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Opinion

The chromosomal basis of cancer

Peter Duesberg [a,*], Ruhong Li [a], Alice Fabarius [b] and Ruediger Hehlmann [b]
[a] *Department of Molecular and Cell Biology, Donner Laboratory, UC Berkeley, Berkeley, CA 94720, USA*
[b] *III. Medizinische Klinik Mannheim of the University of Heidelberg at Mannheim, Wiesbadener Str. 7-11, 68305 Mannheim, Germany*

Abstract. Conventional genetic theories have failed to explain why cancer (1) is not heritable and thus extremely rare in newborns, (2) is caused by non-mutagenic carcinogens, (3) develops only years to decades after initiation by carcinogens, (4) follows pre-neoplastic aneuploidy, (5) is aneuploid, (6) is chromosomally and phenotypically "unstable", (7) carries specific aneusomies, (8) generates much more complex phenotypes than conventional mutation such as multidrug resistance, (9) generates nonselective phenotypes such as metastasis (no benefit at native site) and "immortality" (not necessary for tumorigenesis), and (10) does not contain carcinogenic mutations. We propose, instead, that cancer is a chromosomal disease. Accordingly carcinogenesis is initiated by random aneuploidies, which are induced by carcinogens or spontaneously. Since aneuploidy unbalances 1000s of genes, it corrupts teams of proteins that segregate, synthesize and repair chromosomes. Aneuploidy is therefore a steady source of chromosomal variations from which, in classical Darwinian terms, selection encourages the evolution and malignant progression of cancer cells. The rates of specific chromosomal variations can exceed conventional mutations by 4–11 orders of magnitude, depending on the degrees of aneuploidy. Based on their chromosomal constitution cancer cells are new cell "species" with specific aneusomies, but unstable karyotypes. The cancer-specific aneusomies generate complex, malignant phenotypes through the abnormal dosages of 1000s of genes, just as trisomy 21 generates Down syndrome. In sum, cancer is caused by chromosomal disorganization, which increases karyotypic entropy. Thus, cancer is a chromosomal rather than a genetic disease. The chromosomal theory explains (1) non-heritable cancer because aneuploidy is not heritable, (2) non-mutagenic carcinogens as aneuploidogens, (3) long neoplastic latencies by the low probability of evolving new species, (4) nonselective phenotypes via genes hitchhiking with selective chromosomes, and (5) immortality because, through their cellular heterogeneity, cancers survive negative mutations and cytotoxic drugs via resistant subspecies.

1. Cause of cancer still a matter of debate

Despite over 100 years of cancer research the cause of cancer is still a matter of debate between theories postulating either mutation or chromosomal alteration or epigenetic events as causes of cancer [43,69,75,77, 79,85,94,102,107,114,137,169,170,185,189,190,215, 225,234,248,251,257,261,264,265,269,273,286,306]. We propose here that the cancer problem is still unsolved, because this debate has been monopolized by conventional gene mutation theories, which hold that cancer is a "genetic disease" [33,104,154,178,210,214, 281,282,287,288].

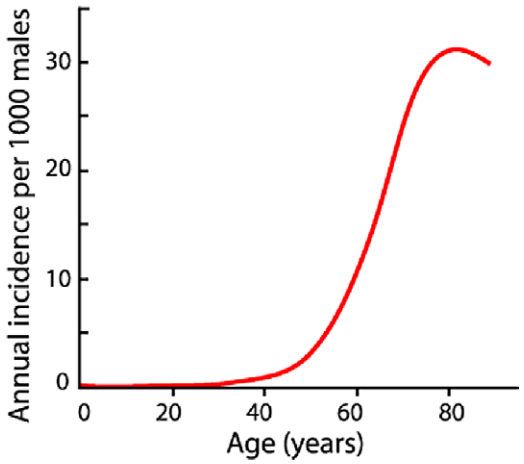
These gene-based theories postulate that cancer is caused by clonal expansion of cells, which have accumulated about 4–7 specific and complementary mutations during the lifetime of a patient [45,108,178, 225,235,286]. In addition, these theories postulate that, once generated by 4–7 mutations, cancer cells independently progress further within "clonal" cancers to form evermore malignant and heterogeneous cancers via evermore spontaneous mutations – while normal cells of the same patient remain un-mutated [32,33, 46,104,108,154,178,214,280,286–288]. But these conventional genetic theories cannot explain the following critical properties of carcinogenesis.

2. Discrepancies between conventional genetic theories and cancer

2.1. Cancer is not heritable

The best news about cancer is that we and other animals are virtually all born cancer-free and typically ac-

*Corresponding author. Tel.: +1 510 642 6549; Fax: +1 643 6455; E-mail: duesberg@berkeley.edu.



Age	Incidence
<1	0.24
1–4	0.22
5–9	0.12
10–14	0.13
15–19	0.21
20–24	0.31
25–29	0.44
30–34	0.59
35–39	0.88
40–44	1.48
45–49	2.70
50–54	5.37
55–59	9.47
60–64	15.41
65–69	22.64
70–74	28.29
75–79	31.23
80–84	30.83
>85	29.77

Fig. 1. Age specific incidence of invasive cancers of males in the United States in 2001. The dominant contributors to the total number of invasive cancers are solid tumors. The growth is approximately exponential until about age 70 and then levels off. Data for the figure, shown in the table at the right, are from the National Program of Cancer Registries at <http://www.cdc.gov/cancer/npcr/index.htm>. Because cancer is primarily a disease of old age it is compatible with an acquired, but not with an inherited disease.

quire cancer, if at all, only at advanced age [12,45,141, 178,198,219]. This bias of cancer for old age is exponential, increasing the cancer risk 300-fold with age, from near-zero rates in newborns and adolescents to rates of 1 in 3 in the last third of a human or animal life span (Fig. 1). Thus cancer is a disease of old age.

But, if the prevailing gene-based cancer theories were correct, the age bias of cancer would be paradoxical. According to the gene mutation theories, cancer should be common in newborns. For example, a baby, which inherits 3 colon cancer mutations from his mother and 2 from his father out of the about 6 that are thought to cause colon cancer [178,189,286], should develop cancer at a very young age from just one more spontaneous mutation in any one of the billions of its colon cells. Indeed many hypothetical cancer-causing mutations, including those thought to cause colon cancer, are heritable in transgenic mice [76,85] and also in humans [154,287] (see Box 1, *The Achilles heels of the mutation-cancer theory*, and Section 2.10). According to Vogelstein and Kinzler, "one of the cardinal principles of modern cancer research is that the same genes cause both inherited and sporadic (non-inherited) forms of the same tumors" [287]. But, there is no colon cancer in newborns [189] (Fig. 1).

The extremely rare cases of cancer in newborns and children shown in Fig. 1 do not save the mutation theory of cancer. Since cancer affects 1 in 3 human lives, large percentages of newborns and children would have cancer, if cancer were heritable. Thus the mutation theory fails to explain the extremely low rates of cancer at young age.

Instead, the extremely low percentages of children with cancer can be explained as the fringes of the probability distribution of those events that cause cancer typically only after very long latencies, which last decades in humans (see below Sections 2.3, 3 and Fig. 3). In addition, rare genetic diseases that increase those events that initiate and eventually cause cancer also explain some of the cancers in children (Sections 2.5, and 4.8.5).

Moreover, if heritable cancer genes existed twins should have very similar cancer rates. But, according to a multi-national epidemiological study of the cancer incidence in twins, "Inherited genetic factors make a minor contribution . . . the environment has the principal role in causing sporadic cancer" [171].

2.2. *Non-mutagenic carcinogens cause cancer*

Carcinogens are either chemical or physical agents that initiate carcinogenesis [45,219]. Both chemical and even physical carcinogens can be either mutagenic or non-mutagenic. Examples of non-mutagenic carcinogens are asbestos, tar, mineral oils, naphthalene, polycyclic aromatic hydrocarbons, butter yellow, urethane, dioxin, hormones, metal ions such as Ni, Cd, Cr, As, spindle blockers such as vincristine

Box 1

The Achilles heels of the mutation-cancer theory

The currently prevailing cancer theory postulates that cancer is caused by clonal expansion of one single cell that has accumulated about 4–7 complementary mutations during the lifetime of a patient [45,108,178,225,235,286]. However, the mutation theory is hard to reconcile with the following list of facts.

(1) *Non-mutagenic carcinogens.* Contrary to the mutation theory, many carcinogens are not mutagens, including some of the most potent ones. Examples are asbestos, tar, mineral oils, naphthalene, polycyclic aromatic hydrocarbons, butter yellow, urethane, dioxin, hormones, metal ions such as Ni, Cd, Cr, As, spindle blockers such as vincristine and colcemid, extranuclear radiation and solid plastic or metal implants [29,44,76,81,172,176,203,219,304] (see Section 2.2).

(2) *No transforming genes.* Despite over 25 years of efforts no genes or combinations of genes from cancers have been shown to transform normal cells to cancer cells [4,169,170] or generate polyclonal tumors in mice carrying such genes in their germ lines [72, 113,114,150,259,286]. In agreement with this lack of function, many presumably cancer-specific mutations are not detectably expressed in cancer cells [85,221, 228,305] (Section 2.10).

(3) *Dependence of cancer on unrealistically high rates of mutation.* The mutation theory explains the exponential increase of the cancer incidence with age (Fig. 1) by the low probability that conventional mutation would generate 4–7 specific mutations in the same cell [108,180,235,286]. This is so improbable, because the spontaneous mutation rates in all species are naturally restricted to about 10^{-7} per dominant gene and to about 10^{-14} per recessive gene per cell generation, in order to maintain the integrity of the genome [133,167,185,271,285]. Indeed, based on these conventional mutation rates, cancer via 4–7 mutations would not even exist [77]. Even the most probable cancer case predicted by the mutation theory, namely cancer via 4 specific dominant mutations, would occur only once in 10^{12} human lifetimes. This is calculated as follows: Since the spontaneous mutation rate per specific, dominant gene is about 10^{-7}, it takes 10^{28} cells to generate one human cell with 4 specific mutations. The expected cancer rate per human lifetime of 1 in 10^{12} is then obtained by dividing 10^{28} by 10^{16}. 10^{16} is the number of cells that correspond to an average human lifetime [45,77]. Thus, in order to explain the current cancer risk of Americans and Europeans of about 1 in 3 lifetimes [141] (Fig. 1) in terms of 4 mutations, the mutation theory has to assume mutation rates, which are about 10^3 times higher than in conventional mutation. In other words the rates of 4 mutations would have to be about 10^{12} times higher and that of a single mutation about 10^3 times higher $[(10^3)^4 = 10^{12}]$ than they are, to generate the known cancer rates.

(4) *No explanation for "neoplastic latency" after a sufficient dose of carcinogen.* The mutation theory has no simple answer to the question why, after a critical dose of carcinogen, carcinogenesis would only occur after exceedingly long "neoplastic latencies" of years to decades (Section 2.3) [286]. Instead evermore complex sequences of mutations [46] and even "transient" mutator genes (undetectable in subsequent cancers) are postulated without functional proof [179,180].

(5) *Dependence of phenotype alterations in cancers on unrealistically high rates of mutation.* The mutation theory has to assume mutation rates of up to 10^{-3} per cell generation to explain the frequent, spontaneous variation of phenotypes in highly aneuploid cancer cells. Examples are the "high rates" (compared to mutation) at which some cancers generate metastatic cells [5,115], or generate drug-resistant variants [83, 84,113,168] (Section 2.6). But the mutation rates of most cancers are not higher than those of normal cells [76,112,133,162,185,203,257,266,273,292].

(6) *Heritable mutations of cancer cells, but no heritable cancer*. The multi-gene mutation theory predicts that subsets of cancer causing mutations should be heritable. Indeed, proponents of the mutation theory have demonstrated that several of the 6 mutations thought to cause colon cancer [286] can be introduced into the germ line of mice without breaching the viability of these animals (see above, point 2 and Section 2.10). According to one study animals with one of these mutations, namely *ras*, were found "without detectable phenotypic abnormalities" [150]. According to another study, "Surprisingly, homozygosity for the Apc1638T mutation [a hypothetical colon cancer suppressor gene] is compatible with postnatal life" [259]. Thus subsets of colon cancer genes are heritable. Therefore, colon cancer should be common in newborns, which are clonal for inherited subsets of these 6 mutations (like transgenic mice). But there is no colon cancer in newborns (Fig. 1) [45,141,189].

and colcemid, extra-nuclear radiation and solid plastic or metal implants [29,44,76,81,172,176,203,219,304] (see also, Box 1).

Moreover the many agents that accelerate carcinogenesis, termed tumor promoters, are all non-mutagenic, or not directly mutagenic, as for example croton oil and phorbol acetate [139,219].

Conventional genetic theories, however, fail to explain carcinogenesis by non-mutagenic carcinogens and non-mutagenic tumor promoters.

2.3. Long neoplastic latencies

Surprisingly, in view of the mutation theory, there are no fast carcinogens. Nevertheless, many carcinogens are very fast mutagens, as for example, X-rays, UV light and alkylating agents. But *all* carcinogens, mutagenic or not, are very slow – causing cancer only after exceedingly long "neoplastic latencies" [90,219] of many months to years in rodents, and of many years to decades in humans [25,26,29,45,77,90,136, 219,286].

Examples are, (i) the solid cancers, which developed in survivors of atomic bombs only 20 years after exposure to nuclear radiation in1945 [45]; (ii) the breast cancers, which developed in former tuberculosis patients only 15 years after treatments with X-rays in the 1950s [36]; and (iii) the lung cancers, which developed in workers of a Japanese mustard gas factory only 30 years after it was closed in 1945 [70]. Similarly, the risk of lung cancer remains about 5–10× higher for ex-smokers than it is for non-smokers, even decades after they stopped smoking [45,46,71, 129]. Thus an initiated cell evolves only gradually to a visible cancer cell, even though it has received sufficient carcinogen for carcinogen-independent carcinogenesis – much like a submarine volcano only gradually becomes a visible island [25,26,90]. By contrast, the mutation theory would have predicted carcinogenesis as soon as the above examples had received the doses of carcinogen that eventually caused their cancers.

Experimental carcinogenesis demonstrates even more directly that, once initiated, the evolution of cancer cells is an autonomous, if slow, process that is independent of further exogenous influences [29,45,90, 219,243]. Nevertheless, experimental carcinogenesis is accelerated by further carcinogens or tumor promoters [45,138,139,219,243] (Section 2.2). This autonomous evolution continues in cancer cells and their descendents both *in vivo* and even *in vitro* [29,90,120,164, 297]. As a result cancer cells progress independently within individual cancers, to form evermore "polymorphic" [49] and phenotypically heterogeneous cancers with evermore exotic karyotypes and phenotypes [90]. Thus "initiation" confers on cells a lifelong variability that can generate new phenotypes and karyotypes many cell generations or decades after it was established.

But, the evolution of new phenotypes many cell generations or decades after mutagenesis is incompatible with conventional mutation, on which genetic theories of cancer are based. Conventional mutation is immediate and just as stable as the parental genotype [94, 104,167,214]. It is for this reason that Cairns wrote in *Cancer: science and society*, "The conspicuous feature of most forms of carcinogenesis is the long period that elapses between initial application of the carcinogen and the time the first cancers appear. Clearly, we cannot claim to know what turns a cell into a cancer cell until we understand why the time course of carcinogenesis is almost always so extraordinarily long" [45].

2.4. Exact correlations between cancer and aneuploidy

Exact correlations between cancer and aneuploidy have been reported since 1890 [17,18,111,124,155, 245]. Likewise, abnormal expression of 1000s of genes, proportional to the abnormal ploidy of the corresponding chromosomes, have recently been detected in all cancer cells that have been tested by hybridizations of cellular RNAs with arrays of cellular genes [2, 95,221,283].

Aneuploidy is defined by losses or gains of intact chromosomes or of segments of chromosomes [167]. Gained segments of chromosomes are typically rearranged either with the same chromosomes from which they derived or with other chromosomes. The resulting hybrid chromosomes are termed marker chromosomes. Owing to their unique structure, marker chromosomes can serve as tracers for the origin of possibly metastatic cancer cells from primary cancers and for the origin of primary cancer cells from possibly aneuploid pre-neoplastic precursors [155,245]. A typical example of a highly aneuploid cancer karyotype with numerous marker chromosomes, that of a breast cancer cell from the cell line MDA 231, is shown in Fig. 2. The figure also shows the karyotype of a normal male, human cell. In addition, cancer cells often include extra-chromosomal forms of aneuploid segments of chromosomes, termed "amplicons", that are either

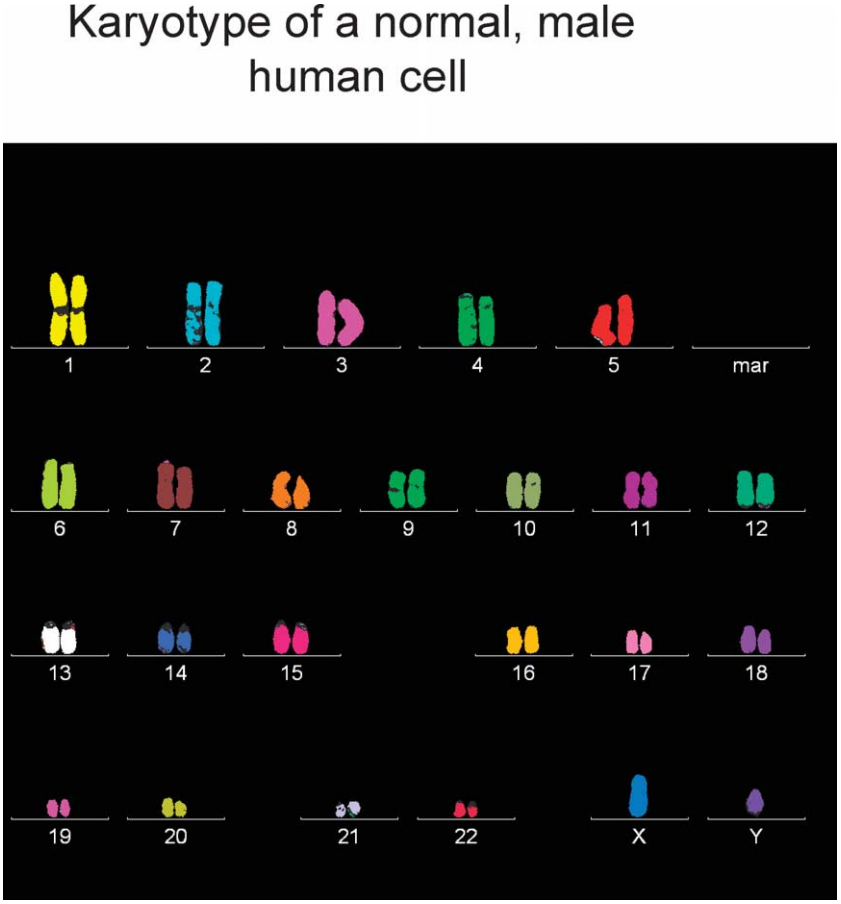


Fig. 2. Metaphase chromosomes of the human breast cancer cell line, MDA 231, and of a normal cell of a human male. The metaphases were prepared and hybridized *in situ* with color-coded, chromosome-specific DNA probes from MetaSystems, Inc., Boston, MA, following published procedures [168]. The numbers identify normal chromosomes. The group labeled "mar" (for marker chromosome) shows structurally abnormal chromosomes, which are either rearranged intra-chromosomally or inter-chromosomally to form various hybrid chromosomes. Such chromosomes are called "marker chromosomes", because they can be used as structurally unique, cytogenetic markers of a given cancer. The numbers above these marker chromosomes identify the chromosomal constituents of hybrid chromosomes in their relative order or signal intra-chromosomal alterations. The comparison of the two karyotypes shows that the cancer cell differs from the normal cell in numerous numerical and structural chromosomal alterations or aneusomies.

microscopically detectable as "double minute" chromosomes [124,245,247] or maybe "submicroscopic" [207], depending on the microscopic technique used, with sizes as low as 1 megabase (Mb) [194,258]. But even extra- and intra-chromosomal amplicons [177] or deletions of only 1 Mb are still nearly as large as an entire *E. coli* chromosome of about 3 Mb. Aneuploidy is thus a much more massive genetic abnormality than the gene mutations that have also been found in cancer cells (Section 2.10 and Box 1).

The ubiquity of aneuploidy in cancer is, however, not postulated nor predicted by the mutation theory. As a consequence, cancer-defining aneuploidy is currently not even mentioned in the cancer chapters of the leading textbooks of biology [7,45,167,178,214].

Nevertheless, exceptions to the coincidence between aneuploidy and cancer have been reported, as for example "diploid" colon cancers with mismatch repair deficiency [162]. But, further analysis of what appeared to be diploid colon cancers by "array-based comparative genomic hybridization" has since indicated that about "5% of their entire genome" is segmentally aneuploid versus 20% of a control group of colon cancers without mismatch repair deficiency [194]. Colon cancers with "normal karyotypes" have also been described by Bardi et al. [21]. But, further scrutiny reveals that these normal karyotypes were either from "hyperplastic polyps" [23] or from "non-neoplastic stromal cells" [22] or were considered to be misidentified tumor cells showing "how dependent

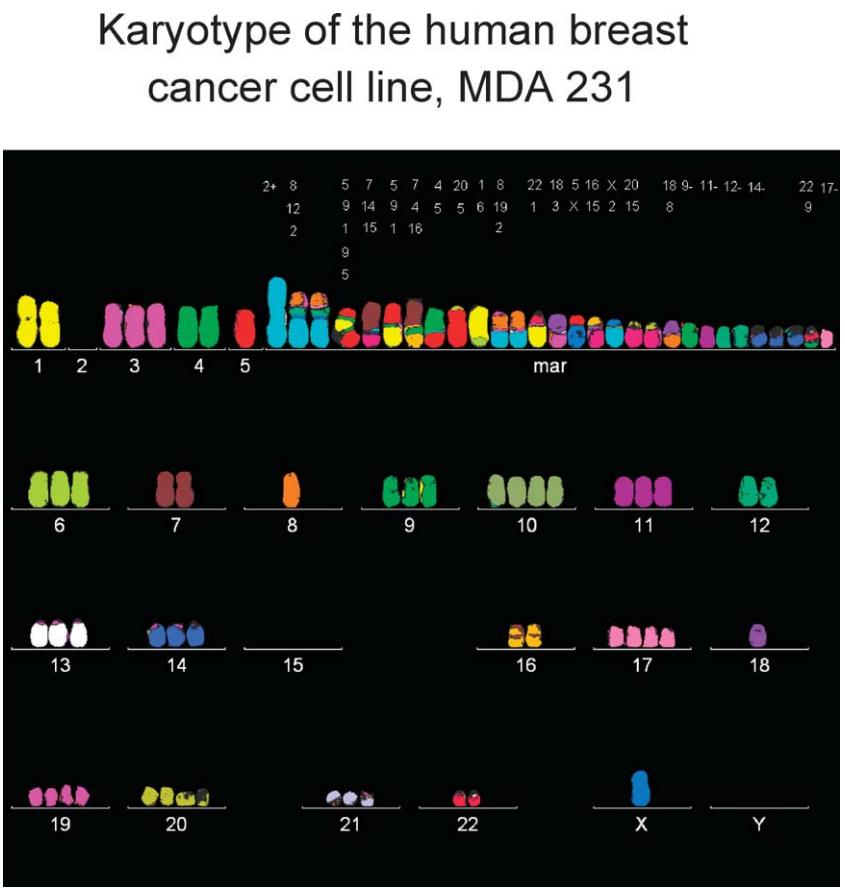


Fig. 2. (Continued.)

findings in solid tumor cytogenetics are on method" [38] (Bardi G., personal communication, 2004). Thus there is currently no unambiguous evidence for diploid cancer.

2.5. *Accidental, genetic and congenital pre-neoplastic aneuploidy*

Intrigued by the aneuploidy in cancer and the long neoplastic latencies, many researchers have analyzed cancer-prone tissues for pre-neoplastic genetic and chromosomal alterations, particularly aneuploidy [16, 76,102,185,257,262].

Accidental pre-neoplastic aneuploidy. The first consistent evidence for pre-neoplastic aneuploidy was obtained in 1960s by Caspersson [48] and Spriggs [262] in cervical tissues [16,114]. Similar studies have since also found aneuploidy prior to carcinogenesis in precancerous tissues and neoplasias of the throat, colon, lung, breast, skin, pancreas, prostate, gonads, esophagus and the cervix [3,20,34,38,125,128,129,181, 182,188,193,196,215,218,223,231,232,241,256,267, 275,296]. Moreover, multinational, epidemiological studies have found that the relative cancer risk of people can be predicted from the degree of chromosomal aberrations of peripheral lymphocytes [39,106].

Experiments undertaken to study the origin of aneuploidy in animals treated with carcinogens, have also found aneuploidy prior to cancer in the liver, skin and subcutaneous tissues of carcinogen-treated rodents [42,56,59,64,184,224,278] (our unpublished observations with Chinese hamsters).

Likewise, treatments of diploid human and animal cells *in vitro* with carcinogens were found to generate aneuploidy long before transformation. Unexpectedly, this pre-neoplastic aneuploidy proved to be variable in subsequent cell generations – creating "delayed" genomic instability or even "delayed reproductive death" [24,27,57,58,60,62,76,80,89,92,134,176, 274,279,290,300]. Aneuploidy also precedes transformation of human and animal cells infected by Simian

Virus 40 and other DNA tumor viruses [170,229,298]. Even spontaneous transformation of cells *in vitro* is preceded by aneuploidy [63,86,122,165].

When we tested pre-neoplastic aneuploidy with regard to its role in cancer, we found that experimental pre-neoplastic aneuploidy always segregated with subsequent morphological transformation and tumorigenicity [80,89]. Based on these data we have concluded that aneuploidy initiates carcinogenesis. This conclusion is directly supported by the high cancer risks of heritable chromosome instability syndromes and of congenital aneuploidies. We show next that in both of these conditions aneuploidy also precedes cancer.

Genetic pre-neoplastic aneuploidy. Heritable diseases that predispose to abnormally high rates of systemic aneuploidy, termed "chromosome instability syndromes", include Fanconi's anemia, Bloom's syndrome, Ataxia Telangiectasia, Xeroderma, Werner's and other syndromes. These chromosome instability syndromes also predispose to high rates of cancer and generate cancers at younger age than in normal controls [245] (see Section 4.8.5). In these syndromes, heritable mutations function as genetic aneuploidogens and carcinogens (Sections 3 and 4.8.5).

Congenital pre-neoplastic aneuploidy. Minor, congenital aneuploidies are viable, while major congenital aneuploidies are lethal [67,118]. The best known examples are Down syndrome, Retinoblastoma, Wilms tumor, Klinefelter's syndrome and others, summarized by Sandberg in 1990 [245]. Just like the chromosomal instability syndromes, the congenital aneuploidy syndromes carry high cancer risks and generate cancers at younger age than in diploid controls [117,155, 245]. The 20-times higher-than-normal incidence of leukemia in Down syndrome is one of the best-studied examples [117,155,245]. The same is true for congenital aneuploidy in mice, in which an artificial duplication of only 1 megabase of chromosome 11 was found to induce lymphomas and other tumors after latencies of several months [177].

However, the mutation-cancer theory does neither postulate nor predict the presence of pre-neoplastic aneuploidy – except, perhaps indirectly, by postulating the generation of cancer genes via chromosomal rearrangements (Section 1). But, again the evidence for cancer-specific mutations is missing [76] (Box 1). According to a recent review by Little, "While radiation-induced cancers show multiple unbalanced chromosomal rearrangements, few show specific translocations or deletions as would be associated with the activation of known oncogenes or tumor suppressor genes" [176].

2.6. *Karyotypic–phenotypic variations of cancer cells at rates that are orders higher than conventional mutation*

The chromosomes of cancer cells are extremely unstable compared to those of normal cells: 1 in 100 highly aneuploid human cancer cells loses or gains or rearranges a chromosome per cell generation [168]. Since humans contain 23 chromosomes, 1 in 23 chromosome alterations can be expected to generate a specific aneusomy. In agreement with this, up to 1 in 1000 aneuploid cancer cells spontaneously generates a specific new phenotype per cell generation – "at frequencies considerably greater than conventional mutation" [300] – as for example drug-resistance [83,84,113,168, 271] or the ability to metastasize at "high rates" [5, 115] or the loss of heterozygosity at rates of 10^{-5} per generation [287].

This inherent karyotypic–phenotypic variability of cancer cells is the reason, why most cancers are "enormously" heterogeneous populations of non-clonal and partially clonal cells, which differ from each other in "bewildering" [155] phenotypic and chromosomal variations [124,248] – even though most cancers are derived from a common, primary cancer cell and thus have clonal origins [45,49,51,90,113,120,124, 151,162,176,199,245].

By contrast, conventional mutation of specific genes is limited to 10^{-7} per cell generation for dominant genes and to 10^{-14} for pairs of recessive genes in all species [133,167,185,270,271,285]. Surprisingly, in view of the genetic theories of cancer, even the gene mutation rates of most cancer cells are not higher than those of normal cells [76,112,133,162,185, 203,257,266,272,273,292]. Thus specific, karyotypic–phenotypic variations of cancer cells are 4 to 11 orders faster than conventional mutation, and therefore not compatible mutational theories.

Following others [185,270,287], we have used here an average, spontaneous mutation rate of 10^{-7} per human/mammalian genetic locus per cell generation. These averages reflect mutation rates that range between 10^{-5} and 10^{-9} [140] and 10^{-5} to 10^{-7} [285] depending on the respective human phenotypes. Lower rates of phenotype variation of 10^{-8} to 10^{-10} are observed in bacteria and yeast [167,214]. The apparently higher mutation rates of humans/mammals compared

to bacteria probably reflect (a) the higher genetic complexity of the respective human loci studied compared to those of bacteria and yeast, and (b) the fact that "the rates in humans are calculated per gamete and several cell divisions are required to produce a gamete" [214]. Take for example the numerous mutant genes and clotting factors that cause the phenotype, hemophilia [285]. Indeed, the mutation rates per unit X-irradiated genetic DNA are the same in all species [1].

2.7. *Cancer-specific chromosomal alterations*

Despite the karyotypic instability of cancer cells and heterogeneity of cancers, partially specific or "nonrandom" chromosomal alterations, also termed aneusomies, have been found in cancers since in the late 1960s [14,15,17,20,22,23,89,124,143,155,204,208, 209,283,301,302,303]. The majority of these nonrandom chromosomal alterations have been detected in cancers since the 1990s by the use of comparative and gene array-based genomic hybridization, rather than by identifying specific aneusomies cytogenetically [68,98,124,127,142,200,205,212,221,234, 236,237,293]. Specific aneusomies have been linked with the following distinct events of carcinogenesis:

(i) Stages of neoplastic transformation in human [68,93,128,132,149,155,205,237,295] and in animal carcinogenesis [89],
(ii) Invasiveness [132,187,295],
(iii) Metastasis [6,11,35,125,152,195,197,213],
(iv) Drug-resistance [168,247,270],
(v) Transplantability to foreign hosts [121],
(vi) Cellular morphologies [289],
(vii) Abnormal metabolism [119,155],
(viii) Cancer-specific receptors for viruses [155, 289].

Moreover, in cases where this has been tested, cancer-specific gene expression profiles are directly proportional to the dosages of the corresponding chromosomes [2,95,178,221,283].

Cancer-specific or "nonrandom" chromosomal alterations, however, are neither postulated nor predicted by mutational theories of cancer. In fact they are a direct challenge of the mutation theory, because specific chromosomal alterations generate specific phenotypes, independent of mutation. The over 71 Down syndrome-specific phenotypes, caused by trisomy 21 without any gene mutation, are a confirmed model [87, 183,230,255].

2.8. *Cancer phenotypes too complex for conventional mutations*

The complexity of most cancer-specific phenotypes far exceeds that of phenotypes generated by conventional mutation. Examples are the gross polymorphisms in size and shape of individual cells within individual cancers [25,49,90]. Moreover, the kind of drug-resistance that is acquired by most cancer cells exposed to a single cytotoxic drug is much more complex than just resistance against the drug used to induce it. Therefore, this phenotype has been termed "multidrug resistance" [84,113,250]. It protects not only against the toxicity of the challenging drug, but also against many other chemically unrelated drugs and is thus probably multigenic.

Cancer-specific phenotypes such as grossly abnormal metabolism, metastasis, transplantability to heterologous species [121] and "immortality" (also Sections 2.9, 4.4 and 4.6) [90,219] are also likely to be multigenic, because all of these phenotypes correlate with altered expressions of thousands of genes [2,95, 178,221,283] and with highly abnormal concentrations of thousands of normal proteins [49,50,190,219]. Immortality is defined as the ability of cancer cells to grow indefinitely in culture or on transplantation [90, 167]. In addition, the number of centrosomes is increased up to five-fold (from a normal of 2 to around 10) in highly aneuploid cancer cells, and their structures are often altered at the same time [100,174,216, 217].

The complexities of these cancer-specific phenotypes, however, can not be achieved by the low, conventional rates of gene mutations during the limited live spans of humans and animals (Section 2.6 and Box 1). For example, it is virtually impossible that the up to five-fold increased numbers of centrosomes, which are observed in highly aneuploid cancer cells [43,174,175, 216], would be the result of mutations that increase the numbers of the 350 different proteins that make up centrosomes [74]. Thus the mutation theory cannot explain the complex phenotypes of cancer.

Contrary to this conclusion, it has been argued that multidrug resistance can be generated by singular genes [148,250]. However, it is biochemically implausible that a single protein could protect against many, biochemically unrelated cytotoxic substances, such as DNA chain terminators, spindle blockers and inhibitors of protein synthesis all at once [148,250]. Moreover it is improbable that only cancer cells would

benefit from such genes, whereas normal cells of cancer patients remain vulnerable [83,84,113].

In view of these discrepancies we have recently proposed that chromosomal alterations are the cause of multidrug resistance [83,84,168]. To test this hypothesis we have carried out two kinds of experiments: First, we have asked whether aneuploid mouse cells, from which multidrug resistance genes had been deleted [8], could still become drug-resistant. In accordance with our prediction we have found that aneuploid cells become multidrug resistant even without all known multidrug resistance genes of mice [83,84]. Second, we have asked whether drug-resistance correlates with resistance-specific chromosomal alterations. Indeed, this too was confirmed recently [168]. In view of this, we conclude that multidrug resistance is chromosomal and thus multigenic (also Section 4.6).

2.9. *Non-selective phenotypes: not helping cancer cells to compete for growth*

Cancer-specific phenotypes can be divided into two classes: Those, which are selective, because they advance carcinogenesis by conferring growth advantages to cancer cells such as invasiveness, grossly altered metabolism and high adaptability via high genomic variability [90,219], and those, which are not selective for growth [30,76].

The non-selective phenotypes of cancer cells include metastasis, multidrug resistance and immortality. Metastasis is the ability to grow at a site away from the primary tumor. Therefore, it is not selective at the site of its origin [30]. Likewise, multidrug-resistance is not a selective advantage for natural carcinogenesis in the absence of chemotherapy. Yet, a high percentage of cancers is intrinsically multidrug-resistant [73, 103]. Moreover, acquired multidrug resistance protects against many more drugs than the cancer was ever exposed to [83,84,250]. Even immortality is not a selective advantage for carcinogenesis, because many types of human cells can grow over 50 generations according to the Hayflick limit [122], and thus many more generations than are necessary to generate a lethal cancer. Consider, that fifty cell generations produce from one single cell a cellular mass equivalent of 10 humans with 10^{14} cells each [77].

Non-selective phenotypes, however, are neither postulated nor predicted by conventional gene mutation-selection mechanisms.

2.10. *No cancer-causing genes in cancer*

Numerous gene mutations have been found in cancer cells since the 1980s [31–33,108,268,280,286]. The prevailing genetic cancer theories postulate that these mutations cause cancer [33,108,178,287,288] (Section 1). But this hypothesis is hard to reconcile with the following facts:

(1) None of the mutations found in cancers are cancer-specific [37,286].
(2) In cases where this information is available, many perhaps most mutations are non-clonal [37,85,147].
(3) Expression of most hypothetically cancer-causing mutations is not even detectable in most human cancer cells without artificial amplification methods [85,221,228,305].
(4) No mutant gene and no combination of mutant genes from cancer cells has been found that converts diploid human or animal cells into cancer cells, despite enormous efforts in the last 25 years [4,76,114,169,170,225,248]. On September 16, 2005, J. Michael Bishop confirmed that there is still no proven combination of mutant genes from cancer cells that is sufficient to cause cancer (at a seminar, “Mouse models of human cancer” at the Lawrence Berkeley Lab at Berkeley).
(5) In contrast to predictions of the mutation theory – mouse strains with hypothetical cancer genes artificially implanted into their germline, and others with hypothetical tumor suppressor genes artificially deleted from the germline have survived many generations in laboratories with either the same or slightly higher cancer-risks than other lab mice [72,76,85,114]. For instance, one group observed that, “Surprisingly, homozygosity for the Apc1638T mutation”, an artificial null mutation of the hypothetical tumor suppressor gene Apc, “is compatible with postnatal life” and that “animals that survive to adulthood are tumor-free” [259] (see also Box 1). Even more surprisingly – some mice with hypothetical cancer genes and others without hypothetical tumor suppressor genes fare even better than un-mutated controls. For example, the authors of one study state that, “Surprisingly”, the “germline expression of an oncogenic erbB2 allele (breast cancer gene, alias Her2 and Neu) ... conferred resistance to mammary tumori-

genesis" [10]. Yet another group reports that "unexpectedly" mice with null mutations of the retinoblastoma gene, rb, "developed fewer and smaller papillomas" than un-mutated controls [235].

There are, however, reports about tumors in mice that can be induced and even reversed experimentally via promoters that switch on and off hypothetical cancer genes, which have been artificially implanted into the germline [252,294]. But the questions, why only local, and thus possibly clonal, tumors appeared in these "transgenic" mice, rather than systemic ones, and whether these reversible tumors were aneuploid or were diploid hyperplasias have not been answered [85, 252].

Twelve years ago, Vogelstein and Kinzler closed an influential review of the mutation theory in 1993 (since cited in text books [284]) as follows, "The genetics of cancer forces us to re-examine our simple notions of causality, such as those embodied in Koch's postulates: how does one come to grips with words like 'necessary' and 'sufficient' when more than one mutation is required to produce a phenotype and when that phenotype can be produced by different mutant genes in various combinations?" [286]. According to the brief summary above and Bishop's seminar in 2005, the answer to Vogelstein and Kinzler's question is still open – 12 years and many studies later.

In sum: In the preceding Sections we have listed 10 features of carcinogenesis that cannot be explained by genetic cancer theories. These and other inconsistencies between carcinogenesis and established genetic theories are the reasons why it is still debated, whether mutations or aneuploidies or epigenetic alterations cause cancer [43,69,75,77,79,85,102,107,108,114,169, 170,185,189,190,215,225,234,248,251,257,264,265, 273,282,286].

3. A new, chromosomal theory of cancer

In view of the many discrepancies between carcinogenesis and conventional genetic theories listed above, we present here a new, chromosomal theory of cancer. *Chromosomal* is defined here primarily by what is seen microscopically by classical cytogenetics [124,245]. It also includes amplicons, or deletions of chromosomes down to about 1 megabase, which are "submicroscopic" according to some [207] but microscopic according to other more recent techniques such as comparative genomic hybridization and gene array-based hybridization [194,221,258] (see also definition of aneuploidy in Section 2.4).

The new chromosomal theory we propose is based on the following data, which were either generated by us recently or were collected from the literature:

(1) *Exact correlations between aneuploidy and cancer*. The literature including that of our own lab shows that chromosomal alterations, alias aneuploidy, are ubiquitous in cancer (Section 2.4). It follows that aneuploidy is necessary for carcinogenesis.
(2) *Carcinogens induce aneuploidy*. To test the chromosomal theory, we have collected studies which have investigated the function of carcinogens and shown that they cause aneuploidy [76,77,80,81,89]. For the same reason, we have collected studies, which have searched for the genetic targets of carcinogens, but have unexpectedly obtained targets over $>1000\times$ larger than a gene, and thus equivalent to the size of chromosomes [76]. It follows that the common, cancer-relevant denominator of carcinogens is aneuploidization.
(3) *Pre-neoplastic aneuploidy*. In preliminary tests of the chromosomal theory we have found that the pre-neoplastic aneuploidy of initiated cells segregates with subsequent malignant transformation [80,89] (Section 2.5). It follows that pre-neoplastic aneuploidy could be the evolutionary precursor of cancer-specific aneuploidy.
(4) *Cancer-specific aneusomies.* Despite the enormous karyotypic heterogeneity of most cancer cells, cancer-specific or "nonrandom" aneuploidies were discovered since the late 1960s (Section 2.7). It follows that specific chromosomal alterations could be sufficient for carcinogenesis.
(5) *Chromosomes of cancer cells vary at rates that are proportional to the degrees of aneuploidy.* In preliminary tests of the chromosomal cancer theory we have observed that aneuploidy catalyzes chromosomal variations in proportion to the degree of aneuploidy [82,83,88,168,228] (Section 2.6). Several labs have recently also found that chromosomal variability of cancer cells is proportional to the degree of aneuploidy [47,51,156,239]. Moreover, we have found that the rates of specific chromosomal variations can go 4–11 orders higher than those of conventional gene mutations [76,88,168]. It follows

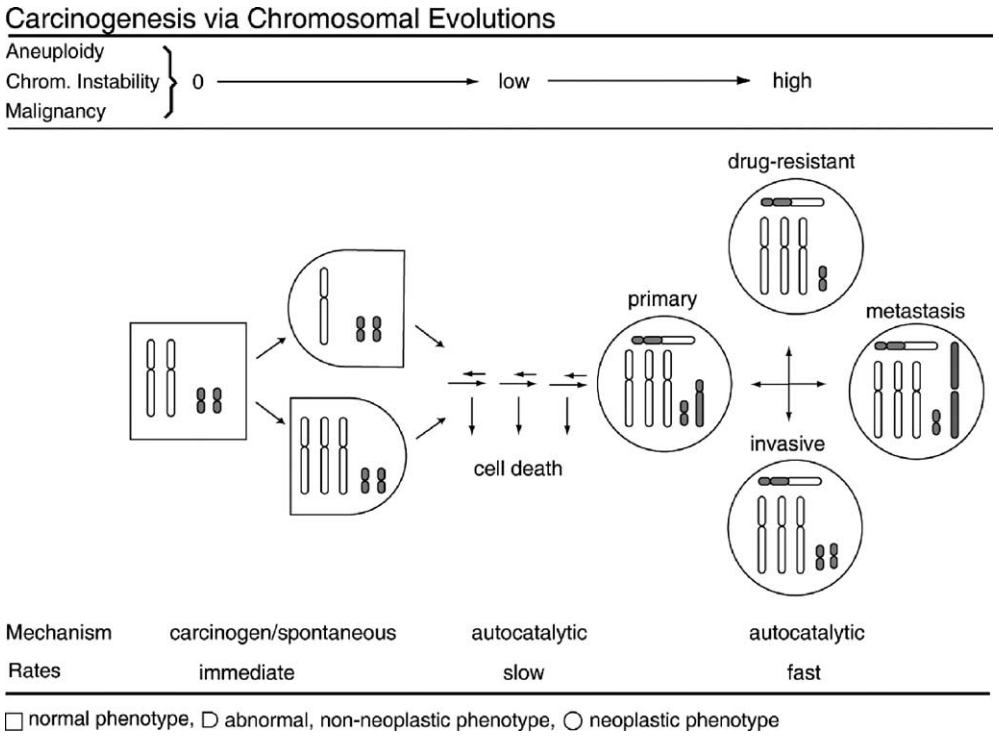


Fig. 3. The chromosomal cancer theory. (i) *Initiation*: A carcinogen or a spontaneous accident induces random aneuploidy either by nondisjunction or by breaking and rearranging chromosomes. (ii) *Pre-neoplastic chromosomal evolutions*: By unbalancing 1000s of genes aneuploidy corrupts teams of proteins that segregate, synthesize and repair chromosomes. Aneuploidy is therefore a steady source of chromosomal variations, from which, in classical Darwinian terms, neoplastic karyotypes eventually evolve. Since pre-neoplastic aneuploidy is typically low, and since pre-neoplastic cells, by definition, do not grow better than normal cells, pre-neoplastic chromosomal evolutions are slow. Many aneuploid cells die because of nullisomies or other non-viable chromosome combinations. (iii) *Neoplastic evolutions*: Once a neoplastic chromosome combination evolves, subsequent karyotypic variations are accelerated, because neoplastic cells are generally more aneuploid and thus more adaptable than pre-neoplastic cells and because they form large pools by outgrowing normal cells. Thus neoplastic cells evolve independently within tumors forming ever-more heterogeneous and malignant phenotypes such as invasiveness, metastasis and drug-resistance at high rates. In sum: Malignancy can be seen as a consequence of autonomous chromosomal evolutions that increase karyotypic entropy to its biological limits, at or near 3n-aneuploidy.

that the karyotypic heterogeneity of cancers is a consequence of the inherent chromosomal instability of aneuploidy.

(6) *The chromosomal theory of cancer proposed by Boveri and von Hansemann over 100 years ago.* Boveri and von Hansemann proposed over 100 years ago that abnormal chromosome numbers were the cause of cancer [40,41,111]. This theory, however, was abandoned in the 1950s and 1960s, because the karyotypic heterogeneity of cancers was interpreted as a consequence of an unknown, clonal cause [65,155, 240]. Ever since, "aneuploidy and other forms of chromosomal abnormality" of cancer cells [113] are generally interpreted as "secondary" events [113,114,124,144,155] – secondary to hypothetical primary mutations [114,123,154, 163,166,189,199,211,226,227,272,306]. It follows that the primary challenge for a new chromosomal theory was to find an explanation for the insidious chromosomal instability of cancer cells.

In an effort to integrate these data into a coherent theory, which would also explain the above-listed discrepancies between carcinogenesis and conventional genetic cancer theories, we arrived at our new, chromosomal theory of cancer (summarized in Fig. 3). According to this theory carcinogenesis is the result of the following chain of events:

- *Carcinogens and spontaneous mitotic errors induce unspecific chromosomal alterations or aneuploidies.*
- *Since chromosomal alterations unbalance 1000s of genes, they corrupt teams of proteins that seg-*

regate, synthesize and repair chromosomes. Aneuploidy is thus a steady source of karyotypic–phenotypic variations from which, in classical Darwinian terms, selection of specific chromosomal alterations encourages the evolution and spontaneous progression of neoplastic cells.

- *The rates of these variations are proportional to the degrees of aneuploidy.*
- *Based on their chromosomal constitution cancer cells are new cell "species" with specific or "non-random" chromosomal alterations and transcriptomes, but unstable karyotypes. This aneuploidy-based, chromosomal uncertainty principle had become the nemesis of the Boveri–von Hansemann theory in the 1950s and 1960s.*
- *The specific chromosomal alterations of cancer cells generate complex, malignant phenotypes via abnormal dosages of 1000s of genes. Down syndrome is a model for how aneuploidy generates complex, abnormal phenotypes.*
- *In sum, cancer is caused by chromosomal disorganization, which increases karyotypic entropy. Thus cancer is a chromosomal rather than a genetic disease.*

Below, we offer a brief explanation of how aneuploidy generates new phenotypes, independent of mutation. By changing the numbers of chromosomes, aneuploidy has the same effects on the phenotypes of cells as changing the assembly lines of a car factory on the phenotypes of an automobile:

Changes of assembly lines that essentially maintain the balance of existing components, alias genes, generate new, competitive car models. For example, the engine could be moved from the back to the front via adjustments in assembly lines without changing the balance of genes. Similarly, phylogenesis generates new species by regrouping old genes of existing species, without changing their balance, into new numbers and structures of chromosomes [201].

However, if changes of assembly lines are made that alter the long-established balance and thus the stoichiometry of many components, alias genes, abnormal and defective products must be expected, as for example cars with three wheels or humans with Down syndrome. The human trisomy 21, which causes Down syndrome, is a classic example [87,255]. Although trisomy 21 is only a tiny aneuploidy compared to that of most cancers [245] (Fig. 2), it generates 71 (!) new, Down-specific phenotypes [183,230]. Likewise, experimentally induced, congenital aneuploidies generate numerous abnormal phenotypes in drosophila, plants and mice, independent of gene mutation [126,173,177, 186]. According to the cancer researcher Vogelstein there is no "normal [animal] cell with an abnormal karyotype" [185]. Thus the complex aneuploidies of cancer cells can be expected to generate numerous abnormal phenotypes including those of cancer.

By contrast, the effects of changing phenotypes of the cell by mutation without touching the karyotype are much more limited than those resulting from changing the karyotype. Mutation without touching the karyotype is analogous to changing specific components of an existing car model: There could either be positive mutations, such as an improved carburetor, or negative mutations such as an unreliable ignition, or neutral mutations such as a new color. None of such mutations would generate an exotic new car model with unpredictable phenotypes. Indeed, none of the 1.42 million gene mutations that distinguish any two humans [244] have generated a new human species, nor have they even been sufficient to cause cancer in newborns. In view of this such mutations are euphemistically called "polymorphisms".

Moreover, the function of genes in biological assembly lines is strongly buffered against mutations: activating mutations are buffered down by normal supplies and inactivating mutations are kinetically activated by increased supplies from un-mutated components of the assembly line [61,116,145]. But, there is no such buffering against aneuploidy.

Thus aneuploidy is inevitably dominant, whereas mutation is nearly always recessive [285]. It is for this reason that gene mutations could never generate new phylogenetic species or even new cancer cell-species – independent of karyotypic alterations.

The following analogy illuminates the differences between mutation and aneuploidy from a slightly different perspective: Consider the cell as a book, the genes as words, and the chromosomes as sentences, paragraphs or chapters. Then most of us would be able to read Hamlet despite hundreds of typos, but the idea of Hamlet would be lost very fast, if sentences, paragraphs and chapters were rearranged, lost and duplicated.

In sum: The chromosomal theory provides a coherent explanation of carcinogenesis that is independent of mutation. Next we show that the chromosomal theory can explain each of the many idiosyncratic features of carcinogenesis that are paradoxical in view of the mutation theory.

Table 1
Features of carcinogenesis, which are paradoxical according to genetic theories but consistent with the chromosomal theory of cancer

Genetic paradox	Chromosomal solution
1 Cancer not heritable	Aneuploidy is not heritable
2 Non-mutagenic carcinogens	Carcinogens function as aneuploidogens
3 Long neoplastic latencies	Autocatalyzed evolutions from cancer-initiating to cancer-specific aneuploidies
4 Exact correlation with aneuploidy	Specific aneuploidies cause cancer
5 Pre-neoplastic aneuploidy	Non-neoplastic aneuploidies that initiate carcinogenesis and evolve toward cancer-causing aneuploidy
6 High rates of karyotypic–phenotypic variations and "immortality"	Aneuploidy catalyzes frequent karyotypic variations: the resulting chromosomal and phenotypic heterogeneity includes subspecies resistant to otherwise lethal conditions
7 Cancer-specific chromosomal alterations	Cancer-specific chromosomal alterations generate cancer-specific phenotypes
8 Complex phenotypes	Cancer-specific aneuploidies alter functions of 1000s of genes via dosages
9 Non-selective phenotypes	Non-selective genes hitchhiking with selective, cancer-specific chromosomal alterations
10 No carcinogenic genes in cancer	Cancer is caused by specific karyotypes

4. Proof of principle: The explanatory value of the chromosomal theory of cancer

The acid test of any theory is its ability to predict and explain a scientific problem. In the following we apply this test to the chromosomal theory of cancer. Table 1 briefly summarizes, how the chromosomal theory explains each of the 10 idiosyncratic features of carcinogenesis that are paradoxical in terms of conventional genetic theories (Section 2). Further commentary is offered in Sections 4.1 to 4.8 on items 1, 3, 4 and 6–10 of Table 1, which are not self-explanatory on the basis of our theory.

4.1. Cancer not heritable

The chromosomal theory predicts no cancer in newborns and non-identical cancer risks in twins (Section 2.1), because aneuploidy is the initiating cause of cancer and is not heritable as originally shown by Boveri [40]. Aneuploidies are not heritable, because they corrupt developmental programs [87,255], which is usually fatal [118,126]. Only some very minor congenital aneuploidies, such as Down syndrome and syndromes based on abnormal numbers of sex chromosomes, are sometimes viable, but only at the cost of severe physiological abnormalities and of no, or very low fertility [26,104,245,285]. Thus ontogenesis is nature's checkpoint for normal karyotypes.

The exponential increase of the cancer risk with age would then reflect the gradual accumulation of non-neoplastic or pre-neoplastic aneuploidy with age, multiplied by the relatively slow, non-selective replication of aneuploid, pre-neoplastic cells (see also Section 4.2).

4.2. Long neoplastic latencies

According to the chromosomal theory the long neoplastic latencies from initiation to cancer reflect the times that are necessary to evolve cancer-specific chromosome alterations from initiating random aneuploidies by autocatalyzed chromosomal variations (Section 3 and Fig. 3).

The theory predicts that pre-neoplastic chromosomal evolutions are slow, because pre-neoplastic aneuploidies are typically minor, i.e. are near-diploid, and thus only weak catalysts of chromosomal variation, and because pre-neoplastic aneuploidy, by definition, has no growth advantages compared to normal cells. Moreover, many non-neoplastic aneuploidies are likely to be fatal due to non-viable chromosome combinations [41,76,118,126,176,245,300] (Fig. 3). Therefore, pre-neoplastic cells would not form large clonal populations that would increase the probability of further evolutions. The non-clonality of the pre-neoplastic aneuploidies also hides any abnormal phenotypes, because phenotypes of single cells are hard to recognize.

By contrast, the chromosomal theory predicts relatively short neoplastic latencies in patients with congenital aneuploidies and with chromosomal instability syndromes and thus cancer at young age. This follows, because the number of aneuploid cells is much higher in these conditions than in normal counterparts (Sections 2.5 and 4.8.5).

Neoplastic "progression" of established cancer cells, however, is predicted to be faster than the chromosomal evolutions during the pre-neoplastic phase for two reasons: (i) Neoplastic cells, through their selective phenotypes, will generate large "clonal" populations with high probabilities of further variations. (ii) The high degrees of aneuploidies of most cancer cells catalyze much higher rates of chromosomal variations than those of non-neoplastic cells (Fig. 3).

The chromosomal theory also predicts a certain endpoint of chromosomal evolutions in carcinogenesis (Fig. 3). This endpoint would be an equilibrium, at which maximal karyotypic disorganization or entropy coincides with maximal variability and adaptability. Karyotypic disorganization and variability are, of course, biologically limited by requirements for essential metabolic functions [47,54,76,265], also termed an "optimized genome" [238]. According to the chromosomal theory maximal chromosomal variability would correspond to near or above triploid chromosome numbers (∼3n) [51,76,228,248]. Near triploid aneuploidy offers an optimal average redundancy of one spare chromosome for each normal chromosome pair, and thus sufficient redundancy to compensate for any losses or genetic mutations of a given chromosome [76]. Accordingly, the karyotypes of most malignant cancer cells are or "converge" [54,131,202] at near 3n [13,76,77,81,101,144,155,157,161,165,238, 239,245,253].

Thus malignancy can be seen as a consequence of autonomous chromosomal evolutions that increase karyotypic entropy to its biological limits – at or near 3n-aneuploidy. The long-established, commercially available human cancer cell lines are models of such *stably unstable* karyotypes with karyotypic entropies close to their biological limits of aneuploidy [47,51,157,238,239,248].

However, it is as yet unclear, why the neoplastic latencies are very species-dependent, namely much shorter (over 10-fold) in rodents than in humans [96, 133,158,260,276,286] (Section 2.3). It is also unclear, what makes the age bias of cancer compatible with the lifespan of an animal, i.e. grants cancer-free decades to humans (Fig. 1), but only a few years to rodents [45,133]. Differential mutation- or growth rates are not the answer, because the rates of conventional mutations are highly conserved in all species [167,285] and the cells of humans and rodents grow at about the same rates. Based on recent studies it appears to us that the low chromosomal stability of aneuploid rodent cells compared to that of equally aneuploid human cells may hold a clue to this puzzle [78,83,84,88, 168]. The evidence obtained so far, suggests that the chromosomal stabilities not only of normal but also of cancer cells are species-specific. In view of these species-specific chromosomal stabilities Holliday proposed that the genetic control of chromosomal stability is at least two times more redundant in humans than in rodents [133].

4.3. Pre-neoplastic aneuploidy

The chromosomal theory predicts that pre-neoplastic aneuploidies are intermediates of the pre-neoplastic chromosomal evolutions that eventually generate cancer-specific aneuploidy.

4.4. High rates of karyotypic–phenotypic variations and "immortality"

The inherent chromosomal instability of aneuploidy is directly predicted by the chromosomal cancer theory. It is confirmed by numerous correlations (Section 2.6) and is mechanistically linked to aneuploidy by the proportionality between the instability and the degree of aneuploidy recently detected by our lab [82, 88]. Further, it is entirely consistent with the critical observation of Holmberg et al. in 1993 that "an increased frequency of sporadic chromosome aberrations was only observed in irradiated cells with aberrant karyotypes and not in irradiated cells with normal karyotypes, which suggests that the 'genomic instability' in these clones is associated with the abnormal karyotype rather than with the radiation exposure as such" [134].

The chromosomal theory also explains the immortality of cancer tissues via the diversity of phenotypes that are constantly generated de novo by the inherent karyotypic instability of aneuploid cells. Owing to the inherent instability of aneuploidy, populations of cancer cells are in fact "polyphyletic" [119] zoos of chromosomally distinct species (species are defined by karyotypes, see Section 3). Such populations of cancer cells are relatively "immortal" via subspecies that can survive mutations or conditions that are lethal to the majority of the cells of a cancer, as for example cytotoxic drugs. By contrast, homogeneous populations of diploid cells would either all survive or all die in a given challenging condition.

An early description of the process of "immortalization" by the cytogeneticist Koller matches this ex-

planation exactly, "It seems that malignant growth is composed of competing clones of cells with different and continuously changing genotypes, conferring the tumor with an adaptable plasticity against the environment. The bewildering karyotypic patterns reveal the multi-potentiality of the neoplastic cell; while normal cells and tissues age and die, through their inherent variability, tumor cells proliferate and survive" [155]. Thus, owing to their cellular heterogeneity cancers survive negative mutations and cytotoxic drugs via resistant subspecies.

4.5. Cancer-specific chromosomal alterations

The presence of specific or "nonrandom" chromosomal alterations in cancer is correlative proof for the chromosomal theory in terms of Koch's first postulate (Section 2.7). Functional proof that cancer-specific aneuploidy generates malignancy in terms of Koch's third postulate could be derived from evidence that the degree of malignancy is proportional to the degree of nonrandom aneuploidy. Indeed, numerous correlations have confirmed the principle that the degree of malignancy of cancer cells is proportional to their degree of aneuploidy since 1930 [20,22,34,51,55,76,77,90, 91,93,105,120,132,143,149,155,192,196,199,209,249, 262,275,295,297].

In addition, gene expression in cancer cells is directly proportional to the gene dosage generated by the respective chromosomal alterations, which indicates that specific aneusomies carry out specific functions [2,19,95,178,191,221,283,305]. It is for this reason that 1000s of metabolic and structural proteins are over- or under-expressed in cancer cells [19,49,50,191, 219,242] (next section).

4.6. Complex phenotypes

Conventional genetic theories cannot explain the generation of the complex, polygenic phenotypes of cancer (Section 2.8). By contrast, the chromosomal theory of cancer explains the complexity of cancer-specific phenotypes by the complexity of the genetic units that are varied, namely chromosomes with 1000s of genes. Accordingly, the complex phenotypes of cancer cells have recently been shown to correlate with over- and under-expressions of 1000s of genes [2,95, 178,221,283,305] (see also Section 4.5). This in turn confirms the long-known over- and under-productions of thousands of normal proteins by cancer cells [49,50, 190,219]. Likewise it explains, why the overproductions of centrosomes by cancer cells are proportional to the degrees of aneuploidy [100,174].

4.7. Non-selective phenotypes

Conventional genetic theories explain the evolution of cancer cells by cancer-specific mutations and Darwinian selections. But this mechanism cannot explain the non-selective phenotypes of cancer cells, such as metastasis, acquired and intrinsic multidrug resistance and immortality.

By contrast, the chromosomal theory of carcinogenesis attributes non-selective phenotypes such as metastasis and intrinsic multidrug-resistance to non-selective genes hitchhiking with selective, cancer-causing aneusomies, because they are also located on these chromosomes. The same would be true for those resistances of acquired multidrug-resistance that are directed against drugs to which the respective cancer was never exposed. (The non-selective phenotype, immortality, has been explained in Section 4.4.)

In sum: The chromosomal theory explains all features of carcinogenesis that are paradoxical in view of the competing genetic theories (Table 1). However, it may still be argued that chromosomal cancer depends on mutation. Therefore, we analyze this question in the next and last chapter of our article.

4.8. Is carcinogenesis dependent on mutation?

Cancer coincides with aneuploidy as well as with mutations [77,102,114,185,248]. In the words of a recent review in *Science*, "Cancer cells are chock-full of mutations and chromosomal abnormalities" [185].

Therefore, it can be argued that:

(1) Chromosomal variations are sufficient for carcinogenesis, as we have proposed here.
(2) Mutations are sufficient to cause cancer, as the prevailing genetic theories propose (Section 1). But this argument must await unambiguous evidence for diploid cancers, which is not available now (Section 2.4) [76,77,81].
(3) Mutations are necessary for chromosomal cancer, as conditional mutation theories propose [114,123,161,163,189,215,226,227,248].

In view of the challenge that chromosomal cancer depends on mutation, we adduce here 4 arguments, which indicate that carcinogenesis (of normal cells in normal organisms) is not dependent on somatic mutation.

4.8.1. *Initiation of carcinogenesis much more probable via direct aneuploidization than via mutation*

Initiation of carcinogenesis by aneuploidy, resulting from chromosomes that have been fragmented or eliminated by mutagenic carcinogens, is about 35,000 times more likely than by aneuploidy resulting from mutations generating specific "aneuploidy genes" [185] or "chromosomal instability genes" [189]. This is because mammals contain about 35,000 genes, and thus only 1 in 35,000 specific mutations would generate a specific chromosomal instability gene [75,159, 201]. Moreover, non-mutagenic carcinogens can neither generate mutations nor aneuploidy by attacking DNA, because they are not "genotoxic". But, non-mutagenic carcinogens, as for example the polycyclic hydrocarbons, cause aneuploidy by corrupting the spindle apparatus (Sections 2.2 and 3). Thus initiation of carcinogenesis is virtually independent of somatic mutation.

4.8.2. *Complex phenotypes of cancer much more probable via chromosomal alteration than via mutation*

Chromosomal alteration is about 1500-times more efficient in generating the complex phenotypes of cancer than mutation. This follows, because mammals, including us, contain about 35,000 genes and thus about 1500 genes per average chromosome in humans (35,000/23) [159,201]. Since the rates of chromosomal variations in aneuploid cells are also many orders higher than mutation (Sections 2.6 and 4.8.3), we deduce that carcinogenesis is not dependent on somatic mutation for the generation of cancer-specific phenotypes.

4.8.3. *Phenotype variation of cancer cells via chromosomal variation is 4–11 orders faster than via mutation*

Chromosomal variation alters cancer-specific phenotypes at rates that are 4 to 11 orders faster than conventional gene mutation (Section 2.6). Indeed cancer, based on spontaneous, somatic mutation would practically not exist (see Box 1, 3). Thus phenotype variation in cancer cells is independent of mutation.

4.8.4. *Mutations of cancer cells as consequences of aneuploidy*

Cancer-specific aneuploidy can generate gene mutations by the same mechanism that varies the structures of chromosomes, e.g. by unbalancing teams of DNA repair enzymes (Section 3). In addition aneuploidy is mutagenic, because it renders DNA synthesis error-prone by unbalancing nucleotide pools [66]. Thus, the simplest explanation of the many mutations of cancer cells would be that these mutations are consequences of aneuploidy and thus not necessary for carcinogenesis. This hypothesis explains, why mutations are frequently not detectable [292] or are non-clonal in cancers [85,147,180], and why they do not transform normal cells to cancer cells, and do not breach the livelihood of transgenic mice (Section 2.10 and Box 1). Thus mutation of cancer cells is a consequence of aneuploidy, rather than a cause.

In sum: Based on the roles of chromosomal variation and mutation in 4 distinct cancer-specific events – initiation, generation of complex phenotypes, high rates of karyotypic–phenotypic variations, and generation of mutations via aneuploidy (4.8.1–4.8.4) – we conclude that chromosomal carcinogenesis does not depend on somatic mutation.

In response to this conclusion, it may be argued that, at least, the cancers associated with heritable cancer-disposition syndromes depend on mutation (Section 2.5) – although sporadic cancers do not. In the following, however, we show that even the heritable mutations of cancer-disposition syndromes cause cancers only via aneuploidy.

4.8.5. *Heritable cancer-disposition syndromes also generate cancer via aneuploidy*

Retinoblastoma, Xeroderma, Bloom syndrome, Fanconi anemia, Gorlin-syndrome, Ataxia Telangiectasia and Mosaic variegated aneuploidy are heritable cancer-disposition syndromes with mutations that generate high levels of systemic aneuploidy [52,53,99,110, 130,135,146,160,178,245,254,263,282,300] and that predispose to high risks for non-systemic cancers with aneuploidy [110,130,153,154,178,245,254,282, 300] (see Section 2.5). In other words, these heritable mutations are genetic equivalents of carcinogens, which increase the cancer risk by inducing random aneuploidy at high rates.

This view is supported by the presence of systemic aneuploidy in patients prior to carcinogenesis [245], as for example in Mosaic variegated aneuploidy [110,146], Retinoblastoma and other chromosomal eye syndromes [52,135,263], Ataxia Telangiectasia and Fanconi anaemia [206,300], Bloom syndrome [99], Gorlin-syndrome [254], and Xeroderma [53,160,282]. This view is further supported by confirmed correlations between aneuploidy and "heritable" cancers of Retinoblastoma- [9,28,97,109,220,222, 277], Fanconi anaemia- [246], Ataxia- [206], Mosaic

variegated aneuploidy- [110,146], Xeroderma- [160, 299] and Bloom syndrome-patients [99]. We conclude that the abnormally high rates of carcinogenesis in heritable cancer disposition syndromes are dependent on the abnormally high rates of systemic aneuploidizations that are generated by these heritable mutations. Thus heritable aneuploidy syndromes confirm and extend the chromosomal theory of carcinogenesis.

The hypothesis that systemic aneuploidy defines cancer risks is also supported by the epidemiological studies described above, which have shown that this risk corresponds directly with the degrees of chromosomal aberrations in peripheral lymphocytes (Section 2.5).

5. Conclusions

We conclude that the new chromosomal cancer theory provides a coherent explanation of carcinogenesis and can resolve all features of carcinogenesis that are paradoxical in terms of the prevailing genetic theories of cancer. Thus cancer is a disease of chromosomal disorganization rather than a genetic disease.

In response to this conclusion it has been pointed out by proponents of the mutation theory such as G. Steven Martin (Berkeley), Manfred Schwab (Heidelberg) and Larry Loeb (Seattle) that the chromosomal theory overlaps with the mutation theory and that aneuploidy is just another, albeit extreme form of mutation. But the following absolute discrepancies between the mutation and chromosomal theories indicate that these ideas fall in the same gaps that they try to bridge:

(1) How would non-mutagenic carcinogens cause cancer?
(2) What kind of mutation would cause cancer only after delays of several decades and many cell generations?
(3) What kind of mutation would alter the phenotype of mutant cells perpetually, despite the absence of further mutagens?
(4) What kind of mutation would be able to alter phenotypes at rates that exceed conventional gene mutations 4–11 orders of magnitude?
(5) What kind of mutation would generate resistance against many more drugs than the one used to select it?
(6) What kind of mutations would change the cellular and nuclear morphologies several-fold within the same “clonal” cancer?
(7) What kind of mutation would alter the expressions and metabolic activities of 1000s of genes, which is the hallmark of cancer cells?
(8) What kind of mutation would consistently coincide with aneuploidy, although conventional gene mutations generate infinite numbers of new phenotypes without altering the karyotype?
(9) Why would cancer not be heritable via conventional mutations by conventional Mendelian genetics?

The chromosomal theory, however, offers answers to each of these questions.

Moreover, if confirmed, the chromosomal theory would have revealed the first Achilles heel of cancer yet: Pre-neoplastic aneuploidy. Pre-neoplastic aneuploidy can be detected in routine biopsies, e.g. Pap smears, cytogenetically and thus offers new chances for cancer therapy. Accordingly a prospective cancer could be detected and removed prior to, perhaps years prior to malignancy via pre-neoplastic aneuploidy. Several long-established, but as yet poorly appreciated studies already prove this principle [34,79, 233,234].

The chromosomal theory could also improve chemotherapy based on the presence or absence of resistance-specific aneusomies [168,291].

Thus, if confirmed, the chromosomal theory is likely to innovate cancer research and improve treatment.

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