ORIGINAL ARTICLE

Basic Study

Gene mutations in stool from gastric and colorectal neoplasia patients by next-generation sequencing

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Abstract

AIM

To study cancer hotspot mutations by next-generation sequencing (NGS) in stool DNA from patients with different gastrointestinal tract (GIT) neoplasms.

METHODS

Stool samples were collected from 87 Finnish patients diagnosed with various gastric and colorectal neoplasms, including benign tumors, and from 14 healthy controls. DNA was isolated from stools by using

the PSP® Spin Stool DNA Plus Kit. For each sample, 20 ng of DNA was used to construct sequencing libraries using the Ion AmpliSeq Cancer Hotspot Panel v2 or Ion AmpliSeq Colon and Lung Cancer panel v2. Sequencing was performed on Ion PGM. Torrent Suite Software v.5.2.2 was used for variant calling and data analysis.

RESULTS
NGS was successful in assaying 72 GIT samples and 13 healthy controls, with success rates of the assay being 78% for stomach neoplasia and 87% for colorectal tumors. In stool specimens from patients with gastric neoplasia, five hotspot mutations were found in *APC*, *CDKN2A* and *EGFR* genes, in addition to seven novel mutations. From colorectal patients, 20 mutations were detected in *AKT1*, *APC*, *ERBB2*, *FBXW7*, *KIT*, *KRAS*, *NRAS*, *SMARCB1*, *SMO*, *STK11* and *TP53*. Healthy controls did not exhibit any hotspot mutations, except for two novel ones. *APC* and *TP53* were the most frequently mutated genes in colorectal neoplasms, with five mutations, followed by *KRAS* with two mutations. *APC* was the most commonly mutated gene in stools of patients with premalignant/benign GIT lesions.

CONCLUSION
Our results show that in addition to colorectal neoplasms, mutations can also be assayed from stool specimens of patients with gastric neoplasms.

Key words: Stool DNA; Next-generation sequencing; Mutations; Gastric neoplasia; Colorectal neoplasia

Core tip: Next-generation sequencing (NGS) was successfully applied for detecting cancer gene mutations in stool DNA of patients with different gastrointestinal neoplasms. Using a gene panel, comprising up to 50 cancer genes, it was found that mutations not only could be detected in stool DNA from colorectal cancer patients but also in patients with stomach cancer and those with benign or premalignant lesions. No hotspot mutations were detected in healthy controls. Our results show that NGS could be useful in screening for neoplastic changes of the gastrointestinal tract.

INTRODUCTION
Gastrointestinal tract (GIT) malignancies are a diverse group of neoplasms with diverse epidemiology and incidences that affect different regions of the GIT, from the stomach to the large intestine. The value of somatic mutations in GIT malignancies is recognized, (1) as markers for early detection; (2) as markers that predict drug resistance, and (3) for follow up of cancer treatment[1-6]. In global terms, gastric carcinoma is the fifth most common cancer and the third most common cause of cancer-related mortality. Adenocarcinoma is its most typical subtype and present in 90% of all cases[7]. *TP53, PIK3CA, ARID1A* and cell adhesion pathway genes have been found to be the most frequently mutated genes in gastric adenocarcinomas[8]. The *CDH1* gene is described as being involved in the pathogenesis of diffuse gastric carcinoma[9]. In sporadic colorectal cancer (CRC), *APC, TP53, KRAS, PI3CA, FBXW7, SMAD4* and *BRAF* are the most commonly mutated genes[10].

One of the major issues in GIT malignancies, specifically gastric carcinoma, is that they are usually detected at an advanced stage, due to late diagnosis[11]. Moreover, recent studies have demonstrated the diversity of morphological (intestinal and diffuse subtypes) and molecular subtypes (mesenchymal-like type, microsatellite-unstable tumor type, and *TP53* tumor type) of gastric carcinoma, which contributes to the challenge of optimizing proper diagnosis and treatment[12]. The principal problem hindering early detection of gastric and colorectal neoplasia is the lack of symptoms; even when symptoms are present, they tend to be mild and nonspecific, which may delay subjecting the patient for endoscopic examination. Exfoliation of cells, whether premalignant or malignant, is continuously occurring from epithelial layer into the digestive lumen[13,14]; these display various genetic changes that have occurred in these cells, and can provide evidence of tumor pathogenesis[15]. Testing DNA abnormalities in stool specimens from GIT carcinoma patients represents a promising noninvasive approach for early cancer detection and for treatment follow-up. Multi-target stool DNA test is currently being used for CRC screening[16].

We have previously shown that next-generation sequencing (NGS) can be successfully applied for investigating mutations in stool DNA obtained from patients with CRC[13]. In the current study, we applied the NGS method to determine whether cancer mutations could also be detected in stool samples from patients with other GIT tumors, including both diffuse and intestinal subtypes of gastric adenocarcinoma, gastric dysplasia, colorectal adenocarcinoma and adenoma, and colorectal leiomyoma.

MATERIALS AND METHODS
Patients
During the period from April 2015 to May 2017, 79 gastric cancer, 38 gastrointestinal stromal tumor

Table 1 Summary of stool samples collected and analyzed

Tumor location	Total collected samples	Successful DNA extraction	Successful sequencing	Number of cases with only known mutations	Number of cases with Mutations, all
Stomach	41	35	32 (78)	5 (15.6)	8 (25)
Carcinoma	38	32	29	3	6
Intestinal type	18	17	15	1	1
Diffuse type	20	15	14	2	5
Benign	3	3	3	2	2
Hamartoma	1	1	1	0	0
Dysplasia	1	1	1	1	1
NET	1	1	1	1	1
Colorectal	46	42	40 (87)	12 (30)	12 (30)
Carcinoma	40	37	35	9	9
Benign	6	5	5	3	3
Adenoma	4	3	3	2	2
Dysplasia	1	1	1	0	0
Leiomyoma	1	1	1	1	1
Healthy controls	14	13	13 (92.9)	0	2 (15)
Total	101	90	85	17	22

Data are presented as *n* or *n* (%). NET: Neuroendocrine tumor.

(GIST), 669 colon cancer and 271 primary rectal cancer patients were referred to three hospitals in Finland: Kirurgi, Meilahti and Jorvi. Three authors (Kokkola A, Carpelan-Holmström M and Koskensalo S) collected stool samples from patients who were referred to them for surgery. Stool specimens were collected from 87 patients with stomach or colorectal neoplasia, representing 41 stomach neoplasia (18 intestinal type, 20 diffuse type, 1 neuroendocrine tumor, 1 gastric dysplasia and 1 hamartoma) and 46 colorectal lesions (40 adenocarcinoma, 4 adenoma, 1 dysplasia and 1colorectal leiomyoma), as well as from 14 healthy individuals (Table 1). A total number of 21 patients had received treatment before the time of sampling (10 patients with stomach neoplasia and 11 with colorectal lesions). Treatments were in the form of chemotherapy (17 patients), radiotherapy (1 patient), or antibiotics for *Helicobacter pylori* infection (3 patients).

Patients were diagnosed and treated in Meilahti Hospital in Helsinki. The study was approved by the Hospital District of Helsinki and Uusimaa (HUS) Review Board (Ethical permission number 351/13/03/02/2014). Written informed consent was obtained from all subjects.

Stool specimens
Stool specimens were collected in special collection tubes provided in the extraction kit (PSP® Spin Stool DNA Plus Kit; Stratec Biomedical, Berlin, Germany). These tubes are prefilled with 8 mL of stool DNA stabilizer to allow collection, transport and storage of the samples without DNA degradation. One spoon of stool specimen (spoon provided with the collection tubes) was transferred to the tube and mixed thoroughly to obtain a stool homogenate. The samples

were stored at -20 ℃ until analysis, for an average of 7 d before extraction.

DNA extraction
Before starting the DNA isolation, each stool specimen tube was vortexed vigorously to ensure proper mixing of the contents with the stabilizer liquid provided in each collection tube. A volume of 1.4 mL of the stabilized stool specimens was transferred to 2 mL tubes. Then, DNA was extracted from each stool specimen using the PSP® Spin Stool DNA Plus Kit (Stratec Biomedical) according to the manufacturer's instructions. Extracted DNA was eluted in 50 μL of elution buffer, and then DNA was quantified by a Qubit® 2.0 Fluorometer (Life Technologies, Carlsbad, CA, United States) using the Qubit® dsDNA BR Assay Kit. The extracted DNA was stored at -20 ℃.

NGS
Library preparation: Twenty nanograms of stool DNA was used for preparing amplicon libraries using Ion AmpliSeq™ Library Kit 2.0 (Life Technologies) according to the manufacturer's guidelines. Gene panels comprising pools of primer mixes were used to amplify templates. The gene panels used were one of the following: (1) Ion AmpliSeq Cancer Hotspot Panel v2 (Life Technologies), consisting of a primer pool for 207 amplicons from an average of 2800 mutational hotspot regions in 50 genes, including *KIT* and *PDGFRA* mutations. The genes included in the panel are *ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAS, GNAQ, HNF1A, HRAS, IDH1, JAK2, JAK3, IDH2, KDR, KIT, KRAS, MET, MLH1, MPL, NOTCH1, NMP1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1,*

SMO, SRC, STK11, TP53, and *VHL*; (2) Ion AmpliSeq Colon and Lung Cancer panel v2 (Life Technologies), consisting of a primer pool for 92 amplicons from 504 hotspot regions in 22 genes frequently mutated in CRC. The genes included in this panel are *AKT1, ALK, BRAF, CTNNB1, DDR2, EGFR, ERBB2, ERBB4, FBX7, FGFR1, FGFR2, FGFR3, KRAS, MAP2K1, MET, NOTCH1, NRAS, PIK3CA, PTEN, SMAD4, STK11*, and *TP53*.

All specimens from patients with gastric neoplasms and 19 specimens from patients with colorectal neoplasia were assayed using Ion AmpliSeq Cancer Hotspot Panel v2, while the remaining specimens from colorectal neoplasia patients were studied by Ion AmpliSeq Colon and Lung Cancer panel v2. The major reason for using two different platforms was that Ion AmpliSeq Cancer Hotspot Panel v2 contains several genes that are commonly mutated in gastric neoplasia.

The amplified libraries were purified using Agencourt AMPure XP beads (Beckman Coulter Genomics, High Wycombe, United Kingdom). The concentration of the purified libraries was measured on the Qubit® 2.0 Fluorometer, using the Qubit® dsDNA HS Assay Kit. The DNA libraries were stored at -20 ℃ until further use.

Template preparation and sequencing: The amplified and purified libraries were diluted to 100 pmol/L, and the templates were prepared and enriched using the Ion OneTouch™ 2 System (Life Technologies), an automated emulsion PCR system. Finally, sequencing was carried out on the Ion Personal Genome Machine System (PGM™; Life Technologies) using Ion 316™ Chips and the Ion PGM™ Sequencing Hi-Q Kit v2.

Data analysis

The Torrent Suite Software v.5.2.2 (Life Technologies) was used to assess run performance and data analysis, and Integrative Genomics Viewer (IGV v 2.2; Broad Institute, Cambridge, MA, United States) was used for visual inspection of the aligned reads.

Variants were further filtered based on quality score (score of 15 or more) and mutant allele frequency (more than 3%). Only single nucleotide variants (SNVs) resulting in a nonsynonymous amino acid change, or a premature stop codon, and all short indels resulting in either a frameshift or insertion/deletion of amino acids were selected. All SNVs were analyzed for previously reported hotspot mutations (somatic mutations reported in the COSMIC database) and novel variations, *i.e.* new mutations detected by NGS but not reported in either COSMIC or dbSNP (build 150) databases.

RESULTS

Success rate

Successful DNA extraction was performed on 77/87

patient stool samples, while NGS assay was successfully carried out on 72 patient stool DNA samples. Five samples were removed from NGS assay due to poor DNA quality (too little or degraded DNA). Of the 14 controls, DNA could be isolated from 13 samples and all were successfully sequenced. The success rates of sequencing stool samples for stomach and colorectal neoplasia were rather similar (78% and 87% respectively) (Table 1).

Hotspot (COSMIC) and novel mutations

In patients' stool samples, a total of 25 hotspot mutations were found (20 in patients with colorectal neoplasia and 5 in patents with stomach neoplasia), while 9 novel mutations were detected (7 in patients with stomach neoplasia and 2 in control samples).

Thirteen control samples from healthy individuals did not reveal any hotspot mutations, but two novel mutations were observed in *ALK,* and *STK11* genes in two subjects.

Mutations in patients with stomach neoplasms

A total number of five hotspot mutations that had been reported earlier in the COSMIC database were detected in *APC, CDKN2A* and *EGFR* genes in stool specimens from 3 gastric adenocarcinoma patients, 1 patient with neuroendocrine tumor, and 1 patient with gastric dysplasia (Table 2). Four samples from patients with adenocarcinoma (diffuse type) revealed a total of seven novel mutations that led to an amino acid change and which had not been reported previously in either the COSMIC or dbSNP databases. The detected novel mutations were found in seven genes, that included *APC, CDH1, DDR2, HRAS, NRAS, PTEN,* and *SMARCB1* (Table 2).

Mutations in patients with colorectal tumors

Twenty hotspot mutations in *AKT1, APC, ERBB2, FBXW7, KIT, KRAS, NRAS, SMARCB1, SMO, STK11,* and *TP53* were seen in 9 patients with adenocarcinomas, 2 with benign adenoma, and 1 with leiomyoma. *APC* was the most frequently mutated gene with five mutations, followed by *TP53* (five mutations), and *KRAS* (two mutations) (Table 2). One case of benign leiomyoma revealed a *TP53* mutation (R306*) mutation, which had been reported as a germline mutation associated with the hereditary cancer predisposing syndrome. Additionally, this mutation has also been reported as a somatic pathogenic mutation in COSMIC in tumors of the colon and other parts of the digestive tract (Table 2). No novel mutations were found in our study in stool samples from colorectal cases.

DISCUSSION

We are one of the first groups that applied NGS to

Table 2 COSMIC and novel mutations detected by Ion Torrent sequencing

Histopathology	Cases ID	Age, yr	Sex	TNM staging	Gene	Mutation type	COSMIC No.
Stomach							
AC (D)	3	66	F	T2N0	SMARCB1	p.T72K	Novel
AC (D)	14	80	F	T4aNxM1	APC	p.D1570N	Novel
					CDH1	p.V82A	Novel
					HRAS	p.V44M	Novel
					NRAS	p.K135R	Novel
AC (D)	39	65	F	T1aN0M0	EGFR	p.A750T	COSM1651572
					DDR2	p.E523K	Novel
AC (D)	43	43	M	T4bNxM1	PTEN	p.E256G	Novel
AC (D)	77	78	M	TxNxM1	APC	p.A1582P	COSM4170230
AC (I)	100	69	M	T3N3M1	APC	p.A1582P	COSM4170230
NET	11	67	M	5% PR	CDKN2A	p.V126I	COSM13778
Dysplasia	78	77	M	T0N0M0	APC	p.A1582P	COSM4170230
Colorectal							
AC	20	70	M	T3N0M0	KIT	p.N564S	COSM30732
AC	12	71	F	T3N1bM0	APC	p.A1582P	COSM4170230
					TP53	p.P72A	COSM3738520
AC	21	79	M	T2N0	APC	p.A1582P	COSM4170230
AC	22	64	F	T2N0	STK11	p.F354L	COSM21360, COSM4169323
					SMO	p.N202S	COSM5979442
AC	23	53	M	TxNxM1	APC	p.A1582P	COSM4170230
AC	31	64	M	T2N0	TP53	p.Y205D	COSM43844
AC	55	69	M	T3N0M0	KRAS	p.G12V	COSM520
					TP53	p.T172fs	COSM44371
AC	68	63	M	T3N0M0	TP53	p.Y163H	COSM43846
AC	28	77	F	T1N0	KRAS	p.G12D	COSM521
					AKT1	p.E17K	COSM34142, COSM33765
Adenoma	24	81	M		APC	p.E1295*	COSM18961
Adenoma	19	64	F		NRAS	p.Q61R	COSM584, COSM28048
					FBXW7	p.R505C	COSM22975, COSM33844
					APC	p.S1465fs	COSM18873, COSM19688 COSM19332, COSM18931, COSM13864
					ERBB2	p.V842I	COSM14065, COSM1666633
					SMARCB1	p.R377C	COSM3972885
Leiomyoma	94	69	F		TP53	p.R306*	COSM10663, COSM145026
Healthy controls							
	C 2	F	27		ALK	p.L1190P	Novel
	C 8	F	33		STK11	p.Y36H	Novel

AC: Adenocarcinoma; D: Diffuse type; I: Intestinal type; NET: Neuroendocrine tumor; PR: Proliferative rate.

detect mutations in DNA isolated from stool samples of colorectal carcinoma patients[13]. We have now applied NGS analysis on stool samples of not only malignant colorectal carcinoma but also demonstrated that it is possible to detect mutations in stool specimens from patients with gastric neoplasms, and also from patients with benign colorectal tumors. Moreover, we observed mutations in stool from patients with early tumor stages, with no hotspot mutations in stools of healthy subjects. As in our previous study, we set the threshold for variant quality score at 15 and the mutant allele frequency cutoff at 3%, and when using these threshold values, no COSMIC hotspot mutations were found in the 13 control specimens.

Mutations in patients with colorectal neoplasms
The overall success rate of NGS for colorectal neoplasm patients was 87%, which is similar to the 80% reported in our earlier study[13]. In our previous study, the patients were of Iranian origin, whereas in the current study, the patients were from Finland; nonetheless, the mutation types were similar in both of these ethnic groups. In the current series, the most common mutated genes were *APC, TP53* and *KRAS*, while in the earlier study, the top mutated genes were *TP53, KRAS, FBXW7, EGFR* and *SMAD4.*

The most recurrently occurring mutation in colorectal carcinoma cases was *APC* mutation (A1582P), which was found in 3 patients. In our series, four *TP53* mutations were seen in stool samples from CRC patients.

We detected *KRAS* codon 12 mutations (G12V and G12D) in two specimens and *NRAS* codon 61 (Q61R) in one specimen from colorectal carcinoma patients. Similar to our present results, codon 12 mutations were the most common *KRAS* mutations found in our previous study on Iranian samples, although other *KRAS* mutations at codons 12, 13, 20, 63, 117, 146 and 43 were also found previously[13]. Additionally,

a recent study demonstrated the detection of *KRAS* G12D mutation in stool samples from patients with colorectal carcinoma by using droplet PCR[17]. Clinical data available from those patients for who *KRAS* testing in tumor tissue was carried out correlated to *KRAS* mutation status in stool. In patient number 55, the presence of *KRAS* G12V mutation was confirmed in tumor tissue specimens with 20% mutant allele fraction. The same mutation (*KRAS* G12V) was detected in the stool DNA from the same patient with 13% mutant allele fraction. Moreover, tissue samples from patient number 23 revealed no *KRAS* or *NRAS* mutations, and the same negative findings for those two mutations were also observed in the stool DNA specimen from this case.

Among the patients with benign colorectal tumors, *APC* mutations were most common and found in two samples with colorectal benign adenoma. Adenomas with *APC* mutations have been reported more likely to progress into large adenomas and invasive carcinomas[18,19]. Inactivation of the *APC* gene and the subsequent activation of Wnt signaling pathway are key factors in the initiation of tumorigenesis of CRC[20,21]. The R505C mutation in *FBXW7* seen in a colorectal adenoma patient in the present study was also reported in our previous study in a colorectal carcinoma patient[13].

TP53 is another gene commonly mutated in CRC, and plays a crucial role in the adenoma to carcinoma transition during carcinogenesis, and may have an impact on cancer prognosis[22]. A patient with leiomyoma revealed a nonsense *TP53* mutation (R306*), which has been reported as a somatic mutation in colon tumors and also considered as a germline mutation associated with hereditary cancer-predisposing syndrome and Li-Fraumeni syndrome in CRC, although not in gastric carcinoma[23]. In our case, this *TP53* mutation is apparently somatic, as the allele fraction was 5.5%. A meta-analysis of studies carried out on stool DNA testing has shown an overall sensitivity of 68% and 93% specificity in the diagnosis of advanced colorectal adenoma[24].

Mutations in patients with stomach neoplasms

As far as we aware, this is the first study to have utilized stool samples from patients with stomach neoplasia. Eight out of 32 patients' samples (25%) with stomach neoplasia revealed 12 mutations (both hotspot COSMIC and novel).

In gastric neoplasia, the *APC* gene mutations were those most frequently encountered. Four *APC* mutations were detected in patients with gastric neoplasia (three mutations in gastric carcinoma, and one in benign gastric dysplasia). *APC* is a tumor suppressor gene that has a key role in several molecular processes, such as suppression of canonical

Wnt signaling[25], and the presence of *APC* mutations have been demonstrated in gastric adenocarcinoma samples[26,27]. *APC* gene mutations have been reported in both intestinal and diffuse types of gastric carcinoma with a higher frequency in the intestinal subtype of the disease[28,29]. The adenoma to carcinoma transition pathway has a 20% *APC* mutation in the intestinal type of gastric carcinoma[30]. In our study, an *APC* A1582P mutation was seen in both the intestinal and diffuse types, and an *APC* D1570N mutation was seen in the diffuse type. Furthermore, we detected the same *APC* mutation (A1582P) in a stool specimen from a patient with benign gastric dysplasia. This is in concordance with an earlier study that identified the presence of *APC* mutations in tumorous tissue in cases with gastric adenomas or flat dysplasia, and also in benign cases associated with adenocarcinoma[5,31].

In the diffuse type of gastric carcinoma, *CDH1* is reported to be commonly mutated[32], and *CDH1* germline mutations have also been reported to play an important role in diffuse gastric carcinoma development[33]. *EGFR* mutations are also commonly encountered in the diffuse subtype[34-36] of gastric neoplasia, although their role is still controversial. We found the E-cadherin gene (*CDH1*) V82A mutation in a diffuse gastric carcinoma patient, and also found the exon 19 *EGFR* (A750T) mutation in another case with diffuse gastric carcinoma.

Novel mutations were found in *NRAS* (K135R), *DDR2* (E523K) and in exon 7 of *PTEN* (E256). Codon 12 or 13 *NRAS* mutations in tumor tissues have been reported to be associated with a poor prognosis in metastatic stomach carcinoma[37,38], whereas *DDR2* expression in gastric tumor tissues has been described to be associated with an increased risk of peritoneal dissemination[39]. Despite the low frequency of *PTEN* mutations in gastric malignant tumors, they tend to be associated with poorly differentiated gastric carcinoma, TNM staging and resistance effect to chemotherapy[40,41]. Interestingly, the novel *PTEN* (E256G) mutation seen in our study was found in a gastric cancer case with an advanced tumor stage (T4bNxM1).

In conclusion, our results demonstrate that NGS technology can be applied for detection of gene mutations in stool specimens from not only colorectal cancer patients but also from patients with stomach neoplasms, as well as those with benign tumors of the gastrointestinal tract.

ARTICLE HIGHLIGHTS

Research background
Stool DNA sample is a simple, noninvasive source for studying genetic markers of diagnostic/prognostic or predictive significance in colorectal cancer. The significance of stool DNA testing is, however, not well known for stomach cancers and for benign tumors. Current assays screen individual or few mutations only, and do not cover all important cancer mutations. Amplicon-

based NGS could, thus, provide a sensitive method for DNA testing from stool samples in gastrointestinal (GIT) malignancies.

Research motivation

The main challenge in stool DNA-based genetic testing is that only a small proportion of stool DNA is of human origin, thus requiring a very sensitive test. We, therefore, hypothesized that diagnostic value of stool-based DNA testing could be enhanced by applying sensitive amplicon–based next-generation sequencing (NGS) to stool DNA. With the application of NGS we could screen all important mutations in 50 genes from a small amount of input DNA in a single test.

Research objectives

The objective of the study was to apply NGS for screening hotspot mutations in commonly mutated genes in GIT malignancies from stool DNA. The aim was also to see if mutations could be detected in patients with gastric cancer and in patients with early neoplasms, in addition to those with colorectal cancer.

Research methods

Mutation detection was performed by amplicon-based NGS using the Ion AmpliSeq Cancer Hotspot Panel v2 and Ion AmpliSeq Colon and Lung Cancer panel v2. Template preparation was done using the Ion OneTouch™ 2 System and sequencing was performed on Ion PGM (Thermo-Fisher Scientific). Sequencing data analysis and variant calling was performed by using the Torrent Suite Software v.5.2.2 with variant caller plugin. All single nucleotide variants were analyzed for previously reported hotspot mutations (reported in the COSMIC database) and novel variations, *i.e.* not reported in either COSMIC or dbSNP databases.

Research results

Hotspot mutations in stool DNA were found in *APC, CDKN2A* and *EGFR* in patients with stomach neoplasms and in *AKT1, APC, ERBB2, FBXW7, KIT, KRAS, NRAS, SMARCB1, SMO, STK11* and *TP53* in patients with colorectal neoplasia. *APC* was the most commonly mutated gene in stools of patients with premalignant/benign GIT lesions.

Research conclusions

This study demonstrates that NGS-based mutation screening can be successfully applied to stool DNA from patients with GIT neoplasms. In addition to mutation detection in stool DNA from colorectal cancer patients, mutations can also be detected from gastric cancer patients, as well as from patients with premalignant or benign neoplasms.

Mutation testing from stool DNA is mainly carried out for individual gene mutations by PCR-based methods for colorectal cancer screening. Since the amount of DNA of human origin is very low in stool, it was hypothesized that amplicon-based NGS could be highly sensitive and suitable for studying a large number of mutations, which could greatly enhance the diagnostic value of stool DNA testing. The methods used in this study require low input of DNA, can amplify around 200 targeted regions of important cancer genes, and together with the high sensitivity of NGS provide a great advantage over prevailing methods for mutation detection from stool DNA.

This study showed that mutations can also be detected in stool DNA from patients with stomach neoplasms. Detection of mutations in stool DNA of patients with premalignant neoplasm, and also in patients with stage I and II of tumor, demonstrates its application for early detection of GIT neoplasms.

The results of this study could have implication in future NGS-based stool DNA diagnostic tests that could be useful for screening of GIT malignancies and for detection at the premalignant stage. It could also act as a guide in a targeted therapy regimen, and to ease follow up of the treatment.

Research perspectives

Genetic mutations can be detected by amplicon–based NGS in stool DNA from patients with GIT tumors other than colorectal cancer also. Moreover, early neoplastic changes in GIT can also be detected in stool DNA. These results open up possibilities of development of NGS-based stool DNA test. Further testing of this method on a larger number of samples from patients with different GIT malignancies, premalignant lesions and healthy individuals is needed to

fully assess it applicability in cancer diagnostics.

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