Whole-exome Sequencing Analysis for Mutations Characteristics in Recurrent or Metastatic Gastrointestinal Stromal Tumors

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Research

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Abstract

Background: Surgical resection for the metastasis and recurrence of GIST was controversial. It is increasingly important to identify clinical factors related with survival and explore the driver genes and mutations in GIST.

Methods: GIST patients who received two surgery for primary and recurrent and/or metastatic tumors between January 2003 and December 2018 were reviewed. Primary outcome was overall survival after reoperation. Kaplan-Meier, Cox proportional hazard regressions, and mean survival time were used to evaluate outcomes. Paired PT(primary tumor), RMT(recurrent and/or metastatic tumor) and normal DNA was whole-exome sequenced to generate comparable data for those specific 8 GIST cases.

Results: We identified 39 eligible patients with a median overall survival time of 56.7 months(IQR:9.6-190.3months). Regular TKI(Imatinib) after primary tumor resection (HR:0.568; 95% confidence interval(CI):0.211-0.874; P=0.043) was associated with better OS, while presence of liver metastasis were prognostic for worse OS(HR:1.45; 95% CI:1.13-2.02;P=0.032) for those GIST patients who received re-surgery due to recurrent and/or metastatic tumor. Compared with normal tissue, we detected mutation on *MUC* family both in 8 PT and 7 RMT among the 8 patients. Only in irregular(TKI) group, the *KIT* mutations between PT and RMT contain correlations and differences, while its influence exist less on other cases. We also found that 31 genes which were direct correlation with coding regions may associated with RMT. We attained that the Spatial heterogeneity and temporal heterogeneity of the tumor reflected on mutation signature and subclone.

Conclusions: The *MUC* mutations were supposed to be a potential predict to recurrent and/or metastatic GIST. The treatment of TKI could influence the *KIT* mutations on RMT of GIST. As the heterogeneity exist in PT and RMT, the direction of tumor evolution and progression were not stable and regular.

1. Background

Gastrointestinal stromal tumors(GIST) are the most common sarcoma subtype and originate from the intestinal cells of Cajal (ICCS), most commonly in the stomach and small bowel which are characterized by activating mutations of the KIT or PDGFRA receptor tyrosine kinases in nearly 85-90% of cases¹⁻³. At present, the mainstay of treatment is surgery for patients with clearly resectable primary GIST⁴. When the FDA approved imatinib(IM), a receptor tyrosine kinase inhibitor of KIT, for the management of patients with advanced GIST in 2002, the treatment of GIST entered the era of imatinib and the prognosis of the GIST patients were significantly increasing, as GISTs generally resistant to conventional chemoradiotherapy⁵⁻⁷. And current recommended treatment for GIST patients with a high risk for recurrence is adjuvant imatinib for 3 years at least⁸. Patients with *KIT* exon 11 deletion mutations were verified to benefit most from imatinib^{6,9}. But in confirmed cases of *KIT* exon 9 mutation, raising the dose of imatinib could significantly improve the PFS¹⁰.

However, a number of GIST cases still suffered from recurrence and/or metastasis, leading to patients' death despite comprehensive treatment. It has been reported that surgical resection of residual lesions after disease control with imatinib was beneficial to patients with recurrent or metastatic cases^{11,12}. Previous studies have showed that *KIT* exon 11 mutations in GISTs were significantly higher response to imatinib than other mutations in *KIT*, while *PDGFR* exon 18 *D842V* mutation in GIST was resistant to IM¹³. Some studies revealed that the larger tumor volumes were correlated with adverse outcomes, the chance of molecular evolution and secondary clonal resistance¹⁴⁻¹⁶. As the heterogeneity and IM resistance of GISTs, it is difficult to predict the prognosis of the recurrence and metastasis cases. Therefore, the aims of this study were to evaluate the prognosis factors correlated with surgery for the metastasis and recurrence of GIST patients and explore the gene mutations and tumor evolution between primary tumors and recurrence and/or metastasis tumors by Whole genome sequence (WGS).

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 surgery at Ruijin Hospital, Shanghai Jiao Tong University school of Medicine, from January 2003 to December 2018. Diagnosis was confirmed using standard histology as well as immunohistochemistry for CD117 (KIT) and sometimes DOG-1.

In the second part, we selected 8 clinical cases of the 39 patients on the basis of different groups. The reasons for the 8 patients as candidates: 1) based on prognostic factors attained through first part; 2) the time of two surgeries were all after 2010 on account of the degradation of DNA and RNA. The samples contain primary tumor, recurrent or metastasis tumor and normal appearing tissue. All tissues were manually microdissected from unstained, 10-μm-thick formalin-fixed, paraffin-embedded (FFPE) sections. The integral process was showed in Figure.1.A. The study was reviewed and approved by Shanghai Jiaotong University School of Medicine Ruijin Hospital Ethics Committee.

2.2 Data Collection and Follow-up

Demographic and clinicopathological data were reviewed retrospectively, including as following age, sex, characteristics of the primary tumor (PT) and recurrent and/or metastasis tumor (RMT)(size, site or recurrence/metastasis site, mitotic rate), tumor growth pattern(Invasive, Dilative), histologic variant (spindle, epithelioid, or mixed), margins from surgery 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 the experienced pathologist to determine mitotic rate. Recurrence interval time (RIT) represents the period from the first surgery to recurrence. Overall survival was defined as the time between recurrent surgery and death of any cause.

2.3 Statistical Analysis

For statistical analysis, qualitative data are presented as number (%). Continuous variables are expressed as mean with standard deviation (SD). Actuarial 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 univariate analysis were entered into multivariate analysis. Multivariate analysis was performed with the Cox proportional hazard regression model. Hazard ratio (HR) for comparison of the 2 groups was summarized with its 95% confidence interval (CI) and P-value using logistic regression. Statistical analysis and calculations were done by using IBM SPSS statistics 24 performing independent samples t-test and Kaplan-Meier survival analysis. A p-value less than 0.05 was considered statistically significant.

2.4 Genomic analysis, Bioinformatic Analysis and annotation

We adopted the Agilent_60M exon targeting sequence enrichment system to capture human exon sequences in our study. Firstly, we tested the purity and concentration of captured hybridization DNA library, and illumina HiSeq Platform sequencing (GeneX Health Co. Ltd, Beijing, China) was performed. Taking the accuracy of sequencing data into account, we sequenced each sample two times, then cleared the repetition by merging two sequencing data, and constructed the sequencing results of the case at that time. The average sequencing depth of the target regions was >100x.

Take the Raw data into bioinformatics analysis process①②Sequencing quality assessment : collect the sequencing error rate, data volume, comparison rate; evaluate whether the library sequencing meets the standard, then perform the following analysis ; 2) Information mining and analysis: detect Somatic SNV(single number variation)③CNV(copy number variation); annotate and analyze each database; explore and compare gene mutation spectrum and mutation characteristics, CNV distribution, Tumor evolution, Tumor heterogeneity between PT and RMT.

In our study, small variants include single nucleotide variants (SNVs) and insertions or deletions. We took Control-FREEC, CNVkit, Contra to detect Somatic Copy Number Variant(sCNV), hyperdiploid tumor samples and tumor samples mixed with normal cells were analyzed. Functional annotation and deleterious alterations of germline variants were performed using ANNOVAR¹⁷. Additional annotation of somatic SNVs to 30 COSMIC (Catalogue Of Somatic Mutations In Cancer) mutational signatures¹⁸was performed using Somatic Signatures in R package¹⁹. To the heterogeneity of tumor, we took three samples in different site of the primary and recurrent tumor in case 6, then analyzed the mutations and CNV of each sample. For Somatic SNVs, analyses were conducted on mutation spectrum and mutation signature. Based on Bayesian clustering method, we chose PyClone which could take the complexity of influencing factors containing the mutation's frequency into account to classify Somatic mutations in one or multiple points into clone clusters, then estimated the rate of cell morbidity, and gave the explanation for the Allelic imbalance caused by CNV and normal cell contamination ultimately.

3. Results

3.1 Patient population

Among 1942 patients with GIST treated at our hospital between January, 2003 and December, 2018, there were 1547 patients who underwent surgery. A total of 70 patients who 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 received surgery for primary and recurrence or metastasis tumor all at our institution. Imatinib treatment for the primary GIST was given to 20 patients regularly and 14 irregularly, the other 5 patients not taking IM (patients with high-risk GIST were recommended for 3 years²⁰). After being diagnosed as recurrence and/or metastasis, 12 patients chose increasing the dose of IM (400mg/d-600mg or 800mg/d), 6 patients selected Sunitinib instead; the all 18 patients mentioned above received surgery within 6-12 months; the left 21 patients accepted surgery directly. The median follow-up period was 62.7 months (IQR:44.2-81.1).

3.2 Clinicopathologic features and outcomes

For the primary surgery , the median age of the patients at the time of diagnosis was 56.2 years (range:35.1-75.3). As the tumor location, the small intestine presented the most common primary site④n=28,71.8%⑤followed by the stomach④n=4,10.3%⑤and colorectum (n=4,10.3%). The median tumor size was 9.18 cm (range:3.2-17.9). Mitotic rate of 12 patients with primary tumor was less than 5/50, those of 5 patients more than 25/50.The median RDT was 41.3 months (IQR:33.5-55.7). 37 patients underwent radical surgery, and the other 2 patients only underwent R1 surgery.

After the primary surgery, all patients had recurrence, metastasis or disease progression in 41.3 months (range :12.3-117.5)⑥On account of recurrent site, peritoneum was involved in 28 cases (17 patients with local recurrence, the others with new lesions)⑦liver in 9 cases (4 patients only with liver metastasis, the other 5 patients with two or more sites), rare sites including lymph nodes⑧1 patient⑧, cutaneous of chest wall and inguinal(1 patient). All the cases received surgery within 6-12 months⑧34 patients accepted the radical surgery, 2 patients' pathology revealed the margins was positive, the other 3 patients only received palliative resection(Table 1).

In our study, 23 cases died from the tumor and 6 cases lived with neoplasm until the last follow-up(2020-12-31)⑨excepted for 2 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). On survival analysis, liver metastasis and without IM treatment predicted poor prognosis (Figure1-B,C). On univariate analysis, recurrent site and IM treatment were associated with overall survival. Taking the two into multivariate analysis, they were proved to be independent factors associated with prognosis.(Table 2)

4. Whole-genome Analysis

4.1 *KIT* mutations

Of the 8 clinical cases, 5 harbored mutations in *KIT*, which containing two with both primary and recurrent tumor mutations, the other three only had primary tumor mutation; 1 harbored mutations in *PDGFRA* among the RMT, the two remaining patients were wild type. We discovered that the *KIT* mutations between

primary tumor and recurrent tumor not only had correlation that all had same mutations in exon 11 clustered in the proximal part between codons 568 and 576 and consisted of small in-frame deletions and point mutations; but also had difference that as Table 3 shows. In the remaining three patients, two detected mutations in exon 11 and one harboured in exon 17, these mutations were only detected in PT. In the mutated exons of the *KIT* gene, the types mainly included point mutations, base deletions and amino acid substitution.

4.2 CNV variations

The 8 patients were grouped according to IM treatment after primary surgery (Table 4).

All samples were obtained deletions in chromosome 1p, 14, 15; while deletions in the chromosome 13q were frequent especially in metastatic and/or recurrent tumors (Fig. 2A,B). In PT samples (Fig. 2A), the deletions in chromosome 10 and 22 were detected in 7 cases, the left one was in No drug group. While in RMT samples (Fig. 2B), the amplification in chromosome 5, especially in 5p, was generally detected (6/8) and the two in No drug group were also not detected variation mentioned above.

We could not obtain any deletion in chromosome 10 of correspondent cases in RMT compared with PT. However, new CNVs were detected in chromosome 22 in 2 cases, and the deletions of chromosome 22 attained in PT did not exist in RMT upon the irregular group. Only in the liver metastasis group, we acquired deletions in chromosome 18 both in PT and RMT, and its feature was consistent in two tumors. which only occurred in patients receiving IM treatment.

4.3 Somatic SNV results

Firstly, High frequency mutations (top 20) in different code genes were filtrated among all the samples, while one sample was excluded because it did not contain those genes (Fig. 2C1). Surprisingly, the mutations on the *MUC* family were obtained both in each sample of PT and RMT, such as *MUC4*, *MUC6*, *MUC16*, *MUC17* et al, except one sample of RMT. We also found the frameshift mutation on *FRG1* and *ZNF717* had common tendency. The remained mutation tendency between PT and RMT existed correlations and distinctions (Fig. 2C2, C3).

We filtrated out new mutations of the recurrent or metastatic tumors compared with the sequencing data of primary tumors. The statistic results of each pair of tumor tissue occurred repeatedly in 182 genes and the variation types contained Exonic, Downstream, Upstream, UTR, Intergenic, and ncRNA_exonic. Among those genes, we found that 31 genes 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), *TMPRSS13* (Transmembrane Serine Protease 13) and so on. And many genes including *AJAP1*, *C2CD4D*, *EMX2*, *FBNP4*, *GGT1*, *GIGYF1*, *HDGFRP2*, *KCNN3*, *KRT13*, *LILRB1*, *LRRK1*, *MUC19*, *PHF24*, *RAC1*, *RAD54L2*, *RBPJ*, *SETD1B*, *SMG7*, *SRRT*, *VGLL3*, *WDR8*, *ZNF358* did involve in multiple bases deletion (Fig. 3A, B, C, D).

Two recurrent tumors of the 8 patients were detected mutation in *AJAP1* resulting in the loss of two amino acids of its encoding product. In two samples of RMT (25%), we observed the mutation in *RPL19* encoding an essential structural constituent of ribosome. Additionally, mutations of coding region of the LOC101927550 (ncRNA) were harbored in three cases. which influences cell proliferation and growth velocity by affecting the synthesis of the protein.

4.4 Mutations among clinical group

We detected mutations occurred on UTR region of the *MTMR11* and *GOLGA6L4* only in regular treatment group (Fig. 3A1, B1). Interestingly, we acquired frameshift mutations in the *EMX2*, *C2CD4D* gene besides above-mentioned in liver metastasis group with regular treatment (Fig. 3A2, B2). Mutations in *FAM101A* (*RFLNA*) were only found in the PT of the group. In regular group, we detected the mutations in *MTMR11*, *GOLGA6*. And in No Drug group (Figure 3-A3, B3), we detected mutations in the coding region of *PTPRG* gene. Besides, mutations occurred on UTR region of *STK35*/*FANCD2* gene were also harbored. Only in irregular treatment group (Fig. 3A4, B4), frameshift mutations were obtained repeatedly in *SMG7*, *RAD54L2*, *RBPJ* genes.

4.5 Tumor heterogeneity and evolution

4.5.1 Somatic SNV

To obtain the characteristics of tumor in point mutation level, we analyzed Somatic SNV variation in multiple angles, including mutation spectrum and mutation signature. Point mutation contains 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 the type of GIST, the mutation of T>G/A>C accounted for the high proportion of the 6 types, and the proportion of each mutation was significantly different in three spatial locations, while its tendency and feature in primary and recurrent tumor was similar.

To infer the mutation pattern of recurrent and/or metastasis tumor, bases at upper and downstream positions of 1bp of point mutation were taken into consideration and we classified it into 96 varieties according to Mutational Signatures (Figure 4-B)., Mutational characteristics were obtained through those varieties. As it (Figure 4-C) shows, the difference of pattern between the primary and recurrent tumor was mainly reflected on 8 signatures (Signature1, Signature2, Signature3, Signature5, Signature11, Signature12, Signature20, Signature30).

4.5.2 Somatic CNV

The loss of copy number at Chr1, Chr2, Chr13, Chr15 was detected both primary and recurrent tumors. Besides, we also obtained increase at Chr5 and decrease at Chr11, Chr14 in RMT, while decrease was detected in Chr4, Chr10, Chr12, Chr22, ChrX only in PT. As it (Fig. 4D) shows, the CNVs were not only existed different expects, but also had correlations at different points.

4.5.3 The subclones, evolution and heterogeneity of tumor cells

Compared with primary tumor, the number of subclone and the cluster of low prevalence were decreased significantly in recurrent tumor. But tumor in different sites in the same sample, the number and the cluster were almost conformable both in two tumors (Fig. 4E-1). Take the all samples of PT and RMT into subclone analysis (Figure4-E3), 3 subclones cluster were divided. The prevalence of three clusters in recurrent tumor was quietly higher than it in primary tumor, while the difference in the same tumor was not significant both in the two tumors respectively (Figure4-E2). To know the evolution of different tumor, we attained that the evolutionary relationship in PT was $P3 > P2 > P1$ and in RMT was $R3 > R2 > R1$ in Clonal Phylogeny (Fig. 4F1). Compared with PT, the samples of RMT were in the end of evolutionary tree (Fig. 4F2). And the correlation between P2 and P1 was low in PT, while it existed close correlation between R2 and R1 in RMT. The relatedness was quietly low among P3, R3 and other correspondent samples.

There existed the same and different mutation sites in different locations of the same tumor through comparing the functional mutations of each sample, and it was confirmed that the same results were acquired both PT and RMT (Fig. 4G). It was attained that the mutation frequency of some genes was presented to be significantly different between the each sample of the two tumors, especially in *MUC19*, *KIT*, *PABPC3*, *ZNF208*, *TOP3A*, *RGS19*. Those genes were taken into functional enrichment, and we found that the genes involving in biology process and its biological functions were closely connected with tumor cell growth regulation extensively, such as influencing cell differentiation, regulating the expression of nuclear genes, and regulating translation level.

5. Discussion

Prior to the era of imatinib, there was no effective treatment for recurrent or metastatic GIST and surgery was often attempted in the absence of any alternative or as an emergency for bleeding or gastrointestinal perforation or obstruction. It was previously reported that the rate of complete gross resection was low and the median survival was only 15-19 months^{21,22}. And many research^{23,24} revealed that the prognosis of patients diagnosed with advanced or metastatic GIST was significantly raised 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 after the second surgery for recurrent t and/or metastatic tumors. But the survival outcome in patients who took IM regularly after resection for primary tumor was significantly better than those who did not take IM or take it irregularly ($p=0.043$), and was also better than previous research in terms of OS. The importance of IM and radical surgery resection should be highlighted at the maximal clinical response of GIST that may be associated with survival benefits^{16,26,27}. Surgical resection, however, improved the prognosis of the patients who were resistant or unresponsive to IM that may contributed to secondary gene mutation to it²⁸.

We presented the result of the surgery resection for liver metastasis of GISTs that the OS was obviously lower than those with other sites recurrence or metastasis ($p=0.021$). As liver was reported to be the most common site of the GIST metastasis, 50-60 % of patients were found to have a liver involvement during the disease process^{25,29,30}. Hou et al.³¹ revealed that there was no statistical difference in the survival of patients who underwent the radical surgery with different metastasis or recurrence only through IM therapy. A multicenter prospective study³² got a rough view that liver metastasis of gastrointestinal stromal tumor may not be controllable by surgery alone and require concomitant imatinib therapy. Thus, the therapeutic model and surgical opportunity of those patients should be seriously considered.

Based on the clinical results, we used deep Whole-genome sequence (WGS) to profile genomic variation in both primary tumors and recurrent and/or metastatic tumors. As we all know, the majority of GIST (75-80%) harbor gain of function *KIT* mutation in exons 9,11,13,14,17, and 5-10 of GIST have mutations in platelet-derived growth factor receptor α (*PDGFRA*) gene in exons 12, 14, 18³³. As the amount of our samples are small, we observe an interested result that the *KIT* mutations existed correlations and difference between PT and RMT in Irregular Medication group, and in Regular-medication group, the *KIT* mutations only were found in PT. With the use of IM, the mutations in *KIT* could not been attained in the RMT. While the mutations were actually harbored both PT and RMT in irregular group, but these mutations were not completely equal. Miselli FC³⁴ raised that KIT amplification may be a mechanism of drug resistance in GIST. As to reactivation of KIT signaling by tumor subclones with heterogeneous secondary KIT mutations, it resulted in oncogenically-activated *KIT* to be the key driver of GIST proliferation and survival³⁵⁻³⁷. Serrano et al.³⁸ reported on 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, also showed that rapid alternation of sunitinib and regorafenib is more effective than monotherapy using either drug in vitro. Mutations in *PDGFRA* were only detected in the RMT of the regular group. The difference of *KIT*/*PDGFRA* mutations between PT and RMT was likely to be caused by secondary mutation or tumor evolution.

MUC4 was verified 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, resulting in repression of apoptosis and stimulation of proliferation^{39,40}. The mutations in *ASH1L*, *MUC4* and *KMT2D* seemed to have coordinated variation between PT and RMT. *ASH1L* is a histone methyltransferase of lysine specifically trimethylating 'Lys-36' of histone H3 forming H3K36me3⁴¹; *MUC4* was supposed to affect the intracellular regulation of *ASH1L* and *KMT2D* through intracellular cascade. The decrease of *FRG1* expression may influence tumor progression by regulating cell migration and invasion⁴², and *ZNF717*, as a transcription factor, involves in nucleic acid binding and DNA-binding⁴³. We had reasons to believe that *ZNF717* promoted the progression of tumor through the combination with *FRG1*. The tumor cell growth mainly depended on the *MUC* family in the two tumors (mentioned above), while the effect of *KIT*/*PDGFRA* on RMT decreased significantly. And the mutation of *ASH1L* and *KMT2D* tended to occur on RMT.

In regular group, we detected the mutations in *MTMR11*, *GOLGA6*. *MTMR11* is one of Myotubularin Related Protein, involving in process of glycerophosphatidylinositol dephosphorylation. The high frequency mutations of *GOLGA6L4* and the expression of *MTMR11* were found to be obvious abnormal in many cancers⁴⁴. The mutations in *EMX2*, *FAM101A* were only attained in liver metastasis group with regular treatment. *EMX2* encodes a homeobox-containing transcription factor which could influence the growth and development of cells and animals⁴⁵; and it was proved to interact with *FLNA* to regulate filamin framework around nucleus and the shape of the nucleus. Consequently, we inferred that the mutations had correlation with these patients.

In irregular group, the mutations on *SMG7*, *RAD54L2* and *RBPJ* were detected. We acquired that *SMG7* and *SMG5* were involved in cell nonsense-mediated mRNA decay⁴⁶. *RAD54L2* is one of DNA helicases, and *RBPJ* is a transcriptional activator for the Notch signal pathway⁴⁷. The frameshift mutations of the two genes may result in escaping from the effect of IM on tumors. In No-Drug group, mutations in *PTPRG*, *STK35/FANCD2* were detected. As we know, the encoding conduct of *PTPRG* constitutes Tyrosine protein phosphatase receptor activating a series of signaling cascade to affect protein synthesis, which was confirmed to play a key role in the development and progression of many cancers⁴⁸. Therefore, its mutation could result in the abnormality of the activation activity, thus influencing subsequent signaling cascade. *STK35* is one of serine/threonine protein kinase and *FANCD2*, as one of histones, prevents DNA breakage and loss of Chromatin and participants in the stabilization of chromosomes and DNA damage repair⁴⁹.

As an adhesion protein, *AJAP1* may be translocated to the nucleus, via its interaction with β -catenin complexes, where it can regulate gene transcription, then possibly have a potent impact on cell cycling and apoptosis and also participates in tumor cell adhesion and intercellular metastasis^{50,51}, implying that its mutation may have effect on tumor metastasis or recurrence.

The mutations of coding region of the LOC101927550 existed overlap with *SMURF1* encoding a ubiquitin ligase that is specific for receptor-regulated SMAD proteins in the bone morphogenetic protein (BMP) pathway. So we speculated that LOC101927550 influenced the targeted combination with miRNA of *SMURF1* that regulated the expression of *SMURF1*, and affected the development of tumors⁵².

For the tumor heterogeneity and evolution, we obtained that the mutations and variants of different sites in the same tumor were significantly different. And the differences between PT and RMT were greatly discrepant, mainly reflecting in point mutations and subclones, which were related with the grade malignancy and IM resistance in RMT.

Heidi M⁵³ demonstrated that mutational analysis by use of liquid biopsies can capture the molecular heterogeneity of the whole tumour, and also revealed that multiple resistance mutations were synchronously present. It has been shown that imatinib-resistant disease frequently harbours up to two resistance mutations within a single tumour or metastasis, or up to five mutations in separate metastases from one patient⁵⁴⁻⁵⁶.

As B Liegl et al⁵⁵ also showed that extensive intra- and inter-lesional heterogeneity of resistance mutations and gene amplification in patients with clinically progressing GIST and also underscore the heterogeneity of clinical TKI resistance, and highlight the therapeutic challenges involved in salvaging patients after clinical progression on TKI monotherapies. Thus, we deduce that the spatial and temporal heterogeneity contributes to the occurrence or metastasis of tumor and resistance to TKI.

6. Conclusions

Although our study is limited by small population size and its retrospective 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;

Declarations

Acknowledgements

Not applicable

Authors' contribution

WTL, ZGZ, and LY contributed to conceptualization; CY, MY and CL contributed to formal analysis; ZTN, YM, contributed to investigation; JX contributed to methodology and resources; LQW, WX and WJC contributed to writing—original draft; LQW, WX, XFW and WJC contributed 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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Tables

Table 1. Description of GIST patients who received two surgery for primary and recurrent and/or metastatic tumors

Variable			Variable		
First surgery n=39			Recurrence or metastasis surgery(n=39)		
Age	Median(range)	56.2(12.1)	Interval time(months)	Median(range)	41.3(26.5)
Sex	Male	19	Second age	Median(range)	59.8(11.9)
	Female	20	Recurrence site	Local recurrence	17
Tumor site	Stomach	4		Liver(only)	4
	Small intestine	28		Peritoneum	11
	Colorectum	4		Liver+any organs	5
	Others	3		Others	2
Tumor size(cm)	<5	6	Tumor size(cm)	<5	14
	5-10	13		5-10	17
	>10	20		>10	8
Mitotic rate	<5/50	12	Mitotic rate	<5/50	5
	5-10/50	9		5-10/50	8
	10-25/50	9		10-25/50	19
	>25/50	5		>25/50	3
Risk(NIH criterion)	High	29	Risk of recurrence	High	29
	Moderate	3		Moderate	4
	Low	2		Low	1
	Unclassifiable	1		Unclassifiable	5
Histologic type	Spindle	25	Histologic type	Spindle	21
	Epithelioid	11		Epithelioid	13
	Mixed	3		Mixed	5
Curative resection	R0	37	Curative resection	R0	34
	R1	2		R1	2
	R2	0		R2	3
TKI treatment	Regular	20	Combined surgery	No	36
	Irregular	14		Yes	3
	Non drug	5			

Table 2. Univariate and multivariate analysis of prognostic factors for overall survival(OS) after the second surgery for RMT.

Variable	Univariate Analysis					Multivariate Analysis	
	Class value	Reference	HR	95% CI	P-Value	HR(95% CI)	P-Value
Gender	Male	Female	0.51	0.35-1.14	0.181	-	-
Age(years)	<60	≥60	0.82	0.98-2.01	0.089	-	-
Tumor location	Small intestine	others	0.71	0.20-2.57	0.192	-	-
Recurrence site	Liver	Others	1.64	1.28-2.27	0.011	1.45(1.13-2.02)	0.032
Interval time(Month)	<41.3	≥41.3	1.53	0.75-3.96	0.342	-	-
Histologic type	Epithelioid	Spindle(PT)	0.35	0.09-1.36	0.126	-	-
	Mixed		1.29	0.63-2.65	0.492	-	-
Histologic type	Epithelioid	Spindle(RMT)	1.48	0.22-2.38	0.602	-	-
	Mixed		1.10	0.48-2.02	0.649	-	-
Tumor size(cm)	5.1 to 10	<5(PT)	1.30	0.56-2.67	0.887	-	-
	>10.1		1.25	0.62-2.33	0.527	-	-
	5.1 to 10	<5(RMT)	1.52	0.73-2.59	0.184	-	-
	>10.1		1.29	0.85-1.97	0.198	-	-
Mitotic rate(5/50)	≥5/50	<5 /50(PT)	0.91	0.86-2.04	0.107	-	-
	≥5/50	<5 /50(RMT)	0.74	0.25-2.28	0.387	-	-
TKI treatment	Irregular	Regular	1.28	1.05-1.91	0.039	1.09(1.02-1.37)	0.045
	No drug		1.44	1.25-2.59	0.023	1.16(1.08-1.79)	0.030

Abbreviations: CI, confidence interval; GIST, gastrointestinal stromal tumor; PT, primary tumor; RMT, recurrent and/or metastatic tumor; TKI, tyrosine kinase inhibitors.

Table 3. The mutational profile contained *KIT*/*PDGFRA* gene of 6 GIST cases

Case	Gene	Exon	Nucleotide	Codon	Case	Exon	Nucleotide	Codon
CJP6-P	<i>KIT</i>	11	c.1700_1721del		CJP6-R	11	c.1700_1721del	
		11	c.1723C>G	p.Q575E		11	c.1723C>G	p.Q575E
		11	c.1726C>T	p.L576V		11	c.1726C>T	p.L576V
		18	c.2540 C>T					
WZH5-P		11	c.1696_1704 AACAAATTAT>A		WZH5-R	11	c.1696_1704 AACAAATTAT>A	
		11	c.1707delinsT>TG			11	c.1707delinsT>TG	
		11	c.1708_1725TACATAGACCCAACACAA>T			11	c.1708_1725TACATAGACCCAACACAA>T	
						17	c.2466T>A	p.N822S
SGQ8-P		17	c.2459 A>G	p.D820G	SSL7-P	11	c.1665_1671CAGTGGA>G	
YXH2-P		11	c.1673_1676AGGT>A			11	c.1672A>G	p.K558E
QDH1-R	<i>PDGFRA</i>	11	c.1630 G>A	p.V544I				
QDH1-R		20	c.1630 G>A	p.G898S				

Table 4. Clinical presentation, sequencing statistics and genomic variation of 8 cases

	QDH-1	YXH-2	WQW-3	ZXZ-4	WZH-5	CJP-6	SSL-7	SGQ-8
Group(IM)	Regular(Liver)		Regular		Irregular		No drug	
Clinical presentation at surgery								
Age (years)	44	56	58	61	52	53	55	49
Interval time(months)	31.2	48.3	21.2	33.9	28.9	49.4	29.5	35.6
Risk(PT)	High	High	High	High	Middle	High	Middle	Low
PT site	Jejunum	Ileum	Stomach	Jejunum	Jejunum	Duodenum	Rectum	Stomach
OSMonths	29.8	21.3	116.9	148.6	89.4	31.7	26.8	22.4
Outcome	Death	Death	Lived	Lived	Lived	Death	Lived	Death
WGS statistics								
Normal coverage	97X	112X	88X	260X	95X	185X	125X	340X
Tumor coverage(PT)	80X	67X	71X	91X	77X	76X	85X	86X
Tumor coverage(RMT)	110X	135X	210X	76X	182X	97X	58X	65X
Single Nucleotide variants (total number) ²								
RT/RMT	1478	456	559	421	559	1764	1069	934
SNVTotal	159	252	326	407	439	535	75	271
Downstream	2	6	9	12	16	8	13	9
	6	1	12	1	21	6	1	7
Exonic	1146	315	251	203	16	1367	663	689
	90	117	49	312	19	376	43	48
Intergenic	142	84	174	91	255	185	136	94
	37	92	188	24	241	76	15	88
ncRNA_exonic	56	4	10	12	24	25	17	17
	2	1	3	13	13	1	1	15
Upstream	21	9	21	18	29	23	35	19
	3	6	19	8	27	10	4	26
UTR3	58	21	51	61	102	121	132	70
	11	19	40	25	98	44	7	60
UTR5	51	15	28	21	21	28	69	34
	3	13	14	22	19	20	3	25
Other	2	2	1	3	1	7	4	2
	7	3	1	2	1	2	1	2

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 occurred on PT. C-3, The top 10 mutational genes cover 7 patients.

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 lithotripsis (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)