

Cancer Stem Cells: Targeting the Roots of Cancer, Seeds of Metastasis, and Sources of Therapy Resistance

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Abstract

With the goal to remove the roots of cancer, eliminate metastatic seeds, and overcome therapy resistance, the 2014 inaugural International Cancer Stem Cell (CSC) Conference at Cleveland, OH, convened together over 320 investigators, including 55 invited world-class speakers, 25 short oral presenters, and 100 poster presenters, to gain an in-depth understanding of CSCs and explore therapeutic opportunities targeting CSCs. The meeting enabled intriguing discussions on

several topics including: genetics and epigenetics; cancer origin and evolution; microenvironment and exosomes; metabolism and inflammation; metastasis and therapy resistance; single cell and heterogeneity; plasticity and reprogramming; as well as other new concepts. Reports of clinical trials targeting CSCs emphasized the urgent need for strategically designing combinational CSC-targeting therapies against cancer. *Cancer Res*; 75(6); 924–9. ©2015 AACR.

Cancer Stem Cell Overview

As early as 1937, Furth and Kahn successfully transplanted leukemia with a single mouse leukemic cell (1), showing the first evidence of stem cell-like cancer cells, now termed cancer stem cells (CSC) or tumor-initiating cells (TIC). Dr. John Dick's group transplanted and identified human leukemic stem cells (LSC) in the 1990s (2, 3). The continued cornerstones of identifying CSCs in human solid tumors, breast (4) and brain (5), led to the emerging field of cancer stem cell research with new prospects to understand and the hope of eliminating cancer (6, 7).

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The 2014 inaugural International Cancer Stem Cell (CSC) Conference was held from August 17 to 20, 2014, in Cleveland, OH. A complete list of speakers and their presentation titles is provided in Supplementary Table S1.

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At the opening session, Dr. Jeremy Rich (Cleveland Clinic, Cleveland, OH) introduced the concept of tumor heterogeneity and presented the evolution of the CSC model as being driven by key regulatory factors such as genetic diversity, epigenetics, and pathways, and tumor microenvironment (8). He explained the required functional characteristics of CSCs—self-renewal, proliferation, and tumor initiation/propagation, as well as the common, but not defining, characteristics of CSCs such as rarity, stem cell markers, and differentiation. In this meeting, scientists explored CSCs in many tumor types including brain tumors, epithelial cancers, and leukemia.

As a keynote speaker, Dr. Irving Weissman (Stanford University, Stanford, CA) emphasized that the exclusive characteristic of stem cells and CSCs is self-renewal (9). His group reported preleukemic mutations in the otherwise normal hematopoietic stem cells (10), and identified CD47 as an important CSC marker of immune evasion from macrophage-mediated phagocytosis (11) as well as a therapeutic target in human primary acute myeloid leukemia (AML) and breast cancer cell xenografts. Dr. Michael Clarke (Stanford University, Stanford, CA), also a keynote speaker, presented his work on the genetic regulations of stem cells and cancer stem cells. He showed that regulation can be determined by two properties, sufficient self-renewal promoters such as Bmi1, and lack of drivers of differentiation, apoptosis, and senescence. He demonstrated that USP16 inhibits self-renewal with Cdkn2a activation, thereby causing a stem cell defect in neural stem cell (NSC) as well as mammary epithelial stem cells in Down syndrome (12).

Genetics, Epigenetics, and RNA Regulators of CSCs

Genetics and epigenetics are two major regulatory mechanisms underlying the diversity and heterogeneity of CSCs. Lineage tracing has been commonly used in stem cell and CSC studies

to explore the cell of origins. Dr. Luis Parada (University of Texas Southwestern Medical Center, Dallas, TX) reported on his work that focuses on the early genetic events and cell of origin of mouse gliomas, and demonstrated that a subset of endogenous quiescent glioma stem cells (GSC) was able to propagate the tumor after chemotherapy by lineage tracing (13). Dr. Michael M. Shen (Columbia University Medical Center, New York, NY) showed that luminal cells are favored as the cell of origin for mouse prostate cancer by lineage tracing (14). Dr. Helen Salz (Case Western Reserve University, Cleveland, OH) presented her work describing the tumor-suppressing role of the female-specific RNA-binding protein SXL and the oncogenic role of testis-specific chromatin reader Phf7 in *Drosophila* ovary stem cells (15). Dr. Zhenghe John Wang (Case Western Reserve University, Cleveland, OH) reported that the loss of the tumor suppressor tyrosine phosphatase PTPRT in intestinal stem cells makes these cells more proliferative through activating STAT3 (pY705; ref. 16). In glioblastoma multiforme (GBM), Dr. Antonio Iavarone (Columbia University, New York, NY) identified novel oncogenic tyrosine kinase fusion proteins (FGFR-TACC3) as genetic drivers and targets of CSCs (17), in addition to the epigenetic regulation by transcriptional factors such as STAT3 and C/EBP β . In short talks, Dr. Jennifer Yu (Cleveland Clinic, Cleveland, OH) showed the semaphorin family member SEMA3C as a GSC-specific target, and Dr. Craig Horbinski (University of Kentucky, Lexington, KY) reported on the role of proapoptotic prostate apoptosis response 4 (PAR4) in GSCs (18).

Dr. Bradley Bernstein (Massachusetts General Hospital, Boston, MA) discovered a core set of neurodevelopmental transcription factors (POU3F2, SOX2, SALL2, and OLIG2) essential for CSC reprogramming and GBM propagation (19), and presented the intratumoral heterogeneity of primary GBM by single cell RNA-seq with one tumor showing an immune cell expression signature (20). Dr. Catriona Jamieson (University of California, Moores Cancer Center, San Diego) presented that in replating and in humanized mouse model assays, RNA recoding by adenosine deaminases acting on dsRNA (ADAR1) is essential for blast crisis chronic myeloid leukemia stem cells to be maintained (21).

Dr. Michael Taylor (University of Toronto, Toronto, ON) discussed the CpG island methylator phenotype (CIMP) in the posterior fossa (PF) ependymoma, the only recurrent cancer with no known recurrent mutations but with silenced genes controlled by the polycomb-related complex 2 (22). Dr. Jeongwu Lee (Cleveland Clinic, Cleveland, OH) reported that noncanonical signaling by EZH2 in regulating methylation of STAT3 was required for glioblastoma stem cell maintenance (23). Dr. Elizaveta Benevolenskaya (University of Illinois at Chicago, Chicago, IL) demonstrated that the targets of H3K4 demethylase KDM5A were significantly elevated and the targets of H3K27 methyltransferase EZH2 were significantly decreased in the basal subtype of breast cancer. These results suggest that targeting the aberrant epigenetic landscape of aggressive tumors may be an effective treatment strategy.

Microenvironment, Metabolism, Inflammation, and New Concepts

Dr. Jeremy Rich suggested that CSCs are products of their environments in which conventional therapies (chemotherapy and radiotherapy) stimulate cancer-associated fibroblasts (24) to

secrete cytokines (TGF β , IL6, IL17A) and promote stemness of brain CSCs. Dr. Arnold Caplan (Case Western Reserve University, Cleveland, OH) presented his work demonstrating that mesenchymal stem cells (MSC) originate *in situ* as perivascular cells called pericytes (25). The pericyte serves as gate-keepers for metastasizing tumor cells like those from melanoma. The CD146 antigen on both the pericyte and the melanoma plays a crucial role in bringing the MSCs into the developing tumor to assist in tumor expansion (26). Dr. Justin Lathia (Cleveland Clinic, Cleveland, OH) discussed that the interaction of CSCs with immune cells results in immune suppression and tumor progression in GBM.

Dr. Jan Lötval (University of Gothenburg, Gothenburg, Sweden) reported on an emerging mechanism of microenvironmental cross-talk conducted via exosomes (40–100 nm in diameter), which are secreted by various cell types and play roles in epithelial-to-mesenchymal transition (EMT), tumor hypoxia, cancer invasiveness, metastasis, angiogenesis, and tolerance to chemotherapy (27).

Metabolites and inflammatory cells/cytokines are part of the environmental regulation of CSCs. Featured speaker Dr. Celeste Simon (University of Pennsylvania, Philadelphia, PA) reported that depletion of fructose-1,6-bisphosphatase promotes clear cell renal cell carcinoma growth, regulates carbon metabolism, and inhibits hypoxia-inducible factors (HIF1 α , HIF2 α ; ref. 28), in accordance with miR-218 to inhibit HIF2 α in GBM (29). Dr. Rolf Bjerkvig (University of Bergen, Bergen, Norway) presented an innovative GBM model system of biopsy spheroids, which can be implanted in a nude rat brain, and reported on the cellular plasticity of the CD133^{+/-} cells as well as the metabolomics adaptability of the GBM in response to anti-VEGF treatment (30, 31).

Dr. Ofer Reizes (Cleveland Clinic, Cleveland, OH) demonstrated that leptin and leptin receptor, a JAK/STAT cytokine receptor expressed in breast CSCs (BCSC), is necessary for maintaining the NANOG⁺ BCSC phenotype (32). Dr. Emina Huang (Cleveland Clinic, Cleveland, OH) showed that elevated IL8 levels secreted from stromal fibroblasts cause a substantial increase in tumorigenicity induced by aldehyde dehydrogenase (ALDH)-1⁺ colon cancer-initiating cells within colitis-associated cancer (33).

Dr. Yue Xiong (University of North Carolina, Chapel Hill, NC) reported two regulatory mechanisms of the Tet DNA dioxygenases, by metabolites and by ubiquitylation. These mechanisms eventually influence DNA methylation and epigenetic modifications of embryonic stem cells and CSCs (34). Dr. Alex Huang (Case Western Reserve University, Cleveland, OH) established intravital 2-photon microscopy to directly observe how immune cells traffic and interact with various cells in the tumor microenvironment in real time with single-cell resolution (35). In a short talk, David Schonberg's (Cleveland Clinic, Cleveland, OH) work suggested that glioblastoma stem cells require increased ferritin, and that targeting ferritin inhibits GSC tumorigenicity *in vitro* and *in vivo*. Dr. Masahiro Hitomi (Cleveland Clinic, Cleveland, OH) described a critical role for cell–cell communication in GBM CSCs and suggested a novel therapeutic option for treating GBM. Kelli Pointer (University of Wisconsin, Madison, WI) briefly discussed the role of collagen in GBM tumor invasion and patient survival.

Epithelial Tumor Stem Cells and Metastasis

Epithelial tumors are the most frequent tumor types (breast and prostate) as well as the leading cause of cancer deaths

(lung, colon, and breast), of which metastasis accounts for 90%. Dr. Carla Kim (Boston Children's Hospital and Harvard Medical School, Boston, MA) identified Sca-1⁺ lung tumor-propagating cells in the murine genetic models. Her work demonstrated that BDNF-TrkB promotes lung adenocarcinoma metastasis and that the BMP4 signaling drives differentiation (36, 37). Dr. Catherine O'Brien (University of Toronto, Toronto, ON, Canada) reported that human colorectal cancer-initiating cell (CIC) functions are dependent on the canonical self-renewal regulator, BMI-1, by regulating β -catenin and TOP/LEF activity (38).

Dr. Jeffrey Rosen (Baylor College of Medicine, Houston, TX) described that Wnt signaling and Ror2 depletion regulated both normal mammary stem cells and BCSCs, and that there was a cooperative interplay between CSCs and non-CSCs in mouse breast tumor models. Dr. Ruth Keri (Case Western Reserve University, Cleveland, OH) discussed the distinct transcriptional controls of breast cancer epithelial cell states by FOXA1 and FOXC1 as well as their signaling pathways related to BET and MMP7 (39, 40).

Dr. John Condeelis (Albert Einstein College of Medicine, Bronx, NY) introduced multiphoton imaging technology to examine invasion and intravasation of tumor cells, demonstrating that macrophages enhance breast tumor cell migration and intravasation upon physical contact (41, 42). Dr. Huiping Liu (Case Western Reserve University, Cleveland, OH) reported a surprising differentiation and nonmalignancy phenotype of lung metastasis compared with primary tumors with upregulated miR-30c and miR-138 that target TWF1/VIM/IL-11 and EZH2 (43, 44). Dr. Ruggero De Maria (Regina Elena National Cancer Institute, Rome, Italy) reported on the CSC targeting strategies he has developed, including locked nucleic acid to inhibit oncomiRs (miR-197; ref. 45), new therapeutic antibodies against GBM CSCs, and strategies to prevent colon cancer metastasis by targeting CD44v6⁺ cells (46). Dr. Yibin Kang (Princeton University, Princeton, NJ) characterized the interactions between the metadherin and Staphylococcal nuclease domain-containing 1 (SND1) in tumor initiation and metastasis of breast and prostate cancers (47).

In short talks, Dr. Xiling Shen (Cornell University, Ithaca, NY) demonstrated that miR-34a regulates Notch1, symmetric divisions, and differentiation of colon colorectal CSCs (48). Dr. Min Yu (University of Southern California, Los Angeles, CA) reported on the genomic mutations in ESR1 and other proliferative signaling genes of captured circulating tumor cells from patients with breast cancer. Dr. Yu showed novel drug sensitivities in these mutant cell lines (49). Dr. Sunitha Nagrath (University of Michigan, Ann Arbor, MI) presented the results of her analysis on circulating tumor cells and CSCs using microfluidic chips. Benjamin Bryson (Case Western Reserve University, Cleveland, OH) showed that Oncostatin M (OSM) drives EMT and breast CSC phenotypes in a MYC-dependent manner. Mary Doherty (Case Western Reserve University, Cleveland, OH) discussed a potential inhibitory role of IFN β in breast CSC transition and Ilana Chefetz Menaker (University of Michigan, Ann Arbor, MI) reported ALDH inhibition-induced death of ovarian CSCs. Dr. Diana Azzam (University of Miami, Coral Gables, FL) identified distinct subsets of CSCs within triple-negative breast cancers with varying metastatic potential and differential response to epigenetic targeting agents.

Evolution, Heterogeneity, and Therapeutic Resistance

Dr. Stanton Gerson (Case Western Reserve University, Cleveland, OH) proposed a preleukemic stem cell (pre-LSC) model in MSH2^{-/-} mice in which MSH2^{-/-} pre-LSCs are able to initiate normal hematopoiesis and develop transplantable lymphomagenesis within 5 to 7 months. Using high-content single cell analysis with single cell genomics, Dr. Peter Kuhn (University of Southern California, Los Angeles, CA) presented the mathematical modeling for a spatiotemporal understanding of cancer progression, as well as an experimental mapping of disease response in patients (50). Dr. Dean Tang (University of Texas MD Anderson Cancer Center, Houston, TX) demonstrated that a population of phenotypically undifferentiated prostate cancer cells (low or negative for prostate-specific antigen and androgen receptor) is highly resistant to castration and other clinical therapies, and may also represent a cell-of-origin for castration-resistant prostate cancer (51).

Using the lineage mapping of NSC clones in glioblastoma-prone mouse brains in their premalignant state, Dr. Evan Snyder's (Sanford-Burnham Medical Research Institute, and University of California-San Diego, La Jolla, CA) work suggested that many different cells, under the right conditions, can give rise to a neoplasm, and that the "fate choice" of a given member of a stem cell clone to become a normal brain constituent or a GBM cell may pivot on a developmentally inappropriate neurogenic-to-gliogenic switch compounded by cell nonautonomous factors (and not solely on genetic predisposing factors). Subsequently, Dr. Claudia Petritsch (University of California, San Francisco, CA) described the presence of asymmetry-defective cells with oligodendrocyte progenitor cell characteristics in malignant astrocytomas modeled by BRaf^{V600E} expression and Cdkn2a deletion (52). Dr. Tannishtha Reya (University of California, San Diego, CA) suggested that regulators of asymmetric division can be effective targets for tumor resolution (53). Using fluorescence-guided multiple sampling, Dr. Colin Watts (University of Cambridge, Cambridge, England) demonstrated the clonal evolutionary dynamics of glioblastomas in patients having multiple GBM subtypes (54, 55).

Dr. Ichiro Nakano (Ohio State University, Columbus, OH) found that radiosensitive proneural glioma with differentiation capacity is converted by radiation to mesenchymal glioma, which is resistant to radiation with elevated ALDH (ALDH-1A3 in particular) activity in GSCs and a worse prognosis (56). Dr. Jacob Scott (H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL) presented a mathematical model of rapid selective evolutionary dynamics in hypoxic GSC niches (57). With identification of four factors (CD15, Notch, CD90, and EGFR) that can distinguish NSCs from neural progenitor cells (NPC), Dr. Siddhartha Mitra (Stanford University, Stanford, CA) demonstrated that NPCs were more likely to form gliomas than NSCs *in vivo*. And, in the final talk of this session, Dr. Yu Shi (Southwest Hospital of Third Military Medical University, Chongqing, China) described the tumor-suppressing role of miR-663 in glioblastoma, which is down-regulated along with increased glioma grade (58).

Dual Role Enigmas of CSC Regulators in Tumor Progression

Dr. Bingcheng Wang (Case Western Reserve University, Cleveland, OH) reported the dual roles of EphA2 as both a

ligand-dependent tyrosine kinase tumor suppressor protein and a ligand-independent promoter of GSCs in response to AKT activation (59). Dr. William Schiemann (Case Western Reserve University, Cleveland, OH) introduced an EGFR-assisted TGF β -driven EMT model (NMuMG cells) and suggested that both EMT and MET phenotypes contribute to metastasis. Dr. Jianjun Chen (University of Chicago, Chicago, IL) reported a dual role of miR-126 in the development and progression of AML (60) to either promote or inhibit AML1-ETO/*t(8;21)*-mediated leukemogenesis with different gene signatures.

In a short talk, Dr. Géraldine J. Guasch (Cincinnati Children's Hospital Medical Center, Cincinnati, OH) suggested that transition zones are associated with tumor formation in humans upon loss of TGF β signaling. Jacob Smigiel (Case Western Reserve University, Cleveland, OH) reported a role of GREM-1 as inhibiting EMT and the cytokine Oncostatin M-induced breast CSC transition. However, GREM-1 may show different effects in brain tumor cells as discussed by Drs. Lathia and Rich.

Clinical Trials of CSC Targeting Therapeutics

In addition to Dr. Irving Weissman's ongoing anti-CD47 trials, this meeting witnessed the growth of emerging clinical trials that target CSCs. Dr. David Wald (Case Western Reserve University, Cleveland, OH) induced differentiation of AML stem cells by using the GSK3 inhibitor lithium in synergy with all-trans-retinoic acid (ATRA) now in a phase I clinical trial (61). Dr. Yogen Sauntharajah (Cleveland Clinic, Cleveland, OH) reported on his exploration of a novel epigenetic regulation approach using a DNMT1 inhibitor decitabine in a nontoxic dosing scheme to induce differentiation with cytogenetic remissions in AML patients, reporting 84% responded with stable disease (62), without affecting normal hematopoietic stem cells (63). Dr. Kenneth Nephew (Indiana University, Bloomington, IN) also investigated the effects of a new DNMT inhibitor, SGI-110, in eliminating ovarian cancer stem cells that are enriched in residual, platinum-resistant tumors (64).

Dr. Austin Gurney (OncoMed Pharmaceuticals, Redwood City, CA) targeted the stem cell pathways of Notch, Wnt, and RSPO in cancer treatment using Demcizumab (anti-Notch ligand DLL4 antibody), OMP-52M51 (anti-Notch1), and OMP-18R5 (anti-Wnt receptor FZD monoclonal antibody) in combination with chemotherapeutic agents on cancers of pancreas, lung, breast, and colon (65). Dr. Sanford Markowitz (Case Western Reserve University, Cleveland, OH) identified the TGF β -regulated metabolic tumor suppressor 15-prostaglandin dehydrogenase (15-PGDH) pathway in colon tumorigenesis and discussed its clinical translation. Dr. Lyndsay Harris (Case Western Reserve University, Cleveland, OH) and her team discovered a basal-like group of HER2 tumors with a stem cell-like, EMT phenotype that are more

resistant to Herceptin. Her laboratory also showed that stem cells in HER2 tumors are associated with resistance to Herceptin.

There were a few clinical trials suggesting that combination therapies might be necessary to target both CSCs and non-CSCs. Dr. Andrew Sloan (University Hospitals Case Medical Center & Case Western Reserve University, Cleveland, OH) presented data from his randomized controlled phase II trial that vismodegib alone had biologic activity targeting the sonic hedgehog-signaling pathway, but was not sufficient as a single agent to improve survival in patients with recurrent GBM. On the basis of a phase II clinical trial, Dr. Manmeet Ahluwalia (Cleveland Clinic, Cleveland, OH) concluded that a potent selective inhibitor of γ secretase, R04929097, by itself is inefficient in inducing progression-free survival in GBM. Dr. Ira Steinberg (Boston Biomedical, Cambridge, MA) summarized the promising antitumor effects of the cancer stemness inhibitors, including BBI608 (inhibitor of Stat-3 and β -catenin pathways) in synergy with paclitaxel and BBI503 (inhibitor of stemness kinases).

Conclusions

The research presented in this conference demonstrated an increasing contribution from the expanding CSC field to a better understanding of cancer biology, and showed a growing demanding of bench-to-bedside translations. In addition to scientific sessions, a grant and career development workshop with six panelists (Dr. Huiping Liu, Dr. Suresh Mohla of NIH/NCI, Dr. Angela Ng of NIH/CSR, Dr. Ming Lei of NIH/NCI, Dr. Justin Lathia, and Dr. Jill Barnholtz-Sloan of Case Western Reserve University) was offered to inspire young generations. Dr. Stanton Gerson suggested creating an International Society of Cancer Stem Cells by the second CSC meeting in 2016.

Disclosure of Potential Conflicts of Interest

A.I. Caplan has provided expert testimony for CWRU royalties shared from Osiris Therapeutics. J. Rich is a consultant/advisory board member for Curtana Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

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