Cancer: Cell Structures, Carcinogens and Genomic Instability
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Aneuploidy, stem cells and cancer

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Summary. Telomeres which protect the individual chromosomes from disintegration, end-to-end fusion and maintain the genomic integrity during the somatic cell divisions play an important role in cellular aging. Aging and cancer development are linked with each other because cancer is considered a group of complex genetic diseases that develop in old cells and, in both, telomere attrition is involved. Numeric chromosome imbalance also known as aneuploidy is the hallmark of most solid tumors, whether spontaneous or induced by carcinogens. We provide evidence in support of the hypothesis that telomere attrition is the earliest genetic alteration responsible for the induction of aneuploidy. Dysfunctional telomeres are highly recombinogenic leading to the formation of dicentric chromosomes. During cell divisions, such complex chromosome alterations undergo breakage fusion bridge cycles and may lead to loss of heterozygosity (LOH) and gene amplification. Furthermore, we have provided evidence in support of the hypothesis that all types of cancer originate in the organ- or tissue-specific stem cells present in a particular organ. Cancer cells and stem cells share many characteristics, such as, self-renewal, migration, and differentiation. Metaphases with abnormal genetic constitution present in the lymphocytes of cancer patients and in some of their asymptomatic family members may have been derived from the organ-specific stem cells. In addition, evidence and discussion has been presented for the existence of cancer-specific stem cells. Successful treatment of cancer, therefore, should be directed towards these cancer stem cells.

Key words: Aneuploidy, dysfunctional telomeres, fluorescence *in situ* hybridization, genetic instability, stem cell.

Introduction

Cancer is not a single disease. It comprises a group of complex genetic diseases of uncontrolled cell division and is also one of the characteristics of aged cells. Aging and cancer development, therefore, are linked with each other. Most cancers are caused by chromosome and gene mutations that accumulate in a specific tissue or organ during the cellular aging. Genetic instability is exhibited by aging cells, both *in vitro* and *in vivo*, in the form of numerical (aneuploidy) and structural chromosomal alterations (translocation, deletion, amplification and inversion) [1]. More than 90% cancers are caused by exposure to environmental carcinogens. The insult inflicted by carcinogens are first faced by the termini of chromosomes, called telomeres, which are attached to the inner nuclear wall. Among their many functions, telomeres determine the domain and stability of individual chromosomes within the nucleus and serve as guardian of the genome [2]. Functional telomeres are essential for the normal segregation and maintenance of chromosomes during mitotic and meiotic

divisions [3]. More recent information has shown that the maintenance of the telomere depends on interactions with an enzyme, telomerase, with telomeric proteins, and with some still undiscovered factors regulating the telomeric functions. Dysfunctional telomeres support the survival of aneuploid cells, a characteristic of many human and murine cancers.

The single unifying cellular mechanism that influences both aging and cancer development is the telomere dynamics [4, 5]. Cancer cells stabilize their telomere repeats either by a telomerase-dependent pathway or by the telomere-independent or alternate lengthening of telomere (ALT) pathway [6]. Unlike the murine somatic cells, human somatic cells lack or have diminished telomerase activity. This major difference in human and murine cells can easily explain why it is difficult to transform normal human cells, but easy to transform mouse cells, *in vitro*. Mouse and human somatic cells differ in many other respects, for example, in their responses to oxidative stress [7].

Most hematological neoplasms are known to arise from stem cells, whereas epithelial malignancies are generally considered to originate in differentiated organ- or tissue-specific somatic cells. Recently, a hypothesis was proposed that not only the hematological malignancies but also all solid tumors originate in organ- or tissue-specific stem cells or their immediate progeny (progenitor) cells [5, 8, 9]. This hypothesis is based mainly on two recent observations: the presence of stem cells in each and every human organ or tissue [10–12], and the presence of poorly differentiated cancer cells signaling a poor prognosis for patients. Since stem cells, especially embryonic stem (ES) cells, have the potential to differentiate into all three major tissue lineages, ectoderm, mesoderm and endoderm and their derivative organs [13–15], it is not unreasonable to propose that organ- or tissue-specific cancers originate in the organ- or tissue-specific stem cells [2, 8, 9]. Human ES cells have four unique characteristics: (1) self renewal, (2) differentiation into other cell types, (3) migration *in vivo*, and (4) cell death under unfavorable conditions [16, 17].

The purpose of this chapter is to discuss, in brief, the relationships between aneuploidy, stem cells and cancer development. That stem cells and aneuploidy play crucial roles in cancer development and metastasis will also be discussed in some detail under separate subtitles.

Aneuploidy and carcinogenesis

Mammalian species, in general, contain a diploid (2n) complement of chromosomes in the somatic cells of both sexes. However, cancer cells originating from the same diploid cells are mostly aneuploid, especially the solid tumors. Aneuploid constitutions are generally due to random chromosome losses/gains from non-disjunctions and multipolar mitoses, mostly originating from tetraploid (4n) cells [18–21]. Tetraploid cells are formed either by fusion of two diploid (2n) cells or due to the endomitosis of a diploid cell. In addition, segmental chromosomal losses or gains are due to structural chromosome

alterations, including translocations, amplifications or deletions of certain segments. Because of these inherent characteristics, most cancer cells have genomic heterogeneity. In other words, each cell of a given tumor has its own chromosomal features except for certain specific common marker chromosomes. Inherent chromosomal instability, which can be due to the telomere erosion, plays a major role in causation of most cancers [22–24].

As early as 1890, von Hansemann [25] first suggested that cancer originates in an alteration in the genetic content of a cell. Later, Theodore Boveri [18, 26], while working on chromosomes of *Ascaris* and *Paracentrotus* sea urchin eggs, proposed his famous theory of malignancy. According to Boveri's theory, the neoplastic properties of a cancer are the consequence of chromosomal aberrations, and a malignant transformation results from the clonal expansion of a single genetically altered somatic cell. In our earlier publications we asked [9], among several questions, 'Could this somatic cell undergoing neoplastic transformation be an organ- or tissue-specific stem cell?'. Boveri, who first introduced the term centrosome [27], postulated that cancer cells are formed due to abnormal chromosome distribution originating as the consequence of multipolar mitosis, caused by the formation of multiple centrosomes. Recent molecular studies have provided strong evidence in support of Boveri's theory of malignancy [28–37]. The centrosome is an important actor of the cell division machinery. Its malfunction may cause abnormal chromosome segregation or no segregation at all, resulting in aneuploidy, the hallmark of cancers [37–46].

The original definition of aneuploidy was deviation of one or more chromosomes from the haploid (1n) state [47], which in the human is 23 chromosomes, in mouse 20 chromosomes, in rat 21 chromosomes, in cat 19 chromosomes, in Syrian hamster 22 chromosomes and in Chinese hamster 11 chromosomes. Presence of an extra copy (trisomy) or the absence of a chromosome (monosomy) is generally considered an example of aneuploidy. However, recently, the term aneuploidy has been used even for the presence of an extra segment or the deletion of a segment from a chromosome without a gain or loss in the total chromosome/centromere numbers. Currently, this term is used ambiguously to encompass all kinds of structural and numerical chromosome instabilities. Is aneuploidy that is caused by a dysfunctional centrosome, an early genetic change that initiates cancer formation? Some researchers, including the present authors, favor the opinion that aneuploidy indeed is the first causal step in tumor development. According to the strict classical definition of aneuploidy, primary leukemia and lymphomas, which do not show numerical anomalies but are characterized by their specific structural alterations, including reciprocal translocations and inversions, should not be considered aneuploid [47]. Presence of t(9;22) in chronic myelogenous leukemia, and t(8;14) and t(14;18) in different lymphomas are typical examples of human cancers. Only in the blast phase or at advance stage of the disease have numerical (aneuploidy) and additional structural anomalies been reported. Practically all cancer types, hematological and solid, contain structural anom-

alies in their genomes [2, 5, 45, 46, 48, 49]. That structural chromosome anomalies precede aneuploidy has even been reported in immortal fibroblast cultures of Li-Fraumani syndromes [50]. The important question is: which comes first, the numerical alterations (aneuploidy) or the structural modifications in cancer initiation? The obvious reply is: structural alteration due to telomere erosion comes first in the multistage carcinogenic process (Fig. 1).

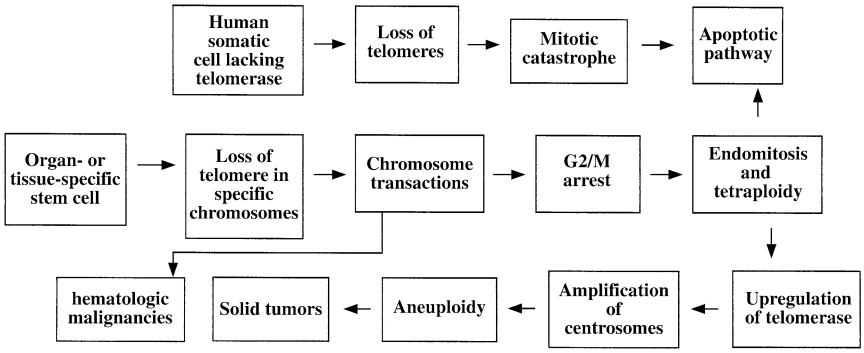

Figure 1. Pathways for apoptosis, aneuploidy and cancer initiation. Reprinted with permission and modified after [9].

Telomeres and chromosomal instability

Chromosomal anomalies have long been associated with, and are considered causal for, congenital birth defects and cancer initiation. The only difference is that children born with birth defects have minimal chromosomal alteration, whereas cancer cells and especially epithelial malignancies, have numerous defects. These abnormalities include numerical as well as structural alterations of different chromosomes in various neoplasias. This finding was possible due to the discovery of various chromosome banding techniques in early 1970s and the fluorescence *in situ* hybridization (FISH), comparative genomic hybridization (CGH) and spectral karyotyping (SKY) procedures later on. These molecular cytogenetic techniques have helped in dissecting break points of translocations, inversions, duplication and deletions, thus providing the pathological consequences of specific chromosome defects.

As mentioned earlier, the discovery of the Philadelphia (Ph) chromosome, t(9;22), in chronic myelogenous leukemia (CML) was the first reported cancer-specific cytogenetic defect [51, 52]. Since then, a number of cancer-specific chromosomal lesions (locations of oncogenes) have been identified [45, 46]. Chromosomal breakage and fusion (translocation) have been observed in all cancer cells. But, why do only certain chromosomes break and rejoin in a given cancer type? There is no satisfactory and definite answer available to such a question. We have, recently, hypothesized that only those chromosomes

that have partially dysfunctional telomeres undergo such genetic changes. In other words, telomeres serve as the guardian of individual chromosomes and protect them from cellular challenges [2, 5]. It is also reported that not all chromosomes or both arms of the same chromosome have a similar number of telomeric repeats [53].

Telomeres are special DNA-protein structures present at the ends of linear eukaryotic chromosomes. Since the pioneer research of Muller and McClintock, the telomere has been recognized as protecting the chromosomal ends from degradation and fusion to other broken ends [54–57]. The telomeric DNA consists of G-rich sequences, for example, T_2AG_3 repeated many times in all vertebrate species (reviewed in [58]). In humans, all chromosome ends have approximately 5 kb of telomeric DNA [53]. Telomerase, a ribonucleoprotein reverse transcriptase, is responsible for stabilization of telomeres in cancer and germ cells. This enzyme is composed of an RNA subunit, hTERC, and a catalytic protein subunit, hTERT, in humans.

Chromosome instability and replication senescence

In most mammalian somatic cells, telomeres shorten with each round of cell division. In normal human somatic cells, the telomere length progressively decreases owing to an end replication problem, and the cell population undergoes either senescence or neoplastic transformation depending on the telomerase status. Upregulation/activation of the telomerase in human aged somatic cells may help stabilize the telomeres or cap them as functional. Under such conditions, these cells with genetic instability may get transformed and initiate cancer development. On the other hand, in the absence of telomerase activity, aged cells with continued telomere attrition may lead to apoptosis.

As shown diagrammatically in Figure 2, organ- or tissue-specific stem/progenitor cells, when insulted by clastogens, may either readily undergo altruistic apoptosis or may undergo chromosome rearrangements, gene amplification and aneuploidy, finally resulting in cancer formation, especially solid tumors. In the case of hematological malignancies, only specific translocations or inversions are sufficient to activate proto-oncogene(s) and the emergence of neoplastic cells. Aneuploidy in the strict classical sense may not be the primary cause of initiation of hematological malignancies. It may, however, be necessary at late stage during the blast phase of the disease. In solid tumors, the classical aneuploidy definitely plays an important role in initiation/promotion of the disease in which loss of heterozygosity (LOH) of tumor suppressor gene(s) is required [59, 60]. The presence of a high degree of aneuploidy is not uncommon among cancer cells [61]. Approximately 88% of all colon cancers are characterized by chromosome instabilities [62].

In the absence of telomerase, continued somatic cell division is accompanied by progressive shortening of telomeres. When the telomere lengths reach a critical stage, cells stop dividing and enter senescence. It was Hayflick who

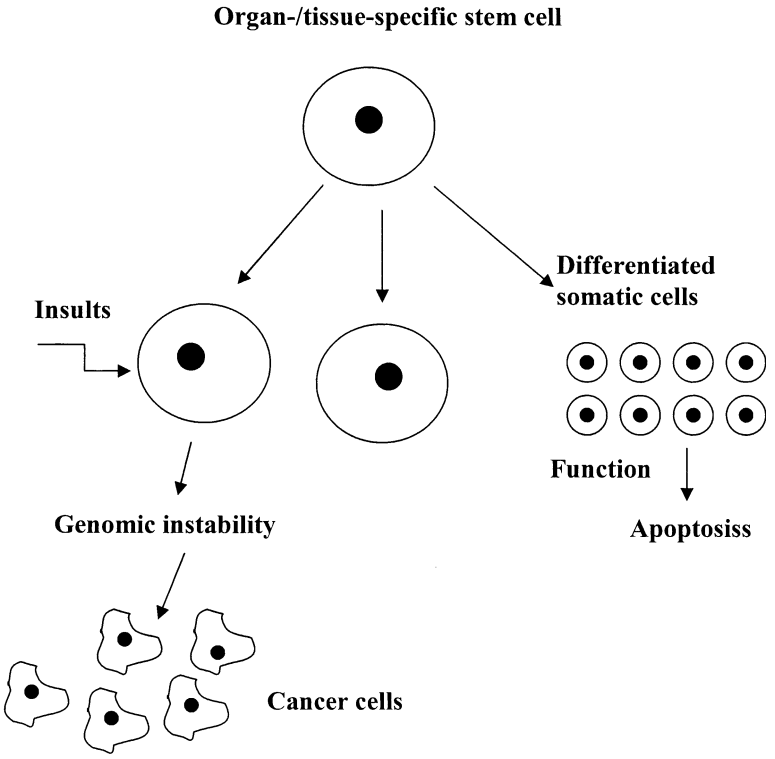

Figure 2. Diagram showing cancer formation from organ-specific stem cells.

first suggested that diploid human fibroblasts have a limited number of replication in culture, after which they stop dividing [63], and reach mortality stage 1 (M1) [64]. However, viral oncogenes, e.g., AgTsv40, may transform such cells even with critically shortened telomeres. Such cells may enter another mortality stage, stage 2 (M2), and in the senescent stage may remain metabolically active but without undergoing DNA synthesis. These cells with drastically changed morphology become large and express β-galactosidase activity [65]. Replicative senescence is also a genetically dominant phenotype [66].

The question here is: What factor(s) are responsible for driving primary somatic cells to enter replicative senescence? One of the answers lies in telomere erosion and the absence of telomerase. In mTERC$^{-/-}$ mutant mice, telomere attrition has been shown to cause genomic instability, progressive infertility and even the induction of epithelial malignancies in late generation animals [67–69]. Primary murine embryonic fibroblasts (MEFs) from the mTERC$^{-/-}$ mouse were used to study the mechanism of dysfunctional telomeres and a number of telomere-associated proteins (Tab. 1). These mice are telomerase null because they lack the gene that encodes for telomerase RNA. MEFs derived from such mutant mice, have reduced ability to immortalize sponta-

Table 1. Localization of telomerase gene and telomere-associated proteins on human chromosomes*

Name	Interaction	Functions at telomeres	Chromosome localization	References
Telomerase	With T_2AG_3 overhang,	Telomere elongation	5p15	[70]
hTERT		RNA subunit		
hTERC	Telomerase		3q26	[71]
Specific proteins				
Pot1	With G-rich strand	Length maintenance	7	[72]
TRF1	T loops	Negative length regulator (dependent on telomerase)	8q13	[73]
TRF2	T loops	Negative length regulator (independent of telomerase)	16q22	[74]
TANK1/2	With telomere	Positive length regulator	8p23/10q23	[75, 76]
TIN2	With TRF1	Positive length regulator	14q11	[77, 78]
RAP1	With TRF2	Length regulator	16	[79]
PINX1	With TRF1/Pin2	Telomerase inhibitor	8p23	[80]
Nonspecific proteins				
Ku70/Ku86	With telomeric repeats	Negative length regulator	2q35/22q11	[81, 82]
DNA-PKCA	With telomeric DNA	Capping of telomere	8q11	[83]
Rad50 NSB/ MRE11	With TRF2	T-loop sterilization	5q31	[84]
Rad51	In ALT cells	Recombination in ALT cells	15q15	[85]
WRN/BLM	With 3' overhang	Telomere structure maintenance	8p12/15q26	[86, 87]
p53	With single-strand T-loop	Telomere structure	17p13	[88, 89]
ATM	With TRF1	Telomere chromatin structure	11q22	[90, 91]

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neously in culture. These findings indicate that telomere attrition limits the replicative potential of MEF *in vitro*. A typical metaphase spread from a fifth generation (G5) $mTERC^{-/-}$ mouse fibroblast revealing a chromosome fusion product and 41 chromosomal arms is shown in Figure 3.

Stem cell characteristics of cancer and metastatic cells

As listed in Table 2, stem cells and cancer cells have many characteristics in common. Organ-specific stem cells are known to participate in organ or tissue homeostasis by constantly replacing differentiated somatic cells lost as a result

Figure 3. A typical metaphase spread from a fifth generation (G5) mTERC$^{-/-}$ mouse fibroblast showing a fusion product (arrow) and 41 chromosomal arms, an example of aneuploidy.

Table 2. Common characteristics of stem cells and cancer cells*

	Stem cells	Cancer cells
1	Proliferate indefinitely	Proliferate indefinitely
2	Self renewal by similar signals	Self-renewal by similar signals
3	Are heterogeneous, with different phenotypes	Are heterogeneous, with different phenotypes
4	Migrate	May metastasize (migrate)
5	Express telomerase	Express telomerase
6	Have extended telomere repeats	Metastatic cells have extended telomeric repeats
7	Differentiate	Differentiate
8	Can be tissue-specific	Can be tissue-specific
9	Undergo organogenesis	Undergo limited organogenesis
10	Undergo apoptosis	Undergo apoptosis

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of cellular aging, injury, or disease. A cancer is essentially an aberrant organ or tissue that acquires the characteristics for unregulated proliferation through the accumulation of genetic mutations. Like stem cells, cancer cells are able to

proliferate indefinitely and in some cases may lead to metastasis. Cancer metastasis, which is analogous to species migration [93], is achieved by a series of genetic mutations and amplification of telomereic DNA in the cancer cells [94]. Some of these sequential steps are: dissociation from the primary tumor mass, extensive vascularization, invasion, detachment, embolization, extravasation into the organ parenchyma, and, finally, vascularization and proliferation within the organ site [95]. Almost all of these steps involve cell migration. That stem cells can migrate *in vivo* explains most of these steps, once we consider that cancers originate in the stem cells and that the poorly differentiated cancer cells still retain some characteristics of the stem/progenitor cells.

Angiogenesis or the formation of new blood vessels is a pre-requisite for cancer cell growth and metastasis to distant organs [96]. Cancer cells have the potential to form not only their own blood channel [97, 98] and mosaic-blood vessels [99], but also a variety of blood cell types [10]. It is therefore clear that stem cells are pluripotent and can differentiate into various cell types, including lymphocytes. That solid tumors arise from organ- or tissue-specific stem cells has profound implications for cancer treatment. The target for successful cancer treatment and chemoprevention must be the cancer-specific stem cells.

Do cancer cells have their own stem cells?

Stem cells, especially the ES cells, have infinite proliferative and developmental potentials. Self renewal, migration and differentiating characteristics of stem cells make them suitable as a source for organ and tissue regeneration. We have previously proposed that all cancers originate in organ- and tissue-specific stem cells [5, 8]. Stem cells, sometimes also called master cells, are known to participate in organ or tissue homeostasis by replacing aged somatic cells lost as a result of aging, injury or disease. It has been shown that mouse neural stem cells can differentiate into brain cells (astrocytes, oligodendrocytes and neurons) and can form a variety of blood cell types, including hematopoietic cells [10–12]. Neural stem cells from adult mouse brain are capable of forming chimeric chick and mouse embryos, and give rise to all germ layers and cell types [11]. These observations taken together suggest that adult neural stem cells have a pluripotent phenotype, and may have potential to produce a variety of cell and organ types for transplantation. Although the molecular mechanism of the neural stem cell proliferation and differentiation is not fully resolved, the phosphatase and tensin gene (PTEN) homolog, also known to be mutated in multiple advanced cancers (MMACI), from human chromosome 10q22-24 is a candidate tumor suppressor gene implicated in such cellular phenotypes [100].

The burning question is: Do cancer cells have their own stem cells? As early as in 1956, a famous cytogeneticist, Sajiro Makino from Japan, proposed that cancer cells may have their own stem cells [101]. This idea was has received

further support [102, 103]. Recently, Reya and associates [104] and Kondo et al. [105] have brought this hypothesis into the limelight by presenting molecular evidence in support of this concept. The later group has isolated "cancer stem cells", as a small side population (SP), even from the long-term established tumor cell lines including C6 glioma, MCF-7 breast cancer, B104 neuroblastoma and HeLa cell lines [105].

Evidence that cancer develops from stem cells, not differentiated somatic cells

There is plenty of evidence to support the statement that dividing (cycling) cells are more susceptible than the quiescent (non-dividing) cells to accumulate mutations when challenged by the environmental mutagens. Most differentiated somatic cells perform their functions in an organ- and tissue-specific environment and are then replaced by proliferation of specialized stem or progenitor cells [106]. During wound healing and in disease conditions, organ- or tissue-specific stem cells or their progenitors divide, migrate and help in repair. The stem or progenitor cells form one cell that remains a stem cell and another cell that differentiates into the specialized function-oriented mature cell. Fully differentiated somatic cells perform their functions and then undergo apoptosis (Fig. 2). They are replaced by the newly differentiated somatic cells in the organ. Since cancer cells originate by the interactions of environmental carcinogens and the genetic make-up of the person, it is worth considering that stem cells, being poorly differentiated somatic cells with the phenotypes of their progenitors, also having telomerase activity, are the target of neoplastic transformation [104, 105, 107–109]. In a recent book chapter, Sell [110] speculated that most carcinomas and adenocarcinomas originate in the organ-specific stem cells. Of course, most hematological malignancies have their origin in stem cells. In fact, cancer originates from an imbalance between the rate of cell division and the rate of differentiation and cell death. Also, maturation arrest of stem cell differentiation has been considered a common pathway for the origin of teratocarcinoma and epithelial malignancies [111]. Inherently, the stem cells are characterized by substantial longevity, replication potential and telomerase activity. In addition, they are also known to have much longer mean telomere length, which is a survival factor for the cells [2].

There is mounting evidence to suggest that practically every organ has its own stem cell reservoir. Although these specialized cells are present in a small pocket in the organ-specific environment, their potential to replace damaged cells is controlled by the cell requirement. This is analogous to the "Seed and Soil" hypothesis proposed by Paget [112] for metastatic breast cancer cells. The presence of organ-specific stem cells has been reported in lung, mammary gland, liver, pancreas, kidney, heart, retina, muscle, skin and brain of mammalian species including human. Even during angiogenesis, angioblasts,

which are the precursors of endothelial cells, act as progenitor cells with several stem cell characteristics.

Are circulating abnormal metaphases derived from organ- or tissue-specific stem cells of cancer patients?

The initial (primary) genetic (chromosomal) alterations associated with cancer development do not necessarily occur in every somatic cell [113]. It has been proposed that predisposed individuals inherit susceptibility traits that makes their specific chromosomes prone to breaking at a particular loci [49]. The chromosomes of a cancer-predisposed individual may undergo specific alterations at relatively low frequency in all tissues, including peripheral blood lymphocytes (PBL). Could this trait be the attrition of telomere in those chromosomes? Clastogens that are able to induce chromosome-specific aberrations have been described previously in a separate report [2].

Specific cytogenetic alterations were first identified in PBL as being associated with chromosome 13 in retinoblastoma, with chromosome 11 in Wilms' tumor, with chromosome 3 in renal cell carcinoma, with chromosomes 2, 5 and 11 in colorectal cancer, with chromosomes 1, 6 and 9 in melanoma, with chromosome 1 in endometrial cancer, and chromosomes 5, 7, 8, 10, and 16 in prostate cancer (see reviews [2, 49]). Subsequent reports have shown specific chromosomal changes in a small percentage (1–3%) of phytohemagglutinin-stimulated lymphocytic metaphases of various epithelial malignancies, including breast, lung, prostate and other adenocarcinomas [114, 115]. These circulating aberrant metaphases are not cancer cells because: (1) they have mainly chromatid breaks, simple translocations or deletions; (2) the patients whose tumor cells and PBL were both analyzed cytogenetically had mainly chromatid breaks in the PBL but stable marker chromosomes involving the same chromosomes in their tumor cells [116]; and (3) such types of chromosomal alterations are present even in the PBLs of some asymptomatic family members [117]. Could these rare abnormal metaphases be coming from the tissue- or organ-specific stem cells? Undoubtedly, more research in future is required to substantiate this hypothesis.

Conclusion

In conclusion, we provide evidence for the proposal that telomere attrition (dysfunction) is the earliest genetic alteration in the organ- or tissue-specific stem cells, which then is responsible for aneuploidy and is the cause of all types of cancer. Metaphases with abnormal genetic constitutions present in the PBL of cancer patients and some of their asymptomatic family members might be derived from the organ-specific stem cells. Successful treatment of cancer by different modalities and chemoprevention strategies should be directed

towards these cancer stem cells. It would be most rewarding to develop isolation procedures for these organ-specific stem cells for their further biological characterization and to elucidate the mechanism of proliferation, progression and metastasis of cancer.

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