Developmental Origins of Cancer

Kimberly J. Johnson,1 Nathan M. Springer,2 Anja-Katrin Bielinsky,3,4 David A. Largaespada,4,5 and Julie A. Ross1,4

1Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota; 2Department of Plant Biology, University of Minnesota, St. Paul, Minnesota; and 3Department of Biochemistry, Molecular Biology & Biophysics, 4Masonic Cancer Research Center, and 5Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, Minnesota

Introduction

Laboratory and epidemiological studies on childhood and adult malignancy support a role for the developmental origins of cancer. The Developmental Origins of Cancer Symposium hosted by the University of Minnesota (February 28, 2009 to March 1, 2009) brought together individuals from diverse disciplines to discuss and prioritize steps needed to advance the research area. Information on key speakers and their recommended reading list is provided in the Appendix. The symposium format allowed for considerable audience discussion and brainstorming; speakers presented five or fewer slides in 15 minutes. From the discussions it became clear that (a) developmental origins of childhood and adult malignancies exist, (b) establishment of many critical epigenetic patterns occurs in utero and is sensitive to environmental perturbations, (c) failure to establish proper epigenetic patterns may increase the risk of developing cancer, and (d) improved methods to evaluate environmental exposures and proper establishment of epigenetic patterns could provide important tools to better understand the risks of specific in utero environmental exposures. Highlights are provided below along with recommendations for the future.

Epigenetic Evidence

Dr. Randy L. Jirtle (Duke University, Durham, NC) opened the symposium describing the concept of epigenetically labile genes or genes from identical cells that can produce heritable variation in phenotype. He illustrated this idea with his experimental data from the agouti mouse model, in which the coat color phenotype provides a visual representation of the epigenetic state of a transposon that is located in the promoter of the agouti allele (A<sup>y</sup>). When the transposon is not silenced expression of the downstream agouti gene occurs and results in yellow coat color. However, epigenetic silencing of the transposon via DNA methylation results in brown coat color. Intriguingly, the coat color distribution can be shifted toward brown with maternal dietary methyl-donor (folic acid, vitamin B12, choline, and betaine) supplementation. This coat color variation indicates that the epigenetic state at the A<sub>y</sub> allele is sensitive to in utero environmental exposures. Although no epigenetically labile genes have yet been discovered in humans, he suggested that strong candidates include imprinted genes in which parent-of-origin expression is dictated through epigenetic mechanisms. Using an in silico approach involving pattern recognition algorithms, Dr. Jirtle's group has predicted that there may be 600 imprinted genes in mice and >200 in humans. Notably, only ~30% of the genes are predicted to be imprinted in both mice and humans giving rise to the question of whether mice are suitable models for the study of human diseases arising from epigenetic dysregulation.

Dr. Nathan M. Springer (University of Minnesota, St. Paul, MN) followed by discussing epigenetics in plants and gave an overview of technologies used to detect DNA methylation. A key point was that many genes that are sensitive to epigenetic regulation in plants have nearby transposons, which agrees with the characteristics of the three known epigenetically labile genes in mice (A<sup>y</sup>, Axin<sup>Pa</sup>, Caby<sup>AP</sup>). Interestingly, in corn, imprinting in offspring genes can be modified by maternal environmental conditions (e.g., amount of sunlight), which also parallels the nutritional data from mice. Dr. Springer emphasized that although DNA methylation can alter gene activity, the exact mechanisms of its sensitivity to the environment are unclear.

Epidemiological Evidence

Dr. Karin B. Michels (Harvard University, Boston, MA) presented evidence for an association between high birth weight and breast cancer, childhood leukemia and brain tumors, testicular cancer, and possibly prostate cancer. Although the epidemiological evidence is fairly strong, especially for breast cancer and childhood leukemia, she pointed out that birth weight is only a marker for other in utero events such as hormones, growth factors, and other epigenetic changes (in addition to genetic variation). To understand the specific mechanisms that explain the association between birth weight and cancer, it is necessary to understand the complex interactions between prenatal exposures, intrauterine hormonal milieu, and epigenetic marks affected by these factors, as well as the role of postnatal exposures (i.e., secondary hits). Key impediments include the lack of prospective studies, lack of stored biospecimens and recorded data from historical cohorts, and the difficulty of measuring intrauterine exposures.

Dr. Logan G. Spector (University of Minnesota, Minneapolis, MN) followed with observational evidence for the developmental origin for many childhood malignancies including (1) a young age at diagnosis, (2) cancer cells that are histologically similar to those in early development, (3) occasional prenatal diagnoses, and (4) twin studies and molecular investigations of translocations that have shown that leukemia can be “backtracked” to birth. He also described the consistent epidemiological evidence supporting a role for prenatal radiation, high birth weight, and congenital abnormalities as risk factors for certain childhood cancers. Lastly, he described the observation that secondary leukemias [mostly acute myeloid leukemia (AML)] with MLL translocations arise in some cancer patients following chemotherapies that inhibit DNA topoisomerase II. Epidemiological studies of infant leukemias, which frequently have an MLL translocation, suggest that maternal

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Requests for reprint: Julie A. Ross, MMC 422 (554 CCRB), 420 Delaware Street, SE, Minneapolis, MN 55455. Phone: 612-626-2902; Fax: 612-626-4842; E-mail: ross0014@umn.edu.

©2009 American Association for Cancer Research.
doi:10.1158/0008-5472.CAN-09-1391


Downloaded from cancerres.aacrjournals.org on April 15, 2017. © 2009 American Association for Cancer Research.
exposure to dietary DNA topoisomerase II inhibitors during pregnancy may increase the risk of infant AML but not infant acute lymphoblastic leukemia (ALL).

**Molecular Evidence**

Dr. Joseph L. Wiemels (University of California, San Francisco, CA) presented data showing that at least 60% of childhood B-cell leukemias are estimated to initiate in utero, which was derived through backtracking studies that have detected common genetic events in B-cell leukemias in neonatal blood spots, including high hyperdiploidy, TEL-AML1 translocations, and MLL translocations. Interestingly, Dr. Wiemels described that ~1% of children have TEL-AML1 translocations at birth, but only one of 2,000 children develop leukemia with these translocations, indicating that the TEL-AML1 is a relatively common event but is insufficient to cause leukemia. He proposed several unanswered questions with regard to understanding the molecular etiology of childhood leukemias including: (1) When do secondary hits occur and by what mechanisms?; (2) What is the influence of the fetal environment?; (3) What is the role of early and inherited epigenetic events in cancer susceptibility?; and (4) How do environmental factors (e.g., viruses) influence genetic and epigenetic changes that increase leukemia risk? He identified a lack of prospective data collection as the largest barrier to answering these questions.

Dr. Barry A. Finette (University of Vermont, Burlington, VT) suggested leukemic translocations may involve V(DJ) recombinase, an enzyme that shuffles variable (V), diversity (D), and joining (J) genes at B- and T-cell receptor loci in lymphocytes to create new sequences required to develop the antigenic diversity of a competent immune system. He provided data to support the hypothesis that the distribution of age at onset in childhood ALL incidence, which peaks at around 3 years, may be explained (at least in part) by errors in developmentally regulated expression of V(DJ) recombinase, which is most active during fetal development and early childhood. V(DJ) recombinase activity has been linked to mutagenic rearrangements at nonimmune sites including the hypoxanthine-guanine phosphoribosyltransferase (HPRT) locus. Dr. Finette suggested that the mutagenic rearrangements at the HPRT locus could serve as an in vivo model system for elucidation of leukemogenic mechanisms in children and as a biomarker for environmental exposure induced mutagenic rearrangements in hematopoietic cells.

Extending the discussion on chromosomal rearrangements, Dr. Anja-Katrin Bielinsky (University of Minnesota, Minneapolis, MN) provided insight into the role of cell cycle checkpoints in maintaining genome stability. S-phase checkpoints that operate during DNA replication play a critical role in suppressing chromosomal breaks; they are required to stabilize stalled replication forks and prevent their collapse. Replication fork collapse is thought to be the primary source of endogenous double strand breaks and can thus cause chromosome fragility and gross chromosomal rearrangements (GCRs). She noted that several human cancer syndromes are mediated by genetic defects in S-phase checkpoint genes, pointing to their functional importance in the prevention of malignancy. Checkpoint activation is an early event in cancer development and can be activated by DNA damage and replication stress. Because most S-phase checkpoint genes are highly conserved between species, gene function can be studied in budding yeast. Here, the frequency of GCRs is greatly increased in S-phase checkpoint and certain replication mutants. She concluded that yeast is a useful model for understanding the S-phase checkpoint pathway and may provide new insight into cancer initiation via GCRs. However, she also noted the lack of animal models, especially for gene defects in replication factors that control fork stability and suppress DNA damage.

**Animal Evidence**

Dr. Leena Hilakivi-Clarke (Georgetown University, Washington DC) presented evidence from rodent models of breast cancer demonstrating that maternal exposures during pregnancy can influence mammary cancer risk in offspring. Maternal high fat diets, estradiol exposure, or a combination, result in offspring with early puberty onset and increased numbers of mammary proliferating cells. These offspring also are at increased risk of developing spontaneous, or carcinogen- or oncogene-induced mammary tumorigenesis compared with control offspring. Although the specific mechanism is unclear, alterations in the number of stem cells (i.e., “cancer initiating cells”) or expression of genes that regulate mammary stem cell behavior may play a role. Challenges to addressing these hypotheses involve the technological hurdles of identifying and quantifying mammary stem cells and studying them in their “natural” environment, along with the key epigenetically regulated genes that modulate stem cell behavior in in utero exposed animals. Another larger challenge is how to translate findings in rodents to cancer prevention in humans.

Dr. John H. Kersey (University of Minnesota, Minneapolis, MN) described four important questions in understanding the molecular mechanisms by which MLL translocations can result in infant leukemia. The first was “what are the functional domains of the MLL protein and what is the nature of the fusion gene that results from the translocation?” The MLL protein is a positive regulator of gene expression, including several HOX genes, through its function in methylaing histone H3 lysine four. The MLL protein has more than 4,000 amino acids and several functional domains including a break point cluster (BCR) region; all known translocations between MLL and other partners (>60 with the most common being AF4 and AF9) have fusions in the BCR region. The second question was “why do the initiating translocations and resultant MLL fusions often occur in utero?” One hypothesis involves DNA topoisomerase II inhibitor exposure as described above. The third was “how many steps or hits are involved in the evolution to frank leukemia and do additional hits result from haploinsufficiency of either MLL or its fusion partner?” Lastly, “what cells are initially transformed by the MLL fusion proteins?” He described research using a mouse model of infant leukemia with the MLL-AF9 translocation and how his laboratory has determined that preleukemic hematopoietic stem cells, rather than committed granulocyte-monocyte progenitors, cause leukemia with highest efficiency.

A major hurdle to understanding the etiology of specific cancer types is the availability of an appropriate animal model. For germ cell tumors this is especially challenging because no cell lines or animal models exist with the exception of mouse models of teratomas. Dr. James F. Amatruda (University of Texas Southwestern Medical School, Dallas, TX) proposed that the zebra fish model may be useful. Using a forward genetic screen in zebra fish, he presented data that identified a germ cell tumor locus. The human homolog of this gene is also expressed in some human GCTs. Zebra fish may also be a useful model in the study of other cancers including hematological malignancies, pancreatic cancer, and melanoma.

Dr. David A. Largaespada (University of Minnesota, Minneapolis, MN) indicated that a key challenge to understanding cancer initiation is the relatively short list of genes known to be involved.
His research in cancer gene discovery uses an unbiased in vivo forward genetic screen in mice that employs the Sleeping Beauty (SB) transposon system. Mapping of transposon insertion sites in mice that are genetically engineered to express tissue-specific SB transposon and transposase activities allows for unbiased site-specific cancer gene discovery. Dr. Largaespada recently published data on colon cancer gene discovery that implicated 77 genes (60 of which have been reported in human colorectal cancer), including the well-known colon cancer gene APC. One barrier to the large gene lists often generated using this approach is developing efficient methods to test their functional significance.

Dr. Jaime F. Modiano (University of Minnesota, Minneapolis, MN) highlighted molecular similarities between dog and human hematological malignancies. Equivalent translocations between dog and human leukemias have been found for BCR-ABL (chronic myelogenous leukemia), IGH-MYC (Burkitt lymphoma), and del(13)(q14.3) (chronic lymphoid leukemia). Two insights into leukemia pathogenesis arise from dog research: (1) rearrangements occur near regions in which genome reorganization occurs to produce species-specific karyotypes, and (2) inherent “fragile sites” such as those adjacent to loci encoding BCR, ABL, RB-1, AML-1, ETO, and others are more likely to undergo aberrant rearrangement. A question that arises is whether these sites are more likely to undergo aberrant rearrangements during development? Dr. Modiano also pointed out that dog models may be especially useful for studying childhood cancers because of their short lifespan and environmental similarities with young children.

Dr. Julie A. Ross (University of Minnesota, Minneapolis, MN) summarized that whereas independent epidemiological and laboratory studies of cancer will continue, there is a need for more interaction among disciplines. She illustrated how such a transdisciplinary study might be developed for childhood cancer (Supplementary Figure). Although epidemiological studies indicate certain associations, most depend on retrospective recall with little or no confirmatory evidence. However, neonatal blood spots collected at birth for universal screening of inborn errors of metabolism could serve as a resource for future case-control studies of childhood cancer to evaluate (as examples) specific maternal exposures, gene methylation and expression, and translocations. Importantly, these investigations would first need to be validated in clinical research studies (such as pregnancy cohorts) that collect biological specimens and questionnaires. Further, animal models could investigate various maternal exposures and their impact on cancer risk in offspring and could be used to help inform human studies with the identification of potentially important genes.

**Summary and Future Recommendations**

There is strong molecular and epidemiological support for in utero cancer initiation. Data from animal and plant systems show that environmental conditions during development can influence offspring phenotypes through epigenetic (and direct genetic) mechanisms. Several important areas for future research were identified and include:

1. Identifying epigenetically labile human genes and their molecular characteristics (e.g., is a transposon located nearby?)
2. Developing a simple and reliable assay to detect exposures that alter genes epigenetically during development (e.g., what is the epigenetic “Ames test?”)
3. Exploiting genetically engineered animal models for identification of human cancer genes
4. Identifying creative ways of using human data for studies trying to understand developmental origins of cancer including registry linkages, and assay development using neonatal blood spots to identify molecular markers of premalignancy and environmental exposures
5. Collecting prospective data during pregnancy and at birth through establishment of large birth cohorts. These data could be used in the study of intermediate markers (i.e., between birth and disease development) and could serve as a data repository for future generations of cancer researchers
6. Inviting surgical pathologists and clinicians to these discussions because they are critical in tissue procurement for research in this area

In conclusion, interdisciplinary cancer research settings that foster transfer of knowledge are needed to further our understanding of the developmental origins of cancer.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

Received 4/23/09; revised 5/28/09; accepted 6/9/09; published OnlineFirst 8/4/09.

**Grant support:** We would like to thank the Office for the Vice President for Research, University of Minnesota, for funding this symposium.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Developmental Origins of Cancer


Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-09-1391

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2009/07/30/0008-5472.CAN-09-1391.DC1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.