

Research Article

Genome-Wide Catalogue of Chromosomal Aberrations in Barrett's Esophagus and Esophageal Adenocarcinoma: A High-Density Single Nucleotide Polymorphism Array Analysis

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Abstract

To better understand the molecular mechanisms behind esophageal adenocarcinoma (EAC) tumorigenesis, we used high-density single nucleotide polymorphism arrays to profile chromosomal aberrations at each of the four sequential progression stages, Barrett's metaplasia (BM), low-grade dysplasia (LGD), high-grade dysplasia (HGD), and EAC, in 101 patients. We observed a significant trend toward increasing loss of chromosomes with higher progression stage. For BM, LGD, HGD, and EAC, respectively, the average numbers of chromosome arms with loss per sample were 0.30, 3.21, 7.70, and 11.90 (P for trend = 4.82×10^{-7}), and the mean percentages of single nucleotide polymorphisms with allele loss were 0.1%, 1.8%, 6.6%, and 17.2% (P for trend = 2.64×10^{-6}). In LGD, loss of 3p14.2 (68.4%) and 16q23.1 (47.4%) was limited to narrow regions within the *FHIT* (3p14.2) and *WWOX* (16q23.1) genes, whereas loss of 9p21 (68.4%) occurred in larger regions. A significant increase in the loss of other chromosomal regions was seen in HGD and EAC. Loss of 17p (47.6%) was one of the most frequent events in EAC. Many recurrent small regions of chromosomal loss disrupted single genes, including *FHIT*, *WWOX*, *RUNX1*, *KIF26B*, *MGC48628*, *PDE4D*, *C20orf133*, *GMD5*, *DMD*, and *PARK2*, most of which are common fragile site regions in the human genome. However, *RUNX1* at 21q22 seemed to be a potential tumor suppressor gene in EAC. Amplifications were less frequent than losses and mostly occurred in EAC. 8q24 (containing *Myc*) and 8p23.1 (containing *CTSB*) were the two most frequently amplified regions. In addition, a significant trend toward increasing amplification was associated with higher progression stage. *Cancer Prev Res*; 3(9): 1176–86. ©2010 AACR.

Introduction

Esophageal cancer is the eighth most common and the sixth most lethal cancer in the world (1). In the United States, an estimated 16,470 new cases and 14,530 deaths from this disease were expected in 2009 (2). Esophageal cancer tends to have a very poor prognosis because approximately two thirds of patients who are diagnosed have advanced-stage disease, at which point current therapies are largely ineffective (3). The overall 5-year survival rate

in the Surveillance, Epidemiology, and End Results database is 16.8% (3). These dismal statistics highlight the need to develop methods to detect esophageal cancer in its early stages and to identify biomarkers that can predict clinical outcomes.

More than 90% of esophageal cancers are either esophageal squamous cell carcinomas or esophageal adenocarcinoma (EAC; ref. 3). Once considered a rare tumor (representing less than 5% of esophageal cancers in the United States), EAC is the cancer with the fastest increasing incidence in the past three decades and currently accounts for more than 60% of new esophageal cancer cases in this country (4, 5). Most EAC cases arise from Barrett's esophagus, or Barrett's metaplasia (BM), a precursor lesion in which the squamous epithelium of the esophagus is replaced by a metaplastic columnar epithelium. BM is estimated to be present in 1% to 2% of the general population and confers a 30- to 125-fold increased risk of developing EAC. However, in patients with BM, the absolute risk of developing EAC is only ~0.5% per patient-year, which calls for more accurate and robust prediction of who may develop EAC to increase the cost-effectiveness of surveillance strategies such as routine

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endoscopy among high-risk BM patients (6, 7). The malignant progression of BM follows a generally accepted series of stages, from metaplasia, to low-grade dysplasia (LGD), to high-grade dysplasia (HGD), and finally, to adenocarcinoma. The risk of developing EAC in patients with HGD may be higher than 10% per patient-year (7, 8). However, this risk has been hard to assess because the grading of dysplasia is subjective and there is relatively high interobserver variability in its diagnosis. Independent objective biomarkers thus may improve the assessment of EAC risk by complementing pathologic grading.

The accumulation of genetic aberrations plays a pivotal role during the malignant progression from BM to EAC. Previous studies using candidate region analysis, low-resolution conventional comparative genomic hybridization, and low-density single nucleotide polymorphism (SNP) arrays have identified many of the chromosomal aberrations involved in the progression of EAC (9–15). A number of well-known tumor suppressor genes (TSG) and oncogenes have been implicated in EAC, including *p16*, *p53*, *p21*, *APC*, *Rb*, *SMAD4*, *Myc*, *K-ras*, *EGFR*, *cyclins*, and *CDKs* (16–18). However, except for the most consistent deletion of 9p21 across different histologic stages and the loss of heterozygosity (LOH) of *p53* in the later stages, the results for other chromosomal aberrations are highly heterogeneous in terms of stage, frequency, and size. A high-resolution genome-wide profiling of chromosomal aberrations in different stages from BM to EAC may not only elucidate the mechanisms of tumorigenesis of EAC but also identify predictors of malignant progression, biomarkers of prognosis and treatment response, and potential targets for prevention and therapy.

Whole-genome high-density SNP array analysis is a powerful new technology for detecting both copy number variations and LOH events (19). Other notable advantages include its high resolution, its capability of profiling both physical and genetic aberrations, its low levels of required input DNA (as little as 10 ng), and its high sensitivity in the presence of normal cell contamination (allowing the detection of LOH in paired samples with ~67% normal background cells; ref. 19). Nancarrow et al. (20) recently performed a high-density SNP array analysis of 23 primary biopsies of EAC tumors. In this study, we used Illumina's 317K SNP array to profile and compare genome-wide chromosomal aberrations in the four stages of EAC tumorigenesis, BM, LGD, HGD, and EAC.

Materials and Methods

Tissue samples and DNA extraction

A total of 101 (20 BM, 19 LGD, 20 HGD, and 42 EAC) disease tissues and their paired normal tissues obtained from The University of Texas M.D. Anderson Cancer Center were included in this study. All tissues were snap frozen at the time of diagnostic or therapeutic endoscopic biopsies using a tissue collection protocol approved by the institutional review board. Experienced gastrointestinal

pathologists at M.D. Anderson Cancer Center performed the histologic readings of the corresponding juxtaposed paraffin-fixed specimens. The diagnoses of LGD and HGD were based on each biopsy using the criteria previously described (21). All disease tissues in this cross-sectional study were from separate patients diagnosed as having EAC. A corresponding normal squamous tissue sample was collected from a healthy-appearing mucosa at least 3 cm from the edge of the apparent tumor from each patient by an expert gastroenterologist. The tumors were staged according to the American Joint Commission on Cancer Staging Manual (22). DNA was extracted using QIAamp DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's instructions.

SNP array analysis

SNP array analysis was done using Illumina's Human-Hap300 BeadChip array according to the manufacturer's protocol (Illumina). Briefly, 200 ng of genomic DNA extracted from the tissues were denatured and amplified at 37°C overnight. The amplified DNA was fragmented and precipitated at 4°C, resuspended in hybridization buffer, and hybridized to HumanHap300 chips at 48°C overnight. The unhybridized and nonspecifically hybridized DNA was then washed away, and the captured DNA was used as template for one-base extension of the locus-specific oligos on the BeadChips. All SNP data were analyzed and exported by BeadStudio 2.0 (Illumina), which generated whole-genome profiles for disease and normal tissues based on the \log_2R ratio and allele frequency of each SNP. We used paired analysis (each disease tissue versus its own adjacent normal tissue reference) instead of a single normal reference because paired analysis offers higher sensitivity, better quality of data, and lower variation of \log_2R ratio in the case of limited input DNA (19). We tested the built-in autoscoring algorithms of BeadStudio to identify LOH, homozygous deletion, and amplification. The \log_2R ratio between tumor and normal samples and the absolute value of the difference between B allele frequency in tumor and normal samples ($|dAllelefreq|$) were obtained using the BeadStudio software. In paired analysis, both disease and normal samples came from the same patients, and deviations of $|dAllelefreq|$ from zero indicated regions with chromosomal aberrations. We first performed a filtering process to select the candidate points for analysis using B allele frequency (between 0.4 and 0.6) of normal samples following the BeadStudio LOH User Guide. We then applied a circular binary segmentation algorithm (23) implemented within the R software environment to identify change points for the regions of aberration using $|dAllelefreq|$ on the candidate points. Regions that were bound by these change points and that deviated from zero were identified as regions of chromosomal aberrations. For these regions of chromosomal aberrations, we then determined whether it represented a chromosomal loss or gain using the \log_2R ratio. The resulting annotations were compared with the annotations from the built-in BeadStudio algorithms and further confirmed by manually inspecting the BeadStudio

Genome Viewer plots of tumor B allele frequency, normal B allele frequency, |dAllelefreq|, and \log_2R ratio. We grouped LOH (including copy-neutral LOH) and homozygous deletion together as chromosome loss for two reasons: (a) homozygous deletion was a rare event; (b) when there were high levels of normal cells in disease tissues, it was difficult to differentiate among LOH, copy-neutral LOH, and homozygous deletion because the \log_2R was ambiguous, although it was clear from the |dAllelefreq| plot that the region had one of these events. We manually inspected the X chromosome from each tissue and identified high-frequency aberrations; however, we only counted auto-chromosomes in the analyses of total number, size, and percentage of aberrations. We excluded Y chromosomes from the analysis because there are only two representing SNPs on it.

Statistical analyses

For all analyses, loss and amplification were analyzed separately. Fisher's exact test was used to test for differences in the frequency of aberrations (loss and amplification) by each chromosome arm among BM, LGD, HGD, and EAC tissues. The Kruskal-Wallis and nonparametric trend tests were used to compare the sizes of aberration at each chromosome arm among BM, LGD, HGD, and EAC tissues. For each tissue sample, we also tallied the total number and total size of chromosome aberrations, and the percentage of SNPs with aberrations among all the analyzed SNPs (317K). The Kruskal-Wallis and nonparametric trend tests were used to compare the mean total number, mean total size, and mean

percentage of SNPs with aberrations among BM, LGD, HGD, and EAC tissues. All analyses were done using the Stata 8.0 statistical software package (Stata Co.). All tests were two-sided, and $P < 0.05$ was considered statistically significant.

Results

Patient and tumor characteristics

A total of 101 (20 BM, 19 LGD, 20 HGD, and 42 EAC) disease tissue specimens were included in this study. Table 1 lists selected characteristics of patients from whom these tissues were obtained. BM tissues were from patients who were younger (mean age \pm SD: 54.60 ± 10.34 years) and included a higher percentage (40%) of female subjects than patients with the other three histologic stages. The mean age was similar among patients with LGD (62.21 ± 9.82), HGD (61.70 ± 12.62), and EAC (57.62 ± 9.51). More than 90% of patients in each of these latter three stages were men and more than 80% were Caucasian. The distribution of EAC patients by stage was as follows: 17 with stage II, 20 with stage III, 2 with stage IV, and 3 unspecified; 21 patients had moderately differentiated and 20 had poorly differentiated tumors.

Genome-wide catalogue of chromosomal aberrations

We profiled genome-wide chromosomal aberrations using Illumina's HumanHap300 Beadchips, which contained ~317K haplotype tagging SNPs. SNPs on the HumanHap300 chips represent even coverage by each chromosome (except the Y chromosome) and are evenly spaced across

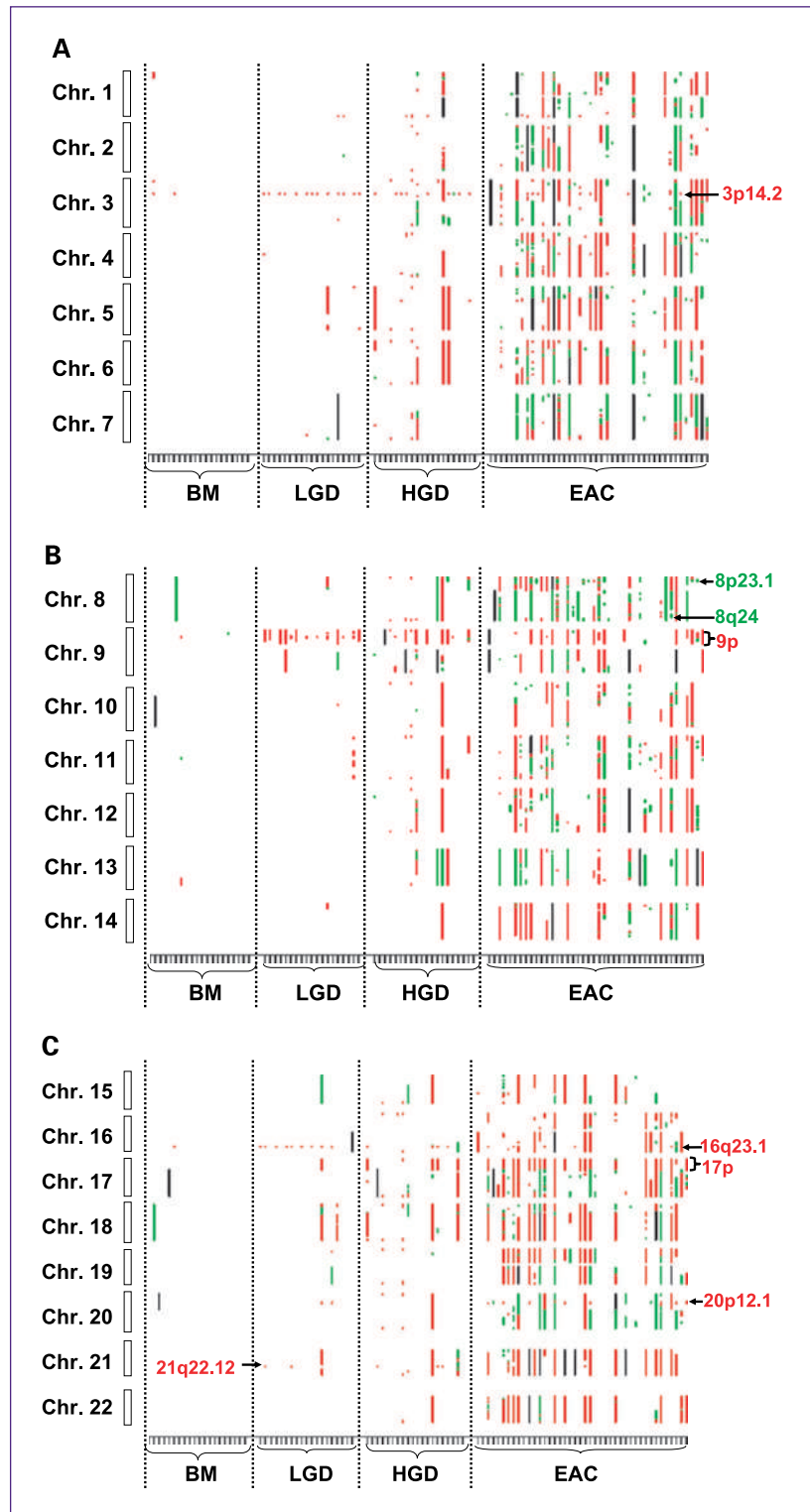
Table 1. Selected host and tumor characteristics

Variables	BM, n (%)	LGD, n (%)	HGD, n (%)	EAC, n (%)	P
Age (y), mean (SD)	54.60 (10.34)	62.21 (9.82)	61.70 (12.62)	57.62 (9.51)	0.067*
Sex					
Male	12 (60.0)	18 (94.7)	18 (90.0)	39 (92.9)	0.006†
Female	8 (40.0)	1 (5.3)	2 (10.0)	3 (7.1)	
Ethnicity					
Caucasian	19 (95.0)	18 (94.7)	16 (80.0)	34 (81.0)	0.275†
Other	1 (5.0)	1 (5.3)	4 (20.0)	8 (19.0)	
Stage					
II				17 (40.5)	
III				20 (47.6)	
IV				2 (4.8)	
Stage Unspecified				3 (7.1)	
Grade					
Moderately differentiated				21 (50.0)	
Poorly differentiated				20 (47.6)	
Unspecified				1 (2.4)	

*P from ANOVA test.

†P from Fisher's exact test.

Fig. 1. Genome-wide catalogue of chromosomal aberrations on each chromosome. X axis denotes tissue samples, grouped by histologic stages. Y axis denotes chromosomes, oriented with the p-arm at the top and q-arm at the bottom for each chromosome. Red represents loss, green represents amplification, and black represents ambiguous aberrations. A, chromosomes (Chr.) 1 to 7; B, chromosomes 8 to 14; C, chromosomes 15 to 22. Some of the most frequent losses and amplifications are marked.



the whole genome to ensure comprehensive coverage. On average, there is one SNP every 9 kb across the genome, and the median spacing is 5 kb. The 90th percentile spacing between loci, indicating the largest intervals in content

on the chip, is 19 kb (24). With a mean SNP spacing of 9 kb and a 10 SNP smoothing window, the effective resolution of HumanHap300 SNP array for chromosomal aberration analysis is ~90 kb (19).

Table 2. Frequent loss of individual chromosome arm (%)

Chromosome arm	BM	LGD	HGD	EAC	P*
3p	10.0	68.4	70.0	47.6	1.85E-04
4p	0.0	0.0	10.0	38.1	5.38E-05
4q	0.0	5.3	20.0	42.9	1.19E-04
5q	0.0	10.5	35.0	38.1	1.09E-03
6p	0.0	0.0	30.0	40.5	3.33E-05
8p	0.0	5.0	20.0	38.1	6.08E-04
9p	5.0	68.4	50.0	38.1	2.35E-04
11p	0.0	5.3	20.0	38.1	6.08E-04
11q	0.0	5.3	20.0	35.7	1.25E-03
12q	0.0	0.0	20.0	35.7	2.27E-04
16q	5.0	47.4	25.0	42.9	5.52E-03
17p	0.0	5.3	25.0	47.6	2.14E-05
18q	0.0	10.5	25.0	35.7	3.59E-03
19p	0.0	0.0	15.0	38.1	5.38E-04
21q	0.0	15.8	35.0	35.7	4.05E-03
22q	0.0	0.0	10.0	35.7	1.37E-04

NOTE: Only losses with >35% frequency in EAC were included.

*Fisher's exact test.

Figure 1 shows the genome-wide distribution of losses and amplifications by chromosome in each sample. A visual examination of the figure reveals that there is a gradual increase in chromosome aberrations with the increase in histologic stages. In BM samples, there were only scattered chromosome losses at 3p14.2 (10%), 9p21 (5%), and 16q23.1 (5%); these three events were the most frequent aberrations in LGD samples (68.4%, 68.4%, and 47.4%, respectively; Table 2). There were dramatic increases in the numbers of aberrations in the HGD and EAC stages (Fig. 1; Table 2). Furthermore, the 3p14.2 (68.4%) and 16q23.1 (47.4%) losses seen in the LGD stage were exclusively limited within genes *FHIT* (3p14.2; Figs. 2A and 3A) and *WWOX* (16q23.1; Supplementary Fig. S1A), two of the most frequently activated common fragile site (CFS) regions in the human genome.

In the HGD and particularly EAC stages, the sizes of 3p loss (Fig. 2A) and 16q loss (Supplementary Fig. S1A) increased. In contrast, 9p loss occurred in larger regions from LGD to EAC (Fig. 2B), and homozygous deletion of the 9p21 locus was observed in the context of large region of 9p LOH (Fig. 3B).

We then computed the overall levels of chromosomal aberration in each sample by three parameters (total number, total size, and percentage of SNPs with aberrations) and compared the values among the four different stages. As shown in Fig. 4, there was a significant trend of increasing number and size of chromosomal aberrations and increasing percentage of SNPs with aberrations associated with increasing histologic stages. The average total numbers of chromosome arms with losses per sample were 0.30,

3.21, 7.70, and 11.90 for BM, LGD, HGD, and EAC, respectively (P for trend = 4.82×10^{-7} ; when analyzing BM, LGD, and HGD only, P for trend = 4.44×10^{-5} ; Fig. 4A). The mean total sizes of chromosome losses per sample were 2.33, 39.10, 167.87, and 449.90 Mb (P for trend = 8.13×10^{-8} ; Fig. 4B), and the mean percentages of SNPs with allele losses were 0.1%, 1.8%, 6.6%, and 17.2% for BM, LGD, HGD, and EAC, respectively (P for trend = 2.64×10^{-6} ; Fig. 4C).

Similar analyses were done for amplifications, which were less frequent than losses and mostly occurred in EAC. The average numbers of chromosome arms with amplifications per sample were 0.30, 0.42, 1.90, and 8.50 for BM, LGD, HGD, and EAC, respectively (P for trend = 1.35×10^{-9}); however, the trend was not significant for amplification in pre-malignant (BM, LGD, and HGD) stages (P for trend = 0.147) due to the rarity of amplifications in these stages. The mean total sizes of amplifications per sample were 11.00, 9.41, 37.70, and 215.74 Mb (P for trend = 1.23×10^{-8} ; Fig. 4B), and the mean percentages of SNPs with amplifications were 0.5%, 0.4%, 1.5%, and 8.0% for BM, LGD, HGD, and EAC, respectively (P for trend 1.26×10^{-8} ; Fig. 4C).

Individual chromosome aberrations and small overlapping regions of aberrations

Chromosome losses in LGD samples were mostly confined to three regions (3p14.2, 9p21, and 16q23.1). However, there was a significant increase in chromosome losses and gains in the HGD and EAC stages (Fig. 1; Table 2). The most frequent chromosome arm losses in the HGD stage were 3p (70%), 9p (50%), 5q (35%), 21q (35%), 6p (30%), 16q, 17p, and 18q (all 25%), and 4q, 8p, 11p, 11q, and 12q (all 20%). In EAC, the frequent (>35%) chromosome losses included 17p (47.6%), 3p (47.6%), 16q (42.9%), 4q (42.9%), 6p (40.5%), 9p (38.1%), 8p (38.1%), 5q (38.1%), 19p (38.1%), 11p (38.1%), 4p (38.1%), 11q (35.7%), 18q (35.7%), 21q (35.7%), and 18q (35.7%; Table 2). All of these frequent individual chromosome losses showed significant associations with higher stages; the most significant individual associations were 17p ($P = 2.14 \times 10^{-5}$), 6p ($P = 3.33 \times 10^{-5}$), and 4p ($P = 5.38 \times 10^{-5}$; Table 2). The 17p loss (all including *p53* locus) occurred predominantly in either a large portion of (>20 Mb) or the entire 17p arm, with only one exception involving a small focal LOH at 17p13.1 (323 kb, containing *p53* and 22 other genes; Supplementary Fig. S1B).

There were many small recurrent regions of loss that disrupted single genes, including *FHIT* (3p14.2), *WWOX* (16q23.1), *RUNX1* (21q22.12), *KIF26B* (1q44), *MGC48628* (4q22.1), *PDE4D* (5q11.2), *C20orf133* (20p12.1), *GMDS* (6p25.3), *DMD* (Xp21.2), and *PARK2* (6q26), the majority of which were CFS regions in the human genome (Table 3). Of particular interest was *RUNX1* at 21q22.12; the focal loss of this gene occurred in two cases of LGD and three cases of HGD, and an additional HGD case lost the entire 21q arm. 21q loss was also a frequent event in EAC (occurring in 35.1% of cases), suggesting that *RUNX1* is a potential TSG involved in the development of EAC.

The small overlapping regions of amplifications contained from 4 to nearly 100 genes, among which there were many known oncogenes and proliferation genes. The high-frequency small overlapping regions of amplifications (occurring in more than 15% of samples) and candidate genes in these regions included 8q24.21 (36.6%, *MYC* and 4 other genes), 8p23.1 (31.7%, *CTSB* and 15 others), 7q21 (30.8%, *CDK6* and 4 others),

20q13.2 (28.6%, *ZNF217* and 3 others), 7p11.2 (25.6%, *EGFR* and 33 others), 3q26 (20.5%, *PIK3CA* and 93 others), 12p12.1 (19.5%, *KRAS* and 6 others), 18q11.2 (17.5%, *CTAGE1* and 3 others), and 11q13.2-q13.3 (16.7%, *CCND1* and 10 others; Table 4). Of particular interest, the majority of amplifications at 8p23.1 were focal amplifications encompassing the *CTSB* gene (Figs. 1B and 3C).

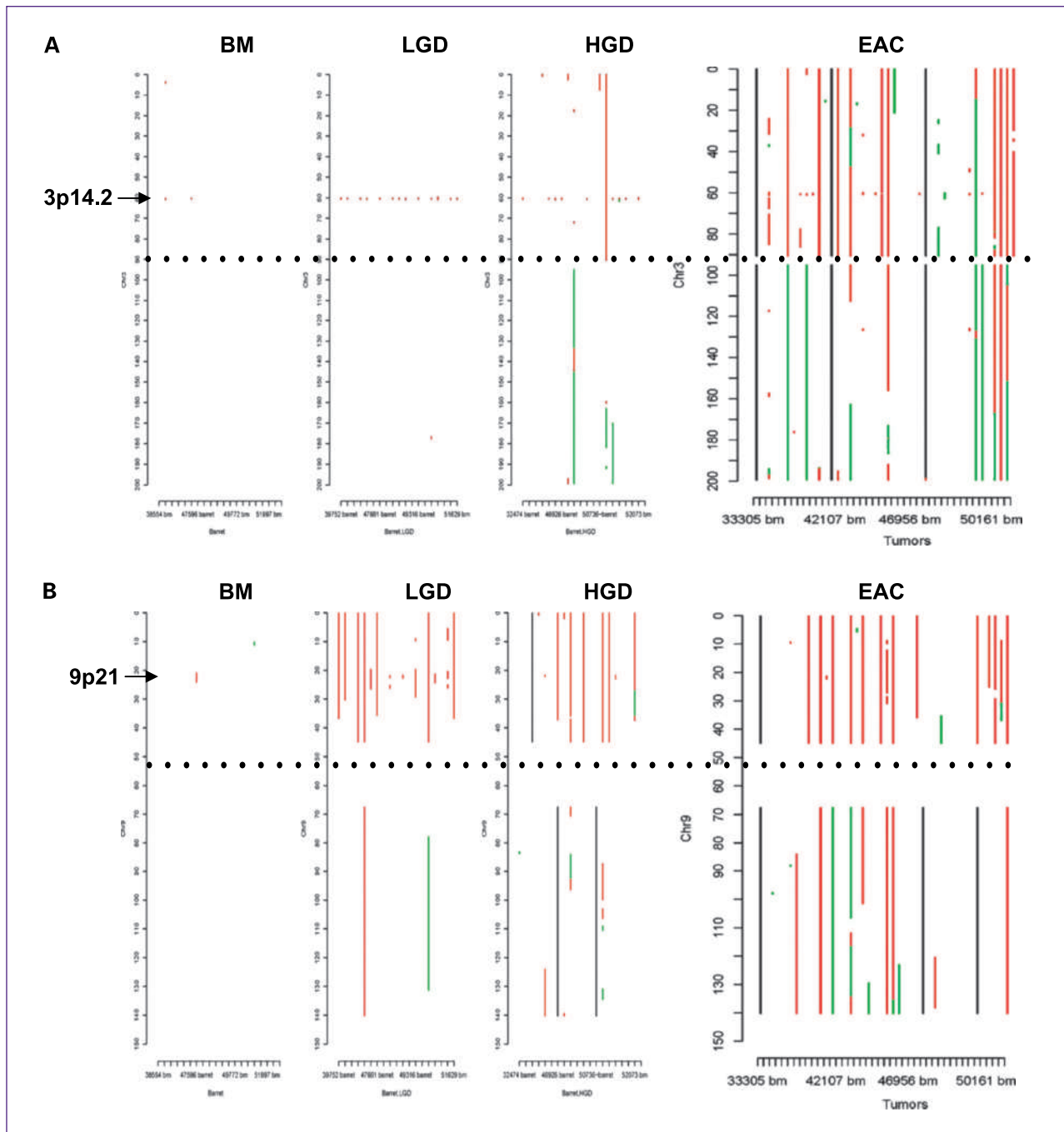


Fig. 2. Distribution of aberrations at chromosomes 3 (A) and 9 (B) in different stages from BM to EAC. X axis denotes samples, Y axis denotes chromosome position in Mb. Red, loss; green, amplification; black, aberrations ambiguous; and dotted black, centromere position.

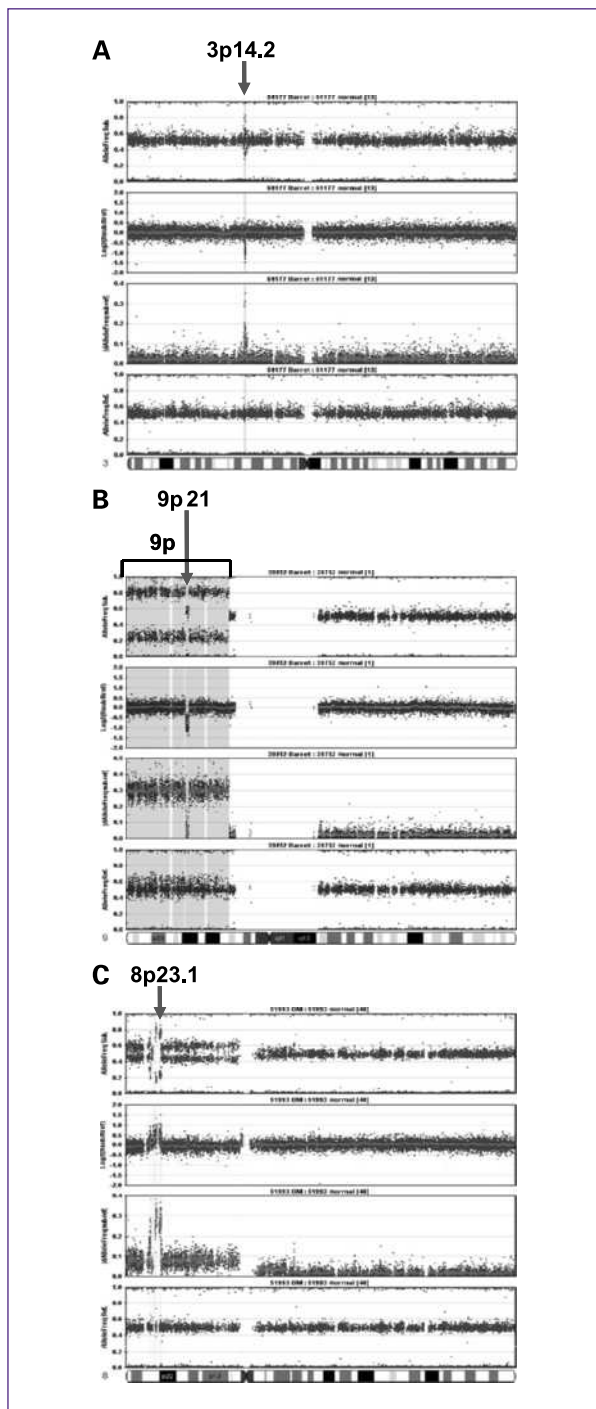


Fig. 3. Representative pictures of chromosome loss and amplification. A, 3p14.2 loss; B, 9p LOH and 9p21 homozygous deletion; C, 8p23.1 amplification. Panel 4 shows the allele frequencies of normal DNA. Panel 1 shows the allele frequencies of disease tissue. Panel 3 shows the absolute allele frequency differences between disease (panel 1) and normal (panel 4) tissues (ldAllelefreq). Regions of ldAllelefreq > 0 indicate regions of aberrations. Panel 2 shows the log₂R ratio of disease to normal tissues (measuring changes in intensity from what is expected for two copies of each locus). The regions without aberrations should have log₂R values of 0. A negative log₂R value indicates chromosome loss and a positive value suggests chromosome amplification.

Discussion

Many previous studies have shown an increasing accumulation of chromosomal aberrations during the metaplasia-dysplasia-carcinoma sequence of EAC development (9–14) and the pathogenesis of other malignancies (25–27). The distribution and frequency spectrum of each chromosomal aberration in the literature has been inconsistent (17, 20) for a number of reasons, including assay technology, normal tissue contamination, small sample sizes, tumor heterogeneity, different environmental exposures, gene/environment interactions, and population stratifications. Using a large series of premalignant and malignant tissues, the current study applied high-density SNP array technology to produce the highest-resolution genome-wide catalogue of chromosomal alterations in BM through EAC to date. The clear advantage of our high-resolution arrays was shown when the losses of the 3p14.2 and 16q23.1 regions in samples from the BM and LGD stages were observed exclusively within the two most frequently activated CFS regions in the human genome. In contrast, 9p deletions in both dysplastic (LGD and HGD) and malignant (EAC) biopsies often involved large regions surrounding the p15 and p16 loci. The predominant occurrence of these three chromosomal losses in the LGD stage supports the notion that *p15* and/or *p16* genes and genetic instability are early driving forces in the development of dysplasia. With further malignant progression to the HGD and EAC stages, the spectrum and size of chromosomal aberrations gradually increased. By the EAC stage, approximately a quarter (17% losses and 8% amplifications) of the whole genome exhibited aberrations.

CFS regions are large, unstable genomic regions that exhibit increased chromosomal gaps and breaks when DNA replication is partially inhibited (28–31). The single genes identified from this study (Table 3) were mostly large genes (from ~500 kb to over 2 Mb) that included the majority of previously confirmed CFS genes (*FHIT*, *PARK2*, *IMMP2L*, *CNTNAP2*, *CTNNA3*, *WWOX*, and *DMD*; refs. 30, 31). In addition, our data indicated several potential new CFS genes, including *KIF26B* at 1q44, *MGC48628* at 4q22.1, *GMDS* at 6p25.3, and *C20orf133* at 20p12.1. Others have previously hypothesized *MGC48628* to be a CFS gene (28). Homozygous deletion of ~500 kb within *MGC48628* was recently reported to be found in EAC (20). We confirmed the same deletion in both dysplastic and EAC tissues. Whether these CFS genes are specifically involved in the development of a subset of EACs or are only “hitchhikers” (32) remains to be studied. To our knowledge, the occurrence of CFS regions in BM and EAC is more extensive than in other cancers, which biologically may be attributable to the constant bile acid exposure during gastrointestinal reflux. Previous studies have shown that bile acid induces inflammation and oxidative stress, which in turn activates CFS regions (33, 34).

Chromosomal loss at 5q occurs frequently in EAC (10–16). The *APC* gene at 5q21 has been hypothesized to be the candidate TSG in EAC (35); however, we found that most of the 5q losses were around the *PDE4D* gene

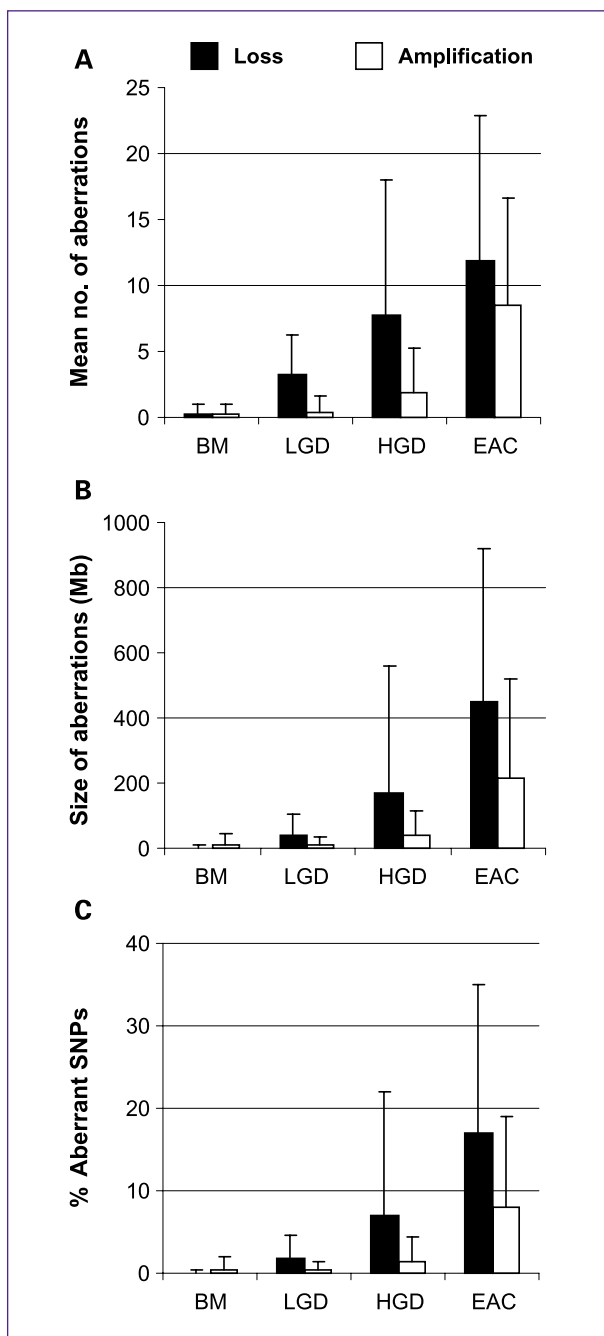


Fig. 4. Increased level of chromosomal aberrations (losses and amplifications) with increasing histologic stage. A, mean numbers of chromosome arm aberrations (P for trend = 4.82×10^{-7} for loss and P for trend = 1.35×10^{-9} for amplification). B, mean total sizes (Mb) of chromosome aberrations (P for trend = 8.13×10^{-8} for loss and P for trend = 1.23×10^{-8} for amplification). C, mean percentage of SNPs with aberrations per tissue in each stage (P for trend = 2.64×10^{-6} for loss and P for trend = 1.26×10^{-8} for amplification).

(encoding a cyclic AMP phosphodiesterase) at 5q11.2, including focal losses within *PDE4D* in two LGD samples and five EAC samples. Nancarrow et al. (20) reported similar deletions in EAC samples. Interestingly, Weir et al.

(36) found homozygous focal deletions within *PDE4D* in lung adenocarcinoma. The underlying biological mechanism of this homozygous deletion, its functional consequence, and its pathologic impact on esophageal and lung adenocarcinoma warrant further investigation.

The second potential candidate TSG in EAC is *RUNX1* at 21q22.12. Focal loss of *RUNX1* occurred in two LGD and three HGD samples, and 21q loss was frequent in EAC (Table 2). *RUNX1* belongs to the Runt domain family of transcription factors consisting of three DNA binding α subunits (*RUNX1*, *RUNX2*, and *RUNX3*), each of which forms heterodimers with the common β subunit CBF β and plays pivotal roles in neoplastic progression (37). *RUNX1* (*AML1*) is a well-established TSG in leukemia (37, 38), whereas *RUNX3* is an established TSG in gastric, esophageal, and other solid tumors (37, 39). A previous report showed a dramatic downregulation of *RUNX1* and *RUNX3* in gastric tumors compared with adjacent normal tissues (40). Taken together, these data suggest that *RUNX1* may have tumor suppressor functions in solid tumors, including gastric and esophageal cancers.

We found loss of the 9p21 region to occur in the early stages (BM and LGD), whereas loss of the *p53* locus at 17p13 occurred in the later stages and was one of the most frequent events in the EAC stage. These data are consistent with previous reports that p16 drives the early progression of metaplasia and that p53 inactivation is a late event that permits further genomic instability and promotes aneuploidy (41).

The sizes of the small overlapping regions of amplification ranged from <500 kb to >10 Mb. These regions contained some well-characterized oncogenes or proliferation genes, such as *Myc*, *CDK6*, *ZNF217*, *KRAS*, *CCND1*, *EGFR*, *PIK3CA*, and *VEGF*. The most frequent regions of amplification in EAC were 8q24 (containing *Myc*) and 8p23.1 (containing *CTSB*; Table 4). *Myc* is the most overexpressed oncogene in human cancers, whereas *CTSB* encodes a lysosomal cysteine proteinase. *CTSB*-deficient mice have been shown to have reduced tumor cell proliferation, and cancer cells lacking *CTSB* have been shown to exhibit resistance to apoptosis (42). Furthermore, the overexpression of *CTSB* mRNA and protein has been previously observed in EAC compared with normal tissues (43). Therefore, *CTSB* is a candidate oncogene for EAC.

We found a gradual increase in chromosomal aberrations during the metaplasia-dysplasia-carcinoma sequence, consistent with reports in the literature (9–16). However, there were large intersample variations in chromosomal aberrations among tissues with the same histologic stage (Fig. 1), with some samples having no or very few aberrations and some with large numbers of aberrations. For example, among 42 EAC samples, 3 samples did not have any aberrations and 1 sample had only one aberration on chromosome 8q (Fig. 1). Akagi et al. (15) recently reported that some of the EAC samples did not have any chromosome aberrations and suggested that the low tumor content in these samples was a possible reason for the lack of detectable aberrations. In our study, most EAC samples have

Table 3. Recurrent small overlapping regions of chromosome loss

Chromosome	Start	End	Size (kb)	Genes	Gene size	Fragile site
1q44	243529398	243765708	236.3	<i>KIF26B</i>	548,142	FRA1I
3p14.2	60383444	60475589	92.1	<i>FHIT</i>	1,502,089	FRA3B
4q22.1	91643255	91880768	237.5	<i>MGC48628</i>	546,974	FRA4F
5q11.2	58523521	58711295	187.8	<i>PDE4D</i>	1,019,680	
6p25.3	1206805	1658389	451.6	<i>GMD5</i>	621,806	FRA6B
6q26	161958628	162243987	285.4	<i>PARK2</i>	1,380,352	FRA6E
7q31.1	110645504	111017480	372.0	<i>IMMP2L</i>	899,238	FRA7G
7q35	145302806	145820585	517.8	<i>CNTNAP2</i>	2,304,634	FRA7I
9p21.3	21987872	22122076	134.2	<i>CDKN2B</i>	6,411	
10q21.3	68040186	68345373	305.2	<i>CTNNA3</i>	1,776,019	FRA10D
16q23.1	77146033	77164753	18.7	<i>WVVOX</i>	1,113,014	FRA16D
17p13.1	7413608	7736254	322.6	<i>p53</i> and 22 others	19,198 (p53)	
20p12.1	14825654	15018049	192.4	<i>C20orf133</i>	2,057,697	FRA20B
21q22.12	35077882	35179895	102.0	<i>RUNX1</i>	261,544	
Xp21.1	31612560	31712320	99.8	<i>DMD</i>	2,214,919	FRAXC

NOTE: Only includes regions of ~500 kb and smaller.

abundant tumor cells (>50% tumor cells in the specimen). We believe that the lack of chromosomal aberrations in some EAC samples are likely due to inherent tumor heterogeneity; however, we cannot rule out that low tumor content in occasional tumor samples may have resulted in no detectable aberrations.

There are a few limitations to this study. This is a cross-sectional study in which all the disease samples were from

separate individuals; therefore, we could not test the hypothesis that chromosomal aberrations may be useful biomarkers for predicting the development of EAC in patients with dysplasia. Another related limitation is that all the premalignant tissues (BM, LGD, and HGD) in this study were obtained from patients who had EAC. Other cross-sectional biomarker studies (e.g., using SNP array analysis and gene expression array) have been conducted in the

Table 4. Recurrent small overlapping regions of amplification

Chromosome	Start	End	Size (kb)	Frequency (%)		Genes
				HGD	EAC	
3q26	173230050	186580274	13,350	15.0	20.5	<i>PIK3CA</i> and 93 genes
5p13.2-p12	36348269	44184542	7,836	0.0	20.0	53 genes
6p21.33	30559883	31040288	480	0.0	16.7	24 genes
6p21.1	41746737	44753714	3,007	0.0	16.7	<i>VEGF</i> and 69 genes
7p22.2	795019	2280133	1,485	0.0	25.6	25 genes
7p11.2	54471518	56699692	2,228	0.0	25.6	<i>EGFR</i> and 33 others
7q21.2-q21.3	92102346	92744573	642	0.0	30.8	<i>CDK6</i> and 4 others
8p23.1	11194870	11897260	702	15.0	31.7	<i>CTSB</i> and 15 others
8q24.21	127660810	129220171	1,559	5.0	36.6	<i>MYC</i> and 4 others
11q13.2-q13.3	69152144	69957414	805	0.0	16.7	<i>CCND1</i> and 10 others
12p12.1	25029283	25546789	518	5.0	19.5	<i>KRAS</i> and 6 others
12q14.3	65978417	67060279	1,082	5.0	19.5	<i>MDM1</i> and 8 others
13q14.3-q21.32	53356272	65178858	11,823	10.0	22.5	30 genes
17q21.2	36045007	36600822	556	10.0	19.5	38 genes
18q11.2	17931784	18382880	451	15.0	17.5	<i>CTAGE1</i> and 3 others
20q13.2	50951694	51913648	962	0.0	28.6	<i>ZNF217</i> and 3 others

NOTE: Only include regions with >15% frequency in EAC.

esophagus and other organs (15, 44, 45); like ours, some involved assessments of various tissues of cancer patients. It can be argued that this approach increases the potential to identify high-risk markers because it detects molecular changes (including frequency of a change) in premalignancy that accompanies cancer (versus premalignant molecular changes in noncancer patients). Thus, the approach can complement cross-sectional studies of premalignancy biomarkers (in premalignancy-only patients) versus cancer biomarkers and can complement prospective studies of cancer risk in Barrett's or other premalignancy patients by identifying candidate high-risk markers for such studies (46).

An additional limitation is that we grouped genetic events that may be biologically distinct into two categories, losses and amplifications, because we did not have an estimate of the fraction of normal tissues present in each sample and it is difficult to separate different aberration types (e.g., copy neutral LOH, LOH, or homozygous deletion) based on \log_2R ratio when there is substantial normal cell contamination. However, it is not likely that the increasing trend of overall chromosomal aberrations was due to higher percentages of normal tissues in the lower histologic stages because the specific losses of 3p, 9p, and 16q were actually higher in LGD than EAC tissues in this study. Nevertheless, biologically, it would be important to differentiate different types of aberrations to determine the molecular mechanism behind the devel-

opment of EAC. Future studies are needed to distinguish each specific chromosomal aberration.

In conclusion, the present study provided a high-resolution genome-wide catalogue of chromosomal aberrations at different stages of histologic progression from BM to EAC. This study also provided strong evidence that genetic instability, as evidenced by the early and extensive occurrence of microdeletions in CFS regions, and 9p21 loss drive the early progression from metaplasia to dysplasia and that *p53* is critical in carcinoma development. Additionally, we identified RUNX1 as a potential TSG for EAC. Finally, we showed that overall chromosomal instability index and specific chromosome aberrations may be associated with neoplastic progression in BM patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

- Garcia M, Jemal A, Ward EM, et al. Global cancer facts & figures 2007. Atlanta (GA): American Cancer Society; 2007.
- Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009;59:225–49.
- Horner MJ, Ries LAG, Krapcho M, et al. SEER cancer statistics review, 1975–2006. Bethesda (MD): National Cancer Institute; 2009. Available from: http://seer.cancer.gov/csr/1975_2006/, based on November 2008 SEER data submission, posted to the SEER web site.
- Lagergren J. Adenocarcinoma of oesophagus: what exactly is the size of the problem and who is at risk? *Gut* 2005;54 Suppl 1:i1–5.
- Enzinger PC, Mayer RJ. Esophageal cancer. *N Engl J Med* 2003;349:2241–52.
- Reid BJ, Li X, Galipeau PC, Vaughan TL. Barrett's oesophagus and oesophageal adenocarcinoma: time for a new synthesis. *Nat Rev Cancer* 2010;10:87–101.
- Shaheen NJ, Richter JE. Barrett's oesophagus. *Lancet* 2009;373:850–61.
- Schnell TG, Sontag SJ, Chejfec G, et al. Long-term nonsurgical management of Barrett's esophagus with high-grade dysplasia. *Gastroenterology* 2001;120:1607–19.
- Wu TT, Watanabe T, Heitmiller R, Zahurak M, Forastiere AA, Hamilton SR. Genetic alterations in Barrett esophagus and adenocarcinomas of the esophagus and esophagogastric junction region. *Am J Pathol* 1998;153:287–94.
- Riegman PH, Vissers KJ, Alers JC, et al. Genomic alterations in malignant transformation of Barrett's esophagus. *Cancer Res* 2001;61:3164–70.
- Walch AK, Zitzelsberger HF, Bruch J, et al. Chromosomal imbalances in Barrett's adenocarcinoma and the metaplasia-dysplasia-carcinoma sequence. *Am J Pathol* 2000;156:555–66.
- Li X, Galipeau PC, Sanchez CA, et al. Single nucleotide polymorphism-based genome-wide chromosome copy change, loss of heterozygosity, and aneuploidy in Barrett's esophagus neoplastic progression. *Cancer Prev Res* 2008;1:413–23.
- Lai LA, Paulson TG, Li X, et al. Increasing genomic instability during premalignant neoplastic progression revealed through high resolution array-CGH. *Genes Chromosomes Cancer* 2007;46:532–42.
- Paulson TG, Maley CC, Li X, et al. Chromosomal instability and copy number alterations in Barrett's esophagus and esophageal adenocarcinoma. *Clin Cancer Res* 2009;15:3305–14.
- Akagi T, Ito T, Kato M, et al. Chromosomal abnormalities and novel disease-related regions in progression from Barrett's esophagus to esophageal adenocarcinoma. *Int J Cancer* 2009;125:2349–59.
- Paulson TG, Reid BJ. Focus on Barrett's esophagus and esophageal adenocarcinoma. *Cancer Cell* 2004;6:11–6.
- Koppert LB, Wijnhoven BP, van Dekken H, Tilanus HW, Dinjens WN. The molecular biology of esophageal adenocarcinoma. *J Surg Oncol* 2005;92:169–90.
- Fitzgerald RC. Molecular basis of Barrett's oesophagus and oesophageal adenocarcinoma. *Gut* 2006;55:1810–20.
- Peiffer DA, Le JM, Steemers FJ, et al. High-resolution genomic profiling of chromosomal aberrations using Infinium whole-genome genotyping. *Genome Res* 2006;16:1136–48.
- Nancarrow DJ, Handoko HY, Smithers BM, et al. Genome-wide copy number analysis in esophageal adenocarcinoma using high-density single-nucleotide polymorphism arrays. *Cancer Res* 2008;68:4163–72.
- Montgomery E, Bronner MP, Goldblum JR, et al. Reproducibility of the diagnosis of dysplasia in Barrett esophagus: a reaffirmation. *Hum Pathol* 2001;32:368–78.
- Singletary SE, Greene FL, Sobin LH. Classification of isolated tumor cells: clarification of the 6th edition of the American Joint Committee on Cancer Staging Manual. *Cancer* 2003;98:2740–1.

23. Olshen AB, Venkatraman ES, Lucito R, Wigler M. Circular binary segmentation for the analysis of array-based DNA copy number data. *Biostatistics* 2004;5:557–72.
24. Illumina Technical Bulletin. Whole-genome genotyping with the Sentrix® HumanHap300 Genotyping BeadChip and the Infinium™ II Assay. Available from: www.servicexs.nl/blobs/illumina/HUMANHAP300TechBull.pdf.
25. Rajagopalan H, Nowak MA, Vogelstein B, Lengauer C. The significance of unstable chromosomes in colorectal cancer. *Nat Rev Cancer* 2003;3:695–701.
26. Weir B, Zhao X, Meyerson M. Somatic alterations in the human cancer genome. *Cancer Cell* 2004;6:433–8.
27. Bell DW. Our changing view of the genomic landscape of cancer. *J Pathol* 2010;220:231–43.
28. Durkin SG, Glover TW. Chromosome fragile sites. *Annu Rev Genet* 2007;41:169–92.
29. Lukusa T, Fryns JP. Human chromosome fragility. *Biochim Biophys Acta* 2008;1779:3–16.
30. McAvoy S, Ganapathiraju SC, Ducharme-Smith AL, et al. Non-random inactivation of large common fragile site genes in different cancers. *Cytogenet Genome Res* 2007;118:260–9.
31. Smith DI, Zhu Y, McAvoy S, Kuhn R. Common fragile sites, extremely large genes, neural development and cancer. *Cancer Lett* 2006;232:48–57.
32. Maley CC, Galipeau PC, Li X, Sanchez CA, Paulson TG, Reid BJ. Selectively advantageous mutations and hitchhikers in neoplasms: p16 lesions are selected in Barrett's esophagus. *Cancer Res* 2004;64:3414–27.
33. Zhu Y, McAvoy S, Kuhn R, Smith DI. RORA, a large common fragile site gene, is involved in cellular stress response. *Oncogene* 2006;25:2901–8.
34. Coquelle A, Toledo F, Stern S, Bieth A, Debatisse M. A new role for hypoxia in tumor progression: induction of fragile site triggering genomic rearrangements and formation of complex DMs and HSRs. *Mol Cell* 1998;2:259–65.
35. Huang Y, Boynton RF, Blount PL, et al. Loss of heterozygosity involves multiple tumor suppressor genes in human esophageal cancers. *Cancer Res* 1992;52:6525–30.
36. Weir BA, Woo MS, Getz G. Characterizing the cancer genome in lung adenocarcinoma. *Nature* 2007;450:893–8.
37. Ito Y. RUNX genes in development and cancer: regulation of viral gene expression and the discovery of RUNX family genes. *Adv Cancer Res* 2008;99:33–76.
38. Perry C, Eldor A, Soreq H. Runx1/AML1 in leukemia: disrupted association with diverse protein partners. *Leuk Res* 2002;26:221–8.
39. Li QL, Ito K, Sakakura C, et al. Causal relationship between the loss of RUNX3 expression and gastric cancer. *Cell* 2002;109:113–24.
40. Sakakura C, Hagiwara A, Miyagawa K, et al. Frequent downregulation of the runt domain transcription factors RUNX1, RUNX3 and their cofactor CBFβ in gastric cancer. *Int J Cancer* 2005;113:221–8.
41. Barrett MT, Sanchez CA, Prevo LJ, et al. Evolution of neoplastic cell lineages in Barrett oesophagus. *Nat Genet* 1999;22:106–9.
42. Vasiljeva O, Korovin M, Gajda M, et al. Reduced tumour cell proliferation and delayed development of high-grade mammary carcinomas in cathepsin B-deficient mice. *Oncogene* 2008;27:4191–9.
43. Hughes SJ, Glover TW, Zhu XX, et al. A novel amplicon at 8p22-23 results in overexpression of cathepsin B in esophageal adenocarcinoma. *Proc Natl Acad Sci U S A* 1998;95:12410–5.
44. Wang S, Zhan M, Yin J, et al. Transcriptional profiling suggests that Barrett's metaplasia is an early intermediate stage in esophageal adenocarcinogenesis. *Oncogene* 2006;25:3346–56.
45. Tsafrir D, Bacolod M, Selvanayagam Z, et al. Relationship of gene expression and chromosomal abnormalities in colorectal cancer. *Cancer Res* 2006;66:2129–37.
46. Maley CC, Galipeau PC, Finley JC, et al. Genetic clonal diversity predicts progression to esophageal adenocarcinoma. *Nat Genet* 2006;38:468–73.

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Genome-Wide Catalogue of Chromosomal Aberrations in Barrett's Esophagus and Esophageal Adenocarcinoma: A High-Density Single Nucleotide Polymorphism Array Analysis

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