Defining the steps that lead to cancer: Replicative telomere erosion, aneuploidy and an epigenetic maturation arrest of tissue stem cells

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Summary   Recently, an influential sequencing study found that more than 1700 genes had non-silent mutations in either a breast or colorectal cancer, out of just 11 breast and 11 colorectal tumor samples. This is not surprising given the fact that genomic instability is the hallmark of cancer cells. The plethora of genomic alterations found in every carcinoma does not obey the 'law of genotype–phenotype correlation', since the same histological subtype of cancer harbors different gene mutations and chromosomal aberrations in every patient. In an attempt to make sense out of the observed genetic and chromosomal chaos in cancer, I propose a cascade model. According to this model, tissue regeneration depends on the proliferation and serial activation of stem cells. Replicative telomere erosion limits the proliferative life span of adult stem cells and results in the Hayflick limit (M1). However, local tissue exhaustion or old age might promote the activation of M1-deficient tissue stem cells. Extended proliferation of these cells leads to telomere-driven chromosomal instability and aneuploidy (abnormal balance of chromosomes and/or chromosome material). Several of the aforementioned steps have been already described in the literature. However, in contrast to common theories, it is proposed here that the genomic damage blocks the epigenetic differentiation switch. As a result of aneuploidy, differentiation-specific genes cannot be activated by modification of methylation patterns. Consequently, the phenotype of cancer tissue is largely determined by the epigenetic maturation arrest of tissue stem cells, which in addition enables a fraction of cancer cells to proliferate, invade and metastasize, as normal adult stem cells do. The new model combines genetic and epigenetic alterations of cancer cells in one causative cascade and offers an explanation for why identical histologic cancer types harbor a confusing variety of chromosomal and gene aberrations. The Viennese Cascade, as presented here, may end the debate on if and how 'tumor-unspecific' aneuploidy leads to cancer.
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Introduction

In the US today, the lifetime probability of developing cancer is 46% for men and 38% for women [1], and the mortality rate from cancer has changed

very little over the past 50 years [2,3]. Carcinomas, malignant epithelial tumors, account for 80% of cancer-related deaths in the western world [4]. Therefore, the focus of this paper is on epithelial cancer that currently represents an 'epidemic' in the middle-aged and elderly population.

In the past, enthusiasm for tumor cytogenetics gradually faded due to a confusing plethora of chromosomal aberrations found in human cancers (reviewed in Ref. [5]). As a consequence, the cause of cellular transformation was thought to have a mutational basis. Ironically, molecular geneticists have been confronted with an even larger variety of 'cancer-causing' gene mutations [2,6]. This is not surprising given the fact that genomic instability is the hallmark of cancer cells [7,8]. Thousands of genomic alterations [9] found in every cancer patient do not obey the 'law of genotype—phenotype correlation', since the same histological subtype of cancer harbors different mutations in every patient [10—12]. Tumor cell heterogeneity with no two individual cells harboring identical mutations [13] has further impeded progress in the field of molecular genetics. The purpose of this article is to present a model in which the observed chaotic variety of chromosomal aberrations and gene mutations leads to similar histologic subtypes of cancers (Fig. 1).

Historical background

In 1867, Cohnheim hypothesized that all tissues are renewed by cells in the blood stream [14] and that cancer in adults develops from embryonic cells that are produced in excess and remain in fully mature organs [14]. In retrospect, both predictions are chillingly accurate. A large body of evidence now supports the idea that adult human stem cells circulate in the blood stream and participate in tissue regeneration of many, if not all, organs [15—17]; but the full extent of their regeneration potential is still unknown [18]. Following the classic 'initiation—promotion' experiments [19], many cancers are considered a disease of stem cells, in which (cancer) stem cells renew (tumor) tissue [20]. The observation that only a minority of leukemia or tumor cells can initiate cancer [21,22], and that these cells display many stem cell characteristics resurrected the 'cancer stem cell hypothesis' [23,24], originally proposed in 1961 [25,26]. In the 1970s, Pierce suggested that cancers represent a maturation arrest of stem cells. He stated that all of the characteristics associated with malignancy are expressed during some stage of development, suggesting that the normal genome contains the

information necessary for malignant expression [27]. Yet, certain characteristics of malignancy are clearly not physiological (e.g. anaphase bridges and atypical mitoses), but can be attributed to critically short telomeres and aneuploidy [28]. Aneuploidy usually means an abnormal number of chromosomes, deviating from euploidy. In recent literature, this term has expanded to include all unbalanced chromosomal aberrations, including both structural and numerical defects.

Almost 80 years ago, Mueller and McClintock proposed the concept of protective caps at the end of eukaryotic chromosomes [29], called telomeres. In 1961, Hayflick reported an elegant experiment demonstrating that normal human cells are capable of a limited number of cell divisions [30]. Subsequently, Olovnikov suggested a biological explanation for this phenomenon, namely incomplete DNA replication [31,32]. It later became known as 'the end-replication problem of linear DNA molecules' [32]. Based on experimental findings, telomeres shorten every time a somatic cell divides, and subsequently limit the proliferation potential of normal human cells, constituting the so-called mitotic clock [33]. Once a telomere is critically short, it can no longer form the functional t-loop structure [34]. Human cell proliferation usually stops before telomeres reach this critically short length, a phenomenon called the Hayflick limit, replicative senescence, or M1. If cells are M1-deficient (e.g. via deregulation of the p53 and RB1 pathways), proliferation continues and short non-functional telomeres lead to chromosome end fusion and to breakage—fusion—bridge cycles during cell division [29]. This results in crisis-stage, which is characterized by chromosomal instability and aneuploidy (Fig. 1) [35,36]. In vitro experiments with simian virus 40 (SV40) genes suggest that spontaneous telomerase expression may rescue some human cells from crisis by stabilizing the telomeres [37,38]. Thus elevated telomerase expression is thought to induce a state of cellular immortality.

In 1890, von Hansemann concluded that malignant tumor cells are defined by abnormal chromatin content [39]. More than 20 years later, Boveri suggested that tumors might arise as a consequence of abnormal segregation of chromosomes to daughter cells. He postulated that tumor growth is based on a particular, incorrect chromosome combination which is the cause of abnormal growth characteristics passed on to daughter cells [40,41]. Until quite recently, these ideas have received only limited attention, because the primary focus of cancer genetics has been mainly on the role of individual oncogenes and tumor suppressor genes [42,43]. A somewhat collective disappointment was that more than 40 years of cancer cytogenetic
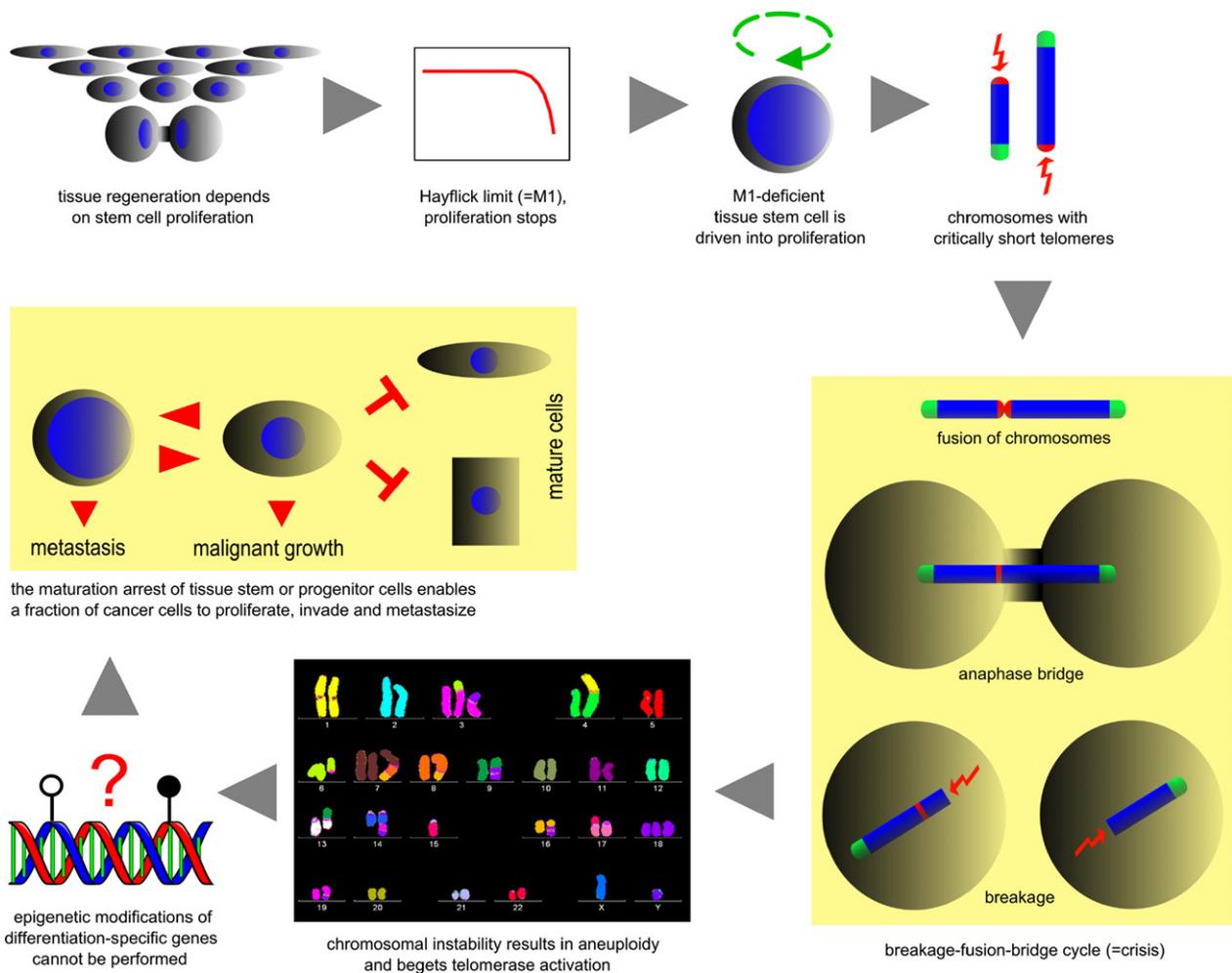
Figure 1 The Viennese Cascade.

research resulted in just a handful of tumor-specific chromosomal aberrations (reviewed in Ref. [5]). Among these findings, approximately 40% of published recurrent balanced aberrations were found to be biased [44]. A confusing combination of apparently meaningless abnormalities in numerous solid tumors were regarded as epiphenomena incurred during tumor progression [5]. Despite the fact that chromosomal instability is an early event in tumorigenesis [45,46] and that there is abundant evidence of correlation between increasing aneuploidy and greater malignancy in tumors [47—49]. Indeed human adult stem cells, after acquiring aneuploidy *in vitro*, have been shown to transform spontaneously [50].

Every normal somatic cell contains the same set of chromosomes and genes, yet the human body consists of hundreds of different cell types. Differentiation of distinct cell types requires transcriptional activation of differentiation-specific genes and the suppression of genes associated with the progenitor cell. Consequently, tissue-specific dif-

ferentiation can be linked to epigenetic mechanisms. More than 20 years ago, patterns of genomic 5-methyl-cytosine levels in tissue were observed [51]. Since then, it has been clearly shown that DNA methylation represents a mechanism for the regulation of gene expression in vertebrates [52,53]. Demethylation of so-called 'CpG islands' in promoter regions occurs during differentiation and is a required step in the process of transcriptional activation [53—55].

The author's quest

Duesberg proposed that the cancer phenotype is a direct consequence of aneuploidy changing the dosage of thousands of genes [56—58]. According to him, the phenotype is the result of a genotype—phenotype correlation, just as trisomy of chromosome 21 generates Down syndrome [56]. As a postdoctoral fellow in his laboratory (1999—2002) I was skeptical about aneuploidy and its
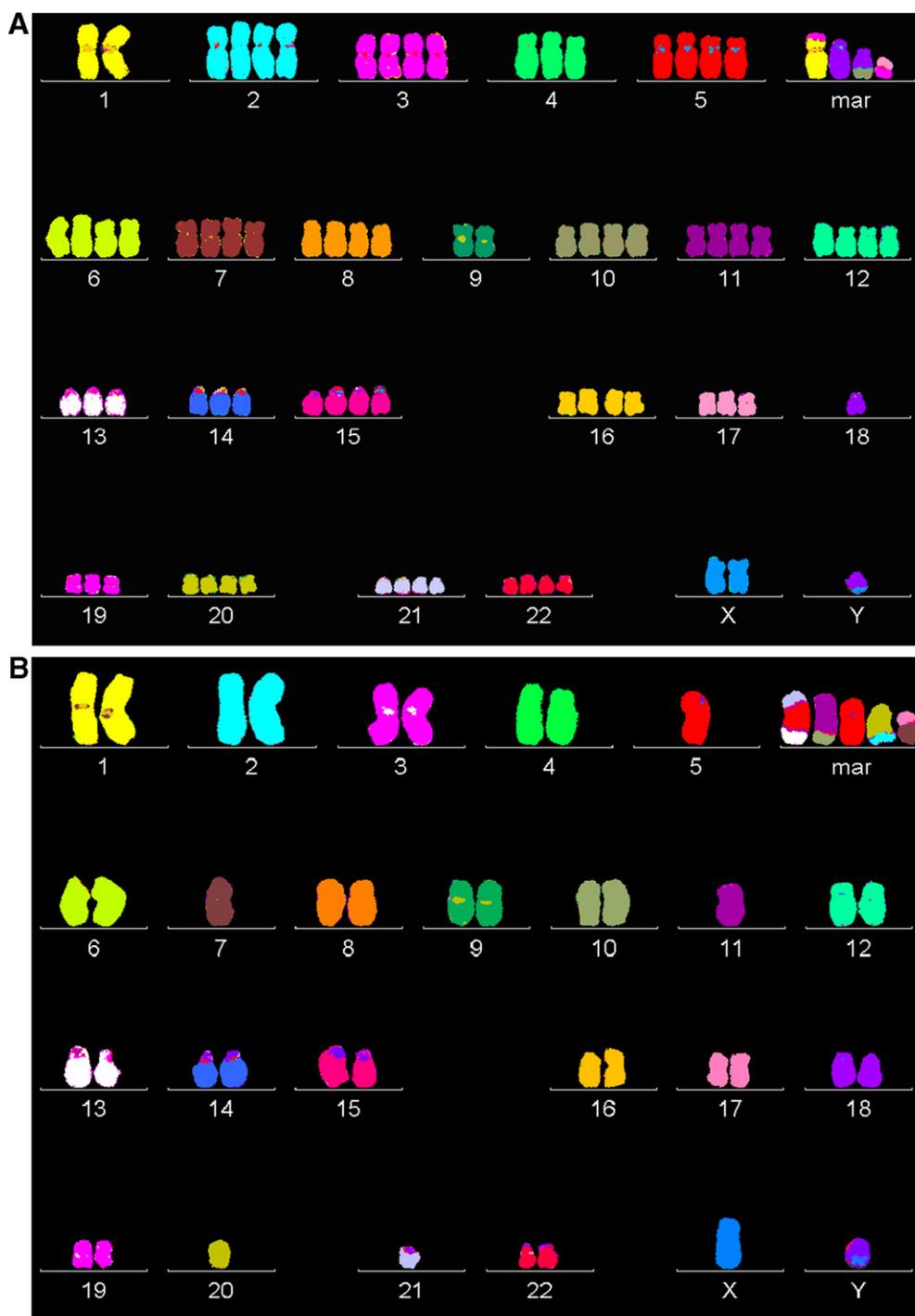
Figure 2 Grossly different forms of chromosomal imbalance without any impact on the malignant phenotype: (A) A representative karyotype of the grossly aneuploid cell line HA1ER (=human embryonic kidney cells transfected with SV40 early region, *hTERT* and *H-ras* [64]), and (B) the pseudo-diploid subclone HA1ER-3 [59]. Both cell lines give rise to similar numbers of colonies in soft agar [59] and result in indistinguishable phenotype and growth characteristics *in vitro*. Although this is a highly artificial setting, the same is true for *in vivo* cancer. Similar histologic subtypes of cancers exhibit different spectrum of genetic aberrations [11]. (Multicolor-FISH was performed according to the supplier's protocol (MetaSystems, Altlussheim, Germany)).

direct effect on the cancer phenotype (Fig. 2A and B) [43,59,60]. Aneuploidy is one of the most common properties of cancers [61], but it has not been entirely clear whether it is essential for tumorigenesis or a coincidence due to oncogene deregulation [62]. In a milestone paper, Weinberg and colleagues claimed that three defined genetic elements (later four [63]) suffice to produce a

human malignant tumor [59,64], providing the ultimate support to the somatic mutation model. However, I received the supposedly diploid cells [59] from Hahn in January 2001 and based on multicolor-FISH analysis I could not find one diploid cell in any of those after only 1 week in culture (Fig 2B) [60]. Identical chromosomal rearrangements were present in a large fraction (up to 89%) of the cells (my unpublished letter to *Cancer Research*), which cannot be explained by the ''outgrow of aneuploid variants'' (authors reply [60]).

Ironically, large-T antigen, one of the 'defined genetic elements' inserted by the Weinberg group, has been known to induce chromosomal instability in human cells [38]. Several years ago, the Bacchetti group actually studied large-T antigen and chromosomal instability in the same embryonic kidney cells (HA1) [38]. Another blow to the 'defined genetic elements' model is that hyperproliferative tumors in transgenic mice (large-T antigen Tet on/off system [65]) can only be switched off at an early stage. After several months of large-T antigen (TAg) activity, tumors became irreversible, although TAg expression was silenced. Most importantly, tumor cells were found to be polyploid [65]. However, a chromosome analysis was not undertaken in this study published in *Science*. The authors concluded that ''…cumulative changes occur…that prohibit reversal…The nature of these changes remains to be identified.'' [65].

Beside the observation that carcinoma cells are always aneuploid (structurally and/or numerically), there are several other unresolved issues regarding the somatic gene mutation theory. Four of them are listed below:

1. As Duesberg pointed out in a letter to *Science*, viral promoters that are used in most current gene transfection experiments result in oncogene expression rates of up to 100 times stronger than cellular promoters [57]. Furthermore, the expression rate of a gene under the control of a viral promoter is stable and cannot be fine-tuned. Hence, the insertion of oncogenes with viral promoters and/or the cultivation of human cells might occasionally result in unexpected effects on carcinogenesis [66], including induction of aneuploidy [43].
2. Somatic gene mutations are thought to largely determine the phenotype and the behavior of cancer cells (genotype—phenotype correlation), but it has been found that tumors have diverse mutational profiles [10,67]. Thousands of mutations per cancer cell [9,62,68] would make histopathology a hopeless endeavor; but this is not the case. Metastatic liver cancer in the lung can be easily recognized by a histopathologist. Hepatocellular carcinoma cells almost always retain some characteristics of normal liver tissue. However 'transdifferentiation' should occur commonly, if the observed ~11,000 mutational hits [9,68] per cancer cell have a direct effect on the cellular phenotype. Yet, precancerous epithelial tissue seemingly dedifferentiates, but it usually does not transdifferentiate.
3. Knudson's two-hit model is based on an inherited mutation or loss of a gene (RB1) causing a rare form of cancer [69]. However, cancer gene mutations in germ cells play only a marginal role in the genesis of common cancers [70], and the vast majority of cancers are sporadic [70]. Yet, if mutations in a defined number of genes cause cancer, and if >1% of all human genes are cancer genes [2,6], inherited forms of epithelial cancers (=carcinomas) should occur commonly in young adults. As has been pointed out by Duesberg, inherited mutations in cancer genes would reduce the number of additional mutational hits required and would significantly reduce the age of onset [56]. However, carcinomas display a characteristic age range with practically no cases arising in young people (Figure 11.1 of Ref. [4]).
4. Cancer incidence increases exponentially beyond the age of 40 (Fig. 3A), and this increase is caused almost exclusively by epithelial cancers. One out of every two men and one out of every three women develop cancer during their lifetime [1]. This is a remarkably efficient pathological mechanism, which is difficult to explain by the requirement of 4—5 collaborating mutations in a cell [4] based on somatic mutation rates in normal cells [8]. Therefore a mutator phenotype was proposed [8]. However, what causes these highly elevated mutation rates in all carcinoma patients remains a mystery.

In an attempt to shed light on these unresolved issues, I present here a causative cascade. Most of the mechanisms in this cascade have been already associated with cancer progression in some way. Yet, the combination and series of events described here is unique and should be considered an important shift in thinking. The challenge was to explain how the (observed) chaotic variety of chromosome and gene aberrations [12] can lead to identical cancer phenotypes.

The Viennese Cascade

The human body undergoes an extensive process of tissue regeneration, replacing many billion cells

each day. The task is accomplished by highly regulated proliferation [71] and serial activation of tissue stem cells [72]. Senescent tissue stem cells are replenished by adult stem cells circulating in the bloodstream and residing in the bone marrow, and potentially other organs [73]. Exhaustion of replicative capacity and a limited supply of fresh stem cells may contribute to tissue aging [74]. Telomerase activity measured in adult stem cells is not sufficient to prevent telomere loss and suggests that cells with insufficient telomere length undergo senescence [74,75]. This situation is in stark contrast to embryonic stem cells that express high levels of telomerase, exhibit remarkable genomic stability, are capable of unlimited self-renewal, and are pluripotent [74]. Under physiological conditions, the differentiation potential of adult stem cells is much more limited [76], being multipotent at most [71]. Consequently, cells give rise to one or multiple cell lineages of one particular tissue only [74,77,78]. Circulating adult stem cells are recruited to a specific organ and reside there as tissue stem cells. Subsequently, committed progenitors are derived from tissue stem cells.

It is proposed here that in the elderly, the number of adult stem cells is already limited, and deregulated tissue stem cells undergo proliferation. These cells are M1-deficient (*e.g.* have deregulated *p53* and *RB1* pathways) and continue to multiply beyond their limit [79], causing critically short telomeres in proliferating progenitor cells. Subsequently, non-functional telomeres lead to chromosome fusion. During cell division, fused chromosomes eventually get pulled to opposite poles (Fig. 1), resulting in anaphase bridges [28], and eventual chromosome breakage [80]. The process starts again with fusion of the broken parts (breakage—fusion—bridge cycle, Fig. 1). Breakage—fusion—bridge cycles lead to unbalanced translocations of chromosome fragments [28,81]. In addition, dysfunctional telomeres result in tetraploidization [82] and losses of whole chromosomes [28]. These structural and numerical chromosome aberrations (=aneuploidy) affect the integrity, location, and copy number of hundreds or thousands of genes.

Cancer cells are often characterized by a hypermethylated state of gene promoters and a decreased global level of DNA methylation compared to normal cells [54,55,83,84]. Global hypomethylation seems to have no direct oncogenic effect [85] and might simply be associated with proliferation state [86]. Promoter hypermethylation is thought to inactivate tumor suppressor genes; but it is not entirely clear why many gene promoters in cancer cells contain hypermethylated

CpG islands (compared to their differentiated counterparts) [87]. The Viennese Cascade provides an explanation for aberrant methylation patterns found in most human cancers [88]. Based on the current view that the differentiation switch modifies expression patterns of several genes on many chromosomes [89], it is proposed here that the intactness, correct location and correct copy number of differentiation-specific genes are critical. Hence, genetic damage caused by chromosomal instability may impair epigenetic modifications of differentiation-specific genes (Fig. 1). Due to extensive aneuploidy, the demethylation machinery cannot activate tissue-specific patterns of gene expression and the committed progenitor cell remains in its current differentiation state, resulting in epigenetic maturation arrest. Although my proposed mechanism focuses mainly on methylated DNA modifications, the perturbation of histone methylation/acetylation and other yet unknown epigenetic mechanisms that are involved in differentiation may also be causative.

Depending on the pattern and the extent of aneuploidy, immature cells might be blocked at different developmental stages, causing variable degrees of malignancy. Accordingly, it has been shown that increasing aneuploidy is mirrored by morphological dedifferentiation [90]. For example, advanced epithelial cancer is characterized by an immature phenotype and severe aneuploidy [47]. It is an essential part of the hypothesis that different aneuploidy/mutation patterns give rise to identical phenotypes, because differentiation depends on the epigenetic modification of differentiation genes, whereas alterations in all other genes would have no direct effect on the maturation arrest. Therefore, different patterns of chromosomal and gene aberrations that have been found in identical histologic subtypes of human tumors can lead to identical cancer phenotypes. In addition, a particular combination of genomic alterations may cause transformation of a particular tissue or cell type only, since differentiation-specific genes are tissue-specific [89].

A critical step in the cascade involves telomerase reactivation. Increased telomerase activity stabilizes short telomeres, leading to decreased chromosomal instability in cancer cells [36]. It is tempting to suggest that the proliferation of stem cells with unstable chromosomes begets high levels of telomerase activity. Telomerase activation may represent an attempt to heal stem cells with unstable chromosomes. Chromosome healing has been described to be restricted to the embryonic development stages in maize [91], which might explain why high levels of telomerase expression occur in

'embryonic-like' cancer stem cells. Unfortunately, the rescue mechanism sometimes fails and produces immortal 'aneuploid monster cells'. Accordingly, clinical elevation of telomerase activity positively correlates with pathological stage and poor prognosis in many cancer patients [92].

Metastases recapitulate the organization of their primary tumors [93], and the probability of metastasis suggests that nongenetic mechanisms are involved [94]. My proposed model is not based on mutated metastasis genes [95], since migration is inherent to normal adult stem cells [96]. Several studies have suggested that tumor cell migration and metastasis are a consequence of cancer stem cells responding to chemokines from other tissues [97−99], as normal adult stem cells do [78,97,100].

Despite the similarities between cancer stem cells and normal adult stem cells, there are three fundamental differences:

1. The progeny of cancer stem cells cannot fully differentiate. Although, in most cancers differentiation occurs in a fraction of cells and tumor tissue is characterized by a varying mixture of partly- and fully-differentiated cells with some characteristics of the tissue of origin. The lack of cellular differentiation is accompanied by cytological evidence of short telomeres, chromosomal instability and aneuploidy, including anaphase bridges and nuclear abnormalities.
2. Cancer progenitor cells usually do not respond to signals to stop proliferation. This might be a direct consequence of maturation arrest, or a consequence of tissue cells that continue to send regeneration signals (=lack of negative feedback due to the production of undifferentiated progeny). The role of oncogene activity and impaired intercellular communication might also play a role.
3. Cancer stem cells can migrate to and grow in the wrong organs, resulting in metastasis.

The metastatic potential of a tumor may depend on the nature of the maturation arrest, which itself is determined by the pattern and amount of genetic damage, as well as the tissue type. It has been proposed by others that tumors derived from early tissue stem cells have an increased tendency to metastasize, whereas a maturation arrest of committed progenitor cells may only result in local invasive growth [96]. According to my model, maturation arrest is neither 100% effective nor is it fixed, but rather is seen as a continuous dynamic state, where a fraction of cells is able to escape and differentiate.

Discussion

The Viennese Cascade combines genetic and epigenetic alterations of cancer cells in one causative cascade (Fig. 1) and offers an explanation for how different patterns of gene and chromosomal aberrations can lead to identical histologic phenotypes. Several steps in this cascade have already been investigated by numerous leaders in the field. Duesberg resurrected the aneuploidy-cancer-theory [57], and subsequent papers dealt with the direct effect of aneuploidy on the cancer phenotype [101]. It has been recently proposed by Vogelstein and others that chromosomal instability (CIN) can initiate tumorigenesis [46,61]. DePinho developed a theory of carcinogenesis based on telomere erosion and chromosomal instability, in which transformation and metastasis are a consequence of additional gene-specific mutations [36]. DePinho's model does not include the cancer stem cell concept or the differentiation block. Pathak proposed that a telomere erosion/aneuploidy/transformation sequence occurs in tissue-specific stem cells [102,103]. However Pathak's theoretical work does not provide a mechanism to explain how aneuploidy leads to stem cell transformation, it does not include epigenetic aberrations, and metastasis is seen as a consequence of a series of genetic mutations and amplifications of telomeric DNA [103]. Matzke and coworkers showed that trisomy in plants can lead to increased DNA methylation in regulatory regions and hypothesized that aneuploidy predisposes DNA to epigenetic modifications [104]. Yet, the Viennese Cascade proposes that aneuploidy results in aberrant methylation patterns because epigenetic modifications that are essential for normal tissue differentiation cannot be performed.

Supporters of the somatic mutation cancer theory might argue that there is no need for an alternative model. However, the facts remain that the age-adjusted cancer mortality rate has remained relatively stable in the last half century and we have yet to fulfill the expectations for a new era of molecularly-based cancer research and therapy [2]. Indeed, the utility of any individual gene therapeutic approach may be negligible in light of the ever-growing perplexing diversity of cancer-causing gene mutations [12]. The fact that every tumor harbors its own unique set of cancer gene mutations [10,12] is a staggering scenario for molecular geneticists. Currently, there are 23,544 somatic mutations reported in ∼2000 original publications with respect to the *p53* tumor suppressor gene alone (http://www-p53.iarc.fr/Statistics.html). A recent sequencing study, lead by Vogelstein, found that 1718 genes,

representing 9.4% of all known coding genes in the human genome, had at least one non-silent mutation in either a breast or colorectal cancer (out of 11 breast and 11 colorectal tumor samples). On average, the number of non-silent gene mutations per tumor was shown to be between 77 and 101 [12]. Accordingly, the authors concluded that the genomic landscapes of (breast and colorectal) cancers are composed of a handful of commonly mutated gene ''mountains'' and a much larger number of gene ''hills'' that are mutated at low frequency. The Viennese Cascade should be considered an important shift in thinking as it can explain how this staggering variety of ''hills'', representing different chromosome and gene aberration patterns, results in identical cancer phenotypes.

The current scientific knowledge of carcinogenesis is extensive, and any new theories must explain experimental results and cancer-related phenomena better than the current somatic gene mutation model does. This is outlined in the following sections.

Oncogenes and tumor suppressor genes

The role of somatic gene mutation in carcinogenesis is a subject of debate [62]. Oncogenes are usually activated by gene mutations, gene amplifications or balanced chromosomal aberrations. Tumor suppressor genes are deactivated by gene mutations or unbalanced chromosomal aberrations such as deletions (loss of heterozygosity). In a recent issue of *PNAS*, the article by Feng et al. [105] provides compelling evidence that tumor suppressors, like *p53*, remain structurally intact but decline in functional activities during aging. Hence, it is tempting to suggest that nonmutational declines in tumor suppressor functions might be intrinsic to adult stem cells at old age. It has been shown that normal human mammary epithelial cells spontaneously escape senescence and acquire genomic changes [79]. Indeed, the prevailing characteristic of epithelial carcinogenesis is chromosomal instability [106]. In accordance with the Viennese Cascade, the aberrant activity of oncogenes and tumor suppressor genes might either increase the proliferation rate of a particular tissue or the proliferation potential of a particular stem cell (by inactivating M1), and eventually drive stem cells into telomere crisis. However, most of the gene mutations found in human tumors [12] might be a consequence of increased proliferation rates and decreased repair activity in transcriptional silent genes [107]. It has been proposed that gene mutations in somatic cells would preferentially occur in already highly methylated regions [107].

Benign tumors — a different entity

According to the somatic gene mutation model, every benign tumor represents an early stage of cancer, since those cells already harbor proliferation-inducing mutations. Several cycles of proliferation and selection should increase the occurrence of malignant cell formation. However, this is not the case, and many types of benign tumors never undergo malignant transformation.

Benign adult tumor cells are characterized by diploid or near-diploid karyotypes [48], intact replicative limits (M1) [108] and functional telomeres [35]. Telomerase activity is absent or low in benign adult tumors [109,110]. Benign tumor cells are capable of differentiation, do not grow invasively, do not metastasize and do not show any signs of chromosomal instability (*e.g.* anaphase bridges or nuclear anomalies) [35]. This type of benign tumor rarely transforms into malignant cancer. Accordingly, early human colon adenomas are diploid [111] and the large majority of polyps never become malignant. Dysplastic lesions are another group of putative benign tumors, which represent the initial stage of premalignant growth. Aneuploidy has been reported in dysplastic lesions [112—114], and cells display variability in nuclear size and shape, increased nucleus-to-cytoplasm size ratio, increased mitotic activity, and lack of the cytoplasmic features associated with differentiation. Furthermore, the relative numbers of the variety of cell types seen in normal tissue are no longer observed [4]. Cells of dysplastic lesions and carcinoma *in situ* do not penetrate the basement membrane. Interestingly, the level of dysplasia correlates directly with polyp size [115]. Dysplastic adenomas of the colon are not clonal [116,117] and greater genetic clonal diversity is associated with higher risks for transformation [118,119]. In accordance with my model, advanced chromosomal instability produces cells with a variety of aneuploidy patterns, increasing the probability of maturation arrest.

It is known that the heterogeneity characteristic of early colon carcinomas is lost in late-stage cancer [120], which in my view is suggestive of the emergence of an immortal clone with active telomerase. In conclusion, benign tumors are the local result of excessive proliferation of diploid, mortal stem cells, whereas dysplasia and carcinoma *in situ* represent initial growths of M1-deficient, aneuploid stem cells.

Cell fusion experiments and their unexpected results

In a number of experiments, when tumor cells were fused with normal cells, the resulting tetra-ploid cell hybrids lost the ability to form tumors [4]. These unexpected results led to the concept of tumor suppressor genes. Inactivating mutations in these genes seem to be recessive in the presence of an intact, wild-type allele. The introduction of wild-type alleles during cell fusion was thought to directly suppress the tumor phenotype. However, an alternative explanation may be that the new set of intact chromosomes from the normal cell can be targeted by the differentiation machinery, and promoters of differentiation-specific genes can be demethylated. Once the genes are switched on, the (hybrid) cell differentiates and loses its stem cell behavior and ability to form tumors. Since cell hybrids are not stable and are known to lose chromosomes, the reoccurrence of malignancy after several rounds of multiplication [121] can be easily explained by the Viennese Cascade.

The phenomenon of multifocality

The concept of field cancerization [122] refers to the frequent presence of premalignant, genetically altered, epithelial cells in the surrounding mucosa of a carcinoma [114]. Fields with dimensions of >7 cm in diameter [123] containing multiple pre-cancerous lesions have been detected in the muco-sa of e.g. the esophagus. Cytogenetic studies reveal that most bilateral breast carcinomas arise independently [124]. The same phenomenon has been described for thyroid cancer: individual tumor foci in patients with multifocal papillary thyroid cancer often arise as independent tumors [125]. Hence, these clinical observations do not support the argument of somatic mutation very well, since the probability of acquiring 3—6 cancer mutations (the original Vogelstein model) [126] in several distinct somatic cells is very, very low. A mutator phenotype [8] might lead to a chaotic mixture of mutations, but cancer-promoting mutations would also be neutralized by cancer-inhibiting mutations. Most importantly, these proposed mechanisms do not reflect the current situation where every second man and every third woman are developing cancer [1]. Yet, tumor multifocality can be easily explained by tissue exhaustion and multiple recruitment of aneuploid stem cells.

Tissue exhaustion as a consequence of aging and chronic wounds

Aging is the most prevalent risk factor for cancer. In accordance with my proposed theory, the aging phenotype is largely a result of replicative senescence and generalized tissue exhaustion. A shortage of stem cells in many aged tissues leads to the recruitment of M1-deficient and aneuploid stem cells, and would explain the exponential increase of cancer incidence in the elderly.

| Table 1	Hallmarks of epithelial cancer and proposed causative mechanisms
Chromosomal instability and aneuploidy	M1-deficiency and extended proliferation of cells with critically short telomeres leads to breakage—fusion—bridge cycles
Limitless replicative potential	Telomerase up regulation in aneuploid cancer stem cells
Uncontrolled growth	Direct consequence of maturation arrest and/or tissue regeneration cannot be completed due to the production of undifferentiated, non-functional cells, eliminating negative feedback in cancer stem cells
Immature phenotype	Aneuploidy inhibits epigenetic modifications of differentiation-specific genes
Sustained angiogenesis	Proliferation of cancer stem cells, like normal stem cells, activates a pro-angiogenic switch in the surrounding connective tissue
Tissue invasion	Cancer stem cells migrate like normal adult stem cells do
Metastasis	Cancer stem cells respond to regeneration signals (i.e. chemokines) from other organs
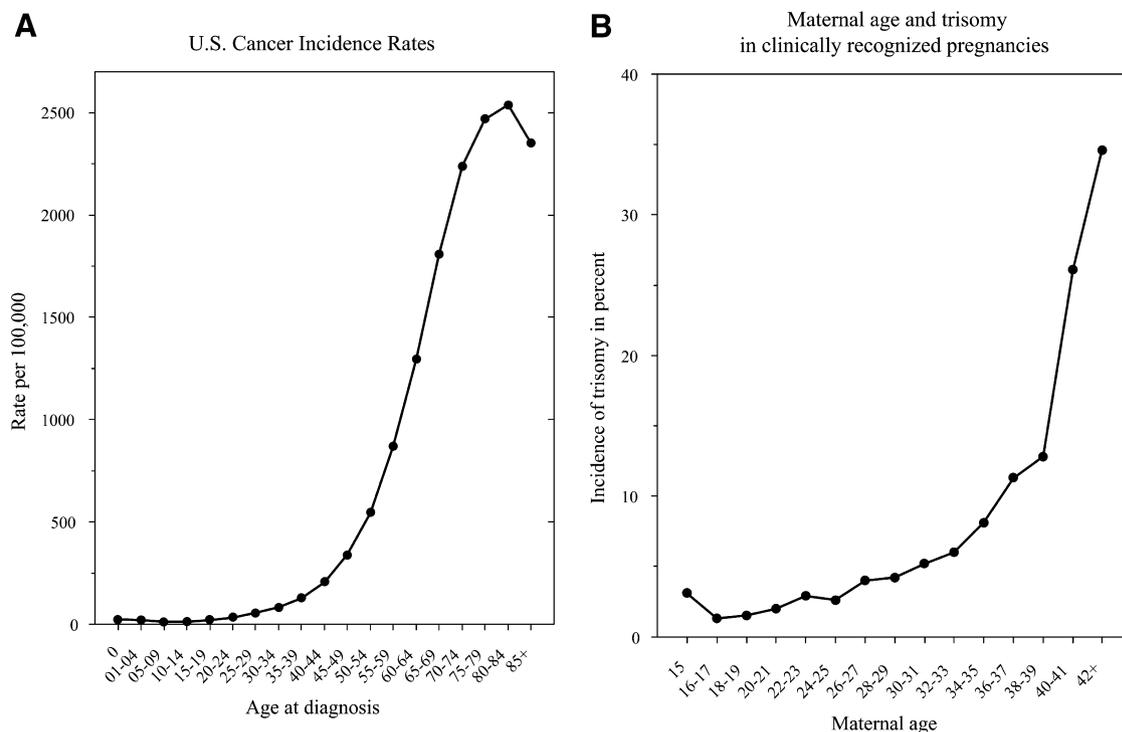
Figure 3 Age-association of cancer and aneuploidy syndromes. (A) Age is the most prevalent risk factor for cancer. Cancer incidence rates increase steadily with advancing age (source: SEER1992-2002). Most intriguingly, there is an exponential growth in incidence rates leading to an explosion in absolute numbers of newly diagnosed cancers beyond the age of 40 (in both sexes). (B) Incidence rates of trisomic pregnancies (aneuploidy syndromes, *e.g.* Morbus Down) increase exponentially starting at maternal age of 35 (data taken from Ref. [129]), known as the maternal-age effect. The similarity between both incidences is striking. By definition, aneuploidy syndromes are caused by chromosomal imbalance and it has been shown that the majority of oocytes in women aged 40 years or older are aneuploid [129–131]. I propose a similar age-association of aneuploidy in tissue stem cells.

Chronic injury can be associated with an elevated cancer risk, as in the case of acid reflux disease and esophageal adenocarcinoma [127]. Dvorak described cancer as wounds that do not heal [128]. Others have speculated that cancer represents the continuous and unregulated state of tissue repair [127]. According to the Viennese Cascade, chronic wounds might eventually lead to tissue exhaustion and tissue stem cells proliferating beyond their limit (Table 1).

Age-association of cancer and aneuploidy syndromes

As proposed, cancer initiation starts with the activation of M1-deficient tissue stem cells relatively late in life, with the exception of local tissue exhaustion (*e.g.* smoking, alcoholism, chronic inflammation). A stem cell hierarchy may exist, where healthy, intact cells are utilized first, giving way to genetically-damaged cells. This may explain the exponential increase of cancer observed in middle-aged humans (Fig. 3A). Another pathological condition based on aneuploidy displays similar kinetics (Fig. 3B). The incidence of Down syndrome births and other aneuploidy syndromes increases exponentially with the mother's age, starting at middle age [129]. Oogenesis may represent the ultimate model of tissue regeneration. A defined number of oocytes are produced in the fetal ovaries and the eggs are activated in a serial manner, like adult tissue stem cells. It has been shown that the majority of oocytes in women 40 years or older are aneuploid [129–131]. In a parallel manner, an accumulation of M1-deficient and aneuploid stem cells may contribute to age-dependent increase of cancer incidence.

Resistance to therapy

Chemoresistance is considered to be an acquired feature of cancer cells. Numerous mutations in 'chemoresistance genes' have been described [132], even against the new-age drug Gleevec [133]. Surprisingly, chemoresistance is an inherent feature of normal adult stem cells [134–136]. Protection of the most vital, regeneration-competent

cells by specific mechanisms such as transmembrane pumps is rational, and may explain the resistance of cancer stem cells to therapies [135]. Chromosomal instability, gene amplification and gene mutation can be involved in the development of chemoresistance, but may play only a marginal role in epithelial cancers. For example, if chemotherapy selects for a small number of cancer cells that contain a 'resistance mutation', then tumors would shrink by 99.9% and reappear. However in most cases, carcinomas shrink partially, and remaining cells are resistant to the applied drug, and most other drugs [135]. This observation is incompatible with the mutation-selection model, but is compatible with the concept of cancer stem cells. Accordingly, malignant tumors consist of a small number of cancer stem cells, a fraction of proliferating cells and a fraction of differentiated cells. Cytotoxic chemotherapy just hits the amplifying cells, but after the first round of therapy, these cells eventually get replenished by cancer stem cells.

Implications of the Viennese Cascade for research, prevention and therapy

A side effect of current chemotherapy regimens is the destruction of cycling healthy progenitor cells, which accelerates tissue aging. It is not surprising that chronic health problems of childhood cancer survivors are therapy-related [137]. Yet, chemotherapy usually spares dormant tissue stem cells. The common side effect of hair loss after chemotherapy is a result of therapy-related toxicity to cycling progenitor cells of the hair bulb. Once the cytotoxic treatment stops the remaining dormant stem cells get activated and the hair regrows. Analogously, cancer regrows from dormant cancer stem cells [135]. A thorough understanding of stem cell dynamics and regeneration mechanisms will help pave the way for novel and effective cancer treatments. Whereas, molecular targeted therapy is doomed to fail, since the hallmark of cancer cells is genetic instability, which produces just too many 'targets'.

A potential curative strategy may be to force malignant cells to differentiate, and lose their ability to migrate and proliferate. The molecular machinery behind the differentiation switch [89] needs to be explored further. Drugs that induce global DNA demethylation have been tested with various successes [55,87]. Undifferentiated cancer cells seem to be in a steady state of tension, and some of them undergo apoptosis while others eventually differentiate. Accordingly, a biochemical ''push'' may have beneficial effects. The

well-known example of retinoic acid-induced differentiation therapy for acute promyelocytic leukemia might serve as a model for future therapies [23]. In the famous Mintz–Illmensee experiments, teratocarcinoma cells participated in the normal development of a mouse [138]. Other researchers tried to reproduce the results and did not succeed. The cells Illmensee used in the experiments were near-diploid [138,139], whereas other researchers might have experimented with grossly aneuploid ones. Nevertheless, the embryonic environment seems to be beneficial for the differentiation process [140] and yet unknown embryonic factors might lead to the development of new drugs.

One obvious option to prevent cancer would be to lengthen telomeres in healthy diploid stem cells, so that their increased replicative potential ensures lifelong tissue regeneration. For decades, doctors have been telling us that a change in lifestyle is the most promising prevention strategy. According to the model presented here, they might be right, since persistence of particular unhealthful habits (i.e. smoking, heavy drinking) may exhaust local tissues and accelerated tissue aging of afflicted tissues (i.e. lung, liver, respectively). Accordingly, a switch from chain-smoking to alcoholism may be better than continuation of either habit. Once exhausted, tissue stem cells must be replenished, and the remaining adult stem cells are likely to be limited in numbers and in their replicative capacity.

In my view, resistance to cancer may depend on the regenerative capacity of a particular tissue [141], based on numbers of recruitable stem cells and average telomere length. Both telomere length and stem cell capacity seem to be heritable traits. Future technologies might enable us to predict the remaining regenerative potential of an organ and the probability of malignant transformation.

Conclusion

The proposed key steps of carcinogenesis are exhausted tissues, M1-deficient tissue stem cells, a telomere crisis, chromosomal instability and aneuploidy that finally leads to the perturbation of epigenetic differentiation mechanisms. It is an essential part of the Viennese Cascade that the cancer phenotype is caused by the inaccessibility of genes involved in tissue-specific differentiation, rather than the direct effect of mutated oncogenes. Future experiments to evaluate my proposed model should involve experimental investigation of two

crucial components of the theory: that advanced aneuploidy disturbs epigenetic modifications of genomic DNA, and that differentiation-specific genes are silenced in aneuploid cancer cells. If it is experimentally verified, the Viennese Cascade may end the debate on if and how 'tumor-unspecific' aneuploidy leads to cancer [62].

Acknowledgements

References

[1] Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, et al. Cancer statistics, 2005. CA Cancer J Clin 2005;55:10–30.

[2] Varmus H. The new era in cancer research. Science 2006;312:1162–5.

[3] Kiberstis PA, Travis J. Celebrating a glass half-full. Science 2006;312:1157.

[4] Weinberg RA. The biology of cancer. New York: Garland Science, Taylor & Francis Group; 2006.

[5] Heim S, Mitelman F. Cancer cytogenetics. New York: Wiley-Liss; 1995.

[6] Futreal PA, Coin L, Marshall M, Down T, Hubbard T, Wooster R, et al. A census of human cancer genes. Nat Rev Cancer 2004;4:177–83.

[7] Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. Nature 1998;396:643–9.

[8] Loeb LA. A mutator phenotype in cancer. Cancer Res 2001;61:3230–9.

[9] Stoler DL, Chen N, Basik M, Kahlenberg MS, Rodriguez-Bigas MA, Petrelli NJ, et al. The onset and extent of genomic instability in sporadic colorectal tumor progression. Proc Natl Acad Sci USA 1999;96:15121–6.

[10] Kerangueven F, Noguchi T, Coulier F, Allione F, Wargniez V, Simony-Lafontaine J, et al. Genome-wide search for loss of heterozygosity shows extensive genetic diversity of human breast carcinomas. Cancer Res 1997;57:5469–74.

[11] Thiagalingam S. A cascade of modules of a network defines cancer progression. Cancer Res 2006;66:7379–85.

[12] Wood LD, Parsons DW, Jones S, Lin J, Sjoblom T, Leary RJ, et al. The genomic landscapes of human breast and colorectal cancers. Science 2007;318:1108–13.

[13] Klein C, Blankenstein T, Schmidt-Kittler O, Petronio M, Polzer B, Stoecklein N, et al. Genetic heterogeneity of single disseminated tumour cells in minimal residual cancer. Lancet 2002;360:683.

[14] Cohnheim J. Ueber Entzuendung und Eiterung. Virchows Arch Pathol Anat Physiol 1867;40:1–79.

[15] Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:41–9.

[16] Herzog EL, Chai L, Krause DS. Plasticity of marrow-derived stem cells. Blood 2003;102:3483–93.

[17] Grove JE, Bruscia E, Krause DS. Plasticity of bone marrow-derived stem cells. Stem Cells 2004;22:487–500.

[18] Wagers AJ, Sherwood RI, Christensen JL, Weissman IL. Little evidence for developmental plasticity of adult hematopoietic stem cells. Science 2002;297:2256–9.

[19] Stenback F, Peto R, Shubik P. Initiation and promotion at different ages and doses in 2200 mice. I. Methods, and the apparent persistence of initiated cells. Br J Cancer 1981;44:1–14.

[20] Alison MR, Lovell MJ, Direkze NC, Wright NA, Poulsom R. Stem cell plasticity and tumour formation. Eur J Cancer 2006;42:1247–56.

[21] Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001;414:105–11.

[22] Mackenzie IC. Stem cell properties and epithelial malignancies. Eur J Cancer 2006;42:1204–12.

[23] Sell S. Stem cell origin of cancer and differentiation therapy. Crit Rev Oncol Hematol 2004;51:1–28.

[24] Pardal R, Clarke MF, Morrison SJ. Applying the principles of stem-cell biology to cancer. Nat Rev Cancer 2003;3:895–902.

[25] Till JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res 1961;14:213–22.

[26] Wicha MS, Liu S, Dontu G. Cancer stem cells: an old idea—a paradigm shift. Cancer Res 2006;66:1883–90. discussion 1895–6.

[27] Pierce GB, Johnson LD. Differentiation and cancer. In Vitro 1971;7:140–5.

[28] Stewenius Y, Gorunova L, Jonson T, Larsson N, Hoglund M, Mandahl N, et al. Structural and numerical chromosome changes in colon cancer develop through telomere-mediated anaphase bridges, not through mitotic multipolarity. Proc Natl Acad Sci USA 2005;102:5541–6.

[29] McClintock B. The significance of responses of the genome to challenge. Science 1984;226:792–801.

[30] Hayflick L, Moorhead PS. Serial cultivation of human diploid cell strains. Experimental Cell Research 1961;25:585–621.

[31] Olovnikov AM. Principle of marginotomy in template synthesis of polynucleotides. Dokl Akad Nauk Sssr+ 1971;201:1496–8.

[32] Olovnikov AM. Telomeres, telomerase, and aging: origin of the theory. Exp Gerontol 1996;31:443–8.

[33] Harley CB. Telomere loss: mitotic clock or genetic time bomb? Mutat Res 1991;256:271–82.

[34] Griffith JD, Comeau L, Rosenfield S, Stansel RM, Bianchi A, Moss H, et al. Mammalian telomeres end in a large duplex loop. Cell 1999;97:503–14.

[35] DePinho RA, Polyak K. Cancer chromosomes in crisis. Nat Genet 2004;36:932–4.

[36] DePinho RA. The age of cancer. Nature 2000;408:248–54.

[37] Counter CM, Avilion AA, LeFeuvre CE, Stewart NG, Greider CW, Harley CB, et al. Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. Embo J 1992;11:1921–9.

[38] Stewart N, Bacchetti S. Expression of SV40 large T antigen, but not small t antigen, is required for the induction of chromosomal aberrations in transformed human cells. Virology 1991;180:49–57.

[39] von Hansemann D. Ueber asymmetrische Zellteilung in Epithelkrebsen und deren biologische Bedeutung. Virchows Arch Pathol Anat Physiol 1890;119:299–326.

[40] Boveri T. Zur Frage der Entstehung Maligner Tumoren. Jena: Gustav Fischer; 1914.

[41] Balmain A. Cancer genetics: from Boveri and Mendel to microarrays. Nat Rev Cancer 2001;1:77—82.

[42] Li R, Yerganian G, Duesberg P, Kraemer A, Willer A, Rausch C, et al. Aneuploidy correlated 100% with chemical transformation of Chinese hamster cells. Proc Natl Acad Sci USA 1997;94:14506—11.

[43] Li R, Sonik A, Stindl R, Rasnick D, Duesberg P. Aneuploidy vs. gene mutation hypothesis of cancer: Recent study claims mutation but is found to support aneuploidy. Proc Natl Acad Sci USA 2000;97:3236—41.

[44] Mitelman F, Mertens F, Johansson B. Prevalence estimates of recurrent balanced cytogenetic aberrations and gene fusions in unselected patients with neoplastic disorders. Genes Chromosomes Cancer 2005;43:350—66.

[45] Shih IM, Zhou W, Goodman SN, Lengauer C, Kinzler KW, Vogelstein B. Evidence that genetic instability occurs at an early stage of colorectal tumorigenesis. Cancer Res 2001;61:818—22.

[46] Michor F, Iwasa Y, Vogelstein B, Lengauer C, Nowak MA. Can chromosomal instability initiate tumorigenesis? Semin Cancer Biol 2005;15:43—9.

[47] Ried T, Heselmeyer-Haddad K, Blegen H, Schrock E, Auer G. Genomic changes defining the genesis, progression, and malignancy potential in solid human tumors: a phenotype/genotype correlation. Genes Chromosomes Cancer 1999;25:195—204.

[48] Buchner T, Hiddemann W, Wormann B, Kleinemeier B, Schumann J, Gohde W, et al. Differential pattern of DNA-aneuploidy in human malignancies. Pathol Res Pract 1985;179:310—7.

[49] Wolman SR. Karyotypic progression in human tumors. Cancer Metastasis Rev 1983;2:257—93.

[50] Rubio D, Garcia-Castro J, Martin MC, de la Fuente R, Cigudosa JC, Lloyd AC, et al. Spontaneous human adult stem cell transformation. Cancer Res 2005;65:3035—9.

[51] Ehrlich M. Cancer-linked DNA hypomethylation and its relationship to hypermethylation. Curr Top Microbiol Immunol 2006;310:251—74.

[52] Hashimshony T, Zhang J, Keshet I, Bustin M, Cedar H. The role of DNA methylation in setting up chromatin structure during development. Nat Genet 2003;34:187—92.

[53] Brandeis M, Ariel M, Cedar H. Dynamics of DNA methylation during development. Bioessays 1993;15:709—13.

[54] Jones PA, Laird PW. Cancer epigenetics comes of age. Nat Genet 1999;21:163—7.

[55] Gilbert J, Gore SD, Herman JG, Carducci MA. The clinical application of targeting cancer through histone acetylation and hypomethylation. Clin Cancer Res 2004;10:4589—96.

[56] Duesberg P, Li R, Fabarius A, Hehlmann R. The chromosomal basis of cancer. Cell Oncol 2005;27:293—318.

[57] Duesberg PH. Oncogenes and cancer. Science 1995;267:1407—8.

[58] Duesberg P. Chromosomal chaos and cancer. Sci Am 2007;296:52—9.

[59] Zimonjic D, Brooks MW, Popescu N, Weinberg RA, Hahn WC. Derivation of human tumor cells in vitro without widespread genomic instability. Cancer Res 2001;61:8838—44.

[60] Li R, Rasnick D, Duesberg P. Correspondence re: D. Zimonjic et al., Derivation of human tumor cells in vitro without widespread genomic instability. Cancer Res., 61: 8838—8844, 2001. Cancer Res 2002;62:6345—8. author reply 6348—9.

[61] Rajagopalan H, Lengauer C. Aneuploidy and cancer. Nature 2004;432:338—41.

[62] Marx J. Debate surges over the origins of genomic defects in cancer. Science 2002;297:544—6.

[63] Hahn WC, Dessain SK, Brooks MW, King JE, Elenbaas B, Sabatini DM, et al. Enumeration of the simian virus 40 early region elements necessary for human cell transformation. Mol Cell Biol 2002;22:2111—23.

[64] Hahn WC, Counter CM, Lundberg AS, Beijersbergen RL, Brooks MW, Weinberg RA. Creation of human tumour cells with defined genetic elements. Nature 1999;400:464—8.

[65] Ewald D, Li M, Efrat S, Auer G, Wall RJ, Furth PA, et al. Time-sensitive reversal of hyperplasia in transgenic mice expressing SV40 T antigen. Science 1996;273:1384—6.

[66] Check E. Gene-therapy trials to restart following cancer risk review. Nature 2005;434:127.

[67] Hahn WC, Weinberg RA. Rules for making human tumor cells. N Engl J Med 2002;347:1593—603.

[68] Boland CR, Ricciardiello L. How many mutations does it take to make a tumor? Proc Natl Acad Sci USA 1999;96:14675—7.

[69] Nowell PC, Rowley JD, Knudson Jr AG. Cancer genetics, cytogenetics—defining the enemy within. Nat Med 1998;4:1107—11.

[70] Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, et al. Environmental and heritable factors in the causation of cancer — analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 2000;343:78—85.

[71] Bryder D, Rossi DJ, Weissman IL. Hematopoietic stem cells: the paradigmatic tissue-specific stem cell. Am J Pathol 2006;169:338—46.

[72] Kim KM, Shibata D. Methylation reveals a niche: stem cell succession in human colon crypts. Oncogene 2002;21:5441—9.

[73] Borue X, Lee S, Grove J, Herzog EL, Harris R, Diflo T, et al. Bone marrow-derived cells contribute to epithelial engraftment during wound healing. Am J Pathol 2004;165:1767—72.

[74] Krtolica A. Stem cell: balancing aging and cancer. Int J Biochem Cell Biol 2005;37:935—41.

[75] Vaziri H, Dragowska W, Allsopp RC, Thomas TE, Harley CB, Lansdorp PM. Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. Proc Natl Acad Sci USA 1994;91:9857—60.

[76] Vickers MA, Canning SJ, Craig WL, Masson NM, Wilson IJ. X inactivation patterns of closely, but not distantly, related cells are highly correlated: little evidence for stem cell plasticity in normal females. Stem Cells 2006;24:2398—405.

[77] Slack JM. Stem cells in epithelial tissues. Science 2000;287:1431—3.

[78] Kucia M, Ratajczak J, Ratajczak MZ. Are bone marrow stem cells plastic or heterogenous — that is the question. Exp Hematol 2005;33:613—23.

[79] Romanov SR, Kozakiewicz BK, Holst CR, Stampfer MR, Haupt LM, Tlsty TD. Normal human mammary epithelial cells spontaneously escape senescence and acquire genomic changes. Nature 2001;409:633—7.

[80] Gisselsson D, Pettersson L, Hoglund M, Heidenblad M, Gorunova L, Wiegant J, et al. Chromosomal breakage—fusion—bridge events cause genetic intratumor heterogeneity. Proc Natl Acad Sci USA 2000;97:5357—62.

[81] Artandi SE, Chang S, Lee SL, Alson S, Gottlieb GJ, Chin L, et al. Telomere dysfunction promotes non-reciprocal

translocations and epithelial cancers in mice. Nature 2000;406:641–5.

[82] Gisselsson D. Chromosome instability in cancer: how, when, and why? Adv Cancer Res 2003;87:1–29.

[83] Issa JP, Aging. DNA methylation and cancer. Crit Rev Oncol Hematol 1999;32:31–43.

[84] Lengauer C, Kinzler KW, Vogelstein B. DNA methylation and genetic instability in colorectal cancer cells. Proc Natl Acad Sci USA 1997;94:2545–50.

[85] Laird PW, Jackson-Grusby L, Fazeli A, Dickinson SL, Jung WE, Li E, et al. Suppression of intestinal neoplasia by DNA hypomethylation. Cell 1995;81:197–205.

[86] Rojas MV, Galanti N. Relationship between DNA methylation and cell proliferation in Trypanosoma cruzi. FEBS Lett 1991;295:31–4.

[87] Nephew KP, Huang TH. Epigenetic gene silencing in cancer initiation and progression. Cancer Lett 2003;190: 125–33.

[88] Ongenaert M, Neste LV, Meyer TD, Menschaert G, Bekaert S, Criekinge WV. PubMeth: a cancer methylation database combining text-mining and expert annotation. Nucleic Acids Res 2008;36:D842–6.

[89] Xia K, Xue H, Dong D, Zhu S, Wang J, Zhang Q, et al. Identification of the proliferation/differentiation switch in the cellular network of multicellular organisms. PLoS Comput Biol 2006;2:e145.

[90] Wilkens L, Flemming P, Gebel M, Bleck J, Terkamp C, Wingen L, et al. Induction of aneuploidy by increasing chromosomal instability during dedifferentiation of hepatocellular carcinoma. Proc Natl Acad Sci USA 2004;101: 1309–14.

[91] McClintock B. The behavior in successive nuclear divisions of a chromosome broken at meiosis. Proc Natl Acad Sci USA 1939;25:405–16.

[92] Chen KY, Lee LN, Yu CJ, Lee YC, Kuo SH, Yang PC. Elevation of telomerase activity positively correlates to poor prognosis of patients with non-small cell lung cancer. Cancer Lett 2006;240:148–56.

[93] Brabletz T, Jung A, Spaderna S, Hlubek F, Kirchner T. Opinion: migrating cancer stem cells – an integrated concept of malignant tumour progression. Nat Rev Cancer 2005;5:744–9.

[94] Michaelson JS, Cheongsiatmoy JA, Dewey F, Silverstein MJ, Sgroi D, Smith B, et al. Spread of human cancer cells occurs with probabilities indicative of a nongenetic mechanism. Br J Cancer 2005;93:1244–9.

[95] Woodhouse EC, Chuaqui RF, Liotta LA. General mechanisms of metastasis. Cancer 1997;80:1529–37.

[96] Tu SM, Lin SH, Logothetis CJ. Stem-cell origin of metastasis and heterogeneity in solid tumours. Lancet Oncol 2002;3:508–13.

[97] Gazitt Y. Homing and mobilization of hematopoietic stem cells and hematopoietic cancer cells are mirror image processes, utilizing similar signaling pathways and occurring concurrently: circulating cancer cells constitute an ideal target for concurrent treatment with chemotherapy and antilineage-specific antibodies. Leukemia 2004;18: 1–10.

[98] Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature 2001;410:50–6.

[99] Liotta LA. An attractive force in metastasis. Nature 2001;410:24–5.

[100] Wright DE, Bowman EP, Wagers AJ, Butcher EC, Weissman IL. Hematopoietic stem cells are uniquely selective in their migratory response to chemokines. J Exp Med 2002;195:1145–54.

[101] Duesberg P, Li R, Fabarius A, Hehlmann R. Aneuploidy and cancer: from correlation to causation. Contrib Microbiol 2006;13:16–44.

[102] Pathak S, Multani AS, Furlong CL, Sohn SH. Telomere dynamics, aneuploidy, stem cells, and cancer (Review). Int J Oncol 2002;20:637–41.

[103] Pathak S, Multani AS. Aneuploidy, stem cells and cancer. EXS 2006;96:49–64.

[104] Matzke MA, Mette MF, Kanno T, Matzke AJ. Does the intrinsic instability of aneuploid genomes have a causal role in cancer? Trends Genet 2003;19: 253–6.

[105] Feng Z, Hu W, Teresky AK, Hernando E, Cordon-Cardo C, Levine AJ. Declining p53 function in the aging process: a possible mechanism for the increased tumor incidence in older populations. Proc Natl Acad Sci USA 2007;104: 16633–8.

[106] Desmaze C, Soria JC, Freulet-Marriere MA, Mathieu N, Sabatier L. Telomere-driven genomic instability in cancer cells. Cancer Lett 2003;194:173–82.

[107] Prehn RT. Cancers beget mutations versus mutations beget cancers. Cancer Res 1994;54:5296–300.

[108] Michaloglou C, Vredeveld LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM, et al. BRAFE600-associated senescence-like cell cycle arrest of human naevi. Nature 2005;436:720–4.

[109] Hiyama E, Kodama T, Shinbara K, Iwao T, Itoh M, Hiyama K, et al. Telomerase activity is detected in pancreatic cancer but not in benign tumors. Cancer Res 1997;57: 326–31.

[110] Sugino T, Yoshida K, Bolodeoku J, Tahara H, Buley I, Manek S, et al. Telomerase activity in human breast cancer and benign breast lesions: diagnostic applications in clinical specimens, including fine needle aspirates. Int J Cancer 1996;69:301–6.

[111] Haigis KM, Caya JG, Reichelderfer M, Dove WF. Intestinal adenomas can develop with a stable karyotype and stable microsatellites. Proc Natl Acad Sci USA 2002;99: 8927–31.

[112] Rabinovitch PS, Dziadon S, Brentnall TA, Emond MJ, Crispin DA, Haggitt RC, et al. Pancolonic chromosomal instability precedes dysplasia and cancer in ulcerative colitis. Cancer Res 1999;59:5148–53.

[113] Lofberg R, Brostrom O, Karlen P, Ost A, Tribukait B. DNA aneuploidy in ulcerative colitis: reproducibility, topographic distribution, and relation to dysplasia. Gastroenterology 1992;102:1149–54.

[114] Smith AL, Hung J, Walker L, Rogers TE, Vuitch F, Lee E, et al. Extensive areas of aneuploidy are present in the respiratory epithelium of lung cancer patients. Br J Cancer 1996;73:203–9.

[115] Ioachim HL. Morphology of human carcinogenesis. Anticancer Res 1999;19:4627–43.

[116] Bjerknes M, Cheng H, Kim H, Schnitzler M, Gallinger S. Clonality of dysplastic epithelium in colorectal adenomas from familial adenomatous polyposis patients. Cancer Res 1997;57:355–61.

[117] Shibata D, Schaeffer J, Li ZH, Capella G, Perucho M. Genetic heterogeneity of the c-K-ras locus in colorectal adenomas but not in adenocarcinomas. J Natl Cancer Inst 1993;85:1058–63.

[118] Shibata D. Clonal diversity in tumor progression. Nat Genet 2006;38:402–3.

[119] Maley CC, Galipeau PC, Finley JC, Wongsurawat VJ, Li X, Sanchez CA, et al. Genetic clonal diversity predicts progression to esophageal adenocarcinoma. Nat Genet 2006;38:468–73.

[120] Losi L, Baisse B, Bouzourene H, Benhattar J. Evolution of intratumoral genetic heterogeneity during colorectal cancer progression. Carcinogenesis 2005;26:916—22.

[121] Harris H. A long view of fashions in cancer research. Bioessays 2005;27:833—8.

[122] Slaughter DP, Southwick HW, Smejkal W. Field cancerization in oral stratified squamous epithelium; clinical implications of multicentric origin. Cancer 1953;6:963—8.

[123] Braakhuis BJ, Tabor MP, Kummer JA, Leemans CR, Brakenhoff RH. A genetic explanation of Slaughter's concept of field cancerization: evidence and clinical implications. Cancer Res 2003;63:1727—30.

[124] Pandis N, Teixeira MR, Gerdes AM, Limon J, Bardi G, Andersen JA, et al. Chromosome abnormalities in bilateral breast carcinomas. Cytogenetic evaluation of the clonal origin of multiple primary tumors. Cancer 1995;76:250—8.

[125] Shattuck TM, Westra WH, Ladenson PW, Arnold A. Independent clonal origins of distinct tumor foci in multifocal papillary thyroid carcinoma. N Engl J Med 2005;352: 2406—12.

[126] Vogelstein B, Kinzler KW. The multistep nature of cancer. Trends Genet 1993;9:138—41.

[127] Beachy PA, Karhadkar SS, Berman DM. Mending and malignancy. Nature 2004;431:402.

[128] Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med 1986;315:1650—9.

[129] Hassold T, Chiu D. Maternal age-specific rates of numerical chromosome abnormalities with special reference to trisomy. Hum Genet 1985;70:11—7.

[130] Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. Nat Rev Genet 2001;2:280—91.

[131] Pellestor F, Andreo B, Arnal F, Humeau C, Demaille J. Maternal aging and chromosomal abnormalities: new data drawn from in vitro unfertilized human oocytes. Hum Genet 2003;112:195—203.

[132] Aas T, Borresen AL, Geisler S, Smith-Sorensen B, Johnsen H, Varhaug JE, et al. Specific P53 mutations are associated with de novo resistance to doxorubicin in breast cancer patients. Nat Med 1996;2:811—4.

[133] McCormick F. New-age drug meets resistance. Nature 2001;412:281—2.

[134] Chaudhary PM, Roninson IB. Expression and activity of P-glycoprotein, a multidrug efflux pump, in human hematopoietic stem cells. Cell 1991;66:85—94.

[135] Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer 2005;5:275—84.

[136] Donnenberg VS, Donnenberg AD. Multiple drug resistance in cancer revisited: the cancer stem cell hypothesis. J Clin Pharmacol 2005;45:872—7.

[137] Skinner R, Wallace WH, Levitt GA. Long-term follow-up of people who have survived cancer during childhood. Lancet Oncol 2006;7:489—98.

[138] Illmensee K, Mintz B. Totipotency and normal differentiation of single teratocarcinoma cells cloned by injection into blastocysts. Proc Natl Acad Sci USA 1976;73:549—53.

[139] Martin GR. Teratocarcinomas and mammalian embryogenesis. Science 1980;209:768—76.

[140] Pierce GB, Speers WC. Tumors as caricatures of the process of tissue renewal: prospects for therapy by directing differentiation. Cancer Res 1988;48: 1996—2004.

[141] Prehn RT. Regeneration versus neoplastic growth. Carcinogenesis 1997;18:1439—44.