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Cancer Biology and Genetic Heterogeneity

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Cancer is not a single disease but a group of complex genetic diseases of aged cells. Therefore, aging and cancer development are interlinked phenomena. There is growing evidence that most cancers are caused by mutations that accumulate in many genes during cellular aging. Aging cells, which are known to have reduced amounts of telomeric DNA, may give rise to neoplastic cells due to the induction of genetic instability. The unifying single mechanism in both aging and cancer development is, therefore, the telomere dynamics. The number of telomeric repeats, which are guardians of the chromosome (genome), is stabilized in neoplastic cells by the activation of the enzyme telomerase that keeps on adding nucleotides at the ends of each chromosome. Telomere erosion is responsible for genetic instability, which can activate or inactivate certain genes responsible for immortalization and neoplastic transformation. Amplification of telomeric repeats is characteristic of metastatic cells, which can develop resistance to various treatment modalities. Since telomere dynamics is so important in the survival of cells (both normal and cancerous), it should be studied on a population-based research in most countries, including India. Based on the quantitation of telomeric repeats and specific structural and numerical cytogenetic abnormalities present in the peripheral blood lymphocytes, individual family members, in most cases, can be identified for cancer predisposition, nature and aggressiveness of the disease and in discovering new drugs for cancer treatment with minimal or no side effects. The future of telomere biology is bright and has much to offer in understanding human aging, cancer research, treatment and prevention.

Key Words: Genetic heterogeneity, Telomere, Fluorescence in situ hybridization, Amplification, Clastogens, Instability, Lifestyle, Transformation, Aneuploidy, Apoptosis, Metastasis

Introduction

Cancer is a group of complex genetic diseases of uncontrolled cell division. To understand the difference between normal and cancer cells is to understand an automobile with an accelerator and brakes (normal cells) and an automobile with accelerator only and no brakes (cancer cells). Cancer, a global problem, is one of the leading causes of death throughout the world. Annual deaths from cancer exceed 4.5 million. One type of cancer may be more prevalent in a particular region or country than other types, which could be more prevalent in another country. Cancer has a geographical distribution. More than 150 different cancer types have been recognized. Of all cancer types, hematologic malignancies (leukemia and lymphoma) represent only 10%, whereas 90% of cancers are of solid tumor types. The most common cancers, ranked by annual worldwide incidence, are breast, colorectal, lung, stomach, head and neck, prostate, cervical and skin cancer, of which melanoma is the most lethal. Of all these cancer types, the majority are the results of an interaction between the person's genotype and environmental exposure. Differences in the ethnicity of human populations and differences in their country-specific environments may explain the geographical distribution of this group of dreadful diseases that we call cancer.

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The purpose of this mini-review is to discuss certain general aspects of cancer biology and heterogeneity and then to provide possible mechanisms of genetic susceptibility, cancer predisposition, treatment modalities and ultimately prevention of these diseases, which are as old as the history of mankind. This review is prepared with the hope of providing a general knowledge of the genetics of cancer. It is not intended for those readers who are on the cutting edge of molecular biology of cancer research and oncologists involved in the treatment of cancer patients, but for the general public who have some knowledge of cell biology.

Facts about Cancer

Most cancers originate from a single cell, meaning they are monoclonal in origin. Somatic cells in our body are programmed to die after a certain number of cell divisions. Cancer cells, originating from the same kind of somatic cells, program themselves to survive (Pathak et al. 2000a, Pathak 2001). The neoplastic transformation to and the progression of cancers are multistep processes in which sequentially acquired and/or constitutional genetic alterations confer uncontrolled growth and proliferation advantages for individual tumors (Foulds 1954, Nowell 1976,1986, Pathak 1989a). All types of cancer cells are characterized by the presence of abnormal genotypes compared to their normal counterparts. The presence of identical and structurally altered chromosomes, which we call markers, in the majority of cells of a tumor indicates that most neoplasms are monoclonal in origin. Monoclonality may be a characteristic of the early phases of the primary neoplasm and the metastatic cancer cells. During the late stages of progression and, in some cases resistance to treatment, most tumors become heterogeneous, meaning having multiple cell populations differing in their genetic constitutions and biological behavior.

Cancer cells have only one aim and that is to SURVIVE (Pathak 2001). Contrary to general belief, cancer cells do not thirst to kill their host, but rather to consume growth factors (nutrients) and angiogenic factors (formation of new blood vessels) from the host cells and to survive. The best indication that cancer cells thirst only to survive is that when put in tissue culture they proliferate indefinitely, long after the cancer patient may have already died. For instance, the first-ever established human cancer cell line, HeLa, derived from the cancer biopsy of a 31-year-old African American woman, the late Henrietta Lacks, in the 1950s by George Otto Grey and associates (1952), is still surviving today in culture vessels and in freezers all over the world.

As mentioned earlier, cancer development is a multistage carcinogenesis which is caused by gene mutations. One set of genes may be responsible for neoplastic transformation or cancer development and other sets, for local and distant metastases. Primary tumor cells are generally not capable of invading lymph nodes or other distant body organs. It is only the metastatic cells that are capable of aggressive behaviors including dissociation, invasion, embolism, extravasation and proliferation, caused by the accumulation of additional genetic abnormalities. Genetic instability, which is the cause of heterogeneity, contributes significantly to the development and metastasis of cancer (Pathak 1989a, Fidler 1990). In simple terms, metastasis is the spread of cancer cells from the primary tumor to local and distant-organ sites and proliferation there. Recently, we have reported that cancer cells are not only capable of dividing in the distant-organ environment, but are also capable of transforming adjacent stromal cells (Pathak et al. 1997,1998a). Because of these unexpected observations, the biology and treatment of metastatic cancer cells must be considered more complex than is currently thought. Some investigators believe that metastasis is a non-random process, consisting of a series of sequential genetic steps that must be acquired by cancer cells if a metastasis is to develop. Just like primary cancer, metastasis is also the result of clonal expansion of single cells. The interaction between the "visiting" tumor cells and the organ environments determines the fate of metastasis and provides credence to the "Seed and Soil" hypothesis of Paget (1889), which he proposed to explain the organ-specific metastasis. Paget examined the autopsy reports of 735 women who died with mammary cancer and concluded that metastasis is caused by only certain cancer cells (the seeds) that can divide only in certain organs (the soil).

Cancer Development and Lifestyle

Cancer is a group of complex diseases of old age. Some cancers, particularly lung and breast, are more common in older people. This may be due to smoking, poor diet, or exposure to environmental (internal and external) carcinogens. Previously, it was suggested that environment and lifestyle are the major causes of cancer development. About 90% of cancers occur in epithelial tissues and clearly represent the result of interactions between host factors (genetic constitution, health, age and nutritional status) and the environment. Genetic abnormalities by themselves are believed to be responsible for only a small fraction of neoplasms. By contrast, an estimated 80-90% of cancers are thought to be preventable through the identification and control of environmental factors exerting their clastogenic effects on the genetic and acquired traits that modify individual response (see, Pathak & Dhaliwal 1991). Environmental carcinogens, or cancer-causing substances, appear to be the "wild card" in cancer causation. In all reality, genetics loads the gun but environment pulls the trigger. Carcinogens can be broadly classified into three groups: a) Chemical agents, such as hazardous chemicals; b) Physical agents, such as X-ray and ultraviolet radiation and, c) Biological agents, such as viruses, bacteria etc. In a country like India, the incidence of esophageal and head and neck cancer is more prevalent as compared to other cancer types. It could very well be due to the lifestyle (chewing tobacco, heavy automobile pollution exposure) that people are having in that country. Some cancers in our society are "self-inflicted," caused by unhealthy habits of smoking, drinking alcohol, and eating too little fibrous food and too many fast foods high in animal fat.

The high incidence of specific cancers in particular professions may indicate a close relationship between occupational agents and the occurrence of cancer. Percival Pott was the first to discover excessive cancers of the scrotum among chimney sweepers (Pott 1775). Benzo(a) pyrene, present in coal tar, is one of the most potent carcinogens and may be responsible for the induction of skin cancer in individuals exposed to coal tar. Air pollution from motor vehicles, hazardous waste products, logging sites, agricultural fertilizers and pesticides (causing water pollution), fire fighting exposure, laundries, the transportation industry, construction sites, power generators and toxicological research laboratories are well-known primary sources of exposure to occupational and environmental carcinogens (Pathak & Dhaliwal 1991).

Most cancers have a geographical distribution. The World Health Organization (WHO) in Geneva maintains a data bank for cancer incidence and mortality from which information can be obtained, but for only 50% of the world population (WHO 1999). According to a WHO report, smoking eventually will kill about 500 million people alive in the world today. Tobacco-related deaths are expected to increase from 4 million per year today to about 10 million per year by the 2030s, with 70% of those deaths occurring in developing countries including India. Smoking is increasing in developing countries by about 3.4% per year and India is playing a leading role. The estimated 1.2 billion smokers in the world today consume an average 14 cigarettes per day. Secondhand or environmental tobacco smoke (ETS) contains numerous carcinogens for which a safe level of exposure is not known. Approximately 3000 nonsmokers die each year from lung cancer as a result of breathing secondhand smoke (US,EPA, 1992). According to an American Cancer Society (ACS) report about 1,268,000 new cancer cases are expected to be diagnosed in 2001. These estimates do not include noninvasive cancers and basal and squamous cell skin cancers. More than 1 million cases of basal and squamous cell skin cancers are expected to be identified in 2001. About 553,400 Americans are expected to die of cancer this year, roughly more than 1,500 patients per day. Cancer is the second leading cause of death in the US, exceeded only by heart disease. In the USA, 1 of every 4 deaths is from cancer- related disease.

There is not only a geographical but also an ethnic distribution of various cancers. Overall, African Americans are more likely to develop cancer than persons of any other racial and ethnic group. During 1990-1997, incidence rates were 444.6 per 100,000 among blacks, 402.1 per 100,000 among whites, 272.9 per 100,000 among Hispanics, 279.3 per 100,000 among Asian/Pacific Islanders, and 152.8 per 100,000 among American Indians. Reported

rates of female breast cancer are highest among white women (114.0 per 100,000) and lowest among American Indian women (33.4 per 100,000). Among women, African Americans have the highest incidence rates of colon and rectum (45.2 per 100,000) and lung and bronchus cancer (45.8 per 100,000) followed by whites, Asian/Pacific Islanders, Hispanics, and American Indians, respectively, for both cancer types. African American men have the highest incidence rates of prostate (225.0 per 100,00), colon and rectum (58.3 per 100,00), and lung and bronchus cancers (111.1 per 100,000). Similar to American Indian females, American Indian males have lower reported rates of cancer incidence than men of other racial and ethnic groups (ACS Publication, 2001). Are these differences due to their genetic make-ups, lifestyles or some yet unknown reasons? Answers to some of these crucial epidemiologic questions are not easily determined and much more research is needed in this area.

Chromosomes and Cancer

Chromosomal catastrophes have long been associated with and are considered causal for congenital birth defects in children. Tumor-specific genes that have been cloned and are now well-studied were first identified in the key family members who had both birth defects and cancer development. Two childhood neoplasia-related tumor suppressor genes, retinoblastoma (Rb) and Wilm's tumor (WT), are well-known examples. There is a plethora of evidence to support the statement that chromosomal abnormalities are prerequisites for neoplastic transformation, cancer progression and metastases (see, Pathak 1989a,b, 2001, Pathak & Dhaliwal 1991, Popescu 1994, Heim & Mitelman 1995). These abnormalities include numerical as well as structural alterations of different chromosomes in various neoplasms. This finding was possible due to the development of various chromosome banding techniques and molecular biological procedures. These have helped in dissecting break-points of translocations, inversions, duplications and deletions, thus providing the pathological consequences of specific chromosomal abnormalities. Figure 1 shows a Giemsa (G)- banded karyotype of a human melanoma with structural and numerical

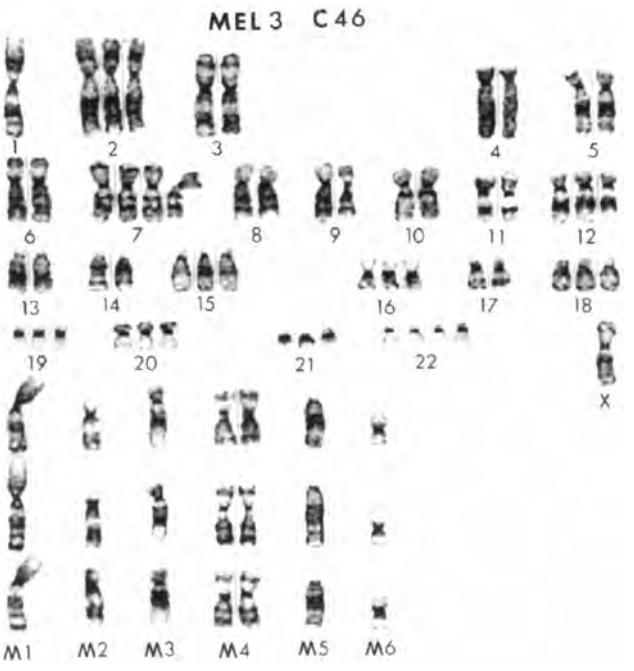

Figure 1 A Giemsa (G-)-banded karyotype of a human female melanoma clone (MEL. 3 C46) showing structural and noncrucial abnormalities. Structurally altered marker (M1 to M6) chromosomes from this spread and two additional metaphases are shown on the bottom three rows. (For the identification of these markers refer to our paper; Pai et al. 1999).

abnormalities. This melanoma biopsy was obtained from a white woman who came to The University of Texas M.D. Anderson Cancer Center at Houston, Texas, in 1977 for evaluation. We have generated a number of clones from the parental melanoma cell line and have studied their biological properties (Pai et al. 1999). Chromosomes 1,3,5,9,17 and 22 were non-randomally involved in structural alterations. Lately, aneuploidy has attracted the attention of some researchers as the initial genetic defect responsible for neoplastic transformation (Li et al. 1997). What causes such defects in specific chromosomes is not fully understood. Recently, we have reported that loss of telomeric repeats arrests cells in the G2-M-phase. Chromosomes in such cells divide but the cytoplasm does not, resulting in endoreduplication and tetraploid cell formation. These cells, with their chromosomes undergoing continued loss of telomeres, have shown severe DNA fragmentation and ultimately cell death (Multani et al. 2000). Telomere erosion under certain unknown conditions also induces genetic instability which, in turn, may activate the reverse transcriptase

enzyme telomerase, oncogenes, and the loss of tumor suppressor genes (Artandi et al. 2000, Hanahan 2000). Roles of telomeres in cell death and causation of genetic instabilities will be discussed in detail later.

Since telomeres àre the guardians of the chromosomes and, therefore, of the genome, their erosion resulting in ring and dicentric chromosome formation may cause telomere dysfunction (Pathak 2001). Such complex configurations follow breakage - fusion - bridge – cycles during subsequent limited cell divisions (McClintock 1941). Since the development of the quantitative fluorescence in situ hybridization (Q-FISH) technique, now it is possible to quantitate the telomeric DNA, not only in a given nucleus but also in given chromosome arms. Martens and associates (1998) have reported short telomeres on the short (p) arm of human chromosome 17 by a new Q-FISH technique. It is quite possible that specific chromosome translocations involved in human leukemias, lymphomas and many solid neoplasms could be due to the erosion of telomeric repeats in the chromosomes involved. For example, in the translocation t(9:22) in chronic myelogenous leukemia, the two chromosomes involved have smaller telomeric repeats as compared to the intact homologs of chromosomes 9 and 22 (our unpublished data). Fusion of two acrocentric chromosomes, popularly called Robertsonian translocations, in the human karyotype could be due to the loss or reduced number of telomeric repeats present in the p arms of these chromosomes. Of course, fusion of acrocentric chromosomes in mice, cattle and dog cells are well-known. New evidence from Q-FISH indicates that their chromosomes have much fewer telomeric repeats in their p arms as compared to their long (q) arms (our unpublished data). Based on these observations one can conclude that most structural alterations of chromosomes, whether in cancer cells or in normal cells, are caused by a reduced number of telomeric repeats. Because of this deficiency, either inherent or induced, telomeres become dysfunctional and chromosomes undergo structural and numerical alterations. That telomere dysfunction promotes non-reciprocal translocations and epithelial cancer in mice has just been reported by Artandi and associates (2000), and in a commentary by Hanahan (2000). Therefore, it is important to explore the dynamics of telomeres in aging, preneoplastic cancer and metastatic lesions of various malignancies. Our preliminary data have already indicated that highly metastatic tumor cells of various histopathologic epithelial malignancies have an amplification of telomeric DNA without an increase in the number of chromosomes as compared to their non-metastatic counterparts (Multani et al. 1999a). On the other hand, hematologic malignancies, such as chronic myelogenous and acute lymphocytic leukemias, have shown a reduced amount of telomeric DNA in the blast phase of the disease as compared to the non-blast (Pathak et al. under preparation).

Chromosomal Abnormalities in Cancer: Causal or Consequential

Most mammalian species (and this could be true for other groups of vertebrates also) have two types of chromosomal constitutions: (a) the unique type and (b) the shared type. Based on their unique chromosomal characteristics, individual species or their cell cultures can be positively identified, but not by their shared chromosomal features. A similar situation exists when chromosomes of human and any other mammalian tumors are carefully analyzed. The idea that most neoplasms have primary (unique) and secondary (shared) types of cytogenetic abnormalities came to me from my life-long research in animal cytogenetics. Live animal tissues (bone marrow, peripheral blood, testis, and fibroblasts from different organs) belonging to more than 100 mammalian species were studied for their karyotypic evaluation using various banding techniques (Pathak 1976, 1990). Since at that time cancer cytogenetic literature was flooded with identical marker chromosomes, even in histopathologically different human epithelial neoplasms, a reputed American cancer cytogeneticist told me that a non-random (unique) chromosomal alteration such as t(9;22) Philadelphia (Ph) chromosome, which is the primary cytogenetic characteristic of chronic myelogenous leukemia (CML), could not be found in other malignancies, especially of epithelial origin. Most cancer cytogeneticists studying epithelial malignancies, of which there were only a handful at the time, were

finding common identical markers in tumors of different organs and tissues, suggesting no definitive role of such genetic changes in cancer development. Of course, their observation was correct but the interpretation was not right. In reality, they were identifying secondary/tertiary types of shared chromosome anomalies which were responsible for metastasis in tumors of different histopathologic origin. For the identification of primary (unique) chromosomal defects, we decided to evaluate normal cells such as, peripheral blood lymphocytes (PBLs). Primary (unique) chromosomal defects are much more commonly observed at diagnosis in leukemia than in solid malignancies. This is possible due to the fact that the biological manifestations of leukemia (blood cancer) become apparent at an earlier stage of the disease with minimal chromosome alterations than do those of solid cancers. In most solid neoplasms, when clinically visible, the chromosomal abnormalities are often complex and numerous and tend to obscure the primary genetic defects. This inherent problem in solid tumor cytogenetics pressed the need of an alternative approach, which is to look for chromosomal (primary) anomalies in PBLs and in normal somatic cell cultures.

There is plenty of data confirming that the primary chromosomal changes associated with solid cancers were first detected in PBLs (see Dave et al. 1995, Pathak & Goodacre 1986, Pathak & Dhaliwal 1989, Pathak et al. 1989,1991). Pathak (1989a,b) and Pathak et al.(1989) had proposed that an individual predisposed to cancers might be genetically mosaic and the primary genetic defects associated with neoplastic transformation may not be necessarily inherited in every somatic cell but may be harbored in only a few somatic cells. Mosaicism has been recognized frequently in two childhood cancers: retinoblastoma and Wilm's tumor (Cecilia & Riberio 1988, Dave et al. 1995). An example of chromosomal mosaicism showing instability of specific chromosomes 1, 9 and 13 in the PBLs culture of a male patient with basal cell carcinoma (a form of skin cancer) is shown in figure 2. In only two out of 50 PBL metaphases of the patient examined were these anomalies present. Structural anomalies of chromosomes 1 and 9 have been implicated in skin carcinomas, especially in human melanomas, in the

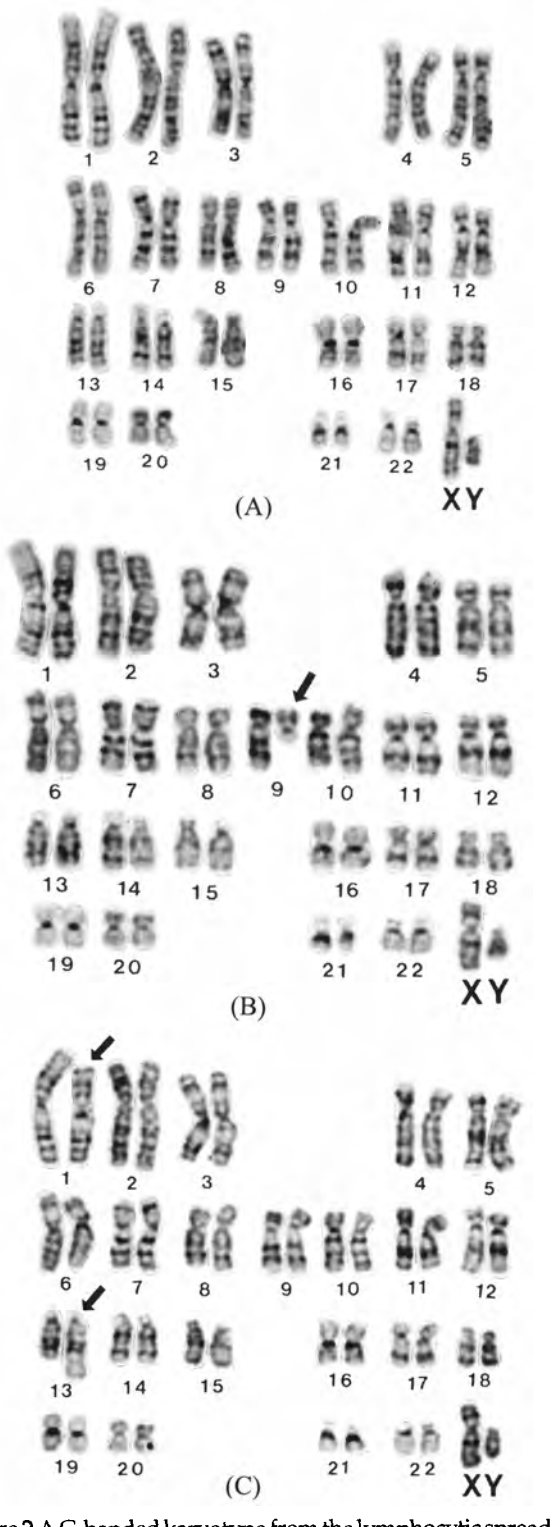

Figure 2 A G-banded karyotype from the lymphocytic spreads of a patient with basal cell carcinoma showing chromosomal mosaicism. (A) a normal male karyotype showing 46, XY chromosome constitution; (B) a karyotype from the same patient showing deletion in the long arm (q) of a chromosome 9; (C) a karyotype from the same patient showing a translocation between the short arm (p) of chromosome 1 and the long arm of 13. Altered chromosomes 1, 9 and 13 are marked by arrows in figure 2B and C.

literature (figure 1). In figure 3 is shown a lymphocytic karyotype with inversion in a chromosome 10 from a prostate cancer patient. Two other brothers of this patient who had prostate cancer also showed chromosome 10 alterations of different types in their PBL cultures. Of course, the frequency of such metaphases was not more than 2 to 3 percent. What are these cells with cancer-specific chromosomal alterations? Could these be the stem cells of a particular organ in which a cancer might develop? Can these lymphocytic chromosomal lesions form stable markers in the cancer cells (Ghayee et al. 1997)? Are these stem cells produced in the bone marrow or in that particular organ? Answers to some of these questions are not known.

Telomere Dynamics in Cancer

Because of my life-long research in the field of mammalian cytogenetics, I have a special "love" for telomere research. The word telomere was coined in 1938 by H J Muller stated that a chromosome must have telomeres at both ends for its protection and, therefore, telocentric chromosomes can not exist in nature. McClintock (1941), provided experimental evidence for Muller's hypothesis of telomeres protecting chromosomes. Conventional and molecular cytogenetics techniques have identified

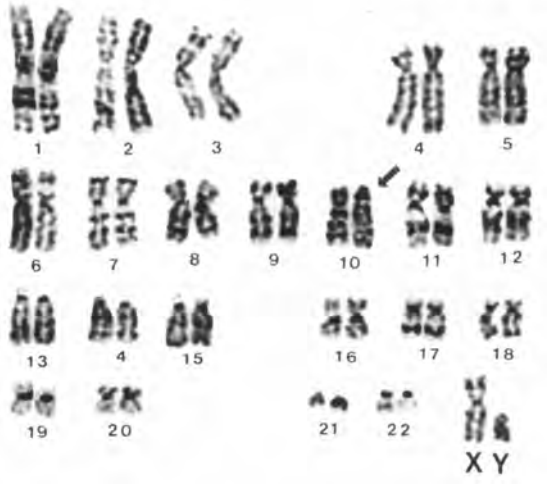

Figure 3 A Giemsa-banded karyotype from the lymphocytic metaphase spread of a prostate cancer patient showing inversion in a chromosome 10 (arrow). All other chromosomes showed normal banding patterns. Two brothers of this patient who were diagnosed with prostate cancer also showed chromosome 10 alterations in their lymphocytes.

the existence of telocentric chromosomes in many mammalian species, including humans, which have shown centric fission (division of a bi-armed chromosome into two telocentrics) in their karyotypes (Pathak 2001). Telomeres, consisting of several kilobase TTAGGG repeat sequences in vertebrates, protect the ends of eukaryotic chromosomes, serve as protein binding sites (Bouffler 1998), guard the ends from nuclease-mediated degradation and end-to-end chromosome fusion (Bourgain & Katinka 1991, van Steensel et al. 1998), and are capable of inducing unique chromatin structure (Tommerap et al. 1994). In a human at birth, telomeres consist of 15000 base pairs (bp) of (TTAGGG)n DNA repeat sequences. Every time a cell divides, it loses 25-200 DNA base pairs off the telomere ends. When this pruning takes place about 100 times, a cell ages and stops dividing. Telomere erosion is directly implicated in cellular and individual aging processes. Older people have a reduced amount of telomeric DNA in their cells. FISH preparations of telomeric DNA in the lymphocyte nuclei from a 17-year-old and an 83-year-old male are shown in figure 4. The cells in the older male have significantly less telomeric DNA compared to the cells in the younger male. Telomeric proteins play a crucial role in the separation of telomeres during mitosis and meiosis (Kirk et al. 1997), and chromosomal attachment to the nuclear matrix (Luderus et al. 1996). In addition, several other functions related to cell biology and physiology have been assigned to telomeres (Pathak et al. 1996, Pathak 2001). Aging in humans is associated with reduction in telomeric DNA and slowing in cell division (Holt et al. 1996).

Telomere repeats are not only confined to the chromosomal termini but are also found at intrachromosomal and pericentromeric sites in many eukaryotic species including human (de la Sena et al. 1995, Azzalin et al. 1997, Meyne et al. 1990, Pathak et al. 1998b, Multani et al. 2001). Telomeres also influence gene expression, cell replication and recombination in subtelomeric regions. In humans, meiotic recombination is upregulated near telomeres (Ashley 1994). Recently, Kilburn and associates (2001) demonstrated that introduction of 800 bp of functional vertebrate telomere sequence into the second intron of the adenosine

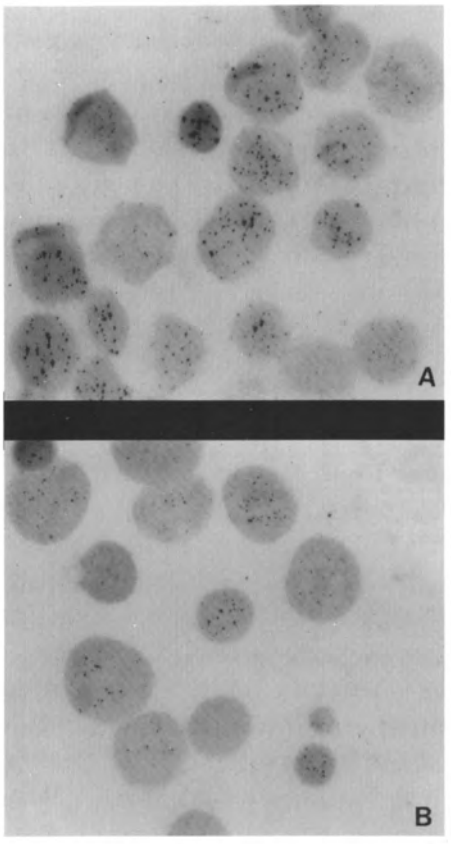

Figure 4 FISH preparations using telomeric DNA probe in the lymphocyte culture of a 17 – year-old and an 83-year-old male. Nuclei of the young male (A) have more telomeric DNA as compared to the nuclei of the older male (B). Reduction in the telomeric DNA is a natural process of aging. It may also contribute to neoplastic transformation and programmed cell death (apoptosis) under different conditions.

phosphoribosyltransferase (APRT) gene in CHO (Chinese hamster ovary) cells significantly destabilized the interior of chromosomes. In other words the telomeric DNA repeat, by virtue of its functionality, can induce genetic instability in normal and cancer cells (Multani et al. 2001, Pathak 2001). In a recent article involving mice which were lacking the RNA component of telomerase (mTERC), Artandi and associates (2000) reported progressive loss of telomere resulting in chromosomal instability, reduced fertility and the development of epithelial malignancies. Recent data collected in our group have shown that some infertile human azoospermic males have reduced telomeric DNA in their lymphocytes and have developed prostate cancer (Pathak et al. under preparation). These reports together support the hypothesis that erosion of telomeric DNA precedes chromosomal instability (formation of ring, fragments and multicentric chromosomes) and their amplification serves as a "survival factor" for metastatic cancer cells (Multani et al. 1999a, Pathak 2001).

Telomeres and Apoptosis

Individual chromosomes have their own domains within the cell nucleus. These domains are determined for each chromosome by the telomeric DNA that attaches to the nuclear wall. Any physical, chemical and/or biological agents which are capable of inducing chromatid- and/or chromosome-breaks are called clastogens. When a clastogen enters the cell nucleus, it first comes in contact with the telomere, the "body guard" of the chromosomes. Because of the location and structural biology, telomeres are hit first by the clastogens. It is, therefore, reasonable to consider that telomeres must be playing a role in spontaneous or clastogen-induced cell death (Pathak et al. 2000a and b, Ishibashi & Lippard 1998). In a series of publications, we and others have shown that telomeric DNA becomes the target when cancer cells are treated with known clastogens and chemotherapeutic drugs (Multani et al. 1999b & 2000, Pathak et al. 1998, 2000b, Izbicka et al.1999, Ishibashi & Lippard 1998). In addition, telomeric DNA is being used as a target for discovering new plant products that can be used as supplements with commonly used cancer treatment modalities (Pathak et al. 2000b). This structure-based approach is now being used for the discovery of compounds which can be used for cancer treatment, because this is the way to go in future. Izbicka and associates (1999) have said, "The fact that DNA (telomeric), not telomerase protein or RNA, can be a target for rational drug design has important implications for understanding the telomerase mechanism of action." Therefore, it is important and sorely needed to focus on and discover telomere-interactive agents that can preferentially kill the cancer cells with minimal or no side effects.

Cancer Treatment and Future Directions

When cancer patients die, it is usually due to the spread of cancer cells to other organs (metastasis) (Fidler 1990). The prognosis and successful

treatment of human cancers are based on the correct identification of the disease stage and its metastatic potential. While treating metastasis, physicians must consider not only the original tumor cells that have metastasized to distant organs, but also their capability to transform the organ-specific cells (Pathak et al. 1997). Primary cancer cells generally do not kill the host. Only metastatic cells are capable of amplifying their telomeres, forming new blood capillaries (angiogenesis), developing resistance to treatment, and killing their host due to their selfish motive of surviving. Cancer treatment options include surgery, chemotherapy, radiotherapy, immunotherapy, and gene therapy in combination with cord blood or bone marrow transplants. Since chemotherapy has severe side effects, it is important to use alternative medicines such as homeopathic and Ayeurvedic medicines, acupuncture, hydrotherapy, massage, yoga and other ancient treatment modalities in combination with current practice for the treatment of cancer. Gene therapy, though popular, has its limitations because most cancers are not caused by a single gene mutation. Targeting telomeres with G-quadruplex interactive drugs would be beneficial because the highest active gene concentrations, recombinations, and gene transcriptions in the human genome are in the sub-telomeric regions of chromosomes. Further research into telomere dynamics is needed for the successful treatment and prevention of cancer.

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