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AIDS-related Kaposi’s Sarcoma Is a Clonal Neoplasm

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Abstract
Kaposi’s sarcoma is generally believed to be a non-neoplastic hyperproliferation because it may regress spontaneously and its spindle cells lack features of typical tumor cells, such as aneuploidy, nuclear atypia, and permissive growth in cell culture. A fundamental characteristic of neoplasms is clonality, in that they arise from clonal replication of a single cell whereas reactive processes are derived from polyclonal proliferation. We used an X chromosome inactivation assay to determine the clonality of Kaposi’s sarcoma nodules from patients with AIDS-related disease. The assay is based on a methyl-sensitive restriction digest followed by PCR amplification of the highly polymorphic androgen receptor gene. Two of three evaluable cases had a monoclonal pattern of inactivation, and the third case had a clonal expansion of cells with an altered microsatellite repeat sequence. These data suggest that Kaposi’s sarcoma (at least in the AIDS setting) is a clonal neoplasm.

Introduction
Kaposi’s sarcoma is the most frequently occurring AIDS-associated tumor. It has a spectrum of clinical appearance, ranging from patches or plaques to nodular disease. Kaposi’s sarcoma affects predominantly the skin and may also involve mucous membranes, lymph nodes, and internal organs. The histological picture of overt disease has four characteristic features: thin-walled vascular structures, extravasated erythrocytes, infiltrating lymphocytes, and proliferating spindle cells. The tumor compartment is thought to be the spindle cells; their derivation is enigmatic, although current data support an endothelial origin (1). Cells similar to spindle cells can be isolated from the blood of HIV type 1 infected and normal individuals (2), but a relationship of these circulating cells to the spindle cells of Kaposi’s sarcoma has not been established. The stromal content is higher in patch or plaque lesions, while nodular disease has the highest concentration of spindle cells. Kaposi’s sarcoma also occurs in non-AIDS settings, in association with iatrogenic immunosuppression (e.g., in transplant recipients), in an endemic form in central and eastern Africa, and in a classical form (in eastern European and Mediterranean populations). The epidemiology and clinical behavior differ among these settings, but similar histopathological findings suggest a common pathogenesis.

The nature of Kaposi’s sarcoma is controversial, with long-standing disagreement regarding whether it is a neoplastic lesion or represents a reactive process (3). The spindle cells contain an apparently normal chromosomal complement, which would favor hyperplasia rather than neoplasia (4, 5). Other evidence cited in support of a reactive process includes the absence of nuclear atypia (6) and the occasional spontaneous regression of individual lesions (and rarely of all disease) (7), and the dependence on cytokines, such as basic fibroblast growth factor, oncostatin M, and interleukin 6, for growth of Kaposi’s sarcoma-derived cells in vitro (8). On the other hand, a Kaposi’s sarcoma-derived cell line, KS Y-1, induces tumors resembling Kaposi’s sarcoma in nude mice (9), and has other neoplastic characteristics, including tetraploidy (3).

Clonality is a distinguishing characteristic of neoplastic lesions. The parenchyma of a neoplasm is derived from uncontrolled replication of a clone of cells, which can be traced to a common progenitor. Conversely, absence of clonality is indicative of reactive, rather than autogenous, replication. A useful marker of clonality is the inactivation pattern of the X chromosomes. In females, one of the two X chromosomes in each cell is inactivated by methylation, which provides dosage compensation for sex-linked genes. In an early stage of embryonic development, one of the two X chromosomes in each cell is methylated at random, and the same methylation pattern is thereafter passed to daughter cells in somatic replication (10). Normal somatic tissues in females are composed of a mosaic in which the paternally derived X chromosome is methylated in some cells and the maternally derived X chromosome is methylated in other cells. Clonally related daughter cells, on the other hand, all share the same methylation pattern on the X chromosome. Identity of the active allele of an X-linked gene was originally exploited to demonstrate the clonality of Burkitt’s lymphoma (11), and this strategy was advanced with a RFLP-based technique for direct detection of methylated DNA by methylation-sensitive restriction enzymes (10). The X-linked human androgen receptor gene contains a highly polymorphic trinucleotide repeat sequence that has been utilized in a PCR-based variation of the RFLP assay (12). The locus is heterozygous in upwards of 90% of females and hence highly informative for determinations of clonality. This assay is extremely useful when dealing with small quantities of DNA, not amena-
ble to Southern-based RFLP techniques. Accordingly, we have used a similar approach to examine the clonality of Kaposi’s sarcoma in women with nodular, AIDS-related disease.

Materials and Methods

Eight HIV type 1-seropositive women with nodular Kaposi’s sarcoma lesions were recruited during July 1993 from among patients of the Dermatovenerology Department, University Teaching Hospital, Lusaka, Zambia. One patient (Patient 1) had never been treated for Kaposi’s sarcoma and the other seven had previously received only i.v. vincristine. All patients gave informed consent for their participation. The study was reviewed and approved by the Institutional Review Boards of the University Teaching Hospital and the National Cancer Institute.

Superficial cutaneous nodules were excised and immediately snap frozen in liquid nitrogen; paired normal skin biopsies were obtained from nonadjacent sites. Biopsies were mounted in O.C.T. compound (Miles, Inc., Elkhart, IN) and cut on a cryostat. One section was stained with hematoxylin and eosin. Only lesions estimated by light microscopy to have less than 20% contaminating normal cells were used for further analysis. Three biopsies (from Patients 1–3) met this criterion. The sections from these lesions were incubated in SDS/proteinase K at 48°C for 24 h. DNA was then extracted as described previously (13).

Ten μl of the resuspended DNA were incubated with 10 units of HhaI (GIBCO-BRL) in a 20-μl reaction volume. Simultaneously, in a mock reaction, 10 μl of the same DNA were incubated with 10 μl water. Four μl of the reaction mixture were used in each case as the template for PCR. The oligonucleotide primers for PCR were designed from exon 1 of the human androgen receptor gene (HUMARA01; GenBank accession no. M35844):

- Androgen S: 5'-GCGCGAAGTGATCCAGAAC-3'
- Androgen AS: 5'-CCAGGACCAGGTAGCCTG-3'

One of the primers was end labeled with [γ-32P] ATP using T4-polynucleotide kinase (New England Biolabs). Reactions were carried out at 95°C for 30 s, 60°C for 60 s, and 70°C for 60 s, for 30 cycles. Negative water controls were always used, and all reactions were repeated by redigestion and reamplification. Products were separated by denaturing gel electrophoresis, followed by autoradiography.

Results and Discussion

Only one X chromosome in each female cell is active. The inactivation of the other X chromosome occurs early in embryogenesis, remains stable throughout the lifetime of the cell, and the same pattern is passed on to its daughter cells. The active and inactive X chromosomes differ in their methylation status, with numerous restriction sites being methylated in the inactive chromosome. Therefore, after digestion with a methyl-sensitive restriction enzyme and amplification by PCR, it is possible to distinguish between the active and inactive alleles in informative (heterozygous) females. In cells that compose normal tissue, approximately half have inactivated the maternal X chromosome and half the paternal chromosome, since X chromosome inactivation is a random process. Therefore, both maternal and paternal alleles are digested equally with methyl-sensitive enzymes, and there is an equal reduction in signal from both alleles following PCR amplification. In contrast, tumor cells are monoclonal because they arise from a single precursor cell, and hence have the same parental allele inactivated. After digestion and amplification, the signal from the inactive allele remains unchanged, while the signal from the active allele nearly disappears. Because this technique is PCR based, there is always some residual signal from contaminating normal tissue. We have consistently seen a monoclonal pattern in head and neck neoplasms, whereas normal tissue from the same patients demonstrate a polyclonal non-neoplastic pattern of inactivation.4

Three Kaposi’s sarcoma lesions and paired normal skin controls were analyzed. All three patients were heterozygous at the androgen receptor locus, as demonstrated by the presence of two bands on autoradiography. In Patients 1 and 2, DNA from normal skin yielded equal bands after restriction digestion with HhaI (cut), indicating that the frequency of methylation was similar for the maternal and paternal alleles (i.e., a characteristic polyclonal pattern). In contrast, tumor DNA from these two patients yielded unequal bands (i.e., a clonal pattern) after HhaI digestion, indicating preferential methylation of one of the two alleles (Fig. 1A). A residual band was present at the other allele, as expected from residual normal cells within the tumor tissue. Tumor DNA from Patient 3 had two major alleles of equal intensity after HhaI digestion; however, both with and without digestion an additional minor band was present in the tumor DNA but not in the normal DNA (Fig. 1B). This larger allele was again present only in tumor DNA after repeated amplification.

We have shown that Kaposi’s sarcoma lesions in two of the three patients studied displayed a monoclonal pattern of X chromosome inactivation. Since methylation occurs at random and was evenly distributed in normal tissue from these patients, our findings indicate that the Kaposi’s sarcoma lesions are derived from single clones, providing strong evidence that Kaposi’s sarcoma is a neoplastic process.

The novel, tumor-associated band in the third patient indicates the presence of a clonal population of cells that share a microsatellite expansion at the androgen receptor trinucleotide repeat. Such expansions have been observed in hereditary nonpolyposis colorectal cancer and other tumors and have been attributed to replication errors during tumorigenesis (14, 15). Although hereditary nonpolyposis colorectal cancer tumors display widespread microsatellite instability, single alterations are not uncommon at the androgen receptor locus in other cancer types (16). An error in replication that occurred during early cell division is propagated in daughter cells during clonal evolution. These microsatellite alterations have proven useful as clonal markers for the detection of human cancer, even in dilute clinical samples (16). In addition, it has been shown recently that such microsatellite instability is common in some neo-
plasms from HIV-seropositive patients (17). Due to the presence of the novel allele, it is not possible to draw any conclusions from the X chromosome inactivation assay because it is not known (a) from which (i.e., methylated or unmethylated) allele the novel allele was derived, (b) whether methylation status is retained after repeat expansion, or (c) what percentage of cells carry this third allele (i.e., when did the replication error arise during clonal expansion). This issue is further complicated by the presence of some contaminating normal tissue (which contributes an equal ratio of alleles after digestion) and the non-linear amplification efficiency of each allele by PCR itself. The pattern we observed in the parental alleles after digestion is not consistent with simple assumptions and is not evidence for or against clonality per se. However, the presence of the novel larger allele implies clonal expansion in at least a fraction of the tissue from this patient (16).

We have demonstrated the clonal, and thus neoplastic, nature of spindle cells, confirming their central role in Kaposi’s sarcoma. Additional studies are needed to determine whether multiple lesions in an individual patient arise from the same clone, as in metastatic growth, or from independent clones. Clonality also remains to be confirmed for the other histological variants, which have a lower content of spindle cells and may be more difficult to assay. Nodular lesions may be at a later stage than patch or plaque disease, and the initial stages of Kaposi’s sarcoma may be polyclonal until monoclonal outgrowth occurs. Our findings may not apply to Kaposi’s sarcoma outside of the AIDS setting. The cellular alterations that distinguish the expanded clone and promote its growth remain to be determined, as do the timing of these changes relative to the cytokine abnormalities that may be required for tumor maintenance. Understanding of these complex events will assist in developing better diagnostic, therapeutic, and preventive approaches to Kaposi’s sarcoma.

References


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