



LUND UNIVERSITY

Genetic profiles of gastroesophageal cancer: combined analysis using expression array and tiling array-comparative genomic hybridization

Isinger Ekstrand, Anna; Johansson, Jan; Ohlsson, Mattias; Francis, Princy; Staaf, Johan; Jönsson, Mats; Borg, Åke; Nilbert, Mef

Published in:
Cancer Genetics and Cytogenetics

DOI:
[10.1016/j.cancergencyto.2010.03.013](https://doi.org/10.1016/j.cancergencyto.2010.03.013)

2010

[Link to publication](#)

Citation for published version (APA):

Isinger Ekstrand, A., Johansson, J., Ohlsson, M., Francis, P., Staaf, J., Jönsson, M., Borg, Å., & Nilbert, M. (2010). Genetic profiles of gastroesophageal cancer: combined analysis using expression array and tiling array-comparative genomic hybridization. *Cancer Genetics and Cytogenetics*, 200(2), 120-126. <https://doi.org/10.1016/j.cancergencyto.2010.03.013>

Total number of authors:
8

General rights

Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00



LUND UNIVERSITY
Faculty of Medicine

LUP

Lund University Publications

Institutional Repository of Lund University

This is an author produced version of a paper published in Cancer Genetics and Cytogenetics. This paper has been peer-reviewed but does not include the final publisher proof-corrections or journal pagination.

Citation for the published paper:
Anna Ekstrand, Jan Johansson, Mattias Ohlsson,
Princy Francis, Johan Staaf, Mats Jönsson, Åke Borg,
Mef Nilbert

"Genetic profiles of gastroesophageal cancer:
combined analysis using expression array and tiling
array--comparative genomic hybridization."

Cancer Genetics and Cytogenetics 2010 200, 120 -
126

<http://dx.doi.org/10.1016/j.cancergencyto.2010.03.013>

Access to the published version may require journal
subscription.

Published with permission from: Elsevier

Genetic Profiles of Gastroesophageal Cancer

combined analysis using expression array and tiling array-CGH

Ekstrand-Isinger. A^{a*}, Johansson J^b, Ohlsson M^c, Francis P^a, Staaf J^a, Jönsson M^a, Borg Å^a
and Nilbert M^{a,d}

^aDepartment of Oncology, Clinical Sciences, Lund University, Lund, Sweden

^bDepartment of Surgery, Clinical Sciences, Lund University, Lund, Sweden

^cDepartment of Theoretical Physics, Lund University, Lund, Sweden

^dClinical Research Centre, Hvidovre Hospital, Copenhagen University, Hvidovre, Denmark

* Corresponding author. Barngatan 2b, SE-22185 Lund, Sweden. Tel. +46 46 178524. Fax. +46 46 147327. E-mail. anna.ekstrand@med.lu.se.

Abstract

We aimed to characterize the genomic profiles of adenocarcinomas in the gastroesophageal junction in relation to cancers in the esophagus and the stomach. Profiles of gains/losses as well as gene expression profiles were obtained from 27 gastroesophageal adenocarcinomas using 32k high-resolution array-based comparative genomic hybridization (aCGH) and 27k oligo gene expression arrays and putative target genes were validated in an extended series. Adenocarcinomas in the distal esophagus and the gastroesophageal junction showed strong similarities with the most common gains at 20q13, 8q24, 1q21-q23, 5p15, 13q34, and 12q13, whereas different profiles with gains at 5p15, 7p22, 2q35, and 13q34 characterized gastric cancers. CDK6 and EGFR were identified as putative target genes in cancers of the esophagus and the gastroesophageal junction with upregulation in one quarter of the tumors. Gains/losses and gene expression profiles show strong similarity between cancers in the distal esophagus and the gastroesophageal junction with frequent upregulation of CDK6 and EGFR, whereas gastric cancer displays distinct genetic changes. These data suggest that molecular diagnostics and targeted therapies can be applied to adenocarcinomas of the distal esophagus and gastroesophageal junction alike.

1. Introduction

Gastroesophageal cancers are genetically complex and carry a serious prognosis with 5-year overall survival rates below 20%. Refined diagnosis and new treatment options, e.g. biological therapies directed at critical signaling pathways, are therefore needed. Worldwide, gastroesophageal cancer affects 1.5 million individuals annually [1]. Gastric adenocarcinomas (GA) show a decreasing incidence in the western world and are associated with *Helicobacter Pylori* infection, whereas adenocarcinomas in the distal esophagus (EA) constitutes one of the most rapidly increasing tumor types linked to overweight and gastroesophageal reflux [2]. Tumors that arise within the gastroesophageal junction (JA) may cause diagnostic dilemmas. Though correct classification has implications for the choice of treatment strategy, the current classification is based on the anatomical location and fails to account for differences in etiology and tumor biology [3-5].

Genetic changes have been closely linked to the metaplasia-dysplasia sequence of gastroesophageal cancer. EA as well as GA have demonstrated complex genetic alterations and gene expression changes [6, 7]. Though multiple genetic similarities have been recognized between EA and JA, differences with losses of 5q, 8p, 14q and gains of 2q, 6p, 12p and 20q to be discriminative have also been suggested [8, 9, 10-12]. With the aim to obtain a detailed genetic picture of gastroesophageal adenocarcinomas and to identify key genes and pathways herein, we applied a combined array-based gene expression and genomic profiling analysis to adenocarcinomas in the esophagus, the gastroesophageal junction, and the gastric body.

2. Materials & Methods

2.1. Materials

Tumor tissue from 27 primary gastroesophageal and gastric adenocarcinomas were snap frozen at -80°C. The mean age of the 18 males and 9 females was 65 (range 39-84) years and none of the patients had received neoadjuvant radiotherapy or chemotherapy. The tumors were classified according to the Siewert classification [5] and included 10 EA (located 1-5 cm above the gastroesophageal junction), 9 JA (located within 1 cm above and 2 cm below the gastroesophageal junction), and 8 GA (located in the gastric fundus-corpus with 5 being of the intestinal type and 3 being diffuse GA). Tumor stage was I in 2 tumors, II in 13, III in 5, and IV in 7. Barrett's esophagus was diagnosed in 7/10 tumors in the distal esophagus and in 1/9 tumors in the gastroesophageal junction and thus occurred at a higher frequency in the former subset ($p=0.02$). Ethical approval for this study was obtained from the Lund University ethics committee and the patients provided informed consent for participation.

2.2. Target preparation and hybridization

High-resolution tiling 32k BAC microarrays with complete genome coverage were produced by the Swegene DNA Microarray Center, Lund University, using the BAC Re-Array set Ver. 1.0 (BACPAC Resource Center, Children's Hospital Oakland Research Institute, Oakland, CA, USA (<http://bacpac.chori.org/>) [13]. BAC clones were mapped to the hg 17 build from the UCSC Genome Browser (<http://genome.ucsc.edu/>). Genomic DNA was extracted using proteinase K treatment followed by phenol chloroform purification. 2 µg of tumor DNA and 1.5 µg of a pool of male reference DNA (Promega, Madison, WI, USA), were differentially labeled with Cy3-dCTP and Cy5-dCTP (Amersham Biosciences, UK) using the Bioprime Genomic labeling system (Invitrogen Life Technologies, Carlsbad, CA, USA). Labeled DNA was pooled, mixed with 100 µg human Cot-1 DNA (Invitrogen Life Technologies), and dried in a Thermo Savant DNA SpeedVacTM (Thermo Electron Corporation, Waltham, MA, USA)

and thereafter re-suspended in 57 µl of hybridization buffer, denatured, re-annealed and applied to the arrays where after washing and scanning was performed [14].

27k oligonucleotide arrays printed from the Human Genome Oligo Set Version 2.1 (21, 329; 70-mer probes) and the Version 2.1 upgrade (5, 462 probes) were produced at the Swegene DNA Microarray Resource Centre [15]. RNA was extracted from 80-120 mg freshly frozen tumor tissue using TRizol (Invitrogen Life Technologies) and the RNeasy Midi Kit (Qiagen, Valencia, CA, USA) according to the manufactures' recommendation. Target preparation was performed using the Pronto!™ Plus System (Corning Incorporated, Corning, NY, USA), in which 5 µg of tumor RNA and reference RNA (Stratagene Universal Reference, Stratagene, La Jolla, CA, USA) was incubated with a mix of random primers and oligo(dT). cDNA was labeled with Cy3 dCTP (tumor) and Cy5 dCTP (reference). Purified labeled tumor cDNA and reference cDNA was combined and dried in a Thermo Savant DNA SpeedVac™ and resuspended in Universal Hybridization Solution. Including 10 alien RNA sequences and 10 *Arabidopsis thaliana* derived RNA sequences without any homology validated the quality of the arrays (Stratagene, La Jolla, CA, USA).

RNA/DNA quality was assessed using an Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) and RNA/DNA quantity using a NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Arrays were scanned using an Agilent G2565AA Microarray Scanner (Agilent Technologies).

2.3. Data analysis

GenePixTMPro 4.1 (Axon Instruments Inc., Union City, CA, USA) was used for identification of individual spots. Gene expression data were uploaded into the Bio Array Software Environment (<http://base.thep.lu.se>) for further analysis [16]. Correction of background intensities of Cy3 and Cy5 were calculated using a median-feature and median-local background intensities of the uploaded files. The intensity ratios for the individual probes were calculated as intensity of the background corrected tumor channel (Cy3) divided with the intensity of the background corrected reference channel (Cy5). Low quality spots flagged during image analysis and spots with a signal-to-noise ratio (SNR) <1.5 and >10% saturation were excluded from further analysis. To compensate for dye bias and local background effects, data were normalized using a lowess algorithm in BASE [17]. The uncertainty of a spot u was estimated using $SNR1-2 + SNR2-2$, where SNR_i corresponds to the signal-to-noise ratio for channel i . Replicates assays were merged and represented by a weighted mean. Expression values were modified according to an error model in that merged genes with large uncertainties u_i were moved closer to a weighted mean m across assays for that gene, as described [18]. Genes missing in more than 20% of the samples or with a standard deviation of the modified log ratios ≤ 0.3 was excluded. After these steps, 93,777 spots representing 3,535 genes remained for further processing. Hierarchical cluster analysis was performed using Pearson correlation. Significant Analysis of Microarrays (SAM) was used to identify significant genes with 1,000 permutations (cut-off level of median 0 false significant genes, corresponding to a false discovery rate of 0) [19]. Cluster analysis and SAM analysis were performed using the MeV application from the TM₄ microarray software suite, freely available at www.tigr.org/software/tm4/ [20]. The Expression Analysis Systematic Explorer (EASE) software (<http://david.niaid.nih.gov/david/ease.htm>) was used for the interpretation of biological function and the genes were classified into groups according to the Gene Ontology

Consortium (GO Biological Process) and the KEGG pathway. Functional groups with EASE score less than 0.05 are included in the results.

Copy number data were processed and handled as described in the gene expression section with the following modifications. In BASE, individual spots with a SNR ≤ 5 were removed from further analysis and were handled as missing values. The X and the Y chromosomes were excluded from the normalization step using a pin-based lowess algorithm. After these steps, 645,690 spots representing 29,780 BAC-clones remained. Copy number data was loaded into Nexus software, v.3 (Biodiscovery) for visualization. Data was segmented using a rank based segmentation algorithm with at least 5 probes per segment. Gains and losses were defined as \log_2 (ratios) of ≥ 0.2 and ≤ -0.3 respectively, with high gains defined as \log_2 (ratio) of ≥ 0.8 . Copy number variation frequencies and comparison between groups were estimated using Nexus software.

2.4. Correlation between copy number and gene expression

After filtering and normalization of gene expression data, the remaining 14,000 genes were used to create a “map-list” with start and end positions of each 70-mer oligo in relation to position of the BAC-clones. Since most genes were covered by more than one BAC-clone a weighted mean copy number, derived from the size of the overlap, was used. Genes with expression values that varied between assays with a standard deviation of the modified log ratios ≥ 0.2 were further used for correlation analysis. Relative gene expression and copy number correlations were calculated using Spearman rank correlation. Plots showing the overall relative correlation between expression and copy number were generated using the \log_2 of the mean of expression/copy number in each tumor group (figure 1). The data were

filtered using a sliding window to reduce noise. Each plot was normalized to a maximum absolute value of 1.

2.5. Immunohistochemistry

Immunohistochemical validation of EGFR and CDK6 was applied in an extended set of 47 tumors, which represented a consecutive series of patients with EA/JA that had undergone surgery. Immunohistochemical stainings for EGFR (clone E30, 1:50, DAKO A/S, Glostrup, Denmark) and CDK6 (clone DCS-83, 1:25, Progen Heidelberg, Germany) were performed using 4-µm sections of formalin-fixed, paraffin embedded tissue, which were mounted on DAKO ChemMate Capillary Gap Microscope Slides (DAKO). Tissue sections were deparaffinized in xylol, and rehydrated through descending concentrations of alcohol. Sections for EGFR staining were pretreated with Proteinase K for 10 minutes. For CDK6 staining, antigen retrieval was performed in DAKO Target Retrieval Solution, pH 9 (DAKO), in a pressure cooker (Histolab, Gothenburg, Sweden). Immunohistochemical staining was performed using LSAB+ (EGFR) or the Envision method (CDK6) (DAKO) in an automated immunostainer (TechMate 500 Plus, DAKO). Membranous EGFR staining was scored as 0 (no expression), 1 (expression in few cells), 2 (moderate expression), and 3 (strong expression). Nuclear CDK6 expression was scored according to the fraction of stained tumor cells; 0 (0% of the cells), 1 (5-30%), 2 (31-60%) and 3 (61-100%). The staining was independently evaluated by two of the coauthors and a score of 2 or 3 was defined as strong expression. Fisher's exact t-test was used for categorical analysis of gene expressions, copy number and protein expressions.

3. Results

3.1. Gene expression profiling

Unsupervised cluster analysis based on the gene expression data (3,535 reporters) identified several sub-clusters, one of which contained 5/8 GA and the other containing a mixture of JA, EA and GA (supplemental data, figure 1). A similar pattern was observed when a less stringent variation filter (resulting in 7,451 genes) was applied (data not shown). The gene expression profiles in EA and JA showed strong similarity, whereas SAM-analysis identified 38 (FDR of 0%) and 164 (FDR of 5%) genes that were significantly upregulated in EA/JA compared to GA (supplemental data, table 1). Overexpressed genes were by EASE analysis found to be involved in cell cycle regulation, proliferation, MAP kinase pathway, stress response, biotic stimulus, and apoptotic response

3.2. Copy number analysis

High-quality copy number profiles were obtained from 23/27 tumors and showed multiple gains/losses and several recurrent alterations. Gains were more frequent than losses and similar to the expression profiles, the gains/losses identified in EA and JA were highly similar (figure 2) with the most common gains at 20q13.33 (88%), 8q24.3 (75%), 1q21.3-q23.1 (69%), 5p15.33 (69%), 13q34 (62%), 12q13.31 (56%), 12q13.13 (56%), 18q11.1-q11.2 (56%), 2q14.1 (50%), 1p36.33 (44%), 1p36.31 (44%), 6p21.1 (44%), 2q35 (38%) and 16q24.2-24.3 (38%). Genes located within these regions were preferentially involved in transcription, defense response and RNA elongation. The only significant loss (present in 69% of the tumors) was located on the Y-chromosome (Yq11.1-q11.23). Gains in GA affected 5p15.33 (86%), 7p22.3 (87%), 2q35 (57%) and 13q34 (57%) and these regions contained several genes involved in regulation of proteolysis, B cell proliferation and defense response. Multiple high-level amplifications (HLAs) with \log_2 ratios >0.8 were identified in

the majority of JA and EA but were rarely observed in GA. Candidate genes linked to these HLAs were *VEGFA* (6p21.1), *EGFR* (7p11.2), *CDK6* (7q21.2) and *ERBB2* (7q12).

Differential analysis between EA and GA identified 2,205 significantly different BAC clones (Mann-Whitney U-test, $p < 0.05$). Supervised cluster analysis applied on all three sub-sets identified two sub-clusters with a close relation between EA and JA (data not shown). Application of the Nexus software identified only two significantly gained/lost regions between EA and JA with gain of 12q23.3-q24.21 and gain of 18p11.32, respectively.

3.3. Correlation between gene expression and copy number and protein expression

Overexpression and copy-number gains were highly correlated for the majority of the regions identified, which supports biologically relevant roles (figure 1). Based on concordant amplification and over expression in EA/JA, and previous links to gastroesophageal tumorigenesis and application of targeted therapies, CDK6 and EGFR were chosen for immunohistochemical validation in an extended sample set of 47 EA/JA. Gain/amplification and upregulated gene expression of CDK6 was identified in 4/14 (29%) and 2/14 (14%) tumors, respectively. Immunostaining of CDK6 showed strong expression in 11/47 (23%), weak in 18/47 (38%) and no expression in 19/47 (40%) (figure 3a). Amplification and upregulated gene expression of EGFR was found in 7/14 (50%) and in 1/14 (7%) EA/JA, respectively, and strong immunohistochemical expression in 11/46 (24%), weak in 11/46 (24%) and no expression was found in 24/46 (52%) EA/JA (figure 3b). No correlations with gene expression/copy number and protein expression was found.

4. Discussion

Combined gene expression and copy number analysis demonstrated extensive similarity between adenocarcinomas in the distal esophagus and the gastroesophageal junction, whereas adenocarcinomas in the gastric body revealed different profiles. All three tumor types showed multiple gains and losses, including gains at 20q13 and 8q, which have been recognized as recurrent in gastroesophageal tumors [9, 11, 12]. Gain of 20q13 has also been described in other tumor types and has been suggested to be of prognostic value in e.g. breast cancer and colorectal cancer [21, 22]. The genomic profiles of EA and JA showed extensive similarity, though gain of 12q was overrepresented in EA and gain of 18q in JA. A number of genetic regions have been reported to discriminate between EA and JA, but none of these have been validated and overall the similarities by far outnumber the differences, suggesting similar and to a large extent shared tumorigenic pathways [8-10, 12].

The gene expression profiles in EA/JA demonstrated upregulation of several genes linked to gastroesophageal tumorigenesis, e.g. *ECGF1*, *H19*, *S100A10*, *MYC*, *MMP11*, and *CTSB* [23-25]. In EA, both *ECGF1* and *CTSB*, which are linked to tumor development and suggested to represent a diagnostic marker, showed a high degree of over expression and amplification [26, 27]. Upregulation of MAP-kinase genes, i.e. *PLA2G2A*, *DUSP1*, *MYC*, *FOS* and *GADD45B* were identified in EA/JA and is intriguing since *in vitro* data suggest an anti-proliferative effect from MAPK inhibition in Barrett's adenocarcinoma and because of promising therapeutic strategies aimed at that downregulating MAPK signaling [28].

Two markers of potential diagnostic, prognostic and treatment predictive impact were further evaluated using immunostaining. The cell cycle regulator CDK6, which has been linked to prognosis in medulloblastoma, has been suggested to represent the target gene in the 7q21

amplicon in EA [29, 30]. We identified gain/amplification of the *CDK6* locus in 29%, upregulated gene expression in 14%, and increased immunostaining in 23% of EA/JA. EGFR represents a major target for amplification and overexpression in several cancer types, which is exploited in targeted therapies using monoclonal antibodies as well as tyrosine kinase inhibitors. Amplification of the *EGFR* locus was found in 50% of EA/JA, upregulated gene expression in 7%, and immunohistochemical overexpression in 24%. This finding is in line with e.g. data from fluorescence in situ hybridization showing *EGFR* amplification in 8-31% of EA [31-33] and upregulation of *EGFR* has been suggested to correlate with poor prognosis [34].

In summary, gastroesophageal cancers are characterized by genetic complexity but herein show concordant copy-number gains and upregulation of target genes suggesting that molecular diagnostics and targeted therapies can be applied to EA and JA alike. The correlations between genomic and expression-based signatures highlight involvement of central signaling pathways, including MAPK, *CDK6* and *EGFR* that may be of relevance for refined diagnostics, prognosis and targeted treatment in a tumor type with considerable resistance to the therapeutic options currently available.

Acknowledgements

The study was supported by grants from the Knut and Alice Wallenberg Foundation via the Swegene program, the Swedish Cancer Society, the Nilsson Cancer Research Fund, the Kamprad Research Fund, the Region Skåne Research Funds and the Inga-Britt and Arne Lundberg Foundation.

We thank Anna Laurell for technical assistance.

References

- [1] Lambert R, Hainaut P. The multidisciplinary management of gastrointestinal cancer. Epidemiology of oesophagogastric cancer. *Best Pract Res Clin Gastroenterol* 2007;21:921-45.
- [2] Forman D. Review article: oesophago-gastric adenocarcinoma -- an epidemiological perspective. *Aliment Pharmacol Ther* 2004;20 Suppl 5:55-60; discussion 1-2.
- [3] Kalish RJ, Clancy PE, Orringer MB, Appelman HD. Clinical, epidemiologic, and morphologic comparison between adenocarcinomas arising in Barrett's esophageal mucosa and in the gastric cardia. *Gastroenterology* 1984;86:461-7.
- [4] Ruol A, Parenti A, Zaninotto G, Merigliano S, Costantini M, Cagol M, Alfieri R, Bonavina L, Peracchia A, Ancona E. Intestinal metaplasia is the probable common precursor of adenocarcinoma in barrett esophagus and adenocarcinoma of the gastric cardia. *Cancer* 2000;88:2520-8.
- [5] Siewert JR, Stein HJ. Classification of adenocarcinoma of the oesophagogastric junction. *Br J Surg* 1998;85:1457-9.
- [6] Riegman PH, Vissers KJ, Alers JC, Geelen E, Hop WC, Tilanus HW, van Dekken H. Genomic alterations in malignant transformation of Barrett's esophagus. *Cancer Res* 2001;61:3164-70.
- [7] 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.
- [8] El-Rifai W, Frierson HF, Jr., Moskaluk CA, Harper JC, Petroni GR, Bissonette EA, Jones DR, Knuutila S, Powell SM. Genetic differences between adenocarcinomas arising in Barrett's esophagus and gastric mucosa. *Gastroenterology* 2001;121:592-8.
- [9] van Dekken H, Geelen E, Dinjens WN, Wijnhoven BP, Tilanus HW, Tanke HJ, Rosenberg C. Comparative genomic hybridization of cancer of the gastroesophageal junction:

deletion of 14Q31-32.1 discriminates between esophageal (Barrett's) and gastric cardia adenocarcinomas. *Cancer Res* 1999;59:748-52.

[10] Menke-Pluymers MB, van Drunen E, Vissers KJ, Mulder AH, Tilanus HW, Hagemijer A. Cytogenetic analysis of Barrett's mucosa and adenocarcinoma of the distal esophagus and cardia. *Cancer Genet Cytogenet* 1996;90:109-17.

[11] Moskaluk CA, Hu J, Perlman EJ. Comparative genomic hybridization of esophageal and gastroesophageal adenocarcinomas shows consensus areas of DNA gain and loss. *Genes Chromosomes Cancer* 1998;22:305-11.

[12] Varis A, Puolakkainen P, Savolainen H, Kokkola A, Salo J, Nieminen O, Nordling S, Knuutila S. DNA copy number profiling in esophageal Barrett adenocarcinoma: comparison with gastric adenocarcinoma and esophageal squamous cell carcinoma. *Cancer Genet Cytogenet* 2001;127:53-8.

[13] Jonsson G, Staaf J, Olsson E, Heidenblad M, Vallon-Christersson J, Osoegawa K, de Jong P, Oredsson S, Ringner M, Hoglund M, Borg A. High-resolution genomic profiles of breast cancer cell lines assessed by tiling BAC array comparative genomic hybridization. *Genes Chromosomes Cancer* 2007;46:543-58.

[14] Snijders AM, Nowak N, Segreaves R, Blackwood S, Brown N, Conroy J, Hamilton G, Hindle AK, Huey B, Kimura K, Law S, Myambo K, Palmer J, Ylstra B, Yue JP, Gray JW, Jain AN, Pinkel D, Albertson DG. Assembly of microarrays for genome-wide measurement of DNA copy number. *Nat Genet* 2001;29:263-4.

[15] Jonsson G, Naylor TL, Vallon-Christersson J, Staaf J, Huang J, Ward MR, Greshock JD, Luts L, Olsson H, Rahman N, Stratton M, Ringner M, Borg A, Weber BL. Distinct genomic profiles in hereditary breast tumors identified by array-based comparative genomic hybridization. *Cancer Res* 2005;65:7612-21.

- [16] Saal LH, Troein C, Vallon-Christersson J, Gruvberger S, Borg A, Peterson C. BioArray Software Environment (BASE): a platform for comprehensive management and analysis of microarray data. *Genome Biol* 2002;3:SOFTWARE0003.
- [17] Yang YH, Dudoit S, Luu P, Lin DM, Peng V, Ngai J, Speed TP. Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. *Nucleic Acids Res* 2002;30:e15.
- [18] Fernebro J, Francis P, Eden P, Borg A, Panagopoulos I, Mertens F, Vallon-Christersson J, Akerman M, Rydholm A, Bauer HC, Mandahl N, Nilbert M. Gene expression profiles relate to SS18/SSX fusion type in synovial sarcoma. *Int J Cancer* 2006;118:1165-72.
- [19] Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci U S A* 2001;98:5116-21.
- [20] Saeed AI, Sharov V, White J, Li J, Liang W, Bhagabati N, Braisted J, Klapa M, Currier T, Thiagarajan M, Sturn A, Snuffin M, Rezantsev A, Popov D, Ryltsov A, Kostukovich E, Borisovsky I, Liu Z, Vinsavich A, Trush V, Quackenbush J. TM4: a free, open-source system for microarray data management and analysis. *Biotechniques* 2003;34:374-8.
- [21] Aust DE, Muders M, Kohler A, Schmidt M, Diebold J, Muller C, Lohrs U, Waldman FM, Baretton GB. Prognostic relevance of 20q13 gains in sporadic colorectal cancers: a FISH analysis. *Scand J Gastroenterol* 2004;39:766-72.
- [22] Isola JJ, Kallioniemi OP, Chu LW, Fuqua SA, Hilsenbeck SG, Osborne CK, Waldman FM. Genetic aberrations detected by comparative genomic hybridization predict outcome in node-negative breast cancer. *Am J Pathol* 1995;147:905-11.
- [23] Hibi K, Nakamura H, Hirai A, Fujikake Y, Kasai Y, Akiyama S, Ito K, Takagi H. Loss of H19 imprinting in esophageal cancer. *Cancer Res* 1996;56:480-2.

- [24] Hourihan RN, O'Sullivan GC, Morgan JG. Transcriptional gene expression profiles of oesophageal adenocarcinoma and normal oesophageal tissues. *Anticancer Res* 2003;23:161-5.
- [25] Tselepis C, Morris CD, Wakelin D, Hardy R, Perry I, Luong QT, Harper E, Harrison R, Attwood SE, Jankowski JA. Upregulation of the oncogene c-myc in Barrett's adenocarcinoma: induction of c-myc by acidified bile acid in vitro. *Gut* 2003;52:174-80.
- [26] Hughes SJ, Glover TW, Zhu XX, Kuick R, Thoraval D, Orringer MB, Beer DG, Hanash S. 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.
- [27] Mitas M, Almeida JS, Mikhitarian K, Gillanders WE, Lewin DN, Spyropoulos DD, Hoover L, Graham A, Glenn T, King P, Cole DJ, Hawes R, Reed CE, Hoffman BJ. Accurate discrimination of Barrett's esophagus and esophageal adenocarcinoma using a quantitative three-tiered algorithm and multimarker real-time reverse transcription-PCR. *Clin Cancer Res* 2005;11:2205-14.
- [28] Holcombe RF, Marsh JL, Waterman ML, Lin F, Milovanovic T, Truong T. Expression of Wnt ligands and Frizzled receptors in colonic mucosa and in colon carcinoma. *Mol Pathol* 2002;55:220-6.
- [29] Mendrzyk F, Radlwimmer B, Joos S, Kokocinski F, Benner A, Stange DE, Neben K, Fiegler H, Carter NP, Reifenberger G, Korshunov A, Lichter P. Genomic and protein expression profiling identifies CDK6 as novel independent prognostic marker in medulloblastoma. *J Clin Oncol* 2005;23:8853-62.
- [30] van Dekken H, van Marion R, Vissers KJ, Hop WC, Dinjens WN, Tilanus HW, Wink JC, van Duin M. Molecular dissection of the chromosome band 7q21 amplicon in gastroesophageal junction adenocarcinomas identifies cyclin-dependent kinase 6 at both genomic and protein expression levels. *Genes Chromosomes Cancer* 2008;47:649-56.

- [31] al-Kasspooles M, Moore JH, Orringer MB, Beer DG. Amplification and over-expression of the EGFR and erbB-2 genes in human esophageal adenocarcinomas. *Int J Cancer* 1993;54:213-9.
- [32] Miller CT, Moy JR, Lin L, Schipper M, Normolle D, Brenner DE, Iannettoni MD, Orringer MB, Beer DG. Gene amplification in esophageal adenocarcinomas and Barrett's with high-grade dysplasia. *Clin Cancer Res* 2003;9:4819-25.
- [33] Rygiel AM, Milano F, Ten Kate FJ, Schaap A, Wang KK, Peppelenbosch MP, Bergman JJ, Krishnadath KK. Gains and amplifications of c-myc, EGFR, and 20.q13 loci in the no dysplasia-dysplasia-adenocarcinoma sequence of Barrett's esophagus. *Cancer Epidemiol Biomarkers Prev* 2008;17:1380-5.
- [34] Wang KL, Wu TT, Choi IS, Wang H, Resatkova E, Correa AM, Hofstetter WL, Swisher SG, Ajani JA, Rashid A, Albarracin CT. Expression of epidermal growth factor receptor in esophageal and esophagogastric junction adenocarcinomas: association with poor outcome. *Cancer* 2007;109:658-67.

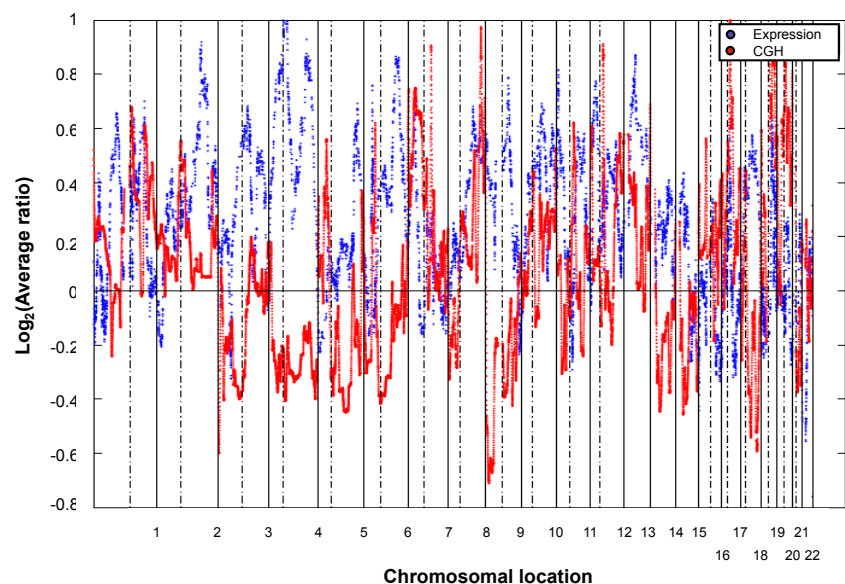
Titles and legends to figures

Figure 1 - Correlation between the relative mean expression and copy number data (log2 values and normalized to a maximum absolute value of 1) in the different tumor types; A, esophageal adenocarcinoma (EA); B, esophagogastric junction (JA) and C, gastric adenocarcinoma (GA). The p- and q-arms of the chromosomes are separated with dotted lines.

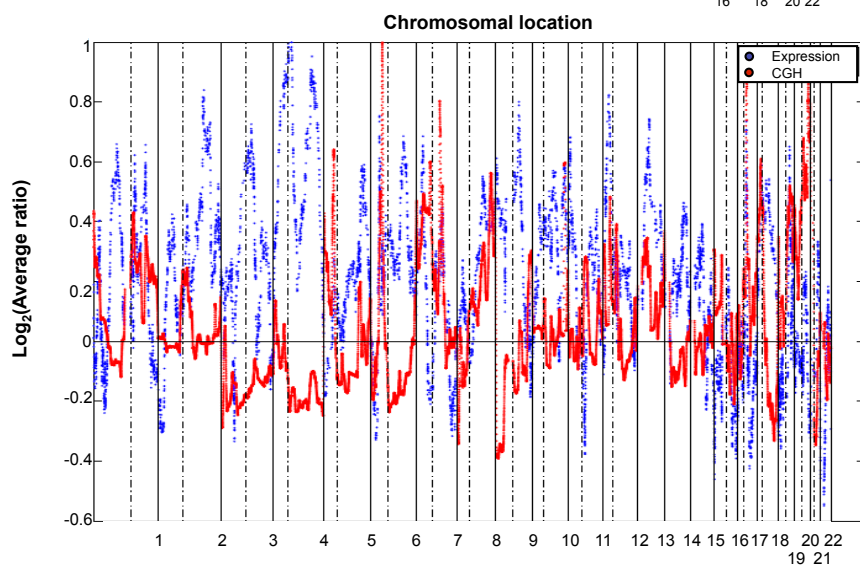
Figure 2 - Frequency plots demonstrating genomic gains and losses in distal esophageal tumors (EA), gastric tumors (GA) and esophagogastric junction tumors (JA) respectively. The plot at the top summarizes the frequencies of gains/losses for all tumors.

Figure 3 - Genomic profiles of two gastroesophageal tumors, with high level amplification peaks corresponding to CDK6 (a) and EGFR (b) respectively, and with corresponding immunohistochemical photo of protein expression.

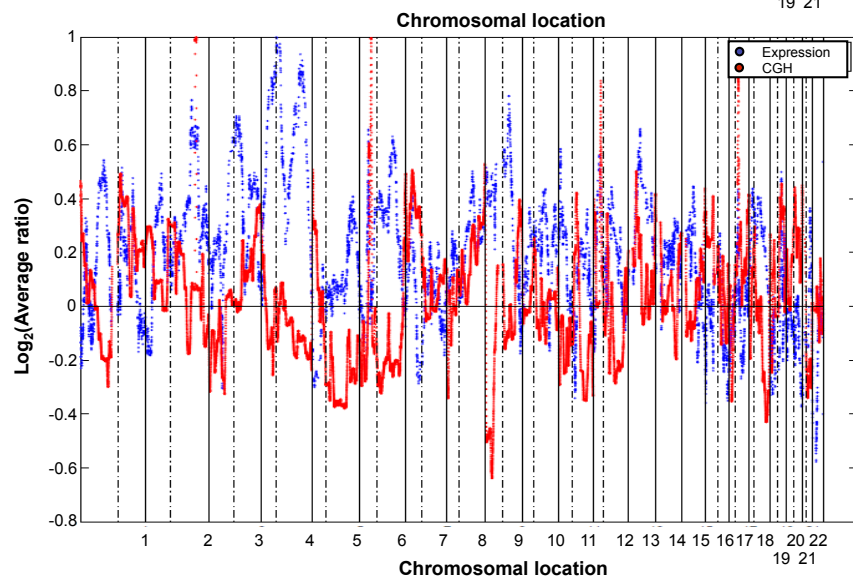
A

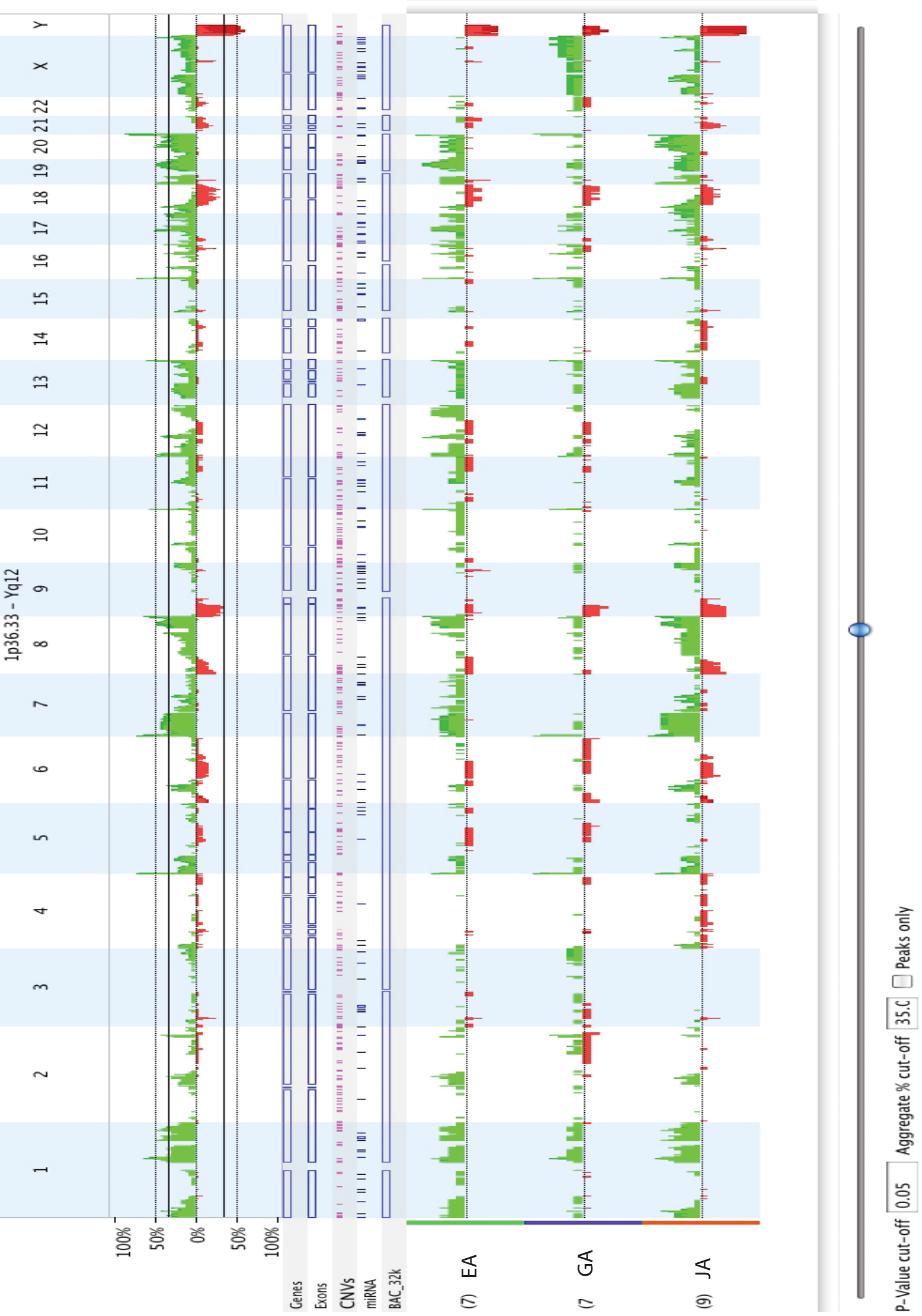


B

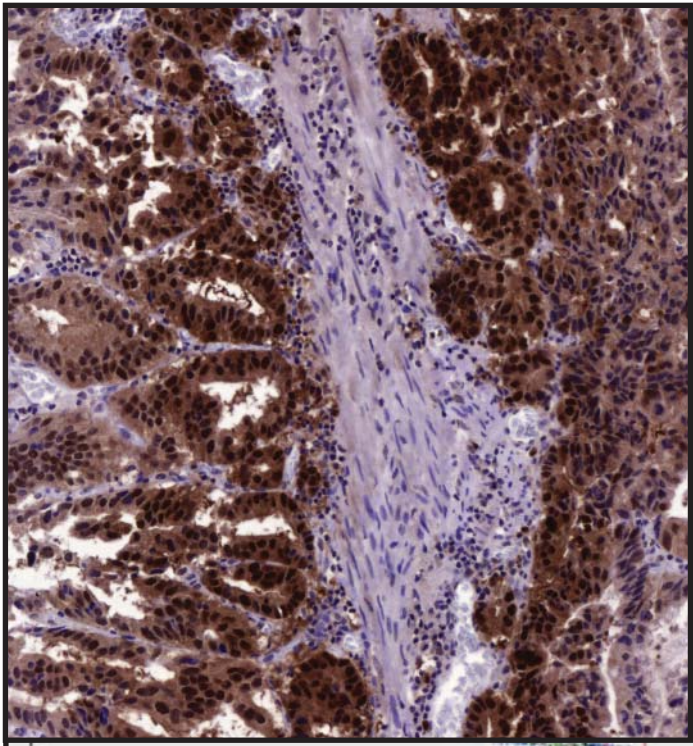
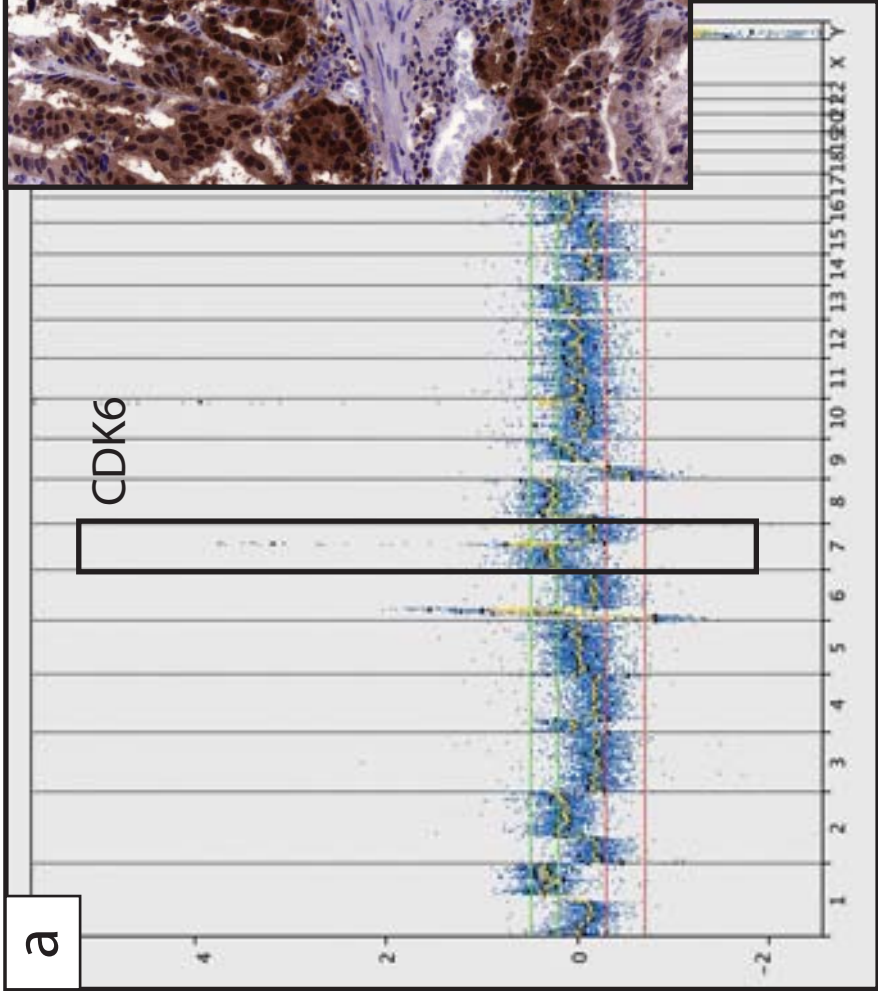


C

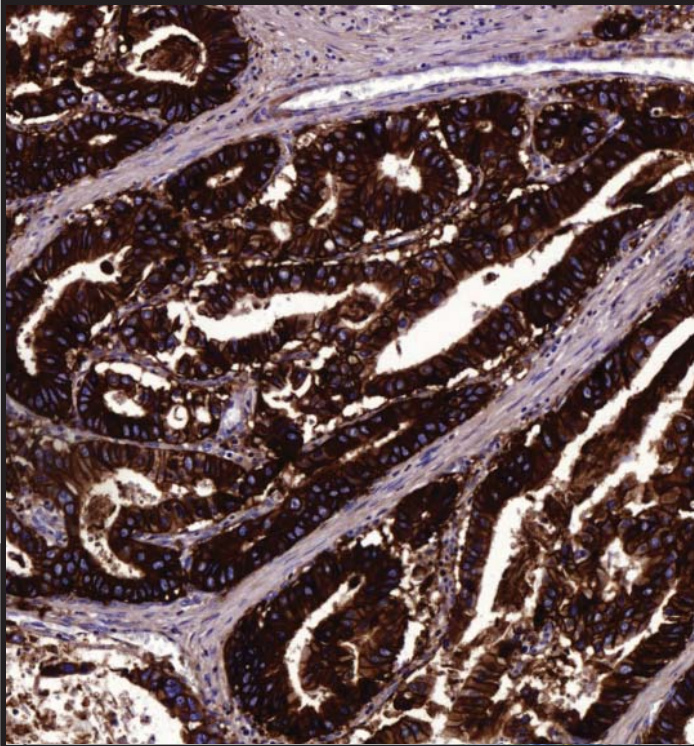
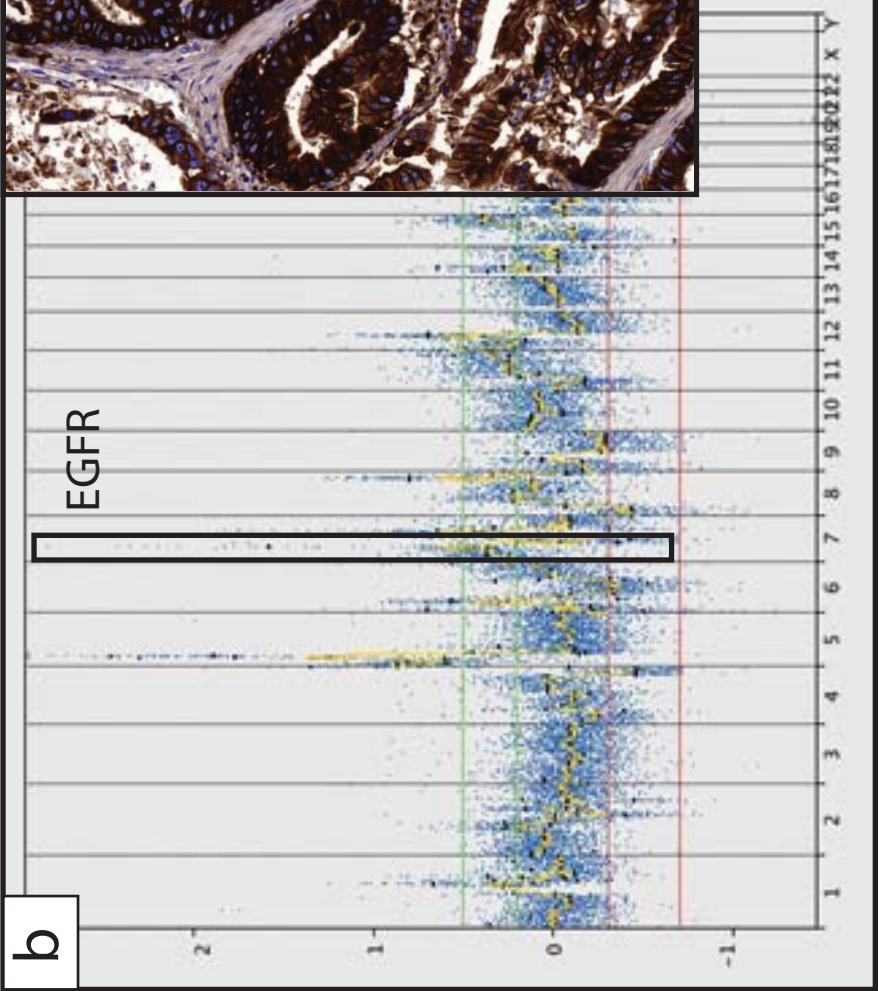


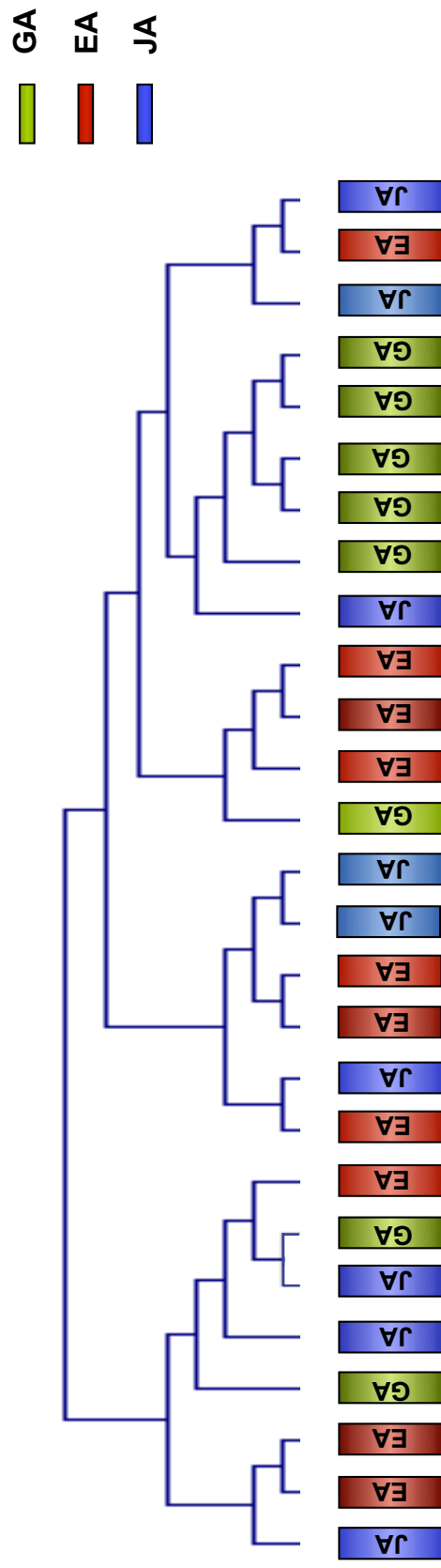


a



b





Supplemental figure 1 - Unsupervised hierarchical clustering of esophagogastric adenocarcinomas based on ~3,500 genes in which 5/8 GA cluster together, whereas the 10 EA and the 9 JA fall into several sub-groups.

Supplemental table 1 - Upregulated genes in the distal esophageal adenocarcinomas (EA) and the gastroesophageal junction adenocarcinomas (JA) compared to the gastric adenocarcinomas (GA). The table includes 164 genes with a FDR of 5%, identified by two-paired SAM-analysis whereas genes showed in bold have an FDR of zero.

| Gene symbol | Chromosome band | Locus link | Fold change |
|-----------------------|-----------------|------------------|-------------|
| <i>CD53</i> | 1p13 | 963 0.5950888 | |
| <i>DNAJB4</i> | 1p31.1 | 11080 0.6175997 | |
| <i>LAPTM5</i> | 1p34 | 7805 0.5276802 | |
| <i>PLK3</i> | 1p34.1 | 1263 0.7473697 | |
| <i>PLA2G2A</i> | 1p35 | 5320 0.30144104 | |
| <i>CA6</i> | 1p36.2 | 765 0.74980944 | |
| <i>CDA</i> | 1p36.2-p35 | 978 0.58441144 | |
| <i>TNFRSF1B</i> | 1p36.3-p36.2 | 7133 0.75916666 | |
| <i>S100A10</i> | 1q21 | 6281 0.45890749 | |
| <i>S100A2</i> | 1q21 | 6273 0.14996576 | |
| <i>S100A9</i> | 1q21 | 6280 0.28657454 | |
| <i>ADAMTS4</i> | 1q21-q23 | 9507 0.6469072 | |
| <i>FCGR3A</i> | 1q23 | 2214 0.5993047 | |
| <i>FCGR2B</i> | 1q23 | 2213 0.3723505 | |
| <i>IER5</i> | 1q25.3 | 51278 0.63266563 | |
| <i>RGS2</i> | 1q31 | 5997 0.5641359 | |
| <i>CFH</i> | 1q32 | 3075 0.68747014 | |
| <i>NUAK2</i> | 1q32.1 | 81788 0.7528812 | |
| <i>CAPG</i> | 2p11.2 | 822 0.67054206 | |
| <i>DUSP2</i> | 2q11 | 1844 0.6577075 | |
| <i>ARID5A</i> | 2q11.2 | 10865 0.67046505 | |
| <i>AFF3</i> | 2q11.2-q12 | 3899 0.65619636 | |
| <i>MGAT4A</i> | 2q12 | 11320 0.6667216 | |
| <i>DPP4</i> | 2q24.3 | 1803 0.64388 | |
| <i>TFPI</i> | 2q32 | 7035 0.7909449 | |
| <i>HSPD1</i> | 2q33.1 | 3329 0.61402315 | |
| <i>FN1</i> | 2q34 | 2335 0.56064487 | |
| <i>FN1</i> | 2q34 | 2335 0.76720315 | |
| <i>COL6A3</i> | 2q37 | 1293 0.6679635 | |
| <i>ARL4C</i> | 2q37.1 | 10123 0.5192106 | |
| <i>CMKOR1</i> | 2q37.3 | 57007 0.64132357 | |
| <i>ITIH1</i> | 3p21.2-p21.1 | 3697 0.7313921 | |
| <i>FBLN2</i> | 3p25.1 | 2199 0.62631726 | |
| <i>COPG</i> | 3q21.3 | 22820 0.3697191 | |
| <i>PLSCR1</i> | 3q23 | 5359 0.7631207 | |
| <i>HOP</i> | 4q11-q12 | 84525 0.4049343 | |
| <i>TRIO</i> | 5p15.1-p14 | 7204 0.7274474 | |
| <i>NKD2</i> | 5p15.3 | 85409 0.7764414 | |
| <i>F2R</i> | 5q13 | 2149 0.6378974 | |
| <i>ARRDC3</i> | 5q14.3 | 57561 0.78025544 | |
| <i>SEPP1</i> | 5q31 | 6414 0.5310646 | |
| <i>EGR1</i> | 5q31.1 | 1958 0.5816082 | |
| <i>GM2A</i> | 5q31.3-q33.1 | 2760 0.73493916 | |
| <i>PDGFRB</i> | 5q31-q32 | 5159 0.7776797 | |

| | | |
|------------------|-----------------------|-------------------|
| CDX1 | 5q31-q33 | 1044 0.45628867 |
| DUSP1 | 5q34 | 1843 0.56651855 |
| TREM2 | 6p21.1 | 54209 0.7435078 |
| NFKB1E | 6p21.1 | 4794 0.6824272 |
| CDKN1A | 6p21.2 | 1026 0.69051194 |
| HSPA1B | 6p21.3 | 3303 0.34192654 |
| HSPA1A | 6p21.3 | 3303 0.35465878 |
| LSM2 | 6p21.3 | 57819 0.7099511 |
| MICA | 6p21.3 | 4276 0.602144 |
| C6orf149 | 6p25.1 | 57128 0.7735012 |
| CD109 | 6q13 | 135228 0.5931137 |
| C6orf150 | 6q13 | 115004 0.6841717 |
| GJA1 | 6q21-q23.2 | 2697 0.72992593 |
| MTRF1L | 6q25 | 54516 0.7133811 |
| SOD2 | 6q25.3 | 6648 0.6384444 |
| UPP1 | 7p12.3 | 7378 0.4879369 |
| GPNMB | 7p15 | 10457 0.47042316 |
| RALA | 7p15-p13 | 5898 0.731414 |
| SCAP2 | 7p21-p15 | 8935 0.6974611 |
| YWHAG | 7q11.23 | 7532 0.7440039 |
| GTF2I | 7q11.23 | 2969 0.63876134 |
| SERPINE1 | 7q21.3-q22 | 5054 0.49055758 |
| PODXL | 7q32-q33 | 5420 0.70612633 |
| CTSB | 8p22 | 1508 0.536777 |
| ATP6V1B2 | 8p22-p21 | 526 0.7464556 |
| GEM | 8q13-q21 | 2669 0.74662995 |
| CTHRC1 | 8q22.3 | 115908 0.6243986 |
| TNFRSF11B | 8q24 | 4982 0.4656863 |
| MYC | 8q24.12-q24.13 | 4609 0.6064123 |
| TATDN1 | 8q24.13 | 83940 0.74204445 |
| GRINA | 8q24.3 | 2907 0.6829245 |
| BNC2 | 9p22.3-p22.2 | 54796 0.7179303 |
| ANXA1 | 9q12-q21.2 9q12-q21.2 | 301 0.18238491 |
| CTSL | 9q21-q22 | 1514 0.5519466 |
| GADD45G | 9q22.1-q22.2 | 10912 0.76934904 |
| ZNF33A | 10p11.2 | 7581 0.52114326 |
| ZNF438 | 10p11.23 | 220929 0.8107101 |
| BAMBI | 10p12.3-p11.2 | 25805 0.5222711 |
| SH3PXD2A | 10q24.33 | 9644 0.7740955 |
| KIAA1754 | 10q25.1 | 85450 0.749174 |
| GSTO1 | 10q25.1 | 9446 0.75374824 |
| BAG3 | 10q25.2-q26.2 | 9531 0.5450204 |
| H19 | 11p15.5 | 283120 0.20831887 |
| MUC2 | 11p15.5 | 4583 0.37577808 |
| STIP1 | 11q13 | 10963 0.7423256 |
| FTH1 | 11q13 | 2495 0.6611367 |
| YAP1 | 11q13 | 10413 0.7424274 |
| CENTD2 | 11q13.4 | 116985 0.7427585 |
| SERPINH1 | 11q13.5 | 871 0.5462724 |
| PRCP | 11q14 | 5547 0.6991295 |
| CHORDC1 | 11q14.3 | 26973 0.5871089 |
| ENDOD1 | 11q21 | 23052 0.6716178 |
| REXO2 | 11q23.1-q23.2 | 25996 0.7803356 |
| ETS1 | 11q23.3 | 2113 0.6304317 |
| EMP1 | 12p12.3 | 2012 0.26516217 |

| | | |
|------------------|-----------------|-------------------|
| FLJ22662 | 12p13.1 | 79887 0.58745563 |
| CSDA | 12p13.1 | 8531 0.6497142 |
| MGP | 12p13.1-p12.3 | 4256 0.92134434 |
| CD9 | 12p13.3 | 928 0.63136715 |
| A2M | 12p13.3 | 2 0.6739991 |
| FKBP4 | 12p13.33 | 2288 0.52674276 |
| CLEC2B | 12p13-p12 | 9976 0.660021 |
| KRT5 | 12q12-q13 | 3852 0.15863033 |
| RND1 | 12q12-q13 | 27289 0.5358515 |
| KRT8 | 12q13 | 3856 0.06145612 |
| LOC144501 | 12q13.13 | 144501 0.50792146 |
| PHLDA1 | 12q15 | 22822 0.70168537 |
| NID2 | 14q21-q22 | 22795 0.72342676 |
| PYGL | 14q21-q22 | 5836 0.70001274 |
| AHSA1 | 14q23.3-31 | 10598 0.7698342 |
| LTBP2 | 14q24 | 4053 0.58221406 |
| FOS | 14q24.3 | 2353 0.41947952 |
| HSP90AA1 | 14q32.33 | 3320 0.5715347 |
| ITPKA | 15q14-q21 | 3706 0.49442717 |
| THBS1 | 15q15 | 7057 0.59211385 |
| NOD27 | 16q13 | 84166 0.74626994 |
| IRF8 | 16q24.1 | 3394 0.6988805 |
| CLDN7 | 17p13 | 1366 0.5413264 |
| KRT17 | 17q12-q21 | 3872 0.058498725 |
| COL1A1 | 17q21.33 | 1277 0.4859523 |
| MRC2 | 17q23.2 | 9902 0.754025 |
| C1QTNF1 | 17q25.3 | 114897 0.75775164 |
| TUBB6 | 18p11.21 | 84617 0.58213055 |
| RAB31 | 18p11.3 | 11031 0.68956786 |
| IFI30 | 19p13.1 | 10437 0.61414695 |
| GADD45B | 19p13.3 | 4616 0.6593149 |
| WDR18 | 19p13.3 | 57418 0.76591265 |
| ICAM1 | 19p13.3-p13.2 | 3383 0.5397866 |
| ACP5 | 19p13.3-p13.2 | 54 0.72082096 |
| PEPD | 19q12-q13.2 | 5184 0.69930196 |
| TOMM40 | 19q13 | 10452 0.27576482 |
| TOMM40 | 19q13 | 10452 0.57135195 |
| ZFP36 | 19q13.1 | 7538 0.51085496 |
| TYROBP | 19q13.1 | 7305 0.6816054 |
| ITPKC | 19q13.1 | 80271 0.75168335 |
| PPP1R15A | 19q13.2 | 23645 0.4729918 |
| APOC2 | 19q13.2 | 344 0.43337035 |
| KLK1 | 19q13.3 | 3816 0.38010582 |
| FLJ20512 | 19q13.32 | 54958 0.7608311 |
| NOL5A | 20p13 | 10528 0.74574405 |
| C20orf96 | 20p13 | 140680 0.7309621 |
| CPXM | 20p13-p12.3 | 56265 0.7354351 |
| PROCR | 20q11.2 | 10544 0.5665474 |
| CPNE1 | 20q11.22 | 8904 0.7290372 |
| MAFB | 20q11.2-q13.1 | 9935 0.6669973 |
| TGM2 | 20q12 | 7052 0.5686818 |
| PLTP | 20q12-q13.1 | 5360 0.6612585 |
| CEBPB | 20q13.1 | 1051 0.75960183 |
| TMEPAI | 20q13.31-q13.33 | 56937 0.62337506 |
| UCKL1 | 20q13.33 | 54963 0.78323305 |

| | | |
|----------------|-------------------|------------------|
| <i>SAMSN1</i> | 21q11 | 64092 0.76850903 |
| <i>ADAMTS1</i> | 21q21.2 | 9510 0.6576979 |
| <i>ITGB2</i> | 21q22.3 | 3689 0.6448108 |
| <i>BID</i> | 22q11.1 | 637 0.7016404 |
| <i>MMP11</i> | 22q11.2 22q11.23 | 4320 0.616754 |
| <i>HMOX1</i> | 22q12 22q13.1 | 3162 0.44399348 |
| <i>LGALS2</i> | 22q12-q13 22q13.1 | 3957 0.58973575 |
| <i>ECGF1</i> | 22q13 22q13.33 | 1890 0.5001053 |
| <i>TIMP1</i> | Xp11.3-p11.23 | 7076 0.51190704 |
| <i>BGN</i> | Xq28 | 633 0.52467614 |