

Human rRNA Gene Clusters Are Recombinational Hotspots in Cancer

Dawn M. Stults,^{1,8} Michael W. Killen,^{2,8} Erica P. Williamson,³ Jon S. Hourigan,^{4,7,8}
H. David Vargas,^{4,7,8} Susanne M. Arnold,^{5,7,8} Jeffrey A. Moscow,^{6,7,8} and Andrew J. Pierce^{1,2,7,8}

¹Graduate Center for Toxicology, Departments of ²Microbiology, Immunology and Molecular Genetics and ³Biology, ⁴Section of Colon and Rectal Surgery, Division of General Surgery; Departments of ⁵Internal Medicine and ⁶Pediatrics, Division of Hematology-Oncology; ⁷Markey Cancer Center; and ⁸College of Medicine, University of Kentucky, Lexington, Kentucky

Abstract

The gene that produces the precursor RNA transcript to the three largest structural rRNA molecules (rDNA) is present in multiple copies and organized into gene clusters. The 10 human rDNA clusters represent <0.5% of the diploid human genome but are critically important for cellular viability. Individual genes within rDNA clusters possess very high levels of sequence identity with respect to each other and are present in high local concentration, making them ideal substrates for genomic rearrangement driven by dysregulated homologous recombination. We recently developed a sensitive physical assay capable of detecting recombination-mediated genomic restructuring in the rDNA by monitoring changes in lengths of the individual clusters. To prove that this dysregulated recombination is a potential driving force of genomic instability in human cancer, we assayed the rDNA for structural rearrangements in prospectively recruited adult patients with either lung or colorectal cancer, and pediatric patients with leukemia. We find that over half of the adult solid tumors show detectable rDNA rearrangements relative to either surrounding nontumor tissue or normal peripheral blood. In contrast, we find a greatly reduced frequency of rDNA alterations in pediatric leukemia. This finding makes rDNA restructuring one of the most common chromosomal alterations in adult solid tumors, illustrates the dynamic plasticity of the human genome, and may prove to have either prognostic or predictive value in disease progression. [Cancer Res 2009;69(23):9096–104]

Introduction

The human rRNA gene clusters (rDNA clusters) produce 45S precursor transcripts, subsequently processed to make three of the four structural RNA components of the ribosome, and are critically required for protein synthesis and cellular viability. The rDNA clusters comprising collectively hundreds of gene copies are located on the short arms of the five human acrocentric chromosome pairs (13p12, 14p12, 15p12, 21p12, 22p12: OMIM 180450-180454) and are generally organized with individual genes in head-to-tail tandemly repeated arrays (1). Each individual gene is each 43-kb in length (2), with near perfect sequence conservation. Extremely high sequence similarity combined with the length of the individual repeats makes both shotgun-based and directed sequencing approaches to genome sequence assembly of the rDNA impossible. As a result, the rDNA clusters are one of the largest

remaining components of the human genome with no representation in the Human Genome Project.

The highly repetitive nature of these clustered genomic repeats also prevents the application of commonly used techniques to investigate genomic instability such as array-based comparative genome hybridization, single nucleotide polymorphism (SNP) analysis, and high-throughput paired end sequencing approaches. Nevertheless, the very considerations that make the rDNA clusters difficult to study by conventional means also make the rDNA biologically interesting from a genomic stability perspective. The sequence conservation, repeat length, and high relative local concentration of the repeats involving multiple chromosomal loci are, in principle, conducive to cluster restructuring by homologous recombination. We previously physically characterized human rDNA cluster lengths, finding an average of 600 repeats per diploid genome spread over the 10 rDNA clusters, subject to wide person-to-person variability driven by strong meiotic recombination; the number of gene repeats per cluster changes with a frequency of ~10% per cluster per meiosis (3). The high meiotic recombination frequency phenotype of the rDNA leads us to consider whether the rDNA clusters could serve as sentinel biomarkers for dysregulated mitotic recombination in cancer.

Dysregulated recombination has the potential to mediate genomic restructuring in cancer (reviewed in ref. 4). We recently showed that changes in rDNA cluster lengths in cultured mitotic human cells are potential indicators of recombination-mediated genomic destabilization by showing a 10- or 100-fold increase in the frequency of spontaneous rDNA cluster restructuring in cells deficient for the ataxia-telangiectasia mutated (ATM) protein or the Bloom syndrome protein, respectively (5), in line with the increased prevalence of malignancy in ATM (6) and Bloom syndrome (7) patients. To determine whether the rDNA clusters are restructured in sporadic cancers generally as well as in cancer predisposition syndromes such as ATM and Bloom syndrome, we prospectively recruited lung cancer, colorectal cancer, and pediatric leukemia patients for molecular analysis of their disease.

Lung and colorectal cancers are the leading and second leading cause of American deaths from cancer, accounting for 28% and 9% of all cancer deaths, respectively (8). Notably, both lung and colorectal cancers derive from tissues with potentially substantial exposure to environmental genotoxic agents. Many potential environmental carcinogens, including specifically those in tobacco smoke, are potent inducers of sister chromatid exchange (9–11), which is thought to occur by a recombination-based mechanism (12). Ordinarily, mammalian cells efficiently suppress recombination-mediated genomic rearrangements (13, 14), but damage or loss of this control of recombination, combined with protracted recombination induction by environmental agents, may significantly impact the etiology of tumors in these organs. Evidence suggesting potential cancer etiology relevance for dysregulated recombination, particularly in the

Requests for reprints: Andrew J. Pierce, University of Kentucky, 207 Combs Research Building, 800 Rose Street, Lexington, KY 40536-0096. Phone: 859-323-1455; Fax: 859-257-8940; E-mail: Andrew.Pierce@uky.edu.
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case of lung cancer, includes both the overexpression of recombination genes (15) and ATM defects commonly found in this tissue (16). Conversely, pediatric leukemia is thought to be driven by specific chromosomal events, rather than the gradual accumulation of malignant features caused by many years accumulating molecular genomic insults (17). This work reports the result of testing the hypothesis that dysregulated recombination is a significant feature of primarily sporadic adult lung and colorectal cancers rather than pediatric leukemias.

Materials and Methods

Human investigations were performed after approval by the University of Kentucky Institutional Review Board and Markey Cancer Center Protocol Review Committee, and in accordance with an assurance filed with and approved by the Department of Health and Human Services. Informed consent was obtained from University of Kentucky Medical Center patients undergoing surgical resection for either lung or colorectal cancer, or from the parents of pediatric patients being treated for leukemia. For patients with solid tumors, peripheral blood was drawn before surgery. After surgery, resected specimens were examined by the Division of Surgical Pathology, and pieces of both tumor and surrounding nontumor tissue were removed for analysis. Leukemia patients had either peripheral blood or bone marrow aspirates removed for analysis periodically throughout the course of their treatment. All patient samples are referred to in this work by study code names.

Sample processing. Solid tissues were rinsed on ice in RPMI1640 containing antibiotics and 10% fetal bovine serum (RPMI/FBS). Tissues were then disaggregated to single cells mechanically either by gentle scraping and mincing, or by mincing in RPMI/FBS and processing in a 50- μ m pore size Medicon unit in a MediMachine followed by debris removal by filtration with a 70- μ m pore size Filcon (all from Becton Dickinson). For whole blood and bone marrow aspirates, leukocytes were isolated by ammonium chloride lysis. No digestive enzymes were used in the preparation of any of the single-cell suspensions. Single-cell suspensions of either leukocytes or solid tissues had DNA-containing cells quantified by vital staining with 2 μ g/mL acridine orange followed by flow cytometric analysis with a 488-nm argon-ion laser triggering acquisition on green fluorescence (DNA content) essentially as described (18). Single-cell suspensions were adjusted to 1×10^7 cells/mL final concentration in 0.8% low-melting-temperature agarose and allowed to solidify. High-molecular-weight genomic DNA was prepared from this solid phase cell suspension by treatment with 1% sarkosyl/500 mmol/L EDTA/0.5 mg/mL proteinase K at 50°C for at least 16 h, followed by treatment with phenylmethylsulfonyl fluoride, extensive rinsing, and final equilibration in 50% glycerol/10 mmol/L Tris/1 mmol/L EDTA (pH 8.0) and long-term storage at -20 C.

Southern blot analysis. The rDNA clusters were analyzed by pulsed-field gel electrophoresis and Southern blotting as described (3). Approximately 1 μ g genomic DNA in a 10- μ L solid-phase agarose slice was equilibrated with appropriate restriction digestion buffer and was digested by EcoRV (New England Biolabs) to liberate intact rDNA clusters from bulk genomic DNA. Agarose slices containing digested genomic DNA were sealed into the wells of a 1% Pulse Field Certified agarose (Bio-Rad) gel in 44.5 mmol/L Tris/44.5 mmol/L boric acid/1.0 mmol/L EDTA [pH 8.0; 0.5 \times Tris-borate EDTA (TBE)]. The gels were run using a CHEF-MAPPER (Bio-Rad) in 0.5 \times TBE for 24 h at 6 V/cm field strength, switching between 120° separated field vectors (frequency, 3–90 s) using a “ramp factor” of 0.357 (Bio-Rad) at a constant temperature of 14°C to achieve approximately linear size resolution of between 30 kb and 1 Mb.

Following electrophoresis, gels were equilibrated to 0.5% final concentration glycerol and dried at 65°C. Dried gels were rehydrated with water, denatured with 0.4 N NaOH/0.8 mol/L NaCl, neutralized with 0.5 mol/L Tris (pH 8.0)/0.8 mol/L NaCl, and prehybridized at 65 C in 2 \times SSC (300 mmol/L NaCl/30 mmol/L Na-citrate) with 7% SDS and 0.5% casein. Gels were probed overnight at 65°C in fresh prehybridization solution using the rDNA-specific radiolabeled PCR products of primers 5'-GGGCTCGA-

GATTGGGACGTCAGCTTCTG and 5'-GGGTCTAGAGTGCTCCCTTCCTCTGTGAG on pBSrDNA11, a pBluescript-based (Stratagene) plasmid containing a subcloned and sequenced segment of the human rDNA intergenic spacer, generated by using the aforementioned primers to amplify human genomic DNA (3). Gels were rinsed at 65°C with 2 \times SSC/0.1% SDS followed by 0.5 \times SSC/0.1% SDS, briefly equilibrated with 2 \times SSC, and developed by exposing a PhosphorImager cassette with subsequent imaging in a PhosphorImager (Molecular Dynamics). Raw data were obtained in TIFF format and processed using Adobe Photoshop. All images were processed en bloc. In no case did image processing either obscure bands from the raw data, or cause bands not apparent in the raw data to appear.

Results

We assay for dysregulated recombination in the rDNA by examining changes in the physical length of individual rDNA clusters (Fig. 1). Every person has a unique configuration of these rDNA clusters (3), so the cluster lengths vary from person to person. Where possible, we compare the rDNA cluster lengths found in surgically excised nearby nontumor tissue to the gene cluster lengths found in the tumor itself, and to gene cluster lengths from the patients' peripheral blood as an additional control (*lanes N, T, and B, respectively*). Our prior experiences with both human blood and cell lines indicated that analysis of gene cluster lengths from 50 kb to ~1 Mb is the most informative range for detecting recombination-mediated alterations (3, 5), so this is the methodology we followed in this present work. Gene clusters larger than 1 Mb all run

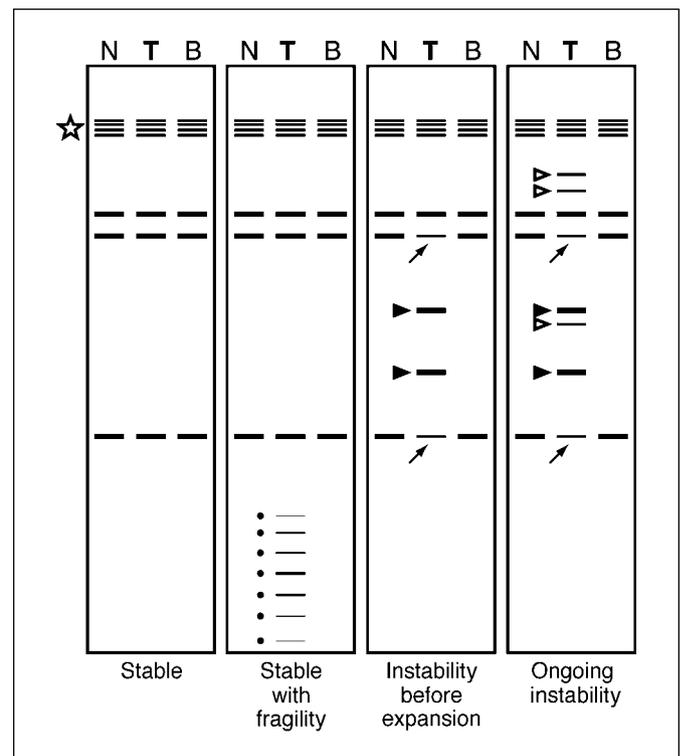


Figure 1. Patterns of genomic instability in the rDNA. In each case lanes are nontumor tissue (*N*), tumor tissue (*T*), and peripheral blood (*B*). The grouped bands at the star represent unresolved gene clusters larger than 1 Mb. The lower limit of resolution of the gel is 50 kb. ☆, breakage from fragile DNA not involving actual chromosomal restructuring. ▶, new bands found in tumors and not in nontumor tissue. ▷, new bands found in a fraction of tumor tissue but not nontumor tissue. Arrows, bands found in nontumor tissue but underrepresented in tumor tissue.

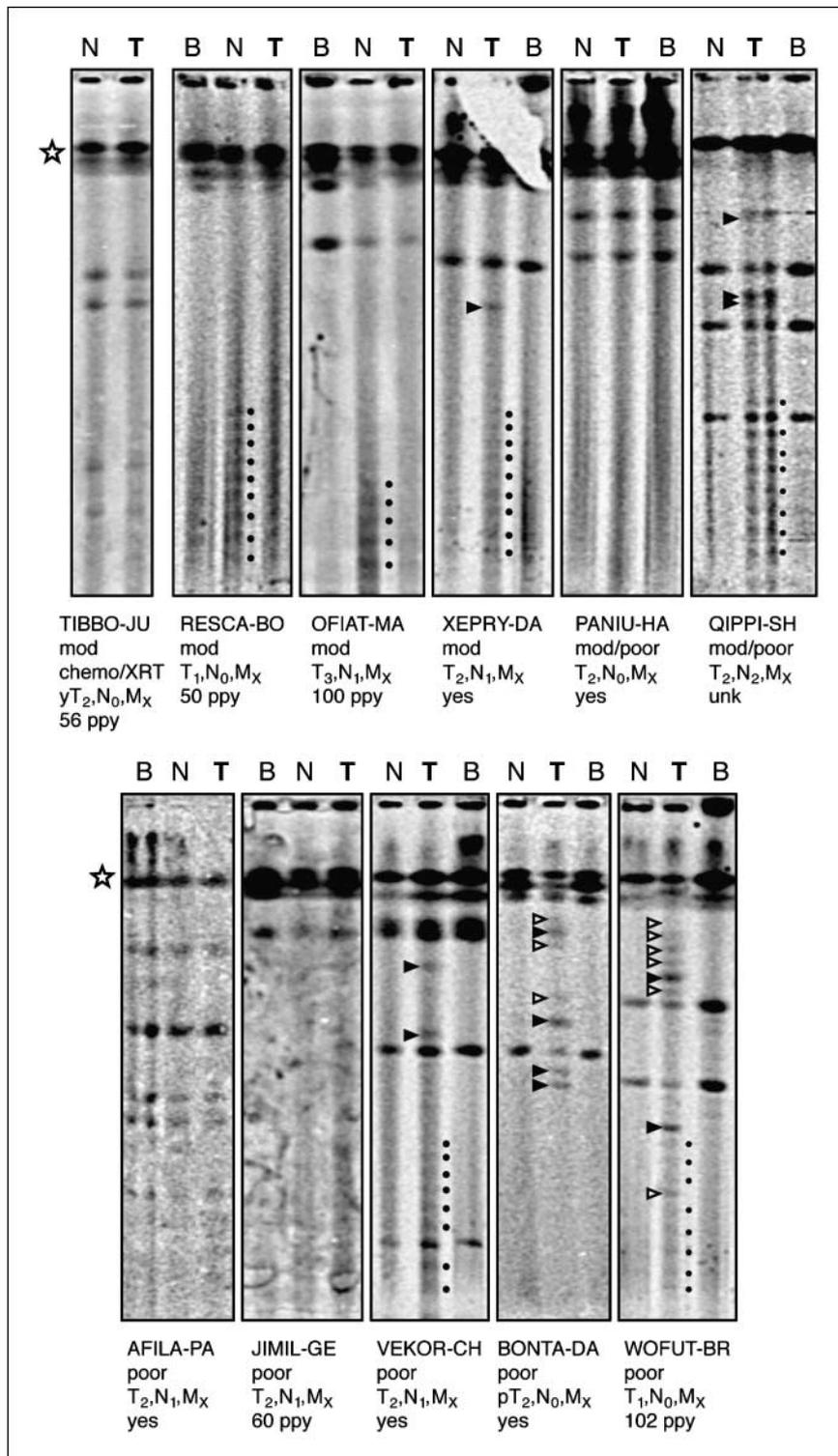


Figure 2. Lung squamous cell carcinoma rDNA stability analysis. rDNA clusters resolved up to 1 Mb in length (★). *N*, nontumor tissue; *T*, tumor tissue; *B*, peripheral blood. ●, sample fragility; ▶, new major bands found in tumors; ▷, new minor bands found in tumors. Coded patient names are indicated below each gel. Differentiation status of tumors: *mod*, moderately differentiated; *mod/poor*, moderately to poorly differentiated; *poor*, poorly differentiated. Any clinical pretreatment is indicated as chemotherapy and radiation therapy (*chemo/XRT*). Pathologic staging of tumors is indicated. Smoking history is given in person-pack-years (*ppy*) or when the patient is a smoker but smoking history has not been obtained (*yes*); *unk*, unknown.

together unresolved at the top of the gel in the region (indicated by the ★) under these electrophoretic conditions, otherwise all unique gene clusters shorter than 1 Mb are resolved as individual bands. If the rDNA clusters are stable under mitotic cellular expansion, the banding pattern found in tumor cells will match that found in nontumor cells (Fig. 1, *Stable*). Occasionally, we observe a relatively low-molecular-weight ladder of bands at the bottom of the gel in tumor and normal tissues (Fig. 1, *Sample fragility*), consistent

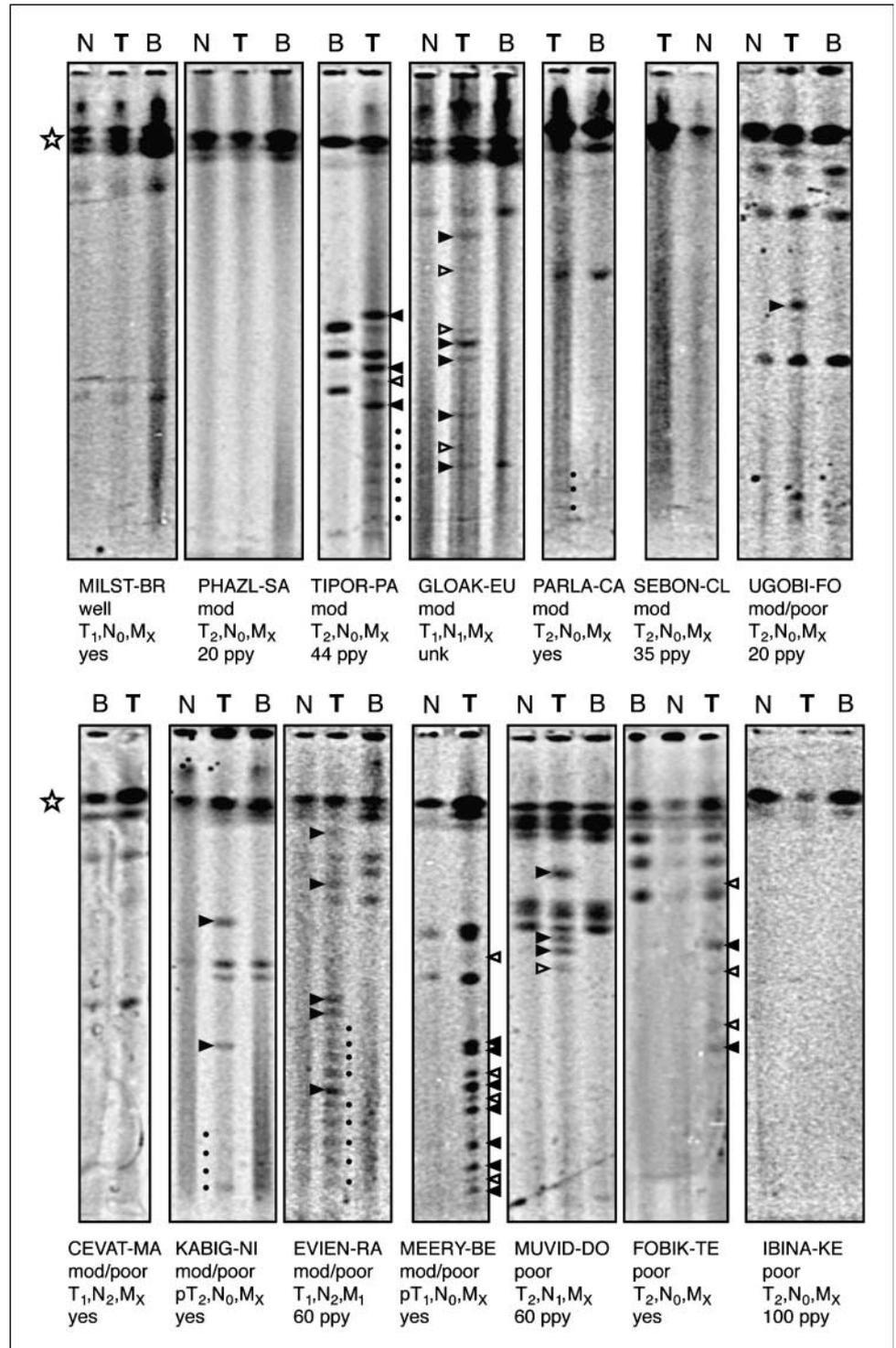
with the 43 kb rDNA individual gene repeat length. These bands appear to arise from stochastic, low-frequency fragile-site breakage in individual rDNA repeats during the genomic DNA isolation procedure itself rather than from *bona fide* chromosomal alterations in cells, since the intensities of these bands follows a smooth Gaussian distribution and is not dependent on liberation of the gene clusters from bulk genomic DNA by restriction enzyme digestion. Although this fragility may contribute to initiating chromosomal alterations

in gene cluster lengths, we do not consider a low-molecular-weight banding pattern on its own to be indicative of genomic instability.

On the other hand, if a tumor has lost control over recombination, the configuration of gene cluster lengths in the tumor will be different, relative to nontumor cells (5). If the rDNA cluster length restructuring occurred before clonal expansion of the prototumor cell, for example in the lungs of a heavy smoker, the altered length gene clusters will be present in the entirety of the subsequent tumor. In this case, some bands found in nontumor tissue may be

lost in the tumor, and new bands become evident (Fig. 1, *Instability before expansion*). These new bands (Fig. 1, \blacktriangleright) show intensities proportional to their length. Tumor samples are commonly contaminated with nontumor tissue, so fainter bands in the tumor sample occurring at the same position as bands found in normal tissue likely represent contaminating normal tissue rather than heterogeneity of cluster lengths within the tumor itself. Finally, gene cluster lengths may have changed both before clonal expansion of the prototumor cell and also during the clonal expansion

Figure 3. Lung adenocarcinoma rDNA stability analysis. rDNA clusters resolved up to 1 Mb in length (\star). *N*, nontumor tissue; *T*, tumor tissue; *B*, peripheral blood. \bullet , sample fragility; \blacktriangleright , new major bands found in tumors; \blacktriangleright , new minor bands found in tumors. Coded patient names are indicated below each gel. Differentiation status of tumors: *mod*, moderately differentiated; *mod/poor*, moderately to poorly differentiated; *poor*, poorly differentiated. Pathologic staging of tumors is indicated. Smoking history is given in person-pack-years (*ppy*), or when the patient is a smoker but smoking history has not been obtained (*yes*); *unk*, unknown.



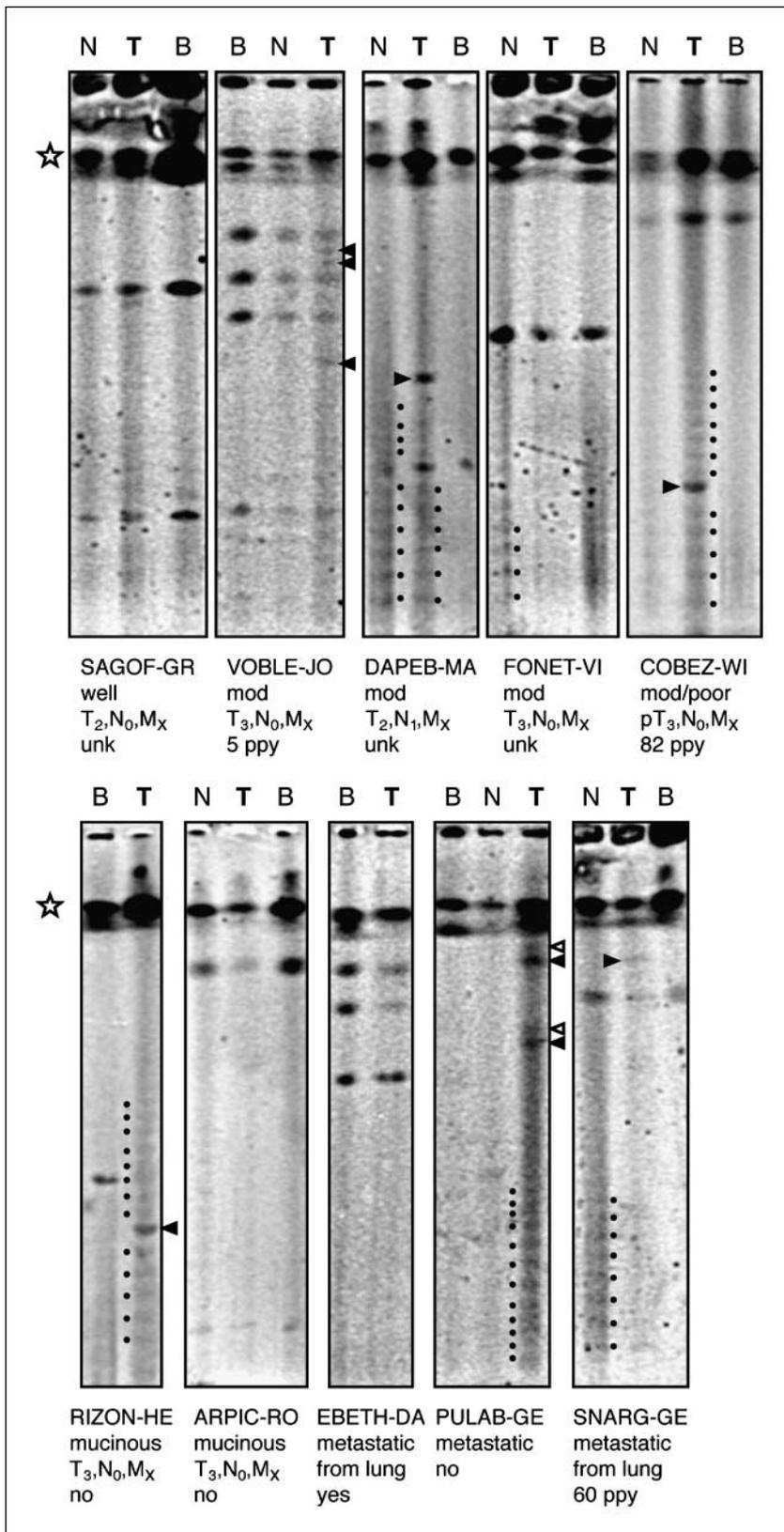


Figure 4. Colon adenocarcinoma rDNA stability analysis. rDNA clusters resolved up to 1 Mb in length (★). *N*, nontumor tissue; *T*, tumor tissue; *B*, peripheral blood. ●, sample fragility; ▲, new major bands found in tumors; ▼, new minor bands found in tumors. Coded patient names are indicated below each gel. Differentiation status of tumors: *mod*, moderately differentiated; *mod/poor*, moderately to poorly differentiated; alternatively, tumors are classified as either mucinous or metastatic. Pathologic staging of nonmetastatic tumors is indicated. *From lung*, likely metastatic colon adenocarcinoma surgically excised from lung tissue. Smoking history is given in person-pack-years (*ppy*), or when the patient is a smoker but smoking history has not been obtained (*yes*); *no*, for a nonsmoker, *unk*, unknown.

of the new tumor. Alterations that occurred after the tumor began clonal expansion will manifest as new bands, but with reduced intensity (Fig. 1, *Ongoing instability*, ▼). The degree to which the intensity of these “minor bands” is reduced relative to the intensi-

ties of the “major new bands” in the tumor is determined by the fraction of the tumor made up of cells with these alterations; the later in the clonal expansion the gene cluster alteration occurred, the fainter the minor intensity bands will be. In contrast

to the "Sample fragility" bands, new minor bands in the tumor occur at high molecular weights, do not vary smoothly in intensity, and are dependent on liberation from bulk genomic DNA by restriction digestion before visualization on the gels.

We first analyzed lung squamous cell carcinoma (Fig. 2). Four of 11 patients, TIBBO-JU, PANIU-HA, AFILA-PA, and JIMIL-GE show no differences between observed gene cluster lengths in tumor relative to nontumor tissues indicating rDNA cluster stability. Two more patients, RESCA-BO and OFIAT-MA, show the same gene cluster profiles between tumor and nontumor cells, with the exception of an even laddering of bands in the lower part of the gel (•) consistent with fragility in the isolated genomic DNA. We therefore also consider these tumors to be gene cluster stable. The stability of TIBBO-JU is particularly remarkable because this tumor was a recurrence following both chemotherapy and radiation treatment. Clearly, the intense genomic damage from treatment does not necessarily cause loss of gene cluster stability, nor is loss of gene cluster stability necessarily required for poor response to therapy.

The remaining five lung squamous cell carcinomas all show evidence of structural alteration in the rDNA. XEPHY-DA, QIPPI-SH, and VEKOR-CH show bands found in tumor cells not present in either blood or surrounding nontumor cells (Fig. 2, ♣). In XEPHY-DA and VEKOR-CH, each novel band is ~30% the intensity of the bands found in nontumor tissue. This is consistent with either the rearrangements occurring early in the clonal expansion of the initial prototumor cell, or in the pretumor lung tissue before the initiation of clonal expansion, in which case the tumor would make up 30% of the cells in the tumor sample, with the remaining 70% being normal tissue. The novel bands in QIPPI-SH are on the order of intensity of bands in nontumor tissue, suggesting that these genomic alterations preceded clonal tumor cell expansion.

Finally, two of the poorly differentiated lung squamous cell carcinomas, BONTA-DA and WOFUT-BR, show evidence of extensive and ongoing recombinational instability. In both cases, there are new major bands in the tumors not found in nontumor tissue (Fig. 2, ♠). Significantly, there are additionally several high-molecular-weight minor intensity bands (Fig. 2, ♣). The presence of both new major and minor intensity bands indicates that not only were the rDNA clusters altered in the pretumor tissue as might be expected for cells under genotoxic pressure from tobacco exposure, but that these gene clusters continued to structurally rearrange in the early history of the tumor after the prototumor cells had already become committed to clonal expansion.

We see a similar level of gene cluster restructuring in lung adenocarcinomas (Fig. 3). In 6 of 14 cases, there is no evidence for rDNA cluster rearrangement. In PHAZL-SA, SEBON-CL, and IBINA-KE gene, cluster lengths in all of nontumor, tumor, and blood samples are longer than the ~1-Mb resolution limit of the gels. This absence of resolved size differences in the gene clusters from the tumor samples compared with the nontumor controls indicates that these tumors did not undergo recombination-mediated gene cluster alterations, although we cannot preclude alterations in the unresolved gene cluster lengths larger than 1 Mb in these patients. The banding patterns in MILST-BR, PARLA-CA, and CEVAT-MA are also the same between tumor and either peripheral blood or normal lung, indicating gene cluster stability; however, UGOBI-FO, KABIG-NI, and EVIEN-RA show evidence of pretumor alteration with new major bands, but no new minor banding. The remaining five tumors, TIPOR-PA, GLOAK-EU, MEERY-BE, MUVID-DO, and FOBK-TE, all show both new major and minor intensity bands, consistent with a period of dysregu-

lated recombination both before and immediately following commitment to clonal tumor cell expansion.

The colorectal tumor patients show a similar distribution of rDNA cluster instability as the lung cancer patients. Of the 10 colon cancer samples (Fig. 4), 4 appear stable (SAGOF-GR, FONET-VI, ARPIC-RO, and EBETH-DA), 5 more show preclonal expansion alterations (VOBLE-JO, DAPEB-MA, COBEZ-WI, RIZON-HE, and SNARG-GE), whereas 1 shows both new major and minor bands indicative of gene cluster restructuring both before and after tumor cell commitment (PULAB-GE). The process of metastasis does not necessarily involve recombinational gene cluster restructuring, as seen in the colon tumor from EBETH-DA, which was surgically recovered from a colon metastasis to lung. Likewise, it is notable that unlike the lung cancer patients, in nearly all of whom have a smoking history, rDNA cluster alterations are also seen in colon cancer patient tumors from either nonsmokers (RIZON-HE, PULAB-GE) or individuals with a relatively modest smoking history (VOBLE-JO).

The rectal cancers follow a similar pattern (Fig. 5). Two of four patients (CLIMP-HA and DOOVA-CL) show light stochastic breakage but are otherwise gene cluster stable. Two other patient tumors show evidence of either pre-expansion (BINTA-CH) or preclonal and postclonal expansion (WIVIT-HE) gene cluster instability. Our experience was that nontumor rectal tissue was difficult to work with; so generally, we compared tumor gene cluster structure to that from peripheral blood.

Architectural rearrangements in the rDNA clusters are less frequent in the 12 pediatric leukemias we have examined (Fig. 6).

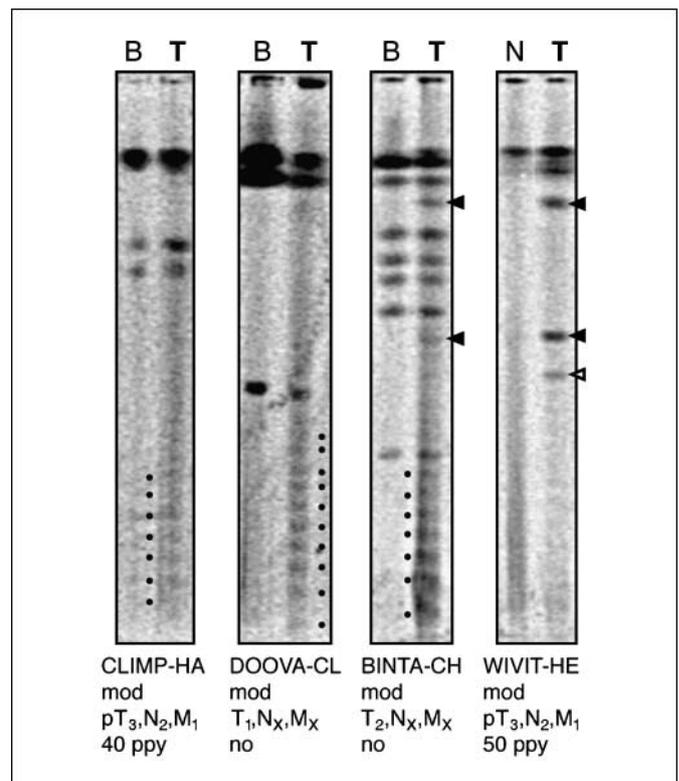


Figure 5. Rectal adenocarcinoma rDNA stability analysis. rDNA clusters resolved up to 1 Mb in length (★). N, nontumor tissue; T, tumor tissue; B, peripheral blood. •, sample fragility; ♠, new major bands found in tumors; ♣, new minor bands found in tumors. Coded patient names are indicated below each gel. Differentiation status of tumors: mod, moderately differentiated. Pathologic staging of nonmetastatic tumors is indicated. Smoking history is given in person-pack-years (ppy) or for a nonsmoker (no).

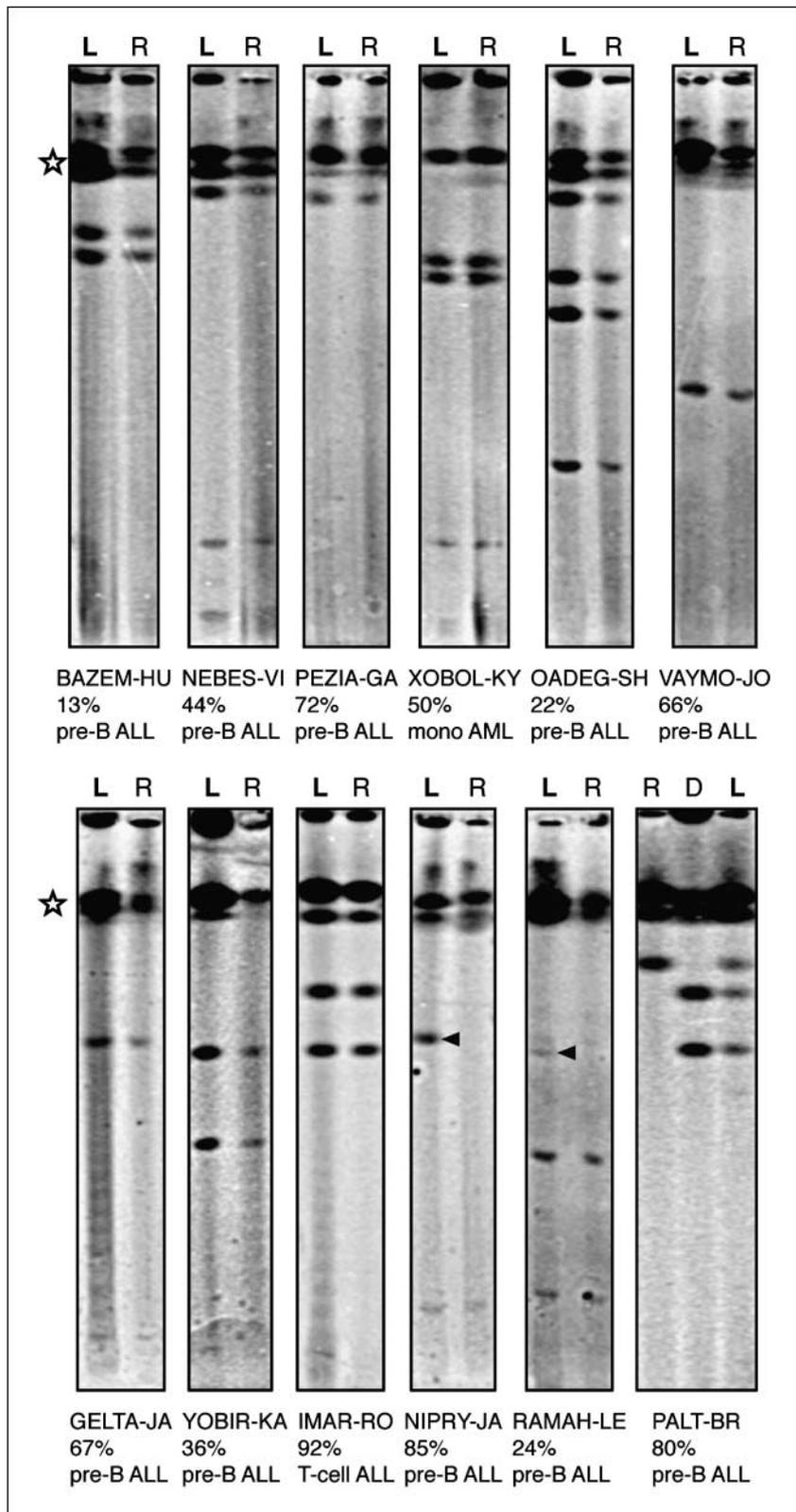


Figure 6. Pediatric leukemia rDNA stability analysis. rDNA clusters resolved up to 1 Mb in length (☆). *L*, leukemia: peripheral blood containing blasts; *R*, remission: peripheral blood posttreatment with no detectable blasts; *D*, donor: peripheral blood following engraftment of transplanted bone marrow from an allogeneic donor. Coded patient names are indicated below each gel. The percentage of blasts in leukemia samples is indicated. Leukemic classification: *pre-B ALL*, pre-B-cell acute lymphocytic leukemia; *mono ALL*, monocytic acute myelogenous leukemia; *T-cell ALL*, T-cell acute lymphocytic leukemia.

Comparing peripheral blood containing blasts before or shortly after the initiation of treatment to peripheral blood from the same patients in remission with no residual detectable blasts after recovery from induction chemotherapy shows the same pat-

tern of bands in 10 of the 12 different pediatric patients. Evidence of pre-expansion rearrangement is detected in NIPRY-JA and RAMAH-LE, with novel band intensities proportional to the fraction of blasts found in these patients' peripheral blood. PALT-BR is an

interesting, if tragic, case. We first encountered this 5-year-old boy in remission 3 years after a diagnosis of and treatment for pre-B-cell acute lymphocytic leukemia before his allogeneic stem cell transplant. After his transplant, his peripheral blood shows the rDNA cluster pattern of the engrafted donor marrow. Shortly after the transplant, the patient relapses and we see a re-establishment of his pretransplant rDNA cluster pattern 2 weeks before his death. Although this individual underwent years of intensive chemotherapy and full body irradiation, his disease shows no evidence of rDNA cluster instability (remission versus posttransplant leukemia rDNA cluster pattern, ignoring the bands from the donor marrow).

Discussion

Of the solid tumors analyzed for rDNA cluster instability, the overall frequency of any observed rDNA cluster alteration is 54% (21 of 39; 95% confidence interval, 39–68% by adjusted Wald method). Thirty-one percent (12 of 39) showed alterations consistent with preclonal expansion alterations only, and a further 23% (9 of 39) showed evidence of recombination-mediated rDNA cluster alterations both before the start of clonal tumor expansion as well as in the several rounds of cell division immediately following commitment to clonal expansion. No evidence of rDNA cluster instability was observed in 46% (18 of 39) of cases. Because the rDNA clusters range in size up to 6 Mb and we only analyzed clusters smaller than 1 Mb, the frequency of 54% observed gene cluster alterations is likely an underestimate.

Considering that the rDNA clusters only make up ~0.5% of the diploid human genome, this alteration frequency of over 50% in human solid tumors indicates that the rDNA clusters are extraordinarily sensitive indicators of the capacity for human cancer to use recombination to alter submicroscopic genomic structure, and that this submicroscopic genomic restructuring is one of the most frequent chromosomal aberrations found in nonhereditary solid tumors. In comparison, rDNA alteration in pediatric leukemia is only found in ~15% of cases. This frequency difference in cluster recombinational restructuring between pediatric leukemia and adult solid tumors ($P=0.04$ Fisher's two-tailed exact test) may reflect the adult tumors' progressive accumulation of genomic insults from both time and genotoxic environmental exposure, compared with more simple specific translocation-driven disease in the pediatric patients.

The rDNA clusters are critical components of cellular metabolism, so recombinational dysregulation in the rDNA may directly alter the capacity for tumor cell growth. The hypermetabolic nature of cancer cells requires expanded capacity for protein synthesis. Because rRNA production is rate limiting for the construction of new ribosomes, instability in the rDNA clusters will allow for positive selection of subpopulations of cancer cells that have expanded their rDNA complement. Upregulation of rRNA expression through epigenetic derepression is already known in lung cancer (19), making selectable gene amplification of the rRNA an additional effective mechanism for producing the large number of required ribosomes in hypermetabolic, relatively rapidly dividing tumor cells.

In addition to its direct contribution to tumor cell metabolism, instability of the rDNA may be indicative of broader recombination-based genomic instability in repetitive genomic elements and may serve as a sentinel biomarker for genomic alteration mediated by recombination between other high-similarity low-copy repeated sequences (20). For example, dysregulated recombination causing genomic restructuring of centromeric α -satellite repeats may result in centromeric dysfunction, contributing to the aneu-

ploidy commonly seen in adult solid tumors (21). Intriguingly, other highly self-similar clustered gene loci in the human genome (22) express protein products found with relative specificity only in cancer and in highly recombination-active tissues such as testis: the so-called "CT" genes (23). We are actively pursuing whether these loci are also recombinationally destabilized in cancer and with what potential clinical relevance.

From a predictive standpoint, because DNA double-strand breaks are potent inducers of homologous recombination in human cells (24) and many chemotherapeutic agents exert their genotoxic effects through DNA double-strand breaks, the use of these agents in tumors with dysregulated recombination may prove to be problematic. Dysregulated recombination in tumor cells may facilitate large-scale genomic restructuring in response to radiomimetic chemotherapy accelerating establishment of chemotherapeutic resistance. Similarly, exposure to genotoxic agents from tobacco in potentially numerous precancerous cells with dysregulated recombination may contribute to the synchronous dissimilar primary lung tumors sometimes found in heavy smokers.

We are continuing to follow and expand our enrolled patient population to ascertain whether assaying rDNA restructuring in tumor cells has prognostic and/or predictive value. Specifically, now that we can divide solid tumor cancer patients into two approximately evenly populated cohorts, based on whether tumors either do or do not display evidence of dysregulated recombination, we will determine whether dysregulated recombination is informative with regard to risk of tumor recurrence, with the disease-free survival interval postsurgery, and with overall survival. For patients that undergo adjuvant chemotherapy or radiation therapy postsurgery, we will also determine whether the degree of responsiveness to adjuvant therapy is influenced by the presence of dysregulated recombination in the primary tumors. The dysregulated recombination cohort can be further subdivided, based on the presence or absence of minor banding in the tumor sample, into patients for whom the tumor was still actively recombining the rDNA clusters during the initial phase of tumor clonal expansion and into those for whom dysregulated recombination appeared to be restricted to the interval before primary tumor clonal expansion, allowing further fine-tuning of the potential clinical application of the assay.

In summary, we have detected structural alterations to the rRNA gene clusters in over 50% of the adult solid tumors examined, making these genomic rearrangements one of the most common specific chromosomal alterations in adult solid tumors. The functional significance of the rDNA for the capacity of tumor cells to produce protein, as well as the potential sentinel biomarker nature of the rDNA for recombination-driven genomic alterations involving other highly conserved low-copy repetitive genomic elements, suggests assaying rDNA cluster instability in tumors may prove to have prognostic and/or predictive value.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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