Pancreatology 19 (2019) 419–423

Contents lists available at ScienceDirect

Pancreatology

journal homepage: www.elsevier.com/locate/pan

Analysis of BRCAness with multiplex ligation-dependent probe amplification using formalin-fixed and paraffin-embedded pancreatic ductal adenocarcinoma tissue obtained via endoscopic ultrasound-guided fine-needle aspiration biopsy

Kosuke Okuwaki [a, *], Hironori Masutani [a], Hiroshi Imaizumi [a], Tsutomu Yoshida [b], Mitsuhiro Kida [a], Tomohisa Iwai [a], Hiroshi Yamauchi [a], Masayoshi Tadehara [a], Kai Adachi [a], Masafumi Watanabe [a], Takahiro Kurosu [a], Wasaburo Koizumi [a]

[a] Department of Gastroenterology, Kitasato University School of Medicine, 1-15-1 Kitasato, Minami, Sagamihara, Kanagawa, 252-0375, Japan
[b] Department of Pathology, Kitasato University School of Medicine, 1-15-1 Kitasato, Minami, Sagamihara, Kanagawa, 252-0375, Japan

ARTICLE INFO

Article history:
Received 7 January 2019
Received in revised form
18 February 2019
Accepted 19 February 2019
Available online 20 February 2019

Keywords:
Anticancer drug sensitivity
EUS-FNAB
MLPA
Pancreatic carcinoma
Precision medicine

ABSTRACT

Background/Objectives: A breakthrough in chemotherapy for pancreatic ductal adenocarcinoma (PDAC) may be achieved using precision medicine, which involves identifying cases that are highly likely to respond to a certain treatment and then performing that treatment. BRCAness has been receiving attention as a novel predictor of anticancer drug sensitivity in PDAC, making the screening of BRCAness paramount.

Methods: We conducted the first-ever examination of the feasibility of analyzing BRCAness using multiplex ligation-dependent probe amplification (MLPA). Formalin-fixed paraffin-embedded (FFPE) tissue samples obtained via endoscopic ultrasound-guided fine-needle aspiration biopsy (EUS-FNAB) from 20 patients with the highest pancreatic carcinoma cell counts in tissue samples out of 40 consecutive PDAC patients who underwent EUS-FNAB at our hospital were analyzed by MLPA for BRCAness.

Results: We were able to accurately analyze BRCAness in 75% of the 20 cases of PDAC using FFPE tissue obtained by EUS-FNAB. BRCAness was observed in one of the 20 cases.

Conclusions: In PDAC, analyzing BRCAness by MLPA using FFPE tissue obtained by EUS-FNAB offers the remarkable benefit of yielding results in a short period of time and at a low cost. In addition, this method of BRCAness analysis may prove to be a feasible and effective approach for performing precision medicine.

Introduction

Despite the advanced medical technology that is currently available, pancreatic ductal adenocarcinoma (PDAC) remains diagnosed as an unresectable advanced cancer in approximately 80% of cases [1] and has a poorer prognosis than most other carcinomas [2,3]. Despite the recent development of a novel chemotherapy regimen for advanced PDAC [4,5], response rates remain insufficient. One possible breakthrough that is foreseeable in the near future regarding the approach in chemotherapy for PDAC is precision medicine, which involves identifying cases that are highly likely to respond to a particular treatment and then performing that suitable treatment. The breast cancer susceptibility gene 1 (BRCA1)/BRCA2 mutation is involved in the onset of cancer by impairing homology-directed repair of DNA. The concept of BRCAness has been proposed as a pathology that is triggered by an abnormality in the BRCA pathway, in addition to the BRCA1/2 mutation [6]. Thus, BRCAness indicates not only the BRCA1/2 mutation but also changes in chemically modified proteins that regulate qualitative and quantitative genome abnormalities and gene expression.

BRCAness has been receiving attention as a novel predictor of anticancer drug sensitivity in breast cancer [7–9]. Platinum agents

* Corresponding author.
E-mail address: kokuwaki@kitasato-u.ac.jp (K. Okuwaki).

https://doi.org/10.1016/j.pan.2019.02.010
1424-3903/

and poly (ADP-ribose) polymerase (PARP) inhibitors are expected to be effective for PDAC with BRCAness, just as they are for BRCA-mutated breast cancers. However, searching for abnormalities in driver genes such as BRCA1/2 is time-consuming and expensive. Fortunately, BRCAness can also be diagnosed using multiplex ligation-dependent probe amplification (MLPA), which can be performed in a relatively short period of time and at a low cost [10,11]. We therefore chose to examine the feasibility of analyzing BRCAness in PDAC by MLPA on small tissue fragments obtained via endoscopic ultrasound-guided fine-needle aspiration biopsy (EUS-FNAB) on patients with PDAC.

Methods

Patients

The current study was approved by the Ethics Committee of Kitasato University prior to any of the specific study procedures being started. The initial candidates for the study consisted of 40 consecutive patients from July 2016 to April 2017 who underwent EUS-FNAB at Kitasato University Hospital due to suspicion of PDAC. Three patients for whom a histological diagnosis of PDAC was not obtained were excluded from the study. From the remaining 37 patients, a pathologist with no knowledge of the clinical information of the patients used light microscopy to examine hematoxylin-eosin (HE) stained aspirate specimens to a histological diagnosis PDAC and to select the 20 patients whose specimens demonstrated the highest pancreatic carcinoma cell counts. The specimens from these 20 patients were included in the study for analysis of BRCAness using MLPA.

EUS-FNAB

In the current study, EUS-FNAB was indicated for patients suspected of PDAC and who required pathological diagnosis as part of the normal protocol to determine the most appropriate treatment strategy. The aspiration needle in all cases was a 22-gauge needle with excellent maneuverability (EZ Shot 3 Plus™, Olympus Medical, Tokyo, Japan). After the endoscope was inserted, the pancreatic carcinoma (the aspiration target) was visualized. Upon confirmation of the absence of cysts, blood vessels, or other vessels that would typically not permit aspiration along the aspiration route, the aspiration needle was inserted under ultrasound-guidance into the lesion. Under negative pressure of 20 cc, the needle was agitated inside of the lesion with 10–20 strokes to collect tissue. The aspiration was performed three times for each biopsy. The collected tissue fragments were immediately fixed in 10% neutral-buffered formalin solution.

DNA isolation and MLPA

The formalin-fixed paraffin-embedded (FFPE) tissue obtained by EUS-FNAB as described above was sectioned on a microtome at a thickness of 10 μm and used to prepare 10 unstained slides for each patient. The cancerous portions of the HE stained samples that were used in the pathological diagnosis were marked by the pathologist to be used as references during DNA isolation. DNA was extracted from the tumor biopsy material using a QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany). A lack of isolated DNA in terms of quality or quantity was assessed based on Q-fragments (control peaks) and the concentration of the isolated DNA. If the Q-fragment peak was less than 1/3 of the 92-nt reference, the DNA was assessed as poor-quality. In cases in which DNA isolation was insufficient due to a lack of DNA quantity, additional unstained slides were prepared one additional time and the DNA reexamined.

BRCAness was analyzed using MLPA. The MLPA was carried out at FALCO Biosystems Ltd. and was performed according to the manufacturer's instructions [12]. Classification of BRCAness subtypes was performed using MLPA with a P376-B2 BRCA1ness probemix (MRC-Holland, Amsterdam, Netherlands), as previously reported [13]. Data analysis was performed using the Coffalyser. NET software (MRC-Holland, Amsterdam, Netherlands). The relative copy number for each sample was compared using human genomic DNA (Promega, Madison, WI, USA) as a reference sample and the ratio was calculated using Coffalyser. Net default settings. BRCAness scores were calculated using the relative copy number ratios of various DNA sequences. The relative copy number ratios generated from the Coffalyser. NET calculations for all the target-specific probes were used in the prediction analysis for the microarrays (PAM). The training set generated by MRC-Holland with P376-B2 Lot 0911 was used for the PAM. Each sample was analyzed twice and the mean score was used in the analysis. The BRCAness analysis was performed completely blinded to the clinical information. A sample with a BRCAness score $\geq$0.5 was classified as BRCAness. If the BRCAness score was <0.5, the sample was classified as being non-BRCAness [14].

Results

Among the EUS-FNAB samples obtained from PDAC patients, a pathologist selected the 20 samples with the highest counts of pancreatic carcinoma cells. For these samples, BRCAness was analyzed by MLPA. Table 1 shows the demographics of the 20 patients included in the study. The median age of the patients was 72 years (range, 51–82 years). Fifteen patients (75%) were male. Pancreatic carcinoma was located in the head of the pancreas in seven patients (35%), the body of the pancreas in eight patients (40%), and the tail of the pancreas in five patients (25%). The median greatest tumor dimension was 37 mm (range, 20–55 mm). Based on the International Union Against Cancer TNM classification of malignant tumors (7th edition), two patients (5%) were TNM stage IIA, seven patients (35%) were stage III, and 12 patients (60%) were stage IV. Table 2 shows the results of BRCAness analysis by MLPA. For cases in which reexamination was required due to insufficient DNA (indicated in the table with *), the table reports the results of the reexamination. Initial analysis suggested a poor response due to poor DNA quality in four of the 20 cases (Case Nos. 3, 9, 10, and 15) and due to an insufficient quantity of DNA in another four cases (Case Nos. 1, 5, 7, and 11). For the four cases in which a poor response was suspected to be due to an insufficient quantity of DNA, additional unstained sections were made from FFPE tissue and DNA isolation was re-attempted. In one of the four re-examined cases (Case No. 11), as in the initial examination, an insufficient quantity of DNA was indicated. Results for the five cases with insufficient DNA (indicated in the table with †) are shown for reference. In 15 of the 20 cases (75%), we were able to analyze BRCAness by MLPA using small tissue fragments obtained via EUS-FNAB. Five cases (25%) may have yielded false negatives due to insufficient quantities of DNA. BRCAness was confirmed in one case (5%).

Discussion

Using small tissue fragments obtained via EUS-FNAB, which is performed in the process of diagnosis for most cases of PDAC, we evaluated the feasibility of using MLPA to analyze BRCAness, which has been receiving attention as a novel predictor of anticancer drug sensitivity in PDAC. In doing so, we were able to accurately analyze 75% of the cases in the study. The greatest advantage of MLPA is that it yields results in a short period of time, which is a factor that

Table 1
Patient demographics.

Demographic	
Median Age, y [range]	72 [51–82]
Sex, n (%)	
Male	15 (75)
Female	5 (25)
Location of pancreatic carcinoma, n (%)	
Head of pancreas	7 (35)
Body-Tail of pancreas	13 (65)
Greatest median tumor dimension, mm [range]	37 [20–55]
TNM stage: UICC 7th, n (%)	
IIA	1 (5)
III	7 (35)
IV	12 (60)

Abbreviations: TNM, TNM Classification of Malignant Tumors; UICC, International Union Against Cancer.

makes MLPA a feasible method for analyzing BRCAness in real-world clinical settings. Although there are some issues that must be overcome, the establishment of precision medicine using small tissue fragments obtained via EUS-FNAB may be a breakthrough in PDAC treatment going forward.

One reason that outcomes in PDAC remain poor, despite improved diagnostic technology currently available, is that approximately 80% of cases are diagnosed in an unresectable advanced state [1]. Another reason is that PDAC often recurs quickly, even when it is initially resected. The five-year survival rate for PDAC is 8% in the United States [2] and 7.7% in Japan [3]. Therefore, while improving outcomes in PDAC naturally requires attempts at early detection, it is also necessary to develop chemotherapy-centered treatment for advanced cases. Currently, the most commonly applied chemotherapy regimen is the combination of oxaliplatin, irinotecan, 5-fluorouracil, and leucovorin (FOLFIRONOX); and a combination of gemcitabine and nab-paclitaxel. In the ACCORD trial, which used the FOLFIRONOX regimen, and the MPACT trial, which used the gemcitabine/nab-paclitaxel regimen, overall survival was 11.1 months [4] and 8.5 months [5], respectively. Thus, current chemotherapy fails to yield satisfactory results.

Precision medicine involves a breakthrough in chemotherapy for PDAC, in which a specific treatment that is highly likely to be effective for a given patient is identified. In recent decades, there has been new information available regarding germline and sporadic mutations in the deoxyribonucleic acid (DNA) damage repair pathway in PDAC, leading to the expectation that novel targeted therapies will be developed. This is bolstered by what has been seen for breast cancer. The BRCA1/BRCA2 mutation causes a deficiency in DNA damage repair (DDR) due to inhibition of repair of DNA double-strand breaks by the normal mechanism of homologous recombination [6]. Platinum agents are potentially more effective in patients with DDR mutations due to their cytotoxic effect caused by their binding directly to DNA, causing crosslinking of DNA strands, and thereby inducing DNA double-strand breaks. It has been assumed that BRCA-mutated PDAC is also sensitive to platinum agents [15]. Indeed, favorable results of this nature have been reported, albeit based on retrospective studies [16–18]. Also, inhibiting PARP in cells causes the persistence of DNA lesions normally repaired by homologous recombination [19]. Favorable clinical outcomes have recently been reported for the use of PARP inhibitors in BRCA-mutated PDAC [20]. In addition to BRCA1/2 germline mutations, sporadic mutations may also result in a so-called BRCAness phenotype. Turner et al. define BRCAness in their publication as “traits that usually occur in BRCA1/2 mutation carriers but are also present in some sporadic cancers.” [6] Two clinical trials have recently been conducted with ovarian cancer patients to examine the effects of PARP inhibitors on non-germline BRCA-mutated tumors [21,22]. In both trials, there was a positive response to PARP inhibitors in the BRCA phenotype. Platinum agents and PARP inhibitors are expected to be effective for PDAC with BRCAness as well.

After Vilmann et al. [23] first performed EUS-FNA for pancreatic carcinoma in 1992, which was prior to the ability to biopsy during the procedure, the use of EUS-FNA spread rapidly, particularly in Europe and the United States. Currently, EUS-FNAB is widely used around the world. During this time, in addition to the development of aspiration needles, innovations in aspiration methods [24,25] and in the handling of specimens, such as rapid on-site evaluation (ROSE) [26–29] and macroscopic on-site quality evaluation (MOSE)

Table 2
Result of BRCAness by MLPA.

No.	Age, Sex	Location of tumor	Greatest tumor dimension	DNA			BRCAness score	Result
				Concentration (ng/μL)	Solution volume (μL)	$A_{260/280}$		
1[b]	57, F	B	47	14.7	15	1.84	0.464	non BRCAness
2	57, M	T	46	19.4	15	2.03	0.307	non BRCAness
3	51, M	B	43	6.2	15	2.76	0.313	non BRCAness[a]
4	69, M	H	50	20.1	15	2.04	0.384	non BRCAness
5[b]	75, M	H	27	32.3	60	2.01	0.081	non BRCAness
6	72, F	H	32	17.8	15	2.00	0.371	non BRCAness
7[b]	72, M	T	55	17.1	15	1.82	0.102	non BRCAness
8	71, F	B	34	13.0	15	2.20	0.623	BRCAness
9	76, M	B	23	4.3	15	2.77	0.225	non BRCAness[a]
10	72, M	B	47	3.5	15	3.48	0.115	non BRCAness[a]
11[b]	63, M	T	41	5.6	60	2.69	0.271	non BRCAness[a]
12	72, M	H	24	6.9	15	2.44	0.040	non BRCAness
13	65, F	T	34	27.6	60	2.03	0.392	non BRCAness
14	72, M	B	27	10.1	15	2.27	0.033	non BRCAness
15	79, M	B	42	2.1	60	6.45	0.232	non BRCAness[a]
16	82, M	H	34	30.5	60	2.01	0.050	non BRCAness
17	78, M	B	22	15.4	15	2.05	0.210	non BRCAness
18	62, M	H	20	7.7	15	2.14	0.142	non BRCAness
19	82, F	H	40	15.7	15	2.02	0.071	non BRCAness
20	57, M	T	54	7.7	15	2.29	0.069	non BRCAness

Abbreviations: MLPA, Multiplex Ligation-dependent Probe Amplification; H, head of pancreas; B, body of pancreas; T, tail of pancreas; A260/280, Absorbance 260/280 ratio.
[a] Included as a reference only due to the possibility of poor response stemming from insufficient DNA.
[b] Cases in which re-examination was performed. The results shown are for the second examination.

[30], have improved the diagnostic accuracy of EUS-FNAB. To further improve the diagnostic accuracy for PDAC, attempts have been made in recent years to assess malignancy by also applying molecular methodology to tissue fragments obtained via EUS-FNAB. During pancreatic cancer tumorigenesis, many genetic and epigenetic alterations occur. One of the key features of the genetic basis of PDAC is the point mutation of the KRAS oncogene, which occurs in over 90% of its pathogenesis [31]. Many studies have reported that analysis of the KRAS mutation using EUS-FNAB appears to be highly accurate at differentiating between benign and malignant pancreatic lesions [32–35]. Due to progress in molecular biology, many studies regarding markers related to the development of PDAC have been reported. KRAS mutations, allelic losses of tumor suppressor p16, and deleted in pancreatic cancer locus 4 (DPC4) are highly sensitive markers [36]. Other markers include mucine (MUC) expression [37] and plectin-1 [38]. The results of these studies have been useful in reducing the false-negative rates and the frequency of radical surgery. However, patients must be identified who are most likely to respond to specific agents using small EUS-FNAB specimens of unresectable pancreatic cancer, which accounts for the majority of pancreatic cancer cases. In other words, we still have a significant way to go in order to make precision medicine a reality. Ashida et al. [39] have already reported that deoxycytidine kinase mRNA expression in EUS-FNAB samples of unresectable PDAC may predict the efficacy of gemcitabine.

Multiplex ligation-dependent probe amplification is a method that is applicable for the efficient detection of exon units, large gene deletions, and multiple mutations. To understand the characteristics of BRCAness as genomic copy number abnormalities, Joosse et al. [14] first used breast cancer tissue with germline mutations in BRCA1 in a comprehensive analysis using array comparative genomic hybridization (aCGH). In doing so, they identified copy number abnormalities in specific genomic areas, a profile which they termed “BRCA1-like.” Vollebergh et al. [40] obtained a therapeutic effect from platinum-based chemotherapy for BRCA-1 like tumors, a result that may reflect the fact that “BRCA1-like” also captures the characteristics of BRCAness as well. As part of subsequent translational research to make assessments of “BRCA1-like,” i.e., BRCAness easy to introduce into routine clinical testing, the BRCA1-like profile in aCGH was translated to MLPA. In this research, the concordance between aCGH and MLPA was 94% [41]. The MLPA-BRCAness kit used in the current study has also been used in several recent studies on breast cancer [7–9]. Because MLPA enables efficient analysis of all the necessary information, it can be performed more simply, more quickly, and more cheaply than aCGH or gene sequencing [42,43]. The process, from DNA isolation to BRCAness analysis, takes roughly 2–3 days. Going forward, after platinum agents and PARP inhibitors have been proven effective for PDAC with BRCAness, the rapid results of BRCAness analysis by MLPA will be extremely important in selecting a first-line agent for patients with advanced PDAC. However, in our study, the possibility of a poor response due to insufficient DNA occurred in 1/4 of all the specimens. A quantitative lack of DNA may possibly be resolved by using a 19-gauge needle, which is thicker than the needle used in EUS-FNAB. However, as is understood by many endoscopists, that it is difficult to perform EUS-FNAB for every case with a 19-gauge needle. Therefore, the present study included only cases in which the widely employed 22-gauge needle was used. As another method of resolving the quantitative lack of DNA, it may be important to perform aspiration more frequently and to collect additional samples in advance. The present study included specimens with the highest number of carcinoma cells. However, despite a high quantity of DNA, some cases produced a poor response due to poor DNA quality. Going forward, in preparation for analysis with DNA isolated from FFPE tissue as in the present study, there is a need to minimize the breakdown of nucleic acids, proteins, and other biomolecules in the process of FFPE preparation and to prepare specimens with a constant, or even better, level of quality of specimens that more closely resemble fresh specimens. Specific methods that may help accomplish these goals include the following: use of 10% neutral-buffered formalin solution for specimen fixation; use of a volume of formalin 10-times larger than the volume of tissue; avoiding nucleic acid fragmentation and chemical modification of the nucleotide bases associated with excessive fixation; and inhibiting the degradation of nucleic acids associated with cryopreservation after FFPE preparation [44,45]. Another possibility in order to perform precision medicine is to isolate high-quality DNA from frozen specimens, rather than formalin-fixed specimens.

In BRCAness analysis of PDAC with MLPA using small tissue fragments obtained via EUS-FNAB, issues remain that must be overcome. However, the ability of BRCAness analysis with MLPA to yield results in a short time and at a low cost may make it a feasible and important method in the future for performing precision medicine.

Acknowledgments

The authors would like to extend their heartfelt gratitude to Atsuko Takeuchi for providing valuable technical assistance throughout the course of the study. This work was supported by the 2018 Research Grant for young medical doctors and healthcare professionals from SRL, Inc. The authors have no financial relationships or conflicts of interest to disclose.

References

[1] Koorstra JB, Hustinx SR, Offerhaus GJ, Maitra A. Pancreatic carcinogenesis. Pancreatology 2008;8:110–25.
[2] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA A Cancer J Clin 2016;66:7–30. https://doi.org/10.3322/caac.21332.
[3] Monitoring of cancer incidence in Japan - survival 2006-2008 report. Center for Cancer Control and Information Services, National Cancer Center; 2016.
[4] Conroy T, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med 2011;364:1817–25.
[5] Von Hoff DD, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med 2013;369:1691–703.
[6] Turner N, Tutt A, Ashworth A. Hallmarks of 'BRCAness' in sporadic cancers. Nat Rev Canc 2004;4:814–9.
[7] Akashi-Tanaka S, Watanabe C, Takamaru T, Kuwayama T, Ikeda M, Ohyama H, et al. BRCAness predicts resistance to taxane-containing regimens in triple negative breast cancer during neoadjuvant chemotherapy. Clin Breast Canc 2015;15:80–5.
[8] Tanino H, Kosaka Y, Nishimiya H, Tanaka Y, Minatani N, Kikuchi M, et al. BRCAness and prognosis in triple-negative breast cancer patients treated with neoadjuvant chemotherapy. PLoS One 2016;11:e0165721.
[9] Mori H, Kubo M, Nishimura R, Osako T, Arima N, Okumura Y, et al. BRCAness as a biomarker for predicting prognosis and response to anthracycline-based adjuvant chemotherapy for patients with triple-negative breast cancer. PLoS One 2016;11:e0167016.
[10] Slater HR, Bruno DL, Ren H, Pertile M, Schouten JP, Choo KH. Rapid, high throughput prenatal detection of aneuploidy using a novel quantitative method (MLPA). J Med Genet 2003;40:907–12.
[11] Hömig-Hölzel C, Savola S. Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. Diagn Mol Pathol 2012;21:189–206.
[12] Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res 2002;30:e57.
[13] Oonk AM, van Rijn C, Smits MM, Mulder L, Laddach N, Savola SP, et al. Clinical correlates of 'BRCAness' in triple-negative breast cancer of patients receiving adjuvant chemotherapy. Ann Oncol 2012;23:2301–5.
[14] Joosse SA, van Beers EH, Tielen IH, Horlings H, Peterse JL, Hoogerbrugge N, et al. Prediction of BRCA1-association in hereditary non-BRCA1/2 breast carcinomas with array-CGH. Breast Canc Res Treat 2009;116:479–89.
[15] van der Heijden MS, Brody JR, Dezentje DA, Gallmeier E, Cunningham SC, Swartz MJ, et al. *In vivo* therapeutic responses contingent on Fanconi anemia/BRCA2 status of the tumor. Clin Cancer Res 2005;11:7508–15.

[16] Golan T, Kanji ZS, Epelbaum R, Devaud N, Dagan E, Holter S, et al. Overall survival and clinical characteristics of pancreatic cancer in BRCA mutation carriers. Br J Canc 2014;111:1132–8.
[17] Lowery MA, Kelsen DP, Stadler ZK, Yu KH, Janjigian YY, Ludwig E, et al. An emerging entity: pancreatic adenocarcinoma associated with a known BRCA mutation: clinical descriptors, treatment implications, and future directions. Oncol 2011;16:1397–402.
[18] Kondo T, Kanai M, Kou T, Sakuma T, Mochizuki H, Kamada M, et al. Association between homologous recombination repair gene mutations and response to oxaliplatin in pancreatic cancer. Oncotarget 2018;9:19817–25.
[19] Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 2005;434:917–21.
[20] O'Reilly EM, Lee JW, Lowery MA, Capanu M, Stadler ZK, Moore MJ, et al. Phase 1 trial evaluating cisplatin, gemcitabine, and veliparib in 2 patient cohorts: germline BRCAmutation carriers and wild-type BRCA pancreatic ductal adenocarcinoma. Cancer 2018;124:1374–82.
[21] Swisher EM, Lin KK, Oza AM, Scott CL, Giordano H, Sun J, et al. Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial. Lancet Oncol 2017;18:75–87.
[22] Mirza MR, Monk BJ, Herrstedt J, Oza AM, Mahner S, Redondo A, et al. Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. N Engl J Med 2016;375:2154–64.
[23] Vilmann P, Jacobsen GK, Henriksen FW, Hancke S. Endoscopic ultrasonography with guided fine needle aspiration biopsy in pancreatic disease. Gastrointest Endosc 1992;38:172–3.
[24] Bang JY, Magee SH, Ramesh J, Trevino JM, Varadarajulu S. Randomized trial comparing fanning with standard technique for endoscopic ultrasound-guidedfine-needle aspiration of solid pancreatic mass lesions. Endoscopy 2013;45:445–50.
[25] Nakai Y, Isayama H, Chang KJ, Yamamoto N, Hamada T, Uchino R, et al. Slow pull versus suction in endoscopic ultrasound-guided fine-needle aspiration of pancreatic solid masses. Dig Dis Sci 2014;59:1578–85.
[26] Chang KJ, Katz KD, Durbin TE, Erickson RA, Butler JA, Lin F, et al. Endoscopic ultrasound-guided fine-needle aspiration. Gastrointest Endosc 1994;40:694–9.
[27] Klapman JB, Logrono R, Dye CE, Waxman I. Clinical impact of on-site cytopathology interpretation on endoscopic ultrasound-guided fine needle aspiration. Am J Gastroenterol 2003;98:1289–94.
[28] Cleveland P, Gill KR, Coe SG, Woodward TA, Raimondo M, Jamil L, et al. An evaluation of risk factors for inadequate cytology in EUS-guided FNA of pancreatic tumors and lymph nodes. Gastrointest Endosc 2010;71:1194–9.
[29] Iglesias-Garcia J, Dominguez-Munoz JE, Abdulkader I, Larino-Noia J, Eugenyeva E, Lozano-Leon A, et al. Influence of on-site cytopathology evaluation on the diagnostic accuracy of endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) of solid pancreatic masses. Am J Gastroenterol 2011;106:1705–10.
[30] Iwashita T, Yasuda I, Mukai T, Doi S, Nakashima M, Uemura S, et al. Macroscopic on-site quality evaluation of biopsy specimens to improve the diagnostic accuracy during EUS-guided FNA using a 19-gauge needle for solid lesions: a single-center prospective pilot study (MOSE study). Gastrointest Endosc 2015;81:177–85.
[31] Almoguera C, Shibata D, Forrester K, Martin J, Arnheim N, Perucho M. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. Cell 1988;53:549–54.
[32] Tada M, Komatsu Y, Kawabe T, Sasahira N, Isayama H, Toda N, et al. Quantitative analysis of K-ras gene mutation in pancreatic tissue obtained by endoscopic ultrasonography-guided fine needle aspiration: clinical utility for diagnosis of pancreatic tumor. Am J Gastroenterol 2002;97:2263–70.
[33] Bournet B, Souque A, Senesse P, Assenat E, Barthet M, Lesavre N, et al. Endoscopic ultrasound-guided fine-needle aspiration biopsy coupled with KRAS mutation assay to distinguish pancreatic cancer from pseudo tumoral chronic pancreatitis. Endoscopy 2009;41:552–7.
[34] Reicher S, Boyar FZ, Albitar M, Sulcova V, Agersborg S, Nga V, et al. Fluorescence in situ hybridization and K-ras analyses improve diagnostic yield of endoscopic ultrasound-guided fine-needle aspiration of solid pancreatic masses. Pancreas 2011;40:1057–62.
[35] Ogura T, Yamao K, Hara K, Mizuno N, Hijioka S, Imaoka H, et al. Prognostic value of K-ras mutation status and subtypes in endoscopic ultrasound-guided fine-needle aspiration specimens from patients with unresectable pancreatic cancer. J Gastroenterol 2013;48:640–6.
[36] Salek C, Benesova L, Zavoral M, Nosek V, Kasperova L, Ryska M, et al. Evaluation of clinical relevance of examining K-ras, p16 and p53 mutations along with allelic losses at 9p and 18q in EUS-guided fine needle aspiration samples of patients with chronic pancreatitis and pancreatic cancer. World J Gastroenterol 2007;13:3714–20.
[37] Wang Y, Gao J, Li Z, Jin Z, Gong Y, Man X. Diagnostic value of mucins (MUC1, MUC2 and MUC5AC) expression profile in endoscopic ultrasound-guided fine-needle aspiration specimens of the pancreas. Int J Cancer 2007;121:2716–22.
[38] Park JK, Paik WH, Song BJ, Ryu JK, Kim MA, Park JM, et al. Additional K-ras mutation analysis and Plectin-1 staining improve the diagnostic accuracy of pancreatic solid mass in EUS-guided fine needle aspiration. Oncotarget 2017;8:64440–8.
[39] Ashida R, Nakata B, Shigekawa M, Mizuno N, Sawaki A, Hirakawa K, et al. Gemcitabine sensitivity-related mRNA expression in endoscopic ultrasound-guided fine-needle aspiration biopsy of unresectable pancreatic cancer. J Exp Clin Canc Res 2009;28:83.
[40] Vollebergh MA, Lips EH, Nederlof PM, Wessels LF, Schmidt MK, van Beers EH, et al. An aCGH classifier derived from BRCA1-mutated breast cancer and benefit of high-dose platinum-based chemotherapy in HER2-negative breast cancer patients. Ann Oncol 2011;22:1561–70.
[41] Lips EH, Laddach N, Savola SP, Vollebergh MA, Oonk AM, Imholz AL, et al. Quantitative copy number analysis by Multiplex Ligation-dependent Probe Amplification (MLPA) of BRCA1-associated breast cancer regions identifies BRCAness. Breast Cancer Res 2011;13:R107.
[42] Slater HR, Bruno DL, Ren H, Pertile M, Schouten JP, Choo KH. Rapid, high throughput prenatal detection of aneuploidy using a novel quantitative method (MLPA). J Med Genet 2003;40:907–12.
[43] Hömig-Hölzel C, Savola S. Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. Diagn Mol Pathol 2012;21:189–206.
[44] Srinivasan M, Sedmak D, Jewell S. Effect of fixatives and tissue processing on the content and integrity of nucleic acids. Am J Pathol 2002;161:1961–71.
[45] Sato M, Kojima M, Nagatsuma AK, Nakamura Y, Saito N, Ochiai A. Optimal fixation for total pre analytic phase evaluation in pathology laboratories: a comprehensive study including immunohistochemistry, DNA, and mRNA assays. Pathol Int 2014;64:209–16.