Whole-exome sequencing analysis of mutation characteristics in recurrent or metastatic gastrointestinal stromal tumors

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Whole-exome sequencing analysis of mutation characteristics in recurrent or metastatic gastrointestinal stromal tumors

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Abstract

Purpose: Surgical resection for the metastasis and recurrence of gastrointestinal stromal tumors (GISTs) is controversial. Therefore, it is increasingly important to identify clinical factors related to survival and explore the driver genes and mutations in GIST.

Patients and Methods: GIST patients who received two surgeries for primary and recurrent and/or metastatic tumors between January 2003 and December 2018 were reviewed. Paired PT (primary tumor), RMT (recurrent and/or metastatic tumor), and normal DNA were whole-exome sequenced to generate comparable data in eight GIST cases.

Results: We identified 39 eligible patients with a median overall survival time of 56.7 months (IQR: 9.6-190.3 months). Regular TKI (Imatinib) treatment after primary tumor resection was associated with better OS, while presence of liver metastasis was prognostic for worse OS in GIST patients who received re-surgery due to recurrent and/or metastatic tumors. Compared with normal tissue, mutation of the *MUC* family in eight PTs and seven RMTs among the eight patients was detected. In the irregular (TKI) group, correlations and differences were noted in *KIT* mutations between the PT and RMT, while its influence was lower in the other cases. We also found that the mutation signature and subclone indicated the spatial and temporal heterogeneity of the tumor.

Conclusions: *MUC* mutations may be a potential predictor of recurrent and/or metastatic GIST. Treatment with TKI influenced *KIT* mutations in RMT of GIST patients. Due to the heterogeneity in the PT and RMT, the direction of tumor evolution and progression were not stable and regular.

Keywords: Gastrointestinal stromal tumor (GIST), recurrent and/or metastatic tumor, re-surgery, whole exome sequencing, somatic mutations, heterogeneity

1. Introduction

Gastrointestinal stromal tumors (GISTs) are the most common sarcoma subtype. GISTs originate from the interstitial cells of cajal (ICCS), most commonly in the stomach and small bowel. These are characterized by activated mutations of the KIT or PDGFRA receptor tyrosine kinases in nearly 85–90% of cases [1–3]. At present, the mainstay of treatment for patients with clearly resectable primary GIST is surgery [4]. In 2002, the FDA approved the KIT inhibitor imatinib (IM) for the management of patients with advanced GIST, and the prognosis of these patients significantly increased, as GISTs are generally resistant to conventional chemoradiotherapy [5–7]. The current recommended treatment for GIST patients with a high risk for recurrence is adjuvant therapy with imatinib for at least 3 years [8]. Patients with *KIT* exon 11 deletion mutations have been shown to benefit most from imatinib[6,9]. However, in confirmed cases of *KIT* exon 9 mutation, increasing the dose of imatinib significantly improved PFS[10].

However, many GIST patients continue to suffer from recurrence and/or metastasis leading to death, despite comprehensive treatment. It has been reported that surgical resection of residual lesions after disease control with imatinib is beneficial to patients with recurrent or metastatic cancer [11,12]. Previous studies have shown that *KIT* exon 11 mutations in GISTs resulted in a significantly higher response to imatinib than other *KIT* mutations, while *PDGFR* exon 18 *D842V* mutations were resistant to IM[13]. Some studies have revealed that a larger tumor volume is correlated with adverse outcomes such as molecular evolution and secondary clonal resistance[14–16]. Owing to the heterogeneity and IM resistance of GISTs, it is difficult to predict the prognosis of recurrent and metastatic cases. Therefore, the aims of this study were to evaluate the prognostic factors correlated with surgery for the metastasis and recurrence of GIST patients, and to explore gene mutations and tumor evolution between primary tumors and recurrent and/or metastatic tumors by whole-exome sequencing (WES).

2. Materials and methods

2.1 Patients and tumor samples

This study was divided into two parts. In the first part, we retrospectively analyzed 39 patients who had recurrence or metastasis after the first surgery and underwent one or multiple surgeries at Ruijin Hospital, Shanghai Jiao Tong University school of Medicine, between January 2003 and December 2018. Diagnosis was confirmed using standard histology and immunohistochemistry for CD117 (KIT) and sometimes DOG-1.

In the second part, we selected eight clinical cases from the 39 patients based on different groupings. The eight patients were chosen as candidates: 1) based on prognostic factors attained through part 1; 2) the timing of the two surgeries was after 2010, due to the degradation of DNA and RNA. The samples contained primary tumor tissue, recurrent or metastatic tumor tissue, and normal tissue. All tissues were manually micro-dissected from unstained, 10-μm-thick formalin-fixed, paraffin-embedded (FFPE) sections. The integral process is shown in Fig. 1.A.

2.2 Data Collection and Follow-up

Demographic and clinicopathological data were reviewed retrospectively and included age, sex, characteristics of the primary tumor (PT) and recurrent and/or metastatic tumor (RMT) (size, site, recurrence/metastasis site, mitotic rate), tumor growth pattern (Invasive, Dilative), histologic variant (spindle, epithelioid, or mixed), margins from the surgical resection (R0, R1/2), and significant dates (date of diagnosis, surgery time, recurrence interval time, death, last follow-up). Fifty high-powered fields (HPF) were counted by an experienced pathologist to determine mitotic rate. Recurrence interval time (RDT) represents the period from the first surgery to recurrence. Overall survival was defined as the time between recurrent surgery and death from any cause.

2.3 Statistical Analysis

Qualitative data are presented as a number (%). Continuous variables are expressed as the mean plus standard deviation (SD). Recurrence-free survival was calculated using the Kaplan–Meier method. Factors associated with recurrence were tested by univariate log-rank analysis. Variables that were significant in the univariate analysis were entered into the multivariate analysis. Multivariate analysis was performed using the Cox proportional hazard regression model. Hazard ratio (HR) for comparison of the two groups was summarized with 95% confidence interval (CI) and p-value using logistic regression. Statistical analysis and calculations were done using IBM SPSS statistics 24. Independent *t*-tests and Kaplan–Meier survival analysis were performed. A p-value less than 0.05 was considered statistically significant.

2.4 Genomic analysis, Bioinformatic Analysis and annotation

The Agilent_60M exon targeting sequence enrichment system was used to capture human exon sequences. First, we tested the purity and concentration of the captured hybridization DNA library, and Illumina HiSeq Platform sequencing (GeneX Health Co., Ltd., Beijing, China) was performed. Considering the accuracy of the sequencing data, we sequenced each sample twice, cleared the repetition by merging the sequencing data, and constructed the sequencing results of the case at that time. The average sequencing depth of the target regions was >100×.

The bioinformatic analysis of the raw data was done in two steps. For sequencing quality assessment, the sequencing error rate, data volume, and comparison rate were collected and whether the library sequencing met the standard was evaluated. For information mining and analysis, somatic SNV (single number variation) and CNV (copy number variation) were detected. Each database was annotated and analyzed, whereupon gene mutation spectrum and mutation characteristics, CNV distribution, tumor evolution, and tumor heterogeneity were explored and compared between PT and RMT samples.

In our study, small variants included single nucleotide variants (SNVs) and insertions or deletions. We used Control-FREEC, CNVkit, and Contra to detect somatic copy number variants (sCNV) in hyperdiploid tumor samples and tumor samples mixed with normal tissue cells. Functional annotation and identification of deleterious alterations of germline

variants were analyzed using ANNOVAR[17]. Additional annotation of somatic SNVs in 30 COSMIC (Catalogue Of Somatic Mutations In Cancer) mutational signatures[18] was performed using the somatic signatures in the R package[19]. For tumor heterogeneity analysis, we took three samples from different sites of the primary and recurrent tumors in Case 6 and analyzed the mutations and CNV of each sample. For somatic SNVs, mutation spectrum and mutation signature analyses were conducted. Based on the Bayesian clustering method, we used PyClone, which considers the complexity of influencing factors on mutation frequency, to classify somatic mutations in one or multiple points into clone clusters. We then estimated the rate of cell morbidity and determined the allelic imbalance caused by CNVs and normal cell contamination.

2.5 Validation in Common data base

To testify our results obtained by WES, we used cBioPortal (https://www.cbioportal.org/) to download clinical and genomic data from TCGA (PanCancer Atlas) and MSK-IMPACT (2022). But we could only acquire the relative data for Sarcoma that contained GIST. First, we downloaded the original data and the mutation(Mutations, Structural Varient, Putative copy-number alterations from GISTIC) distribution map, then, to sum up the number of the mutation selected from our analysis. Survival analysis were performed by using R package, The patients were divided into two group: Altered group and Unaltered group. RFS and OS were used as study endpoint.

3. Results and genome analysis

3.1.1 Patient population

Among the 1,942 GIST patients treated at our hospital between January 2003 and December 2018, 1,547 patients underwent surgery. A total of 70 patients underwent surgery for recurrent or metastatic GIST, with a median age of 59.8 years (range: 31.2–88.6). Only 39 patients, including 20 males (51.3%) and 19 females (41.7%), received surgery for primary and recurrent or metastatic tumors at our institution. Imatinib treatment for the primary GIST was given to 20 patients regularly and 14 irregularly, the other five patients were not treated with IM. Patients with high-risk GIST are recommended to receive this treatment for 3 years[20]. After diagnosis with recurrence and/or metastasis, 12 patients chose to increase the dose of IM (400–600 mg/d or 800 mg/d), six patients selected Sunitinib instead, and all 18 patients mentioned above received surgery within 6–12 months; the left 21 patients underwent surgery directly. The median follow-up period was 62.7 months (IQR:44.2–81.1).

3.1.2 Clinicopathologic features and outcomes

In primary surgery patients, the median age at the time of diagnosis was 56.2 years (range: 35.1–75.3). Regarding tumor location, the small intestine presented as the most common primary site (n = 28, 71.8%), followed by the stomach (n = 4, 10.3%) and colorectum (n = 4, 10.3%). The median tumor size was 9.18 cm (range: 3.2–17.9). The mitotic rate of 12 patients with primary tumors was less than 5/50, and in five patients it was more than 25/50. The median RDT was 41.3 months (IQR: 33.5–55.7). Thirty-seven patients had radical surgery and two patients only underwent R1 surgery.

After the primary surgery, all patients had recurrence, metastasis, or disease progression within 41.3 months (range: 12.3–117.5). Due to the site of recurrence, the peritoneum was involved in 28 cases (17 patients with local recurrence, the others with new lesions), the liver in nine cases (four patients with liver metastasis only, the other five patients with two or more sites), rare sites including lymph nodes (one patient), cutaneous mass of the chest wall and inguinal lymph nodes (one patient). All the cases received surgery within 6–12 months; 34 patients underwent radical surgery and the pathology from two patients revealed positive margins, where the other three patients only received palliative resection (Table 1).

In our study, 23 patients died from the cancer and six lived with the neoplasm until the last follow-up (2020-12-31), except for two cases who were lost to follow-up. The median overall survival of the entire GIST cohort was 56.7 months (range: 9.6–190.3). Survival analysis revealed that liver metastasis and no IM treatment predicted poor prognosis (Fig. 1-B, C), univariate analysis showed that recurrent site and IM treatment were associated with overall survival, and multivariate analysis identified these as independent factors associated with prognosis (Table 2).

3.2.1 *KIT* and *PDGFRA* mutations

Of the eight clinical cases, five harbored *KIT* mutations, of which two had primary and recurrent tumor mutations where the other three only had primary tumor mutations. One harbored mutations in *PDGFRA* in the RMT, and the two remaining patients had wild type mutations. The *KIT* mutations in the primary and recurrent tumors all had the same mutations in exon 11 (clustered in the proximal part between codons 568 and 576 and consisting of small in-frame deletions and point mutations). There were also differences, which are shown in Table 3. In the remaining three patients, two had mutations in exon 11 and one harbored a mutation in exon 17; these mutations were only detected in the PT. In the mutated exons of the *KIT* gene, the types mainly included point mutations, base deletions, and amino acid substitutions.

3.2.2 CNV variations

The eight patients were grouped according to IM treatment after primary surgery (Table 4). All samples had deletions in chromosomes 1p, 14, and 15, as well as in chromosome 13q were frequent, especially in metastatic and/or recurrent tumors (Fig. 2A, B). In PT samples (Fig. 2A), deletions in chromosomes 10 and 22 were detected in seven cases, the other one was in the No drug group. In RMT samples (Fig. 2B), amplification in chromosome 5, especially in 5p, was detected in six of the eight samples; the two samples from the No drug group did not have the variation mentioned above.

No deletion in chromosome10 was detected in the correspondent RMT cases compared with the PT. However, new CNVs were detected in chromosome 22 in two cases, and the deletions of chromosome 22 in the PT did not exist in the RMT in the irregular treatment group. Deletions in chromosome18 both in the PT and RMT were only detected in the liver

metastasis group, and the above-mentioned feature was consistent in two tumors. This only occurred in patients receiving IM treatment.

3.2.3 Somatic SNV results

The top 20 high-frequency mutations in different coding genes were filtered among all the samples; one sample was excluded because it did not contain those genes (Fig. 2C1). Surprisingly, mutations in the *MUC* family genes, such as MUC4, MUC6, MUC16, and MUC17, were obtained in all but one PT and RMT sample. We also found a common tendency for a frameshift mutation of *FRG1* and *ZNF717*. The remaining mutation correlations and distinctions between PT and RMT are shown in Fig. 2C2, C3.

We filtered out new mutations of the recurrent or metastatic tumors as compared with the sequencing data of primary tumors. In each tumor pair, statistically significant differences occurred in 182 genes and the variation types included exonic, downstream, upstream, UTR, intergenic, and ncRNA_exonic. Among the genes, 31 were directly correlated with coding regions such as *AJAP1* (Adherens Junctions Associated Protein 1), *RPL19* (Ribosomal Protein L19), *PTPRG* (Protein Tyrosine Phosphatase Receptor Type G), and *TMPRSS13* (Transmembrane Serine Protease 13). Many genes, including *AJAP1, C2CD4D, EMX2, FNBP4, GGT1, GIGYF1, HDGFRP2, KCNN3, KRT13, LILRB1, LRRK1, MUC19, PHF24, RAC1, RAD54L2, RBPJ, SETD1B, SMG7, SRRT, VGLL3, WDR8,* and *ZNF358,* involved multiple base deletions (Fig. 3A, B, C, D).

Two recurrent tumors from the eight patients had mutations in AJAP1, resulting in the loss of two amino acids from its encoded product. In two RMT samples (25%), we observed a mutation in PRL19, which encodes an essential structural constituent of ribosomes. Additionally, mutations in the coding region of LOC101927550 (ncRNA), which influences cell proliferation and growth velocity by affecting the synthesis of the protein, were identified in three cases.

3.2.4 Mutations among the clinical group

We detected mutations occurring in the UTR region of *MTMR11 and GOLGA6L4* only in the regular treatment group (Fig. 3A1, B1). Interestingly, we identified frameshift mutations in the *EMX2 and C2CD4D* genes in addition to the above-mentioned mutations found in the liver metastasis group receiving regular treatment (Fig. 3A2, B2). Mutations in *FAM101A (RFLNA)* were only found in the PT of this group. In the regular group, we detected mutations in *MTMR11* and *GOLGA6*, and in the No Drug group (Fig. 3-A3, B3), we detected mutations in the coding region of the *PTPRG* gene. In addition, mutations occurred in the UTR region of the *STK35/FANCD2* gene. Frameshift mutations were detected repeatedly in the *SMG7, RAD54L2,* and *RBPJ* genes only in the irregular treatment group (Fig. 3A4, B4).

3.2.5 Tumor heterogeneity and evolution

3.2.5.1 Somatic SNV

To obtain the tumor characteristics at the point mutation level, we analyzed somatic SNV

variation from multiple angles, including mutation spectrum and mutation signature. Point mutations contain 6 types: C>A/G>T, C>G/G>C, C>T/G>A, T>A/A>T, T>C/A>G, T>G/A>C. Cluster analysis was performed on the number and type of point mutation in each tumor sample, and we obtained the preference and the similarity degree of point mutation in GIST (Fig. 4A). In GIST, T>G/A>C mutation accounted for the high proportion of the six types, and the proportion of each mutation was significantly different in three spatial locations, while its tendency and feature in the primary and recurrent tumors was similar.

To infer the mutation patterns of recurrent and/or metastatic tumors, bases at the upper and downstream positions 1 bp of the point mutation were taken into consideration and classified into 96 varieties according to mutational signature (Fig. 4-B); mutational characteristics were obtained through those varieties. As Fig. 4-C shows, the differences in pattern between the primary and recurrent tumors were mainly reflected in eight signatures (Signature 1, Signature 2, Signature 3, Signature 5, Signature 11, Signature 12, Signature 20, Signature 30).

3.2.5.2 Somatic CNV

The loss of copy number at Chr1, Chr2, Chr13, and Chr15 was detected in both primary and recurrent tumors. Additionally, an increase at Chr5 and a decrease at Chr11 and Chr14 was detected in RMT, while a decrease was detected in Chr4, Chr10, Chr12, Chr22, and ChrX only in the PT. As Fig. 4D shows, the CNVs not only had different aspects, but also correlations at different points.

3.2.5.3 Subclones, evolution, and heterogeneity of tumor cells

Compared with primary tumors, the number of subclones and the cluster of low prevalence were significantly lower in recurrent tumors. However, in different sites in the same sample, the number and cluster were almost conformable in PT and RMT (Fig. 4E-1). All PT and RMT samples were used in the subclone analysis (Fig. 4-E3) and divided into 3 subclone clusters. The prevalence of three clusters in recurrent tumors was higher than in the primary tumors, while within tumor differences were not significant (Fig. 4-E2). In determining the evolution of different tumors, we found that the evolutionary relationship in the PT was P3>P2>P>1 and in RMT it was R3>R2>R1 for Clonal Phylogeny (Fig. 4F1). Compared with PT, the RMT samples were at the end of the evolutionary tree (Fig. 4F2,). In addition, the correlation between P2 and P1 was low in PT, while a close correlation was seen between R2 and R1 in RMT. The relatedness was low among P3, R3, and other correspondent samples.

Through comparing the functional mutations of each sample, we identified similar and different mutation sites in different locations of the same tumor, and the same results were acquired for both PT and RMT samples (Fig. 4G). The mutation frequency of some genes was significantly different between each sample from the two tumors, especially in *MUC19, KIT, PABPC3, ZNF208, TOP3A,* and *RGS19*. These genes were used for functional enrichment, and we found that the genes involved in biological processes and biological functions such as influencing cell differentiation, regulating the expression of

nuclear genes, and regulating translation level, were closely connected with tumor cell growth regulation.

3.2.6 Verification in Common data base

We chose the top 10 high-frequency mutations in different coding genes(*MUC4*、*MUC6*、*MUC16*、*MUC17*、*FRG1*、*TTN*、*ASH1L*、*WNK1*、*ZNF717*、*CD55*) to verify in TCGA (PanCancer Atlas) and MSK-IMPACT (2022). Unfortunately, there exist not any mutation in the Sarcoma from MSK-IMPACT. And the mutation distribution in the Sarcoma from TCGA were shown in supplementary Table1 and supplementary Figure1. To take those genes mutation into survival analysis, there was no difference between Altered group and Unaltered group supplementary Table2.

4. Discussion

Prior to the era of imatinib, no effective treatment for recurrent or metastatic GIST existed, and surgery was often attempted in the absence of alternative treatments or as an emergency response to bleeding, gastrointestinal perforation, or obstruction. Previous reports showed that the rate of complete gross resection was low and median survival was only 15–19 months [21,22]. Research [23,24] revealed that the prognosis of patients diagnosed with advanced or metastatic GIST was significantly increased by the optimal use of imatinib. Kang [12,25] retrospectively analyzed the correlation between IM and surgery in patients with metastatic and/or recurrent GISTs and concluded that surgical resection of residual lesions after disease control with imatinib is likely to be beneficial to patients. In our series, the OS of the entire group was 56.7 months following the second surgery for recurrent and/or metastatic tumors. However, the survival outcome in patients who took IM regularly after resection for the primary tumor was significantly better than those who did not take IM or took it irregularly ($p = 0.043$), although it was better than previous research in terms of OS. The importance of IM and radical surgical resection should therefore be highlighted for a maximal clinical response that may be associated with survival benefits[16,26,27]. However, surgical resection has been shown to improve the prognosis of patients who are resistant or unresponsive to IM, which may contribute to secondary gene mutations[28].

Our results showed that in patients who underwent surgical resection for liver metastasis of GIST, OS was significantly lower than in patients with other recurrence or metastasis sites ($p = 0.021$). The liver is reported to be the most common site of GIST metastasis, as 50–60% of patients have liver involvement during the disease process[25,29,30]. Hou et al.[31] reported no statistical difference in the survival of patients who underwent radical surgery for metastasis or recurrence compared to those that underwent IM therapy only. A multicenter prospective study[32] reported that liver metastasis of gastrointestinal stromal tumors may not be controllable by surgery alone and requires concomitant imatinib therapy. Thus, the therapeutic model and surgical opportunity for these patients should be seriously considered.

Based on the clinical results, we used deep whole-genome sequencing (WGS) to profile genomic variation in both primary tumors and recurrent and/or metastatic tumors. It is well known that the majority of GIST tumors (75–80%) harbor gain of function *KIT* mutations in exons 9, 11, 13, 14, and 17, and 5–10% of GIST tumors have mutations in the platelet-derived growth factor receptor a (PDGFRA) gene in exons 12, 14, and 18 [33]. Because our sample size was small, we observed correlations and differences in *KIT* mutations between the PT and RMT in the irregular medication group. In the regular-medication group, the *KIT* mutations only were found in the PT. Mutations in *KIT* were not observed in the RMT following IM use where mutations were identified in both the PT and RMT in the irregular group, however, these mutations were not completely equal. Miselli FC[34] raised the idea that KIT amplification may be a mechanism of drug resistance in GIST. Reactivation of KIT signaling by tumor subclones with heterogeneous secondary KIT mutations results in oncogenic activation of *KIT* and is the key driver of GIST proliferation and survival [35–37]. Serrano et al.[38] tested the activity of nine TKIs which have either been approved or are under clinical investigation as KIT inhibitors for GISTs, against imatinib-resistant GIST cell lines with different secondary *KIT* mutations. They showed that rapid alternation of sunitinib and regorafenib was more effective than monotherapy of either drug in *vitro*. Mutations in *PDGFRA* were only detected in the RMT of the regular group. The difference in *KIT*/PDGFRA mutations between the PT and RMT was likely caused by secondary mutation or tumor evolution.

MUC4 is known to play an important role in cell proliferation and differentiation of epithelial cells by inducing specific phosphorylation of ERBB2 and affecting the formation of a MUC4-ERBB2-ERBB3-NRG1 complex, leading to down-regulation of CDKN1B and resulting in repression of apoptosis and stimulation of proliferation[39,40]. Mutations in *ASH1L, MUC4,* and *KMT2D* exhibited coordinated variation between PT and RMT. ASH1L is a histone methyltransferase of lysine, specifically trimethylating 'Lys-36' of histone H3 to form H3K36me3[41]. MUC4 was hypothesized to affect the intracellular regulation of *ASH1L* and *KMT2D* through an intracellular cascade. A decrease in *FRG1* expression may influence tumor progression by regulating cell migration and invasion[42], and the transcription factor ZNF717 is involved in nucleic acid binding and DNA-binding[43]. We hypothesized that ZNF717 promoted tumor progression through combination with FRG1. Tumor cell growth was mainly dependent on the *MUC* family in the two tumor types (mentioned above), while the effect of *KIT/PDGFRA* on RMT decreased significantly, and the mutation of *ASH1L* and *KMT2D* tended to occur in RMT. Though we could successfully verified our results in Common data base, the main reason were that there exist no independent classification for GSIT. And we need to handle large-scale research to validate the results.

In the regular group, we detected mutations in *MTMR11 and GOLGA6*. MTMR11 is a Myotubularin Related Protein, involved in the process of glycophosphatidylinositol dephosphorylation. High-frequency mutations of *GOLGA6L4* and the expression of *MTMR11* have been reported to be abnormal in many cancers[44]. Mutations in *EMX2 and FAM101A* were only detected in the liver metastasis group with regular treatment. *EMX2* encodes a homeobox-containing transcription factor which can influence the growth and

development of cells and animals[45] and has been proved to interact with FLNA to regulate the filamin framework around the nucleus and the shape of the nucleus. Consequently, we inferred that the mutations were correlated in these patients.

In the irregular group, mutations in *SMG7, RAD54L2,* and *RBPJ* were detected. We determined that *SMG7* and *SMG5* were involved in cell nonsense-mediated mRNA decay[46]. *RAD54L2* is a DNA helicase, and *RBPJ* is a transcriptional activator of the Notch signaling pathway[47]. The frameshift mutations of the two genes may result in tumor escape from the effect of IM. In the No-Drug group, mutations in PTPRG and STK35/FANCD2 were detected. It is known that the encoding product of PTPRG is a Tyrosine protein phosphatase receptor which activates a series of signaling cascades to affect protein synthesis. This has been confirmed to play a key role in the development and progression of many cancers[48]. Therefore, its mutation could result in abnormal activation and activity, thus influencing subsequent signaling cascades. STK35 is a serine/threonine protein kinase, and the histone FANCD2 prevents DNA breakage and loss of chromatin, participating in the stabilization of chromosomes and DNA damage repair[49].

As an adhesion protein, AJAP1 is translocated to the nucleus via its interaction with β-catenin complexes where it can regulate gene transcription, potentially exerting a potent impact on the cell cycle and apoptosis. AJAP1 also participates in tumor cell adhesion and intercellular metastasis[50,51], implying that its mutation may influence tumor metastasis or recurrence. The mutations in the coding region of LOC101927550 overlap with *SMURF1*, which encodes a ubiquitin ligase that is specific to receptor-regulated SMAD proteins in the bone morphogenetic protein (BMP) pathway. Therefore, we speculated that LOC101927550 influences the targeted combination of SMURF1 miRNA, regulating the expression of SMURF1 and affecting tumor development[52].

In analyzing tumor heterogeneity and evolution, we determined that the mutations and variants of different sites in the same tumor were significantly different. Additionally, differences between PT and RMT were greatly discrepant, mainly reflecting in point mutations and subclones which were related with the grade of malignancy and IM resistance in RMT. Heidi M[53] demonstrated that mutational analysis via liquid biopsies can capture the molecular heterogeneity of the whole tumor, and revealed that multiple resistance mutations were synchronously present. It has been shown that imatinib-resistant disease frequently harbors up to two resistance mutations within a single tumor or metastasis, or up to five mutations in separate metastases from one patient[54–56]. B Liegl et al. [55] also reported extensive intra- and inter-lesional heterogeneity of resistance mutations and gene amplification in patients with clinically progressing GIST, underscoring the heterogeneity of clinical TKI resistance and highlighting the therapeutic challenges involved in salvaging patients after clinical progression on TKI monotherapies. Thus, we deduce that spatial and temporal heterogeneity contributes to the occurrence or metastasis of tumors and resistance to TKI.

Although our study is limited by a small population size and its retrospective design, it had several strengths. First, this was the first genome sequencing of GIST within PT and RMT. Second, we used different modes of TKI treatment and deep whole-genome sequencing to analyze the correlations and distinctions within PT and RMT. Third, we analyzed tumor evolution and heterogeneity among different times and sites. The different TKI treatment modes may induce different mutations in coding genes, and we found that irregular treatment with TKI was more likely to contribute to various mutations. We also highlight TKI treatment for high risk GISTs after resection for primary tumors. We determined that mutations in the *MUC* family were very likely to have a close connection with the recurrence and metastasis of GIST. Thus, we suggest that genotyping and the use of precision medicine for GIST is significant and meaningful[57,58].

5. Conclusions

Although our study is limited by small population size and its restrospective design, it had several strengths, including the first genome sequencing for GIST within PT and RMT, different modes of TKI treatment, deep whole genome sequencing to analyze the correlations and distinctions within PT and RMT, and the analysis of the tumor evolution and heterogeneity among different times and sites. The different treatment modes of TKI may induce different mutations in code gene, while the irregular group is more likely to contribute to various mutations. And we also highlight the TKI treatment for high risk GISTs after resection for primary tumor. We acquire that mutations in *MUC* family were very likely to have close connection with the recurrence and metastasis of GIST. Thus, we insist that the genotype and precision medicine for GIST is quietly significant and meaningful[57,58].

Abbreviations

GIST: gastrointestinal stromal tumors; PT:primary tumor; RMT:recurrent and/or metastatic tumor;TKI: Tyrosine kinase inhibitors;IM: Imatinib;RDT:recurrence interval time OS:overall survival; HPF: high-powered fields; SD:standard deviation;HR: hazard ratio; CI:confidence interval; WES: whole-exome sequencing; FFPE: formalin-fixed, paraffin-embedded; SNV: single number variation; CNV: copy number variation; PDGFRA: platelet-derived growth factor receptor;

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Authors' contribution

WTL,ZGZ, and LY contributed to conceptualization; CY, MYand CL contributed to formal analysis; ZTN, YM,contibuted to investigation; JX contributed to methodology and resources; LQW,WX and WJC contributed to writing—original draft; LQW,WX,XFWand WJCcontributed to writing—review and editing. All authors read and approved the final

manuscript.

Availability of data and materials

The datasets used and analyzed during the current study are available within the manuscript and its figures and tables.

Ethical approval and consent to participate

This research was approved by the Ruijin Hospital Ethics Committee of Shanghai jiaotong University of medicine and written informed consent was obtained from all patients before enrolling in the research program.

Consent for publication

All patients involved in our study obtained written consent for publication.

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Conflict of Interest

All authors declare no competing interests.

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Figures

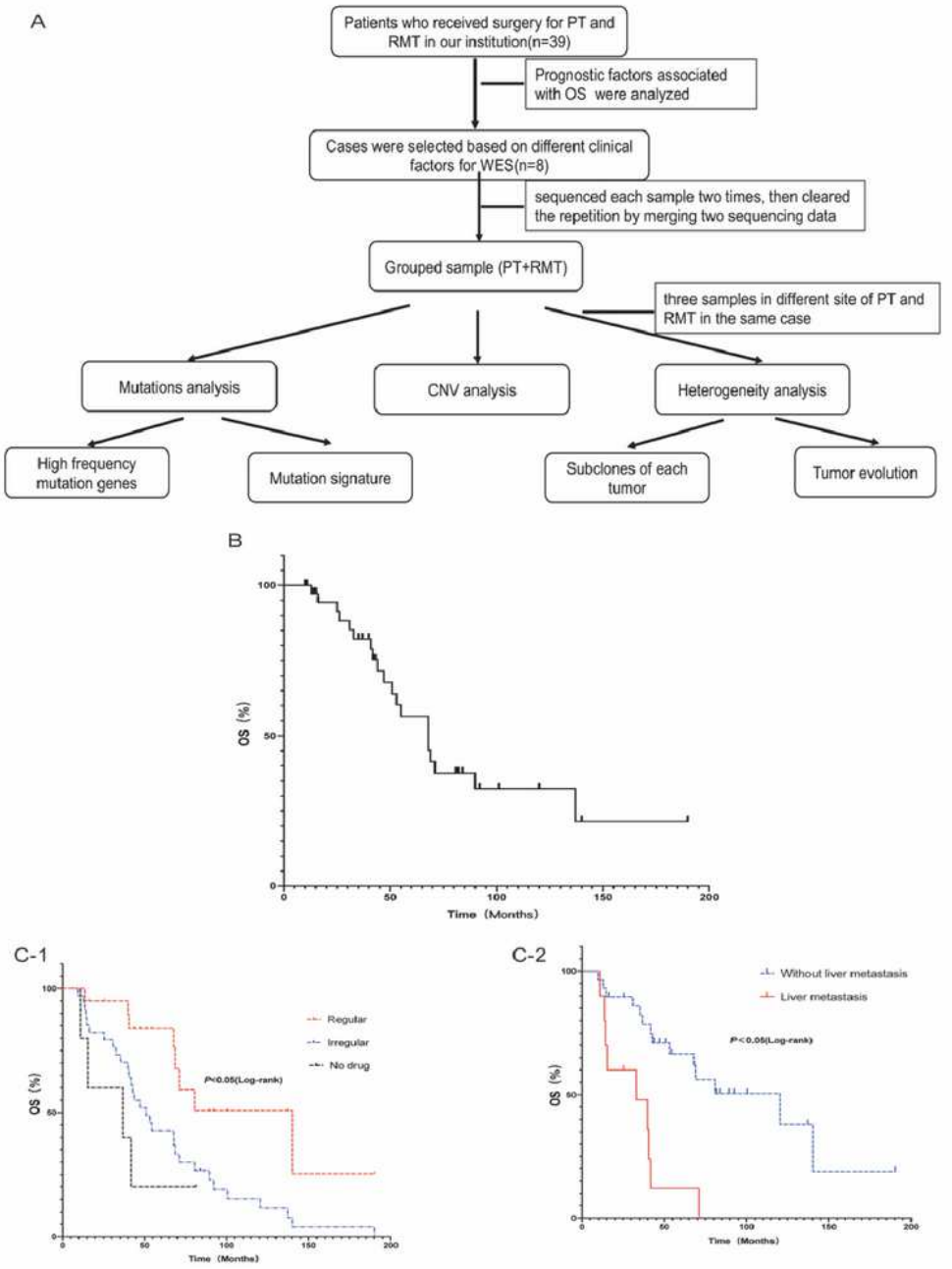

Figure 1

(A) The whole research process contained clinical factors and sequencing analysis; (B) OS is shown for the whole group; (C-1) OS by IM use-pattern is shown for Regular, Irregular and No drug. (C-2) OS by metastasis whether contained liver is shown for with or without liver metastasis.

Figure 2

The mutations contained SNVs and CNVs among PT and RMT. A,The CNVs(Copy Number Variant) detected on PT, each column represents a genomic region. B, The CNVs acquired on RMT. C-1, The top 20 mutational genes existed on PT and RMT. C-2, The top 10 mutational genes ooured on PT. C-3, The top 10 mutational genes cover 7patients.

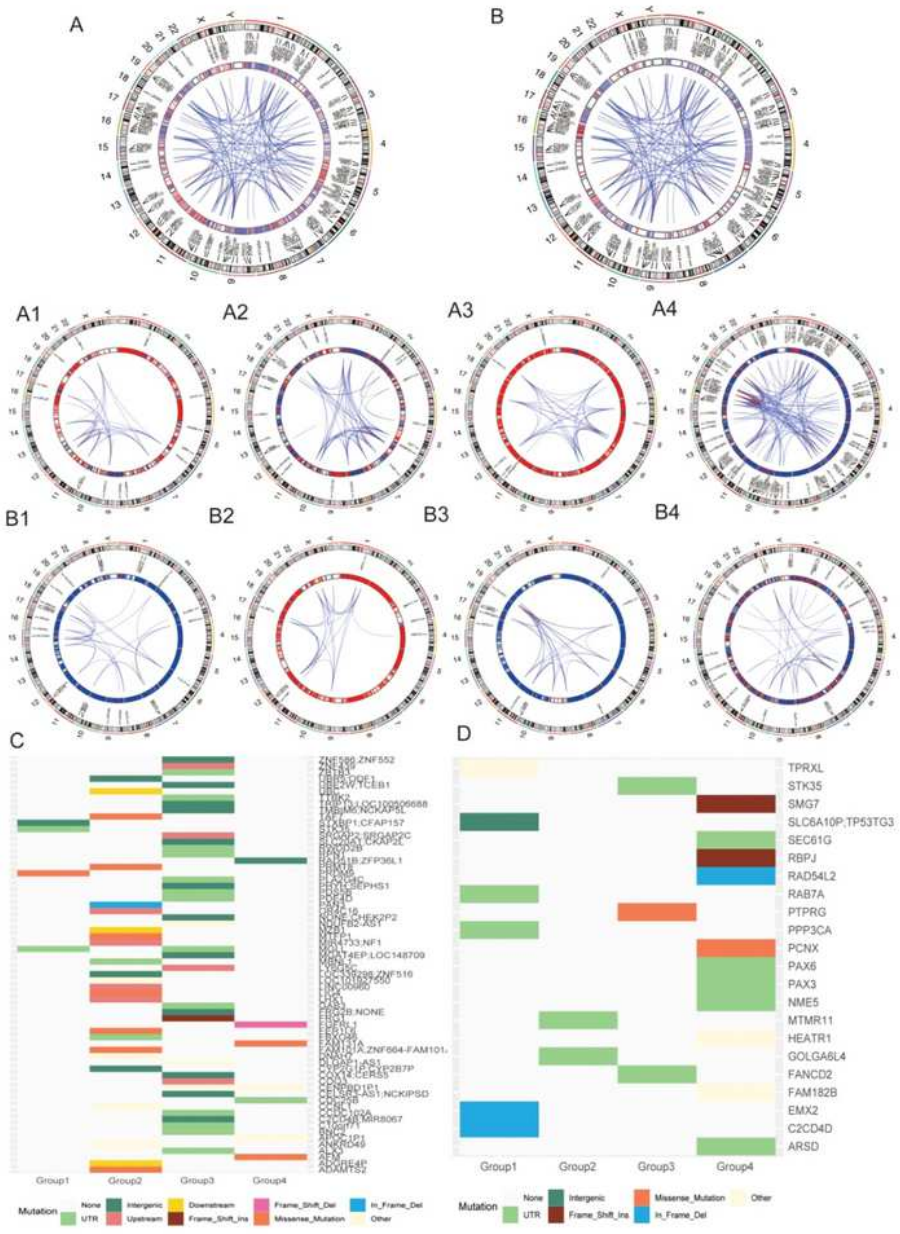

Figure 3

Cricos plots depicting total tumor mutational burden and spectrum of somatic genomic variation in PT and RMT. The outermost(first) track represents autosome ideograms(chromosomes 1 to Y), the different color represents the information of G line. The second and third tracks represents the mutational genes(the mutation occurred on the coding region, and happened on at least three samples). The fourth track is a heat-map that show the value equals to the largest proportion of those genes which contained

multiple mutations. The innermost area shows the existence of interaction between different genes acquired in the second and third tracks through STRING website （https://string-db.org）, the red line represents the connection between gene fragments was on the same chromosome, while the blue line represents the connection was on the different chromosome. A, The PT of the eight cases. B, The RT of the 8 patients; the somatic aberrations within PT (C)and RMT(D) in different group. There may existing significantly mutated genes among the two tumors, the genes affected are shown next to form. Group1(A1,B1):regular medication(IM)+liver metastasis;Group2(A2,B2):regular medication(IM);Group3(A3,B3):No drug; Group4(A4,B4): irregular medication）

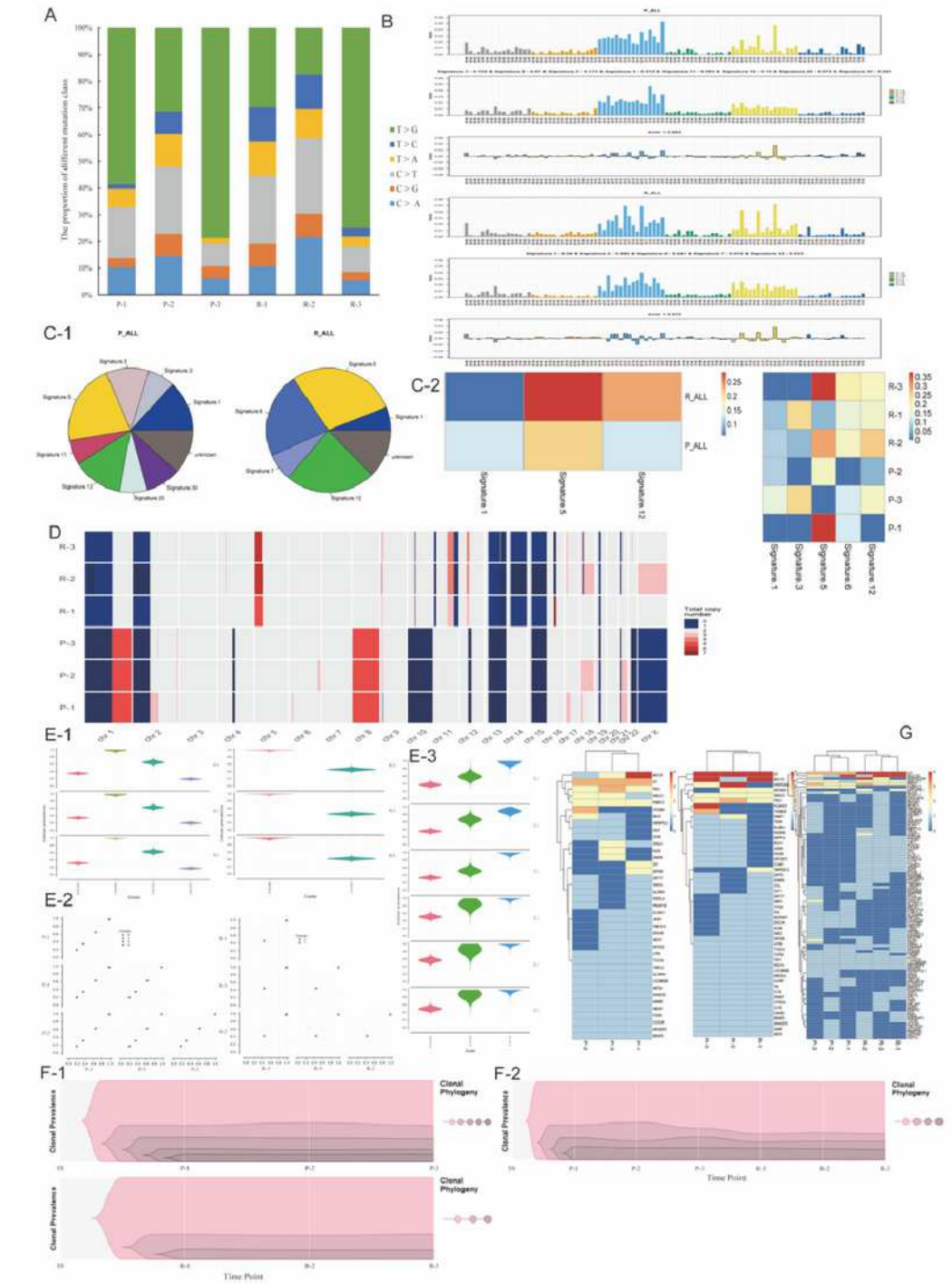

Figure 4

The deduction of tumor heterogeneity and evolution; A. The 6 types of point mutation of different sites in the same tumor among two tumors; B. The two estimated mutation patterns of PT(upper figure) and RMT(middle figure), the difference of the two patterns（below figure）;C-1. The two pie charts reveal the contribution of different signature on the mutation pattern in PT and RMT; C-2.the two heat maps show the contribution of different signature on the mutation pattern in the different sites of the same tumor

and two total tumors; D. The distribution of different CNVs on each chromosome among every sample, red stripe represents increase of copy number, while blue stripe represent decrease of copy number; E. Phylogenies revealing difference between PT and RMT for clone and subclone clusters (E-1), the lithotripses (E-2) show the prevalence of cell in different samples among PT and RMT. Take the 3 clusters into calculation for all samples of PT and RMT□E-3□ F-1. The evolutionary tree of RT and RMT F-2. The evolutionary tree of the two tumors G. The number of mutated genes in different location of PT and RMT as left and middle heat-figure shows, add up those mutations from different sites in PT and RMT (right figure). (P-1,P-2,P-3: 3 different site of primary tumor; R-1,R-2,R-3: 3 different site of Recurrent tumor)

Supplementary Files

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- SupplementaryTable12andFigure1.docx