general features at the gene expression level of invasiveness. It will be interesting to convert these tumorigenic gene signatures into their corresponding biological networks so as to begin to understand the unique and shared features of cancer stem cells.

## **Emerging technologies for proteome characterization**

While transcriptomic approaches have proven useful for identifying informative molecular signatures for cancers and cancer stem cells, proteomic characterizations have lagged behind. The reason for this is clearly the more difficult challenge of measuring proteins compared to transcripts. Emerging proteomics technologies hold the promise of greatly improving our ability to make detailed assessments of protein-based molecular signatures, similar to those that have had success thus far for gene expression. One key advantage of protein signatures relative to gene expression is that proteins can be found in the blood and other accessible bodily fluids more readily due to slower degradation rates than their mRNA counterparts. Thus, protein signatures represent an important class of molecular signature for disease diagnosis. For cancer stem cells, these approaches will help to elucidate the differences between the proteome of cancer stem

cells relative to their non-tumorigenic counterparts. Ultimately, the reconstruction of protein and gene regulatory networks of cancer stem cells holds the promise of rationally-designed therapeutics for personalized medicine by allowing the therapeutic chosen to be governed by informative molecular signatures measured from each patient at the time of diagnosis. A few promising proteomics technologies are described below (figure 1.).

### ***Isobaric tagging for relative and absolute quantitation (iTRAQ)***

Stable isotope labeling enables the quantitative analysis of protein concentrations through mass spectrometry (MS). One state-of-the-art technique for quantitative MS is iTRAQ [81], which uses stable isotope labeling of proteolytic peptides. This technique modifies primary amino acid groups of peptides by linking a mass balance group (carbonyl group), and a reporter group (based on N-methylpiperazine) by forming an amide bond. The iTRAQ reagents are designed to be isobaric, which enables differentially labeled peptides to appear as single peaks in MS, which is important for reducing peak overlapping in the MS scans. When MS/MS is used for analysis with iTRAQ-tagged peptides, the mass balancing carbonyl moiety is released as a neutral fragment, thereby liberating isotope-encoded reporter ions that provide relative quantitative information on protein abundance. Because four different iTRAQ reagents are currently available, comparative analysis of a set of two to four samples is feasible within a single MS run [82]. The iTRAQ technology represents the state-of-the-art in quantitative proteomics and represents a promising technology for using proteomics to differentiate key differences in protein networks of cancer stem cells from normal stem cells or other cells in the tumor.

### ***Glyco-peptide capture***

Mass spectrometry-based methods will allow for the identification of proteins spanning approximately three orders of magnitude in concentration from a given sample. Therefore, methods that can select specified fractions of the proteome are important for simplifying the sample sufficiently to identify the proteins of interest. One recently developed approach is the shotgun glycopeptides capture approach [83]. This approach selects for N-linked or O-linked glycosylated peptides, which are enriched for secreted proteins and cell-surface markers. Thus, this approach could be used to identify candidates for unique cell-surface markers for cancer stem cells that differ from the bulk tumor by comparing the glyco-captured proteomes from both sample sets.

### ***Antibody microarrays using surface plasmon resonance imaging (SPRI)***

Protein chip methods hold potential for broad quantitative screens of proteins, and a variety of techniques have been developed based on antibody binding [84-86]. Antibody microarrays have been used for biomarker discovery and protein profiling of serum from patients with prostate, lung, pancreas and bladder cancer [87-90]. One emerging approach with tremendous promise is SPRI [82, 91, 92], which enables real-time, label-

free measurement of protein expression. SPR-based chips have a detection sensitivity of 10-100 times less than ELISA [82], but have a spatial resolution down to approximately 4  $\mu\text{m}$  [93]. It is thus possible to print up to 800 unique antibodies on Lumera Nanocapture Gold<sup>TM</sup> microarray slides and monitor the abundance of the target proteins in real time [82]. Because the same slide can be regenerated for reuse many times (Z. Hu, C. Lausted, unpublished observations), this means that the approach has the capacity necessary to screen through hundreds of patient samples. Thus, this approach holds tremendous promise in the case of cancer stem cells to be able to screen through large numbers of proteins, including secreted proteins and cell surface markers and not only measure their presence, but also abundance and the dynamics of their binding. The limitation of this technique is its dependence on the affinity and specificity of the antibodies it employs for detection—cross reactivities in complex protein mixtures (like blood) can pose significant problems.

### ***DNA-encoded antibody libraries (DEAL)***

One recently developed technique that offers great potential for analyses of cancer stem cells is DEAL, which enables cell localization and single-cell measurements of protein, RNA, and single-stranded DNA simultaneously on a single chip [94]. DEAL is a highly sensitive measurement technique, with a reported detection limit of 10 fM for the protein IL-2 – 150 times more sensitive than ELISA. This sensitivity can be applied to the isolation of rare cells based on combinations of cell-surface markers, enabling the isolation and addressing of individual cancer stem cells. DEAL can also be used to make single cell measurements of secreted proteins from each of these isolated single cells. Thus, DEAL offers superb sensitivity and the ability to perform spatially multiplexed detection for characterization of cancer stem cells.

### **Biomolecular networks in cancer**

Systems approaches to cancer [95-99] require not only the identification of the key components of a system through global analyses, but also information about how these components interact in biological networks. Network models of multiple types have been applied to cancer systems. The most commonly applied to cancer are interaction networks, including protein-protein interaction networks, protein-DNA interaction networks and so forth. Gene expression data can be used to identify differentially expressed genes in which can then be visualized on interaction networks, as has been done for lung cancer [100]. Various properties of these networks have been studied [99], with reported findings including, for example, the enrichment of cancer-related genes among the “hubs” of the networks. While these interaction networks are very useful tools for visualizing large data sets, they are not computable, predictive network models, which are those that hold the most promise for predictive medicine and drug development. Predictive models stemming from mathematical descriptions of biochemical reaction networks and statistical influence models should prove highly useful [101].

Another area of network modeling that should prove very beneficial in research of cancer and cancer stem cells is that of metabolic networks. Key metabolic differences have been shown to exist in cancers which can be exploited using Positron Emission Tomography (PET) to do *in vivo* imaging of tumors [102] and even to predict treatment response [103, 104]. If key metabolic differences can be found between cancer stem cells and the rest of the tumor, such approaches could potentially even be used to identify the location of cancer stem cell populations *in vivo*. One enabling resource for large-scale quantitative modeling of metabolic networks in cancer is the recent stoichiometric reconstruction of known human metabolism at the genome-scale [105]. With this global reconstruction, gene expression and other data can be used to create initial models of the genome-scale metabolic networks of a variety of human cell types, including for cancer stem cells. These biochemical reaction networks can be used to make numerous quantitative simulations that have been shown previously to match well with experimental data in model organisms [106]. These successes with model organisms have also been extended to models of simple human systems such as the erythrocyte [107, 108] and mitochondria [109], with the global metabolic reconstruction poised to allow for larger human metabolic networks to now be modeled. These studies may well provide insights into the unique metabolic features of cancer cells—allowing one to identify both metabolic features that are shared among cancer cells and features that are unique to individual types of cancer.

More detailed dynamic models of specific biochemical networks in cancer have been made for important signaling networks in cancer, leading to insightful biological observations for, among many others, the NF- $\kappa$ B signaling network [110-112]. As isolated cancer stem cell populations become better characterized, it will be possible to model these systems to identify differences in their regulation in cancer stem cells and further identify possible therapeutic targets. Dynamic simulations of large-scale signaling networks in cancer cells has also been performed [113].

Large amounts of high-throughput data (i.e. transcriptomes) can also be used to infer networks that can explain statistical dependencies seen in the data, indicate candidate novel interacting partners, and quantitatively predict the gene expression resulting from knockouts or environmental perturbation. For model systems, such approaches are now being successfully applied at the genome-scale for gene-regulatory networks [114, 115]. Such approaches are now also being applied to mammalian systems as was done for normal and cancerous B-cells with the development of an algorithm called Reconstruction of Accurate Cellular Networks (ARACNe) [116]. As cancer stem cell populations are profiled extensively, these same approaches will be useful to identify predictive networks for cancer stem cells. Comparing these networks to those in normal stem cells and other tumor cells should prove highly informative for identifying drug targets unique to the cancer stem cell population of interest. By generating networks of cancer stem cells in particular and comparing them with networks of normal stem/progenitor cells we should be able to greatly enhance our understanding of what could lead to these cells becoming cancerous.

Computational modeling and systems approaches will be key to catalyzing the future of drug discovery [117, 118], and drug discovery focused specifically on cancer stem cells offers tremendous promise for advancing cancer therapies. Thus, computational modeling of cancer stem cell networks to identify potential therapeutic targets and to predict the effect of drug-induced perturbations is critical to this field moving forward.

## **Perspective and concluding remarks**

The identification and prospective isolation of cancer stem cells from leukemia and a number of solid tumors has spawned a new paradigm in cancer research. From the perspective of systems biology—with the goal of predictive, preventive, personalized, and participatory (P4) medicine—we envision increasingly global assessment of cancer stem cells and their microenvironments (niche) at the level of complete transcriptome, proteome, and epigenome, using empowering new high throughput technologies. The resulting gene expression profile signatures of cancer stem cell would serve as more accurate indicatives for cancer diagnosis and prognosis. Emerging proteomic technologies employing mass spectrometry and protein chip platforms would allow for identification of better cell-surface markers and their interaction with the resident stem cell niche and potential diagnostic markers from both body fluids and tumor tissues. Incorporating these data into biological networks will provide fundamental insights into the biology of cancer stem cells and their abilities for renewal and differentiation. These combined efforts will ultimately lead to new therapeutic strategy specifically targeting cancer stem cells for unprecedented personalized cancer therapy.

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## **Figure legends**

Figure 1. Systems biology strategies for studying cancer stem cells.

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