Genetic Mutation Analysis of Human Gastric Adenocarcinomas Using Ion Torrent Sequencing Platform

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Introduction

Gastric cancer is the second most common cancer worldwide with a frequency that varies greatly across different geographic locations. Its incidence is highest in Japan, Eastern Asia, South America, and Eastern Europe, whereas Canada, Northern Europe, Africa, and United States have the lowest incidences [1]. However, it remains the third most common gastrointestinal malignancy in North America after colorectal and pancreatic cancer and commonly occurs after 40 years of age [2]. The Lauren classification divides gastric cancer into two major histologic types: intestinal or diffuse. Diffuse-type cancers have noncohesive cells diffusely infiltrating the stroma of the stomach and often exhibit deep infiltration of the stomach wall with little or no gland formation. Intestinal-type cancers, on the other hand, show recognizable gland formation similar in microscopic appearance to colonic mucosa [3]. Most gastric cancers are sporadic but 8–10% are genetically inherited [2].

Many commonly activated oncogenes have been shown to harbor mutations in gastric cancer. Single or combinatorial therapeutics targeting genetic mutations is becoming attractive options in the treatment of gastric cancers. For example, trastuzumab was approved in combination with chemotherapy for the treatment of ERBB2-positive gastric cancers [4]. EGFR, another receptor tyrosine kinase is noted for its overexpression in some gastric cancers and trials employing the use of EGFR inhibitors are currently underway [5]. Similarly, gastric cancers are associated with the overexpression or amplification of other...
molecules such as MET, MSTIR, and FGFR2, and multiple trials testing the efficacy of inhibitors against these molecular mutations are also ongoing [6,7].

Despite several improvements made in treating and screening for gastric cancers, the prognosis of patients with gastric adenocarcinoma remains poor [8]. To understand and develop new therapeutics and treat patients with gastric adenocarcinoma more effectively, it is essential to profile the individual cancer genome and dissect the oncogenic mechanisms that regulate the progression of gastric cancer, which may form the foundation for individualized, tailored therapy. Next-generation sequencing technologies have revolutionized cancer genomics research by providing an unbiased and comprehensive method of detecting somatic cancer genome alterations [9]. These technologies have several advantages over Sanger sequencing by capillary electrophoresis such as the ability to sequence gigabases of nucleotides to detect genetic mosaicism in depth [10]. However, routine usage of these technologies leaves us with several limitations such as the cost of entry, long processing time, and sample scalability. Recently, a new Ion Torrent sequencing technology based on semiconductor sequencing [11] has substantially circumvented many of these issues. The Ion Torrent method relies on standard DNA polymerase sequencing with unmodified dNTPs but uses semiconductor-based detection of hydrogen ions released during every cycle of DNA polymerization [11]. Each nucleotide incorporation into the growing complementary DNA strand causes the release of a hydrogen ion that is sensed by a hypersensitive ion sensor [11]. Ion Torrent Personal Genome Machine (PGM) can currently generate 10 Mb pairs (Mbp) of sequence data on the first-generation 314 chip within several hours of machine run time. In this study, we have sequenced 238 clinical gastric adenocarcinoma samples to identify genetic mutations in 737 loci of 45 cancer-related genes.

Materials and Methods

Ethics statement

The study has been approved by the Ethical Committee of the Affiliated Nanjing First Hospital, Nanjing Medical University, China. For formalin fixed and paraffin embedded (FFPE) tumor samples, no informed consent was available, therefore all samples and medical data used in this study have been irreversibly anonymized.

Patient information

Tumor samples used in the study were collected from the Affiliated Nanjing First Hospital, Nanjing Medical University, China. A total of 238 FFPE tumor samples from gastric adenocarcinoma patients were analyzed. The mean age of 238 patients was 60 years (range, 28–81). Out of 238 samples, 135 tumors were diffuse and 103 tumors were intestinal.

DNA preparation

DNA was isolated from FFPE samples after deparaffinization and extraction of 3–5 μm thick paraffin sections in xylene and by using the QiAamp DNA Mini Kit (Qiagen) per the manufacturer’s instructions.

Ion Torrent PGM Library Preparation and Sequencing

An Ion Torrent adapter-ligated library was made following the manufacturer’s Ion AmpliSeq Library Kit 2.0 protocol (Life Technologies, Part #4475345 Rev. A). Briefly, 50 ng pooled amplicons were end-repaired, and Ion Torrent adapters P1 and A were ligated using DNA ligase. Following AMPure bead (Beckman Coulter, Brea, CA, USA) purification, adapter-ligated products were nick-translated and PCR-amplified for a total of 10 cycles. The resulting library was purified using AMPure beads (Beckman Coulter) and the concentration and size of the library determined using the Agilent 2100 Bioanalyzer (Agilent Technologies) and Agilent BioAnalyzer DNA High-Sensitivity LabChip (Agilent Technologies).

Sample emulsion PCR, emulsion breaking, and enrichment were performed using the Ion Xpress Template Kit (Part #4467389 Rev. B), according to the manufacturer’s instructions. Briefly, an input concentration of one DNA template copy/Ion Sphere Particles (ISPs) was added to the emulsion PCR master mix and the emulsion was generated using an IKADT-20 mixer (Life Technologies). Next, ISPs were recovered and template-positive ISPs enriched for using Dynabeads MyOne Streptavidin C1 beads (Life Technologies). ISP enrichment was confirmed using the Qubit 2.0 fluorometer (Life Technologies). Sequencing was undertaken using 316 chips on the Ion Torrent PGM for 65 cycles and barcoding was used for these samples. The Ion Sequencing Kit v2.0 was used for sequencing reactions, following the recommended protocol (Part Number 4469714 Rev. B).

Variant Calling

Data from the PGM runs were processed initially using the Ion Torrent platform-specific pipeline software Torrent Suite to generate sequence reads, trim adapter sequences, filter, and remove poor signal-profile reads. Initial variant calling from the Ion AmpliSeq sequencing data was generated using Torrent Suite Software v3.0 with a plug-in “variant caller v3.0” program. In order to eliminate erroneous base calling, several filtering steps were used to generate final variant calling (Fig. S1). The first filter was set at an average depth of total coverage of >100, an each variant coverage of >20, a variant frequency of each sample >5%, and P-value <0.01. The second filter was employed by visually examining mutations using Integrative Genomics Viewer (IGV) software (http://www.broadinstitute.org/igv/) or Samtools software SAMtools software (http://samtools.sourceforge.net), as well as by filtering out possible strand-specific errors, i.e. a mutation only detected in either “+” or “−” strand, but not in both strands of DNA. The third filtering step was set as variants within 727 hotspots, according to the manufacturer’s instructions. The last filter step was to eliminate variants in amplicon AMPL339432 (PIK3CA, exon13, chr3:178938822-178938906), which is not uniquely matched in the human genome. From our sequencing runs using the Ion Ampliseq Cancer Panel, false deletion data were generated from the JAK2 gene locus and thus the sequencing data from this locus were excluded from further analysis.

Somatic mutations

Detected mutations were compared to variants in the 1000 Genomes Project [12] and 6500 exomes of the National Heart, Lung, and Blood Institute Exome Sequencing Project [13] to distinguish somatic mutations and germline mutations.

Bioinformatical and experimental validation

We used the COSMIC (version 64) [14], MyCancerGenome database (http://www.mycancergenome.org/) and some published literatures to assess reappearing mutations (Table S1). Additionally, some detected missense mutations were confirmed by Sanger’s sequencing. (Table S2).
Statistical analysis

We selected reappearing somatic missense/in-del mutations of gastric adenocarcinoma to perform the statistical analysis.

Sequencing data

The dataset has been deposited to the NIH Sequence Read Archive, and the accession number is SRP040898.

Results

Ion Ampliseq sequencing of human gastric adenocarcinomas

A total of 238 human gastric adenocarcinoma samples (Table 1) was analyzed using Ion Ampliseq Cancer Panel to identify mutation in 737 loci of 45 oncogenes and tumor suppressor genes in human gastric adenocarcinomas (Fig. 1A). Due to possible false base calling generated by the Ion Torrent sequencing technology, several sets of filters were used in order to yield reliable variant calling from the initial sequencing data, as described in the Materials and Methods. The mean read length was 75 bp. The average of sequence per sample was approximate 22 Mb. With normalization to 300,000 reads per specimen, there was an average of 1630 reads per amplicon (range, 21 to 4027) (Fig. 1A), 180/189 (95.2%) amplicons averaged at least 100 reads, and 170/189 (91.9%) amplicons averaged at least 300 reads (Fig. 1B).

In this study, we sequenced 238 human gastric adenocarcinoma samples with an average coverage depth of the targeted loci \( \geq 100 \) (Fig. 1A). Tumors in our sample set was classified into diffuse or intestinal type based on Lauren classification (Table 1). Using a strict standard variant calling, we identified mutations in the following genes (Table 2): APC, BRAF, ERBB2 FBXW7, KIT, PDGFRA, PIK3CA, PTEN, RB1, SMAD4, and a high incidence of mutation along the TP53 gene. Detailed frequencies of missense, point mutations, insertions, and deletions profiled on the 737 loci of 45 tumor suppressor genes and oncogenes of 238 gastric adenocarcinoma samples is provided in the Table S1. Our sample set included tumors scored at different stages of disease (Ia, Ib, II, IIIa, IIIb, IV) based on the AJCC/TNM cancer staging system (Table 3), and were also at different differentiation potentials (Table 4). Detailed sequencing analysis in the exons and functional domains of TP53 is outlined below.

Missense mutation distribution in the exons and functional domains of TP53

Abnormality of the TP53 gene is one of the most common events in gastric cancers and plays an important role in the
Table 1. Mutations (including missense point mutations/deletion/insertion) frequencies in 45 genes (737 loci) in intestinal and diffuse GA samples (based on LAUREN classification).

| Genes  | Number of samples with mutations (Mutation frequency in 238 samples) | Number of 1 samples with mutations (Mutation frequency in 103 intestinal GA samples) | Number of 2 samples with mutations (Mutation frequency in 135 diffuse GA samples) |
|--------|---------------------------------------------------------------|---------------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| ABL1   | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| AKT1   | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| ALK    | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| APC    | 1(0.4%)                                                      | 1(1.0%)                                                                         | 0(0.0%)                                                                       |
| ATM    | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| BRAF   | 2(0.8%)                                                      | 1(1.0%)                                                                         | 1(0.7%)                                                                       |
| CD1    | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| CDKN2A | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| CSF1R  | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| CTNNB1 | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| EGFR   | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| ERBB2  | 1(0.4%)                                                      | 0(0.0%)                                                                         | 1(0.7%)                                                                       |
| ERBB4  | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| FBXW7  | 1(0.4%)                                                      | 1(1.0%)                                                                         | 0(0.0%)                                                                       |
| FGFR1  | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| FGFR2  | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| FGFR3  | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| FLT3   | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| GNAS   | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| HNF1A  | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| HRAS   | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| IDH1   | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| JAK3   | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| KDR    | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| KIT    | 2(0.8%)                                                      | 1(1.0%)                                                                         | 1(0.7%)                                                                       |
| KRAS   | 1(0.4%)                                                      | 0(0.0%)                                                                         | 1(0.7%)                                                                       |
| MET    | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| MLH1   | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| MPL    | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| NOTCH1 | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| NPM1   | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| NRAS   | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| PDGFR1 | 1(0.4%)                                                      | 1(1.0%)                                                                         | 0(0.0%)                                                                       |
| PIK3CA | 2(0.8%)                                                      | 2(1.5%)                                                                         | 1(0.7%)                                                                       |
| PTEN   | 1(0.4%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| PTPN11 | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| RB1    | 1(0.4%)                                                      | 0(0.0%)                                                                         | 1(0.7%)                                                                       |
| RET    | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| SMAD4  | 2(0.8%)                                                      | 1(1.0%)                                                                         | 1(0.7%)                                                                       |
| SMARCB1| 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| SMO    | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| SRC    | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| STK11  | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |
| TP53   | 21(9.7%)                                                     | 13(12.6%)                                                                       | 10(7.4%)                                                                      |
| VHL    | 0(0.0%)                                                      | 0(0.0%)                                                                         | 0(0.0%)                                                                       |

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| Genes | Number of samples with mutations (Mutation frequency in 238 samples) | Number of female samples with mutations (Mutation frequency in 51 female samples) | Number of male samples with mutations (Mutation frequency in 187 male samples) |
|-------|---------------------------------------------------------------|------------------------------------------------------------------|------------------------------------------------------------------|
| ABL1  | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| AKT1  | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| ALK   | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| APC   | 0(0.0%)                                                      | 0(0.0%)                                                          | 10(0.5%)                                                         |
| ATM   | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| BRAF  | 20(8.8%)                                                    | 0(0.0%)                                                          | 2(1.1%)                                                          |
| CDH1  | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| CDKN2A| 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| CSF1R | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| CTNNB1| 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| EGRF  | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| ERBB2 | 10(4.4%)                                                    | 1(2.0%)                                                          | 0(0.0%)                                                          |
| ERBB4 | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| FBXW7 | 10(4.4%)                                                    | 0(0.0%)                                                          | 10(0.5%)                                                         |
| FGFR1 | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| FGFR2 | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| FGFR3 | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| FLT3  | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| GNAT  | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| HNF1A | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| HRAS  | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| IDH1  | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| JAK3  | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| KDR   | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| KIT   | 20(8.8%)                                                    | 0(0.0%)                                                          | 2(1.1%)                                                          |
| KRA5  | 10(4.4%)                                                    | 0(0.0%)                                                          | 10(0.5%)                                                         |
| MET   | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| MLH1  | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| MPL   | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| NOTCH1| 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| NPM1  | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| NRAS  | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| PDGFRA| 10(4.4%)                                                    | 0(0.0%)                                                          | 10(0.5%)                                                         |
| PIK3CA| 20(8.8%)                                                    | 12(2.0%)                                                         | 10(0.5%)                                                         |
| PTEN  | 10(4.4%)                                                    | 1(2.0%)                                                          | 0(0.0%)                                                          |
| PTPN11| 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| RB1   | 10(4.4%)                                                    | 12(2.0%)                                                         | 0(0.0%)                                                          |
| RET   | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| SMAD4 | 20(8.8%)                                                    | 0(0.0%)                                                          | 2(1.1%)                                                          |
| SMARC5B1| 0(0.0%)                                                  | 0(0.0%)                                                          | 0(0.0%)                                                          |
| SMO   | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| SRC   | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| STK11 | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |
| TP53  | 239(7.7%)                                                  | 7(13.7%)                                                         | 16(8.6%)                                                         |
| VHL   | 0(0.0%)                                                      | 0(0.0%)                                                          | 0(0.0%)                                                          |

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| Genes     | Number of samples with mutations (in 238 samples) | Number of stage 1A samples with mutations (in 26 samples) | Number of stage 1B samples with mutations (in 34 samples) | Number of stage 2 samples with mutations (in 49 samples) | Number of stage 3A samples with mutations (in 79 samples) | Number of stage 3B samples with mutations (in 29 samples) | Number of stage 4 samples with mutations (in 21 samples) |
|-----------|-----------------------------------------------|--------------------------------------------------------|--------------------------------------------------------|--------------------------------------------------------|--------------------------------------------------------|--------------------------------------------------------|--------------------------------------------------------|
| ABL1      | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| AKT1      | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| ALK       | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| APC       | 1(0.4%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| ATM       | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| BRAF      | 2(0.8%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 1(2.0%)                                                | 0(0.0%)                                                | 1(3.4%)                                                | 0(0.0%)                                                |
| CDH1      | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| CDKN2A    | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| CDK4      | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| CTNNB1    | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| ERBB2     | 1(0.4%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 1(1.3%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| ERBB4     | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| FGFR1     | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| FGFR2     | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| FGFR3     | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| FLT3      | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| GNAS      | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| HNF1A     | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| HRAS      | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| IDH1      | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| JAK3      | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| KDR       | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| KIT       | 2(0.8%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 1(2.0%)                                                | 1(1.3%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| MET       | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| MLH1      | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| NOTCH1    | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| NPM1      | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| NRAS      | 0(0.0%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
| PDGFRA    | 1(0.4%)                                       | 0(0.0%)                                                | 0(0.0%)                                                | 1(2.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                | 0(0.0%)                                                |
Table 3. Cont.

| Genes   | Number of stage 1A samples with mutations | Number of stage 1B samples with mutations | Number of stage 2 samples with mutations | Number of stage 3A samples with mutations | Number of stage 3B samples with mutations |
|---------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|
| PIK3CA  | 2 (0.8%)                                 | 0 (0.0%)                                 | 1 (2.9%)                                 | 0 (0.0%)                                 | 1 (1.3%)                                 |
| PTEN    | 1 (0.4%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 1 (1.3%)                                 |
| PTPN11  | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 |
| RB1     | 1 (0.4%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 |
| RET     | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 |
| SMAD2   | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 |
| SMAD3   | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 |
| SMAD4   | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 |
| SMO     | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 |
| SPC     | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 |
| STK11   | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 |
| TP53    | 23 (9.7%)                                | 3 (11.5%)                                | 3 (8.8%)                                 | 7 (14.3%)                                | 8 (10.1%)                                |
| VHL     | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 | 0 (0.0%)                                 |

Multiple mutations and mutation hot spots in gastric adenocarcinomas

Clinical success with individualized combination therapy relies on the identification of mutational combinations and patterns for co-administration of a single or combination of target agents against the detected mutational combinations. Some of the mutations detected in our tumor group through sequencing analysis were not only recurrent and frequent but also occurred in combination with other mutations. 13.6% of samples had at least one or more missense mutations, 1.70% had at least two or more missense mutations, 0.4% had at least three or more missense mutations and 86.1% of samples incurred no deleterious mutations in any of the screened 737 loci of the potential tumor suppressor genes and oncogenes (Table 5).

Discussion

Gastric adenocarcinoma, the most common type of gastric cancer, is heterogeneous and its incidence and cause varies widely with geographical regions, gender, ethnicity, and diet [20]. The infectious agent *Helicobacter pylori* is associated with chronic atrophic gastritis, an inflammatory precursor of gastric adenocarcinoma [20]. While *H. pylori* colonizes the gastric tract of most of the world’s population and induces mutations and genomic instability in host DNA, only individuals with a complex risk profile tend to develop cancer [21].

In this study we have used Ion Ampliseq Cancer Panel to sequence 737 loci in 45 cancer-related genes, mainly oncogenes and tumor suppressor genes, of 238 human gastric adenocarcinoma samples. 23 out of 238 samples incurred mutations along the TP53 gene. Other genes such as BRAFT, APC, FBXW7, ERBB2, KRAS, PIK3CA, PTEN, RB1, and SMAD4 incurred mutations in only 1–2 out of 238 samples. The incurrence of TP53 mutation and that of other genes such as KRAS less frequently mutated in our sample set was consistent to that of previous reports on...
| Genes   | Number of samples with mutations in 238 samples (Mutation frequency) | Number of low differentiation samples with mutations (Mutation frequency in 125 samples) | Number of middle-low differentiation samples with mutations (Mutation frequency in 14 samples) | Number of middle differentiation samples with mutations (Mutation frequency in 84 samples) | Number of unknown samples with mutations (Mutation frequency in 15 unknown samples) |
|---------|-------------------------------------------------|-----------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| ABL1    | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| AKT1    | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| ALK     | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| APC     | 1(0.4%)                                         | 1(0.8%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| ATM     | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| BRAF    | 2(0.8%)                                         | 1(0.8%)                                                         | 0(0.0%)                                                                         | 1(1.2%)                                                                         | 0(0.0%)                                                                         |
| CDH1    | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| CDKN2A  | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| CSF1R   | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| CTNNB1  | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| EGFR    | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| ERBB2   | 1(0.4%)                                         | 1(0.8%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| ERBB4   | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| FBXW7   | 1(0.4%)                                         | 1(0.8%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| FGFR1   | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| FGFR2   | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| FGFR3   | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| FLT3    | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| GNAS    | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| HNF1A   | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| HRAS    | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| IDH1    | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| JAK3    | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| KDR     | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| KIT     | 2(0.8%)                                         | 2(1.6%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| KRAS    | 1(0.4%)                                         | 1(0.8%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| MET     | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| MLH1    | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| MPL     | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| NOTCH1  | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| NPM1    | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| NRAS    | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| PDGFRA  | 1(0.4%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 1(1.2%)                                                                         | 0(0.0%)                                                                         |
| PIK3CA  | 2(0.8%)                                         | 0(0.0%)                                                         | 1(7.1%)                                                                         | 0(0.0%)                                                                         | 1(6.7%)                                                                         |
| PTEN    | 1(0.4%)                                         | 1(0.8%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| PTPN11  | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| RB1     | 1(0.4%)                                         | 1(0.8%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| RET     | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| SMAD4   | 2(0.8%)                                         | 1(0.8%)                                                         | 0(0.0%)                                                                         | 1(1.2%)                                                                         | 0(0.0%)                                                                         |
| SMARCB1 | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| SMO     | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| SRC     | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| STK11   | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |
| TPS3    | 23(9.7%)                                        | 10(8.0%)                                                       | 0(0.0%)                                                                         | 10(11.9%)                                                                      | 3(20.0%)                                                                        |
| VHL     | 0(0.0%)                                         | 0(0.0%)                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         | 0(0.0%)                                                                         |

Table 4. Mutations (including missense point mutations/deletion/insertion) frequencies in 45 genes (737 loci) of different differentiation types of GAs.
mutation screening for gastric cancers [22–24]. Wang et al. reported that certain subtypes of gastric cancers accumulated mutations in ARID1A gene, but is negatively associated with mutations in TP53 [22]. Another study by Nagarajan et al. reported PAPPA as a recurrently mutated gene in TP53 wild-type gastric cancer [24]. It is reported that mutations in TP53 is not common in ovarian, endometrial, kidney, or pancreatic cancers, but these frequently accumulate mutations in chromatin-modify-
mutations on an individual basis by DNA sequencing remained impractical. The recent advance of new next-generation DNA sequencing technologies, such as the semiconductor-based Ion Torrent sequencing platform, makes DNA sequencing cheaper, faster, and more reliable. Our study shows distinctive patterns and combinations of mutations in the gastric adenocarcinoma genome of these Chinese patients. Genomic profiling and identification of specific mutation patterns and designing personalized drug targets and treatment regimens against those cancer mutations can be very useful for personalized therapy. There are many novel compounds available today targeting different molecular pathways of gastric cancer such as HER2, EGFR, MET, FGFR, and PI3K/mTOR, which could potentially be used for the treatment of gastric adenocarcinomas. Hence, we believe that a faster and more cost-effective, accurate high-throughput genomic profiling tool such as Ion Torrent sequencing employed in our current studies will facilitate the implementation of tailored and personalized therapies in the near future.

Supporting Information

Figure S1 Filter process of variants. (a) Strand-biased variants were eliminated using Integrative Genomics Viewer (IGV) software (http://www.broadinstitute.org/igv); (b) Variants in AMPL339432 should be eliminated, because this amplicon is not unique matched to PIK3CA in human genome; (c) All of our statistical analysis was based on the data in blue box. (DOCX)

Table S1 Frequencies of point mutations, insertion, and deletion mutations in 737 loci of 45 genes in 238 GAs. (DOCX)

Table S2 Confirmation of missense mutations by Sanger sequencing. (DOCX)

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Author Contributions

Conceived and designed the experiments: ZX X. Huo SYC JC. Performed the experiments: HY Chuanming Tang FL DZ DH HS SJ GZ ZL ZD BG YH CY LW ZS YL DG XX X. Wu X. Wei LH YZ JY YG Cuiju Tang. Analyzed the data: ZX X. Huo Chuanming Tang SYC JC. Contributed reagents/materials/analysis tools: ZX X. Huo SYC JC. Wrote the paper: ZX X. Huo VN Chuanming Tang IJ X. Huang SYC JC.

Table 5. Single and multiple mutations in 238 GAs.

| Mutations combination (including missense point mutations/deletion/insertion) | Number of samples with mutation combinations | Percentage in all sequenced samples |
|---|---|---|
| Single and more | 33 | 13.60% |
| Double and more | 4 | 1.70% |
| Three and more | 1 | 0.40% |
| Four and more | 0 | 0.00% |
| Five and more | 0 | 0.00% |
| No. missense, deletion, insert or substitution-nonsense | 205 | 86.10% |

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References

1. Davis PA, Sano T (2001) The difference in gastric cancer between Japan, USA and Europe: What are the facts? What are the suggestions? Critical reviews in oncology/hematology 40: 77-94.

2. Borch K, Jonsson B, Tarplia E, Franzen T, Berglund J, et al. (2000) Changing pattern of histological type, location, stage and outcome of surgical treatment of gastric carcinoma. British Journal of Surgery 87: 618-626.

3. Hartgrink HH, Putter H, Klein Kranenburg E, Boumaenck Jj, van de Velde CJH (2002) Value of palliative resection in gastric cancer. British Journal of Surgery 89: 1438-1443.

4. Okines AFC, Cunningham D (2010) Trastuzumab in gastric cancer. European journal of cancer (Oxford, England: 1990) 46: 1949-1959.

5. Arkenau H-T (2009) Gastric cancer in the era of molecularly targeted agents: current drug development strategies. Journal of Cancer Research and Clinical Oncology 135: 853-866.

6. Kuniyasu H, Yasui W, Kitadai Y, Yokozaki H, Ito H, et al. (1992) Frequent amplification of the c-met gene in scirrhous type stomach cancer. Biochemical and Biophysical Research Communications 189: 227-232.

7. Hattori Y, Itoh H, Uchino S, Hosokawa K, Ochiai A, et al. (1996) Immunohistochemical detection of K-sam protein in stomach cancer. Clinical Cancer Research 2: 1373-1381.

8. Khosravi Shahi P, Díaz Munoz de la Espada VM, García Alfonso P, Encina García S, Izarzugaza Perón Y, et al. (2007) Management of gastric adenocarcinoma. Clinical and Translational Oncology 9: 438-442.

9. Metzker ML (2010) Sequencing technologies [miash] the next generation. Nat Rev Genet 11: 31-46.

10. Suzuki S, Ono N, Furusawa C, Ying B-W, Yomo T (2011) Comparison of Sequence Reads Obtained from Three Next-Generation Sequencing Platforms. PLoS ONE 6: e15354.

11. Pourmand N, Karhanek M, Persson HHJ, Webb CD, Lee TH, et al. (2006) A map of human genome variation from population-scale sequencing. Nature 440: 1169-1172.

12. (2010) Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA. http://evsdb.org/EVS/.

13. (March 19, 2013 accessed) Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA. http://evsdb.org/EVS/.

14. Bamford S, Dawson E, Forbes S, Clements J, Pettett R, et al. (2004) The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website. British journal of cancer 91: 355-358.

15. Sigal A, Rotter V (2000) Oncogenic Mutations of the p53 Tumor Suppressor: The Demons of the Guardian of the Genome. Cancer Research 60: 6788-6789.

16. Pfeiffer R, Besaratinia A (2009) Mutational spectra of human cancer. Human Genetics 123: 493-506.

17. Ory KLY, Augun C, Sousi T. (1994 Aug 1) Analysis of the most representative tumour-derived p53 mutants reveals that changes in protein conformation are not correlated with loss of transcription or inhibition of cell proliferation. EMBO J 13: 2596-2604.

18. Peart MJ, Prives C (2006) Mutant p53 gain of function: the N-F-Y connection. Nature 467: 1061-1073.

19. (March 2013 accessed) Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA. http://evsdb.org/EVS/.

20. Bamford S, Dawson E, Forbes S, Clements J, Pettett R, et al. (2004) The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website. British journal of cancer 91: 355-358.

21. Sigal A, Rotter V (2000) Oncogenic Mutations of the p53 Tumor Suppressor: The Demons of the Guardian of the Genome. Cancer Research 60: 6788-6789.

22. Pfeiffer R, Besaratinia A (2009) Mutational spectra of human cancer. Human Genetics 123: 493-506.

23. Ory KLY, Augun C, Sousi T. (1994 Aug 1) Analysis of the most representative tumour-derived p53 mutants reveals that changes in protein conformation are not correlated with loss of transcription or inhibition of cell proliferation. EMBO J 13: 2596-2604.

24. Peart MJ, Prives C (2006) Mutant p53 gain of function: the N-F-Y connection. Nature 467: 1061-1073.

25. Petitjean A, Achatz MI, Borresen-Dale AL, Hainaut P, Olivier M (2007) TP53 mutations in endometriosis-associated ovarian carcinomas. N Engl J Med 356: 1532-1543.

26. Guan B, Mao TL, Panuguanti PK, Kuhn E, Kurman RJ, et al. (2011) Mutation and loss of expression of ARID1A in uterine low-grade endometrioid carcinoma. Am J Surg Pathol 35: 625-632.

27. Varela I, Tarpey P, Raine K, Huang D, Ong CK, et al. (2011) Exome sequencing identifies frequent mutation of the SWI/SNF complex gene PBRM1 in renal carcinoma. Nature 469: 339-342.

28. Jiang Y, Shi C, Edil BH, de Wilde RF, Klimstra DS, et al. (2011) DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. Science 331: 1199-1203.

29. Deng N, Gohl IK, Wang H, Das K, Tao J, et al. (2012) A comprehensive survey of genomic alterations in gastric cancer reveals systematic patterns of molecular exclusivity and co-occurrence among distinct therapeutic targets. Gut 61: 673-684.

30. Corso G, Velso S, Paredes J, Pedrazzani C, Martins D, et al. (2011) Oncogenic mutations in gastric cancer with microsatellite instability. European Journal of Cancer 47: 445-451.

31. Wang K, Kan J, Yuen ST, Shi ST, Chu KM, et al. (2011) Exome sequencing of gastric carcinoma reveals somatic mutations relevant to gastric cancer. Cancer Cell 10: 173-174.

32. Ilson DH, Kelsen D, Shah M, Schwartz G, Levine DA, et al. (2011) A phase 2 trial of erlotinib in patients with previously treated squamous cell and adenocarcinoma of the esophagus. Cancer 117: 1409-1414.

33. Chan JA, Blazkowsky LS, Enzinger PC, Ryan DP, Abrams TA, et al. (2011) A multicenter phase II trial of single-agent cetuximab in advanced esophageal and gastric adenocarcinoma. Annals of Oncology 22: 1567-1573.

34. Ferry DR, Anderson M, Bedield K, Tomlinson S, Atherfold P, et al. (2007) A Phase II Study of Gefitinib Monotherapy in Advanced Esophageal Adenocarcinoma: Evidence of Gene Expression, Cellular, and Clinical Response. Clinical Cancer Research 13: 5069-5075.

35. Vidal O, Menges JP, Elizalde I, Valente M, Volant A, et al. (2009) High preoperative serum vascular endothelial growth factor levels predict poor clinical outcome after curative resection of gastric cancer. British Journal of Surgery 96: 1413-1431.

36. Chen J, Li T, Wu Y, He L, Zhang L, et al. (2011) Prognostic significance of vascular endothelial growth factor expression in gastric carcinoma: a meta-analysis. Journal of Cancer Research and Clinical Oncology 137: 1799-1812.

37. Ilson DH, Kelsen D, Shah M, Schwartz G, Levine DA, et al. (2011) A phase 2 trial of erlotinib in patients with previously treated squamous cell and adenocarcinoma of the esophagus. Cancer 117: 1409-1414.

38. Corso G, Velso S, Paredes J, Pedrazzani C, Martins D, et al. (2011) Oncogenic mutations in gastric cancer with microsatellite instability. European Journal of Cancer 47: 445-451.

39. Chen J, Li T, Wu Y, He L, Zhang L, et al. (2011) Prognostic significance of vascular endothelial growth factor expression in gastric carcinoma: a meta-analysis. Journal of Cancer Research and Clinical Oncology 137: 1799-1812.

40. Ilson DH, Kelsen D, Shah M, Schwartz G, Levine DA, et al. (2011) A phase 2 trial of erlotinib in patients with previously treated squamous cell and adenocarcinoma of the esophagus. Cancer 117: 1409-1414.

41. Varela I, Tarpey P, Raine K, Huang D, Ong CK, et al. (2011) Exome sequencing of gastric carcinoma reveals somatic mutations relevant to gastric cancer. Cancer Cell 10: 173-174.

42. Ilson DH, Kelsen D, Shah M, Schwartz G, Levine DA, et al. (2011) A phase 2 trial of erlotinib in patients with previously treated squamous cell and adenocarcinoma of the esophagus. Cancer 117: 1409-1414.

43. Jackson JR, Gilmartin A, Imburgia C, Winkler JD, Marshall LA, et al. (2000) An Indolecarbazole Inhibitor of Human Checkpoint Kinase (Chk1) Abrogates Cell Cycle Arrest Caused by DNA Damage. Cancer Research 60: 366-372.

44. Graves PR, Yu L, Schwarz JK, Gales J, Sausville EA, et al. (2000) The Chk1 Protein Kinase and the Cdc25C Regulatory Pathways Are Targets of the Anti-cancer Agent UCN-01. Journal of Biological Chemistry 275: 5690-5695.