Genomic Alterations in Gastrointestinal Stromal Tumors as Revealed by Conventional and Array-based Comparative Genomic Hybridization

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Abstract. Gastrointestinal stromal tumor (GIST) is the most commonly occurring mesenchymal neoplasm of the gastrointestinal tract, accounting for 80 percent of these tumors. GIST is highly unresponsive to standard chemotherapy, particularly in patients with advanced or metastatic disease. Recent molecular studies have shown that activating c-kit (KIT) mutations are detectable in a large proportion (>75%) of tumors, between 78% (1) and 89% (2). Approximately 30% of tumors without an identifiable KIT mutation exhibit PDGFRA mutations (3). Furthermore, the KIT mutations are heterogeneous, some being known to confer a relatively better prognosis than others (1). Gross cytogenetic abnormalities associated with GIST appear to be similar regardless of whether a KIT mutation is identified. The molecular genetic alterations associated with multistep GIST tumorigenesis, particularly those which confer intrinsic or acquired resistance to both standard as well as targeted therapeutic approaches, however, are not fully recognized. As an initial approach to identify chromosomal sites of candidate gene(s), which may predict overall clinical and biologic behavior of GISTS, as they relate to response to the specific therapeutic drug Gleevec, we analyzed six GIST samples using both conventional as well as array-based Comparative Genomic Hybridization (CGH). The common abnormalities detected by CGH in low and high grade tumors included loss of all or part of chromosome 14; an entire chromosome 14 was lost in four tumor samples, with the remaining two samples exhibiting loss of the 14q22-q32.3 region. Other recurrent abnormalities included loss of the 1p chromosomal region (four tumor samples), loss of part or entire chromosome 9 (only in metastatic tumors), loss of chromosomes 15 and 22, and a gain of the chromosome 3q region in three samples each. Array-based CGH performed using human BAC arrays (1400V11 Spectral Genomics Chip) on the other hand, not only detected recurrent abnormalities of the chromosomes and chromosomal sites mentioned above, but also identified losses of additional chromosomal sites on chromosomes 6q and 13q. Array-based CGH further delineated the regions of loss or gain to specific chromosome bands or sub bands based on location of chromosomal site-specific BACs. The specific regions of losses are located at 1p36.2-36.3, 6q12, 9p13-ter, 13q33.33-q34, and gain or amplification of chromosomal DNA at bands 3q26-27. It is interesting to note that some of the chromosomal sites of losses and gains also harbor gene(s) such as AFAR, RIZ1, p73(p35-36), Akt-1(14q32), p14 and p16(9p21), NF-2(22q12), ZASCI, p63 and PIK3CA oncogenes (3q26-27), all of which are known to play a major role in solid tumor pathogenesis. The role of these genes in GIST, however, remains to be seen. Thus, the results of our current study demonstrate that such a combined approach to detect global genomic alterations in GIST is significant in providing information related to chromosomal sites of potential tumor suppressor genes associated with multistep tumorigenesis; some of which also may be predictive of prognosis and clinical outcome following specific targeted therapy, which is the focus of future studies.

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the digestive tract, accounting for approximately 80 percent of these tumors, but are considered rare as they only constitute less than three percent of all gastrointestinal malignancies (4). The exact incidence of GIST is unknown, but estimates of at
least 1,000 new cases per year have been published (5). GISTs are believed to arise from the interstitial cells of Cajal, the cells that are responsible for muscle contraction in the stomach and intestinal tract (6).

Extensive studies on GIST, especially in the areas of cytogenetics and molecular genetics are lacking, which may be due to the fact that, like many solid tumors which grow at a fast rate, such tumors are not easily amenable to cytogenetic analyses because of their high rate of necrosis. Recently, new technologies have emerged in the area of cytogenetics and molecular genetics; two of these state-of-the-art technologies are comparative genomic hybridization (CGH) and genome-wide screening of DNA copy number by microarray CGH. The former technique has been extensively used in characterizing genetic imbalances in solid tumors that would otherwise be unsuitable for analysis, partly due to the usually poor quality of the samples or very low mitotic index (7-9).

The lower limit of detection for conventional CGH is the chromosomal segment comprising approximately 10-20 Mb of DNA (8). The advantage of array-based CGH on the other hand is in its ability to detect gains and losses at a much higher level of resolution, which could be at approximately 2 Mb of DNA.

CGH has recently been employed to characterize chromosomal gains and losses in GIST; two such studies (10,11) revealed similar results. The most common findings between these two studies included losses of part or all of chromosome 14, loss of chromosome 22 and loss of 1p. Other areas of copy number change included losses of part or all of chromosomes 9, 13 and 15 and gain of chromosomal regions of 3, 8q and 17q. While the two most common changes in GIST, losses of 14 and 22, were seen in both benign and malignant GIST, other changes such as loss of 1p and loss of 9q were seen mostly in malignant GIST (11, 12). In an earlier study by Marci et al. (13), the authors suggested that the cytogenetic monosomies of chromosomes 14 and 22 detected in GIST may represent the earliest changes necessary for malignant transformation of these tumors; a later study by Brenier et al. (14) further supported this premise.

El-Rifai et al. (15) reported deletions of 14q in 75% of GISTs based on loss of heterozygosity (LOH) for markers located at the region 14q11.1-12 and 14q23-24.3. In a similar study by Debiec-Rychter et al. (16), fluorescence in-situ hybridization was used to identify deletions of part or entire chromosome 14 in 81 percent of samples studied with common deleted regions localized to 14q12-13, 14q22-23 and 14q24.3. Both groups suggested that a tumor suppressor gene(s) located in one of these regions may play a significant role in pathogenesis of GISTs; the nature of such a gene, however, is not known. El-Rifai et al. (10) further suggested that benign tumors are characterized predominantly by losses of genetic material, while malignant tumors show losses, gains and high-level of amplifications.

It is now well established that GISTs are characterized by expression of the CD117 antigen for the KIT protein. Constitutive expression of the KIT gene is normally essential in the development and differentiation of the interstitial cells of the Cajal network, which regulates peristalsis in the stomach (17). In recent years, it has been recognized that in GISTs, the KIT protein is over-expressed due to activating mutations in over 75% of cases (5). These mutations result in uncontrolled signaling which leads to the abnormal proliferation of cells in GIST. It is, however, worth noting that even with such mutations, most GISTs are benign, as only an estimated 10-30% of tumors transform to a malignant state (18). Furthermore, recent studies on the identification of the nature of such mutations have revealed that the spectrum of KIT activating mutations detected in GISTs may correlate with various clinocopathological features (1,3,19). Also, the expression profiling studies revealed a remarkable homogeneity in GISTs with KIT mutations (20).

Until recently, the only effective treatment for GIST was complete surgical resection. While this treatment was successful for patients with benign GISTs, patients with malignant GISTs often experienced recurrence, especially to the liver and peritoneal surfaces. Once metastasis had occurred, survival was limited. Survival rates for patients with unresectable GIST were also low, with most patients having a poor response to conventional chemotherapy and radiation (4).

A recent development in cancer treatment is the use of the target-specific drug Gleevec (Novartis, Basel, Switzerland), which specifically targets a tyrosine kinase activity of Bcr-Abl protein, that is produced as a result of a chromosomal translocation (21). Joensuu et al. (22) first reported the results of using Gleevec for the successful treatment of a patient with malignant GIST who was non-responsive to traditional chemotherapy. In GIST, Gleevec targets the aberrant protein formed in patients with mutated and/or over-expressed KIT gene. Current literature indicates that Gleevec has been effective in Phase II trials on patients with advanced unresectable or metastatic GIST (23). In addition, it is also known that different types of KIT mutation may have varied responses to the drug (1, 17). Therefore, it is, conceivable that additional genetic alterations contribute both primary and acquired resistance observed in some advanced stage GIST.

Based on the brief review provided above of various studies on GISTs, it is not unreasonable to suggest that extensive studies are needed relating to the genomic alterations associated with the clinical and biologic behavior of these tumors. Such studies are expected to be effective if the analysis is performed on a large population of samples from patients uniformly treated under clinical trials. As a prelude to seek answers to some of the questions raised...
above, we have recently performed preliminary experiments comparing conventional and microarray CGH, to identify genetic alterations associated with GIST pathogenesis and clinical outcome following therapy.

Materials and Methods

**DNA preparation.** Genomic DNA was isolated from snap-frozen tumor tissue samples from six gastrointestinal stromal tumors (GIST) and was obtained using a standard phenol-chloroform organic extraction protocol. The DNA samples used for microarray-based CGH analysis were treated to remove RNA contamination; 150µL of each sample was treated with 10µL of RNase A Solution from the Puregene DNA extraction kit (Gentra Systems). The samples were then incubated at 37°C for one hour. After the incubation, 75µL of protein precipitation solution and 300µL of 100% ethanol were added to each sample. Each sample was then inverted 50 times to mix the reagents and precipitate the DNA. The samples were then incubated at room temperature for 15 minutes. Following this, the samples were centrifuged at 14000 rpm for five minutes, the supernatant was decanted from each sample and each DNA pellet was washed with 450µL of 70% ethanol. The samples were again centrifuged at 14000 rpm for one minute, the supernatant was decanted and the pellets were allowed to air dry for approximately 30 minutes. Each DNA sample was then resuspended in 150µl of DNA Hydration Solution. The samples were incubated at 65°C for one hour and then left at room temperature. The DNA concentration was assessed using a one percent agarose gel and the samples were aliquoted so that the final concentration of sample was 1µg/µl of solution.

**Conventional CGH.** For conventional CGH, approximately 2.5µg DNA from each sample was labeled using nick translation (Life Technologies, Inc., Rockville, MD, USA) with fluorescein-12-dUTP. An equivalent amount of placental DNA was labeled with Texas red-5-dUTP (New England Nuclear, Dupont, Boston, MA, USA) as reference DNA. CGH was performed using routine methods as described previously (9). For each sample, at least 10 different metaphases were captured and processed using the quantitative Image Processing System (Metasystems). The mean values of individual ratio profiles were calculated from at least 10 metaphases from each sample to identify gains, losses and amplification of specific chromosomal regions. The composite CGH profile was generated for each sample using 0.8 and 1.2 as the lower (loss) and upper (gain) limits, respectively. Overrepresentation, defined by a sharp peak, was considered as amplification of DNA sequences.

**Microarray-based CGH.** The microarray-based CGH is performed using Human BAC Arrays-1400V11 (Spectral Genomic (G) Chip™, Spectral Genomics Inc., Houston, TX, USA) following the protocol provided by the manufacturer. The arrays supplied by the Spectral Genomics contain 1403 human BACs spanning the genome at approximately a 2-4 megabase interval. These BAC arrays are pre-treated and are ready for direct hybridization. The protocol includes five major steps: pretreatment of DNA samples, differential random prime labeling of DNA with Cy3-dCTP and Cy5-dCTP, hybridization of DNA to the slides post-hybridization washer, and finally scanning and analysis of DNA using a scanner and the Spectral Ware™ software. Details of the various steps are available on the Spectral Genomics, Inc. website www.spectralgenomics.com.

A unique feature of this method is the use of two separate hybridization arrays employed for each sample with reverse labeling to confirm the accuracy of the results. The high resolution genomic profiling analysis is performed using the scanner and software (Spectral Ware™) developed by the Spectral Genomics, Inc. Such an analysis results in each ratio plot comprised of normalized data from two independent arrays such that the normalized data from the array in which the test sample was labeled in Cy3 is shown in red, while the normalized data from the array in which the test sample was labeled in Cy5 is shown in blue. Individual spots along the ratio plot represent the normalized ratio of individual clones linearly ordered such that the left-most clone is consistent with the p-arm terminus while the right-most clone is consistent with the q-arm terminus. Since the normalized Cy3/Cy5 ratio was computed for both arrays, a loss of a particular clone is manifested as the simultaneous deviation of the ratio plots from a modal value of 1.0, with the red ratio plot showing a positive deviation (upward) while the blue ratio plot shows a negative deviation at the same locus (downward). Conversely, DNA copy number gains show the opposite pattern.

**Results**

Cytogenetic analysis was successful in only 2/6 samples studied. Clonal abnormalities were detected in two samples; one showed a monosomy of chromosome 14 as the sole abnormality, while the other sample exhibited a complex abnormality including several numerical and structural abnormalities. The clonal abnormalities detected in this sample with the chromosome constitution as: 41-45, XY, +del(1)(q12), del(6)(q22q25), der(9)(9;11)(p12;q13), del(11)(q22), der(12)(8;12)(p11;q24), +18. The clinical, histological, immunohistochemical, KIT mutation status and clinical outcome of the tumor samples derived from patients with GISTs are summarized in Table I. A positive correlation was not seen between any of the parameters and the clinical outcome.

Conventional CGH analysis results are summarized in Table II. Using conventional CGH, changes in DNA copy number were detected in all six samples. Chromosome 18 was the only chromosome in which no copy number change was seen. Overall, losses were slightly more frequent than gains and high-level amplifications (30 losses to 28 gains), but gains/high-level amplifications were present more often in metastatic malignant than low-grade GISTs, with only one gain of 3q seen in low-grade GIST in patient 5. For the purpose of this analysis, recurrent abnormalities detected in three or more samples were considered significant. The most common copy number change was loss of entire or part of chromosome 14, which was seen in all six samples (Figure 1A). An entire chromosome 14 was lost in four out of six samples, with the remaining samples exhibiting loss of 14q22-q32.3. Other frequent copy number changes included loss of
chromosome 1p, found in four out of six samples; loss of part or all of chromosome 9, seen in three out of six samples; loss of part or all of chromosome 22, found in three out of six samples; and gain of chromosome 3q material, found in three out of six samples (Figure 1A). Changes found in both low-grade and high-grade malignant tumors included loss of 1p, loss of chromosome 14, loss of chromosome 15, loss of chromosome 22 and gain of 3q. Figure 1A and 1B show the representative profiles of recurrent losses and gains, while Figure 2 represents the copy number changes for all samples compiled in a graphical format. A complete summary of gains, losses and amplifications, including borderline changes that may not be visible in the CGH profiles detected in all six tumor samples, is presented in Table II.

Microarray-based CGH results. As summarized in Table III, only those recurrent abnormalities detected in three or more samples are included and considered for comparison with the conventional CGH results. In general we found a close correlation between the genetic alterations detected by conventional and micrarray-based CGH. While the microarray-based approach identified a smallest region of deletion on chromosome 1p (Figure 3A), it did not further delineate the region of chromosome deletions on chromosomes 14 (Figure 3B) and 22. In addition, such an analysis also revealed two additional sites of deletions on chromosomes 6q12 and 13q33.3-q34, which were not detected by conventional CGH. Array-based analysis further delineated a specific site of amplifications at chromosomal region 3q26-27 (Figure 3C). Results of such studies, therefore, clearly demonstrate the potential application of micrarray-based CGH in identifying finer genetic alterations. 

Discussion

The results of this study are consistent with previous reports of frequent losses and gains in GIST. Chromosome 14 was the most frequently affected chromosome with complete or partial loss detected in all samples. Other concordant findings include frequent losses of 1p, gains of 3q, loss of all or part of chromosome 9p, loss of chromosome 15 and loss of chromosome 22. Array-based CGH further detected specific regions of losses localized to 1p36, 6q12, 13q33.3-q34 and amplification of DNA located at 3q26-27.

Recent studies to determine specific regions of loss on chromosome 14 in GIST have shown similar results to those described here. El-Rifai et al. (15) reported detection of two possible tumor suppressor loci at 14q11.2 and 14q23. Similarly, Debiec-Rychter et al. (24) identified common deletion regions at 14q12-13, 14q22-23 and 14q24.3. However, these studies did not present loss of 14q32 as a common finding. Another study by Debiec-Rychter et al. (16), on the other hand, detected loss of 14q32 in both benign and malignant GISTs using the locus specific probe for the IgH gene located at 14q32. The authors suggested that loss of this locus may represent an early step in GIST tumorigenesis.

While CGH analysis has shown complete or partial loss of chromosome 14 in all six samples included in our study, loss at 14q12-24.2 and 14q32.1-32.3 is of particular interest. Thorstensen et al. (25) identified two possible target regions at 14q13-21 and 14q24-31 for tumor suppressor genes in colorectal carcinomas. A study reported by Hu et al. (26) identified 14q22.3-32.1 and 14q32.1-qter as possible target regions of tumor suppressor genes in astrocytoma. Loss of heterozygosity was detected in 80 percent of nasopharyngeal
Table II. Summary of genomic alterations in GIST as revealed by conventional Comparative Genomic Hybridization (CGH).

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>CGH findings losses</th>
<th>CGH findings gains</th>
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<tr>
<td>1</td>
<td>1p,3p,4p,9p,9q,10,13q,14,22q</td>
<td>1q,3q,5p,5q*,6p,8q*,11,15,16p*,17q*,19,20,21</td>
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<td>2</td>
<td>9p,11p,14</td>
<td>2q</td>
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<tr>
<td>3</td>
<td>1p,9,12p,14,15</td>
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</tr>
<tr>
<td>4</td>
<td>1p,2p,6q,10p,13,14q,15,22</td>
<td>1q,2q,3q,4q,5q,7q,12p,20,21*</td>
</tr>
<tr>
<td>5</td>
<td>1p,14,15,22</td>
<td>3q</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>None</td>
</tr>
</tbody>
</table>

* - indicates high level amplification

carcinomas studied by Shao et al. (27), with the most common markers of loss located at 14q11-q13, 14q21-24 and 14q32. Ihara et al. (28) also found loss of heterozygosity at 14q32 in 38.5 percent of esophageal tumors studied. It should be noted, however, that the nature of potential tumor suppressor gene(s) located at this site has not been revealed as yet. The AKT-1 gene located at 14q32, however, may be of interest, as this gene is involved in some of the signal transduction pathways known to be deregulated in cancer cells (29).

Chromosome 1 was also frequently affected in GISTs. While four out of six samples showed loss of complete 1p by conventional CGH, array-based CGH detected the smallest region of deletion located at 1p36.2-p36.3. In an earlier study (30), a common deletion region was identified at 1p36.1-p36.22, in hepatocellular carcinoma. In another study by Valimaki et al. (31) two separate regions, on proximal 1p and one on distal 1p, were identified in parathyroid adenomas and carcinomas. Both regions were hypothesized to contain tumor suppressor genes. Recurrent loss of 1p21-22 has also been identified in malignant mesothelioma (32). Furthermore, losses of 1p, specifically the region 1p35-36 is known to be deleted in a variety of tumors such as colon, breast, liver, lung, pancreas, brain, neuroblastoma (reviewed in 33), suggesting that tumor suppressor gene(s) located at 1p35-36 may be important in the development of a variety of tumors.

While the nature of genes located at 1p35-36, which are associated with tumorigenesis, is not known, genes such as AFAR, RIZ1 and p73 (33-35) may be of interest. Aflatoxin B1-Aldehyde Reductase gene is known to inhibit AFB1-induced tumorigenesis in rats by detoxifying cytotoxic and genotoxic aldehydes, but its role in humans is currently unknown. The retinoblastoma protein-interacting zinc finger gene RIZ1 located at 1p36, is a known tumor suppressor gene, which is inactivated either by CpG island promoter DNA methylation or missense inactivating mutations in a variety of solid tumor types (reviewed in 34), is another attractive candidate gene, which deserves investigation for its role in GIST. The p73 gene located at 1p36, which is also known to share considerable homology with the p53 gene, is yet another candidate gene that deserves further study in GIST (35), as the p73 gene is monoallelically expressed in neuroblastoma and is known to interact with p53 protein and activate its target genes.

Loss of part or all of chromosome 22, which was seen in three out of six samples tested, detected as the second most common aberration in GIST. Using locus-specific FISH probes, Debiec-Rycter et al. (36) identified 22q13 as a common region of loss in GIST. A number of other studies on GIST have also identified loss of part or all of chromosome 22 as a common abnormality by both cytogenetic and molecular methods (6, 12-14, 17). Studies on a number of other solid tumor cancers have found frequent loss of chromosome 22 regions. In a study of sporadic colorectal carcinomas, Zhou et al. (37) identified regions 22q12.2-12.3 and 22q13.32-13.33 as possible locations for a tumor suppressor gene. In their study on squamous cell carcinomas of the head and neck, dos Reis et al. (38) also identified two separate regions on chromosome 22 as locations of two distinct tumor suppressor genes at 22q11.2-12.1 and 22q13.1-13.31. In another study of pancreatic endocrine tumors by Wild et al. (39), two key regions of LOH were identified at 22q12.1 and 22q12.3. These results further support the idea that one or more tumor suppressor genes located at 22q11-12 may play a key role in the pathogenesis of GISTs.

One of these genes, neurofibromatosis type 2 (NF2), has been shown to be lost in multiple meningiomas (40). The MYO18B gene located at 22q12.1, which was lost or mutated in a number of different types of human lung cancers, is another very strong candidate as a tumor suppressor gene (41). Similarly PDGF-B gene, located at 22q12, is known to be deleted in some tumors. To date specific studies on GIST
Figure 1. Composite CGH ratio profile to show recurrent losses at 1p, whole chromosomes 14, 15, 22 and X. A gain of 3q region is also shown in this Figure (A). Figure B shows a recurrent deletion of 9p; other losses and gains in both profiles are not recurrent.
Figure 2. Frequencies of losses (BOTTOM) and gains (TOP) detected in six GIST samples included in the study. Please note that p and q arm gains on chromosome 5 were detected in the same two samples.

Figure 3. Genomic profiles to show deletion of specific BACs located at 1p36.2-36.3 (A) loss of entire chromosome 14 (B) and smallest region of gain on chromosome 3q (C).
have not identified loss or mutations of any of these genes, but the common finding of loss of chromosome 22 in GIST raises the possibility that one or all of these genes may play a role in GIST development.

Chromosome 9p was completely or partially lost in three of the six samples tested, with all three of these samples showing loss of 9p13-pter. This loss of 9p has been a very common finding in a number of other cancers, with 9p21 as the most commonly deleted region of the chromosome. CDKN2A, located at 9p21 encodes the p16 and p14 tumor suppressor genes, both of which are homozygously deleted in a variety of tumor types (reviewed in 42) and are known to collectively affect both p53 and pRb-dependent growth regulatory pathways. The role of these genes in GIST pathogenesis, therefore, needs to be determined.

In our study, loss of part or all of chromosome 9 was seen only in malignant GISTs, supporting the earlier findings that chromosome 9 loss is seen mostly in malignant GISTs (10,12). This suggests that the genetic events occurring within this chromosome region may occur later in disease and, therefore, may be associated with malignant transformation or progression.

The nature of potential tumor suppressor genes located at 6q12 and 13q33.33-q34 is currently unknown, although LOH at these regions is known to occur in a variety of lymphohematopoietic as well as solid tumors (reviewed in 42, 43).

Complete loss of chromosome 15 was seen in three out of six samples tested in this study. While this chromosome has not yet been recognized as a possible chromosome of interest in GIST pathogenesis, previous studies have identified this as a chromosome of frequent loss in GIST (6,10,16). Loss of 15q material has been identified in other cancers, such as squamous cell carcinomas of the head and neck, sporadic colorectal cancer, small cell lung cancer and mesothelioma (32, 44-46). Once again, potential tumor suppressor gene(s) located on chromosome 15 have not been identified as yet. The only frequent DNA gain seen within the six GIST samples tested in this study was a gain of 3q material seen in three samples, with a common region of amplification located at 3q26.1-q27. Although this chromosome aberration has not been seen as commonly as the others previously discussed, significant amplification of this region raises the possibility that one or more oncogenes located at this region may play an important role in GIST tumorigenesis. Again squamous cell carcinoma of the head and neck is one of the cancers in which this abnormality has been found very frequently, with two studies identifying the gain of 3q in these tumors as a very early event, often leading to a poor prognosis (47, 48). The oncogenes namely PIK3CA (49), ZASC1 (50) and p63 (51), located at 3q26-27 are known to play an important role in tumorigenesis of other solid tumors. PIK3CA is known to be amplified in ovarian, cervix and head and neck cancers (49). PIK3CA encodes the p110∞ catalytic sub-unit of phosphatidylinositol 3'-kinase, which has oncogenic potential. Similarly ZASC1, a novel member of Kruppel-like zinc finger molecules, which is involved in proliferation, differentiation and cell death, is also located at 3q26, the amplification of which is frequently seen in various squamous cell carcinomas (50). In addition, this gene is frequently co-amplified with PIK3CA. Yet another gene, p63 located at 3q26 with significant homology to p53 gene, appears to be highly amplified in lung carcinoma and over-expression of this gene confers better survival (51).

The cytogenetic findings of monosomy 14 in GIST #6 correlates well with the CGH result of loss of chromosome 14. Interestingly, the cytogenetic findings for GIST #2 do not correlate with the CGH results of this study. With this complex karyotype some expected CGH results would be loss of 1q, loss of 6q22-q25, gain of 9q, gain of 11q13-q22, loss of 12q24-q24.3 and gain of 8p11-p23. However, the CGH results for GIST #2

<table>
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<th>Chromosome Number</th>
<th>N</th>
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<td>1</td>
<td>5</td>
<td>Loss</td>
<td>RP3-491M17 and RP3-438L4(1p36.2-36.3)</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>Loss</td>
<td>RP11-80L16(6q12)</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>Loss</td>
<td>cM39.00-pter(9p13-ter)</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>Loss</td>
<td>RP-98F14 and 245B11(13q33.33-q34)</td>
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<td>14</td>
<td>6</td>
<td>Loss</td>
<td>Mosaic loss of entire chromosome</td>
</tr>
<tr>
<td>15</td>
<td>3</td>
<td>Loss</td>
<td>Mosaic loss of entire chromosome</td>
</tr>
<tr>
<td>22</td>
<td>3</td>
<td>Loss</td>
<td>Mosaic loss of entire chromosome</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Gain</td>
<td>cM142.20-145.80 and cM176.40-186.70(3q26-q27)</td>
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N= number of samples with the given abnormality
showed gain of 2q, loss of 9p, loss of 11p and loss of chromosome 14. These results demonstrate some important limitations of the conventional cytogenetic analysis as it relates to solid tumors. As previously mentioned, the quality of cytogenetic preparations of solid tumor samples is often very poor. The number of analyzable metaphases is usually low and the morphology of these chromosomes is often less than adequate for compete analysis, which in turn may lead to incorrect classification of chromosomal abnormalities, especially in complex karyotypes that are often detected in solid tumors, as we detected in patient 2. Another limitation of cytogenetic analysis for solid tumors is that the results are only representative of the population of cells that are dividing at the time of chromosome preparation. This may be a very small proportion of total cells, which can again lead to inaccurate results. The discordance between the cytogenetic and CGH results for patient 2, therefore, clearly demonstrates the significance of CGH in identifying global abnormalities, which are representative of the composition of the tumor as a whole.

It is important, however, to realize that while CGH is a power full DNA-based approach to identify deletions or gains of larger chromosomal regions, it is only able to identify such alterations involving 10-12 Mb DNA. Array- based CGH on the other hand, identified regions of deletions and gains to specific BACs, as in the case of chromosomes 1p36.2-36.3, 6q12, 13q33.33-34 and 3q26-27. Such an approach, therefore, is highly significant in identifying sites of candidate gene(s) associated with multistep GIST tumorigenesis, which is the ultimate goal of such studies. With the recent finding that Gluevecs has been successful as a targeted therapy for the treatment of a sub-set of unresectable or metastatic GIST, the need for identification of genetic abnormalities in GIST has greatly increased. The results of this study strongly suggest that a key tumor suppressor gene(s) is located on 14q32 and that loss of this gene(s) is an early step in the tumorigenesis of GIST. Other tumor suppressor genes that may be involved in the progression of GIST are located at chromosome sites 1p36, 9p21, 22q11-12, with the possibility that the gene located on 9p may be involved in the malignant transformation of these tumors. In addition a key oncogene involved in some GISTs may be located on chromosome 3q. Thus, the results of our analysis presented above clearly demonstrate that multi-step tumorigenesis in GIST may involve a number of tumor suppressor genes and at least an oncogene, which in turn may serve as markers of prognosis, targeted therapy and ultimately clinical outcome. The number of tumors analyzed in this study, however, are not sufficient to draw any conclusions relating to clinical outcome.

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References


