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Abstracts from the 3rd Conference on Aneuploidy and Cancer: Clinical and Experimental Aspects

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Overview of the 3rd Conference on Aneuploidy and Cancer in Berkeley, CA USA in 2017

(Organized by Peter Duesberg, Department of Molecular and Cell Biology, University of California at Berkeley and David Rasnick, Oakland CA; sponsored by philanthropist Robert Leppo, San Francisco CA USA)
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David von Hansemann noticed in 1890 that all of the cancer cells that he examined were aneuploid and suggested that the aneuploidy might be the cause of the cancer [1]. Based on Hansemann's observations and his fundamental discovery that individual chromosomes have different genetic functions, Theodor Boveri advanced the first genetic cancer theory in 1914: cancer is caused by the loss of specific growth inhibitory or the gain of specific growth stimulatory chromosomes [2]. For half a century this remained the prevailing genetic theory of cancer, despite strong opposition from Thomas Morgan, who considered that no instances of chromosomal faults were known to give rise to uncontrolled growth of cells [3]. After the causative agent of Peyton Rous's chicken sarcoma was discovered to be a retrovirus that inserted an oncogene into the host genome [4], many cancer researchers discarded the aneuploidy theory, considering the dispute to be resolved in favor of oncogenes and viruses. Since then the field has been dominated by the view that cancer is caused by virus-related or virus-unrelated cellular oncogenes.

The difficulty that many researchers had with the chromosomal or aneuploidy theory was that no consistent stimulatory or inhibitory chromosomes could be found in cancers. As a result Boveri's theory seemed to be stranded on the same reef that prevented the theory of continental drift from being accepted by geophysicists, even though the close fit between Africa and South America had been obvious to anyone who looked at a world map since sufficiently accurate maps became available. In the absence of a credible mechanism, the hypothesis was rejected by most geophysicists, and the fact that Alfred Wegener had no recognized expertise in geophysics doubtless contributed to the skepticism. (Actually, Benjamin Franklin suggested a plausible mechanism similar to the modern theory of plate tectonics as long ago as 1782 [5]).

Returning to cancer, there are also serious difficulties with the oncogene theory. The number of cells in the human body is so large, and the frequency of random mutations in somatic cells is so high, that it is certain that any conceivable oncogene, in its supposedly oncogenic state, is already present in every person, but does not lead to cancer. Such difficulties tend to be brushed aside, just as Wegener's evidence for continental drift was brushed aside in favor of supposed land bridges that had disappeared. The second problem with oncogenes is that overexpression of single genes almost never has any metabolic effects, and under-expression usually has only minor effects. That is why about 85% of mutations in, say, yeast, are "silent" [6, 7]: eliminating them from the genome usually produces no change in phenotype. When "knock-out mice" first became available [8] it was expected that the function of any gene could be revealed by observing the effect of eliminating it, but most such experiments led to disappointment. When a mouse completely lacks a protein such as myoglobin, which has a well-understood function in muscles, it can remain healthy, even when exercised [9].

The almost negligible effects of altering the activities of single genes can be easily understood in terms of metabolic control analysis, an approach to metabolic regulation introduced by Henrik Kacser and Jim Burns in 1973 [10]. Before then, and for considerable time afterwards, because the new ideas did not become immediately accepted, it was assumed that each metabolic pathway had a "key enzyme" or "rate-limiting enzyme", and that altering the activity of this enzyme would alter the flux through the pathway in proportion. Kacser and Burns realized, even before many experimental tests were available, that that could not be correct: flux control is shared between all of the enzymes in the system, and if the system is taken to be a whole cell or a whole organism, this means that most shares must be very small, so that altering a typical enzyme activity should have little or no effect. When techniques for genetic manipulation became available, Jürgen Heinisch and colleagues overexpressed phosphofructokinase (widely regarded as the key enzyme in fermentation) in yeast by a factor of 3.5, and observed no effect on the flux to ethanol [11]. This contradicted what was widely expected, but it was not a surprise for people who had understood the principles of metabolic control analysis.

The expectation of no effect, however, is the expectation of what will happen when the activity of a single enzyme is altered. But aneuploidy alters the activities of many enzymes at a time, and the analysis is not so simple. Even in Down syndrome, with fewer than 2% of genes affected by trisomy of the smallest chromosome, the effects are not negligible. Cancer cells are far more aneuploid than those of Down syndrome patients: for example, colon cancer produces cells in which many chromosomes are triploid, some are tetraploid, and some are damaged. This is a vastly larger perturbation than trisomy of one small chromosome, and altering the activities of a vast number of enzymes must inevitably create large metabolic disturbance. Down syndrome illustrates the severe effects that even a minimal degree of aneuploidy can produce, but people who believe that changing a single enzyme activity, or a small number of activities, can lead to cancer need to explain why Down syndrome patients are as normal as they are when they have hundreds of genetic alterations.

I first became conscious of the importance of aneuploidy for cancer when David Rasnick and Peter Duesberg, as well as Harvey Bialy, participated in a meeting that I organized in 1999 [12]. Since then they have used modern concepts of metabolic control as the basis for a quantitative analysis of metabolism in cancer tissue. Their theory goes a long way towards explaining how aneuploidy can be a cause rather than a consequence of cancer. However, there is no longer a reason to doubt that an error in mitosis is the primary event that produces aneuploidy, which eventually leads to cancer. In the first session of the 3rd Conference on Aneuploidy and Cancer, held in Berkeley, California, in January 2017, David Rasnick presented the current state of the theory and compared it with gene-based theories of cancer [13].

This was followed by a lecture in which Henry Heng argued that the chaotic reorganization of the genome that accompanies aneuploidy explains the development of cancer better than a gene- initiated process, and concluded that the survival and adaptive landscapes are different in cancer [14]. Mathew Bloomfield examined the relationships between aneuploidy, karyotypic variability and metastasis, and argued that the appearance of metastases should be regarded as a form of speciation [15]. Mark Vincent continued in this spirit, and discussed why targeted drugs for cancer, with the exception of chronic myeloid leukemia, which he regarded as atypical, have not resulted in the expected benefits. For him carcinogenesis is a form of "de-speciation" rather than speciation [16], but that can be regarded as a matter of definition rather than anything more fundamental.

Much later in the conference Peter Duesberg described many problems with gene-based theories, noting, for example, that in contrast to conventional Mendelian genetics no common cancer-specific karyotypes are known; instead, all cancers have individual karyotypes. He argued that carcinogenesis is a type of speciation [17].

Without rejecting the role of aneuploidy, Marcelo Aldaz argued against a Manichean approach to cancer, retaining a role for oncogenes [18]. David Gisselsson described how high-risk cancer is genetically dynamic, both in space and in time [19]. Likewise, Floris Foijer considered that aneuploidy is characteristic of only two out of three tumors (much less than the 100% that David Rasnick reported), and emphasized that p53-deficient knockout mice displayed highly reproducible aneuploidy induction [20]. In a later presentation, Rüdiger Hehlmann discussed the so-called Philadelphia chromosome, which is regularly associated with a definite form of human leukemia. The oncogene ABL on chromosome 9 appears to act in a cooperative process with aneuploidy and development of leukemia. This sort of observation can help to provide new recommendations for the management of acute myeloid leukemia [21]. Jonathan Pollack continued the discussion of this cancer by examining the cryptic genes harbored by complex karyotypes, and described a new computer-based analytical tool for integrating data for copy numbers and gene expression [22].

Thomas Liehr focused on the copy-number variations of chromosomes with no obvious clinical effects, which have been known for decades but have assumed greater visibility with the sequencing of the human genome. He considered that these need more attention in cancer research [23].

The facial cancer that is devastating the population of Tasmanian devils, the largest carnivorous marsupial, is an interesting example in which an oncogene must be completely excluded as a possibility, because it is contagious, as Jennifer Marshall Graves described, and spreads by biting of an affected animal by a healthy one: the cancer itself is transferred. Both the host and the cancer karyotypes are highly conserved; indeed the cancer karyotypes are all the same. She went on to describe the pathogenesis and molecular biology of the tumor [24].

Aleksei Stepanenko returned to the theme of genomic instability as the driving force of cancer, and specifically the adaptation of cancer cells to drugs and transgenic manipulations, arguing that increased resistance to drug re-challenge was the only predictable phenotypic trait observed in all long-term drug-treated tumor cells [25].

Daniela Cimini discussed the view that tetraploidy of the whole genome of a cell can be the precursor of chromosomal instability in cancer, though tetraploidal cell clones with normal centrosome numbers can also occur. She examined the link between aneuploidy and chromosome instability, focusing especially on breast cancer, in which the spontaneous return to an unbalanced diploid cell is accompanied by errors, with monosomy of some chromosomes and damaged fragments of others [26].

Milena Dürrbaum examined the molecular mechanisms underlying aneuploidy in human cells from the point of view of systems biology. She and her colleagues found that gene expression affected common cellular pathways independently of the cell line, type of aneuploidy, and its origin [27].

Daniele Mandrioli described the work of the Ramazzini Institute in Bologna in showing that aneuploidy offered an evidence-based marker for environmental health [28]. (He also pointed out that Theodor Boveri's theory of cancer [2] was in reality a joint project carried out with his wife, Marcella O'Grady Boveri.)

Rolf Skotheim used computer analysis to study the question of instability of the transcriptome in cancer, with aberrant processing of RNA, specifically in the context of samples of colorectal cancer from 555 patients. They found enormous variation between samples [29].

Alfred Böcking addressed the question of "active surveillance" of localized prostate cancers with a view to avoiding the undesirable side effects that accompany commonly used aggressive therapies, which may well be unnecessary for a substantial proportion of patients, especially in the short term [30].

Andrew Fritz described studies of the spatial arrangement of chromosomes in breast cancer samples, which showed that these arrangements are not random, so that particular "chromosome territories" tend to be associated with particular other ones [31].

Sarantis Gagos discussed the effect of induced replication stress and extreme telomere dysfunction on chromosomal instability and cancer cell stemness. The results from human cell lines with alternative lengthening of telomeres suggested a trend that preserves monoclonality [32].

Eduardo Torres discussed the role of sphingolipids in modulating the fitness of aneuploid cells, because in his view studying the cellular processes affected by aneuploidy can improve our understanding of its role in tumor biology [33]. He concluded that these lipids have important roles in the physiological responses to aneuploidy.

As will be evident from the introductory paragraphs in this report, I am not a great believer in rate-limiting steps in metabolism, and I am not convinced that progression of cancer is exceptional. However, Martha Stampfer took a different view, and argued that immortalization is the rate-limiting step in human carcinogenesis, observing that efficient transformation of normal human mammary epithelial cells does not require gross genomic alterations. She noted that normal cells from small short-lived mammals like mice do not stringently repress telomerase and lack a significant replicative senescence barrier. In consequence, mouse cells are not an adequate model for immortalization in human carcinogenesis [34].

Yi-Hong Zhou studied tumor recurrence after therapy, concluding that tumor heterogeneity could be maintained by missegregation of tumor-specific chromosomes in response to extracellular environmental cues [35]. This interpretation is not necessarily incompatible with Rasnick's view that recurrence is inevitable after any therapy that destroys only 99.9% of cancer cells, leaving millions (0.1%) to restore a tumor [13], but in any case it is important to understand how tumor heterogeneity is established and maintained. The meeting ended with a presentation by Yi-Hong Zhou's colleague Michelle Digman, who discussed how fluorescence lifetime imaging microscopy could be used to identify glioblastoma subpopulations [36], which should allow understanding of the role of tumor heterogeneity in drug resistance.

In summary, the 3rd Conference on Aneuploidy and Cancer offered a unique opportunity to discuss many aspects of the origin of aneuploidy and its role in producing cancer—mainly human, but also other organisms such as Tasmanian devils.

Competing interests
The author declares no competing interests

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Authors' contributions
The author wrote, read and approved the final version of the introduction.

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A1

Metabolic control and chromosomal abnormalities

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A theory of what goes wrong with metabolism in cancer requires an adequate theory of what goes right in healthy cells. This needs to be based on the theories of metabolic control and metabolic regulation. To a first approximation the rate of an isolated enzyme-catalysed reaction is proportional to the enzyme concentration, but that is not necessarily the case for multi-enzyme systems, in which flux control is shared among all the enzymes, and the concentration of a particular enzyme can vary substantially with negligible effects on the flux. For any one enzyme the degree of flux control is defined by a flux control coefficient, which is normally never unity, and for systems of many enzymes it is typically immeasurably small. However, this is theory: does it work in practice? One indication that it is correct comes from many failures to increase production of economically valuable metabolic products by overexpressing the enzymes believed to be rate-limiting. Another is the observation that in a genetic disease such as phenylketonuria, heterozygotes with half of the normal amount of enzyme have no symptoms and are just as healthy as normal homozygotes. Likewise, inheritance of eye colour in humans is explained by the fact that eyes with only half the activity of the enzymes needed to produce brown eyes are barely different from the eyes of brown-eyed homozygotes. These examples involve very small numbers of genes, but in chromosomal disturbances that affect many genes, such as Down syndrome or more severe cases of

aneuploidy, a large number of effects that would be negligible when considered individually, can add up to very large effects.
The theory of metabolic control needs to be supplemented, however, with a theory of metabolic regulation, to explain why unregulated natural systems are very different in behaviour from living systems or systems designed by engineers. A lake will typically have many sources of water, but a maximum of one exit, whereas engineered and living systems allow regulated flow in different directions. A healthy metabolic system has regulated flows at many different points, and allows forward and reverse transformations between, for example, fructose 6-phosphate and fructose 1,6- bisphosphate, to be possible in the same cells with negligible loss of ATP by hydrolysis. In this example regulation is achieved by strong inhibition and activation of phosphofructokinase and fructose bisphosphatase, to ensure that both processes are not simultaneously in operation. The underlying principle of metabolic regulation by feedback effects is that fluxes are determined primarily by the demand for end-products and as little as possible by the supply of starting materials. If the necessary regulatory interactions are impaired, as they may be in cancer and other illnesses, satisfactory metabolism becomes impossible.

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A2

Competing theories of cancer: chromosomal imbalance vs. gene mutation

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Gene mutation theory in decline

In 2002, Robert Weinberg admitted that, "For those who believe in the simplification and rationalization of the cancer process, the actual course of research on the molecular basis of cancer has been largely disappointing. Rather than revealing a small number of genetic and biochemical determinants operating within cancer cells, molecular analyses of human cancers have revealed a bewilderingly complex array of such factors [1]." In 2014, he added, "Moreover, even within a given type of cancer…there were no uniform successions of genetic change. Instead, each tumor seemed to represent a unique experiment of nature, acquiring a unique set of mutant genes and in an unpredictable chronological order." Weinberg concluded, "The coupling between observational data and biological insight is frayed if not broken [2]." November 2016, Bert Vogelstein dealt the mutation theory another blow when he reported that the search for cancer causing genes is "hindered by the lack of a gold standard, that is, bona fide driver gene mutations [3]." It was actually a double blow since driver genes include oncogenes and tumor suppressor genes.

Theory of chromosomal imbalance

In contrast to "bona fide driver gene mutations," which are exceedingly difficult to find, aneuploidy is abundant in cancer cells [4]. Chromosomal imbalance is orders of magnitude more powerful than gene mutations in producing cancer phenotypes [5]. The phenotypes of cancer cells are determined by the fraction of the genome out of balance relative to the euploid cell [6]. Aneuploid cancer cells have substantially greater amounts of DNA, RNA and protein than normal cells [7]. A 40% increase in cellular protein produces a 32-fold elevation in membrane proteins. A 20% increase in cellular protein causes a 30-fold elevation in secreted proteins [8]. Thus the tumor-associated antigens and the high levels of secreted proteins responsible for invasiveness and loss of contact inhibition are the natural consequence of the excess production of protein in cancer cells. The additional ATP required for the synthesis of the extra protein is produced by the aerobic fermentation of glucose [9-11], the so-called Warburg effect.

Chromosomal imbalance disrupts the mitotic machinery leading to the chaotic separation of chromosomes. "The relationship between aneuploidy and chromosomal instability can be envisioned as a 'vicious cycle,' where one potentiates the other [12]." An extra copy of a single chromosome is sufficient to produce chromosomal instability [13]. The greater the imbalance of chromosomes, the greater is the instability [6, 14]. The survival advantage of cells that gained chromosomes, coupled with chromosomal instability, leads to the autocatalyzed progression of aneuploidy during cell division [6, 15, 16].

Much sought-after mechanism of carcinogenesis

Carcinogen-initiated chromosomal imbalance, coupled with the autocatalyzed progression of aneuploidy during cell division, is necessary and sufficient to generate cancer on the rare occasions the cells survive—independent of gene mutation.

Practical utility

The theory of chromosomal imbalance has a number of practical applications [17]. Monitoring aneuploidy is the most accurate and sensitive way of detecting cancer and following its progression [18]. Reducing exposure to aneuploidogens is the best way to reduce the incidence of cancer. Aneuploidy damages a cell and is the reason primary cancer cells tend to die at high rates *in vivo* and in culture [19, 20]. Indeed, the lability inherent in aneuploid cells is the likely reason for the spontaneous remission of all types of cancer. The theory of chromosomal imbalance predicts that a variety of non-toxic perturbations of the host (such as induced fever) may therapeutically nudge the tumor out of its stable, comfortable environment, increasing the natural spontaneous death rate of the cancer cells [17].

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