Repetitive sequences, genomic instability and Barrett's esophageal adenocarcinoma

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Barrett's esophageal adenocarcinoma (BAC) is a cancer associated with heartburn. If gastroesophageal reflux is not treated, the exposure to acid over the years, leads to a premalignant condition known as Barrett's esophagus (BE) which then progresses through low grade and high grade dysplasias to Barrett's adenocarcinoma. Genomic instability, which seems to arise early at BE stage, leads to accrual of mutational changes which underlie the the succession of histological and physiological changes associated with this disease. Genomic instability is therefore an important target for prevention and treatment of cancer and it is important to elucidate the mechanisms associated with this problem. We have shown that elevated/deregulated homologous recombination mediates genomic instability in cancer. Recently we also demonstrated that the mutational rates of individual chromosomes in BAC cells correlate with their ALU frequency. The aims of this article are to briefly discuss different types of repetitive sequences and highlight their importance in physiology of normal and cancer cells, especially BAC.

Introduction

A substantial fraction of eukaryotic genome is comprised of repetitive DNA sequences.¹ These sequences can occur as tandem repeats or interspersed repeat sequences and have high degree of variation.² Tandem repeats are multiple copies of homologous DNA elements inserted next to each other, whereas interspersed repeats are homologous DNA fragments of 20–30 kbp inserted

at random throughout genome. Tandem repeats can be classified as large satellites, minisatellites (1–5 kb, 20–50 repeats) or microsatellites (short tandem repeats, 1–6 bp, repeating 3–100 times), depending on the size of repeat. Interspersed repeats are usually copies of transposition elements, with ability to get reproduced and copies inserted at other places in the genome. These transposon elements can be either autonomous or non autonomous.

Tandem Repeats

The term satellite is derived from specific mobility of repetitive DNA sequences during density gradient centrifugation. When DNA is subjected to density gradient centrifugation, repetitive sequences containing different AT or GC content form satellite bands, distinct from rest of the DNA. Satellites vary in the number of bases being repeated and total length of the repeat. Number of bases in primary repeat unit can vary from 2 to 171. A number of satellites are located at or around centromere and play important role in cell division by helping the assembly of kinetochore. Tandem repeat sequences can be classified as satellites, minisatellites or microsatellites.

Satellite DNA. These are large stretches of repetitive sequences found in centromere region of eukaryotic chromosomes.³ The satellite DNA can vary in size ranging from 100 kb to more than 1 Mb. Centromere related alphoid DNA in humans, with a repeat unit of 171 bp, is a good example of satellite DNA.⁴

Minisatellites. These are highly variable tandem repeats,⁵ smaller in size

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relative to satellites. Typical minisatellites have a repeat unit of 6–100 bp, with total length of 500 bp to several kilo base pairs (kbp). Because of high degree variation in length, minisatellites are also known as VNTR (variable number of tandem repeats) and often used as genetic markers. In human cells, minisatellites are also considered to be “hotspots” of homology based DNA recombination.⁶ Human subtelomeric region, enriched with minisatellites, is “hotspot” for genomic rearrangements associated with homologous recombination.⁷ Similarly, the hexameric TTA GGG repeats of human telomeric DNA are also minisatellites. Telomeres are added by telomerase, a reverse transcriptase with RNA subunit which has a sequence complementary to TTA GGG and is used as template for telomere expansion. Protein subunit of telomerase is a non-LTR retrotransposon type reverse transcriptase.

Microsatellites. These are repetitive sequences with short repeat unit and extremely high variability.⁸ Repeat unit in microsatellite varies from 1–5 bp. The number of these repeats is variable but rarely exceeds hundreds of units and used in genetic identification. Most microsatellites contain 2 bp repeat units, usually comprised of (CA)*n* repeats.

Interspersed Repeats (Transposable Elements)

Interspersed repeats are mostly transposable elements which are capable of moving within genome. There are two types of transposable elements, DNA transposons and retrotransposons.⁹

DNA transposon elements. DNA transposons, although active in evolution millions of years ago, are now inactive and therefore only their ancient remnants can be found in the human genome.^{9–11} Typically, the DNA transposons move within a genome in a “cut and paste” mechanism. They code for a transposase which cuts the transposon out and then ligates the resulting free ends of chromosomal DNA. The excised transposon binds to the transposase and resulting transposon-transposase complex then moves to a new location in the genome and binds to a specific sequence site. The transposase then cuts the host DNA and

inserts the transposon into new location. Recently other types of transposon elements known as Helitrons or rolling-circle and Mavericks or self-replicating transposons have also been identified.¹⁰

Retrotransposons. Retrotransposons are the most abundant and important transposons as $\geq 45\%$ of our genome is made of these elements which are active.^{11,12} These sequences move through an RNA intermediate. The process requires host RNA polymerases (II or III), which transcribe the retrotransposons into RNA which is reverse transcribed and the resulting DNA is then inserted into a new location in the genome. Thus the parent transposon never leaves its original site and these mobile elements get duplicated with each move. However the process of retrotransposition is very commonly associated with mistakes such as point mutations and/or truncations, therefore the newly inserted copies of transposons are mostly inactive.

Retrotransposons containing long terminal repeats (LTRs), known as LTR retrotransposons, are endogenous retroviruses containing env, gag, prt and pol genes localized in between two LTRs, a composition similar to proviruses.⁹ Deletions or other mutations in env or one of the genes required for assembly of infectious viral particles confine their movement to only within a cell. These retrotransposons which comprise about $\sim 8\%$ of the genome are inactive in human although capable of transposition in several other mammals. Although the DNA of intact endogenous retroviruses can range from 7–9 kb in length, they are frequently truncated to various extents. Since homologous recombination between LTRs can excise the internal DNA, they can also exist as LTR alone. Retrotransposons which code for proteins required for transposition are called autonomous whereas those which do not code for any such protein and depend on proteins from host and other transposons, are known as non autonomous.⁹

Long interspersed nuclear elements (LINES). LINES are non-LTR autonomous retrotransposons, comprising $\sim 21\%$ of the genome in humans.⁹ The most common member of this type of retrotransposons is LINE-1 (L-1) with half a million copies interspersed throughout genome.^{9,12}

Around one hundred of the LINE-1 elements are active; they are ~ 6 kbp in length and contain a 5' UTR (untranslated region) with promoter function, two open reading frames (ORF1 and ORF2), and 3' UTR containing polyA signal. L1 is transcribed by host-cell RNA polymerase II, the transcript moves to cytoplasm, both ORF1 and ORF2 (reverse transcriptase and endonuclease) are translated and both proteins bind to L-1 mRNA. Protein-mRNA complex then moves back to nucleus, the ORF2 endonuclease cuts the DNA at a relatively specific AT rich sequence, the reverse transcriptase domain of the enzyme uses free 3'-OH terminus of cleaved DNA to synthesize cDNA from mRNA. Following synthesis of second strand of cDNA, the double-stranded cDNA is inserted into the new site in genome. Transposition of L-1 is associated with several genomic changes including duplication of target site because of the stagger cut made by endonuclease and other mutations due to the inability of reverse transcriptase to proof read.

Short interspersed nuclear elements (SINES). SINES are nonautonomous retrotransposons, less than 500 bp in length and do not code for any protein.¹³ The most prominent members of SINE family are the Alu elements; more than one million alu elements, ~ 300 bp in length, comprise $\sim 11\%$ of the genome. A 282 base pair consensus sequence in Alus is thought to be derived from RNA subunit of signal recognition particle, known as 7SL RNA. Alus, transcribed by cellular RNA polymerase III can bind to signal recognition particles and subsequently to ribosomes, and are also capable of directing ORF2 protein of LINE-1 to reverse transcribe and insert Alu elements instead of LINE1.

Role of Transposable Elements

Transposable elements do not seem to have any specific role in cellular physiology, however contribute to altered gene functions, inherited disorders, evolution and adaptability.^{9,11,12,14,15} Insertion of LTR or 5' UTR of LINE element near a gene can alter its expression as these elements have a strong promoter activity in each direction. In rare instances, a cellular mRNA can also be reverse transcribed and inserted

in the genome by L-1 elements, leading to gene duplication. Duplicated gene is usually inactive as it lacks the promoter but can get activated by subsequent events, although happens very rarely. Inactive transposon elements can also contribute to genome plasticity through interchromosomal or intrachromosomal homologous recombination between them. The fact that transposon expression is increased under stress indicates that transposons may also have specific physiological role¹⁶ which is currently unknown.

Low Copy Repeat Sequences

Low-copy repeats (LCRs) are highly (95% to 97%) homologous sequences, ranging in size from 10 to 300 kb^{17,18} and are believed to arise by segmental duplication in eukaryotic genome.^{18,19} These sequences, because of their homology, become a source of genomic rearrangements. Alignment of non-allelic copies of LCRs instead of the allelic and subsequent recombination between them may lead to unwanted genomic changes. Depending on the locations and orientations of LCRs undergoing recombination, the process can result in duplication, deletion, inversion or translocation.¹⁴ One of the peculiar characteristic of non-allelic homologous recombination is that most strand exchanges occur within a small region known as recombination hotspot and not elsewhere in the LCR.²⁰⁻²² Moreover there seems to be a link between non-allelic homologous recombination involving LCRs and double-strand DNA breaks (DSBs). DNA sequences/structures such as repetitive (containing transposon elements and minisatellites), palindromic and B-conformation DNA, where DSBs are common, are often found close to recombination hotspots.^{23,24}

Homologous Recombination, Repetitive Sequences and Genomic Rearrangements

Homologous recombination, which is based on high degree of sequence homology, uses homologous chromosome (in G₁ phase) or a sister chromatid (in the G₂ phase of the cell cycle) as template to

copy the missing information in genome. Among known repair mechanisms, homologous recombination is probably the most precise and therefore, extremely important in the maintenance of genomic integrity. Intact homologous recombination pathway is necessary for repairing damaged DNA in cells exposed to certain DNA-damaging agents. Homologous recombination is a precise and extremely regulated process in normal cells. Dysregulation or upregulation of such a pathway which involves DNA breakage, homologous base pairing and DNA reunion activities, can have harmful consequences if genomic integrity is compromised. Elevated/dysregulated recombination has been implicated in the generation of large DNA deletions^{25,26} amplifications,²⁷ and loss of heterozygosity,²⁸ and may lead to activation of oncogenes, inactivation of antioncogenes, telomere maintenance and ultimately the development and progression of cancer.²⁹⁻³² Expression and/or mutational changes in *RAD51* and/or other recombination related genes are associated with elevated risk of cancers including thyroid,³³ breast,³⁴ multiple myeloma³¹ and gastrointestinal.³⁵ We have demonstrated that homologous recombination activity and *RAD51* expression are significantly increased in immortal and cancer cells^{31,32,35} and this elevated recombination is implicated in genomic instability in cancer cells including Barrett's adenocarcinoma.³⁵

Homologous recombination between paralogous (non-allelic) sequences is considered to be a major cause of genomic rearrangements, especially those occurring often or repeatedly.¹⁴ Meiotic recombination events in humans and other eukaryotes are clustered in short areas known as recombination hotspots. Analyses of various recombination hotspots have identified a 13 bp motif (CCN CCN TNN CCN C) whose recombinogenic activity depends on specific bases at degenerate sites and specific area in the genome, reviewed in reference 15. This 13 mer motif related to recombination hotspot has been detected in LCRs in case of neurofibromatosis, charcot-Marie-Tooth disease, Smith-Magenis syndrome, Sotos syndrome, X-linked ichthyosis and Williams-Beuren syndrome.¹⁵

Interestingly the recombination hotspot related 13 mer motif has also been found in minisatellites, the repetitive sequences which are highly variable.³⁶ DNA rearrangements, arisen as consequence of homologous recombination between repetitive DNA sequences, have been associated with several genetic diseases.¹⁴ Non allelic homologous recombination hotspot at interspersed repetitive DNA element LINE 2 is associated with Charcot-Marie Tooth Disease whereas a similar hotspot at AluY is associated with Williams-Beuren syndrome. Inter-LINE1 homologous recombination events have been proposed to be responsible for 55 deletions in human genome.³⁷ Homologous recombination at repeat sequences seems to occur at a rate similar to that of point mutation and is a prominent source of genomic evolution in human.¹⁵

Barrett's Adenocarcinoma, Homologous Recombination and Alu Elements

Barrett's adenocarcinoma is a cancer associated with heartburn, caused by acid reflux. Gastroesophageal reflux exposes esophagus to acid and if the exposure continues, it leads to a premalignant condition known as Barrett's esophagus. Untreated Barrett's esophagus may progress to low grade dysplasia, then high grade dysplasia and subsequently to Barrett's adenocarcinoma. It has been shown that acid can cause double-strand breaks in esophagus and these breaks and associated problems may contribute to etiology of Barrett's adenocarcinoma.³⁸ Consistently, we have shown that acid significantly increases homologous recombination activity in normal human cells and both homologous recombination and recombinase (hsRAD51) are significantly upregulated in Barrett's adenocarcinoma cells.³⁵ Acid is probably involved in the initial upregulation of homologous recombination activity. As described above, the repetitive sequences such as Alu are more vulnerable to double strand breaks, and a 13 bp motif and specific bases at degenerate sites in the motif can affect their recombinogenicity. The mutational ability of acid therefore, may affect repetitive sequences more extensively, rendering them more

recombinogenic. These repeat sequences can be used by deregulated homologous recombination activity in Barrett's adenocarcinoma cells contributing to genomic instability and progression of disease.

Conclusion

More than 50% of our genome is comprised of repetitive (highly homologous) sequences. Repetitive sequences have been implicated in evolution/natural selection, gene regulation and genomic rearrangements associated with specific diseases. Homologous recombination, a DNA repair mechanism which uses homologous DNA sequences as template to repair the DNA damage, is extremely important in maintaining genomic integrity of a cell. If homologous recombination is elevated or deregulated, it may use non-allelic homologous sequences distributed throughout genome as substrates, leading to unnecessary genomic rearrangements and subsequent genomic instability. We have shown that homologous recombination activity is significantly upregulated in human immortal and cancer cells.^{31,32,35} More importantly our recent unpublished data show that a number of important genes, from every step of homologous recombination process, are upregulated at premalignant stage. We therefore propose that factors such as deregulated homologous recombination, mutations in recombinase (hsRAD51) and related genes, and possible mutations/polymorphisms in repetitive sequences in cancer or even pre-cancerous cells, cause an enhanced association of homologous recombination proteins with repetitive sequences and contribute to genomic rearrangements and instability. Therefore deregulated homologous recombination, RAD51 related proteins and repetitive sequences such as Alu may be targeted for prevention and/or treatment of Barrett's adenocarcinoma and possibly other cancers. However this model and several important questions related to the role of homologous recombination (probably the most precise repair system) and repetitive sequences (about half of our genome) need to be investigated and explored further.

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References

- Britten RJ, Kohne DE. Repeated sequences in DNA. Hundreds of thousands of copies of DNA sequences have been incorporated into the genomes of higher organisms. *Science* 1968; 161:529-40; PMID:4874239; <http://dx.doi.org/10.1126/science.161.3841.529>
- Debrauwere H, Gendrel CG, Lechat S, Dutreix M. Differences and similarities between various tandem repeat sequences: minisatellites and microsatellites. *Biochimie* 1997; 79:577-86; PMID:9466695; [http://dx.doi.org/10.1016/S0300-9084\(97\)82006-8](http://dx.doi.org/10.1016/S0300-9084(97)82006-8)
- Csank AK, Henikoff S. Something from nothing: the evolution and utility of satellite repeats. *Trends Genet* 1998; 14:200-4; PMID:9613205; [http://dx.doi.org/10.1016/S0168-9525\(98\)01444-9](http://dx.doi.org/10.1016/S0168-9525(98)01444-9)
- Choo KH, Vissel B, Nagy A, Earle E, Kalitsis P. A survey of the genomic distribution of alpha satellite DNA on all the human chromosomes and derivation of a new consensus sequence. *Nucleic Acids Res* 1991; 19:1179-82; PMID:2030938; <http://dx.doi.org/10.1093/nar/19.6.1179>
- Jeffreys AJ. Highly variable minisatellites and DNA fingerprints. *Biochem Soc Trans* 1987; 15:309-17; PMID:2887471
- Wahls WP, Wallace LJ, Moore PD. Hypervariable minisatellite DNA is a hotspot for homologous recombination in human cells. *Cell* 1990; 60:95-103; PMID:2295091; [http://dx.doi.org/10.1016/0092-8674\(90\)90719-U](http://dx.doi.org/10.1016/0092-8674(90)90719-U)
- Linardopoulou EV, Williams EM, Fan Y, Friedman C, Young JM, Trask BJ. Human subtelomeres are hot spots of interchromosomal recombination and segmental duplication. *Nature* 2005; 437:94-100; PMID:16136133; <http://dx.doi.org/10.1038/nature04029>
- Eckert KA, Hile SE. Every microsatellite is different: Intrinsic DNA features dictate mutagenesis of common microsatellites present in the human genome. *Mol Carcinog* 2009; 48:379-88; PMID:19306292; <http://dx.doi.org/10.1002/mc.20499>
- Jurka J, Kapitonov VV, Kohany O, Jurka MV. Repetitive sequences in complex genomes: structure and evolution. *Annu Rev Genomics Hum Genet* 2007; 8:241-59; PMID:17506661; <http://dx.doi.org/10.1146/annurev.genom.8.080706.092416>
- Feschotte C, Pritham EJ. DNA transposons and the evolution of eukaryotic genomes. *Annu Rev Genet* 2007; 41:331-68; PMID:18076328; <http://dx.doi.org/10.1146/annurev.genet.40.110405.090448>
- Konkel MK, Batzer MA. A mobile threat to genome stability: The impact of non-LTR retrotransposons upon the human genome. *Semin Cancer Biol* 2010; 20:211-21; PMID:20307669; <http://dx.doi.org/10.1016/j.semcancer.2010.03.001>
- Cordaux R, Batzer MA. The impact of retrotransposons on human genome evolution. *Nat Rev Genet* 2009; 10:691-703; PMID:19763152; <http://dx.doi.org/10.1038/nrg2640>
- Okada N. SINEs: Short interspersed repeated elements of the eukaryotic genome. *Trends Ecol Evol* 1991; 6:358-61; PMID:21232509; [http://dx.doi.org/10.1016/0169-5347\(91\)90226-N](http://dx.doi.org/10.1016/0169-5347(91)90226-N)

- Gu W, Zhang F, Lupski JR. Mechanisms for human genomic rearrangements. *Pathogenetics* 2008; 1:4; PMID:19014668; <http://dx.doi.org/10.1186/1755-8417-1-4>
- McVean G. What drives recombination hotspots to repeat DNA in humans? *Philos Trans R Soc Lond B Biol Sci* 2010; 365:1213-8; PMID:20308096; <http://dx.doi.org/10.1098/rstb.2009.0299>
- Badyaev AV. Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. *Proc Biol Sci* 2005; 272:877-86; PMID:16024341; <http://dx.doi.org/10.1098/rspb.2004.3045>
- Bailey JA, Eichler EE. Primate segmental duplications: crucibles of evolution, diversity and disease. *Nat Rev Genet* 2006; 7:552-64; PMID:16770338; <http://dx.doi.org/10.1038/nrg1895>
- Stankiewicz P, Lupski JR. Genome architecture, rearrangements and genomic disorders. *Trends Genet* 2002; 18:74-82; PMID:11818139; [http://dx.doi.org/10.1016/S0168-9525\(02\)02592-1](http://dx.doi.org/10.1016/S0168-9525(02)02592-1)
- Shaw CJ, Lupski JR. Implications of human genome architecture for rearrangement-based disorders: the genomic basis of disease. *Hum Mol Genet* 2004; 13:R57-64; PMID:14764619; <http://dx.doi.org/10.1093/hmg/ddh073>
- Bi W, Park SS, Shaw CJ, Withers MA, Patel PI, Lupski JR. Reciprocal crossovers and a positional preference for strand exchange in recombination events resulting in deletion or duplication of chromosome 17p11.2. *Am J Hum Genet* 2003; 73:1302-15; PMID:14639526; <http://dx.doi.org/10.1086/379979>
- Kurotaki N, Stankiewicz P, Wakui K, Niikawa N, Lupski JR. Sotos syndrome common deletion is mediated by directly oriented subunits within inverted Sos-REP low-copy repeats. *Hum Mol Genet* 2005; 14:535-42; PMID:15640245; <http://dx.doi.org/10.1093/hmg/ddi050>
- López-Corraea C, Dorschner M, Brems H, Lázaro C, Clementi M, Upadhyaya M, et al. Recombination hotspot in NF1 microdeletion patients. *Hum Mol Genet* 2001; 10:1387-92; PMID:11440991; <http://dx.doi.org/10.1093/hmg/10.13.1387>
- Lupski JR. Hotspots of homologous recombination in the human genome: not all homologous sequences are equal. *Genome Biol* 2004; 5:242; PMID:15461806; <http://dx.doi.org/10.1186/gb-2004-5-10-242>
- Wells RD. Non-B DNA conformations, mutagenesis and disease. *Trends Biochem Sci* 2007; 32:271-8; PMID:17493823; <http://dx.doi.org/10.1016/j.tibs.2007.04.003>
- Cheng RZ, Shammass MA, Li J, Shmookler Reis RJ. Expression of SV40 large T antigen stimulates reversion of a chromosomal gene duplication in human cells. *Exp Cell Res* 1997; 234:300-12; PMID:9260898; <http://dx.doi.org/10.1006/excr.1997.3649>
- Shammass MA, Xia SJ, Shmookler Reis RJ. Induction of duplication reversion in human fibroblasts, by wild-type and mutated SV40 T antigen, covaries with the ability to induce host DNA synthesis. *Genetics* 1997; 146:1417-28; PMID:9258684
- Windle B, Draper BW, Yin YX, O'Gorman S, Wahl GM. A central role for chromosome breakage in gene amplification, deletion formation and amplicon integration. *Genes Dev* 1991; 5:160-74; PMID:1995414; <http://dx.doi.org/10.1101/gad.5.2.160>
- Bishop AJ, Schiestl RH. Role of homologous recombination in carcinogenesis. *Exp Mol Pathol* 2003; 74:94-105; PMID:12710940; [http://dx.doi.org/10.1016/S0014-4800\(03\)00010-8](http://dx.doi.org/10.1016/S0014-4800(03)00010-8)
- Li J, Ayyadevera R, Shmookler Reis RJ. Carcinogens stimulate intrachromosomal homologous recombination at an endogenous locus in human diploid fibroblasts. *Mutat Res* 1997; 385:173-93; PMID:9506887
- Sengstag C. The role of mitotic recombination in carcinogenesis. *Crit Rev Toxicol* 1994; 24:323-53; PMID:7857521; <http://dx.doi.org/10.3109/10408449409017922>

31. Shamma MA, Shmookler Reis RJ, Koley H, Batchu RB, Li C, Munshi NC. Dysfunctional homologous recombination mediates genomic instability and progression in myeloma. *Blood* 2009; 113:2290-7; PMID:19050310; <http://dx.doi.org/10.1182/blood-2007-05-089193>
32. Xia SJ, Shamma MA, Shmookler Reis RJ. Elevated recombination in immortal human cells is mediated by HsRAD51 recombinase. *Mol Cell Biol* 1997; 17:7151-8; PMID:9372947
33. Bastos HN, Antão MR, Silva SN, Azevedo AP, Manita I, Teixeira V, et al. Association of polymorphisms in genes of the homologous recombination DNA repair pathway and thyroid cancer risk. *Thyroid* 2009; 19:1067-75; PMID:19772428; <http://dx.doi.org/10.1089/thy.2009.0099>
34. Silva SN, Tomar M, Paulo C, Gomes BC, Azevedo AP, Teixeira V, et al. Breast cancer risk and common single nucleotide polymorphisms in homologous recombination DNA repair pathway genes XRCC2, XRCC3, NBS1 and RAD51. *Cancer Epidemiol* 2010; 34:85-92; PMID:20004634; <http://dx.doi.org/10.1016/j.canep.2009.11.002>
35. Pal J, Bertheau R, Buon L, Qazi A, Batchu RB, Bandyopadhyay S, et al. Genomic evolution in Barrett's adenocarcinoma cells: critical roles of elevated hsRAD51, homologous recombination and Alu sequences in the genome. *Oncogene* 2011; 30:3585-98; PMID:21423218; <http://dx.doi.org/10.1038/onc.2011.83>
36. Myers S, Freeman C, Auton A, Donnelly P, McVean G. A common sequence motif associated with recombination hot spots and genome instability in humans. *Nat Genet* 2008; 40:1124-9; PMID:19165926; <http://dx.doi.org/10.1038/ng.213>
37. Han K, Lee J, Meyer TJ, Remedios P, Goodwin L, Batzer MA. L1 recombination-associated deletions generate human genomic variation. *Proc Natl Acad Sci USA* 2008; 105:19366-71; PMID:19036926; <http://dx.doi.org/10.1073/pnas.0807866105>
38. Clemons NJ, McColl KE, Fitzgerald RC. Nitric oxide and acid induce double-strand DNA breaks in Barrett's esophagus carcinogenesis via distinct mechanisms. *Gastroenterology* 2007; 133:1198-209; PMID:17919494; <http://dx.doi.org/10.1053/j.gastro.2007.06.061>