ORGANIZER

Society for Chromosomal Cancer Research (SCCR)

ORGANIZING COMMITTEE

Peter Duesberg, President of the Conference and SCCR
Department of Molecular and Cell Biology, UC Berkeley

David Rasnick, Vice-President of the Conference and SCCR
Boveran, Inc., Fort Lauderdale

CONFERENCE SITE

Waterfront Plaza Hotel
Jack London Square
Ten Washington Street
Oakland, CA 94607
Tel 510.836.3800 Fax 510.839.7548

REGISTRATION

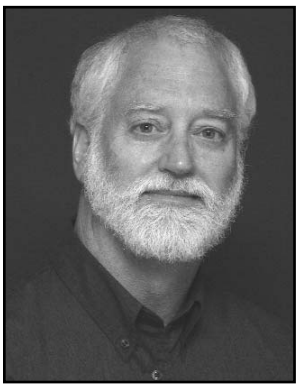
The registration desk is located in the lobby of the Waterfront Plaza Hotel

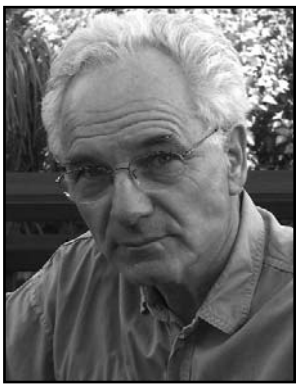
Thursday, January 31 . . . . . 4:00 pm – 7:00 pm
Friday, February 1 . . . . . 8:00 am – 6:00 pm
Saturday, February 2 . . . . . 8:00 am – 6:00 pm
Sunday, February 3 . . . . . 8:00 am – 12:00 pm

For further information about the conference and SCCR, please contact:

Sigrid Duesberg
5373 Thomas Avenue
Oakland, CA 94618
Tel 510.594.8635 Fax 510.594.8635
ssachs@calmail.berkeley.edu

Welcome Address





Welcome to the 2d Conference on Aneuploidy & Cancer here in Oakland. We—our sole sponsor Robert Leppo and us the organizers, Rasnick and Duesberg—are very excited that you came.

It is your presence and participation, and the ever-daunting cancer problem that will hopefully make this conference as exciting to follow as watching the Olympics: Will specific aneuploidies or specific mutations or even epigenetic alterations found to be the causes of cancer?

In the first case, carcinogenesis would be like genesis: A cancer cell would be generated from a normal cell by karyotypic alterations, much like a new phylogenetic species.

In the second case, carcinogenesis would be like mutagenesis: A cancer would be generated by specific mutations of a normal cell, which would maintain its original karyotype. In other words, cancer would be just one special kind of genetic diseases.

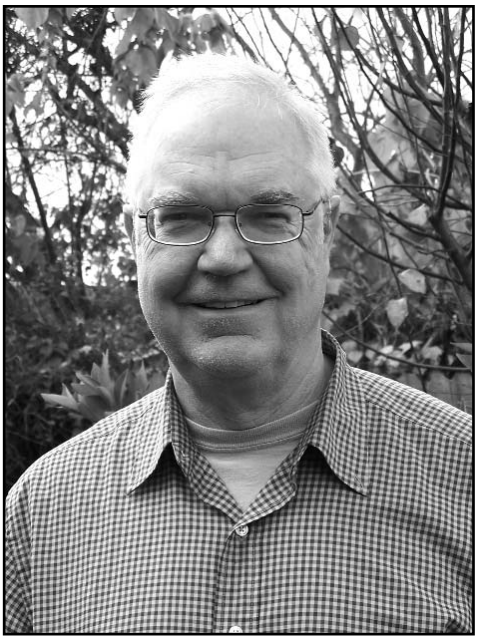
In the third case, neither the chromosomes nor the genes would be changed. Carcinogenesis would be much like development, which is still a very dark box.

The younger ones of us, those under 30, will probably argue that it's one or the other—either a specific aneuploidy or a specific mutation or an epigenetic alteration must be sufficient for cancer. But the more mature researchers among us may argue that aneuploidy and mutations and perhaps epigenetics are all necessary for cancer.

Of course, all of us will know the answer by Sunday afternoon, when this conference comes to an end.

In the meantime it is already clear to us that you all are necessary and sufficient to find that answer.

Thank you and let's go to work …



Robert Leppo

This cancer conference was initiated and is wholly supported by our friend and sponsor, philanthropist Robert Leppo, through the auspices of The Group for Scientific Reappraisals. Leppo's interest and concern was sparked by the idea that cancer has a chromosomal basis, which could serve to improve early detection and therapy, yet is currently disregarded by mainstream cancer research.

In view of the potential merits of this conference for the diagnosis and treatment of cancer, we have dedicated the proceedings of this conference to him.

Peter Duesberg David Rasnick

Scientific Program

OPENING SESSION – Thursday, January 31

Opening Ceremony

6:00 Opening Address
Peter Duesberg (President of the Conference)

6:30 Opening Lecture
1. What causes cancer?
Wayt Gibbs (Bellevue, US)

7:30 **WELCOME COCKTAIL**

SESSION 1 – Friday, February 1

Every talk includes a 5-10-minute discussion at the end.

Chaired by: **Eytan Domany** and **Thomas Ried**

8:45 2. Relationship between the karyotypes and phenotypes of cancer cells
Peter Duesberg (Berkeley, US)

9:15 3. Chromosomal Instability in Oral Cancer Cells: A Generalizable Model System for cancer
Susanne Gollin (Pittsburgh, US)

9:45 4. Patterns of genome dynamics and cancer evolution
Henry Heng (Detroit, US)

10:15 **COFFEE BREAK**

10:45 5. Chromosomal instability and precursors along the path to aneuploidy and cancer in chronic inflammatory gastrointestinal diseases
Peter Rabinovitch (Seattle, US)

11:15 6. Numerical aberrations during the development of cervical carcinogenesis: tetraploidy is an early event that precedes most aneuploidy
Andrew Olaharski (Palo Alto, US)

11:45 **LUNCH**

SESSION 2 – Friday, February 1

Every talk includes a 5-10-minute discussion at the end.

Chaired by: **Peter Rabinovitch** and **Gerrit Meijer**

1:15 7. Genomic instabilities, DNA copy number changes and cancer
Eytan Domany (Rehovot, Israel)

2:15 8. Multiple numerical chromosome aberrations in carcinogenesis: the kidney cancer model
Manuel Teixeira (Porto, Portugal)

2:45 9. The relationship of chromosomal aneuploidy, nuclear structure, and gene expression in cancer cells
Thomas Ried (Bethesda, US)

3:15 **COFFEE BREAK**

3:45 10. Low rates of aneuploidy promote tumorigenesis while high rates of aneuploidy cause cell death and tumor suppression
Beth Weaver (San Diego, US)

4:15 11. Recurrent Genomic Alterations in Prostatic Preneoplasias and in Prostate Cancer
Jeremy Squire (Toronto, Canada)

4:45 12. Genomic instability and clonal outgrowth/ evolution in the upper aerodigestive tract
Walter Hittelman (Houston, US)

5:15 Session ends

5:30 **LIGHTNING TALKS** (5 minutes each)

SESSION 3 – Saturday, February 2

Every talk includes a 5-10-minute discussion at the end.

Chaired by: **Walter Hittelman** and **Peter Duesberg**

8:30 13. D•A•T•E analysis of cancer microarray data
David Rasnick (Fort Lauderdale, US)

9:00 14. Evaluation of DNA-ploidy heterogeneity in gastric cancers
Maria-Chiara Osterheld
(Lausanne, Switzerland)

9:30 15. DNA-aneuploidy: A diagnostic and prognostic marker for tumor cells
Alfred Böcking (Düsseldorf, Germany)

10:00 **COFFEE BREAK**

10:30 16. Missing evidence in cancer genetics: The retinoblastoma paradigm
Domenico Mastrangelo (Siena, Italy)

11:00 17. The genetic basis of Fanconi anemia and other heritable chromosome instability syndromes
Holger Hoehn (Würzburg, Germany)

11:30 18. Integration of DNA copy number and expression microarray data reveals 7 putative oncogenes in 3 amplicons at 20q involved in colorectal adenoma to carcinoma progression
Gerrit Meijer
(Amsterdam, The Netherlands)

12:00 **LUNCH**

SESSION 4 – Saturday, February 2

Every talk includes a 5-10-minute discussion at the end.

Chaired by: **Jeremy Squire** and **Andrew Ray**

1:30 19. TnT: T antigen and telomerase, an explosive route to cancer
Andrew Ray (Fort Collins, US)

2:00 20. Spectrum of chromosomal aneuploidy in lymphocytes of workers exposed to benzene and leukemia risk
Luoping Zhang (Berkeley, US)

2:30 21. Induction of spindle multipolarity by centrosomal cluster inhibition
Alwin Kraemer (Heidelberg, Germany)

3:00 **COFFEE BREAK**

3:30 22. Sporadic ovarian carcinomas show dysregulation of DNA repair and genomic stability pathways associated with complex structural abberrations, chromosomal instability and centrosome aberrations
Jane Bayani (Toronto, Canada)

4:00 23. Cell-to-cell fusion as a link between viruses and chromosomal instability
Yuri Lazebnik (Cold Spring Harbor, US)

4:30 24. Genomic plasticity and its transcriptional consequences in colorectal cancer
Jordi Camps (Bethesda, US)

5:00 Session ends

7:30 **CONFERENCE BANQUET**

SESSION 5 – Sunday, February 3

Every talk includes a 5-10-minute discussion at the end.

Chaired by: **Rüdiger Hehlmann** and **David Rasnick**

8:45 25. The "aneuploidy-modified mutator-phenotype" theory of malignant tumours
Leon Bignold (Adelaide, Australia)

9:15 26. Impact of DNA copy number alteration on transcriptional programs and cancer phenotypes
Jonathan Pollack (Stanford, US)

9:45 27. Genomic instability in context of the chromosomal theory
Alice Fabarius (Mannheim, Germany)

10:15 **COFFEE BREAK**

10:35 28. Lessons in copy number alterations in cancer learned from comparative genomic hybridization (CGH) and fluorescence *in situ* hybridization (FISH)
Nicholas Wang (Berkeley, US)

11:05 29. SV40 Tag/p53 complexes actively promote malignant cell growth of human mesothelial cells
Michele Carbone (Honolulu, US)

11:35 Conference ends

Abstracts

1. What causes cancer?

W. Wayt Gibbs

Executive Editor, Intellectual Ventures, *1756 114th Ave SE, Bellevue, WA 98004*
Contributing Editor, Scientific American; *news@waytgibbs.com*

Of all the myriad toxins, pathogens, and environmental insults commonly accepted as "causes" of cancers—from tobacco smoke to ionizing radiation, oncoviruses to asbestos—none are true causes, because none lead invariably to neoplasia in those they affect. Distinguishing root causes from risk factor is more than mere semantics. Cancer remains one of humanity's largest burdens despite more than a century of work and hundreds of billions of dollars invested in cancer research in large part because our preventions and treatments target risk factors and consequences rather than root causes.

How close is science to identifying the true causes of cancer? A correct and complete theory of tumorigenesis must explain how cancers can be at once so different genetically and yet so similar in phenotype. It should accommodate both the rare congenital cancers of childhood and the common acquired neoplasms of old age. It must admit non-mutagenic carcinogens and other anomalous observations.

The dominant paradigm of the late 20th century—that sequential mutations to key oncogenes and tumor suppressor genes are both necessary and sufficient[1]—now seems clearly inadequate to these challenges. In a 2003 *Scientific American* article[2], I reviewed several newer theories that may do a better job. This talk reviews the progress made on those ideas over the past four years, and offers a challenge to the chromosomal research community to keep its eye on the big picture.

REFERENCES

1. Hahn WC, Weinberg RA. Rules for making human tumor cells. *N Engl J Med*; 2002; 347: 1593-1603.
2. Gibbs WW. Untangling the roots of cancer. *Sci Am* 2003; 289(1): 56-65.

2. *Relationship between the karyotypes and phenotypes of cancer cells*

Alice Fabarius[1], Ruhong Li[2], George Yerganian[3], Ruediger Hehlmann[1], and Peter Duesberg[1,2]

[1] *III. Medizinische Klinik Mannheim of the University of Heidelberg at Mannheim, Wiesbadener Str.7-11, 68305 Mannheim, Germany*
[2] *Department of Molecular and Cell Biology, Donner Laboratory, UC Berkeley, Berkeley, CA 94720*
[3] *Cytogen Research & Development, 89 Bellevue Hill Rd., Boston, MA 02132, and Foster Biomedical Research Laboratory, Brandeis University, Waltham, MA 02254*

Several researchers, including us, have recently proposed that specific karyotypes, rather than specific mutations, generate the "biochemical individuality" of cancers (Foulds, 1969), defined by different growth rates, metabolisms, drug-resistances, metastases and individual cell morphologies. According to our theory independent karyotypic evolutions generate cancers, much like new phylogenetic species. To allow such evolutions in the lifetime of an organism, the normal karyotype must be destabilized, but not the genes. The karyotype is destabilized by aneuploidy, because aneuploidy unbalances conserved teams of proteins that segregate, synthesize and repair chromosomes. And aneuploidy is induced either by carcinogens or spontaneously.

Here we tested this theory using a new system that virtually excludes spontaneous mutation. In this system, 50% of normal human muscle cells became aneuploid and 5 per 10^6 formed foci of transformed cells—only 2 months after transfection with 6 virus-activated cellular genes. Analyses of 10 foci revealed the following: (1) individual karyotypes, consisting of one or more stemlines of spontaneously evolving non-random aneuploidies and some random aneuploidies, and (2) individual phenotypes, such as cell morphologies, growth rates and intrinsic resistance to cytosine arabinoside, shared by 5 foci with a common stemline. Due to the short preneoplastic latencies of Mu6 cells several focus-specific karyotypes were already detectable prior to focus formation. We conclude that specific clones of spontaneously evolving karyotypes, rather than specific mutations, generate the individuality of cancers. This answers the age-old question, why even cancers of the same kind do not have consistent karyotypes.

REFERENCES

Duesberg P, Fabarius A, Hehlmann R. Aneuploidy, the primary cause of the multilateral genomic instability of neoplastic and preneoplastic cells. *IUBMB Life* 2004;56:65-81.

Duesberg P, Li R, Fabarius A, Hehlmann R. The chromosomal basis of cancer. *Cell Oncol 2005*;27:293-318.

Kendall, S.D., Linardic, C.M., Adam, S.J. & Counter, C.M. A network of genetic events sufficient to convert normal human cells to a tumorigenic state. *Cancer Res* 2005; 65:9824-8.

Duesberg P. Chromosomal chaos and cancer. *Sci Am* 2007;296:52-9.

3. *Chromosomal instability in oral cancer cells: A generalizable model system for cancer*

Susanne M. Gollin[1], Rahul A. Parikh[1], Shalini C. Reshmi[1], Xin Huang[1], and William S. Saunders[2]

[1] *Department of Human Genetics, Graduate School of Public Health*
[2] *Department of Biological Sciences, University of Pittsburgh and the University of Pittsburgh Cancer Institute; sgollin@hgen.pitt.edu*

We define chromosomal instability as a scenario in which cells proliferate with increasingly defective genomes in terms of chromosomal gains, losses, rearrangements, and gene amplification, resulting in numerical and structural variations on a background of clonal chromosomal alterations. There are several mechanisms by which chromosomal instability occurs, including but not limited to 1) spindle defects including multipolar spindles resulting from abnormalities in centrosomal clustering or other cellular alterations leading to numerical chromosomal alterations, and 2) chromosome breakage due to environmental exposures like smoking and defects in the DNA damage response, resulting in breakage-fusion-bridge (BFB) cycles, leading to structural chromosomal aberrations including gene amplification.

We physically mapped an 11q13 amplicon in oral cancer cells and hypothesized that 11q13 gene amplification occurred as a result of BFB cycles. We showed that the amplicon occurs in the form of an inverted duplication and is statistically significantly more frequently seen in anaphase bridges between cells with 11q13 amplification compared to cells without 11q13 amplification. Next, we mapped the common fragile site, *FRA11F* to a 7.5 Mb region in 11q14.2, distal to the amplicon, and found that all cell lines with 11q13 amplification in the form of a homogeneously staining region (hsr) either lost part or all of *FRA11F*. This suggests that breakage at *FRA11F* may be the first step in 11q13 amplification. Such breakage would lead to loss or haploinsufficiency for distal 11q, including many genes, several of which are key players in the DNA damage response (*ATM, MRE11A,* and *H2AFX*). We hypothesized and then showed that loss of distal 11q leads to a diminished DNA damage response, measured by H2AX focus formation (a variant phosphohistone) and chromosome aberrations. However, we were surprised that 11q loss resulted in loss of sensitivity to ionizing radiation (IR) in a clonogenic survival assay. This unexpected result led us to further studies of the DNA damage response in oral and other cancer cells with distal 11q loss, leading to a mechanism confirmed by resensitization of the cells to IR using RNA interference. Defects in the DNA damage response appear not only to lead to loss of sensitivity to IR, but to chromosomal instability. These studies are expected to lead to further understanding of the biology of cancer and the development of a test for loss of tumor sensitivity to therapy and a small molecule inhibitor treatment that resensitizes tumors to therapy.

REFERENCES

Gollin SM. Mechanisms leading to chromosomal instability. *Semin Cancer Biol* 2005; 15: 33-42.

Huang X, Godfrey TE, Gooding WE, McCarty KS, Gollin SM. Comprehensive genome and transcriptome analysis of the 11q13 amplicon in human oral cancer and synteny to the 7F5 amplicon in murine oral carcinoma. *Genes Chromosomes Cancer* 2006; 45: 1058-69.

Reshmi SC, Huang X, Schoppy DW, Black RC, Saunders WS, Smith DI, Gollin SM. The relationship between FRA11F and 11q13 gene amplification in oral cancer. *Genes Chromosomes Cancer* 2007; 46: 143-54.

Reshmi SC, Roychaudhury S, Yu Z, Feingold E, Potter D, Saunders WS, Gollin SM. Inverted duplication pattern in anaphase bridges confirms the breakage-fusion-bridge (BFB) cycle model for 11q13 amplification. *Cytogenet Genome Res* 2007; 116: 46-52.

Parikh RA, White JS, Huang X, Schoppy DW, Baysal BE, Baskaran R, Bakkenist CJ, Saunders WS, Gollin SM. Loss of distal 11q is associated with DNA repair deficiency and reduced sensitivity to ionizing radiation in head and neck squamous cell carcinoma. *Genes Chromosomes Cancer* 2007; 46: 761-5.

4. Patterns of genome dynamics and cancer evolution

Henry H.Q. Heng, Joshua B. Stevens, Lesley Lawrenson, Guo Liu, Karen J. Ye, Steven W. Bremer, and Christine J.Ye

Center for Molecular Medicine and Genetics, and Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI 48201; hheng@med.wayne.edu

The importance of the chromosome versus the gene as a causative agent in cancer formation has sparked a heated debate. This issue is directly related to two different schools of thought, namely the gene centric or genome centric paradigms of cancer research. For decades we have essentially ignored the evolutionary nature of complex cancer systems due to the influence of reductionist viewpoints and experimental approaches. Cancer research has focused on identifying and characterizing the linear accumulation of gene mutations and the consequent effects on the corresponding pathways. Despite of the fact that chromosome aberrations are nearly universally detected in cancer cases, the gene–centric viewpoint has driven scientists to conclude that chromosome aberrations are a consequence of gene mutations and therefore must be late events. Furthermore, non-clonal chromosome aberrations (NCCAs), the major form of genome variation and the key index for system instability, have been considered "genetic noise" and have been largely ignored.

The study of chromosomes in cancer has been considered a low resolution approach compared with molecular methods such as DNA sequencing and thus said to not offer causative insight. Mounting evidence (including the failure of identifying a handful of common gene mutations from large scale gene sequencing) shows that the long sought after handful of mutated, cancer causing genes do not exist. Our recent findings demonstrate the stochastic nature of genome variation during cancer progression and have illustrated that cancer progression is mainly mediated by genome variation at the chromosome level.

To support the genome-centric view point, we have shown that chromosomal changes determine a global pattern of gene expression by the use of gene expression patterns coupled with karyotype analysis of an in vitro cancer model. We further introduce the concept that genome context (the highest level of genetic organization which defines the genetic network) and not the gene content defines a given genetic system. We also show a number of new chromosomal aberrations including defective mitotic figures (DMFs) and chromosome fragmentation which illustrate how genome instability (through changed genome context) plays a role in generating population heterogeneity which contributes to cancer evolution. Our data suggests that cancer is a disease of probability, and that cancer evolution is mainly driven by instability mediated genome variation as the somatic evolutionary platform is mainly at the genome level. Comparison of the patterns of somatic evolution and organismal evolution strongly supports this concept.

REFERENCES

Heng HH, Stevens JB, Liu G, et al. Stochastic cancer progression driven by non-clonal chromosome aberrations. *J Cell Physiology* 2006; 208: 461-72.

Heng HH. Cancer genome sequencing: the challenges ahead. *BioEssays* 2007; 29: 783-94.

Ye CJ, Liu G, Bremer SW, Heng HH. The dynamics of cancer chromosomes and genomes. *Cytogenet Genome Res* 2007; 118:237-46.

Heng HH. Elimination of altered karyotypes by sexual reproduction preserves species identity. *Genome* 2007; 50: 517-524.

Stevens JB, Liu G, Bremer SW, Ye KJ, et al. Mitotic cell death by chromosome fragmentation. *Cancer Res* 2007; 67: 7686-94.

5. Chromosomal instability and precursors along the path to aneuploidy and cancer in chronic inflammatory gastrointestinal diseases

Peter S. Rabinovitch

Department of Pathology, University of Washington, Seattle, WA, USA

The gastrointestinal tract provides several examples of diseases in which chronic inflammation, damage and repair are associated with increased cancer risk. Patients with two of these disorders, Barrett's esophagus (BE) and ulcerative colitis (UC) are often followed with endoscopic surveillance to detect cancer or cancer precursors at an early curable stage, however without knowing which subset of patients is at highest risk, this procedure is not time or cost-effective. The histopathological steps of the progression of BE and UC from metaplasia → indefinite for dysplasia → low grade dysplasia → high grade dysplasia → cancer are well known, however, inter-observer variability in a diagnosis of histology less than high grade dysplasia makes these grades less reliable predictors of disease progression.

In order to discover intermediate biomarkers that can accurately assess risk of neoplastic progression, we have been studying the chromosomal instability precursors that ultimately lead to aneuploidy and cancer in these diseases. In both BE and UC, chromosomal instability and telomere shortening are present in large fields of histologically non-dysplastic mucosa in early stages of disease[1,2]. The extent of chromosomal instability is correlated with telomere shortening and anaphase bridges, suggesting that telomere shortening may contribute to chromosomal instability by promoting a bridge-breakage-fusion cycles in these diseases. In UC the rate of telomere shortening is such that after 8 years disease duration, colonic epithelial telomeres are as short as those of 65 year old controls and DNA damage markers, such as γH2AX, are elevated, as are markers of senescence. This disorder might thus be thought of as a disease of accelerated epithelial senescence. That there may also be systemic factors in this process is illustrated by the fact that peripheral blood leukocyte telomere lengths in BE patients are independently predictive of esophageal cancer risk (1st to 4th quartile HR=4.7)[3]. It is possible that reduced telomere length is a reflection of increased oxidative damage and reduced repair capacity. Array CGH demonstrates that the earliest structural abnormalities are small interstitial deletions, and that with advancing disease these expand in size and number, culminating in aneuploidy[4]. In BE the earliest interstitial deletions are mainly at chromosomal fragile sites, perhaps because these, and telomeres, are most sensitive to replicative stress after DNA damage.

REFERENCES

1. O'Sullivan JN, Bronner MP, Brentnall TA, Finley JC, Shen W-T, Emerson S, Emond MJ, Gollahon KA, Moskovitz AH, Crispin DA, Potter JD, Rabinovitch PS. Chromosomal Instability in Ulcerative Colitis is Related to Telomere Shortening. *Nature Genetics*, 32:280-4, 2002.
2. Finley JC, Reid BJ, Odze RD, Sanchez CA, Galipeau P, Li X, Self SG, Gollahon KA, Blount PL, Rabinovitch PS. Chromosomal Instability in Barrett's Esophagus is Related to Telomere Shortening. *Cancer Epidemiol Biomarkers Prev* 15:1451-7, 2006.
3. Risques RA, Vaughan TL, Li X, Odze RD, Blount PL, Ayub K, Gallaher JL, Reid BJ, Rabinovitch PS. Leukocyte Telomere Length Predicts Cancer Risk in Barrett's Esophagus. *Cancer Epidemiol Biomarkers Prev* 16:2649-55, 2007.
4. Lai LA, Paulson TG, Li X, Sanchez CA, Maley C, Odze RD, Reid BJ, Rabinovitch PS. Increasing genomic instability during premalignant neoplastic progression revealed through high resolution array-CGH. *Genes Chromosomes and Cancer* 46:532-542, 2007.

6. Numerical aberrations during the development of cervical carcinogenesis: tetraploidy is an early event that precedes most aneuploidy

Andrew J. Olaharski[1], Maria Gonsebatt[2], and David Eastmond[3]

[1] *Discovery and Investigative Safety, Non Clinical Safety, Hoffman-La Roche, Palo Alto, CA*

[2] *Department of Genomic Medicine and Environmental Toxicology, National Autonomous University of Mexico (UNAM), Mexico City, Mexico*

[3] *Environmental Toxicology Graduate Program, Department of Cell Biology and Neuroscience, University of California, Riverside, CA*

Chromosomal instability as manifested by increases in aneuploidy and structural chromosome aberrations is believed to play a critical role in the development of cervical malignancies. Two studies were designed to determine the role of tetraploidy in the formation of aneuploidy and ascertain the occurrence of these alterations during the earlier stages of cervical carcinogenesis. Cervical cell samples, with diagnoses ranging from normal to high-grade lesions, (HSIL) were obtained from 400 women and were evaluated for chromosomal alterations using dual-probe fluorescence in situ hybridization. Cervical cells from a subset of the group were also evaluated for chromosomal instability in the form of micronuclei. The frequencies of cells exhibiting either tetrasomy or aneusomy for chromosomes 3 and 17 increased significantly with disease progression and displayed distinctive patterns where aneusomy was rarely present in the absence of tetrasomy. The frequencies of micronuclei that formed through either chromosomal loss or breakage increased significantly in both the low-grade and high-grade diagnostic categories and were highly correlated with both the number of tetrasomic and aneusomic cervical cells. Additionally, unique chromosomal alterations were observed where either non-random loss of chromosome 17 specific to near-tetraploid aneusomic cells (trisomy 17 and tetrasomy 3) or non-random gain of chromosome 3 specific to near-diploid aneusomic cells (trisomy 3 and disomy 17).

We conclude that tetraploidy and chromosomal instability are related events occurring during the early stages of cervical carcinogenesis that predispose cervical cells to the formation of aneuploidy frequently involving the loss of chromosome 17.

7. *Genomic instabilities, DNA copy number changes and cancer*

Eytan Domany

Department of Physics of Complex Systems, Weizmann Institute of Science, Rehovot 76100, Israel; fedomany@wisemail.weizmann.ac.il

Most types of cancer were found to exhibit very large-scale genomic instabilities: deletions of amplifications of entire chromosomal regions. The classical model which views single-gene alterations as causative, and the chromosomal instabilities mainly as a side effect[1], has been seriously challenged; claims that view these instabilities as the major cause of cancer are forcefully made[2].

We are participating in several studies that aim at studying the extent to which chromosomal instabilities are prevalent in several kinds of cancer. In addition, the role of DNA copy number changes is also investigated. In particular, we addressed claims[3] about lack of correlations between DNA copy number and mRNA expression levels of the corresponding genes in colon cancer. We found[4] that if the data are smoothed to average out "noise" due to other forms of control of individual genes, correlations between SNP (single nucleotide polymorphism) chip data and expression are high and statistically significant in colon cancer. CGH studies and expression profiling of glioblastoma samples[5], and of data obtained from leukemia patients[6] lead to similar conclusions. These investigations allow a fairly reliable determination of aneuploidy from expression data[6].

I will present results of ongoing research on DNA copy number changes in these three types of cancer. In addition, I will describe preliminary results from an ongoing study of cancer initiation and progression[7] in an in vitro experiment, that allows observation of the timing at which chromosomal instabilities arise in the course of the malignant transformation.

REFERENCES

1. Zimonjic D, Brooks MW, Popescu N, Weinberg RA, Hahn WC. Derivation of human tumor cells in vitro without widespread genomic instability. *Cancer Res* 61: 8838–44 2001.
2. Li, R. and Sonik A, S. R., Rasnick D, Duesberg P. Aneuploidy vs. gene mutation hypothesis of cancer: recent study claims mutation but is found to support aneuploidy. *Proc Natl Acad Sci U S A*, 97: 3236-3241, 2000.
3. Platzer, P., Upender, M. B., Wilson, K., Willis, J., Lutterbaugh, J., Nosrati, A., Willson, J. K., Mack, D., Ried, T., and Markowitz, S. Silence of Chromosomal Amplifications in Colon Cancer. *Cancer Res*, 62: 1134-1138, 2002.
4. Tsafrir D, Bacolod M, Selvanayagam Z, Tsafrir I, Shia J, Zeng Z, Liu H, Krier C, Stengel RF, Barany F, Gerald WL, Paty PB, Domany E, Notterman DA. Relationship of gene expression and chromosomal abnormalities in colorectal cancer. *Cancer Res* 66:2129–2137, 2006; Sheffer, M. et al (manuscript in preparation).
5. Shay T, work in progress, in collaboration with M. Hegi et al, CHUV, Lausanne.
6. Hertzberg L, Betts D. R, Raimondi S C, Schäfer B W, Notterman D A, Domany E, Izraeli S. Prediction of Chromosomal Aneuploidy from Gene Expression Data. Genes, *Chromosomes and Cancer* 46;75-86, 2007.
7. Kogan I, Tabach Y, work in progress, incollaboration with V Rotter et al.

8. *Multiple numerical chromosome aberrations in carcinogenesis: the kidney cancer model*

Manuel R. Teixeira

Department of Genetics, Portuguese Oncology Institute-Porto, 4200-072 Porto, Portugal; mteixeir@ipoporto.min-saude.pt

Several neoplasias are characterized by multiple numerical chromosome abnormalities in their karyotypes. Numerical chromosome changes and ploidy shifts theoretically allow the simultaneous alteration of multiple cancer-relevant genes, thereby reducing the number of independent genomic events necessary for carcinogenesis. The molecular explanation for how these numerical karyotypic alterations arise is very scarce.

The cytogenetic patterns that characterize the various kidney carcinoma subtypes offer an opportunity to study their mechanisms of origin. Papillary and chromophobe renal cell carcinomas are characterized by multiple trisomies and monosomies, respectively. To evaluate the role of mitotic checkpoint defects for the karyotypic patterns characteristic of these two renal cell cancer subtypes, the mRNA expression levels of the major mitotic checkpoint genes of the budding-inhibited by benzimidazole family (*BUB1, BUBR1, BUB3*) and of the mitotic arrest deficiency family (*MAD1, MAD2L1, MAD2L2*) were analyzed by real-time quantitative polymerase chain reaction in 30 renal cell cancer samples (11 chromophobe and 19 papillary) and 36 normal kidney tissue samples. *MAD1, MAD2L1*, and *MAD2L2* showed significant expression differences in tumor tissue compared to controls. Chromophobe tumors presented underexpression of *MAD1* and *MAD2L2*, whereas papillary tumors showed overexpression of *MAD2L1*. The expression level of the BUB gene family did not differ significantly from that of normal kidney. On the other hand, the study of 39 clear cell renal cell carcinomas showed overexpression of *BUB1, BUBR1,* and *MAD2L1* and underexpression of *MAD1*. The degree of genomic complexity of clear cell kidney carcinomas measured by comparative genomic hybridization was associated with *BUB1* and *BUBR1* overexpression, as well as with tumor grade. One can therefore conclude that expression changes in *MAD1, MAD2L1,* and *MAD2L2* play a role in renal carcinogenesis characterized by multiple numerical chromosome abnormalities (chromophobe and papillary carcinomas) and that *BUB1* and *BUBR1* overexpression is associated with karyotypic complexity in conventional renal cell carcinomas.

REFERENCES

Teixeira MR, Heim S (2005). Multiple numerical chromosome aberrations in cancer: what are their causes and what are their consequences? *Seminars in Cancer Biology*, 15:3-12.

Pinto M, Soares MJ, Cerveira N, Henrique R, Ribeiro FR, Oliveira J, Jerónimo C, Teixeira MR (2007). Expression changes of the MAD mitotic checkpoint gene family in renal cell carcinomas characterized by numerical chromosome changes. *Virchows Archiv*, 450:379-385.

Pinto M, Vieira J, Soares MJ, Henrique R, Oliveira J, Jerónimo C, Teixeira MR (2007). Association between BUB1 and BUBR1 expression changes and genomic complexity in clear cell renal cell cancer. Submitted.

9. The relationship of chromosomal aneuploidy, nuclear structure, and gene expression in cancer cells

Thomas Ried, Kundan Sengupta, Jordi Camps, Amanda B. Hummon, Hesed M. Padilla-Nash, Kerstin Heselmeyer-Haddad, B. Michael Ghadimi, Marian Grade, and Michael J. Difilippantonio

Genetics Branch, Center for Cancer Research, National Cancer Institute/NIH, Bethesda, MD 20892

Chromosomal aneuploidies are observed in essentially all sporadic carcinomas. Aneuploidy results in tumor specific patterns of genomic imbalances that are acquired early during tumorigenesis, continuously selected for and faithfully maintained in cancer cells. The presence of these aneuploidies in premalignant, dysplastic precursor lesions is strictly associated with increased progression potential to invasive disease; their detection in routinely collected cytological samples is therefore an important aspect of individualized cancer medicine.

In order to dissect the immediate consequences of genomic imbalances on the transcriptome, we generated artificial trisomies in a karyotypically stable diploid, yet mismatch-repair deficient, colorectal cancer cell line and in telomerase immortalized, cytogenetically normal human breast epithelial cells using microcell mediated chromosome transfer. We then used global gene expression levels to determine what affect chromosome copy number increases have on the average expression levels of genes residing on the trisomic chromosomes as well as how these particular aneuploidies affect the regulation of individual genes throughout the entire genome. Our results show that, regardless of chromosome or cell type, chromosomal trisomies result in a significant increase in the average transcriptional activity of the trisomic chromosome. This increase affects the expression of numerous genes on other chromosomes as well. We therefore postulate that the genomic imbalances observed in cancer cells exert their effect through a complex pattern of transcriptional deregulation. These results were corroborated in primary tumors and tumor derived cell lines and support the interpretation that aneuploidy results in a massive disturbance of the transcriptional equilibrium of cancer cells. After having established this correlation of genome copy number and transcript levels, we were curious as to whether aneuploid chromosomes assume a nuclear position similar to wild-type, endogenous chromosomes, which would suggest a possible correlation with transcriptional activity. Using 3D-FISH and confocal laser scanning microscopy, we show that Chromosomes 7, 18, or 19 introduced via microcell-mediated chromosome transfer into the parental diploid colon cancer cell line DLD-1 maintain their conserved position. Our data is therefore consistent with the model that each chromosome has an associated zip code (possibly gene density) that determines its nuclear localization. Whether the nuclear localization determines or is determined by the transcriptional activity of resident genes has yet to be ascertained.

REFERENCES

Grade M, Hormann P, Becker S, et al. Gene expression profiling reveals a massive, aneuploidy-dependent transcriptional deregulation and distinct differences between lymph node-negative and lymph node-positive colon carcinomas. *Cancer Res* 2007;67(1):41-56.

Sengupta K, Upender MB, Barenboim-Stapleton L, et al. Artificially introduced aneuploid chromosomes assume a conserved position in colon cancer cells. *PLoS ONE* 2007;2(2):e199.

10. Low rates of aneuploidy promote tumorigenesis while high rates of aneuploidy cause cell death and tumor suppression

Beth A. A. Weaver, Alain D. Silk, and Don W. Cleveland

Ludwig Institute for Cancer Research and Department of Cellular and Molecular Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0670, USA; bweaver@ucsd.edu

An abnormal chromosome number, a condition known as aneuploidy, is a common characteristic of tumor cells. Because of this correlation, Boveri proposed aneuploidy to be a cause of tumorigenesis 100 years ago[1,2]. However, this hypothesis remained untested due to the difficulty of selectively generating aneuploidy in the absence of other defects, particularly DNA damage.

We determined that cells and mice with reduced levels of the mitosis-specific, centromere-linked motor protein CENP-E develop aneuploidy and chromosomal instability *in vitro* and *in vivo* in the absence of other defects, including DNA damage. CENP-E reduction causes aneuploidy and chromosomal instability due to the missegregation of one (or a few) whole chromosomes per division[3]. As Boveri had proposed, the low rate of whole chromosome aneuploidy caused by CENP-E heterozygosity in the absence of other defects drives an elevated level of spontaneous spleen and lung tumors. However, aneuploidy due to CENP-E heterozygosity suppressed tumors in three different contexts: spontaneous tumors of the liver, tumors caused by treatment with the carcinogen DMBA, and tumors caused by homozygous loss of the p19/ARF tumor suppressor[4]. All three contexts in which CENP-E heterozygosity suppressed tumors had a pre-existing level of aneuploidy that could be increased by depletion of CENP-E, supporting the hypothesis that high rates of chromosome missegregation promote cell death and tumor suppression. Consistently, additional weakening of the mitotic checkpoint by reduction in Mad2 as well as CENP-E resulted in elevated levels of cell death and decreased rates of tumor development as compared to reduction of CENP-E or Mad2 individually. These findings indicate that while low rates of chromosome missegregation promote tumorigenesis, as Boveri had predicted, higher rates of chromosome missegregation produce cell death and tumor suppression.

REFERENCES

1. Boveri T. Ueber mehrpolige Mitosen als Mittel zur Analyse des Zellkerns. Verhr.d. phys. med. Ges. zu Wurzburg, N.F. (available in English translation at: http://8e.devbio.com/article.php?ch=4&id=24) 1902;Bd. 35.
2. Boveri T. Zur Frage der Entstehung maligner Tumoren. Gustav Fischer, Jena, 1914; English translation *The Origin of Malignant Tumors* by Boveri, M. Williams and Wilkins, Baltimore, 1929.
3. Weaver BA, Bonday ZQ, Putkey FR, Kops GJ, Silk AD, Cleveland DW. Centromere-associated protein-E is essential for the mammalian mitotic checkpoint to prevent aneuploidy due to single chromosome loss. *J Cell Biol* 2003;162(4):551-63.
4. Weaver BA, Silk AD, Montagna C, Verdier-Pinard P, Cleveland DW. Aneuploidy acts both oncogenically and as a tumor suppressor. *Cancer Cell* 2007;11(1):25-36.

11. Recurrent genomic alterations in prostatic preneoplasias and in prostate cancer

Jeremy A. Squire[1,2,3], Anthony Joshua1[4], Bisera Vukovic[1], Simon Hughes[5], Jane Bayani[1,3], Olga Ludlovski[1], Paula Marrano[1], Paulo Nuin[6], Andrew Evans[3,7], and Maisa Yoshimoto[1]

[1] *Division of Applied Molecular Oncology, Ontario Cancer Institute, Toronto, Canada*
[2] *Department of Medical Biophysics, University of Toronto, Toronto, Canada*
[3] *Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada,*
[4] *Department of Medical Oncology, Princess Margaret Hospital, Toronto, Canada*
[5] *Tumour Biology Laboratory, John Vane Science Centre, Cancer Research UK, Clinical Centre, Queen Mary's School of Medicine and Dentistry, UK*
[6] *Division of Cancer Genomics and Proteomics, University Health Network, Toronto, Canada*
[7] *Department of Pathology, University Health Network, Toronto, Ontario, Canada*

Prostate cancer (CaP) is a heterogeneous neoplasm both with regard to its development, molecular abnormalities and clinical course. An understanding of the early molecular events in prostatic carcinogenesis that may underlie both the molecular and clinical heterogeneity is essential for improved diagnosis and therapies.

We, and others, have postulated that age-associated telomere-attrition and subsequent induction of senescence and progression through "crisis" may be an important triggering mechanism for genomic alterations such as translocations in CaP (Vukovic et al, 2003; Joshua et al., 2007). More than 50% of CaP tumors carry a specific gene fusion between the androgen responsive *TMPRSS2* locus to an *ETS* family gene which may involve quite complex genomic alterations (Yoshimoto et al., 2006, 2007a).

We have recently shown that PTEN genomic losses are frequent events in preneoplastic PIN lesions (~20%) of the prostate and in CaP. Interstitial hemizygous genomic deletions of the *PTEN* tumor suppressor gene and neighbouring loci at 10q23 are detected in 40% CaP. Moreover the presence of *PTEN* genomic losses at surgery can be predictive of a shorter time to biochemical recurrence and that homozygous *PTEN* deletions portend early recurrence and metastatic disease (Yoshimoto et al., 2007b). Although *PTEN* inactivation is strongly associated with CaP onset and progression, the genetic and molecular events leading to *PTEN* deletion are poorly understood. We have examined the breakpoint regions associated with *PTEN* deletions and analyzed involvement of flanking genomic regions using FISH analysis of CaP tissue microarrays. Four-color FISH was performed on the TMA using 6 BAC clones spanning both flanking *PTEN* genomic region and the gene locus, and centromeric DNA probe (CEP10) for region 10p11.1-q11.1. These analyses showed that small hemizygous *PTEN* deletion is usually accompanied by a larger second event, which might involve deletion of flanking loci. We also, investigated the location of microhomologies in this region of 10q to determine whether non-allelic homologous recombinational repair errors may initiate these deletion events. In silico analysis identified at least five non-redundant regions of microhomology within chromosome 10q flanked by paired intrachromosomal segmental duplications. These findings draw attention to the impact of the characterization of *PTEN* genomic loss, and complementary or independent candidate flanking genes involved in CaP. The consistent occurrence of genomic abnormalities involving the *TMPRSS2-ETS* and *PTEN* loci and the resulting downstream signaling effects suggest the importance of telomere attrition, and genomic instability as crucial factors in the emergence of the common genomic aberrations in preneoplastic lesions and in CaP.

REFERENCES

Vukovic B, et al. *Oncogene*. 2003; 22(13):1978-87.
Joshua AM, et al. *Neoplasia*. 2007;9(1):81-9.
Yoshimoto M, et al. *Neoplasia*. 2006;8(6):465-9.
Yoshimoto M, et al. *Genes Chromosomes Cancer*. 2007a;46(9):861-3.
Yoshimoto M, et al. *Br J Cancer*. 2007b;97(5):678-85.

12. Genetic instability and clonal outgrowth/ evolution in the upper aerodigestive tract

Walter N. Hittelman

Department of Experimental Therapeutics, University of Texas M. D. Anderson, Houston, Texas 77030; whittelm@mdanderson.org

Aerodigestive tract tumorigenesis is hypothesized to reflect a "field cancerization" process whereby the whole tissue is chronically exposed to carcinogen insult (e.g., tobacco smoke) and accumulates genetic genomic damage[1]. Global tissue injury is postulated to induce proliferative wound healing and inflammatory processes which transform DNA damage into permanent genetic and epigenetic changes. With continued carcinogen insult, the tissue is thought to undergo a multistep process of tumorigenesis in association with multifocal areas of ongoing genetic instability, preferential clonal outgrowth, and clonal evolution[2].

To examine these processes in the lungs of chronic smokers, we obtained bronchial biopsies at the initiation of chemopreventive interventions and carried out biomarker analyses. Using Ki67 as a marker for proliferative status, we found evidence for increased proliferation in the bronchial epithelium of current smokers, the level of which was related to smoking intensity. Using chromosome in situ hybridization as a marker for chromosome copy numbers per cell and spatial analyses to examine localized clonal outgrowths, we found evidence for ongoing genetic instability (related to smoking packs/day) and clonal outgrowth (related to smoking packyears). With smoking cessation, both the levels of ongoing genetic instability and proliferation were found to decrease in most subjects[3,4]. Despite smoking cessation, however, some individuals continued to exhibit increased proliferation and ongoing genetic instability in their bronchial epithelium.

To better understand the molecular determinants of an ongoing process of genetic instability and clonal outgrowth in former smokers, we established three dimensional, organotypic cultures where bronchial epithelial cells at different stages of the multistep tumorigenesis process were grown on a collagen-coated filter at an air-liquid interface. Proliferation and genetic instability were examined in three dimensions using laser scanning confocal microscopy. Preferential clonal outgrowth was examined in this model system by labeling different bronchial epithelial populations with living fluorescence tags (e.g., GFP, YFP, CFP) and monitoring preferential clonal outgrowth using live cell, fluorescence imaging. Normal and immortalized bronchial epithelial cells (transfected with hTERT and cdk4) were found to proliferate only at the basal layer and showed low levels of genetic instability. In contrast, more advanced bronchial epithelial cells continued to proliferate away from the basal layer and showed increased frequencies of mitotic errors and cells exhibiting phospho-H2AX staining of a histone variant. Pulse-chase studies with BrdU labeling indicated that chromosome bridges at anaphase preferentially involved late replicating regions. These results suggest that ongoing genetic instability in the absence of carcinogen exposure may be a result of spatially dysregulated proliferative control. Chemoprevention strategies that re-regulate the spatial aspects of proliferation in the aerodigestive tract might therefore be expected to decrease ongoing genetic instability and clonal evolution, slow the multistep tumorigenesis process, and delay or prevent cancer onset.

REFERENCES

1. Hittelman WN. Genetic Instability in Epithelial Tissues at Risk for Cancer. *Ann NY Acad Sci* 2001;952(1):1-12.
2. Hittelman WN. Clones and Subclones in the Lung Cancer Field. *J. Natl. Cancer Inst.* 1999;91(21):1796-9.
3. Lee JJ, Liu D, Lee JS, Kurie JM, Khuri FR, Ibarguen H et al. Long-Term Impact of Smoking on Lung Epithelial Proliferation in Current and Former Smokers. *J. Natl. Cancer Inst.* 2001;93(14):1081-8.
4. Hittelman WN, Liu DD, Kurie JM, Lotan R, Lee JS, Khuri F et al. Proliferative Changes in the Bronchial Epithelium of Former Smokers Treated With Retinoids. *J. Natl. Cancer Inst.* 2007;99(21):1603-12.

13. D•A•T•E analysis of cancer microarray data

David Rasnick

Boveran, Inc., 4631 NW 31st Avenue, F-510 Fort Lauderdale, FL 33309; drasnick@mac.com

The gene mutation hypothesis of cancer has led to numerous microarray experiments in search of cancer-specific genes. However, the published "genetic signatures" are quite unstable and not reproducible[1]. The aneuploidy theory of cancer predicts that all strategies for coming up with a stable list of cancer genes short of the whole genome will fail because of intrinsic genomic instability[2,3].

The theoretical basis of DATE (**D**ifferentiation, **A**daptation, **T**ransformation, **E**volution) analysis (founded on Metabolic Control Analysis[4]: MCA) sets it apart from all other bioinformatics approaches, which are fundamentally statistical in nature. DATE analysis differs from MCA in that its essential task lies in the *comparison* of phenotypes rather than in the precise definition or description of each. Macroscopic phenotypes are determined by tens of thousands of genes, gene products, and metabolites, each making a small contribution on the order of 10^{-5} to the phenotype[4]. In place of the daunting task of monitoring the kinetics details of thousands of individual cellular components, DATE analysis considers, instead, their aggregate effects[2]. F_a quantifies the relative overall cellular activity of aneuploid cancer cells compared to normal tissue from which they derive. The variable π is the fold-change in the differential expression of aneuploid cells compared to diploid precursors. It is the fraction ϕ (equivalent to the control strength of MCA) of the genome undergoing differential expression–not the magnitude π of the differential expression–that controls phenotypic transformation[2].

The simplifying assumptions of DATE analysis were validated for large data sets. Reproducible values of F_a, RNA index, and ϕ were generated on random subsets of transcript microarray data from as little as 5% of the whole. F_a, RNA index, and ϕ were determined from microarray data for lymphoma and cancers of the breast, colon, kidney, ovary, pancreas, and stomach. Histograms of the distribution of transcripts for the normal tissues were symmetrical with little spread (ϕ=0.03, RNA index=1.03). Histograms for all the cancers, however, were irregular with characteristically large values of ϕ(0.42-0.91) and RNA index (1.5-2.4), indicating advanced malignancies[2]. Current laboratory diagnoses of cancer are based on interpretations that are unavoidably subjective. Consequently, false positive and false negative diagnoses are common. D (based on Shannon entropy of histogram data) and γ (measure of the difference between ordered and random distributions of transcripts) are introduced as quantitative and objective measures of the genetic instability inherent in cancer cells. DATE analysis was performed on the microarray data from 36 invasive ductal carcinomas of the breast which had clinical data[5]. The ductal carcinoma patients were sorted by increasing values of D (2.75-3.05) and γ(2.8-5.8). Grade 3 tumors were concentrated at high values of D and γ. The few examples of Grade 1 favored low values of D and γ. Grade 2 tumors were disperse but tended to low and intermediate values of D and γ. It is likely intermediate Grade 2 is so subjective and uninformative as to be of little value. This was recognized some years ago for cervical cancer when the intermediate category CIN-2 was eliminated. Now there are only low and high grade cervical lesions. The DATE analysis results identified a Grade 1 breast cancer (D=3.05, γ=4.3) that was likely misclassified and probably highly malignant.

REFERENCES

1. Michiels S, Koscielny S, Hill C. Interpretation of microarray data in cancer. *Br J Cancer*. 2007;96(8):1155-8.
2. Rasnick D, Duesberg PH. How aneuploidy affects metabolic control and causes cancer. *Biochem J*. 1999;340(3):621-30.
3. Duesberg P, Li R, Sachs R, Fabarius A, Upender MB, Hehlmann R. Cancer drug resistance: The central role of the karyotype. *Drug Resist Updat*. 2007; 10:51-58.
4. Kacser H, Burns JA. The molecular basis of dominance. *Genetics*. 1981;97:639-66.
5. Zhao H, Langerod A, Ji Y, Nowels KW, Nesland JM, Tibshirani R, et al. Different gene expression patterns in invasive lobular and ductal carcinomas of the breast. *Mol Biol Cell*. 2004;15(6):2523-36.

14. *Evaluation of DNA-ploidy heterogeneity in gastric cancers*

Osterheld MC[1], Laurini RN[2], Saraga EP[3], and Bosman FT[1]

[1] *University Institute of Pathology, Lausanne, Switzerland*
[2] *University Hospital of Lund, Sweden*
[3] *Laboratory Cypa, Ruchonnet 30, Lausanne Switzerland*

Prognostic value of DNA-ploidy in gastric cancers is still a matter of controversy. A possible explanation for the discrepant results reported in the literature could be sampling error in tumours with multiple stemlines differing in DNA-ploidy.

In order to determine wether or not such heterogeneity exists and plays a role in the biology of gastric cancers we have analysed two different types of gastric carcinoma; the early gastric carcinoma (EGC) and the advanced gastric carcinoma (AGC). We have performed DNA-ploidy analysis on multiple samples provided from a group of 17 EGC of which 8 were pure intramucosal and 9 were infiltrating into the sub-mucosa. Then we have analysed 16 AGC, according to the same procedure.

We found an aneuploid DNA-stemline in 8 EGC more often in tumours invading into the sub-mucosa (5/9) than in pure mucosal tumours (3/8). Multiple DNA-stemlines were found more frequently in submucosal infiltrating tumours (4/5). Among the 16 AGC cases, 15 revealed DNA-aneuploid with heterogeneity in 4 cases.

In conclusion we have observed that 53% of EGC were diploid compared to only 6% of AGC. Heterogeneity was found in 13% intramucosal EGC, 44% in submucosal EGC and 26% of AGC.

These results are consistent with the hypothesis of stepwise ploidy progression: from diploid in most EGC to aneuploid but heterogeneous in infiltrating EGC to aneuploid but homogeneous in AGC.

This is in agreement with the notion that the development of a single aneuploid, more aggressive, cell clone is a crucial mechanism in the progression from early to advanced gastric cancer.

REFERENCES

Osterheld MC, Caron L, Demierre M, Laurini R et al.. DNA-ploidy in advanced gastric carcinoma is less heterogeneous than in early gastric cancer. *Cell Oncol* 2004; 26:21-29.

Osterheld MC, Laurini R, Saraga EP. Evaluation of heterogeneity of DNA ploidy in early gastric cancers. *Anal Cell Pathol* 1999; 19:67-72.

Osterheld MC, Laurini R, Saraga EP. Early gastric carcinoma with focal advanced cancer : a particular subtype of gastric carcinoma. *Hum Pathol* 1998; 29: 815-819.

Yonemura Y, Matsumoto H, Ninomiya I et al. Heterogeneity of DNA ploidy in gastric cancer. *Anal Cell Pathol* 1992; 4(2):61-67.

15. DNA-aneuploidy: A diagnostic and prognostic marker for tumor cells

Alfred Böcking and Natalia Pomjanski

Institut für Cytopathologie, Heinrich-Heine-Universität, Düsseldorf, Germany; boecking@uni-duesseldorf.de

DNA-aneuploidy has been defined as a plus or minus of >10% of DNA-mass per nucleus in G0/1-phases of proliferating cells. It is caused by chromosomal aneuploidy (unbalanced numerical and/or structural aberrations) in growing cells. As this phenomenon does neither occur in normal, nor in regenerating or polyploidising cells, it can be used as a sensitive and specific marker to identify cells, that have been transformed either to benign (e.g., adenomas) or to malignant neoplasia (e.g., carcinomas).

Here we report on our experience with the early detection of conjunctival-, oral- and cervical cancers detecting DNA-aneupoidy by image-cytometry on brush biopsies. DNA-aneuploidy can be identified in isolated cells or nuclei by flow- or image-cytometry after specific staining of their DNA and internal calibration with normal reference cells. The degree of DNA-aneuploidy, caused by increasing chromosomal aneuploidy during tumor progression can additionally be used for grading malignancy and for the differentiation between some benign and malignant tumors.

Furthermore, we report on the prognostic significance of DNA-grading for giant cell tumors of the bones, neuroendocrine tumors of the intestines and borderline tumors of the ovary. In salivary gland tumors DNA-aneuploidy can be used to differntiate between benign adenomas and carcinomas.

In conclusion: DNA-aneuploidy, reflecting chromosomal aneuploidy, is a very sensitive and highly specific diagnostic marker for the early identification of tumor cells in human tissues. In most tissues it is specific for (prospective) malignancy. The degree of DNA-aneuploidy, reflecting tumor progression and degree of chromosomal chaos is a suitable marker for grading the malignant potential of most tumors. DNA-image-cytometry can thus be used as an adjuvant method in routine diagnostic cytopathology.

REFERENCES

Remmerbach et al.: Cytologic and DNA-cytometric early diagnosis of oral cancer. *Anal Cell Pathol* 22 (2001) 211-221

Maraki et al.: Cytologic and DNA-cytometric very early diagnosis or oral cancer. *J Oral Pathol Med* 33 (2004) 398-404

Raatz et al.: Prognostic impact of DNA-image-cytometry in neuroendocrine (carcinoid) tumours. *Cell Oncol* 26 (2004) 81-88

Böcking and Nguyen: Diagnostic and prognostic use of DNA-image-cytometry in cervical squamous intraepithelial lesions and invasive carcinoma. *Cancer Cytopathol* 102,1 (2004) 41-54

Grote et al.: Identification of progressive cervical epithelial abnormalities using DNA image cytometry, *Cancer Cytopathol* 102,6 (2004) 373-379

16. *Missing evidence in cancer genetics: The retinoblastoma paradigm*

Domenico Mastrangelo[1], Aldo Di Leonardo[2], Laura Lentini[2], Sonia De Francesco[1], and Theodora Hadjistilianou[1]

[1]*Department of Ophthalmology, Ocular Oncology Unit,University of Siena,Italy*
[2]*Department of Cell and Developmental Biology "A.Monroy," University of Palermo,Italy*

Retinoblastoma (Rb) is the most common primary malignant intraocular tumour in childhood, with an incidence of 1 in 15,000 live births. The "two hit" theory, formulated by Knudson in 1971 to explain the variegated clinical expressions of the disease, led to the theory of the so called tumour suppressor genes and the identification of the Rb1 as the prototype of such genes. Mutations of the Rb1 gene are now commonly believed to be the "cause" of retinoblastoma. During the last three decades or more, little or no doubt has been cast by scientists worldwide on this unproved belief. However, the role of gene mutations in the genesis of cancer has been more recently questioned, and aneuploidy has emerged as the main cause of the disease.

Furthermore, although the "two hit" theory, based on two mutational events affecting the Rb1 gene, is still largely used to explain the genesis of retinoblastoma, it makes predictions, concerning the age distribution of the tumour, its mode of "transmission" (hereditary retinoblastoma), and its pathogenesis, which are not fulfilled by the clinical and epidemiological evidence.

Moreover, a number of other genes and epigenetic mechanisms, and aneuploidy itself seem to be involved in the genesis of retinoblastoma, thus excluding any possible "causative" role of the hypothesized biallelic mutations affecting a single gene (the Rb1).

Overall, epidemiological, clinical, and more recent biological and genetic evidence indicates that the "two hit" theory represents a rather simplistic, outdated, and unreliable model to explain tumour development and clinical evolution of retinoblastoma. In view of this, the authors propose to abandon the mutation model and concentrate research on epigenetic factors and aneuploidy in order to improve diagnosis and treatment of the disease, and the quality of life of the affected patients.

REFERENCES

Knudson AG: Mutation and Cancer: Statistical Study of Retinoblastoma. *PNAS* 1971. 68 (4), 820 - 823

Duesberg P: Chromosomal chaos and cancer. *Sci Am*. 2007;296(5):52-9

Mastrangelo D, De Francesco S, Di Leonardo A, Lentini L, Hadjistilianou T.: Retinoblastoma epidemiology: does the evidence matter? *Eur J Cancer*. 2007;43(10):1596-603

17. The genetic basis of Fanconi anemia and other heritable chromosome instability syndromes

Holger Hoehn[1], Kornelia Neveling[1], Daniela Endt[1], Ralph Melcher[2], and Detlev Schindler[1]

[1] *Department of Human Genetics, University of Wurzburg; hoehn@biozentrum.uni-wuerzburg.de*
[2] *Department of Medicine II, University of Wurzburg; melcher_r@klinik.uni-wuerzburg.de*

Fanconi anemia (FA) is a cancer-prone, multisystem disorder caused by biallelic mutations in at least 13 different genes, including BRCA2, PALB2 and BRIP1. Neoplasias among FA patients include mostly acute myeloblastic leukemia and squamous cell carcinomas of the aerodigestive tract and the anogenital region, but there are also toddlers with medulloblastoma and adults with bilateral breast cancer. Like in any of the other chromosomal breakage syndromes, primary aneuploidy is rare. As a consequence of impaired homologous recombination repair, FA cells are defective in the removal of DNA-interstrand crosslinks and stalled replication forks. This renders these cells exquisitely sensitive towards DNA-crosslinking agents and, most notably, to the DNA-damaging effects of oxygen. Accordingly, mitomycin-C or cisplatinum-treated FA cells typically also display chromatid-type lesions and multiradial exchanges between non-homologous chromosomes. Exposing FA cells to hypoxyic culture conditions all but eliminates their chromosomal instability and restores a normal cell cycle progression[1]. FA may thus represent the only human model of the "free radical" theory of ageing.

Somatic reversion events (due to intragenic crossover, gene conversion, back mutation, or compensating second site mutations) have been observed in patients with Fanconi anemia and in patients with Bloom syndrome[2]. These are highly instructive experiments of nature since a single mutational event suffices to restore the genetic instability cellular phenotype to completely normal. The phenomenon of somatic reversion confirms that single gene mutations cause chromosomal instability, thereby increasing the likelihood of genomic imbalance and, subsequently, the likelihood of neoplastic cell growth.

Other familial, early onset cancers are frequently caused by biallelic inactivation of genomic caretaker genes, examples being TP53, BRCA1, BRCA2, WRN, ATM, BLM and FANCA-I. At the cellular level, inactivation of caretaker genes leads to chromosomal instability, reflecting inability to properly recognize and/or repair genetic damage caused by exogenous or endogenous agents. Chromosomal alterations are strikingly different among caretaker gene defects, indicating involvement of the respective genes in different genomic maintenance functions (e.g., NER, HR, NHEJ, etc). WRN helicase defects cause what we have called "variegated translocation mosaicism" (VTM)[3]: multiple chromosomal rearrangements emerge in a clonal fashion, mostly side by side with diploid cells. During propagation in vitro, a given VTM cell clone may expand or disappear over time (clonal succession and clonal attenuation). The phenomenon of VTM may contribute to the early occurrence of mostly mesenchymal tumors in Werner-syndrome patients.

REFERENCES

1. Schindler D, Hoehn H (2007) *Fanconi Anemia. A paradigmatic disease for the understanding of Cancer and Aging.* Karger, Basel, New York
2. Schindler D, Hoehn H (1988) *Am J Hum Genet* 43:429-35
3. Hoehn H, et al. (1975) *Cytogenet Cell Genet* 15:282-98; Salk D, et al. (1985) *Adv Exp Med Biol* 190:541-6; Melcher R, et al. (2000) *Cytogenet Cell Genet* 9:180-5.

18. Integration of DNA copy number and expression microarray data reveals 7 putative oncogenes in 3 amplicons at 20q involved in colorectal adenoma to carcinoma progression

Beatriz Carvalho[1], Cindy Postma[1], Sandra Mongera[1], Erik Hopmans[1], Sharon Diskin[2], Mark A. van de Wiel[1,3,4], Wim van Criekinge[5], Olivier Thas[6], Anja Matthäi[7], Jeroen Meijerink[8], Jochim Terhaar sive Droste[9], Mike Craanen[9], Evelin Schröck[7], Bauke Ylstra[1], and Gerrit A. Meijer[1]

[1] *Department of Pathology,VU University medical center, Amsterdam 1081HV, The Netherlands*
[2] *Division of Oncology, Children's Hospital of Philadelphia and Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, USA; Penn Center for Bioinformatics (PCBI), University of Pennsylvania*
[3] *Department of Biostatistics, VU University medical center, Amsterdam 1081HV, The Netherlands*
[4] *Department of Mathematics, Free University (VU), Amsterdam 1081 HV, The Netherlands*
[5] *Laboratory of Bioinformatics and Computational Genomics, Department of Molecular Biotechnology, Faculty of Bioscience Engineering, Ghent University, Ghent B-9000, Belgium*
[6] *Department of Applied Mathematics, Biometrics and Process Control, Ghent University, Ghent B-9000, Belgium*
[7] *Technical University Dresden, Medical Faculty Carl Gustav Carus, Institute for Clinical Genetics, 01307 Dresden, Germany*
[8] *Department of Surgery, VU University medical center, Amsterdam 1081HV, The Netherlands*
[9] *Department of gastroenterology, VU University medical center, Amsterdam 1081HV, The Netherlands*

Chromosomal instability (CIN) is a hallmark of colorectal cancer progression, occurring in 85% of colorectal cancers. Gain of the long arm of chromosome 20 is one of the most prominent features of adenoma to carcinoma progression in CIN colorectal cancers, although the oncogene(s) underlying this chromosomal gain are still unknown. In addition, 20q gain is indicative of patient outcome in colorectal cancer. In the present study we investigate the effects of chromosomal instability on gene expression in colorectal adenoma to carcinoma progression, focusing on gain of chromosome 20, with the aim of identifying the oncogenes in this amplicon.

We have analysed two independent series of colorectal tumours, containing 34 non-progressed adenomas, 41 progressed adenomas (i.e., adenomas that harbour already a focus of cancer, also called malignant polyps) and 33 adenocarcinomas, and studied DNA copy number alterations by array CGH and mRNA expression by microarray analysis. Data analysis was done focusing on putative oncogenes whose expression was correlated with DNA copy number gain of the genomic region involved.

Three small regions of overlap (SRO's) of copy number gain were defined and seven genes within these regions showed overexpression in progressed adenomas and carcinomas when compared to non-progressed adenomas.

With this approach seven genes were identified as having a putative oncogenic role in CIN related adenoma to carcinoma progression.

REFERENCES

Ylstra B, van den IJssel I, Carvalho B, Brakenhoff RH, Meijer GA. BAC to the future! or oligonucleotides: a perspective for micro array comparative genomic hybridization (array CGH). *Nucleic Acids Res* 2006;34(2): 445-50.

Coe BP, Ylstra B, Carvalho B, Meijer GA, Macaulay C, Lam WL. Resolving the resolution of array CGH. *Genomics* 2007; 89(5):647-53.

Postma C, Terwischa S, Hermsen MA, van dS, Jr., Meijer GA. Gain of chromosome 20q is an indicator of poor prognosis in colorectal cancer. *Cell Oncol* 2007;29(1):73-5.

Derks S, Postma C, Moerkerk PT, van den Bosch SM, Carvalho B, Hermsen MA, Giaretti W, Herman JG, Weijenberg MP, de Bruine AP, Meijer GA, van Engeland M. Promoter methylation precedes chromosomal alterations in colorectal cancer development. *Cell Oncol* 2006;28(5-6): 247-57.

19. TnT: T antigen and telomerase, an explosive route to cancer

F. Andrew Ray

Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO 80524; fa.ray@colostate.edu

Early this century when Hanahan and Weinberg enumerated the six phenotypes that they defined as the "Hallmarks of Cancer," they segregated out genomic instability as "An Enabling Characteristic" rather than as one of the hallmarks. Reading more carefully, we learn that they have set genomic instability alone as a mechanism "that enables evolving populations of premalignant cells to reach these six biological endpoints." The notion was not new, however, the hypothesis having been elegantly summarized by Peter Nowell in 1976.

Human fibroblasts do not become spontaneously immortal or tumorigenic when they are cultured in vitro. In the 1980's, a number of reports began to emerge that suggested that human cells could be transformed, albeit rarely, by gamma rays, carcinogens or certain viruses. The common denominator of these studies was repetitive treatments, successive for radiation and carcinogens, continuous for viruses. We began working on a model using the SV40 virus. The large T antigen was identified as the suspect carcinogen and constructs were made that allowed us to test the hypothesis that T antigen was necessary and sufficient for immortalization and transformation to tumorigenicity of otherwise refractive human fibroblasts. Furthermore we suspected that the protein performed this role by causing genome instability.

The results were dramatic, when a large T antigen gene was transfected into human diploid fibroblasts virtually every cell expressing the protein had some observable chromosome damage when metaphase spreads were observed using crude Giemsa staining followed by aberration scoring. Little if any transformed traits analogous to the hallmarks of cancer were observed. Some 30-odd populations were expanded to greater than 10 million cells per culture each and then grown, past the normal senescence stage to an apoptotic crisis stage. All but 10 of roughly 300 million cells died. These 10 cells went on to form immortal cell lines that had become telomerase positive. These cell lines, continued to evolve in culture and acquire the "hallmarks" at different rates and eventually several of them became tumorigenic, after several hundred population doublings.

In order to determine how T antigen acted as a genomic destabilizer, we made a series of mutations in the gene and tested them for the ability to cause chromosome damage in human fibroblasts. The effect of these mutations on the ability of T antigen to cause chromosome damage will be discussed in the context of genes known to modulate genomic stability. The overall transformation model is in complete agreement with an enabling role for genomic instability in the process of carcinogenesis.

REFERENCES

Hanahan D, Weinberg RA The hallmarks of cancer. *Cell* 2000; 100: 57-70.

Nowell PC The clonal evolution of tumor cell populations. *Science* 1976; 194: 23-28.

Ray FA, Peabody, DS, Cooper, JL et al. SV40 T antigen alone drives karyotype instability that precedes neoplastic transformation of human diploid fibroblasts. *J. Cell. Biochem.* 1990; 42: 13-31.

Ray FA, Waltman MJ, Lehman JM et al. Identification of SV40 T-antigen mutants that alter T-antigen-induced chromosome damage in human fibroblasts. *Cytometry* 1998; 31: 242-50.

20. Spectrum of chromosomal aneuploidy in lymphocytes of workers exposed to benzene and leukemia risk

Luoping Zhang[1], Q. Lan[2], W. Guo1, A. Hubbard[1], G. Li[3], S.M. Rappaport[1], M. Shen[2], R. Vermeulen[2], S. Yin[3], N. Rothman[2], Martyn T. Smith[1]

[1] *School of Public Health, University of California, Berkeley, CA 94720*
[2] *Division of Cancer Epidemiology and Genetics, NCI, NIH, DHHS, Bethesda, MD 20892*
[3] *Chinese Center for Disease Control and Prevention, Beijing, China*

Aneuploidy, a numerical alteration in chromosomes, is a remarkable common feature (>70%) of human cancer (Weaver, 2006). Chromosomal aneuploidy has been detected not only in benzene-related leukemia and pre-leukemia patients, but also in healthy workers with current exposure to benzene (Zhang, 2002), suggesting that aneuploidy precedes, and may be a potential mechanism underlying benzene-induced leukemia. Previously, we applied a novel fluorescence *in situ* hybridization (FISH) technique called OctoChrome FISH, allowing the detection of aneuploidy of all 24 chromosomes simultaneously, in a small pilot study of benzene-exposed workers (Zhang, 2005a). We reported that benzene produces selective effects on certain chromosomes but not others both in the lymphocytes of exposed workers (Zhang, 2005a) and in human cells treated with benzene metabolites *in vitro* (Zhang, 2005b). In order to address the effects of different benzene exposure levels, we expanded our investigation to a study of 74 Chinese workers in 3 exposure categories (27 unexposed controls, 22 exposed to < 10 ppm, and 25 to ≥10 ppm benzene). There was a statistically significant dose-dependent increase across these exposure categories for monosomy rates of only chromosomes 5, 6, 7, 10, 12, 14, 16 and 19 (p_{trend} < 0.05, incidence rate ratio: IRR ≥ 1.3), and for trisomy rates of only chromosomes 6, 10, 14, 16, 19 and 21 (p_{trend} < 0.05, IRR ≥ 1.5). To directly test the potential selectivity of benzene on chromosomal aneuploidy, we compared effects for chromosomes 5, 6, 7, 10, 12, 14, 16 and 19 combined together as one group to effects in all remaining chromosomes, and found that rates of both monosomy ($p < 0.001$) and trisomy ($p = 0.003$) were significantly different across the two groups. Further, plotting both monosomy and trisomy data for all 24 chromosomes, against continuous benzene exposure levels using a spine analysis, reveals a high variability among individual chromosomes and very different dose-response curves. Finally, when comparing aneuploidy rates in the lower exposed subjects (< 10 ppm benzene) with unexposed controls, statistically significant increases were detected only for trisomy 10 and monosomy 6 and 10 ($p < 0.05$, IRR > 1.5). Overall, our current results suggest that the chromosomes differ in their dose-response to benzene-induced monosomy and trisomy. Chromosomes 5, 6, 7, 10, 12, 14, 16, and 19 comprise the most sensitive group and investigation of their precise roles in benzene-induced leukemogenesis are warranted.

REFERENCES

Weaver BA, Cleveland DW. Does aneuploidy cause cancer? *Curr Opin Cell Biol*. 2006; 18(6):658-67.

Zhang L, Eastmond DA, Smith MT. The nature of chromosomal aberrations detected in humans exposed to benzene. *Crit Rev Toxicol.* 2002; 32(1):1-42.

Zhang L, Lan Q, Guo W, Li G, Yang W, Hubbard AE, et al. Use of OctoChrome fluorescence in situ hybridization to detect specific aneuploidy among all 24 chromosomes in benzene-exposed workers. *Chem Biol Interact*. 2005a; 153-4:117-22.

Zhang L, Yang W, Hubbard AE, Smith MT. Nonrandom aneuploidy of chromosomes 1, 5, 6, 7, 8, 9, 11, 12, and 21 induced by the benzene metabolites hydroquinone and benzenetriol. *Environ Mol Mutagen.* 2005b; 45(4):388-96.

21. *Induction of spindle multipolarity by centrosomal cluster inhibition*

Alwin Krämer[1], Marc S. Raab[2], and Blanka Rebacz[1]

[1] *Clinical Cooperation Unit Molecular Hematology/Oncology, German Cancer Research Center, Heidelberg, Germany; a.kraemer@dkfz.de*
[2] *Dept. of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA.*

The centrosome is a small organelle which consists of two centrioles and a pericentriolar matrix. It functions as the microtubule-organizing center of eukaryotic cells and plays a central role for the formation of the mitotic spindle. Supernumerary centrosomes have been described in almost all human malignancies, including brain, breast, colon, lung, pancreas, and prostate cancers as well as in leukemias and lymphomas. Furthermore, a striking correlation between centrosome aberrations and chromosomal instability and clinical aggressiveness has been described for many tumor entities. Cells with supernumerary centrosomes usually form multipolar spindles which lead to aberrant mitoses and consequently to chromosome missegregation. To regain secondary karyotype stability after clonal selection, tumor cells coalesce their extra centrosomes by a poorly defined mechanism into two spindle poles in order to divide properly and thus to survive.

Here, we describe a screening procedure for the identification of both small molecules and siRNAs that inhibit centrosomal clustering and thus force tumor cells with supernumerary centrosomes to undergo multipolar mitoses and consequently apoptosis. For this purpose, squamous cell carcinoma cells which harbour extra copies of centrosomes (SCC114) and nevertheless divide in a strictly bipolar fashion (Quintyne et al., Science 2005) are used as a model system and treated with either small molecules or a whole genome siRNA library to investigate if they have an effect on spindle polarity. Analysis is performed by high-throughput microscopy, using a SCC114 clone that stably expresses GFP-α-tubulin. Using a genome-wide siRNA library resulted in the identification of ~150 proteins involved in the clustering of supernumerary centrosomes into a bipolar mitotic spindle. From the results of this screening effort a model on the mechanisms leading to centrosomal clustering was derived that will be presented. Screening of a small molecule library led to the identification of several substances which are currently characterized in more detail. One of these substances is the well-known antifungal drug griseofulvin, which led to an increased frequency of multipolar mitoses, mitotic arrest and apoptosis in several different tumor cell lines whereas normal fibroblasts and keratinocytes were not affected (Rebacz et al., Cancer Res 2007; 67: 6342-6350). In addition, a griseofulvin derivative inhibited *in vivo* tumor growth and resulted in prolonged survival in a murine xenograft model of human colon cancer. The identification of proteins that are components of the centrosomal clustering machinery in tumor cells will help to clarify the mechanisms of how tumor cells coalesce supernumerary centrosomes into bipolar spindles. Furthermore, this knowledge will enable us to determine specific targets for anti-cancer therapy and thus for drug development.

REFERENCES

Quintyne NJ, Reing JE, Hoffelder DR., Gollin SM, Saunders WS. Spindle multipolarity is prevented by centrosomal clustering. *Science* 2005; 307: 127-29.

Rebacz B, Larsen TO, Clausen M, Ronnest M, Löffler H, Ho AD, Krämer A. Identification of griseofulvin as an inhibitor of centrosomal clustering in a phenotype-based screen. *Cancer Research* 2007; 67: 6342-50.

22. *Sporadic ovarian carcinomas show dysregulation of DNA repair and genomic stability pathways associated with complex structural abberrations, chromosomal instability and centrosome aberrations*

Jane Bayani[1,2,3], Maisa Yoshimoto[1], Jana Paderova[1], Miltiadis Paliouras[2], Chris Planque[2], Cassandra Graham[1], Shannon Shan[4], Maria Zielenska[3,5], Eleftherios P. Diamandis[2,3], and Jeremy A. Squire[1,3]

1 Department of Applied Molecular Oncology, Ontario Cancer Institute, Princess Margaret Hospital, University Health Network, Toronto, Ontario, Canada

2 Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada

3 Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada

4 The Johns Hopkins University School of Medicine, Baltimore, Maryland

5 Department Laboratory Medicine and Pathobiology, Hospital for Sick Children, Toronto, Ontario Canada.

jane.bayani@utoronto.ca, maisa.yoshimoto@gmail.com, jpaderov@uhnres.utoronto.ca, MPaliouras@mtsinai.on.ca, CPlanque@mtsinai.on.ca, cassy.graham@utoronto.ca, maria.zielenska@sickkids.ca, ediamandis@mtsinai.on.ca, jeremy.squire@utoronto.ca

Ovarian carcinomas are characterized by complex karyotypes and aneuploidy. In this study, chromosomal instability (CIN), characterized by both numerical and structural changes ((S)-CIN) were assessed in 11 untreated, sporadic primary ovarian cancers (8 patients) using advance molecular cytogenetic techniques. To determine whether the associated chromosomal constitution and/or ploidy changes were influenced by mitotic segregation errors, we also conducted centrosome studies.

The findings revealed near-diploid tumors possessed the lowest level CIN and centrosome abnormalities, but possessed the highest level (S)-CIN. However, tetraploid/triploid tumors possessed increasing CIN, increasing centrosome aberrations, and lower-level (S)-CIN. This suggests that in ovarian carcinomas, early karyotypic events are characterized primarily by structural alterations and low-level numerical changes. The consequences of such alterations lead to increasing numerical and ploidy changes. The structural alteration of genomic regions as well as their relative copy number changes can greatly influence gene and protein expression.

In addition to genomic instability studies we investigated the mapping and copy-number status of the kallekrein locus (19q13.3), a 15-gene member family of proteases found to be over-expressed in ovarian carcinomas. We assessed a subset of patients and cell lines and co-related this to protein expression levels and found that over-expression of the protein was associated with either increase copy-number of the KLK locus, or in the translocation of the locus elsewhere in the genome. Thus among other mechanisms that influence gene expression, copy-number imbalances and mapping status may play an important role in aberrant expression.

REFERENCES

Bayani, J., Brenton, J.D., Macgregor, P.F., Beheshti, B., Albert, M., Nallainathan, D., Karaskova, J., Rosen, B., Murphy, J., Laframboise, S., Zanke, B. & Squire, J.A. (2002). Parallel analysis of sporadic primary ovarian carcinomas by spectral karyotyping, comparative genomic hybridization, and expression microarrays. *Cancer Res*, 62, 3466-76.

Bayani, J. & Squire, J.A. (2007). Application and interpretation of FISH in biomarker studies. *Cancer Lett*, 249, 97-109.

Bayani, J., Selvarajah, S., Maire, G., Vukovic, B., Al-Romaih, K., Zielenska, M. & Squire, J.A. (2007). Genomic mechanisms and measurement of structural and numerical instability in cancer cells. *Semin Cancer Biol*, 17, 5-18.

Paliouras, M., Borgono, C. & Diamandis, E.P. (2007). Human tissue kallikreins: the cancer biomarker family. *Cancer Lett*, 249, 61-79.

23. *Cell-to-cell fusion as a link between viruses and chromosomal instability*

Yuri Lazebnik[1] and Dominik Duelli[2]

[1] *Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724; lazebnik@cshl.edu*
[2] *Rosalind Franklin University of Medicine and Science, North Chicago, IL 60064; dominik.duelli@rosalindfranklin.edu*

Chromosomal instability (CIN) is a poorly defined condition that is manifested by karyotypic heterogeneity of cell populations and is common in many solid cancers. The primary cause of CIN in sporadic cancers remains uncertain, with primary suspects being deficiencies in telomere maintenance or in mitosis.

We found that an otherwise harmless virus could rapidly cause massive CIN by fusing cells whose cell cycle is deregulated by oncogenes[1,2]. This synergy between fusion and oncogenes randomized the genomes of the hybrids so extensively that each analyzed cell had a unique karyotype. Some of the cells produced aggressive, invasive, highly aneuploid, heterogeneous, and transplantable epithelial cancers in mice.

These results were consistent with a long-standing hypothesis[3] that in some tumors CIN could result from accidental cell-to-cell fusion, which destabilizes the genome without permanently affecting mechanisms of mitosis or proliferation. Because many viruses, including common human pathogens, fuse cells we proposed[4] that viruses could cause CIN in premalignant lesions by fusing cells thus contributing to carcinogenesis and tumor progression.

We will discuss several pathways by which cell fusion might link viruses to cancer, what types of cancers this mechanism can affect, how the existence of this link can be tested and how the hypotheses that we propose might help the search for human oncogenic viruses.

REFERENCES

1. Duelli DM, Padilla-Nash HM, Berman D, Murphy KM, Ried T, Lazebnik Y. A virus causes cancer by inducing massive chromosomal instability through cell fusion. *Curr Biol* 2007;17(5):431-7.
2. Duelli DM, Hearn S, Myers MP, Lazebnik Y. A primate virus generates transformed human cells by fusion. *J Cell Biol* 2005;171(3):493-503.
3. Duelli D, Lazebnik Y. Cell fusion: a hidden enemy? *Cancer Cell* 2003;3(5):445-8.
4. Duelli D, Lazebnik Y. Cell-to-cell fusion as a link between viruses and cancer. *Nat Rev Cancer* 2007;7(12):968-76.

24. *Genomic plasticity and its transcriptional consequences in colorectal cancer*

Jordi Camps, Amanda B. Hummon, Marian Grade, Quang Tri Nguyen, Georg Emons, Yidong Chen, Hesed M. Padilla-Nash, Michael J. Difilippantonio, and Thomas Ried

Genetics Branch, Center for Cancer Research, National Cancer Institute/NIH, Bethesda, MD 20892

Chromosomal alterations involved in colorectal tumors have been amply described by chromosome banding analysis, conventional CGH and SKY. However, less is known about submicroscopic, subtle genome alterations and the transcriptional output linked to the genes that are affected by them.

The higher resolution of microarray CGH allowed us to identify novel, hitherto undetected chromosomal alterations. In particular, most of the colorectal cancer cell lines analyzed contained structural chromosomal aberrations with subtle deletions or duplications at the sites of the breakpoints. This could point to a yet underestimated mechanism for the generation of allelic imbalances.

In addition, several chromosomal breakpoints identified by SKY and mapped by array-based CGH occurred within genomic regions reported to contain structural chromosomal variants such as segmental duplications and copy number variations (CNVs) in the human population. This suggests that CNVs (including segmental duplications) contribute significantly to the emergence of chromosomal breaks in colon cancer, and hence to the development of genomic imbalances. This phenomenon was corroborated in an independent study of 31 primary colon carcinomas using a high resolution microarray CGH, suggesting that structural variants of the genome might have clear mechanistic implications for the formation of chromosomal translocations, which then lead to genomic imbalances frequently observed in solid tumors. Besides, the comparison between tumor and matched normal mucosa revealed that CNVs can occur somatically in the cancer genome.

Finally, integration of array CGH and gene expression data allowed us to generate a genome-transcriptome correlation map, showing an upward trend in genomic amplifications and overexpression. This intriguing relation was further supported by the identification of candidate oncogenes located at sites of recurrent gains and amplifications of chromosome 13 in colorectal cancer. Functional analyses proved the relevance of several genes for the colorectal tumorigenesis.

REFERENCES

Ried T, Knutzen R, Steinbeck R, et al. Comparative genomic hybridization reveals a specific pattern of chromosomal gains and losses during the genesis of colorectal tumors. *Genes Chromosomes Cancer* 1996;15(4):234-245.

Feuk L, Carson AR, Scherer SW. Structural variation in the human genome. *Nat Rev Genet* 2006;7(2):85-97.

Grade M, Becker H, Liersch T, Ried T, Ghadimi BM. Molecular cytogenetics: Genomic imbalances in colorectal cancer and their clinical impact. *Cell Oncol* 2006;28(3):71-84.

25. *The "aneuploidy-modified mutator-phenotype" theory of malignant tumours*

Leon P Bignold

Institute of Medical and Veterinary Science, Adelaide, South Australia; leon.bignold@adelaide.edu.au

Aneuploidy is thought to be involved in tumour formation for many reasons including (i) its commonness, especially among malignant neoplasms; (ii) cell lines with specific karyotypic changes can be grown from certain types of tumors; and (iii) many immortal tumor cell lines are hyperploid. Mutations are thought to be involved in tumor formation because (i) tumor cells transmit their abnormalities to their descendants; (ii) many hereditary predispositions to tumour types are associated with specific germ-line mutations (iii) many carcinogens are mutagens. In addition to these mechanisms, acquired somatic cell replicative infidelity of DNA ("mutator phenotype") may be a mechanism of tumor formation, because more somatic genomic events are found in malignant tumor cells than could arise either by repeated exogenous mutagenic insults or by aneuploidy alone. Nevertheless, lines of living organisms with "mutator phenotype," sooner or later, might be expected to die out through the accumulation of lethal mutation loads. Despite this, all cases of cancer seem to contain at least some lines of cells which are immortal.

In an earlier somewhat parallel consideration, Muller[1] in the 1960s suggested that populations of living organisms which reproduce asexually are likely to die out because of accumulations of germ-line mutations ("Muller's ratchet"). He further suggested that in sexually-reproducing organisms, two aspects of meiosis—recombination of chromosomes and "crossing-over"—might allow for the formation of occasional gametes in which the accumulated deleterious mutations are significantly reduced by accidental distribution of the majority of such mutations to other gametes. He argued that this would have the effect that at least some progeny of the species do not continue to carry all of the mutation load(s) of their parents.

The present author[2-4] has suggested that in tumour cells—which reproduce asexually—aneuploidy might act in a way analogous to the features of meiosis to correct excess mutational loads caused by "mutator phenotype." A scheme could be: (i) a mutation affects genomic elements for control of growth, and for replicative fidelity of DNA, leading to "mutator phenotype." Then (ii) aneuploidy could develop when "mutator phenotype" results in mutation of genomic elements for mitotic-and-chromosomal stability. And then (iii) an asymmetric mitosis (in the course of the aneuploid phase) could produce occasional cells in which the "bad copy" is lost (or an extra "good copy" is gained) of the original genomic element which had been mutated to provide the "mutator phenotype." The resulting cells would have significantly restored fidelity of replication of DNA, and hence could give rise to populations which are relatively genomically stable, hyperploid and immortal despite having large numbers of alterations in their DNA.

Alternative schemes, for example in which a chromosomal lesion—analogous to the formation of the Philadelphia chromosome—starts the "mutator phenotype" condition, could apply to some tumour types.

REFERENCES

1. Muller HJ. The relation of recombination to mutation advance. *Mutat Res* 1964; 1: 2-9.
2. Bignold LP. Aneuploidy as a counter to "Muller's ratchet" in cancer cells. *Cancer Genet Cytogenet.* 2007; 178: 173-4.
3. Bignold LP. Variation, "evolution", immortality and genetic instabilities in tumour cells. *Cancer Lett.* 2007; 253: 155-69.
4. Bignold LP. Mutation, replicative infidelity of DNA and aneuploidy sequentially in the formation of malignant pleomorphic tumors. *Histol Histopathol.* 2007; 22: 321-6.

26. *Impact of DNA copy number alteration on transcriptional programs and cancer phenotypes*

Jonathan R. Pollack, MD, PhD

Department of Pathology, Stanford University, Stanford, California, USA; pollack1@stanford.edu

Genomic instability is a hallmark of cancer, where a common class of aberrations affects gene dosage through chromosomal imbalances (aneuploidy), segmental gains/losses, or focal amplifications/deletions. Using DNA microarrays to profile gene copy number and expression in parallel, we discovered a sizable impact of DNA copy number alteration (CNA) on mRNA levels in breast cancer[1]. On average, a 2-fold gain/loss resulted in a corresponding 1.5-fold change in mRNA levels, and overall at least 12% of the variation in gene expression among breast tumors was directly attributable to underlying copy number alteration of the genes. The high degree of copy-number dependent gene expression, implying deficient dosage compensation, was unexpected but since observed by us (and others) in additional tumor types including lung, colon, prostate and pancreatic cancers[2,3] (and unpublished data). Our findings suggest the possibility that aneuploidy and the resultant global imbalances in gene expression might more broadly contribute to cancer development or progression, for example by disrupting critical stoichiometric relationships in cell metabolism and physiology, possibly further promoting chromosome instability or predisposing to metastasis or drug resistance.

To begin to address the functional impact of widespread CNA on cancer, we studied a simpler model system, assessing the role of co-amplified genes within tumor amplicons. The known oncogenic receptor tyrosine kinase *ERBB2 (HER2)* at 17q12 is amplified in ~20% of breast cancers, but its neighbors *GRB7* and *STARD3* are also always co-amplified. Using RNA interference, we observed that knockdown of GRB7 or STARD3, like ERBB2, led to decreased cell proliferation[4]. Our findings establish that even within focal tumor amplicons, multiple amplified genes contribute to oncogenic phenotypes, and support the possibility that aneuploidy effects cancer similarly through the altered expression of many, possibly hundreds of genes.

Finally, while the above studies describe the effect of a gene's altered copy number on its own expression levels (i.e., *cis* effect), we have begun to assess the impact of CNA on genes elsewhere in the genome (i.e., *trans* effect). In particular, amplification of transcription factors, such as *MYC* or *TITF (NKX2-1)* in lung cancer[2,5], likely promote oncogenesis through the activation of specific downstream transcriptional programs. More generally, the altered dosage of transcriptional regulators would be expected to dramatically enhance the impact of aneuploidy on gene expression and carcinogenesis.

REFERENCES

1. Pollack JR, Sorlie T, Perou CM, et al. Microarray analysis reveals a major direct role of DNA copy number alteration in the transcriptional program of human breast tumors. *Proc Natl Acad Sci U S A* 2002;99(20):12963-8.
2. Kim YH, Girard L, Giacomini CP, et al. Combined microarray analysis of small cell lung cancer reveals altered apoptotic balance and distinct expression signatures of MYC family gene amplification. *Oncogene* 2006;25(1):130-8.
3. Lapointe J, Li C, Giacomini CP, et al. Genomic profiling reveals alternative genetic pathways of prostate tumorigenesis. *Cancer Res* 2007;67(18):8504-10.
4. Kao J, Pollack JR. RNA interference-based functional dissection of the 17q12 amplicon in breast cancer reveals contribution of coamplified genes. *Genes Chromosomes Cancer* 2006;45(8):761-9.
5. Kwei KA, Kim YH, Girard L, et al. Genomic profiling identifies TITF1 as a lineage-specific oncogene amplified in lung cancer. *Oncogene* In Press.

27. *Genomic instability in context of the chromosomal theory*

Alice Fabarius, Peter Duesberg, Michelle Giehl, Wolfgang Seifarth, Andreas Hochhaus and Rüdiger Hehlmann

III. Medizinische Klinik, Medizinische Fakultät Mannheim, Universität Heidelberg, Mannheim, Germany; ruediger.hehlmann@med3.ma.uni-heidelberg.de; alice.fabarius@med3.ma.uni-heidelberg.de

Cancer cells differ from normal cells in karyotype, cell morphology, invasive or non-invasive growth, development of metastases, primary and acquired drug resistance, and in expression of genes and proteins. The mechanisms of carcinogenesis remain unclear and the prevailing gene mutation theory explaining carcinogenesis by a sequence of mutations has not really solved the problem. Key features of cancer are not explained by mutations such as carcinogenesis by non-mutagenic carcinogens, the absence of carcinogenic genes in cancer in spite of tremendous efforts over the years to show their existence, the lack of explanation for neoplastic latency after carcinogen exposure, dependence on phenotype alterations in cancers of unrealistically high mutation rates or the absence of heritable cancer in spite of heritable mutations in cancer cells.

An explanation of these features is offered by the chromosomal theory[1]. According to this theory, carcinogens induce non-specific chromosomal alterations which unbalance thousands of genes, destabilize the genome and encourage the evolution of neoplastic cells. Induced chromosomal alterations generate abnormal phenotypes via abnormal dosages of genes. Cancer cells, by chromosomal constitution, are new species with non-random specific chromosomal alterations, but unstable karyotypes[2]. The higher the ploidy-factor, the more unstable is the karyotype. Maximal instability is observed with triploidy and decreases towards tetraploidy[3]. Since aneuploidy disrupts interactions of multiple genes, enzymes, and proteins, alters gene dosage effects and is ubiquitous in cancer it is one of the most plausible explanations for the inherent genetic instability of cancer cells.

What are the mechanisms for the induction of such aneuploidies? Centrosome aberrations and defects of spindles have been implicated in the causation of chromosome aberrations. We therefore analyzed chromosomes and centrosomes in CD34 positive CML (Chronic Myelogenous Leukemia) cells along the course of CML. Numerical and structural centrosomal aberrations were observed in chronic phase and decreased in blast crisis. The centrosomal alterations correlate with chromosomal aberrations[4].

Concerning the analysis of spindle defects, the observation was used that tyrosine kinase inhibitors induce spindle aberrations in normal human cells. Using this approach, centrosome and chromosome aberrations were found to correlate with defects of mitotic spindles. In conclusion, alterations of centrosomes and spindles (spontaneous or induced) correlate with chromosomal aberrations and may represent a mechanism for the cause of aneuploidy[5].

The degree of aneuploidy, chromosome non-disjunction or structural alteration is paralleled by increasing genetic instability and by preneoplastic and neoplastic phenotypes of increasing malignancy. Alterations of the structures of centrosomes and of spindles may be mechanisms involved in the generation of aneuploidy. These findings may have far reaching implications for prevention and early diagnosis of cancer.

REFERENCES

1. Duesberg P, Li R, Fabarius A, Hehlmann R: The chromosomal basis of cancer. *Cellular Oncology* 2005;27:293-318.
2. Fabarius A, Willer A, Yerganian G, Hehlmann R, Duesberg P. Specific aneusomies in Chinese hamster cells at different stages of neoplastic transformation, initiated by nitrosomethylurea. *Proc Natl Acad Sci USA* 2002;99:6778-6783.
3. Fabarius A, Hehlmann R, Duesberg PH. Instability of chromosome structure in cancer cells increases exponentially with degrees of aneuploidy. *Cancer Genet Cytogenet* 2003;143:59-72.
4. Giehl M, Fabarius A, Frank O, Erben P, Zheng C, Hafner M, Hochhaus A, Hehlmann R, Seifarth W. Expression of the p210BCR-ABL oncoprotein drives centrosomal hypertrophy and clonal evolution in human U937 cells. *Leukemia* 2007;21:1971-1976.
5. Fabarius A, Giehl M, Frank O, Spiess B, Zheng C, Müller MC, Weiss C, Duesberg P, Hehlmann R, Hochhaus A, Seifarth W. Centrosome aberrations after nilotinib and imatinib treatment in vitro are associated with mitotic spindle defects and genetic instability. *British J Haematol* 2007;138:369-373.

28. *Lessons in copy number alterations in cancer learned from comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH)*

Nicholas J. Wang and Joe W. Gray

Lawrence Berkeley National Laboratory, One Cyclotron Road MS-977R225A, Berkeley, CA 94720

The progression of cells from a normal state towards one characterized by uncontrolled growth and metastatic behavior is caused by the deregulation of key cellular processes and signaling pathways. These alterations in normal cellular behavior are rooted in the accumulation of genomic and epigenomic lesions that impact hallmarks of cancer such as the ability of the cell to control proliferation, undergo apoptosis, increase motility leading to invasion and alter angiogenesis.

A suite of technologies have been developed and are now available to assess genomic and epigenomic aberrations that contribute to cancer progression. The application of these has shown that genome copy number abnormalities (CNAs) are among the most frequent genomic aberrations and can be used as clinical markers such as the case with amplification of the ErbB2 gene in breast cancer. Current advances in microarray technologies are also giving researchers the ability to measure genotypes along with copy number allowing for the detection of loss of heterozygosity (LOH) in tumor samples.

We will review the current technologies available to measure genomic copy number changes and how they have guided us in understanding the pathology of cancer.

REFERENCES

Pinkel, D., Segraves, R., Sudar, D., Clark, S., Poole, I., Kowbel, D., Collins, C., Kuo, W.L., Chen, C., Zhai, Y., Dairkee, S.H., Ljung, B.M., Gray, J.W. & Albertson, D.G. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet* 20, 207-11 (1998).

Snijders, A.M., Nowak, N., Segraves, R., Blackwood, S., Brown, N., Conroy, J., Hamilton, G., Hindle, A.K., Huey, B., Kimura, K., Law, S., Myambo, K., Palmer, J., Ylstra, B., Yue, J.P., Gray, J.W., Jain, A.N., Pinkel, D. & Albertson, D.G. Assembly of microarrays for genome-wide measurement of DNA copy number. *Nat Genet* 29, 263-4 (2001).

Toruner, G.A., Streck, D.L., Schwalb, M.N. & Dermody, J.J. An oligonucleotide based array-CGH system for detection of genome wide copy number changes including subtelomeric regions for genetic evaluation of mental retardation. *Am J Med Genet A* 143, 824-9 (2007).

George, R.E., Attiyeh, E.F., Li, S., Moreau, L.A., Neuberg, D., Li, C., Fox, E.A., Meyerson, M., Diller, L., Fortina, P., Look, A.T. & Maris, J.M. Genome-wide analysis of neuroblastomas using high-density single nucleotide polymorphism arrays. *PLoS ONE* 2, e255 (2007).

Zhao, X., Li, C., Paez, J.G., Chin, K., Janne, P.A., Chen, T.H., Girard, L., Minna, J., Christiani, D., Leo, C., Gray, J.W., Sellers, W.R. & Meyerson, M. An integrated view of copy number and allelic alterations in the cancer genome using single nucleotide polymorphism arrays. *Cancer Res* 64, 3060-71 (2004).

29. *SV40 Tag/p53 complexes actively promote malignant cell growth of human mesothelial cells*

Michele Carbone

Thoracic Oncology Program Cancer Center, and Department of Pathology, Medical School, University of Hawaii

SV40 is a DNA tumor virus that causes malignant transformation of human cells in tissue culture. SV40-transformed human cells contain a large number of chromosomal alterations that may be sufficient to sustain tumor growth even in the absence of viral protein expression[1]. Different types of human cells show different susceptibility to SV40-mediated transformation and mesothelial cells are the most susceptible[2]. SV40 preferentially induces mesothelioma in animals[2]. SV40 has been detected in human mesotheliomas[2], and synergizes with asbestos in carcinogenesis in vitro and in vivo[3]. We previously demonstrated that the unusual high levels of wild-type p53 normally present in mesothelial cells are a critical factor in determining the susceptibility of these cells to SV40-mediated transformation[4]. In cells infected with DNA tumor viruses, p53 is bound to the viral tumor antigens (Tags). The current "dogma" views the Tag-p53 complexes as a way of sequestering and inactivating p53.

Using primary human mesothelial cells and SV40-transformed human cells, we now show that in addition of inactivating p53 tumor suppressor activities, the Tag-p53 complex has growth stimulatory activities that are required for the initial stages of malignant cell growth. We found that in human cells, Tag/p53 complexes regulate transcription of the Insulin-like growth factor 1 (IGF-1) gene by binding to the IGF-1 promoter together with pRb and p300. Depletion of p53 leads to structural rearrangements of this multi-protein complex, resulting in IGF-1 promoter transcriptional repression and growth arrest. Our data provide a novel mechanistic and biological interpretation of the p53/Tags complexes and of DNA tumor virus transformation in general. In the model we uncovered, p53 is not a passive inactive partner of Tag. Instead the p53/Tag complex promotes malignant cell growth through its ability to activate the IGF-1 signaling pathway.

REFERENCES

1. Bocchetta M, Eliasz S, De Marco M, Rudzinski J, Zhang L, Carbone M. The SV40 large T antigen-p53 complexes bind and activate the IGF-1 promoter stimulating cell growth. *Cancer Research*, in press.
2. Gazdar AF, Butel JS, Carbone M. SV40 and human tumours: myth, association or causality? *Nat Rev Cancer* 2002;2:957-64.
3. Krockznyska B, Cutrone R, Bocchetta M, Yang H, Elmishad AG, Vacek P, Ramos-Nino M, Mossman BT, Pass HI, Carbone M. Crocidolite asbestos and SV40 are cocarcinogens in human mesothelial cells and in causing mesothelioma in hamsters. *Proc Natl Aca Sci USA* 2006, 103:14128-14133
4. Bocchetta M, Di Resta I, Powers A, Fresco R, Tosolini A, Testa JR, Pass HI, Rizzo P, and Carbone M. Human mesothelial cells are unusually susceptible to simian virus 40-mediated transformation and asbestos cocarcinogenicity. *Proc Natl Acad Sci USA* 2000, 97:10214-10219.

P1. Hansemann: chromosomes and the origin of the cancerous features of tumor cells

Leon P. Bignold[1], Brian Coghlan[2], and Hubertus Jersmann[3]

[1] *Institute of Medical and Veterinary Science, Adelaide*
[2] *Centre for European Studies and General Linguistics, University of Adelaide*
[3] *Department of Medicine, University of Adelaide, SA 5005, Australia; leon.bignold@adelaide.edu.au*

Chromosomes were described, named and identified as the basis of the cellular hereditary material in the 1870s-80s. Abnormal—including asymmetric—mitoses were discovered in tumor cells at almost the same time. In 1890, David Paul Hansemann (1858-1920) was an *Assistent* to Virchow in Berlin and noted asymmetric mitoses in cancer cells. In addition, he recognised the paramount features of tumors as loss of tissue specialisation and increased capacity for independent existence (i.e., "autonomy"—ability to grow in remote tissues and form metastases).

Probably because Virchow insisted that tumor formation must involve only an abnormality of a "physiological" tissue process (not a new process), Hansemann looked for a cell process which might be a counterpart of this particular combination of changes—i.e., a normal cell process in which changes in chromosomal content, reduction of specialisation and greater autonomy all occurred. Hansemann proposed that oogenesis was the "prototype process" because (i) the egg comes about by reduction divisions (ii) it is less specialised than ovarian epithelial cells and (iii) the egg can survive for days free in the endometrial cavity. Hansemann called the process "anaplasia" and in later works, he suggested that the basis of the cancer cell is loss of the ability to maintain symmetric mitoses, or at least loss of ability to preserve chromosomal integrity. He considered that populations of chromosomally unbalanced cells would arise, some of which have the (ovum-like) anaplastic features, but still have features of the cell type from which they arose. In response to reports that not all tumours exhibit asymmetrical mitoses or chromosomal abnormalities, Hansemann suggested that the chromosomal lesions might simply be too small to be visible.

In the early twentieth century, the directions of cancer research moved towards investigating Mendelian genetics in relation to tumours, and the mechanisms of action of viral, physical and chemical carcinogens. Only in the last 30 or so years, have the roles of chromosomal abnormalities in tumour formation—which were first studied by Hansemann—again received significant attention.

REFERENCE

Bignold LP, Coghlan BLD, Jersmann HPA. David Paul von Hansemann: *Contributions to Oncology. Context, Comments and Translations.* Birkhäuser, Basel, 2007.

P2. Spectral karyotyping and SNP microarray analysis define uniparental disomy (UPD) as novel mutational mechanism in MSI- and CSI-colorectal cancers

Ralph Melcher[1], Holger Hoehn[2], and Michael Schmid[2]

[1] *Department of Medicine II, University of Wurzburg; melcher_r@klinik.uni-wuerzburg.de*
[2] *Department of Human Genetics, University of Wurzburg; hoehn@biozentrum.uni-wuerzburg.de*

Spectral karyotyping greatly improves recognition and definition of chromosomal aberrations[1]. In previous studies, we applied spectral karyotyping to a number of colorectal cancer cell lines derived from metastatic and primary tumors[2]. As expected, we observed complex marker chromosomes and pronounced chromosomal instability (CSI) in tumors devoid of microsatellite instability. In contrast, microsatellite instable (MSI) tumors uniformly displayed stable karyotypes[3]. Likewise, a newly characterized adenoma cell line lacked karyotypic alterations[4].

Recently, we complemented our spectral karyotyping studies by SNP-array analyses of multiple MSI- and CSI-cell lines[5]. Results were verified by the analysis of 15 primary MSI- and 15 CSI-tumors (unpublished data). SNP analysis greatly facilitated the interpretation of complex chromosomal alterations of CSI-cell lines. Monoallelic regions could be correlated with sites of inactivated tumor suppressor genes and activated oncogenes. Some of the genes relevant for colon carcinogenesis are inactivated by allelic loss (e.g., p53, SMAD4). Monoallelic regions with increased copy number may represent oncogene loci activated by allele-specific amplification (e.g., Cyclin D1 in CSI-cell lines). Monoallelic regions without copy number alterations fulfill the criteria of uniparental disomy (UPD). In the tested colorectal cell lines and primary tumors, UPD appears to be instrumental in the inactivation of early-acting tumor supppressor genes, including APC in CSI- and hMLH1/hMSH2 in MSI-cellular phenotypes. Our results suggest that following initial mutational inactivation of one of the APC or hMLH1/hMSH2 alleles the remaining wild-type allele is deleted, concomitant with re-duplication of the mutated allele. Alternatively, UPD may have arisen through some type of gene conversion. In addition to the APC and hMLH1/hMSH2 chromosomal sites, 6pter->p22 was also found to be frequently altered by UPD in primary MSI tumors, suggesting a candidate tumor suppressor gene in this region.

We conclude that the combination of spectral karyotyping and SNP-array analysis permits the detection of UPD. UPD represent a novel type of genetic change that may cause inactivation of early acting tumor suppressor genes involved in the generation of microsatellite- and chromosomal instability of colorectal tumors.

REFERENCES

1. Melcher R, von Golitschek R, Steinlein C, Schindler D, Neitzel H, Kainer K, Schmid M, Hoehn H. Spectral karyotyping of Werner syndrome fibroblast cultures. *Cytogenet Cell Genet* 2000; 91(1-4): 180-5
2. Melcher R, Steinlein C, Feichtinger W, Muller C, Menzel T, Luhrs H, Scheppach W, Schmid M. Spectral karyotyping of the human colon cancer cell lines SW480 and SW620. *Cytogenet Cell Genet* 2000; 88: 145-152
3. Melcher R, Koehler S, Steinlein C, Schmid M, Mueller C, Luehrs H, Menzel T, Scheppach W, Moerk H, Scheurlen M, Koehrle J, Al-Taie O. Spectral karyotype analysis of colon cancer cell lines of the tumor suppressor and mutator pathway. *Cytogenet Genome Res* 2002; 98: 22-8.
4. Menzel T, Melcher R, Koehler S, Dusel G, Backhaus K, Ott G, Breithaupt W, Al-Taie O, Schauber J, Gostner A, Scheppach W, Luhrs, H. Establishment of a colonic adenoma cell line (GEKI-2): spectral karyotype analysis and functional characterization. *Int J Colorectal Dis* 2004; 19: 12-17.
5. Melcher R, Al-Taie O, Kudlich T, Hartmann E, Maisch S, Steinlein C, Schmid M, Rosenwald A, Menzel T, Scheppach W, Luhrs H. SNP-Array genotyping and spectral karyotyping reveal uniparental disomy as early mutational event in MSS- and MSI-colorectal cancer cell lines. *Cytogenet Genome Res* 2007; 118: 214-221.

P3. Meiotic-like division of endopolyploidy preceded by chromosomal instability (CIN) in WI-38 cells

Kirsten H. Walen

Viral & Rickettsial Disease Laboratory, California Department of Public Health, 850 Marina Bay Pky, Richmond, California 94804-6403

Stickiness between chromosomes from senescence-associated heterochromatic changes (e.g., telomeres), caused genome damage from CIN resulting in re-replication of cells with 2n/G2-DNA content (Walen, 2007ab). This shift to mitotic cycling of endopolyploid cells with diplochromosomes (i.e., pairs of sister chromosomes) has as yet only been shown in a relatively unknown, diploid cell strain (L645). The purpose of the present study is, therefore, to strengthen these findings by a re-analysis of the veteran cell strain, WI-38, for evidences of bipolar, reductional division (Hayflick and Moorhead, 1961).

These cells were brought to senescence by progressive cell expansions and all cultural procedures were the same as for L645, including maintenance of spatial relationships between cellular changes by fixation of cells in situ and without special chromosomal treatments. All noted divergence from normal fibroblastic growth was documented by live- and stained-cell (Giemsa, R66) photography.

Briefly, this special type of polyploidy contained diplochromosomes (i.e., pairs of sister chromosomes) from two successive S periods (no mitosis) of arrest-escaped genome damaged cells. These endopolyploid cells could undergo one or two bipolar divisions in succession. One division separated the sister pairs from each other resulting in bichromatid telophase chromosomal products which following G1 and S periods went back to diplo-chromosomes. The first division, bichromatid chromosomes could also divide again by a second division which gave rise to either 3 or 4 nuclei with single chromatids. For endo-tetraploid (4n/8C) and endo-octoploid (8n/16C) (C = 1 haploid complement) cells two successive mitoses would give rise to 2n/2C and 4n/4C products which are normal, single chromatid cycling diploid and tetraploid cells, respectively. The essence of these events is for example, that 2n/4C cells with bichromatid chromosomes from the first division rest in G1 before S with DNA-doubling back to diplochromosomal 4n/8C cells. Currently, there are no known molecular markers for presence of 2n/4C cells in G1 and moreover, cytometric cell-sorting can not distinguish between these cells and 4n/4C (single chromatids) cells.

The present confirmation of diplo-polyploid, genomic reductional division in WI-38 cells, makes this new discovery a more likely general, senescence-associated event. Conclusions that CIN is not a consequence of aneuploidy, but a cause of endopolyploidy with aneuploid potentials, are also now on firmer evidential ground open to further studies.

REFERENCES

Walen K.H. Bipolar genome reductional division of human near-senescent, polyploid fibroblast cells, *Cancer Genet Cytogenet* 2007a, 173: 43-50.

Walen K.H. Origin of diplochromosomal polyploidy in near-senescence: Heterochromatin, telomeres and chromosomal instability (CIN). *Cell Biol Int* 2007b, doi:10,1016/j.cellbi.2007.06.015

Hayflick, L, Moorhead P.S. The serial cultivation of human diploid cell strains. *Exp Cell Res* 1961, 25: 585-621

P4. Stable and highly unstable aneuploidies coexist in cancer: Evidence that transforming function maintains stability

Lin Li[1,2], Amanda McCormack[1], and Peter Duesberg[1]

[1] *Department of Molecular and Cell Biology, Donner Lab, University of California, Berkeley, CA 94720*
[2] *Department of Otolaryngology, Third Hospital, Jilin University, Changchun, PR China, 130033*

The chromosomal cancer theory predicts that cancer-specific karyotypes, consisting of specific aneuploidies and a specific modal chromosome number, cause cancer. To allow the evolution of such cancer-specific karyotypes in a normal somatic cell, at frequencies that are compatible with carcinogenesis, the karyotype must be destabilized. The theory postulates that carcinogens initiate the evolution of cancer karyotypes by inducing aneuploidy. Aneuploidy then destabilizes the karyotype autocatalytically, by unbalancing balance-sensitive teams of proteins that segregate, synthesize and repair chromosomes. Eventually rare cancer-specific karyotypes with specific modal chromosome numbers and specific aneuploidies would evolve—just as new species-specific karyotypes have evolved in phylogenesis. Cancer cells maintain such cancer-specific karyotypes, because they generate cancer-specific phenotypes.

The inherent instability of aneuploidy, however, predicts two groups of aneuploidies in cancer cells: 1) A stable group of clonal aneusomies and marker chromosomes associated with a stable modal chromosome number, which are selected for carcinogenicity, and 2) An unstable group of aneusomies and marker chromosomes, which are not carcinogenic and thus rapidly replaced. Replacement is necessary, because accumulation of unselected aneusomies and markers would ruin the cancer-specific modal chromosome number. The loss of tumorigenicity by ruining the modal chromosome number via fusion of cancer cells with normal cells is a case in point. In addition unselected aneuploidies may be inhibitory or even lethal.

In an effort to test the view that stable aneuploidies have transforming function, rather than other alterations, particularly mutation, we have studied clones of transformed human cells, generated with two highly efficient yet not mutagenic biological carcinogens, namely SV40 virus and a set of 6 cellular genes artificially activated with retrovirus promoters. In these systems about 1 per 10^5 human cells forms a clone of transformed cells within short latent periods of only a few months. The stability of the karyotypes of such clones was determined by comparing karyotypes of consecutive generations of clonal cultures.

All clones of transformed cells were found to have:

1) Stable modal chromosome numbers and several stable or clonal aneusomies and marker chromosomes,
2) Highly unstable and non-clonal aneusomies and marker chromosomes.

The clonal aneusomies and markers have a 0-10% chance of alteration per 20 cell generations. By contrast, their non-clonal counterparts have a 90-100% chance of alteration.

We conclude, that specific karyotypes, defined by specific clonal aneusomies, marker chromosomes and modal chromosome numbers, are necessary, if not sufficient for carcinogenesis.

REFERENCES

Duesberg P, Li R, Fabarius A, Hehlmann R. The chromosomal basis of cancer. *Cell Oncol* 2005;27:293-318.

Duesberg P. Chromosomal chaos and cancer. *Sci Am* 2007;296:52-9.

Fabarius A, Li R, Yerganian G, Hehlmann R, Duesberg P. Specific clones of spontaneously evolving karyotypes generate individuality of cancer. *Cancer Genetics & Cytogenetics* 2008; in press.

Index

Notes

Notes