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Genome-wide profiling of chromosomal alterations in renal cell carcinoma using high-density single nucleotide polymorphism arrays

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Abstract

Purpose—The identification of genetic aberrations may help understand the mechanisms of tumorigenesis and has important implications in diagnosis, prognosis, and treatment.

Methods—We applied Illumina's 317K high-density SNP-arrays to profile chromosomal aberrations in clear cell renal cell carcinoma (ccRCC) from 80 patients and analyzed the association of LOH/amplification events with clinicopathological characteristics and telomere length.

Results—The most common loss of heterozygosity (LOH) were 3p (69 cases) including 38 whole 3p arm losses, 30 large fragment LOH (spanning 3p21-36), and 1 interstitial LOH (spanning 3p12-14, 3p21-22, 3p24.1-24.2, and 3p24.3), followed by chromosome losses at 8p12-pter, 6q23.3-27, 14q24.1-qter, 9q32-qter, 10q22.3-qter, 9p13.3-pter, 4q28.3-qter, and 13q12.1-21.1. We also found several smallest overlapping regions of LOH that contained tumor suppressor genes. One smallest LOH in 8p12 had a size of 0.29 Mb and only contained one gene (*NRG1*). The most frequent chromosome gains were at 5q (32 cases), including 10 whole 5q amplification, 21 large amplifications encompassing 5q32-ter, and 1 focal amplification in 5q35.3 (0.42 Mb). The other common chromosome gains were 1q25.1-qter, 7q21.13-qter, 8q24.12-qter, and whole 7p arm. Significant associations of LOH at 9p, 9q, 14q, and 18q were observed with higher nuclear grade. Significant associations with tumor stage were observed for LOH at 14q, 18p, and 21q. Finally, we found that tumors with LOH at 2q, 6p, 6q, 9p, 9q, and 17p had significantly shorter telomere length than those without LOH.

Conclusion—This is the first study to use Illumina's SNP-CGH array that provides a close estimate of the size and frequency of chromosome LOH and amplifications of ccRCC. The identified regions and genes may become diagnostic and prognostic biomarkers as well as potential targets of therapy.

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Introduction

Renal cell carcinoma (RCC) accounts for about 3% of all new cancer cases and the incidence rates for all stages have been steadily rising over the last three decades.¹⁻³ Approximately one-third of patients present with metastatic disease at the initial diagnosis and more than 40% of patients will eventually die from their cancer.^{4,5} Despite the introduction of new treatment regimens, surgical resection remains the only curative therapy for RCC.⁶ However, up to 50% of patients undergoing nephrectomy for clinically localized RCC will develop local recurrence or distant metastasis.⁷ These statistics highlight the need for detecting RCC at early stages and identifying markers that can predict prognosis and response to therapy. RCC comprises a heterogeneous group of epithelial tumors with various cytogenetic and molecular abnormalities and different histological features. As is the case for most cancers, clinical variables play a major role in prognosis of localized RCC. Several prognostic algorithms and nomograms for RCC survival or recurrence after nephrectomy have been developed that incorporate multiple clinicopathological prognostic factors such as TNM stage, Fuhrman grade, tumor size, performance status, etc.^{5,8} Accumulation of genetic aberrations plays a pivotal role in the initiation and prognosis of RCC and other cancers. Therefore, integration of genetic markers into traditional approaches may allow a more accurate prediction of prognosis.

Clear cell RCC (ccRCC) accounts for 70% to 80% of all RCC and represents the majority of patients who will develop metastatic disease. Loss of chromosome 3p is the most common genetic alteration in ccRCC.^{9,10} The von Hippel Lindau (VHL) gene is a tumor suppressor gene located at 3p25.3 region, and mutation of this gene is associated with development of ccRCC.¹¹ Comprehensive genetic profiling of ccRCC tumors may not only provide insights on the mechanisms of tumorigenesis, but also provide potential prognostic biomarkers for the identification of patients at risk for recurrence or metastasis.

Conventional cytogenetic, allelotyping, and comparative genomic hybridization (CGH) studies have identified a number of chromosome aberrations in development ccRCC.¹²⁻¹⁹ SNP-CGH array is a powerful new technology to detect both copy number variations and LOH events by applying high-density whole-genome SNP genotyping technology to array CGH.²⁰ High resolution SNP-CGH array may enable us to identify chromosome breakpoints and regions of abnormality which will help us locate genes involved in cancer development and progression. In this study, we applied SNP-CGH array to obtain genome-wide LOH and amplification profile of ccRCC tumors. The Illumina's HumanHap300 SNP chip has a 9 Kb mean SNP spacing, enabling an effective resolution of ~90 Kb (with a 10 SNP smoothing). We also analyzed correlations between chromosome aberrations and clinicopathological variables, including tumor stage and nuclear grade.

Materials and Methods

Study population

Tumor samples were obtained from histologically confirmed ccRCC patients who were recruited from the University of Texas M. D. Anderson Cancer Center through a daily review of computerized appointment schedules. We started to recruit patients and collect tumor specimens from 2002. Patients were newly diagnosed and previously untreated. There were no age, gender, and cancer stage restrictions. After signing informed consents, the subjects completed a standard questionnaire through personal interview by trained M. D. Anderson staff interviewers. The information includes demographics, smoking status, alcohol consumption, and medical history. At the end of the interview, 40 ml blood sample was obtained from each subject. This procedure was approved by the Institutional Review Board of M. D. Anderson Cancer Center.

Tumor specimens

Primary tumor specimens were snap-frozen in liquid nitrogen immediately after surgery and stored at -80 °C until DNA extraction. Tumor DNA was extracted using the QIAamp DNeasy Blood and Tissue kit (QIAGEN, Valencia, CA). DNA extracted from peripheral blood of corresponding patient was used as reference normal DNA.

SNP-CGH array

SNP-CGH assay was done using Illumina's HumanHap300 BeadChip arrays, which covers over 317K haplotype tagging SNPs, according to the manufacturer's three-day protocol (Illumina, San Diego, CA). Briefly, 750 ng of genomic DNA was denatured and amplified at 37°C overnight. After overnight incubation, the amplified DNA was fragmented and precipitated at 4°C. The precipitated DNA was resuspended in hybridization buffer and hybridized to the beadchips in the flow-through chamber at 48°C overnight. Unhybridized and non-specifically hybridized DNA was washed away and the captured DNA was used as templates for one base extension of the locus specific oligos on the BeadChips.

Incorporated haptens were converted to fluorescent signals imaged using the BeadArray Reader (Illumina). All of the SNP-CGH array data was analyzed and exported using BeadStudio 2.0 (Illumina). The median call rate for tumor samples was 98.18% (range: 89.91% to 99.96%) and the median call rate for normal samples was 99.87% (range: 97.06% to 99.98%). SNP-based sex determination based on X/Y chromosome ratios were consistent with the gender reported by each patient. The Log R ratio (for copy number changes) and allele frequency (for LOH) were reported for each individual chromosome. If a region in which a stretch of SNPs are heterozygous in normal DNA but become homozygous in the matched tumor DNA, then this region is considered as LOH. The SNP-CGH dataset was deposited into the Gene Expression Omnibus database (accession number GSE15929).

Telomere length assay

The relative telomere length was measured by real-time quantitative PCR as described by Cawthon.²¹ In this method, the ratio of the telomere repeat copy number and the single gene (human β -globin) copy number was determined for each sample using standard curves. The derived ratio is proportional to the overall telomere length. The ratio for each sample was also normalized to a calibrator DNA sample to normalize between different runs. The PCR reaction (15 μ L) consisted of 1 $\times$ SYBR green master mix (Applied Biosystems, Foster City, CA), 200 nM of primers (Tel-1 and Tel-2 for telomere, or Hgb-1 and Hgb-2 for β -globin), and 5 ng of genomic DNA. The thermal cycling conditions were 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 56°C (for telomere amplification) or 58°C (for Hgb amplification) for 1 min. In each run, negative and positive controls, calibrator DNA, and standard curve were included. The positive controls contained a telomere of 1.2 Kb and a telomere of 3.9 Kb from a commercial telomere length assay kit (Roche Applied Science, Indianapolis, IN). For the standard curve, a reference DNA sample was serially diluted 2-fold from 20 ng/ μ L to 0.625 ng/ μ L. The R^2 for standard curve in each run was ≥ 0.99 .

Statistical analyses

Stage was dichotomized into low stage (I and II) and high stage (III and IV) based on clinical characteristics. Pearson χ^2 test or Fisher exact test were used to test for differences between LOH at each chromosome arm and stage. Nonparametric trend test was used to analyze the association of LOH at each chromosome arm with grade. Nonparametric rank sum test was used to test for differences in telomere lengths between tumors with or without LOH at each chromosome arm. Similar analyses were also performed for amplifications at each chromosome arm. Analyses were only performed for chromosome arms with more than

5% LOH or amplification. All tests were two-sided and $P < 0.05$ was considered statistically significant. Stata 8.0 statistical software package (Stata Co., College Station, TX) was used in this study.

Results

Characteristics of subjects

A total of 80 ccRCC tumors were included in the analysis. Supplementary Table S1 shows the host and tumor characteristics. The mean age was 59.88 years (SD: 10.19). Men were slightly overrepresented compared to women (53.75% vs. 46.25%). Based on the Fuhrman grading system of RCC, there were 20 patients with grade 2, 40 with grade 3, and 20 with grade 4 tumors (25%, 50% and 25% respectively). The stage distribution was stage I, 39 (49.37%), stage II, 14 (17.72%), stage III, 13 (16.46%), and stage IV, 13 (16.46%). The proportions of never, former, and current smokers (or recent quitters) were 60.53%, 28.95%, and 10.53%, respectively.

SNP-CGH Analysis

Tumor genomes of ccRCC are often complex with a large number of chromosome aberrations throughout the genome. Of the 80 samples examined, 76 exhibited various chromosome copy number losses and gains with only 4 samples free of any chromosome aberrations. The majority of aberrations encompassed a large segment of chromosome arm or even the whole chromosome. Table 1 shows the frequencies of whole chromosome arm aberrations.

LOH was more frequently detected than amplification at the chromosome arm level (total number of aberrations: 244 vs. 75). The most common chromosome arm losses were at 3p (38 RCC patients, 48.1%). Among those tumors with 3p whole arm losses, 11 also had 3q losses (Figure 1a). Complete losses of chromosome arm were also frequently detected on 8p (24.4%), 14q (19.5%), 9q (19.5%), 6q (15.2%), 9p (14.1%), 6p (13.0%), 4q (11.5%), 13q (10.4%), and 22q (10.3%) (Table 1). The frequencies of chromosome arm amplification were much lower and often the whole chromosome was amplified. For example, 11 tumors showed 5p or 5q amplification, 10 of which had simultaneous amplification of both long and short arms. Six tumors showed gains in the entire chromosome 7 with one additional 7p amplification. Other gains of whole chromosome arms at lower frequencies included 1q (7.8%), 8q (6.5%), 12p and 12q (6.4% and 5.2% respectively), and 16p and 16q (6.4% and 5.1% respectively).

In addition to whole chromosome arm aberrations, we observed a large number of recurrent regional chromosomal losses and gains. One major advantage of SNP-CGH analysis is that it can pinpoint the exact proximal and distal breakpoints of each LOH event on a specific chromosome arm. For example, Fig. 1a shows the detailed distribution of chromosome 3p LOH in each tumor. There were 69 tumors (87.3%) with various LOH at chromosome 3p, including 38 whole 3p arm loss, 30 large fragment LOH (all spanning 3p21-26), and 1 interstitial LOH (spanning 3p12-14, 3p21-22, 3p24.1-24.2, and 3p24.3, but not including the *VHL* gene in 3p25). Of the 30 tumors with large fragment 3p LOH, 28 had LOH extending from 3p telomere to a specific location proximal to centromere, 1 had LOH of 3p12-26, and 1 had LOH of 3p21-26. Fig. 1b shows the distribution of the most frequent chromosome gain event, 5q amplification. Of the 32 tumors with 5q amplification, 10 had the whole chromosome 5 amplification, 21 had large segment amplification encompassing 5q32-telomere, and 1 had a focal amplification in 5q35.3 with a size of 0.42 Mb.

Besides 3p LOH and 5q amplification, there were many other frequent large region aberrations. Table 2 lists the other frequent large regional LOH and amplification with a

minimum size of over 10 Mb. These include LOH at 8p12-pter (32.1%), 6q23.3-27 (30.4%), 14q24.1-qter, (29.9%), 9q32-qter (20.8%), 10q22.3-qter (15.4%), 9p13.3-pter (15.4%), and 4q28.3-qter (15.4%); and amplification at 1q25.1-qter, 7q21.13-qter, and 8q24.12-qter. These large regional LOH events account for the majority of chromosome aberrations in ccRCC.

We also found several smallest overlapping regions of LOH, which ranged in sizes from sub-100kb to 4.5 Mb and contained genes numbered from 1 to 44 (Table 3). One of the smallest LOH in 8p12 has a size of 0.29 Mb and only contains one gene (*NRG1*). Some of the regions harbored known tumor suppressors, for example, *p16* (*CDKN2A*) at 9p21.3 and *TP73* at 1p36.22 - p36.32. The smallest overlapping regions of amplification include 1q32.1, 5p15.33, and 5q35.3.

Correlation with tumor stage and grade

We then analyzed the association of LOH/amplification events at each chromosome arm with clinicopathological characteristics. Significant associations of LOH at 9p ($P=0.015$), 9q ($P=0.041$), 14q ($P=0.024$), and 18q ($P=0.018$) were observed with higher nuclear grade (Fig. 2). In addition, significant associations with tumor stage were observed for LOH at 14q ($P=0.038$), 18p ($P=0.038$), and 21q ($P=0.013$). No significant association was detected between amplification and any of the clinicopathological parameters.

Correlation with telomere length

We measured the overall telomere length in all of the tumor samples and observed significant correlations between LOH and shorter telomere length (Table 4). Tumors with LOH had shorter telomeres than those without respective LOH. These differences reached statistical significance for tumors with LOH at chromosomes 2q ($P=0.003$), 6p ($P=0.011$), 6q ($P=0.034$), 9p ($P=0.023$), 9q ($P=0.048$), and 17p ($P=0.038$), compared to tumors without respective LOH events; and reached borderline significance ($P<0.1$) for tumors with LOH at chromosomes 1p, 3p, 4q, 10q, 18p, 18q, and 22q. There was no significant association between amplification and telomere length by each chromosome (data not show).

Discussion

Illumina's high density SNP arrays have been the choice of platform for genome-wide association study that allowed major advances in identifying germline genetic susceptibility loci to common cancers, including cancers of breast, prostate, colorectum, and lung.²²⁻²⁵ However, there are only a couple of studies that applied Illumina's high density SNP array to query chromosome aberrations in tumor tissues to date.²⁶⁻²⁷ This study is the first such attempt in RCC and provides whole genome LOH profile of ccRCC tumors with the highest resolution to date. Our data showed that LOH are very frequent events in ccRCC tumors, most of which involved large fragments of chromosomes. We also identified focal LOH and amplifications, which may provide clues of new genes involved ccRCC tumorigenesis.

There are four recent studies using Affymetrix's SNP chips, two using 10K chips and two using 100K chips, for copy number aberrations in RCC.²⁸⁻³¹ Our study is the only one using Illumina's 317K SNP chip. We compared the results of these SNP-arrays with ours. There is a complete concordance of the most frequent loss and gain, i.e., the 3p loss and 5q gain. There is also a high concordance of other high frequent regions, for example, chromosome loss at 1p, 4q, 6q, 8p, 9q, 10q, 14q, 19p, and chromosome gain at 7p, 7q, 12q. But we detected more aberrations than other studies and identified some smallest overlapping regions due to the higher density of our SNP chips and larger sample size.

The high density of relatively evenly distributed SNPs enables us to pinpoint the locations of chromosome breakpoints and the sizes of LOH and amplification. The predominant LOH and chromosome gains in ccRCC occur to a large fragment of chromosome arm (from 10 to 100 Mb) and often the whole chromosome arm (Fig. 1a & 1b and Tables 1 & 2). Consistent with literature, the most common LOH was 3p and gain was 5q. Other recurrent large LOH regions (>10%) include 8p21-pter (33.3%), 6q23-27 (30.4%), 14q24.1-qter (29.9%), 9q32-qter (20.8%), 9p21-23 (19.2%), 10q22.3-qter (15.4%), 1p36 (15.8%), 17p, 18q and 22q; and other frequent large regions of chromosome amplification include 1q25.1-qter, 7q21.13-qter, and 8q24.12-qter. These recurrent large chromosome aberrations have mostly been reported previously by different studies with varying frequencies;⁹⁻¹⁰ 12-18; 28-34 however, this present study represents the most complete and detailed account of all the chromosome aberrations.

These large LOH and amplification regions clearly encompass tumor suppressor genes and proto-oncogenes that are involved in ccRCC tumorigenesis, but also include regions that may be caused by general genetic instability, as suggested by the positive associations between LOH events and shorter telomeres. Almost all of the LOH at 3p, except one, encompass *VHL* gene at 3p25, confirming the critical role of *VHL* inactivation in ccRCC tumorigenesis. The only exception (not including *VHL*) is an interstitial LOH, spanning 3p12-14, 3p21-22, 3p24.1-24.2, and 3p24.3. This data is also consistent with a previous observation of the *VHL*-independent carcinogenic pathway in ccRCC.³⁵ The candidate genes in these regions include *FHIT* at 3p14.236 and *RASSF1A* at 3p2137. The second most frequent LOH region is 8p12-pter. This region is one of the most frequently altered regions in human cancers.³⁸ Chromosome breaks that disrupt the *NRG1* gene at 8p12 have been observed in breast and pancreatic cancers and it has been proposed that alteration of the *NRG1* gene occurs through breakage at a non-common fragile site.³⁸⁻³⁹ In this study, one smallest LOH in 8p12 had a size of 0.29 Mb and only contains one gene (*NRG1*) (Table 3). Previous CGH studies have identified 8p12 as a frequent LOH region in ccRCC and validated by fluorescence in situ hybridization (FISH) and chromosome banding.¹⁹⁻⁴⁰ Our study narrowed this region and suggests, for the first time, that 8p12 aberration through breakage of *NRG1* at a fragile site is involved in kidney cancer. Chromosome 6q (6q21 and 6q26) and 9q32 contains three established common fragile sites,⁴¹ which match to two other most frequent LOH at 6q23-27 (30.4%) and 9q32-qter (20.8%). These data, together with a substantial number of breakpoints at 3p14, which harbors the best known fragile site gene (*FHIT*), suggest that many of the observed chromosome aberrations are caused by fragile site instability in tumor cells.

Besides *VHL* and the other genes in chromosome 3, there are other known tumor suppressors that are located in regions of LOH, for example, *p53* at 17p13.1, *p16* or other genes at 9p21,⁴⁴ and *PTEN* at 10q23.3. These genes have been implicated in RCC.⁴²⁻⁴⁵ However, the frequencies of LOH range from 10% to 20%, compared with 86% of *VHL*, indicating a minor role of these tumor suppressors in ccRCC tumorigenesis. The smallest overlapping regions of LOH may contain potential new genes involved in ccRCC. The number of genes in these regions ranges from 1 to 44, some of which encode hypothetical proteins with unknown function. An interesting gene is *RFP2* at 13q14.3, which encodes a transmembrane E3 ubiquitin ligase and is a candidate tumor suppressor.⁴⁶ Future biochemical studies are warranted to measure the expression of these genes and validate their involvement in ccRCC.

Among the four large chromosome amplification regions (Table 2), 5q is the most frequent, including 10 whole 5q amplification, 21 large amplification encompassing 5q32-ter, and 1 focal amplification in 5q35.3 with a length of 0.42 Mb (Fig. 1b). No specific pro-oncogenes have been implicated in ccRCC tumorigenesis in the 5q region. The 0.42 Mb focal

amplification region in 5q35.3 contains 15 genes, one of which is *SQSTM1*, a ubiquitin-binding protein that was shown overexpressed in breast and prostate tumors.^{47,48} The LOH of large region encompassing *SQSTM1* has been validated by FISH analysis and spectral karyotyping,^{19,49} and future functional studies are warranted to evaluate whether *SQSTM1* is involved in ccRCC tumorigenesis.

Amplifications of 1q25.1-qter, 7q21.13-qter, and 8q24.12-qter are common events in a few other cancers. Top candidate genes in these 3 large amplification regions and whole 7p arm include *MDM4* (1q32), which inhibits the tumor suppressor function of p53 and promotes tumorigenesis;^{50,51} *CDK6* (7q21.2), a cyclin-dependent kinase which is amplified and overexpressed in several cancers;⁵²⁻⁵⁴ *MYC* oncogene (8q24.12); and *EGFR* (7p12). In the three smallest overlapping regions of amplification (Table 3), 1q32.1 contains *MDM4* and 17 other genes, 5p15.33 harbors *hTERT* (human telomerase reverse transcriptase) and 15 other genes, 5q35.3 contains *SQSTM1* and 14 other genes. These genes may play important roles in ccRCC tumorigenesis.

In analyses of these chromosome aberrations with clinicopathological features, we found the LOH at 14q was significantly associated with both higher tumor grade and stage. LOH at 14q is one of the most frequent events in ccRCC. Many previous studies have consistently found that this event is associated with higher grade and stage and worse prognosis and survival in ccRCC.^{12,55-58} This region is also frequently deleted in other cancer types and associated with advanced tumors and poor prognosis.⁵⁹⁻⁶² It appears that 14q may harbor a tumor suppressor gene that controls cell proliferation and loss of its function leads to a growth advantage and transformation of low-grade to high-grade tumors. The identity of this gene or genes remains unknown. One interesting note is that *HIF-1α* is located on 14q21-q24 and is lost in a substantial number of ccRCC tumors. It is well established that VHL-HIF-1α pathway is the major driving force for ccRCC tumorigenesis. VHL proteins functions as part of an E3 ubiquitin ligase complex that binds to HIF-1α for ubiquitin-mediated proteasomal degradation. Inactivation of *VHL* by mutation, deletion, or hypermethylation results in the intracellular accumulation of HIF-1α, which in turn increases the expression of many hypoxia-inducible genes essential for cancer cell functions under hypoxic conditions, such as glucose transport, angiogenesis, cell growth, and inflammation.⁶³ It is surprising that HIF-1α as a mediator of VHL function is lost in up to 30% of ccRCC tumors. It was also reported that a large panel of VHL-defective RCC cell lines lacked functional HIF-1α.⁶⁴ The tumorigenesis of these cases may occur through a VHL-independent pathway or an alternative hypoxia inducible factor (e.g., HIF-2α).

We showed that high genetic instability, as evidenced by short telomeres contributes to LOH. It is a general phenomenon the effect of alteration of specific chromosomes and genes, since tumors with LOH at any specific chromosomes had shorter telomeres, 13 chromosomes reached statistical significance or borderline significance, and the other 6 also showed shorter telomeres in tumors with LOH (Table 4). It has been well established that telomere dysfunction leads to genomic instability.^{65,66} Telomere dysfunction via either telomere shortening or disruption of telomere structure causes end-to-end fusion of unprotected chromosomes. The propagation of breakage-fusion-bridge cycles will lead to genomic instability, including aneuploidy, LOH, and chromosome loss or amplification. Several studies showed telomere shortening in RCC tumors compared to normal tissues.⁶⁷⁻⁷¹ In addition, Gisselsson et al.⁷⁰ showed that telomere shortening generated cytogenetic heterogeneity in a subgroup of RCC. Furthermore, Izumi et al.⁷¹ measured telomerase activity, telomere length, chromosome aberrations, and DNA ploidy in ccRCC tumors and divided tumors into high telomerase and low telomerase activity groups. They found that the low telomerase group had shorter telomere length, more chromosome

aberrations, and fewer DNA diploid cells, supporting that shorter telomere length is associated with chromosome instability. Our data is consistent with these findings.

In conclusion, we performed a high density, whole genome SNP array analysis to profile chromosome aberrations in ccRCC. This study provides a close estimate of the size and frequency of chromosome LOH and amplifications of ccRCC and identifies several smallest overlapping regions that harbor potential tumor suppressor genes and oncogenes involved in the development of ccRCC. These regions and genes may become prognostic and therapeutic biomarkers as well as potential target of therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Aberrations Identified in Chromosome 3 (1a) and Chromosome 5 (1b) for 80 RCC Samples (x axis denote subjects, y axis denote chromosome position in Mb. red – LOH/Deletion, green – Amplification, dotted black – centromere)

Fig. 2.
Histogram showing the association of grade and frequencies of LOH at chromosome arms 9p, 9q, 14q, and 18q.

Table 1

Whole Chromosome Arm LOH and Amplification

chromosome arm	number of subjects	LOH		Amplification	
		N	%	N	%
chr1p	76	3	4.0	0	0
chr1q	77	3	3.9	6	7.8
chr2p	76	3	4.0	2	2.6
chr2q	76	1	1.3	1	1.3
chr3p	79	38	48.1	0	0
chr3q	78	11	14.1	0	0
chr4p	77	7	9.1	0	0
chr4q	78	9	11.5	0	0
chr5p	78	2	2.6	11	14.1
chr5q	76	1	1.3	11	14.5
chr6p	77	10	13.0	0	0
chr6q	79	12	15.2	0	0
chr7p	77	0	0	7	9.1
chr7q	77	0	0	6	7.8
chr8p	78	19	24.4	1	1.3
chr8q	77	8	10.4	5	6.5
chr9p	78	11	14.1	0	0
chr9q	77	15	19.5	1	1.3
chr10p	80	5	6.3	0	0
chr10q	79	7	8.9	0	0
chr11p	78	2	2.6	0	0
chr11q	79	2	2.5	0	0
chr12p	78	1	1.3	5	6.4
chr12q	77	1	1.3	4	5.2
chr13p	80	0	0	0	0
chr13q	77	8	10.4	0	0
chr14p	80	0	0	0	0
chr14q	77	15	19.5	0	0

chromosome arm	number of subjects	LOH		Amplification	
		N	%	N	%
chr15p	80	0	0	0	0
chr15q	75	3	4	1	1.3
chr16p	78	3	3.9	5	6.4
chr16q	79	4	5.1	4	5.1
chr17p	77	6	7.8	0	0
chr17q	77	3	3.9	0	0
chr18p	79	5	6.3	0	0
chr18q	79	7	8.9	0	0
chr19p	66	3	4.6	0	0
chr19q	76	3	4.0	0	0
chr20p	79	0	0	2	2.5
chr20q	79	0	0	3	3.8
chr21p	80	0	0	0	0
chr21q	77	5	6.5	0	0
chr22p	80	0	0	0	0
chr22q	78	8	10.3	1	1.3

Table 2

Common Large Region of LOH and Amplifications (>10%)

Chr	Region	Start	End	Size (Mb)	N (%)
LOH					
1	p35.1-36.32	2776953	34105854	31.3	11 (14.5)
2	q36.3-q37.3	229710850	242692820	13.0	11 (14.5)
3	p21.1-p26.1	5866539	52911803	47.0	68 (86.1)
4	p12-pter	63508	45619591	45.6	8 (10.4)
4	q28.3-qter	134301136	191128697	56.8	12 (15.4)
6	q23.3-27	138060132	167464699	29.4	25 (30.4)
8	p12-pter	166818	30505318	30.3	25 (32.1)
9	p13.3-pter	194201	38107647	37.9	12 (15.4)
9	q32-qter	113975797	140128836	26.2	16 (20.8)
10	q22.3-qter	79182925	135284293	56.1	12 (15.4)
13	q12.11-21.1	19688742	53047417	33.4	11 (14.3)
14	q24.1-qter	67823040	106345097	38.5	23 (29.9)
17	p11.2-pter	51088	19639713	19.6	9 (11.7)
18	q12.2-qter	31125880	76116152	45.0	10 (12.7)
22	q11.1-qter	15412698	49524956	34.1	8 (10.3)
Amplification					
1	q25.1-qter	172956548	247177330	74.2	8 (10.4)
5	q32-qter	143278239	178877013	35.6	31 (41.9)
7	q21.13-qter	90338262	158812247	68.5	8 (10.4)
8	q24.12-qter	122063841	146245512	22.4	8 (10.4)

Table 3

Smallest Overlapping Region of LOH and Amplification

Chr	Region	Start	End	Size (Mb)	Genes
			LOH		
1	p36.22-p36.32	2934387	7714998	4.5	<i>TP73</i> & 43 others
2	p12	76540993	77901722	1.4	<i>LOC647278</i> , <i>LRR1M4</i>
4	q34.3-q35.1	182024320	182669289	0.64	<i>LOC728081</i>
4	q28.3	132654783	133220977	0.57	<i>LOC646187</i>
6	q14.3-q15	87409153	88149210	0.74	11 genes
8	p12	32284018	32576053	0.29	<i>NRG1</i>
9	p21.3	19969813	24005138	4.3	<i>p16</i> and 38 others
9	p23	9622921	9710279	0.087	
10	q26.13-q26.2	127094126	127623699	0.53	<i>MMP21</i> , <i>UROS</i> , & 7 others
13	q14.3	49429768	50000059	0.57	<i>RFP2</i> and 8 others
			Amplification		
1	q32.1	202184808	203378384	1.2	<i>MDM4</i> and 17 others
5	p15.33	1105360	2131006	1.0	<i>hTERT</i> and 15 others
5	q35.3	178877013	179301287	0.42	<i>SQSTM1</i> and 14 others

Table 4
The association of LOH and overall telomere length in tumors

	LOH*	N	Telomere Length Median(Range)	P**
chr1p	No	60	1.26(0.76- 5.50)	0.055
	Yes	14	1.08(0.53- 2.19)	
chr2q	No	63	1.35(0.59- 5.50)	0.003
	Yes	11	0.99(0.53- 1.58)	
chr3p	No	10	1.85(0.80- 3.45)	0.084
	Yes	67	1.23(0.53- 5.50)	
chr3q	No	45	1.27(0.59- 3.45)	0.214
	Yes	31	1.19(0.53- 5.50)	
chr4p	No	67	1.23(0.59- 5.50)	0.204
	Yes	8	1.12(0.53- 2.19)	
chr4q	No	61	1.27(0.59- 5.50)	0.051
	Yes	15	1.14(0.53- 2.28)	
chr6p	No	64	1.35(0.59- 5.50)	0.011
	Yes	11	1.08(0.53- 2.19)	
chr6q	No	51	1.37(0.59- 3.45)	0.034
	Yes	26	1.15(0.53- 5.50)	
chr8p	No	50	1.33(0.59- 3.45)	0.562
	Yes	26	1.21(0.53- 5.50)	
chr8q	No	63	1.27(0.59- 3.45)	0.184
	Yes	12	1.15(0.53- 5.50)	
chr9p	No	62	1.33(0.77- 5.50)	0.023
	Yes	14	1.09(0.53- 2.28)	
chr9q	No	60	1.29(0.59- 3.45)	0.048
	Yes	15	0.99(0.53- 5.50)	
chr10q	No	63	1.27(0.59- 5.50)	0.083
	Yes	13	1.13(0.53- 2.28)	
chr13q	No	64	1.24(0.59- 5.50)	0.208
	Yes	9	0.99(0.76- 2.19)	
chr14q	No	52	1.26(0.76- 5.50)	0.219
	Yes	23	1.17(0.53- 2.28)	
chr17p	No	67	1.26(0.59- 5.50)	0.038
	Yes	8	1.06(0.53- 1.87)	
chr18p	No	70	1.29(0.59- 5.50)	0.056
	Yes	7	1.08(0.53- 1.79)	
chr18q	No	68	1.29(0.59- 5.50)	0.073
	Yes	9	1.08(0.53- 1.83)	
chr22q	No	68	1.26(0.53- 5.50)	0.084
	Yes	8	1.12(0.76- 1.44)	

* Frequency > 10%

**
Rank-sum test