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

Lingquan Wang  
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Wei Xu  
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Wenjing Zhang  
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Zhentian Ni  
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Xufeng Wang  
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Yu Mei  
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Chao Yan  
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Chen Li  
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Jing Xie  
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Min Yan  
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Liang Yang  
Tongji University Shanghai First Maternal and Infant Hospital

Zhenggang Zhu  
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Wentao Liu (<wenliu@sjtu.edu.cn>)  
Shanghai Institute of Digestive Surgery, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine  https://orcid.org/0000-0001-6370-4572

Research

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

Posted Date: October 20th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-965024/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
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: 9.6-190.3 months). Regular TKI (Imatinib) after primary tumor resection (HR: 0.568; 95% confidence interval (CI): 0.211-0.874; P = 0.032) 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 cases. At present, the mainstay of treatment is surgery for patients with clearly resectable primary GIST. 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 chemoradiotherapy. And current recommended treatment for GIST patients with a high risk for recurrence is adjuvant imatinib for 3 years at least. Patients with KIT exon 11 deletion mutations were verified to benefit most from imatinib. But in confirmed cases of KIT exon 9 mutation, raising the dose of imatinib could significantly improve the PFS.

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. 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 IM. Some studies revealed that the larger tumor volumes were correlated with adverse outcomes, the chance of molecular evolution and secondary clonal resistance. 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 1A. 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 (RDT) 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 >100×.

Take the Raw data into bioinformatics analysis process: 1) Sequencing 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)||CNV(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 signatures18 was 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 in 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 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). 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). On account of recurrent site, peritoneum was involved in 28 cases (17 patients with local recurrence, the others with new lesions );1 liver in 9 cases (4 patients only with liver metastasis, the other 5 patients with two or more sites), rare sites including lymph nodes(1 patient), cutaneous of chest wall and inguinal(1 patient). All the cases received surgery within 6-12 months;34 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); excepted 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 exon11 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 chromosome1p, 14, 15; while deletions in the chromosome13q were frequent especially in metastatic and/or recurrent tumors!Fig. 2A,B!. In PT samples!Fig. 2A!, the deletions in chromosome10 and 22 were detected in 7 cases, the left one was in No drug group. While in RMT samples (Fig. 2B), the amplification in chromosome5, 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 chromosome10 of correspondent cases in RMT compared with PT. However, new CNVs were detected in chromosome 22 in 2 cases, and the deletions of chromosome22 attained in PT did not exist in RMT upon the irregular group. Only in the liver metastasis group, we acquired deletions in chromosome18 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 filtered among all the samples, while one sample was excluded because it did not contain those genes!Fig. 2C!. Suprisingly, 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), TPMRSS13(Transmembrane Serine Protease 13) and so on. And many genes including AJAP1, C2CD4D, EMX2, FBNP4, GGT1, GIGYF1, HDGFPRP2, KCNN3, KRT13, LRK1, MUC19, PHF24, RAC1, RAD54L2, RBPU, 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 PRL19 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 group. In regular group, we detected the mutations in MTMR11, GOLGA6. And in No Drug group!Figure3-A3,B3!, we detected mutations in the coding region of PTPRG gene. Besides, mutations occurred on UTR region of STK35/FANC2D 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 (Figure4-B), Mutational characteristics were obtained through those varieties. As it(Figure4-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 conformance both in two tumors (Fig. 4E-1). Take the all samples of PT and RMT into subclone analysis (Figure 4E-3), 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 (Figure 4E-2). 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 sites 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. And many research revealed that the prognosis of patients diagnosed with advanced or metastatic GIST was significantly reduced by the limited use of imatinib. Kang retrospectively analyzed the correlation between IM and surgery in patients with metastatic and 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 tumor or metastasis. 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. Surgical resection, however, improved the prognosis of the patients who were resistant or unresponsive to IM that may contributed to secondary gene mutation to IM. 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. Hou et al. 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 study 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 a (PDGFRa) gene in exons 12, 14, 18. As the amount of our samples are small, we observe an interesting 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 be 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 FC revealed that KIT amplification was 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 survival. Serrano et al. reported 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. 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 H3K36me3; 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 invasion, and ZNF717, as a transcription factor, involves in nucleic acid binding and DNA-binding. 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 glycosphingolipidinositol dephosphorylation. The high frequency mutations of GOLGA6L4 and the expression of MTMR11 were found to be obvious abnormal in many cancers. 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 animals, 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 decay. RAD54L2 is one of DNA helicases, and RBPJ is a transcriptional activator for the Notch signal pathway. 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 cancers. 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 repair.

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, 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 tumors.

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 M 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 patient.

As B Liegl et al 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.

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; LQ, WX and WJC contributed to writing—original draft; LQ, WX, XF 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.

Fundings

This work was supported by the Clinical Research Project of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Project Number:2018CR003. This work is also supported by National Natural Science Foundation of China, Project Number: 81972201.

Conflict of Interest

All authors declare no competing interests.

References

1. Min KW, Leabu M. Interstitial cells of Cajal (ICC) and gastrointestinal stromal tumor (GIST): facts, speculations, and myths. J Cell Mol Med 2006;10(4):995-1013.
2. Joensuu H, DeMatteo RP. The management of gastrointestinal stromal tumors: a model for targeted and multidisciplinary therapy of malignancy. Annu Rev Med 2012;63:247-58.
3. Walker SR. Gastrointestinal Stromal Tumour. Eur J Vasc Endovasc Surg 2021;61(5):836.
4. Roberts PJ, Eisenberg B. Clinical presentation of gastrointestinal stromal tumors and treatment of operable disease. Eur J Cancer 2002;38 Suppl 5 S37-8.
5. Croom KF, Perry CM. Imatinib mesylate: in the treatment of gastrointestinal stromal tumours. Drugs 2003;63(5):513-22; discussion 23-4.
6. Joensuu H, Eriksson M, Sundby Hall K, Hartmann JT, Pink D, Schutte J, et al. One vs three years of adjuvant imatinib for operable gastrointestinal stromal tumor: a randomized trial. JAMA 2012;307(12):1265-72.
7. Raut CP, Espat NJ, Maki RG, Araujo DM, Trent J, Williams TF, et al. Efficacy and Tolerability of 5-Year Adjuvant Imatinib Treatment for Patients With Resected Intermediate- or High-Risk Primary Gastrointestinal Stromal Tumor: The PERSIST-5 Clinical Trial. JAMA Oncol 2018;4(12):e184060.
8. Joensuu H, Eriksson M, Sundby Hall K, Reichardt A, Hermes B, Schutte J, et al. Survival Outcomes Associated With 3 Years vs 1 Year of Adjuvant Imatinib for Patients With High-Risk Gastrointestinal Stromal Tumors: An Analysis of a Randomized Clinical Trial After 10-Year Follow-up. JAMA Oncol 2020;6(8):1241-6.
9. Corless CL, Ballman KV, Antonescu CR, Kolesnikova V, Maki RG, Pisters PW, et al. Pathologic and molecular features correlate with long-term outcome after adjuvant therapy of resected primary GI stromal tumor: the ACOSOG Z9001 trial. J Clin Oncol 2014;32(15):1563-70.
10. Debie-Rychter M, Sciot R, Le Cesne A, Schlemmer M, Hohenberger P, van Oosterom AT, et al. KIT mutations and dose selection for imatinib in patients with advanced gastrointestinal stromal tumours. Eur J Cancer 2006;42(8):1093-103.
11. Bauer S, Rutkowski P, Hohenberger P, Miceli R, Fumagalli E, Siedlecki JA, et al. Long-term follow-up of patients with GIST undergoing metastasectomy in the era of imatinib – analysis of prognostic factors (EORTC-STBSG collaborative study). Eur J Surg Oncol 2014;40(4):412-9.
12. Park SJ, Ryu MH, Ryoo BY, Park YS, Sohn BS, Kim HJ, et al. The role of surgical resection following imatinib treatment in patients with recurrent or metastatic gastrointestinal stromal tumors: results of propensity score analyses. Ann Surg Oncol 2014;21(13):4211-7.
13. Heinrich MC, Ozwar K, Corless CL, Hollis D, Borden EC, Fletcher CD, et al. Correlation of kinase genotype and clinical outcome in the North American Intergroup Phase III Trial of imatinib mesylate for treatment of advanced gastrointestinal stromal tumor: CALGB 150105 Study by Cancer and Leukemia Group B and Southwest Oncology Group. J Clin Oncol 2008;26(33):5360-7.
14. Blanké CD, Demetri GD, von Mehren M, Heinrich MC, Eisenberg B, Fletcher JA, et al. Long-term results from a randomized phase II trial of standard- versus higher-dose imatinib mesylate for patients with unresectable or metastatic gastrointestinal stromal tumours expressing KIT. J Clin Oncol 2008;26(4):620-5.
15. Wang D, Zhang Q, Blanké CD, Demetri GD, Heinrich MC, Watson JC, et al. Phase II trial of neoadjuvant/adjuvant imatinib mesylate for advanced primary and metastatic/recurrent operable gastrointestinal stromal tumors: long-term follow-up results of Radiation Therapy Oncology Group 0132. Ann Surg Oncol 2012;19(4):1074-80.
16. Kanda T, Ishikawa T, Kosugi SI, Ueki K, Naito T, Wakai T, et al. Prognostic factors after imatinib secondary resistance: survival analysis in patients with unresectable and metastatic gastrointestinal stromal tumors. Int J Clin Oncol 2016;21(2):295-301.
17. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 2010;38(16):e164.
18. Forbes SA, Beare D, Boutselakis H, Bamford S, Bindal N, Tate J, et al. COSMIC: somatic cancer genetics at high-resolution. Nucleic Acids Res 2017;45(1):D777-D83.
19. Gehring JS, Fischer B, Lawrence M, Huber W. SomaticSignature: inferring mutational signatures from single-nucleotide variants. Bioinformatics 2015;31(22):3673-5.
20. Koo DH, Ryu MH, Kim KM, Yang HK, Sawaki A, Hirota S, et al. Asian Consensus Guidelines for the Diagnosis and Management of Gastrointestinal Stromal Tumor. Cancer Res Treat 2016;48(4):1155-66.
21. Gold JS, van der Zwan SM, Gonen M, Maki RG, Singer S, Brennan MF, et al. Outcome of metastatic GIST in the era before tyrosine kinase inhibitors. Ann Surg Oncol 2007;14(1):134-42.
22. Mudan SS, Conlon KC, Woodruff JM, Lewis JJ, Brennan MF. Salvage surgery for patients with recurrent gastrointestinal sarcoma: prognostic factors to guide patient selection. *Cancer* 2000;88(1):66-74.

23. Ryu MH, Kang WK, Bang YJ, Lee KH, Shin DB, Ryoo BY, et al. A prospective, multicenter, phase 2 study of imatinib mesylate in korean patients with metastatic or unresectable gastrointestinal stromal tumor. *Oncology* 2009;76(5):326-32.

24. Katz D, Segal A, Alberton Y, Jurim O, Weissman P, Catane R, et al. Neoadjuvant imatinib for unresectable gastrointestinal stromal tumor. *Anticancer Drugs* 2004;15(6):599-602.

25. Kim JH, Ryu MH, Yoo C, Chae H, Na H, Beck M, et al. Long-term survival outcome with tyrosine kinase inhibitors and surgical intervention in patients with metastatic or recurrent gastrointestinal stromal tumors: A 14-year, single-center experience. *Cancer Med* 2019;8(3):1034-43.

26. Mussi C, Ronellenfitsch U, Jakob J, Tamborini E, Reichardt P, Casali PG, et al. Post-imatinib surgery in advanced/metastatic GIST: is it worthwhile in all patients? *Ann Oncol* 2010;21(2):403-8.

27. Sym SJ, Ryu MH, Lee JL, Chang HM, Kim TW, Kim HC, et al. Surgical intervention following imatinib treatment in patients with advanced gastrointestinal stromal tumors (GISTs). *J Surg Oncol* 2008;98(1):27-33.

28. Antonescu CR, Besmer P, Guo T, Arkun K, Hom G, Korytowski B, et al. Acquired resistance to imatinib in patients with metastatic gastrointestinal stromal tumor occurs through secondary gene mutation. *Clin Cancer Res* 2005;11(11):4182-90.

29. Nunobe S, Sano T, Shimada K, Sakamoto Y, Kosuge T. Surgery including liver resection for metastatic gastrointestinal stromal tumors or gastrointestinal leiomyosarcomas. *Jpn J Clin Oncol* 2005;35(6):338-41.

30. Roland CL, Bednarski BK, Watson K, Torres KE, Cormier JN, Wang WL, et al. Identification of preoperative factors associated with outcomes following surgical management of intra-abdominal recurrent or metastatic GIST following neoadjuvant tyrosine kinase inhibitor therapy. *J Surg Oncol* 2018;117(5):879-95.

31. Zhu J, Yang Y, Zhou L, Jiang M, Hou M. A long-term follow-up of the imatinib mesylate treatment for the patients with recurrent gastrointestinal stromal tumor (GIST): the liver metastasis and the outcome. *BMC Cancer* 2010;10:199.

32. Kanda T, Masuzawa T, Hirai T, Ikawa O, Takagane A, Hata Y, et al. Surgery and imatinib therapy for liver oligometastasis of GIST: a study of Japanese Study Group on GIST. *Jpn J Clin Oncol* 2017;47(4):369-72.

33. Corless CL, Barretto CM, Heinrich MC. Gastrointestinal stromal tumours: origin and molecular oncology. *Nat Rev Cancer* 2011;11(12):865-78.

34. Miselli FC, Casieri P, Negri T, Orsenigo M, Lagonigro MS, Gronchi A, et al. c-Kit/PDGFRA gene status alterations possibly related to primary imatinib resistance in gastrointestinal stromal tumors. *Clin Cancer Res* 2007;13(8):2369-77.

35. Desai J, Shankar S, Heinrich MC, Fletcher JA, Fletcher CD, Manola J, et al. Clonal evolution of resistance to imatinib in patients with metastatic gastrointestinal stromal tumors. *Clin Cancer Res* 2007;13(18 Pt 1):5398-405.

36. Wardelmann E, Merkelbach-Bruse S, Pauls K, Thomas N, Schildhaus HU, Heinicke T, et al. Polyclonal evolution of multiple secondary KIT mutations in gastrointestinal stromal tumors under treatment with imatinib mesylate. *Clin Cancer Res* 2006;12(6):1743-9.

37. Demetri GD, Heinrich MC, Fletcher JA, Fletcher CD, Van den Abbeele AD, Corless CL, et al. Molecular target modulation, imaging, and clinical evaluation of gastrointestinal stromal tumors patients treated with sunitinib malate after imatinib failure. *Clin Cancer Res* 2009;15(18):5902-9.

38. Serrano C, Marino-Enriquez A, Tao DL, Ketzer J, Eilers G, Zhu M, et al. Complementary activity of tyrosine kinase inhibitors against secondary kit mutations in imatinib-resistant gastrointestinal stromal tumours. *Br J Cancer* 2019;120(6):612-20.

39. Yang Y, Zhang J, Chen Y, Xu R, Zhao Q, Guo W, MUC4, MUC16, and TTN genes mutation correlated with prognosis, and predicted tumor mutation burden and immunotheraphy efficacy in gastric cancer and pan-cancer. *Clin Transl Med* 2020;10(4):e155.

40. Singh AP, Chaturvedi P, Bhat SK. Emerging roles of MUC4 in cancer: a novel target for diagnosis and therapy. *Cancer Res* 2007;67(2):433-6.

41. An S, Yeo KJ, Jeon YH, Song JJ. Crystal structure of the human histone methyltransferase ASH1L catalytic domain and its implications for the regulatory mechanism. *J Biol Chem* 2011;286(10):8369-74.

42. Tiwari A, Mukherjee B, Hassan MK, Pattanaik N, Jaiswal AM, Dixit M. Reduced FRG1 expression promotes prostate cancer progression and affects prostate cancer cell migration and invasion. *BMC Cancer* 2019;19(1):346.

43. Mehrad M, LaFramboise WA, Lyons MA, Trejo Bittar HE, Yousem SA. Whole-exome sequencing identifies unique mutations and copy number losses in gastrointestinal stromal tumors. *Cancer Gene Ther* 2014;21(1):346-58.

44. Gurnani A, Danguch P, Liu B, Dang J, Wu H, et al. Association of common variants in TCF4 and PTPRG with Fuchs' corneal dystrophy: a systematic review and meta-analysis. *PLoS One* 2014;9(10):e109124.

45. Nepal M, Che R, Ma C, Zhang J, Fei P. FANCD2 and DNA Damage. *Int J Mol Sci* 2017;18(8).
50. Zeng L, Fee BE, Rivas MV, Lin J, Adamson DC. Adherens junctional associated protein-1: a novel 1p36 tumor suppressor candidate in gliomas (Review). *Int J Oncol* 2014;45(1):13-7.

51. Han J, Xie C, Pei T, Wang J, Lan Y, Huang K, *et al.* Deregulated AJAP1/beta-catenin/ZEB1 signaling promotes hepatocellular carcinoma carcinogenesis and metastasis. *Cell Death Dis* 2017;8(4):e2736.

52. Xia Q, Li Y, Han D, Dong L. SMURF1, a promoter of tumor cell progression? *Cancer Gene Ther* 2021;28(6):551-65.

53. Namlos HM, Boye K, Mishkin SJ, Baroy T, Lorenz S, Bjerkehagen B, *et al.* Noninvasive Detection of ctDNA Reveals Intratumor Heterogeneity and Is Associated with Tumor Burden in Gastrointestinal Stromal Tumor. *Mol Cancer Ther* 2018;17(11):2473-80.

54. Heinrich MC, Corless CL, Blanke CD, Demetri GD, Joensuu H, Roberts PJ, *et al.* Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. *J Clin Oncol* 2006;24(29):4764-74.

55. Liegl B, Kepten I, Le C, Zhu M, Demetri GD, Heinrich MC, *et al.* Heterogeneity of kinase inhibitor resistance mechanisms in GIST. *J Pathol* 2008;216(1):64-74.

56. Wardelmann E, Thomas N, Merkelbach-Bruse S, Pauls K, Speidel N, Buttner R, *et al.* Acquired resistance to imatinib in gastrointestinal stromal tumours caused by multiple KIT mutations. *Lancet Oncol* 2005;6(4):249-51.

57. Mei L, Smith SC, Faber AC, Trent J, Grossman SR, Stratakis CA, *et al.* Gastrointestinal Stromal Tumors: The GIST of Precision Medicine. *Trends Cancer* 2018;4(1):74-91.

58. Wu CE, Tzen CY, Wang SY, Yeh CN. Clinical Diagnosis of Gastrointestinal Stromal Tumor (GIST): From the Molecular Genetic Point of View. *Cancers (Basel)* 2019;11(5).

Tables

**Table 1.** Description of GIST patients who received two surgery for primary and recurrent and/or metastatic tumors
| Variable | n(%) | Variable                          | n(%) |
|----------|------|-----------------------------------|------|
| First surgery\(n=39\) | | Recurrence or metastasis surgery\(n=39\) | |
| Age      | Median(range) | 56.2(12.1) | 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 | - | - |
| 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 | - | - |
| 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 | KIT  | 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 | KIT  | 11   | c.1696_1704 AACAATTAT>A |           | WZH5-R | 11   | c.1696_1704 AACAATTAT>A |           |
|        |      | 11   | c.1707delinsT>TG  |           | 11     | c.1708_1725TACATAGACCCAACACAA>T |           |
|        |      | 17   | c.2459 A>G        | p.D820G   | SSL7-P | 11   | c.1665_1671CAGTGGG>A |           |
| YXH2-P | KIT  | 11   | c.1673_1676AGGT>A |           |        |                   |           |
|        |      |      |                   |           |        |                   |           |
| QDH1-R | PDGFRA| 11  | c.1630 G>A        | p.V544I   |        |                   |           |
| QDH1-R | PDGFRA| 20  | c.1630 G>A        | p.G898S   |        |                   |           |

**Table 4.** Clinical presentation, sequencing statistics and genomic variation of 8 cases
| Group(IM)   | QDH-1 | YXH-2 | WQW-3 | ZXZ-4 | WZH-5 | CJP-6 | SSL-7 | SGQ-8 |
|------------|-------|-------|-------|-------|-------|-------|-------|-------|
| 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 |
| OS Months  | 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   |
| SNV Total  | 159   | 252   | 326   | 407   | 439   | 535   | 75    | 271   |
| Downstream | 2     | 6     | 9     | 12    | 16    | 8     | 13    | 9     |
| Exonic     | 1146  | 315   | 251   | 203   | 16    | 1367  | 663   | 689   |
| Intergenic | 90    | 117   | 49    | 312   | 19    | 376   | 43    | 48    |
| ncRNA_exonic | 142  | 84    | 174   | 91    | 255   | 185   | 136   | 94    |
| Upstream   | 3     | 9     | 21    | 18    | 29    | 23    | 35    | 19    |
| UTR3       | 58    | 21    | 51    | 61    | 102   | 121   | 132   | 70    |
| UTR5       | 51    | 15    | 28    | 21    | 21    | 28    | 69    | 34    |
| Other      | 2     | 2     | 1     | 3     | 1     | 7     | 4     | 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 represent 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)