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Aneuploidy and Cancer: From Correlation to Causation

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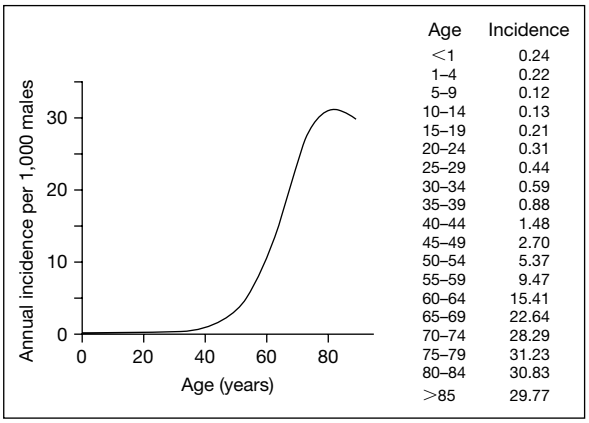
Abstract

Conventional genetic theories have failed to explain why cancer (1) is not found in newborns and thus not heritable; (2) develops only years to decades after 'initiation' by carcinogens; (3) is caused by non-mutagenic carcinogens; (4) is chromosomally and phenotypically 'unstable'; (5) carries cancer-specific aneuploidies; (6) evolves polygenic phenotypes; (7) nonselective phenotypes such as multidrug resistance, metastasis or affinity for non-native sites and 'immortality' that is not necessary for tumorigenesis; (8) contains no carcinogenic mutations. We propose instead that cancer is a chromosomal disease: Accordingly, carcinogens initiate chromosomal evolutions via unspecific aneuploidies. By unbalancing thousands of genes aneuploidy corrupts teams of proteins that segregate, synthesize and repair chromosomes. Aneuploidy is thus a steady source of karyotypic–phenotypic variations from which, in classical Darwinian terms, selection of cancer-specific aneuploidies encourages the evolution and subsequent malignant 'progressions' of cancer cells. The rates of these variations are proportional to the degrees of aneuploidy, and can exceed conventional mutation by 4–7 orders of magnitude. This makes cancer cells new cell 'species' with distinct, but unstable karyotypes, rather than mutant cells. The cancer-specific aneuploidies generate complex, malignant phenotypes, through the abnormal dosages of the thousands of genes, just as trisomy 21 generates Down syndrome. Thus cancer is a chromosomal rather than a genetic disease. The chromosomal theory explains (1) nonheritability of cancer, because aneuploidy is not heritable; (2) long 'neoplastic latencies' by the low probability of evolving competitive new species; (3) nonselective phenotypes via genes hitchhiking on selective chromosomes, and (4) 'immortality', because chromosomal variations neutralize negative mutations and adapt to inhibitory conditions much faster than conventional mutation. Based on this article a similar one, entitled 'The chromosomal basis of cancer', has since been published by us in Cellular Oncology 2005;27:293–318.

Despite over 100 years of cancer research, the cause of cancer is still a matter of debate [1–26]. We propose here that the problem of cancer is still



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.

unsolved, because this debate has been monopolized by conventional genetic theories, which hold that cancer is a 'genetic disease' [27–35]. But these genetic theories cannot explain any of the following properties of carcinogenesis:

Cancer Is Not Heritable

The best news about cancer is that we and other animals are all born cancer-free and typically acquire cancer, if at all, only at advanced age [34, 36–40]. 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).

In view of the prevailing gene-based cancer theory, however, this age bias is paradoxical. This theory holds that cancer is caused by clonal expansion of one single cell that has accumulated about four to seven complementary mutations during the lifetime of a patient [1, 12, 34, 38, 41, 42]. If this theory is correct, 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 presumably 6 that are thought to cause colon cancer [1, 34], 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 (Appendix) and also in humans. According to Vogelstein and Kinzler [43], "one

of the cardinal principles of modern cancer research is that the same genes cause both inherited and sporadic (noninherited) forms of the same tumors".

But there is no colon cancer in newborns (fig. 1). Thus, cancer is somatically generated and not a heritable disease.

Long Neoplastic Latencies

Experimental or accidental carcinogenesis, and the age bias, demonstrate that cancer is a late product of a gradual evolution of somatic cells that may be 'initiated' either by carcinogens or spontaneously [1, 10, 38, 40, 44, 45]. Once initiated, this evolution is autonomous but very slow, generating cancer cells only after lengthy and uneventful 'neoplastic latencies' [40, 45]. These latencies last many months to years in carcinogen-treated rodents and decades in accidentally exposed humans [40, 45–48]. For example, (1) the solid cancers, which developed in human survivors only 20 years after the explosion of atomic bombs in Japan in 1945 [38]; (2) the breast cancers, which developed only 15 years after treatments of tuberculosis with X-rays in the US in the 1950s [49], and (3) the lung cancers, which developed in workers of a mustard gas factory only 30 years after it was closed in Japan in 1945 [50]. The exponential increase of the spontaneous cancer risk of humans with age even implies neoplastic latencies of up to 50 years from a near zero-risk at birth to a one in three risk in the last three decades of a human lifespan of about 80 years (fig. 1). The primary cancer cells that appear after these lengthy pre-neoplastic evolutions continue to progress independently within individuals tumors to form evermore 'polymorphic' [51] and malignant cancers with evermore exotic karyotypes and phenotypes [45].

These long latencies of carcinogenesis, however, are incompatible with the immediate effects of conventional mutation [2, 31, 35, 52]. 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' [38].

Non-Mutagenic Carcinogens Cause Cancer

Both mutagenic and non-mutagenic carcinogens cause cancer. 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, as well as spindle blockers such as vincristine and colcemid, extranuclear radiation and solid plastic or metal implants (Appendix). Conventional genetic theories, however, fail to explain carcinogenesis by non-mutagenic carcinogens.

Karyotype-Phenotype Variations at Rates that Are Orders Higher than Mutation

During the neoplastic phase of carcinogenesis, cancer cells gain or lose chromosomes or segments of chromosomes (fig. 2) and change phenotypes at rates that far exceed those at which genotypes and phenotypes are changed by conventional mutation [53–55]. For example, highly aneuploid cancer cells become drug resistant at rates of up to 10^{-3} per cell generation [53, 54, 56–58] or become metastatic at 'high rates' [59, 60]. As a result of this inherent chromosomal instability most cancers are enormously heterogeneous populations of nonclonal and partially clonal, or sub-clonal cells [13, 61]. Thus, cells from the same cancer differ from each other in 'bewildering' phenotypic and chromosomal variations [62] and in mutations – even though most cancers are derived from a common, primary cancer cell and thus have clonal origins [38, 45, 51, 56, 61, 63–67].

By contrast, the karyotypes of normal cells are stable despite mutational or developmental phenotype variations [31, 34, 52, 68]. And phenotypic variation of normal cells by conventional gene mutation cells is limited to 10^{-7} per cell generation for dominant genes and to 10^{-14} for pairs of recessive genes in all species [6, 47, 52, 57, 68, 69]. Even the mutation rates of most cancers are not higher than those of normal cells [6, 19, 20, 47, 66, 70–75]. Thus, phenotypic variation in cancer cells can be four to eleven orders faster than conventional mutation.

Cancer-Specific Aneuploidies

Despite the karyotypic instability and heterogeneity of cancer cells partially specific or nonrandom aneuploidies have been found in cancers since in the late 1960s [61, 62, 76–87]. Since the 1990s, many more nonrandom aneuploidies have been detected in cancers by the use of comparative genomic hybridization, rather than by identifying specific aneusomies cytogenetically [61, 88–96]. The term aneusomy is used for a specific, aneuploid chromosome. Specific aneuploidies have even been linked with specific stages of carcinogenesis and with specific phenotypes of cancers such as: (1) Distinct stages of neoplastic transformation in human [62, 89, 95–99] and in animal carcinogenesis [84]; (2) invasiveness [97, 98, 100]; (3) metastasis [101–106]; (4) drug-resistance [53, 69, 107]; (5) transplantability to foreign hosts [108]; (6) distinct cellular morphologies [109]; (7) abnormal metabolism [62, 110], and (8) cancer-specific receptors for viruses [62, 109].

Cancer-specific, nonrandom aneuploidies, however, are inconsistent with the conventional mutational theories of cancer. In fact they are a direct challenge of the mutation theory, because specific aneusomies have the potential to generate cancer-specific functions (Appendix). The Down syndrome-specific functions of trisomy 21 are a confirmed model [111–114].

Karyotypes of clonal cultures of the near-diploid human colon cancer line HCT 116 and the hyper-diploid human colon cancer line SW480

HCT 116, mn=45

Chrom.	Metaphases 1 to 29	30
1	2	2
2	2	2
3	2	2
4	2	2
5	2	2
6	2	2
7	2	2
8	2	2
9	2	2
10	1	1
11	2	2
12	2	2
13	2	2
14	2	2
15	2	2
16	1	1
17	2	2
18	1	1
19	2	2
20	2	2
21	2	2
22	2	2
X	1	1
Y	0	0
M1 10^{+}	1	1
M2 8/16	1	1
M3 17/18	1	1
M4 12^{-}	0	***1***

SW480, mn=57

Chrom.	Metaphases 1 to 6				10					15					19
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
7	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	***0***
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	3	3	3	3	3	3	3	3	3	***2***	3	3	3	3	
12	1	1	1	1	1	1	1	***0***	1	1	1	1	1	1	
13	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
14	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
15	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
16	2	2	2	2	2	2	2	2	2	2	2	***1***	2	2	
17	3	3	3	3	3	3	3	3	3	3	***4***	3	3	3	
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
20	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
21	3	3	3	3	3	3	3	3	3	***2***	3	3	3	***1***	
22	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
X	2	2	2	2	2	2	2	2	***1***	2	2	2	2	2	
M1 2/12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
M2 3/12/10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
M3 9/1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
M4 Ω9/1	1	1	1	***0***	***0***	***0***	***0***	***0***	***0***	***0***	1	1	1	1	
M5 3^{+}	1	1	1	1	1	1	***0***	1	1	1	1	1	1	***0***	
M6 8/9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
M7 7/14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
M8 5/20/7	1	1	1	1	1	1	1	1	1	1	1	1	1	***0***	
M9 5/20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
M10 Ω5/20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
M11 3^{-}	1	1	1	1	1	1	1	1	***0***	1	1	1	***2***	1	
M12 12^{-}	1	1	1	1	1	1	1	1	1	1	1	1	***2***	1	
M13 19/8/19/5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
M14 19/8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
M15 15/18	2	***1***	***1***	2	1	2	2	2	***1***	2	2	2	2	2	
M16 16/14/13	0	0	0	0	0	0	0	0	0	***1***	0	0	0	0	
M17 9/5	0	0	0	0	0	***1***	0	0	0	0	0	0	0	0	
M18 2/8	0	0	0	0	0	0	***1***	0	0	0	0	0	0	0	
M19 9/1/11	0	0	0	0	0	0	0	***1***	0	0	0	0	0	0	
M20 12/1	0	0	0	0	0	0	***1***	0	0	0	0	0	0	0	
M21 21/11	0	0	0	0	0	0	0	***1***	0	0	0	0	0	0	

a

Fig. 2. Karyotypes of clonal cultures of human colon cancer and Chinese hamster cell lines. ***a*** Karyotypes of clonal cultures of the near-diploid human colon cancer cell line HCT 116 (modal chromosome number = 45) and of the hyper-diploid human colon cancer cell line SW480 (modal chromosome number = 57). The karyotype of only 1 out of 30 cells of the clonal culture of the near-diploid HCT 116 line was non-clonal, containing an extra, partially deleted chromosome 12, termed marker M4 12^{-} (***bold italic*** number). By contrast, 13 (***bold italic*** numbers) out of 19 cells of the clonal culture of the hyper-diploid SW480 line had nonclonal karyotypes. All 13 nonclonal karyotypes differed from the modal karyotype of this line in the numbers of one or more chromosomes. Four of these 13 nonclonal cells also contained new structurally altered chromosomes, labeled M16 to 21 (***bold italic*** numbers). Chromosomal constituents of the marker (hybrid) chromosomes are indicated following their

Karyotypes of clonal cultures of the near–diploid, hyper-diplod and near-triploid Chinese hamster cells

			Normal chromosomes												Altered chromosomes	
Clone	Meta	Chr No.	1	2	3	4	5	6	7	8	9	10	X	Y	Partially clonal	Non-clonal
B69–1	1 to 17	23										1			ac1–2	
mn = 23	18	24										1			ac1–2	ac101
	19	24										1			ac1–2	ac102
	20	48	4	4	4	4	4	4	4	4	4	2	2	2	ac1[2]–2[2]	ac101[2]
D1	1	30		4			3	4	4						ac1	
mn = 29	2	29		4			3	3	4						ac2	
	3	28		4	3			3	3						ac1	
	4	29		4			3	3	4						ac2	
	5	30		3			3	4	4						ac1–2	
	6	30		4	3			4	4						ac1	
	7	29		4			3	4	4				0		ac1	
	8	29		4				4	4						ac1	
	9	28		4				3	3						ac1–2	
	10	29		4				4	4							ac101
	11	30		4			3	4	4						ac1	
	12	33		4			3	4	4						ac2	ac102–104
	13	30		4				4	4			3			ac1	
	14	27		4				3	3		3					
	15	32		4		3	3	4	4	3						ac105
	16	30		4			3	4	4						ac1	
	17	30		4				4	4						ac1	ac106
	18	29		4			3	4	4							
	19	32		4	4			4	4							ac102, 107
	20	29		4		1	3	4	4						ac1	
B2	1	33							3	3	3				ac1, 5, 7, 12, 21–22	ac201–202
mn = 35	2	34								3					ac1–2, 4–5, 7, 21, 23	ac203–206
	3	34				1									ac1–2, 4–5, 11, 21–22	ac207–212
	4	34													ac1–2, 4–5, 7, 12, 22, 24	ac213–216
	5	33					3								ac2, 4, 5, 7, 22, 24	ac217–220
	6	38					3	1							ac1, 4–5, 7, 21–22	ac221–230
	7	34				3									ac1, 4, 7, 11–12, 22	ac231–235
	8	34			3	3									ac1–4, 7, 12, 21, 24	ac236–237
	9	36					3				3				ac2, 5, 7–8, 11–12, 22	ac238–242
	10	33													ac1, 3, 11–12, 22, 24	ac243–247
	11	32	1	1		1			1						ac1–2, 4–5, 7, 11, 21	ac248–254
	12	36					3		3						ac1–2, 4–5, 11, 21, 24	ac255–259
	13	37				3			3	3					ac1–5, 7[2]–8, 22	ac260–262
	14	38				3									ac1–2, 4–5, 7–8, 12, 21	ac263–269
	15	32				3	3								ac1, 4–5, 7, 22	ac270–272
	16	30	1	1											ac1, 5, 7, 21	ac273–278
	17	32			3										ac1, 4–7, 12	ac279–281
	18	34													ac2–5, 11, 21	ac282–287
	19	36				3					3	4			ac1–2, 4, 12, 21–22	ac288–291
	20	34													ac1–2, 4–5, 12, 21–22	ac292–296
	21 to 26	~66	too complex to analyze													

b

designation, e.g. M1 2/12 for a hybrid of chromosomes 2 and 12. ***b*** Karyotypes of clonal cultures of the near-diploid, hyper-diploid and near-triploid Chinese hamster cell lines B69–1 (modal chromosome number = 21), D1 (modal chromosome number = 29) and B2 (modal chromosome number = 35). No numbers signal normal chromosome numbers. It can be seen that only 3 of 20 cells of the near-diploid line B69–1 had nonclonal karyotypes. Each of these included one new structurally altered chromosome, termed ac101 and ac102. One of these three nonclonal karyotypes also had undergone tetraploidization. By contrast, there were no two identical cells in the clonal cultures derived from the hyper-diploid and near-triploid Chinese hamster cells. Nevertheless, the degrees of both numerical and structural variations were much higher in near-triploid than in hyper-diploid Chinese hamster cells.

Cancers Have Complex Phenotypes

The complexity of most cancer-specific phenotypes far exceeds that of phenotypes generated by conventional mutation. For example, the kind of drug-resistance that is acquired by most cancer cells exposed to a single cytotoxic drug is more complex than just resistance against the drug used to induce it. It protects not only against the toxicity of the challenging drug, but also against many other chemically unrelated drugs [56, 58, 115]. Therefore, this phenotype has been termed 'multidrug resistance'. Thus, drug resistance must be polygenic. The same is likely to be true for the other cancer-specific phenotypes such as grossly altered metabolism, invasiveness, metastasis, and immortality [40, 45], because all of these phenotypes correlate with altered expressions of thousands of genes [34, 87, 116–118] and with highly abnormal concentrations of thousands of normal proteins [16, 40, 51, 119]. Moreover, in highly aneuploid cancer cells the number of centrosomes is increased up to 5-fold – from a normal of two to around ten – and at the same time their structures are often altered [120–123].

The high genetic complexities of most cancer-specific phenotypes, however, are incompatible with accumulations of large numbers of gene mutations generated at conventional rates during the limited live spans of humans and animals. Indeed, it is virtually impossible that the up to 5-fold increased numbers of centrosomes that are observed in highly aneuploid cancer cells [17, 120, 121, 124], would be the result of mutations that increase the numbers of the 350 different proteins that make up centrosomes [125].

Nonselective Phenotypes of Cancer Cells

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 [40, 45], and those, which are not selective for growth [73, 126]. The nonselective, cancer-specific phenotypes include metastasis, drug 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 [126]. Likewise, drug resistance is not a selective advantage for natural carcinogenesis in the absence of chemotherapy. Yet, a high percentage of cancers is a priori or intrinsically drug-resistant [127, 128]. Moreover, the majority of the drug resistances associated with multidrug resistance offer no selective advantages against the drug that induced it. 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 [129], and thus many more generations than are necessary to generate a lethal cancer. Consider that 50 cell generations produce from one single cell a cellular mass equivalent of 10 humans with 10^{14} cells each [10]. Nonselective

phenotypes, however, are entirely inconsistent with conventional gene mutation-selection mechanisms.

No Carcinogenic Genes in Cancer

Numerous gene mutations have been found in cancer cells since the 1980s [1, 29, 42, 130–133], and the prevailing genetic theories of cancer postulate that these mutations are carcinogenic [29, 30, 33, 34, 42].

But none of the mutations found in cancers are cancer-specific [1, 134], and in cases where this information is available many, perhaps most, mutations are nonclonal [8, 134, 135] and are not detectably expressed in human cancer cells in vivo [8, 116, 136, 137]. Despite enormous efforts in the last 25 years, 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 [4, 5, 12, 13, 24, 73, 138]. Moreover, mouse strains with artificially implanted, hypothetical cancer genes, or with artificially deleted tumor suppressor genes have survived many generations in laboratories with either the same or slightly higher cancer risks than other laboratory mice (Appendix) [8, 24, 73].

In view of this, Vogelstein and Kinzler [1] closed a very influential review of the mutation theory in 1993 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?' 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 [1, 3–8, 10–14, 16–22, 24–26, 42].

A New, Chromosomal Evolution Theory of Carcinogenesis

In an effort to resolve the many discrepancies between carcinogenesis and conventional genetic theories listed above, we present here a new, chromosomal evolution theory of carcinogenesis. Our theory is based on: (1) the ubiquity of aneuploidy in cancer [61, 62, 65, 78, 139]; (2) our own data that aneuploidy changes the numbers and structures of chromosomes and phenotypes automatically much faster than and independent of mutation [53–55, 137, 140]; (3) an earlier chromosomal theory of cancer proposed by Boveri and von Hansemann over 100 years ago [141–143]. This theory, however, was abandoned in the 1950s and 1960s in favor of mutation, because instead of the expected cancer-specific aneuploidy, karyotypic heterogeneity was found in most cancers by the methods developed at that time [62, 144, 145]. Ever since, 'aneuploidy and other forms of

chromosomal abnormality' of cancer cells [56] are generally interpreted as 'secondary' events [24, 56, 61, 62, 146] – secondary to presumably primary gene mutations [15, 32, 64, 75, 147–153]; (4) cancer-specific aneuploidies discovered since the late 1960s by many laboratories including ours, particularly by comparative genomic hybridizations [84]. These discoveries, however, are not appreciated as chromosomal causes of cancer because of the prevailing genetic theories.

According to our new chromosomal evolution theory, carcinogenesis is the result of the following chain of events: (1) carcinogens and spontaneous mitotic errors induce unspecific aneuploidies; (2) aneuploidy corrupts teams of proteins that segregate, synthesize and repair chromosomes. Aneuploidy is thus a steady source of karyotypic-phenotypic variations from which, in classical Darwinian terms, selection of cancer-specific aneuploidies encourages the evolution and spontaneous 'progressions' of the malignant phenotypes of neoplastic cells. The rates of these variations are proportional to the degrees of aneuploidy; (3) this chromosomal evolution makes cancer cells new, inherently unstable cell 'species' with distinct, but unstable karyotypes, rather than mutant cells. Owing to this inherent chromosomal instability, cancers are uncertain combinations of random and of relatively specific or 'nonrandom' aneuploidies; (4) the cancer-specific aneuploidies generate complex, malignant phenotypes via abnormal dosages of thousands of genes. Down syndrome is a model for how aneuploidy generates complex, abnormal phenotypes, and (5) 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. According to this mechanism variations of chromosomes have the same effects on the phenotypes of cells as variations of the assembly lines of a car factory on the phenotypes of an automobile. If changes are made that do not alter the balance of components, e.g. moving the engine from the front to the rear, new, competitive car models are generated. Indeed, motor companies change their assembly lines to create a new car model. Likewise, phylogenesis generates new species by changing the numbers and structures of the chromosomes of existing species [154].

If unbalanced, i.e. aneuploid, changes are made, abnormal and defective products must be expected. The human trisomy 21, which causes Down syndrome, is a classic non-neoplastic example [113, 114]. Although trisomy 21 is only a tiny aneuploidy compared to that of most cancers, it generates 71 Down-specific phenotypes [111, 112]. Likewise, experimentally induced, congenital aneuploidies generate numerous abnormal phenotypes in drosophila, plants and mice, independent of gene mutation [155–157]. Thus, the complex aneuploidies of cancer cells can be expected to generate numerous new phenotypes.

By contrast, the power of changing the phenotypes of the cell by gene mutation is comparable to employing a few defective or overactive workers on

the assembly lines of a car factory. Neither of these variables will generate a new car model, except possibly to produce either a defective car or no car at all, if an assembly line comes to a stop [158]. For example, none of the 1.42 million point mutations that distinguish any two humans [159] have generated a new human species, nor have they even been sufficient to cause cancer in newborns.

Instead of being controlled by hypothetical oncogenes or tumor suppressor genes, alias 'gate keepers and caretakers' [75, 160], or being de-controlled by the corresponding mutations, most phenotypes of normal and cancer cells are controlled 'democratically' by hundreds of kinetically linked proteins [161]. Such cooperative assembly lines of gene products are buffered against mutations of single genes by the assembly line principle [161, 162]. According to this principle, unchanging supplies and demands of numerous unmutated genes from upstream and downstream of biochemical assembly lines buffer mutations in two ways. They automatically raise substrate concentrations upstream of slow-working, mutationally compromised genes and restrict by normal supplies of substrates mutationally activated genes [161, 163]. This is indeed the principle that buffers cells of all multicellular organisms against all but knock out mutations that occur during their long lifetimes.

Thus aneuploidization, upsetting the balance of thousands of normal genes, rather than mutation of a few genes, is necessary to generate the complex and dominant phenotypes of cancer cells.

In sum, the chromosomal evolution theory provides a coherent explanation of carcinogenesis that is independent of mutation, and that can explain each of the many idiosyncratic features of carcinogenesis that are paradoxical in view of the mutation theory. However, the chromosomal theory remains challenged by competing claims of the prevailing genetic theories of cancer. In the following we take up this challenge.

Testing Specific Predictions of the Chromosomal Theory against Competing Claims by Genetic Theories of Cancer

According to the prevailing genetic theories of cancer, 'carcinogens are mutagens' [164] initiating carcinogenesis by mutation, and 'initiated' cells then evolve into cancer cells via poorly defined sets of four to seven complementary mutations [1, 29, 34, 35, 38, 41, 42, 52, 134, 165]. Since these claims of the prevailing genetic theories of cancer have monopolized cancer research in the last decades, we have tested the most distinctive predictions of the chromosomal evolution theory: (1) carcinogens initiate carcinogenesis by aneuploidisation; (2) aneuploidy is inherently variable and thus sufficient to catalyze the evolution of cancer-specific chromosome patterns, and (3) carcinogenesis is independent of somatic mutation.

Carcinogens Function as Aneuploidogens

This prediction has been confirmed previously by others [4, 10, 44, 67, 70, 73, 158, 166–168] including Boveri, who first demonstrated that X-rays, several chemicals, heat and physical stress generate aneuploidy, but failed to observe cancer in experimental animals [142, 143]. However, since these studies did not establish pre-neoplastic aneuploidy as the cause of carcinogenesis [6, 7, 24, 25], we have recently retested the question whether carcinogens cause aneuploidy experimentally, using mutagenic [84] and nonmutagenic carcinogens [169, 170], and by reviewing the literature [4, 10, 25, 73, 158]. These tests have shown that mutagenic carcinogens generate aneuploidy either by breaking and rearranging chromosomal DNA or by chromosome nondysjunction owing to alterations of the spindle apparatus. By contrast, nonmutagenic carcinogens would induce aneuploidy primarily via de-polymerization of the proteins of the spindle apparatus or even via physical interference with mitosis as by asbestos [80]. Polycyclic aromatic hydrocarbons and vincristine are examples of carcinogens that cause aneuploidy by depolymerizing protein polymers of the spindle apparatus [70, 158].

Moreover, carcinogens, particularly radiations and mutagenic chemical carcinogens, induce aneuploidy without delay, and thus long before cancer [170–174], as postulated by the chromosomal theory. Most importantly, our own studies have shown that among the many effects that carcinogens have on cells [40], aneuploidy is the one that consistently segregates with subsequent carcinogenesis [84, 170].

A series of recent studies, aiming at the definition of mutations that might 'initiate' carcinogenesis, have instead all pointed to chromosomal initiation [67, 73, 174]. Based on the dosage of a carcinogen delivered to cell cultures, the percentages of 'initiated' cells were found to be >1,000-fold larger than expected for the target gene [73]. Markers identified for the initiation of carcinogenesis were either aneuploidy or chromosomal destabilization or immortalization or 'delayed reproductive death' [67] or transformation of cells in vitro [73]. Since an average human chromosome contains about 1,500 genes – 35,000 genes divided by 23 chromosomes [154] – it follows that the chromosome is the target for the initiation of carcinogenesis [73]. We conclude that carcinogens function as aneuploidogens as postulated by the chromosomal theory.

Aneuploidy Is Inherently Variable and Thus Sufficient to Catalyze the Evolution of Cancer-Specific Chromosome Patterns

We have tested this critical prediction of the chromosomal evolution theory, by measuring the rates at which karyotypes of cancer cells vary spontaneously per cell generation. For this purpose clonal cultures of cancer cells with different degrees of aneuploidy were prepared and the fraction of nonclonal karyotypes in these cultures was determined. The rates of karyotype alteration

per cell generation are then calculated by dividing these fractions by the number of generations of the clonal culture.

Using this method we found karyotypic variation at rates of near 10^{-2} per generation in the hyper-diploid – modal chromosome number = 57 – human colon cancer cell line SW480 [53]. This rate was calculated from the data shown in figure 2a as follows: 6 of the 19 karyotypes were identical and are thus considered the 'stemline' [62] or modal karyotype of this line. But, 13 of 19 'clonal' SW480 cells had non-clonal karyotypes, differing from the predominant 'stemline' in numerical and structural aneusomies, which are identified by bold italic numbers in figure 2a. Since the clone was about 23 generations old by the time it was analyzed, having grown from a single cell to about 10^7, the average rate of karyotype variation per cell per generation is about 3% (13:19:23). Indeed, this is a minimal estimate, because many random chromosomal variations are not viable. A comparison of the karyotypes of an SW480 cell with a normal human foreskin cell is shown in figure 3. The karyotypes were prepared from metaphase chromosomes hybridized in situ with color-coded chromosome-specific DNA probes, as described by us recently [53].

Even higher rates of over 1 chromosomal variation per cell generation were observed in the hyper-diploid and near-triploid Chinese hamster cell lines D1 (modal chromosome number = 29) and B2 (modal chromosome number = 35) [55, 140] (fig. 2b). The normal chromosome number of the Chinese hamster is 22. Not even two of these highly aneuploid Chinese hamster cells were the same [55]. This means that the rates of karyotype variations per cell generation were at least 4% (100%: 23), but probably higher, because most random variations are likely to be lost as fast as they are generated. However, in the case of the near-triploid B2 line the rates of structural chromosomal rearrangements were at least 100% per generation, because each metaphase contained several unique structural chromosome alterations, numbered ac201-ac296 in figure 2b.

As predicted by the chromosomal theory, much lower rates of karyotype variations were observed at low degrees of aneuploidy, namely in the near-diploid human colon cancer cell line HCT 116 (modal chromosome number = 45) and in the near-diploid Chinese hamster line B69–1 (modal chromosome number = 23) [55, 140]. Only 1 of 30 clonal HCT 116 cells contained a new, structurally altered chromosome, again identified by a bold italic number in figure 2a, which corresponds to a rate of only 0.15% karyotypic variations per cell generation. Not even one purely numerical variation was detected in 30 metaphases. Likewise only 3 of 20 clonal B69–1 cells had nonclonal karyotypes (fig. 2b), which corresponds to a rate of 0.65% karyotypic variations per cell generation.

It follows that the degrees of both numerical and structural chromosomal instability of human and Chinese hamster cells are proportional to the degrees

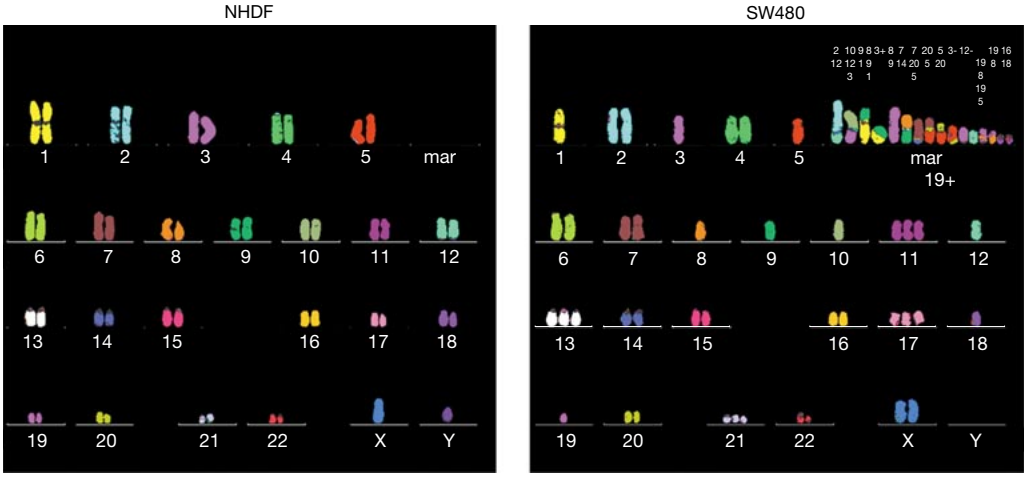


Fig. 3. Metaphase chromosomes of a normal human foreskin cell and of a cell from the human colon cancer cell line SW480. Cytogenetically intact chromosomes are identified by numbers. The group labelled 'mar' (for marker chromosome) shows structurally abnormal chromosomes, which are either rearranged intra-chromosomally or inter-chromosomally to form various hybrid chromosomes. The numbers above these marker chromosomes identify the chromosomal origins of hybrid chromosomes in their relative order or the basis of intra-chromosomal alterations, e.g. 3+ for an amplification of chromosome 3. A comparison of the two karyotypes shows that the cancer cells differ from the normal cell in numerous numerical and structural chromosomal alterations or aneusomies. See online version for color.

of aneuploidy, as postulated by the chromosomal theory. Others have recently described very similar correlations between chromosomal instability and degrees of aneuploidy in human cancer cells including some of those used by us [175–177].

However, the fact that chromosomes are destabilized in proportion to the degree of aneuploidy could also be explained by a series of independent mutations. But, this mutation argument is unlikely, because it is very unlikely that two inherently different kinds of mutations, those that alter the structures and those that alter numbers of chromosomes, would both be equally proportional to the degrees of aneuploidy in all cancers, considering that specific mutations are very rare, even in cancer cells (Appendix). In other words, this argument predicts some cancers with high numerical and no or low structural instability, and others with the opposite distribution, but so far no such cancers have been described.

In sum, the conclusion can be drawn that the inherent variability of aneuploidy is the cause of the chromosomal and phenotypic instabilities of cancer cells and the resulting cellular heterogeneities of cancer, as predicted by the

chromosomal theory. This aneuploidy-specific, chromosomal uncertainty principle had become the nemesis of the Boveri-von Hansemann theory in the 1950s and 1960s.

Carcinogenesis Independent of Somatic Mutation

Cancer coincides with aneuploidy as well as with mutations [6, 7, 10, 13, 24]. In the words of a recent review in *Science*, 'Cancer cells are chock-full of mutations and chromosomal abnormalities' [6]. Therefore, it can be argued that spontaneous and carcinogen-induced aneuploidization is sufficient for the initiation and autocatalytic evolution of carcinogenesis, as we did here. But, it could also be argued that the initial aneuploidization and its subsequent evolution depend on somatic mutations, as others have done recently [13, 14, 26, 150–153, 178].

However, the following 4 arguments indicate that carcinogenesis (of normal cells in normal organisms) is independent of somatic mutation [25]. In fact, cancer cells, via their specific aneuploidy, are even protected against the negative effects of mutation: (1) Initiation of carcinogenesis by aneuploidy, generated by mutagenic carcinogens fragmenting or eliminating chromosomes, is about 35,000 times more likely than by aneuploidy, generated by mutation of a specific mammalian 'aneuploidy-gene' [6]. This is because mammals contain about 35,000 genes, and thus only 1 in 35,000 specific mutations would generate an 'aneuploidy gene' [25, 154], but any mutation leading to a chromosome break or rearrangement generates aneuploidy. Using nonmutagenic carcinogens to generate initiating aneuploidy via the spindle apparatus is in fact infinitely more efficient than via the nontarget gene. Thus, initiation of carcinogenesis is independent of somatic mutation. (2) Generating the complex, cancer-specific phenotypes by chromosomal variation is about 1,500 times more efficient than by mutation. Indeed, it would be almost impossible to generate the complex, polygenic phenotypes of cancer cells in a lifetime of a cancer patient by mutating many genes, considering the complexity of cancer-specific phenotypes and the low rates of spontaneous mutation in normal and most cancer cells (Appendix). By contrast, chromosomal variation is a mechanism that automatically alters the dosages and expressions of thousands of genes. Therefore, aneuploidization is infinitely more efficient in generating the complex phenotypes of cancer cells than mutation. Thus, carcinogenesis is independent of somatic mutation in generating complex, cancer-specific phenotypes. (3) The high rates of cancer-specific karyotype-phenotype variations are irreconcilable with the low rates of conventional mutation. New, cancer-specific phenotypes appear or old ones disappear in highly aneuploid cancer cells at rates of up to 10^{-3} per cell generation, which is four to eleven orders faster than conventional gene mutation (Appendix). Thus phenotype variation in cancer cells is independent

of mutation. (4) The relevance of somatic mutations for carcinogenesis is uncertain. Cancer-specific aneuploidy can generate gene mutations by the same mechanism that varies the structures of chromosomes. In addition, aneuploidy renders DNA synthesis error-prone by unbalancing nucleotide pools [179]. 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 the mutations found in cancer cells are frequently nonclonal in cancers [8, 135], and why they do not transform normal cells to cancer cells and do not breach the livelihood of transgenic mice (Appendix). Indeed, cancer cells are immortal, because frequent, aneuploidy-catalyzed karyotypic variations neutralize all potentially negative mutations at much higher rates than they can be generated.

We conclude that carcinogenesis is independent of somatic mutation, because aneuploidy is much more likely to be generated and varied at the chromosomal level than by mutation. In response to this it has been argued that cancers associated with heritable cancer-disposition syndromes prove that carcinogenesis is dependent on mutation. Examples are the retinoblastoma, xeroderma, Bloom syndrome, and mosaic variegated aneuploidy syndromes [32, 34, 180, 181]. However, these heritable – rather than somatic – mutations are not direct causes of cancer. Instead they initiate carcinogenesis by aneuploidization at much higher rates than it would occur in normal cells by spontaneous or carcinogen-induced aneuploidization [181–183]. According to the chromosomal theory these mutations are genetic equivalents of carcinogens that induce aneuploidy at high rates. This view is supported by the presence of aneuploidy in such patients prior to carcinogenesis, as for example in mosaic variegated aneuploidy patients [183, 184], Bloom patients [182] and xeroderma patients [185], and by the presence of aneuploidy in the cancers of patients with retinoblastoma [186–189], mosaic variegated aneuploidy [183, 184], xeroderma [185, 190] and Bloom patients [182].

We conclude that the abnormally high rates of carcinogenesis in heritable cancer disposition syndromes are dependent on abnormally high rates of aneuploidizations that are generated by these heritable genes. Thus carcinogenesis encouraged by certain heritable mutations confirms and extends the chromosomal theory of carcinogenesis, but does not show that carcinogenesis in normal cells depends on conventional mutation.

Explanatory Value of the Chromosomal Theory of Cancer

In table 1, we have summarized how the chromosomal cancer theory explains each of the idiosyncratic features of carcinogenesis that are paradoxical

Table 1. Features of carcinogenesis

Genetic paradox	Chromosomal solution
1 Cancer not heritable	aneuploidy is not heritable
2 Long neoplastic latencies	autocatalyzed evolution of cancer-specific aneusomies
3 Non-mutagenic carcinogens	carcinogens function as aneuploidogens
4 High rates of karyotype-phenotype variations and the origin of 'immortality'	aneuploidy catalyses karyotype-phenotype variations, including resistance to otherwise lethal conditions, at high rates
5 Cancer-specific aneuploidies	cancer-specific aneuploidies generate cancer phenotypes
6 Complex phenotypes	cancer-specific aneuploidies alter dosages and functions of thousands of genes
7 Nonselective phenotypes	nonselective genes hitchhiking with selective, cancer-specific aneusomies
8 No carcinogenic genes in cancer	cancer is caused by specific aneuploidies

in terms of conventional genetic theories. In the following we offer further commentary on items 1, 2, 5, 6 and 7 listed in table 1, because they are not sufficiently explained by the table and the preceding arguments.

Cancer Is Not Heritable

The chromosomal theory predicts no cancer in newborns, because aneuploidy is not heritable. Aneuploidies are not heritable, because they corrupt embryogenic developmental programs [113, 114], which is usually fatal [157, 191] as originally shown by Boveri [142]. 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 [31, 65, 68, 192]. Thus, ontogenesis is nature's checkpoint for normal karyotypes. The postnatal exponential increase of the cancer risk with age would then reflect the gradual accumulation of non- or preneoplastic aneuploidy with age, multiplied by the relatively slow, nonselective replication of aneuploid, preneoplastic cells (figs 1, 2).

However, it is as yet unclear, why after initiating doses of carcinogens the neoplastic latencies are very species-dependent, namely much shorter in rodents than in humans [1, 46, 47, 193–195]. It is also unclear, why the increase of the cancer risk is proportional to the lifespan of an animal, i.e. is very low for decades in humans (fig. 1), but only for months in rodents [38, 47]. Still, this is unlikely to be due to species-specific mutation rates, because the rates of conventional mutations are highly conserved in all species [52, 68]. However, the

significantly higher chromosomal instability of aneuploid rodent cells compared to equally aneuploid human cells, shown here in figure 2, may offer a different explanation, namely that chromosomal stability of normal and cancer cells is different in different species.

Long Neoplastic Latencies

The chromosomal evolution theory predicts that carcinogenesis is initially very slow, because preneoplastic cells have no growth advantages compared to normal cells and are typically only little aneuploid (fig. 4). Therefore, they 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 of pre-neoplastic cells, because phenotypes of single cells are hard to recognize. By contrast, neoplastic 'progression' of established cancer cells is predicted to be faster than during the pre-neoplastic phase for two reasons: (1) Neoplastic cells, through their selective phenotypes, will generate large 'clonal' populations with high probabilities of further variations. (2) The generally high degrees of most cancer-specific aneuploidies catalyze high rates of chromosomal variations, compared to those of preneoplastic cells (fig. 4).

The chromosomal theory also predicts a certain endpoint of chromosomal evolutions in carcinogenesis. This endpoint would be an equilibrium of aneuploidizations, which is reached once a cancer has maximized cellular variability and adaptability [73] and 'optimized its genome' for essential metabolic functions [196]. According to the chromosomal theory maximal chromosomal variability would correspond to near or above triploid chromosome numbers (>3n) [13, 73, 137]. Near triploid aneuploidy offers an optimal average redundancy of one spare for each normal chromosome pair, and thus sufficient redundancy to compensate for any losses or genetic mutations of a given chromosome [73]. Accordingly, it is the karyotype of most malignant cancer cells [10, 62, 65, 73, 146, 158, 178, 197].

High Rates of Karyotype-Phenotype Variations and the Origin of Immortality

The chromosomal theory attributes the high rates of karyotype-phenotype variations of cancer cells to the inherent variability of aneuploidy. On this basis, the chromosomal theory also explains the notorious immortality of cancer cells as already described in 1972 by the cytogeneticist Koller [62]: '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 multipotentiality of the neoplastic cell; while normal cells and tissues age and die, through their inherent variability, tumor cells proliferate and survive.' Thus, cancers are immortal, because subspecies from within the zoos of their polyphyletic

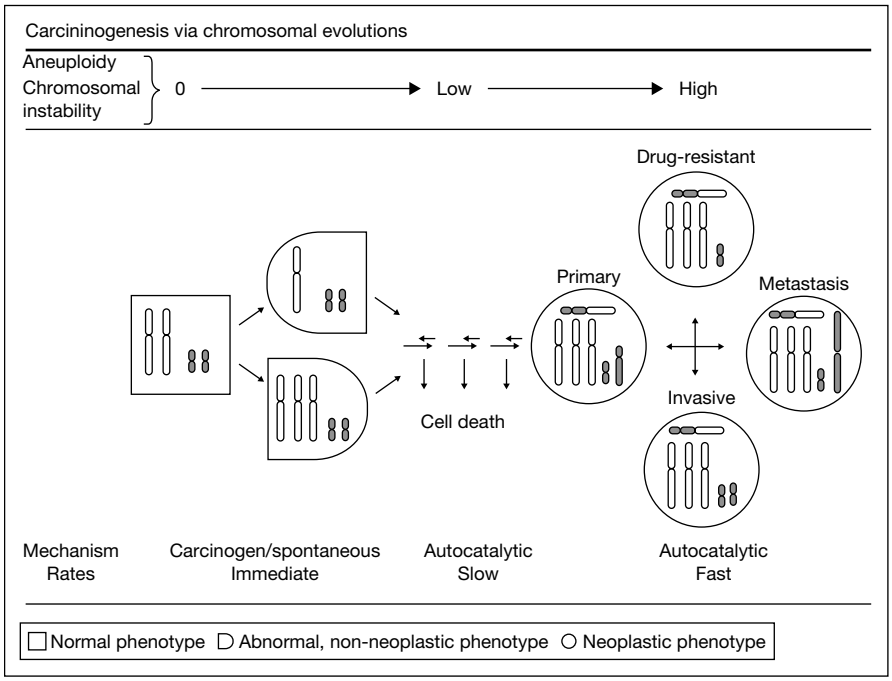


Fig. 4. Carcinogenesis via chromosomal evolutions. According to this mechanism carcinogenesis is initiated by unspecific aneuploidies induced either by carcinogens or spontaneously. Aneuploidy then alters the karyotype automatically at rates that are proportional to the degree of aneuploidy, because it corrupts teams of proteins that segregate, synthesize and repair chromosomes. Aneuploidy is thus a steady source of chromosomal variations from which, in classical Darwinian terms, selection would encourage the evolution and subsequent progressions of neoplastic cell 'species' with cancer-specific aneusomies. This evolution would be slow in the preneoplastic phase, because preneoplastic cells have no growth advantages over normal cells and because the degree of preneoplastic aneuploidy is typically low. By comparison the rate of karyotype variations of most cancer cells would be fast, because cancer cells form large populations by outgrowing normal cells and because the degrees of cancer-specific aneuploidy are typically high. Any kind of cancer could have as many specific aneusomies as there are chromosomes involved in the differentiation of its precursor cell in addition to random aneusomies. Thus cancer-specific phenotypes, such as invasiveness, metastasis, and drug-resistance, are generated by the abnormal dosages of thousands of normal genes. Since aneuploidy is inherently unstable, cancer-specific phenotypes, such as drug-resistance, can be reversible or convertible to other specific phenotypes at the same rates at which they are generated. The chromosomal model predicts the heterogeneous phenotypes and karyotypes of cancers as consequences of independent evolutions of the inherently unstable cancer cells. Since aneuploidy causes dedifferentiation, the model further predicts that the degrees of malignancy of cancer cells are proportional to the degrees of aneuploidy.

cell populations [110] – species are defined by karyotypes – survive conditions that are lethal to the mortal majority of the cells, as for example toxic drugs.

Cancer-Specific Aneuploidies

The presence of cancer-specific or nonrandom aneuploidies is directly predicted by and thus correlative proof for the chromosomal theory in terms of Koch's first postulate. Functional proof that cancer-specific aneuploidy generates malignancy could be derived from evidence that the degree of malignancy is proportional to the degree of aneuploidy. Indeed, numerous correlations have confirmed the principle that the degree of malignancy of cancer cells is proportional to their degree of aneuploidy since the 1930s [10, 45, 62–64, 97, 198–204]. Moreover, other studies have shown that maximal malignancy is, indeed, achieved at maximally stable, near-triploid or hypertriploid aneuploidy [65, 178, 197, 205, 206]. The parallel evolutions of aneuploidy and malignancy in cancer cells are thus functional proof for the chromosomal evolution theory of cancer in terms of Koch's third postulate.

Complex Phenotypes

Conventional genetic theories cannot explain the generation of the polygenic cancer-specific phenotypes such as multidrug resistance, polymorphism, metastasis to non-native sites, and transplantability to heterologous species [108] based on conventional rates of mutation and selection in the lifespan of a human or animal. By contrast, the chromosomal theory of cancer explains the complexity of cancer-specific phenotypes by the complexicity of the genetic units that are varied, namely chromosomes with thousands of genes. Accordingly, the complex phenotypes of cancer cells have recently been shown to correlate with over- and underexpressions of thousands of genes [34, 87, 116–118, 136]. Likewise, cancer cells over- and underproduce thousands of normal proteins [16, 40, 51, 119].

Nonselective Phenotypes`

Conventional genetic theories explain the evolution of cancer cells by cancer-specific mutations and Darwinian selections. But this mechanism cannot explain the nonselective phenotypes of cancer cells, such as metastasis, drug resistance and 'immortality'. By contrast, the chromosomal theory of carcinogenesis attributes nonselective phenotypes such as metastasis and intrinsic multidrug resistance to nonselective genes hitchhiking with selective, cancer-causing aneusomies, because they are all located on the same chromosomes. The same would be true for that part of acquired multidrug-resistance, which is not directed against the selective drug that induced it. The nonselective phenotype immortality has been explained above.

Conclusions

We conclude that the chromosomal 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. In addition, the theory stands out for making new, clinically testable predictions, as for example the prediction that cancer could be detected prior to malignancy via preneoplastic aneuploidy and that chemotherapy could be based on the presence or absence of resistance-specific aneusomies. Thus, if confirmed, the chromosomal theory should become beneficial for cancer research and therapy.

Appendix

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 four to seven complementary mutations during the lifetime of a patient [1, 12, 34, 38, 41, 42]. However, the mutation theory is hard to reconcile with the following list of facts.

1 *Nonmutagenic Carcinogens.* Contrary to the mutation hypothesis, 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 [40, 44, 67, 70, 73, 158, 166, 168].

2 *No Transforming Genes.* Despite years of efforts no genes or combinations of genes from cancers have been shown to transform normal cells to cancer cells [4, 5, 138] or mice carrying such genes in their germ lines into polyclonal tumors [1, 24, 56]. Accordingly, many, presumably cancer-specific mutations are not detectably expressed in cancer cells [8, 116, 136, 137].

3 *Dependence of Cancer on Unrealistically High Rates of Mutation.* The mutation hypothesis explains the exponential increase of the cancer risk with age by the low probability of four to seven specific mutations [1, 41, 42]. However, in order to maintain the integrity of the genome, 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 [6, 47, 52, 57, 68]. Thus, based on these conserved mutation rates cancer via four to seven mutations would not even exist [10]. For example, based on just 4 specific dominant mutations cancer 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 [10, 38]. Thus, in order to explain the current cancer risk of Americans and Europeans of about 1 in 3 lifetimes [39] (fig. 1), the mutation hypothesis has to assume mutation rates, which are 10^3 [$(10^3)^4 = 10^{12}$] times higher than in conventional mutation.

4 *No Explanation for the Long 'Neoplastic Latency' in Carcinogenesis Induced by a Critical Dose of Carcinogen.* The mutation hypothesis has no answer to the question why, after a critical dose of carcinogen, carcinogenesis would only occur after exceedingly long 'neoplastic latencies' of years to decades [1].

5 *Dependence of Phenotype Alterations in Cancers on Unrealistically High Rates of Mutation.* The mutation hypothesis 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 [59, 60], or generate drug-resistant variants [53, 54, 56, 58]. But the mutation rates of most cancers are not higher than those of normal cells [6, 19, 20, 47, 66, 70–74].

6 *Heritable Cancer Genes, but no Heritable Cancer.* The four to seven gene mutation hypothesis predicts that subsets of cancer causing mutations should be heritable. Indeed, proponents of the mutation hypothesis have demonstrated that several of the six mutations thought to cause colon cancer [1] can be introduced into the germ line of mice without breaching the viability of these animals. According to one study, animals with one of these mutations, namely ras, were found 'without detectable phenotypic abnormalities' [207]. Another study reports, "surprisingly, homozygosity for the Apc1638T mutation is compatible with postnatal life" [208]. Thus subsets of colon cancer genes are heritable. Therefore, colon cancer should be common in newborns, which are clonal for inherited subsets of these six mutations (like transgenic mice). But there is no colon cancer in newborns [38, 39].

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