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Original Article

Configuration and rearrangement of the human GAGE gene clusters

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Abstract: The GAGE protein is detected only in cancer and in testis and is expressed from a cluster of nearly identical gene copies on the X-chromosome. We determined the lengths of these GAGE gene clusters from human families, identical twins, and in clinical samples from cancer patients. The GAGE cluster lengths proved to be highly heterogeneous, ranging from 13 to 39 gene copies, with an average content of 20 GAGE genes per cluster. Low levels of meiotic rearrangement in families and mitotic rearrangement in adult solid tumors are detectable. Analysis of Rothmund -Thomson syndrome (RTS) kindreds and probands showed GAGE cluster inheritance and stability indistinguishable from that found in non-RTS individuals. These observations support the concept of evolutionarily rapid rearrangement of clustered repetitive sequences in the human genome.

Keywords: GAGE protein, human, gene clusters, configuration, rearrangement

Introduction

GAGE is a member of the CT family of genes, defined as having expression only in cancer and in testis (reviewed in [1]). The founding member of the class, MAGE, was identified in melanoma cells using a T cell epitope cloning technique [2]. Since that time the list of CT genes has expanded to include over 100 members [3] (http://www.cta.lncc.br/). Importantly, many of the protein products of the CT genes have proven to be immunoreactive. Since testis is an immunoprivileged site, this has opened the door to potential CT protein based immunotherapeutic strategies for cancer treatment. Indeed, several early phase clinical trials have already been conducted with promising results [4-8].

GAGE is a small, acidic protein of 117 amino acids (12.9 kDa) and has been found in 24% of melanoma samples, 25% of sarcomas, 19% of non-small cell lung cancers, 19% of head/neck tumors and 12% of bladder cancers (reviewed in [9]). GAGE transcripts are seen in melanoma [10, 11], lung cancer [10], thyroid carcinoma [12, 13], breast cancer [14], hepatocellular carcinomas [15] and ovarian cancer (30%) [16, 17]. Clinically, GAGE expression has been demonstrated to correlate with poor prognosis in stomach cancer [18], esophageal cancer [19] and neuroblastoma [20]. The GAGE protein has been characterized as having anti-apoptotic activity, conferring resistance to Fas-ligand, taxol and gamma irradiation [21]. GAGE expression also confers cellular resistance to killing by interferon gamma (IFN-γ through post-translational downregulation of interferon regulatory factor 1 (IRF1) with subsequent downregulation of caspases 1 and 7, and through stabilization of nucleophosomin [22].

The GAGE transcript has five spliced exons, the first of which is untranslated. The gene producing the GAGE transcript is multicopy, with a unit

gene repeat of 9,556 bp. Over one third of all CT genes have multiple gene copies in the genome, with 12 of the individual CT proteins expressed from at least six gene copies each [23]. The multiple copies of the GAGE gene are structured as a tandemly repeated cluster of at least 15 gene copies at Xp11.23. The repeated genes in the GAGE cluster are oriented in a head-to-tail manner, without any intervening sequences. The intimately clustered genomic structure is similar in nature to the clustered ribosomal RNA genes (rDNA) we have already characterized [24-26], as well as to other gene clusters elsewhere in the genome [27].

The GAGE cluster is "young," being found only in human, chimpanzee and macaque, is under apparent positive selection for protein function, and has undergone steady copy number expansion over the last 4 million years [28]. Other CT-genes on the X chromosome are similar: recent evolutionary additions diverging faster and under stronger selection than either CT-genes found on autosomes, or non-CT genes on the X chromosome [29]. In this work, we describe a physical assay we developed to measure the number of gene copies in each GAGE cluster. We used this assay with blood samples from human families, human cancer susceptibility (Rothmund-Thomson syndrome) kindreds and probands, and clinical specimens from human cancers to determine the range of human variation of the GAGE cluster, and the stability of the cluster under normal human meiosis and in the genomic pathology of human cancers.

Materials and methods

All human subjects provided informed written consent to participate in a research protocol approved by the Institutional Review Board for Human Subjects Research at the University of Kentucky (Lexington KY) with the exception of Rothmund-Thomson syndrome patients and family members who provided informed written consent to participate in a research protocol approved by the Institutional Review Board for Human Subjects Research at Baylor College of Medicine (Houston TX).

Cell lines derived from Rothmund-Thomson patients and their families were generated as described previously [30]. High molecular weight genomic DNA was prepared in the solid phase from cells, blood and tissues embedded in agarose as described previously [24, 26]. Genomic DNA was subjected to restriction digestion with StuI (New England Biolabs) unless otherwise noted, and loaded onto 1% PFC agarose (Bio-Rad) pulsed field gels. Gels were run in 0.5 x TBE (44.5 mM Tris base, 44.5 mM boric acid, 1 mM EDTA pH 8.0) for 20 hours with 6 V/cm electrical field vectors at 120° separation, with a switching frequency that varied linearly from 10" to 40", at 14C. Following electrophoresis, gels were equilibrated to 0.5% final concentration glycerol, dried, rehydrated and Southern blotted as described previously [25].

Southern probe templates specific to the GAGE cluster were isolated by PCR from human genomic DNA either with the primer set 1: GAGE-1F: GTCCTCCTTCCCTTCACAGG and GAGE-1R: TTCTCGTGATTGCTGCTTTG or with primer set 2: GAGE-2F: AGACCCAGTTCAGAGGAGCA and GAGE -2R: CGTGAAGAACAAAGCACCAA.

Radiolabeled Southern probes were generated by PCR amplification of the template products derived from human genomic DNA described above using the appropriate primer sets. PCR conditions were listed as **Table 1**.

Results

Our experimental strategy is shown in **Figure 1A**.

Table 1. PCR conditions

2.0 µl TAQ buffer (10x)	94°C 30"
2.0 µl PCR product @ 10 pg/µl	65°C 30"
2.0 µl forward primer @ 1 µM	72°C 1' + 2"/cycle
2.0 µl reverse primer @ 1 µM	40 cycles total
5.0 µl α^{32}P-dATP (50 µCi @ 3000 Ci/mmol)	72°C 7'
2.0 µl d(GTC)TP @ 40 µM each	hold at 4C
1.0 µl dATP @ 20 µM (not radioactive)	
0.4 µl TAQ (2U)	
3.6 µl H_2O	

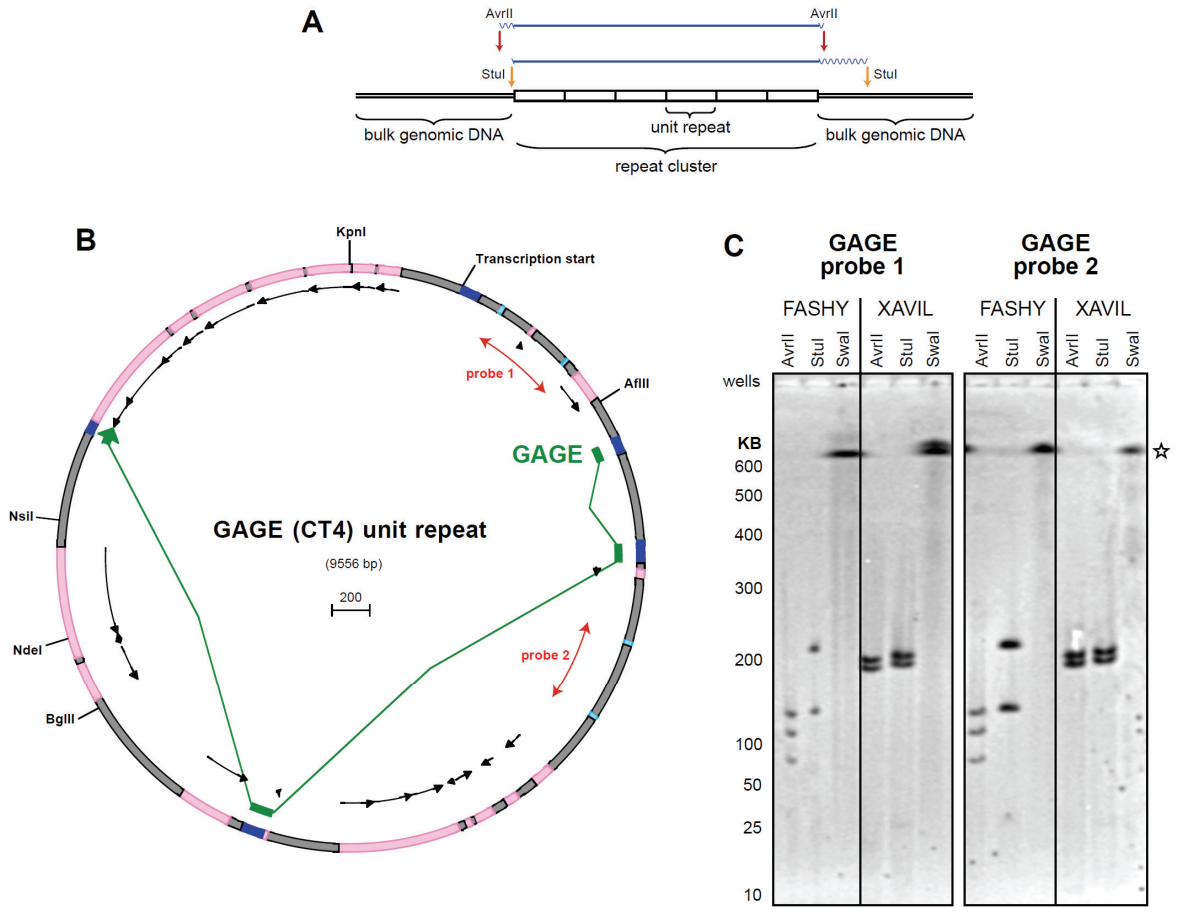

Figure 1. Human GAGE gene clusters. **A**) Experimental schematic showing a model GAGE cluster containing six gene copies. Restriction digestion with enzymes that do not have a recognition site in the unit gene repeat will liberate entire gene clusters (straight blue lines), with a small amount of additional flanking-sequence DNA (wavy blue lines). Open rectangles: unit GAGE clusters; double black lines: non-GAGE genomic DNA. **B**) GAGE unit gene repeat. Transcription proceeds clockwise from the indicated transcription start site. Exons: thick green bars; introns: thin green bars; high copy genomic repeats: pink regions and thin arrows; unique genomic sequences: black bars. The positions of Southern blot probe 1 and probe 2 are indicated by red arrows. **C**) Southern blot of GAGE clusters liberated from genomic DNA by the indicated restriction enzymes and detected with the indicated Southern probe. Digests were overnight: AvrII: 12 units at 37C, StuI: 20 units at 37C, SwaI: 20 units at room temperature. Genomic DNA isolated from the peripheral blood from two different unrelated female donors (FASHY, XAVIL). Open star: unresolved DNA due to inefficient digestion with the SwaI enzyme.

For tandemly repeated genes with very high sequence identity, the gene cluster can be liberated from bulk genomic DNA by digestion with a restriction enzyme that lacks a recognition site in the unit gene repeat. The liberated cluster can be size-resolved by pulsed field gel electrophoresis, detected though Southern hybridization, and the number of gene copies inferred from the length of the cluster with a small correction for flanking non-cluster DNA. Each repeat of the GAGE gene is 9556 bp. We initially selected two different Southern probes with predicted minimal cross-reactivity to the reference human genome, and three different restriction enzymes, AvrII, StuI and SwaI to liberate the GAGE cluster from bulk genomic DNA (**Figure 1B**). Anticipating that evolutionarily rapid GAGE cluster restructuring due to homologous recombination would cause a diversity of cluster lengths in the human population, we assayed genomic DNA from two human females, with the prediction that each X-chromosome would have a different sized GAGE cluster and thereby show two different length

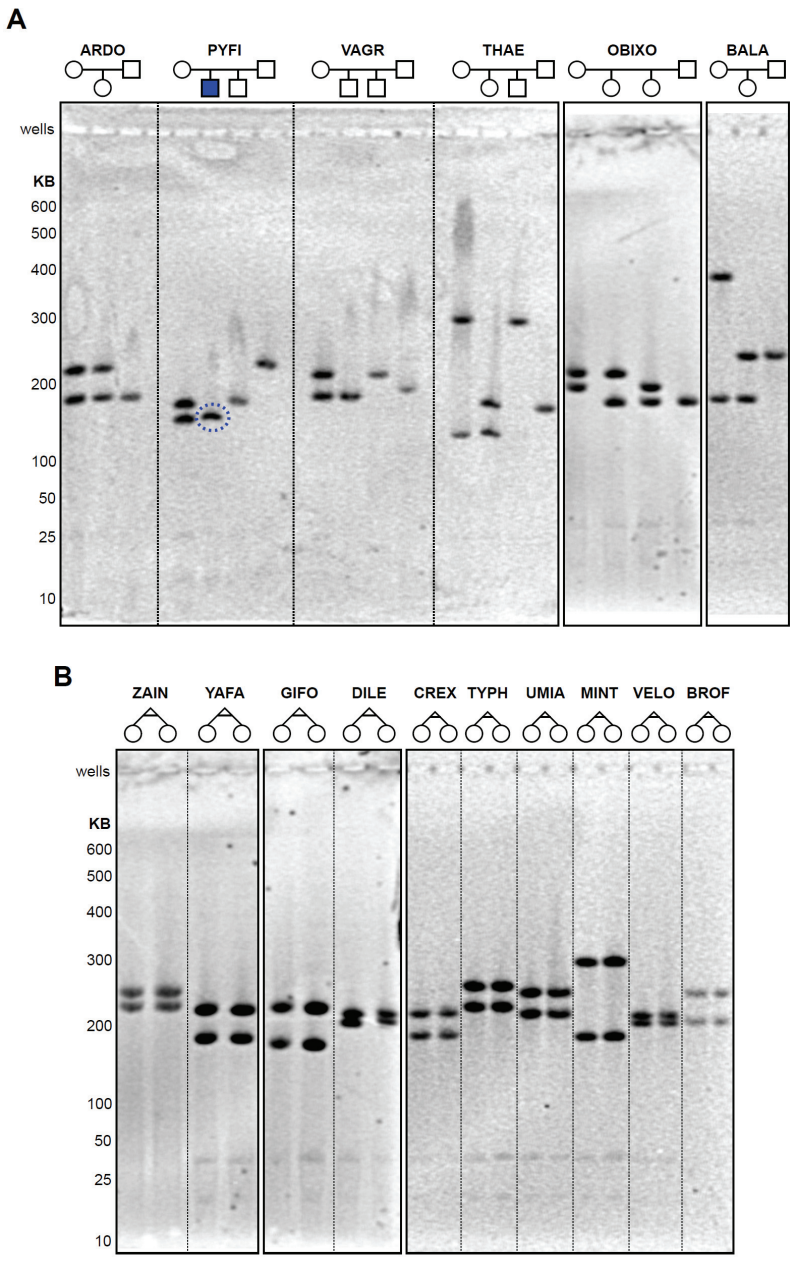

Figure 2. GAGE clusters in families and identical twins. **A**) Families (all names are coded). Circles: females, squares: males. A potential meiotic recombination event (dotted blue oval) is shown in the first PYFI son (blue square). **B**) 10 sets of female identical twins.

clusters, in a manner similar to the 5S rDNA gene cluster at 1q42 [25]. We were able to resolve these two predicted GAGE clusters (**Figure 1C**) using either AvrII or StuI (SwaI failed to efficiently digest the agarose-embedded genomic DNA), and with either Southern probe. The three bands seen with AvrII digestion in the FASHY individual suggests that the GAGE repeats do not share perfect sequence identity with each other, such that a cryptic AvrII site can exist within the cluster, a phenomenon we also observed in analysis of the 5S rDNA clusters. The non-GAGE flanking sequence according to the Human Genome Project is 3859 bp when the clusters are liberated by AvrII digestion (2828 bp telomeric and 1031 bp centromeric with respect to the GAGE cluster) and 9781 bp when liberated by StuI digestion (537 bp telomeric and 9244 bp centromeric with respect to the GAGE cluster). Consistent with the predicted smaller amount of flanking sequence after cluster liberation by AvrII relative to StuI, we observed that the GAGE clusters identified by Southern blotting are slightly shorter when liberated by AvrII than by StuI (**Figure 1C**). After verifying the DNA sequence of probe 2, we selected StuI and probe 2 as an optimal combination for further experiments.

To establish the range of normal human variation in GAGE cluster lengths and to determine the degree of meiotic cluster rearrangement, we analyzed the GAGE cluster lengths in human families (**Figure 2A**). We found strong heterogeneity in the lengths of the GAGE cluster ranging from 135 kb up to 380 kb, corresponding to from 13 to 39 gene copies, after subtracting the predicted 9.7 kb of non-GAGE flanking DNA, with a median cluster length of approximately 20 GAGE repeats. Females generally show two distinct GAGE clusters and males only one, consistent with the X-linkage of the cluster. We observed primarily predicted patterns of Mendelian inheritance without rear-

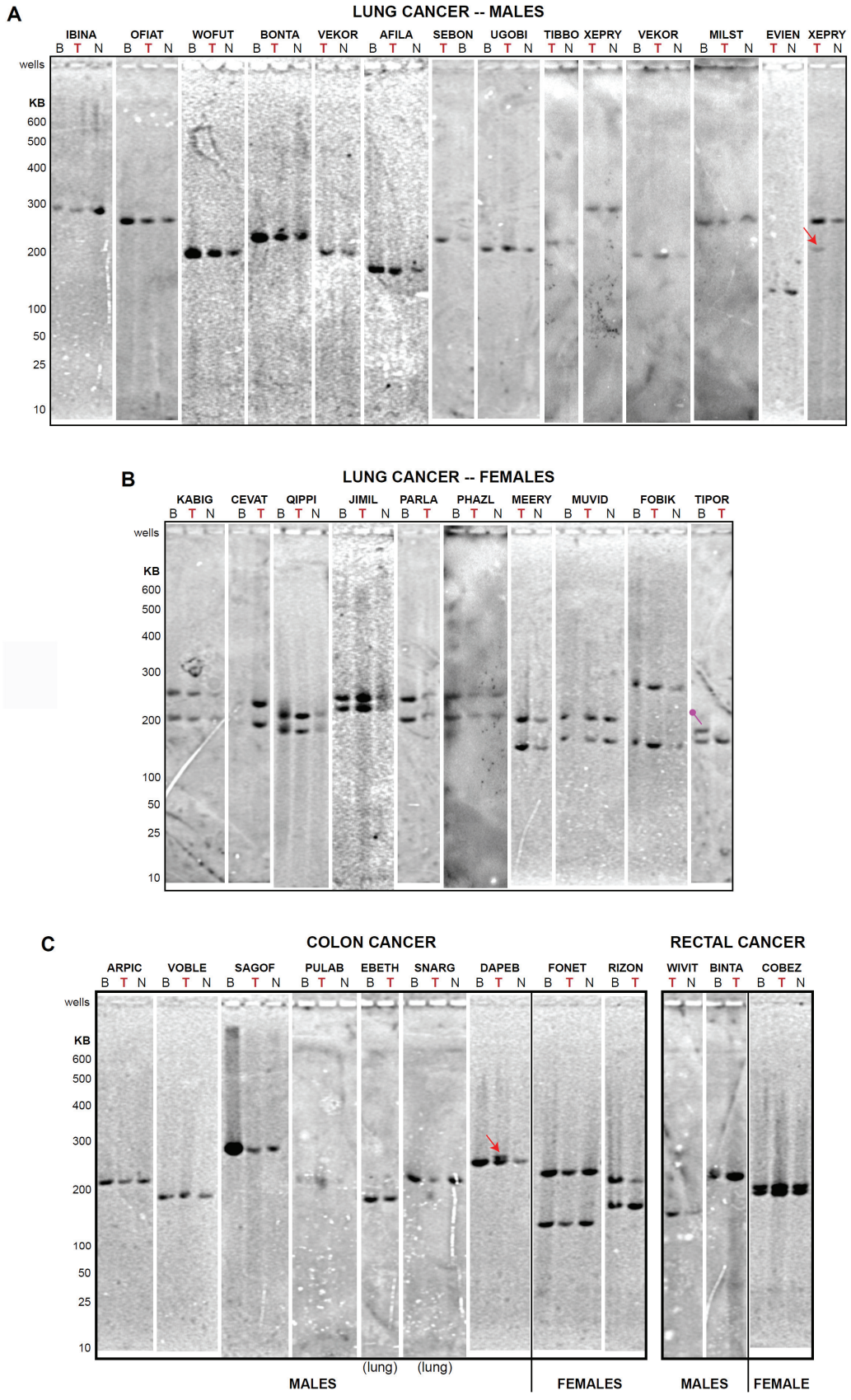

Figure 3. GAGE clusters in adult solid tumors. **A**) Lung cancer in males. B: blood, T: tumor, N: non-tumor surrounding tissue. A mitotic recombination event is shown in the XEPRY tumor sample (red arrow). **B**) Lung cancer in females: B: blood, T: tumor, N: non -tumor surrounding tissue. Loss of one allele seen in the peripheral blood of TIPOR is indicated (red pushpin). **C**) Colorectal cancer. B: blood; T: tumor; N: non-tumor surrounding tissue. A mitotic recombination even that altered GAGE cluster length is seen in the DAPEB tumor sample (red arrow).

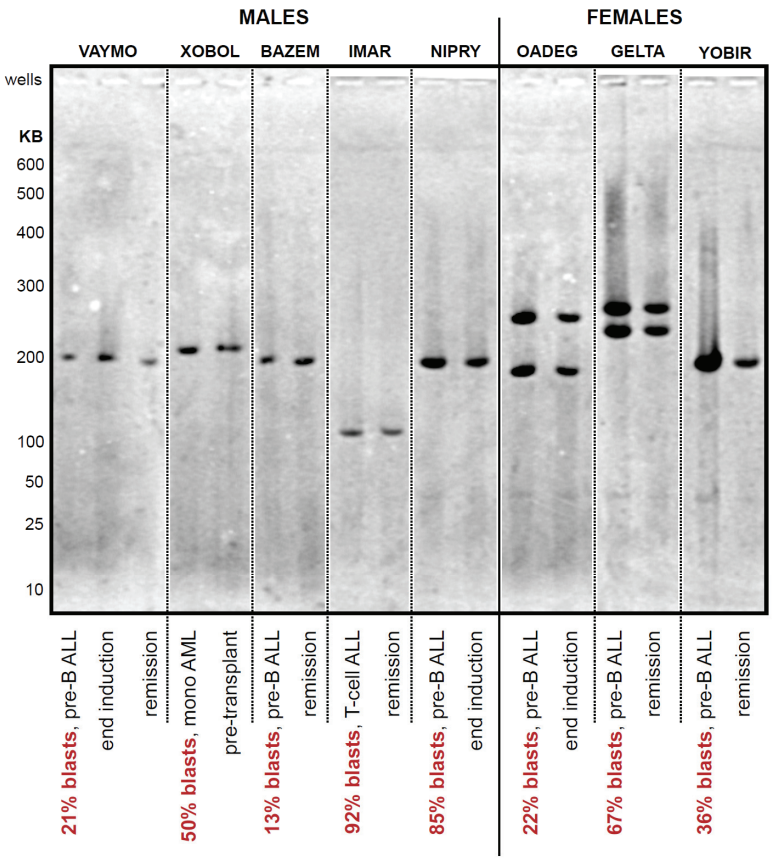

Figure 4. GAGE clusters in pediatric leukemia. Peripheral blood either with or without blasts is analyzed for GAGE clusters by Southern blotting. No alterations between the blasts and the normal cells were detected.

rangement, with the exception of one potential meiotic recombination event in the female germline of the PYFI family (**Figure 2A**, blue dotted circle). To further establish the reproducibility of the assay, we determined GAGE cluster lengths in 10 sets of female identical twins. In all cases, the twins shared the identical GAGE cluster configuration (**Figure 2B**).

Next we sought to determine whether the GAGE cluster was stable under potentially elevated recombination rates in human cancer. We compared the GAGE cluster configuration in individual human lung and colorectal tumors to matched control DNA from the same individuals isolated from either peripheral blood or surgically excised pathologically confirmed non-tumor tissue. In one male lung cancer (**Figure 3A**, 'XEPRY') we observe evidence of mitotic rearrangement generating a new GAGE cluster length in the tumor not seen in the surrounding non-tumor tissue. We observed the same mitotic rearrangement phenomenon in one of the colon cancers (**Figure 3C**, 'DAPEB'). Otherwise the GAGE cluster configuration in tumors was comparable to that in normal tissues, with the exception of loss of one GAGE cluster in a female lung cancer (**Figure 3B**, 'TIPOR'), likely due to X-chromosome aneuploidy. The two confirmed mitotic events in these 36 solid tumor sets would indicate a detectable rearrangement frequency of around 5%.

In order to assess potential GAGE restructuring in pediatric leukemia, in addition to the adult solid tumors described above, we compared the GAGE cluster genomic architecture between peripheral blood containing blasts and peripheral blood from the same patients after their disease was in remission. We detected no differences in the GAGE cluster configurations between disease-containing and disease-free blood sample from eight different pediatric patients (**Figure 4**).

Finally we sought to extend our analysis of GAGE architecture to families with hereditary cancer syndromes. We assayed GAGE architecture in EBV-immortalized lymphocytes from eight kindreds containing Rothmund-Thomson syndrome Type 2 probands, and from four Rothmund-Thomson syndrome Type 1 probands (**Figure 5**). We found that GAGE cluster lengths segregated following the normal Mendelian manner in the kindreds, and that the immortalized cells from Rothmund-Thomson probands displayed well-defined stable banding patterns.

Discussion

We found that the normal human variation in GAGE gene copy number is from 13 to 39 GAGE gene repeats on each X chromosome. This is

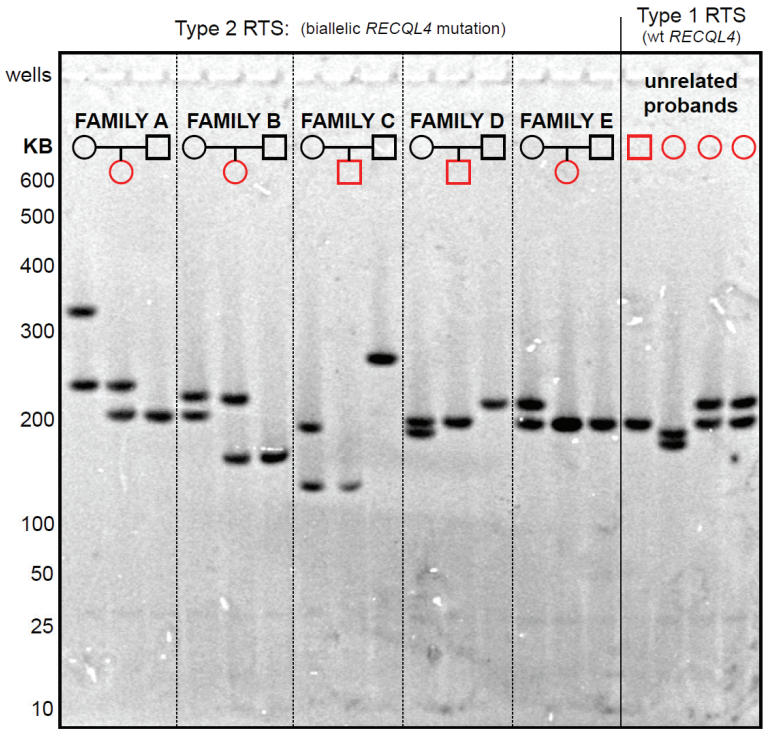

Figure 5. GAGE clusters in Rothmund-Thomson kindreds and probands. Circles: females, squares: males. RTS probands indicated in red.

considerably less variation that we had earlier found in the 45S ribosomal RNA genes that varied from 1 to over 140 copies per cluster [25]. The GAGE clusters also recombine with reduced frequency relative to the rDNA clusters. There are several plausible factors that may account for this reduced frequency of rearrangement: the GAGE unit repeat at 9.6 kb is shorter than the 43 kb rDNA repeat and there are only one or two GAGE clusters per genome (in males and females respectively) compared to the ten rDNA clusters. Nevertheless, the high degree of interperson GAGE cluster heterogeneity indicates that the GAGE clusters are restructuring rapidly in evolutionary time, even if less rapidly than the rDNA. In a similar manner, although we can detect GAGE cluster alterations in adult solid tumors at a frequency of around 5%, this is considerably less than the greater than 50% frequency of rDNA restructuring we observed in human adult solid tumors. Interestingly, both of the cancer patients in which we detected GAGE cluster restructuring also exhibited rDNA cluster restructuring in their disease, suggesting a potential mechanistic link between the two processes [26].

Rothmund-Thomson syndrome arising as a result of mutations in *RECQL4* (RTS type 2) confers an increased cancer risk, particularly of osteosarcoma [30, 31]. As RECQL4 is a member of the RecQ family of proteins associated with altered replication and recombination phenotypes [32], we hypothesized that a potentially recombination-prone genomic locus like GAGE might display accelerated instability in lines defective for RECQL4. Counter to this hypothesis, we found that cells from Rothmund-Thomson probands either with *RECQL4* defects (RTS type 2) or without *RECQL4* defects (RTS type 1) display well-defined discrete banding patterns with no overt evidence of instability. GAGE clusters inherited from parents heterozygous for RECQL4 mutation segregated in the normal Mendelian manner.

Conclusions

The average human GAGE gene cluster length exhibits strong heterogeneity, varying from 13 to 39 gene copies, with an average of approximate 20 copies per X-chromosome. Both meiotic and mitotic alterations to GAGE cluster length occur at detectable frequencies.

Acknowledgments

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References

[1] Scanlan MJ, Simpson AJ and Old LJ. The can-

cer/testis genes: review, standardization, and commentary. Cancer Immun 2004; 4: 1.
[2] van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A and Boon T. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science 1991; 254: 1643-1647.
[3] Almeida LG, Sakabe NJ, deOliveira AR, Silva MC, Mundstein AS, Cohen T, Chen YT, Chua R, Gurung S, Gnjatic S, Jungbluth AA, Caballero OL, Bairoch A, Kiesler E, White SL, Simpson AJ, Old LJ, Camargo AA and Vasconcelos AT. CTdatabase: a knowledge-base of high-throughput and curated data on cancer-testis antigens. Nucleic Acids Res 2009; 37: D816-819.
[4] Davis ID, Chen W, Jackson H, Parente P, Shackleton M, Hopkins W, Chen Q, Dimopoulos N, Luke T, Murphy R, Scott AM, Maraskovsky E, McArthur G, MacGregor D, Sturrock S, Tai TY, Green S, Cuthbertson A, Maher D, Miloradovic L, Mitchell SV, Ritter G, Jungbluth AA, Chen YT, Gnjatic S, Hoffman EW, Old LJ and Cebon JS. Recombinant NY-ESO-1 protein with ISCOMATRIX adjuvant induces broad integrated antibody and CD4(+) and CD8(+) T cell responses in humans. Proc Natl Acad Sci U S A 2004; 101: 10697-10702.
[5] Jager E, Karbach J, Gnjatic S, Neumann A, Bender A, Valmori D, Ayyoub M, Ritter E, Ritter G, Jager D, Panicali D, Hoffman E, Pan L, Oettgen H, Old LJ and Knuth A. Recombinant vaccinia/fowlpox NY-ESO-1 vaccines induce both humoral and cellular NY-ESO-1-specific immune responses in cancer patients. Proc Natl Acad Sci U S A 2006; 103: 14453-14458.
[6] Marchand M, van Baren N, Weynants P, Brichard V, Dreno B, Tessier MH, Rankin E, Parmiani G, Arienti F, Humblet Y, Bourlond A, Vanwijck R, Lienard D, Beauduin M, Dietrich PY, Russo V, Kerger J, Masucci G, Jager E, De Greve J, Atzpodien J, Brasseur F, Coulie PG, van der Bruggen P and Boon T. Tumor regressions observed in patients with metastatic melanoma treated with an antigenic peptide encoded by gene MAGE-3 and presented by HLA-A1. Int J Cancer 1999; 80: 219-230.
[7] Odunsi K, Qian F, Matsuzaki J, Mhawech-Fauceglia P, Andrews C, Hoffman EW, Pan L, Ritter G, Villella J, Thomas B, Rodabaugh K, Lele S, Shrikant P, Old LJ and Gnjatic S. Vaccination with an NY-ESO-1 peptide of HLA class I/II specificities induces integrated humoral and T cell responses in ovarian cancer. Proc Natl Acad Sci U S A 2007; 104: 12837-12842.
[8] Uenaka A, Wada H, Isobe M, Saika T, Tsuji K, Sato E, Sato S, Noguchi Y, Kawabata R, Yasuda T, Doki Y, Kumon H, Iwatsuki K, Shiku H, Monden M, Jungbluth AA, Ritter G, Murphy R, Hoffman E, Old LJ and Nakayama E. T cell immunomonitoring and tumor responses in patients immunized with a complex of cholesterol-bearing hydrophobized pullulan (CHP) and NY-ESO-1 protein. Cancer Immun 2007; 7: 9.
[9] Gjerstorff MF and Ditzel HJ. An overview of the GAGE cancer/testis antigen family with the inclusion of newly identified members. Tissue Antigens 2008; 71: 187-192.
[10] De Backer O, Arden KC, Boretti M, Vantomme V, De Smet C, Czekay S, Viars CS, De Plaen E, Brasseur F, Chomez P, Van den Eynde B, Boon T and van der Bruggen P. Characterization of the GAGE genes that are expressed in various human cancers and in normal testis. Cancer Res 1999; 59: 3157-3165.
[11] Eichmuller S, Usener D, Jochim A and Schadendorf D. mRNA expression of tumor-associated antigens in melanoma tissues and cell lines. Exp Dermatol 2002; 11: 292-301.
[12] Maio M, Coral S, Sigalotti L, Elisei R, Romei C, Rossi G, Cortini E, Colizzi F, Fenzi G, Altomonte M, Pinchera A and Vitale M. Analysis of cancer/testis antigens in sporadic medullary thyroid carcinoma: expression and humoral response to NY-ESO-1. J Clin Endocrinol Metab 2003; 88: 748-754.
[13] Ruschenburg I, Kubitz A, Schlott T, Korabiowska M and Droese M. MAGE-1, GAGE-1/-2 gene expression in FNAB of classic variant of papillary thyroid carcinoma and papillary hyperplasia in nodular goiter. Int J Mol Med 1999; 4: 445-448.
[14] Mischo A, Kubuschok B, Ertan K, Preuss KD, Romeike B, Regitz E, Schormann C, de Bruijn D, Wadle A, Neumann F, Schmidt W, Renner C and Pfreundschuh M. Prospective study on the expression of cancer testis genes and antibody responses in 100 consecutive patients with primary breast cancer. Int J Cancer 2006; 118: 696-703.
[15] Kobayashi Y, Higashi T, Nouso K, Nakatsukasa H, Ishizaki M, Kaneyoshi T, Toshikuni N, Kariyama K, Nakayama E and Tsuji T. Expression of MAGE, GAGE and BAGE genes in human liver diseases: utility as molecular markers for hepatocellular carcinoma. J Hepatol 2000; 32: 612-617.
[16] Gillespie AM, Rodgers S, Wilson AP, Tidy J, Rees RC, Coleman RE and Murray AK. MAGE, BAGE and GAGE: tumour antigen expression in benign and malignant ovarian tissue. Br J Cancer 1998; 78: 816-821.
[17] Russo V, Dalerba P, Ricci A, Bonazzi C, Leone BE, Mangioni C, Allavena P, Bordignon C and Traversari C. MAGE BAGE and GAGE genes expression in fresh epithelial ovarian carcinomas. Int J Cancer 1996; 67: 457-460.
[18] Kong U, Koo J, Choi K, Park J and Chang H. The expression of GAGE gene can predict aggressive biologic behavior of intestinal type of stomach cancer. Hepatogastroenterology 2004; 51: 1519-1523.
[19] Zambon A, Mandruzzato S, Parenti A, Macino B, Dalerba P, Ruol A, Merigliano S, Zaninotto G and Zanovello P. MAGE, BAGE, and GAGE gene

expression in patients with esophageal squamous cell carcinoma and adenocarcinoma of the gastric cardia. Cancer 2001; 91: 1882-1888.
[20] Cheung IY, Chi SN and Cheung NK. Prognostic significance of GAGE detection in bone marrows on survival of patients with metastatic neuroblastoma. Med Pediatr Oncol 2000; 35: 632-634.
[21] Cilensek ZM, Yehiely F, Kular RK and Deiss LP. A member of the GAGE family of tumor antigens is an anti-apoptotic gene that confers resistance to Fas/CD95/APO-1, Interferon-gamma, taxol and gamma-irradiation. Cancer Biol Ther 2002; 1: 380-387.
[22] Kular RK, Yehiely F, Kotlo KU, Cilensek ZM, Bedi R and Deiss LP. GAGE, an antiapoptotic protein binds and modulates the expression of nucleophosmin/B23 and interferon regulatory factor 1. J Interferon Cytokine Res 2009; 29: 645-655.
[23] Hofmann O, Caballero OL, Stevenson BJ, Chen YT, Cohen T, Chua R, Maher CA, Panji S, Schaefer U, Kruger A, Lehvaslaiho M, Carninci P, Hayashizaki Y, Jongeneel CV, Simpson AJ, Old LJ and Hide W. Genome-wide analysis of cancer/testis gene expression. Proc Natl Acad Sci U S A 2008; 105: 20422-20427.
[24] Killen MW, Stults DM, Adachi N, Hanakahi L and Pierce AJ. Loss of Bloom syndrome protein destabilizes human gene cluster architecture. Hum Mol Genet 2009; 18: 3417-3428.
[25] Stults DM, Killen MW, Pierce HH and Pierce AJ. Genomic architecture and inheritance of human ribosomal RNA gene clusters. Genome Res 2008; 18: 13-18.
[26] Stults DM, Killen MW, Williamson EP, Hourigan JS, Vargas HD, Arnold SM, Moscow JA and Pierce AJ. Human ribosomal RNA gene clusters are recombinational hotspots in cancer. Cancer Res 2009; 69: 9096-9104.
[27] Warburton PE, Hasson D, Guillem F, Lescale C, Jin X and Abrusan G. Analysis of the largest tandemly repeated DNA families in the human genome. BMC Genomics 2008; 9: 533.
[28] Liu Y, Zhu Q and Zhu N. Recent duplication and positive selection of the GAGE gene family. Genetica 2008; 133: 31-35.
[29] Stevenson BJ, Iseli C, Panji S, Zahn-Zabal M, Hide W, Old LJ, Simpson AJ and Jongeneel CV. Rapid evolution of cancer/testis genes on the X chromosome. BMC Genomics 2007; 8: 129.
[30] Wang LL, Gannavarapu A, Kozinetz CA, Levy ML, Lewis RA, Chintagumpala MM, Ruiz-Maldanado R, Contreras-Ruiz J, Cunniff C, Erickson RP, Lev D, Rogers M, Zackai EH and Plon SE. Association between osteosarcoma and deleterious mutations in the RECQL4 gene in Rothmund-Thomson syndrome. J Natl Cancer Inst 2003; 95: 669-674.
[31] Siitonen HA, Sotkasiira J, Biervliet M, Benmansour A, Capri Y, Cormier-Daire V, Crandall B, Hannula-Jouppi K, Hennekam R, Herzog D, Keymolen K, Lipsanen-Nyman M, Miny P, Plon SE, Riedl S, Sarkar A, Vargas FR, Verloes A, Wang LL, Kaariainen H and Kestila M. The mutation spectrum in RECQL4 diseases. Eur J Hum Genet 2009; 17: 151-158.
[32] Bohr VA. Rising from the RecQ-age: the role of human RecQ helicases in genome maintenance. Trends Biochem Sci 2008; 33: 609-620.