Meeting Report

Cancer Research

The First AACR Special Conference on Stem Cells, Development, and Cancer: Some of These Cells Are Not Like the Others

David G. Kent[1], Jennifer C. Lin[2], and Geraldine Aubert[3]

Abstract

The American Association for Cancer Research (AACR) held an exciting conference on Stem Cells, Development, and Cancer in Vancouver, British Columbia, Canada (March 3–6, 2011). The meeting was cochaired by Geoffrey Wahl, Connie Eaves, and Hans Clevers and was attended by 250 international researchers, 40% of whom were young investigators. Three key themes emerged: (i) heterogeneity in stem cells and cancer, (ii) solid tissue cancer stem cells, and (iii) lessons from development. The interdisciplinary foundation of this meeting was central to its success and appeal, underscoring the value of juxtaposing and interrelating work from the three topics addressed. *Cancer Res; 71(17); 5616–20. ©2011 AACR.*

Introduction

In Vancouver, tall glass buildings stand alongside the old growth forest of Stanley Park at the foot of snow-capped mountains and forests that rise up directly from the ocean on the north shore. Such a spectacular setting serves as an important reminder of our natural environment, and the lessons we can learn from it. This made Vancouver an excellent setting for a conference on Stem Cells, Development, and Cancer convened by the American Association for Cancer Research (AACR), where researchers from many disciplines came together to be reminded of the enormous impact that developmental biology has, and continues to have, on our understanding of cancer.

Through a series of presentations that crossed multiple disciplines, three major themes emerged. These presentations, coupled with the opportunities afforded by several powerful new technologies highlighted in a special "Emerging Technologies" session, allowed delegates to imagine new approaches to investigate challenging questions within their own specialized fields. In this report, we summarize the salient aspects of these themes and technologies. Perhaps the most important lesson, however, is the importance of convening meetings that bring together scientists working on highly diverse systems who share related questions and tools. At this meeting, a synergy resulted that highlighted cross-disciplinary approaches to some of the most challenging questions in developmental and cancer biology. Regrettably, many excellent meeting presentations are not included in this report in an effort to illustrate succinctly the common interests across the multiple fields merging at the conference.

Heterogeneity in Stem Cells and Cancer

Abstract depictions of stem cells typically assume they comprise biologically homogeneous populations, at least as far as the stem cell properties are concerned. However, there are accumulating examples where all stem cells in a given tissue are not, in fact, intrinsically equal. Much work at this meeting showed the importance of this concept. Three areas of particular relevance to understanding stem cells and cancer were brought forward: (i) heterogeneity in how stem cells respond to oncogenic perturbation, (ii) heterogeneity in the cell types produced during the generation of induced pluripotent stem cells (iPSC), and (iii) intrinsic heterogeneity in tissue stem cell populations.

The theme of heterogeneity was introduced by both opening plenary speakers. Elaine Fuchs (Howard Hughes Medical Institute, The Rockefeller University, New York, NY) began with a reminder of the heterogeneity of stem cells in the skin, while Maarten van Lohuizen (The Netherlands Cancer Institute, Amsterdam, The Netherlands) discussed how the heterogeneity of polycomb gene complexes can elicit different cellular outcomes. For example, BMI1 overexpression leads to cancer in many tissues, whereas overexpression of MEL18 does not, despite the fact that both contribute to the E3 ligase activity of the polycomb complex. Intriguingly, loss of BMI1 causes a G_2M arrest, perhaps due to an accumulation of DNA damage and induction of reactive oxygen species deriving from BMI1 deficiency. Following the opening plenary session, it was queried whether the necessary balance of BMI1

Authors' Affiliations: [1]Department of Haematology, Cambridge Institute for Medical Research, Cambridge, United Kingdom; [2]Gene Expression Laboratory, The Salk Institute for Biological Studies, La Jolla, California; and [3]Terry Fox Laboratory, BC Cancer Research Centre, Vancouver, British Columbia, Canada

Corresponding Author: Geraldine Aubert, BC Cancer Research Centre, Vancouver, BC V5Z 1L3, Canada. Phone: 604-6758000; Fax: 604-8770712; E-mail: gaubert@bccrc.ca

doi: 10.1158/0008-5472.CAN-11-1310

expression was the same in all cells, prompting the audience to consider how particular cell types (e.g., stem versus cancerous versus terminally differentiated) might respond differently to altered levels of BMI1.

Day 2 saw a strong continuation of the heterogeneity theme in the context of the various cell intermediates created in the process of reprogramming to iPSCs, and in the properties of the different iPSC lines ultimately generated (1). Andras Nagy (University of Toronto Mount Sinai Hospital, Toronto, ON, Canada) presented a provocative slide highlighting the three main junctions in the progression of fibroblasts to an iPSC state: (i) the point before which fibroblasts can return to their original fibroblast state (point of no return); (ii) the point after which the embryonic stem (ES)-like state can be achieved (point of commitment); and (iii) the point in the middle of the culture where cells cannot go back to the fibroblast state, but are also not reprogrammed into an ES-like state (Nagy playfully referred to this stage as "Area 51," which like reprogramming, has mysterious attributes and an unknown location).

Heterogeneity in copy number variations (CNV) detectable in many iPSC lines was also highlighted by both Nagy and Athanasia Panopoulos (Salk Institute for Biological Studies, La Jolla, CA) in recently published work (2, 3). These speakers showed that iPSC generation creates a mosaic of genetically distinct cells (large numbers of duplications/losses of genetic material that translate into gains and losses of function). The cells thus produced then compete with one another as they progress to an ES-like state. Interestingly, increased CNVs have been found irrespective of reprogramming method, including those that avoid use of integrating viruses or DNA. This finding highlights the importance of characterizing the basis of the heterogeneity that emerges as cells undergoing reprogramming pass through sequential intermediate states. It also has important implications for future reconsideration of the efficacy and safety of therapies that would use iPSCs or their differentiated derivatives.

Tissue stem cell heterogeneity was also widely discussed. Here, the nature of tissue-specific stem cells (i.e., hematopoietic, lung, mammary, and prostate), and the constellation of cells present in enriched stem cell populations (normal and cancer stem cells) were interrogated. As a prime example, Connie Eaves (BC Cancer Agency, Vancouver, BC, Canada) showed that all blood stem cells do not have the same differentiation potential, and that the distribution of these heterogeneous stem cell types within the stem cell compartment changes as the organism ages. Another important and recurrent point was that even using the most advanced isolation strategies, the resulting populations invariably contain non–stem cells (e.g., the best isolation strategies result in ~50% purity of hematopoietic stem cells). This stresses the importance of functional assessments of stem cell isolates and avoiding overinterpretation of results that could be skewed by non–stem cells in the fractions being subjected to molecular analyses. This is an important consideration as many groups work with populations that are comprised of less than 5% functionally defined stem cells.

Cancer Stem Cells in Solid Tissues

A second theme concerned the stem cell compartments of common solid tissue cancers. These include cancers of the breast, lung, prostate, brain, and skin. Only recently have specific populations been isolated that are enriched for stem cells defined by a developmental endpoint—a capacity to regenerate the full complement of cell types functionally organized to resemble the original tissue and sustain this activity for a substantial fraction of the lifetime of the organism (i.e., as evidence of long-term self-renewal ability). Distinct from normal stem cells, cancer stem cells are most commonly described as those with the ability to regrow a tumor with the cellular heterogeneity found in the original mass. It remains unclear whether these tumor-initiating cells actually contribute to the original tumor, or if they enable its perpetuation in the patient. A number of powerful *in vivo* and *in vitro* assays (elaborated below) that reconstitute basic tissue structures and functions were described that will be instrumental in furthering our knowledge in these areas, including excellent presentations from Brigid Hogan (Duke University Medical Center, Durham, NC) and Peter Dirks (University of Toronto Hospital for Sick Children, Toronto, ON, Canada) on lung and brain, respectively, which will not be discussed further due to space restrictions.

Cells with properties expected of stem cells are being rapidly identified in many normal primary human tissues and in animal model systems. Their location within tissues, their morphology, and their immunophenotype(s) were topics of considerable interest. These cell populations are now being examined to determine their developmental origin, their ability to reconstitute the tissue in the setting of injury and repair, and their role in contributing to the genesis of diseases, including cancer. In particular, mammary and prostate biologists are grappling with detailing the cellular and molecular mechanisms involved in the origin and progression of cancers and whether tumor-initiating cells first seem within normal basal or luminal populations. As Geoffrey Wahl (Salk Institute for Biological Studies, La Jolla, CA) and Inder Verma (also from Salk Institute) both emphasized, it will be critical to determine the molecular programs that drive tumorigenesis in different cancers, as cells that seem "committed" may, nevertheless, already possess or have a latent ability to reactivate stem cell properties and cause tumor regrowth, an important point to consider in the design of future cancer therapeutics.

The reliance of epithelial tumor growth on hijacked and inadequately controlled normal processes that activate normal dormant stem cells was also highlighted in the opening plenary by Elaine Fuchs. She presented evidence of cross-talk between epithelial and mesenchymal cells. She also showed how specific cell positioning and asymmetry at the base of the stem cell niche put the cells in position to receive signals (e.g., WNTs and BMPs) that are important in blocking tissue regeneration pathways. Such pathways are typically absent in cancer, thereby driving proliferation. It was proposed that normal skin stem cells typically only get "used" once to regenerate a hair follicle but that "old" stem cells may be

called upon in case of injury. Fuchs also described a new recently published method to genetically manipulate and track the progeny of embryonic day (E) 9.5 skin epithelial cell precursor (4). Her group injected lentiviral supernatant directly into the amniotic fluid that surrounds the mouse embryo at the E9.5 stage to enable *in vivo* knockdown and rescue experiments. Subsequent analyses of the skin epithelial layer in the embryo and in animals after birth will open new doors for investigating skin development and transformation.

Work in distinct model systems to study tissue-specific stem cells also showed the existence of several different stem and progenitor cell types. Furthermore, an interesting confluence of factors is emerging that has an impact on tissue stem cells, cancer risk, and cancer progression. For example, Owen Witte (University of California, Los Angeles, CA) showed that enhanced expression of the non–receptor tyrosine kinase Src in combination with enhanced expression of the androgen receptor was sufficient to drive the growth of prostate carcinomas in a mouse model using renal capsule tranplantations in immunodeficient mice (5). Similarly, Jane Visvader (Walter and Eliza Hall Institute of Medical Research, Parksville, VIC, Australia) showed that, although a mammary stem cell–enriched basal population lacks estrogen and progesterone receptors, mammary stem cell activity remains highly sensitive to steroid hormones (6). In addition, mammary stem cell–enriched populations isolated from ovariectomized females exhibit reduced mammary repopulating activity in recipients despite normal host hormone levels. This observation provides a tantalizing link to the finding that prior anti-estrogen therapy protects against breast cancer in patients who are no longer on anti-estrogen treatments. Interestingly, in individuals carrying a BRCA1 mutation, the luminal progenitor population seemed to be expanded relative to a reduction in the stem cell/basal progenitor–enriched population (7). Reduced mammary stem cell activity was also detected in pre-neoplastic tissue of BRCA1-deficient individuals. Moreover, the gene expression signature of basal tumors arising in BRCA1-deficient women was found to be more closely related to that of normal luminal progenitor-enriched populations. Although it cannot be excluded that the cell of origin of these basal tumors is a more primitive basal cell, it is interesting to speculate that BRCA1-associated basal tumors may have a luminal cell origin. Further elucidation of the cellular origins of these and other tumors should emerge with improved strategies for purifying stem and progenitor cell populations.

Many of the transformation models presented showed a degree of heterogeneity, and in some cases plasticity, in the behavior of the cells tracked. These observations suggest additional cellular sources of heterogeneity (e.g., microenvironment, epigenetic factors) beyond the mutated tissue stem cells or mutated progenitors that acquire a stem cell phenotype. It also remains possible that spontaneously arising tumors in humans may begin with mutations or epigenetic alterations in stem cells that then create daughter "committed" (or transit-amplifying populations) that are prone to acquire additional changes that, in turn, engender tumor growth as their microenvironment evolves along with the intrinsic properties of the neoplastic cells. These complex possibilities warrant much future consideration, and will also be relevant to the design of future effective cancer treatment strategies.

Lessons from Development

The third theme revolved around the importance of characterizing processes that regulate normal tissue development as a foundation for identifying and understanding perturbations that cause disease. Current studies suggest that the production of differentiated cells in most mammalian tissues involves a hierarchical process originating in a population of competent but undifferentiated stem cells that are responsible for the long-term maintenance of the tissue through a process that balances self-renewal and the production of differentiating daughter cells. Intermediate cells then undergo a series of amplifying divisions to generate the larger numbers of specialized cell types characteristic of the tissue. Current evidence suggests that tumorigenesis can occur when normal developmental pathways are inappropriately reactivated or deregulated. Investigating this possibility first requires identification of critical intrinsic and extrinsic molecular mechanisms that regulate normal stem cell behavior during normal development or in response to injury, well shown in presentations from Margaret Fuller (Stanford University School of Medicine, Stanford, CA) and Hanna Mikkola (University of California, Los Angeles, CA). The second requirement is to identify how these mechanisms may be co-opted or misused by cancer cells during tumor initiation, progression, and metastasis.

Developmental biologists have studied tissue patterning and specification for a long time and have noted the normal activation of a process referred to as epithelial-to-mesenchymal transition during embryogenesis. Cancer biologists find this to be a key component of epithelial tumor metastasis. Of particular interest in this context was the identification and characterization of a mammary stem cell–enriched population isolated from the mouse embryo. By performing *in vivo* mammary transplant studies, Geoffrey Wahl's group showed that the embryonic stage at which fetal mammary stem cells are robustly detectable coincides with the developmental period when mammary epithelial rudiments are rapidly extending into the surrounding stroma. Importantly, this morphologic process resembles the initiation of metastasis (i.e., cancerous epithelial cells invading the surrounding stroma). Cells within the fetal mammary stem cell–enriched population exhibit dual expressions of keratin and mesenchymal markers, a cellular phenotype also detected in some breast cancers. In addition, microarray analyses of the fetal mammary stem- and stromal-enriched populations generated gene signatures that are represented in different breast cancers subtypes. These studies lend strong support to the idea that pathways used in fetal organogenesis and tissue stem cells are reactivated during tumorigenesis. In addition, this property might be exploited for predicting disease progression and perhaps new treatment strategies.

The balance of symmetric and asymmetric division in stem cells to produce progeny with different cell fates is well characterized in invertebrate model systems (e.g., the *Drosophila* nervous and male germline systems). However, insight

into this process in mammalian tissues has been difficult to obtain until recently. As a population, stem cells must strike the correct balance of self-renewal, differentiation, quiescence, and survival, and perturbations in this balance could be key to understanding how cancer clones expand. For example, Tannishtha Reya (Duke University Medical Center, Durham, NC) presented her recently published work in the mouse showing that the NUP98-HOXA9 fusion gene can promote symmetric divisions in hematopoietic progenitor/stem ($Lin^-Sca^+Kit^+$) cells and upregulates *Musashi2* and downregulates *Numb* (8). Both of these changes are also implicated in the transition from the chronic phase of chronic myelogenous leukemia (CML) to the blast crisis phase of this disease. Combined with the known ability of NUP98-HOXA9 to promote expansion of mouse hematopoietic stem cell populations *in vitro*, it is tempting to suggest that the aggressive features of the blast phase of CML may in some instances be mediated by NUP98-HOXA9–driven symmetric renewal divisions and that future therapies that promote asymmetric divisions (or symmetric differentiation divisions) might be useful in treating advanced-phase CML.

Finally, *in vitro* systems were highlighted as useful tools to study organ development in culture. These systems facilitate the understanding of processes that regulate embryonic and normal tissue development. For example, recent advances have allowed the development of 3-dimensional culture systems. Of particular note, James Wells (Cincinnati Children's Hospital Medical Center, Cincinnati, OH) presented his group's recently published *in vitro* system that produces 3-dimensional and functional intestinal organoids from human pluripotent stem cells (9). Human embryonic stem cells or iPSCs were first treated with activin to promote endoderm differentiation, followed by Wnt3a and FGF4 to promote hindgut and intestinal cell types. The cellular structures obtained showed similarities to intestinal organoids and included cells capable of absorptive and secretory functions. This advance sets the stage for *in vitro* investigations of the molecular basis of human intestinal diseases and for the future development of intestinal transplantation-based therapies.

New Technologies

New technologies were an integral element of many presentations but were also the specific focus of one session included to emphasize the important role of new technology in supporting the rapid pace of discovery in stem cell biology. Several new methods likely to have substantial impacts in this regard and described in this meeting were microfluidics, fed batch cultures, mass spectrometry cytometry, and chromosome orientation fluorescence *in situ* hybridization (CO-FISH). Microfluidics systems, as discussed by Carl Hansen and Darek Sikorski (University of British Columbia, Vancouver, BC, Canada), allow large-scale cell monitoring experiments to be done in nanoliter volumes with automated imaging or transcriptome analytical endpoints. These systems constitute powerful additions to approaches to investigate heterogeneity in growth, differentiation, divisional kinetics, and survival.

Peter Zandstra (University of Toronto, Donnelly Centre for Cellular and Biomolecular Research, Toronto, ON, Canada) showed how a fed-batch strategy can be usefully exploited to dilute or concentrate secreted factors to alter the expansion of human hematopoietic stem cells. Such systems also permit expression studies of paracrine and autocrine factors and can connect specific cell types as nodes central to the feedback in the microenvironment. This allows for the definition and analysis of ligand connectivity between stem cells, their progeny, and other cells within their microenvironment and asks if feedback control is occurring within a controlled cellular environment. Combining high-throughput microfluidics with global expression analysis of stem cell culture systems makes it possible to engineer the stem cell niche *in vitro* to identify key positive and negative factors that are produced during a set time frame within the system.

Erin Simonds (Stanford University, Stanford, CA) described a new mass spectrometry–based flow cytometer that can multiplex a range of protein content analyses of single cells that is not possible with traditional flow cytometry methods. Instead of using antibodies conjugated to fluorochromes, mass spectrometry cytometry conjugates antibodies to metals that are subsequently separated by mass spectrometry (10). Current technology allows 34 different antibodies to be detected simultaneously with no need for compensation. In theory, this number could be expanded to 100 antibodies against different proteins.

In the session on epigenetics, Peter Lansdorp (BC Cancer Research Centre, Vancouver, BC, Canada) presented a recently published novel methodology to investigate whether fully replicated sister chromatids segregate in a random or non-random manner, which could be a mechanism used to explain how stem cells execute asymmetric self-renewal divisions (11). Although genetically identical, DNA strands following replication may differ epigenetically, and this could result in a differential activation/repression of cell fate–determining factor(s) in each of the two daughter cells. Non-random segregation of sister chromatids has been identified with CO-FISH labeling of different DNA template strands of a minority of mouse colon epithelial cells labeled *in vivo*. Notably, these studies indicated that non-random segregation did not occur for 100% of chromosome strands between paired cells, which is inconsistent with Cairn's Immortal Strand Hypothesis. A similar strategy can be exploited to investigate strand-specific and direction of replication-specific epigenetic marks by next-generation sequencing. This approach may be useful to investigate the regulation of self-renewal in stem cells and in the cancer setting. It can also be used to address prospectively the question of whether one template strand may be silenced by epigenetic chromatin marks and whether this could be a mechanism in pre- or postmitotic dysregulation of master genes in cancer stem cells.

Summary and Recommendations for Future Research

Heterogeneity in stem cells is complicating our understanding and interpretation of their biology. The solid tissue cancer stem cell field is rapidly catching up to hematopoiesis and leukemia with respect to stem cell purities and robust

functional assays, and developmental biologists continue to inform the frontiers of stem cell and cancer biology with elegant work in model organisms. The disruption that iPSCs have caused to our classical notions of development is having a profound effect on our approaches to understanding disease and new possibilities for treatments of specific conditions. This conference in particular highlighted the importance of understanding the process of reprogramming, identifying the surprising genetic disruption that occurs throughout the culture period. Also, clearly much more work remains to characterize and understand the unanticipated heterogeneity in stem cells and cancer initiating cells, the extent and mechanisms of plasticity that they can exercise, and identifying how this new knowledge will be effectively harnessed to benefit human health. This is indeed a very exciting time to be in the field, and these themes will undoubtedly continue to be central ones at the AACR Annual Meeting and in future meetings dealing with the same topics.

Disclosure of Potential Conflict of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank the AACR staff, Hans Clevers, Connie Eaves, and Geoffrey Wahl for the organization and planning of an excellent meeting. In particular, the authors also thank Geoffrey Wahl and Connie Eaves for critical reading and helpful suggestions regarding this manuscript, for inspiration, and their extraordinary commitment to mentorship and training.

Received April 18, 2011; revised May 27, 2011; accepted June 8, 2011; published OnlineFirst August 30, 2011.

References

1. Ho R, Chronis C, Plath K. Mechanistic insights into reprogramming to induced pluripotency. J Cell Physiol 2011;226:868–78.
2. Hussein SM, Batada NN, Vuoristo S, Ching RW, Autio R, Narva E, et al. Copy number variation and selection during reprogramming to pluripotency. Nature 2011;471:58–62.
3. Gore A, Li Z, Fung HL, Young JE, Agarwal S, Antosiewicz-Bourget J, et al. Somatic coding mutations in human induced pluripotent stem cells. Nature 2011;471:63–7.
4. Williams SE, Beronja S, Pasolli HA, Fuchs E. Asymmetric cell divisions promote Notch-dependent epidermal differentiation. Nature 2011;470:353–8.
5. Cai H, Smith DA, Memarzadeh S, Lowell CA, Cooper JA, Witte ON. Differential transformation capacity of Src family kinases during the initiation of prostate cancer. Proc Natl Acad Sci U S A 2011;108:6579–84.
6. Asselin-Labat ML, Vaillant F, Sheridan JM, Pal B, Wu D, Simpson ER, et al. Control of mammary stem cell function by steroid hormone signalling. Nature 2010;465:798–802.
7. Lim E, Vaillant F, Wu D, Forrest NC, Pal B, Hart AH, et al. Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. Nat Med 2009;15:907–13.
8. Ito T, Kwon HY, Zimdahl B, Congdon KL, Blum J, Lento WE, et al. Regulation of myeloid leukaemia by the cell-fate determinant Musashi. Nature 2010;466:765–8.
9. Spence JR, Mayhew CN, Rankin SA, Kuhar MF, Vallance JE, Tolle K, et al. Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature 2011;470:105–9.
10. Bendall SC, Simonds EF, Qiu P, Amir el AD, Krutzik PO, Finck R, et al. Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. Science 2011;332:687–96.
11. Falconer E, Chavez E, Henderson A, Lansdorp PM. Chromosome orientation fluorescence in situ hybridization to study sister chromatid segregation in vivo. Nat Protoc 2010;5:1362–77.

Cancer Research

Correction: Online Publication Date for *Cancer Research* September 1, 2011, Article

The article by Kent and colleagues (1) in the September 1, 2011, issue of *Cancer Research* was published with an online publication date of August 30, 2011, listed but was actually published online on August 31, 2011.

Reference

1. Kent DG, Lin JC, Aubert G. The First AACR Special Conference on stem cells, development, and cancer: some of these cells are not like the others. Cancer Res 2011;71:5616–20.

Published OnlineFirst October 18, 2011.

doi: 10.1158/0008-5472.CAN-11-3047

The First AACR Special Conference on Stem Cells, Development, and Cancer: Some of These Cells Are Not Like the Others

David G. Kent, Jennifer C. Lin and Geraldine Aubert

Cancer Res 2011;71:5616-5620.