Surgery Today (2019) 49:887–893
https://doi.org/10.1007/s00595-019-01797-7

REVIEW ARTICLE

Clinical assessment of the *GNAS* mutation status in patients with intraductal papillary mucinous neoplasm of the pancreas

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Received: 9 January 2019 / Accepted: 7 February 2019 / Published online: 16 March 2019

Abstract

Intraductal papillary mucinous neoplasm (IPMN) of the pancreas is characterized by cystic dilation of the pancreatic duct, caused by mucin hypersecretion, with slow progression via the adenoma–carcinoma sequence mechanism. Mutation of *GNAS* at codon 201 is found exclusively in IPMNs, occurring at a rate of 41–75%. Recent advances in molecular biological techniques have demonstrated that *GNAS* mutation might play a role in the transformation of IPMNs after the appearance of neoplastic cells, rather than in the tumorigenesis of IPMNs. *GNAS* mutation is observed frequently in the intestinal subtype of IPMNs with MUC2 expression, and less frequently in IPMNs with concomitant pancreatic ductal adenocarcinoma (PDAC). Research has focused on assessing *GNAS* mutation status in clinical practice using various samples. In this review, we discuss the clinical application of *GNAS* mutation assessment to differentiate invasive IPMNs from concomitant PDAC, examine the clonality of recurrent IPMNs in the remnant pancreas using resected specimens, and differentiate pancreatic cystic lesions using cystic fluid collected by endoscopic ultrasound-guided fine needle aspiration (EUS-FNA), duodenal fluid, and serum liquid biopsy samples.

Keywords IPMN · *GNAS* · *KRAS* · Pancreas · Ductal adenocarcinoma

Introduction

Intraductal papillary mucinous neoplasms (IPMNs) of the pancreas progress slowly via the adenoma–carcinoma sequence mechanism. IPMNs, characterized by cystic dilation of the pancreatic duct by mucin hypersecretion are morphologically classified into branch duct (BD), main duct (MD), and mixed type (with features of both BD- and MD-IPMNs) [1–3]. Approximately 60% of MD-IPMNs are malignant, including high-grade dysplasia (non-invasive carcinoma) and invasive carcinoma; thus, MD-IPMNs are a direct indication for resection [1–3]. In contrast, most BD-IPMNs are benign and it is often difficult to distinguish them from other cystic lesions such as mucinous cystic neoplasms (MCNs), macrocystic serous cystic neoplasms (SCNs), and pseudocysts, by radiological assessments alone [1–5]. Moreover, pancreatic ductal adenocarcinoma (PDAC) is often found concomitant with benign BD-IPMN, with a yearly incidence of ~ 1% [1–3], so is now recognized as high risk for the development of PDAC. IPMNs themselves also progress to pancreatic cancer; therefore, investigating their molecular characteristics might lead to a better understanding of the progression of IPMN as well as PDAC, and improve the early diagnosis and treatment of PDAC. Because *GNAS* mutation is found exclusively in IPMNs among the various pancreatic neoplasms [6, 7], numerous studies have attempted to clarify its significance in IPMNs and to apply *GNAS* mutational assessment in daily practice. We review the literature on the clinical application of *GNAS* mutational status during the management of IPMNs.

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GNAS mutation and tumorigenesis

The *GNAS* gene encodes the α-subunit of a stimulatory G-protein (Gαs), which activates adenylate cyclase and increases the level of cyclic adenosine monophosphate (cAMP) [8]. Mutation of *GNAS*, which is frequently detected in codon 201 in various neoplasms, leads to sustained activation of Gαs and an increased level of cAMP, which activates protein kinase A and subsequent cancer-promoting activities [8–12]. McCune–Albright syndrome is an autosomal dominant genetic disease caused by *GNAS* mutation, characterized by fibrosis dysplasia, precocious puberty, and café au lait spots [9, 13]. Neoplasms in the hepatopancreatobiliary system develop in approximately 15% of patients with McCune–Albright syndrome. However, McCune–Albright syndrome is rare, whereas *GNAS* is a well-known important oncogene [14]; therefore, most *GNAS* mutation-related neoplasms are considered to occur through the accumulation of acquired gene mutations.

Methods for assessing *GNAS* mutation

Four major sequencing methods are available for genetic assessment: Sanger sequencing, pyrosequencing, real-time polymerase chain reaction (RT-PCR)-based sequencing, and digital next-generation sequencing (NGS) [15, 16]. Figure 1 shows representative genetic assessment results of IPMN samples for wild-type and mutant *GNAS* using Sanger sequencing and pyrosequencing. The minimum limit of mutation detection for Sanger sequencing is 20–30%, compared with 1–5% for pyrosequencing, 0.1–1% for RT-PCR-based methods, and 0.1% for digital NGS [15, 16]. Although the digital NGS and RT-PCR-based methods show high sensitivity in detecting mutation, there is the possibility for false-positive results because of insufficient quality of purified DNA or errors in the RT-PCR process. The selection of these methods for genetic assessment is based on the type of samples (macro-dissected samples from resected specimens may potentially contain sufficient DNA for Sanger sequencing, whereas liquid biopsy samples with little DNA should be investigated by other more sensitive methods) as well as institutional experience and the investigators' preference.

GNAS mutation and IPMNs

Mutation of the *GNAS* gene at codon 201 is observed in 41–75% of IPMNs [3] and various *GNAS* transgenic mice have been established [17–21]. Taki et al. [17] succeeded in generating a mouse model of the IPMN with the cooperation of $GNAS^{R201H}$ and activated *KRAS* ($KRAS^{G12D}$). Notably, the $GNAS^{R201H}$-only mice failed to show tumorigenesis and *KRAS* co-mutation was essential for the development of IPMNs [17]. In fact, even in the absence of *GNAS* mutation, IPMNs developed in mice harboring $KRAS^{G12D}$ along with the loss of other tumor-suppressor genes such as *activin A*

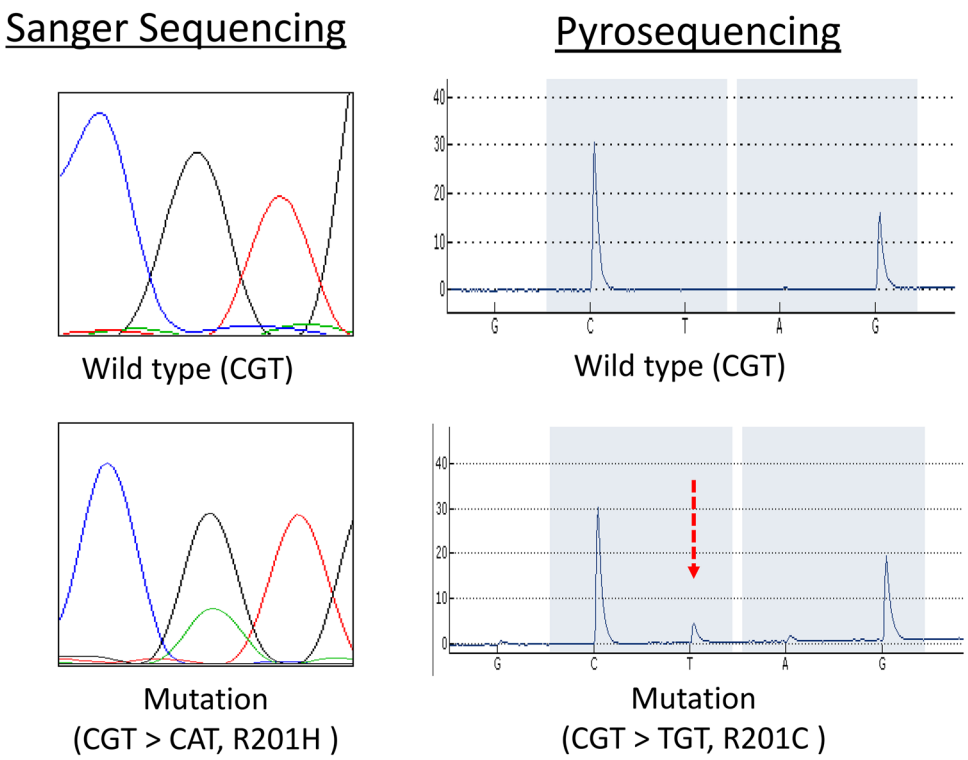

Fig. 1 Representative methods of assessing *GNAS* mutational status. Four major sequencing methods are available for genetic assessment: Sanger sequencing, pyrosequencing, real-time polymerase chain reaction (RT-PCR)-based sequencing, and digital next-generation sequencing (NGS). Representative genetic assessment results of IPMN samples for wild-type and mutant *GNAS* at codon 201 using Sanger sequencing (left panel) and pyrosequencing (right panel) are shown

receptor type IB [18], *Trefoil factor 2* [19], and *PTEN* [20]. These findings could explain the clinical observations that not all IPMNs harbor *GNAS* mutation.

The rate of *GNAS* mutation in resected IPMNs did not vary in accordance with the degree of dysplasia. Tan et al. [22] reported that the prevalence of *GNAS* mutation was 53% in low-grade dysplasia, 59% in high-grade dysplasia and 61% in invasive cancer. Tamura et al. [23] demonstrated that the prevalence of *GNAS* mutation was 64% in low-grade dysplasia, 83% in intermediate dysplasia, 79% in high-grade dysplasia and 56% in invasive carcinoma. Therefore, *GNAS* mutation might be a relatively early event in the progression of IPMNs and a later event than *KRAS* mutation.

GNAS mutation and the intestinal subtype

In addition to morphological classifications, IPMNs are classified pathologically into four subtypes: gastric, intestinal, pancreatobiliary, and oncocytic [24]. The intestinal subtype is characterized by MUC2 expression [24], a dilated orifice of the duodenal papilla by mucin hypersecretion [25], and progression to colloid carcinoma [26, 27]. Conversely, the pancreatobiliary subtype is considered to be a phenotype of the malignant transformation from the gastric subtype with MUC2-negative expression, indicating tubular carcinoma [26, 27]. The oncocytic subtype is rare and only detected in 8% of resected IPMNs [24]. Thus, the alternate classification of IPMN; namely, intestinal (MUC2-positive) and non-intestinal (MUC2-negative) may be practical during the management of IPMNs [26]. The prognosis after resection of intestinal-invasive IPMNs (colloid carcinoma) is better than that after resection of non-intestinal invasive IPMNs (tubular carcinoma) [26, 27]. *GNAS* mutation is more frequently observed in intestinal IPMNs than in the non-intestinal subtype [23, 27–29]; therefore, *GNAS* mutation may play a role in inducing MUC2 expression and less aggressive phenotypes. Although it remains unclear whether the gastric subtype might harbor *GNAS* mutation and subsequently obtain the phenotype of the intestinal subtype with MUC2 expression, *GNAS* mutation is considered to play a role in the transformation of IPMNs after the development of neoplastic cells rather than in the tumorigenesis of IPMNs.

GNAS mutation and concomitant ductal adenocarcinoma

Since its first case report in 1997 [30], concomitant PDAC has become one of the greatest concerns during the management of IPMNs [31–33]. The prevalence of concomitant PDAC in IPMNs is ~1% each year [1–3] and there has been much focus on identifying its risk factors, to promote early diagnosis of concomitant PDAC in the management of IPMNs.

Ideno et al. [34] reported that IPMNs with concomitant PDAC are frequently of the gastric subtype and rarely have *GNAS* mutation. Most PDACs develop in the pancreas with BD-IPMN [1–3], and most BD-IPMNs are of the gastric subtype. In contrast, Tamura et al. [35] demonstrated that concomitant PDACs also occur in the pancreas with MD-IPMNs, and these MD-IPMNs are characterized as having the gastric subtype with wild-type *GNAS*, indicating that concomitant PDACs tend to develop in the pancreas with IPMNs with wild-type *GNAS*, irrespective of the BD- or MD-IPMN subtype. Assessment of the *GNAS* mutation status might be helpful in predicting the development of concomitant PDAC at the time of initial assessment of IPMNs and during surveillance for benign IPMNs or of the remnant pancreas after partial pancreatectomy for an IPMN [36].

Potential clinical applications of *GNAS* mutational assessment

Based on the findings summarized in Table 1, much research has focused on applying the assessment of *GNAS* mutation status in clinical practice using various samples. In this section, we discuss the following five potential clinical applications of *GNAS* mutational samples: Differentiating invasive IPMNs from concomitant pancreatic ductal adenocarcinoma using resected specimens; assessing clonality of recurrent IPMNs in the remnant pancreas using resected specimens; and differentiating pancreatic cystic lesions using cystic fluid collected by endoscopic ultrasound-guided fine needle aspiration (EUS-FNA), duodenal fluid, and serum liquid biopsy samples (Table 2).

Clinical differentiation of invasive IPMNs and concomitant PDAC

During the pathological assessment of resected invasive carcinoma, concomitant PDAC with an IPMN is generally defined as histologically separated from co-existing IPMN

Table 1 Summary of *GNAS* mutation in intraductal papillary mucinous neoplasms

1. *GNAS* mutation at codon 201 is observed exclusively in IPMNs among the various pancreatic neoplasms
2. Frequency of *GNAS* mutation in IPMNs is 41–75% (but not 100%)
3. In transgenic mice, *GNAS* mutation is not always necessary for the induction of IPMN, (see '2' for explanation)
4. *GNAS* mutation is frequently observed in the intestinal subtype of IPMNs

IPMN intraductal papillary mucinous neoplasm

Table 2 Possible clinical application of *GNAS* mutational assessment

Purpose	Sample
Differentiation of invasive IPMNs from concomitant pancreatic ductal adenocarcinoma	Resected specimen
Assessment of clonality of recurrent IPMN in the remnant pancreas	Resected specimen
Differentiation of pancreatic cystic lesions	Cystic fluid collected by EUS-FNA
	Duodenal fluid
	Serum liquid biopsy

IPMN intraductal papillary mucinous neoplasm, *EUS-FNA* endoscopic ultrasound-guided fine needle aspiration

by an uninvolved segment of normal pancreatic duct [34]. Moreover, the lesion from an intestinal IPMN is likely to be colloid carcinoma [37], and *GNAS* mutation is found exclusively in IPMNs. Nevertheless, it is often difficult to distinguish invasive carcinoma derived from an IPMN from concomitant PDAC. In fact, a Japanese multicenter study [38] reported that establishing whether a cancer lesion was derived from IPMN or was concomitant with IPMN by pathological inspection alone was difficult in 30 of 183 (16.4%) patients who underwent pancreatectomy for IPMN.

Tamura et al. [39] assessed the *GNAS*/*KRAS* mutational status of resected specimens with the aim of distinguishing invasive carcinoma derived from IPMN from concomitant PDAC. They demonstrated that the *GNAS*/*KRAS* mutational status of clinically diagnosed concomitant PDAC was different from that of IPMNs in the same pancreas in 17 of 19 cases (89%). They also found that the *GNAS*/*KRAS* mutational status was the same in the invasive and non-invasive components in 18 of 21 (86%) lesions clinically diagnosed as invasive IPMN. Thus, carcinoma derived from an IPMN could be distinguished from concomitant PDAC in 35 of 40 cases (87.5%) by *GNAS*/*KRAS* mutational analyses. Based on these findings, the authors presented a diagnostic strategy to distinguish these two entities [39]:

1. If an undiagnosed lesion has *GNAS* mutation, this is invasive carcinoma derived from IPMN.
2. If an undiagnosed lesion does not have *GNAS* mutation, then the *GNAS* mutational status of the co-existing non-invasive IPMN should be checked; if the co-existing IPMN has *GNAS* mutation, then the undiagnosed lesion is concomitant PDAC.
3. If neither the undiagnosed invasive lesion or the co-existing non-invasive IPMN has *GNAS* mutation, then the *KRAS* mutational status of both lesions should be checked: if the *KRAS* mutational status differs, the undiagnosed lesion is concomitant PDAC.
4. If the *KRAS* mutational status is the same, then the status of *p53* and *p16*/*CDKN2A*, which are frequently mutated in PDAC, should be checked. A different mutation status indicates that the co-existing lesion would be concomitant PDAC, whereas the same mutation status indicates that the co-existing lesion would be invasive carcinoma derived from an IPMN.

Omori et al. [40] recently analyzed resected IPMNs using targeted-NGS and reported three different progression pathways from IPMN to pancreatic cancer; namely, "Sequential", "Branch-off", and "De novo." "Sequential" is for carcinoma derived from an IPMN, 67% of which have *GNAS* mutation, whereas "Branch-off" and "De novo" are for concomitant PDACs, and none of these have *GNAS* mutation. Interestingly, these authors demonstrated that in the "Branch-off" pathway, IPMNs and concomitant PDAC may have the same origin according to the "clonal evolution" theory [40].

Miyasaka et al. recently demonstrated that patients who undergo resection of concomitant PDAC are at high risk of having a second concomitant PDAC in the remnant pancreas, indicating that distinguishing these two entities can be used in postoperative prediction. Distinguishing these two lesions might also lead to a better understanding of the mechanism of the development and natural history of IPMNs as well as PDAC. In contrast, the treatment strategy for invasive IPMNs and concomitant PDAC seems to be almost the same in terms of the planned operation, adjuvant treatment and surveillance protocol. In fact, a Japanese multicenter study suggested the possibility of the same biological behavior in invasive IPMNs and concomitant PDAC, as the 1- and 3-year postoperative survival rates for invasive IPMN (96% and 62%, respectively), were almost the same as those for concomitant PDAC (93% and 60%, respectively). Moreover, these two diseases can be distinguished by detailed examination of the resected specimen. Therefore, how to distinguish these two categories using *GNAS* mutational assessment preoperatively to decide on the best treatment strategy remains unclear.

Assessment of clonality of recurrent IPMNs in the remnant pancreas using resected specimens

Establishing the adequate resection line for MD-IPMN is often difficult and total pancreatectomy is sometimes needed. Although recurrent lesions may develop in the remnant pancreas after partial pancreatectomy for MD-IPMN, even with a negative surgical margin, remnant total pancreatectomy provides a favorable prognosis [35]. Therefore, the international consensus guidelines [3] recommend not to leave high-grade dysplasia or invasive carcinoma at the resection margin in partial pancreatectomy for MD-IPMN, and to avoid total pancreatectomy as much as possible. The morphology of the recurrent lesion is usually similar to that of the initial lesion. Some Japanese groups have shown that recurrent lesions might develop via monoclonal skip progression based on assessment of the pathological subtype, *GNAS*/*KRAS* mutational status, and microarray for mRNA expression [23, 35, 41].

Clinical differentiation of pancreatic cystic lesions by assessing cystic fluid collected by EUS-FNA

Confirming a differential diagnosis of a cystic lesion of the pancreas by radiological investigation alone is often difficult. Among the various cystic lesions, differentiating BD-IPMN, MCN and macrocystic SCN is clinically important because BD-IPMN and MCN must be monitored or resected because of their potential for progression to malignancy, whereas SCN is mostly benign and can be observed without resection [1–5]. Recent studies show that measuring carcinoembryonic antigen (CEA) in the cystic fluid obtained by EUS-FNA can distinguish MCN from other cystic lesions; however, the distinction between IPMN and MCN is still difficult [3, 5].

As described earlier, *GNAS* mutation is observed exclusively in IPMNs among the pancreatic cystic lesions [6, 7]; thus, the assessment of *GNAS* mutational status in cystic fluid obtained by EUS-FNA is expected to be useful for the differential diagnosis and further management of pancreatic cysts [42–45]. Kadayifci et al. [42] investigated 197 patients, including 108 with IPMN and 89 with another pancreatic cystic lesion, and found that the diagnostic accuracy of *GNAS* mutational assessment for IPMNs was 76.6% and the combination of CEA and *KRAS* mutational assessment, in addition to *GNAS* testing, increased the accuracy to 86.2%. Singhi et al. [43] reported that the sensitivity and specificity of *KRAS* and/or *GNAS* mutational assessments for IPMNs by NGS were 100% and 96%, respectively, and this sensitivity was higher than that of Sanger sequencing (72%).

EUS-FNA carries a risk of needle tract seeding or dissemination when performed in patients with malignant neoplasms [46]. The European guidelines [5] recently recommended that EUS-FNA only be performed when the results are expected to change the clinical management and should not be done if the diagnosis has been established by cross-sectional imaging or where there is a clear indication for surgery. Therefore, *GNAS* testing using cystic fluid obtained by EUS-FNA might be useful to make a differential diagnosis of a pancreatic cyst that does not have any sign of malignancy and to plan further surveillance.

Clinical differentiation of pancreatic cystic lesions by assessing duodenal fluid

Various molecular biomarkers have been identified in pancreatic juice, which is in direct contact with the neoplastic cells of IPMNs; thus, the assessment of pancreatic juice is expected to be useful for the diagnosis of various pancreatic diseases including IPMNs. However, performing endoscopic retrograde pancreatography (ERP) to collect pancreatic juice carries a risk in patients with severe pancreatitis, and routine ERP during management of IPMN is not recommended in the guidelines [3, 5]. In contrast, duodenal fluid, including pancreatic juice, can be collected easily during screening of upper gastrointestinal endoscopy [47]. Several investigators [48, 49] have recently evaluated *GNAS*/*KRAS* mutation in duodenal fluid in patients with pancreatic diseases in daily practice.

Kanda et al. [48] reported that *GNAS* mutations were detected in secretin-stimulated duodenal fluid samples from 50 of 78 (64%) patients with an IPMN, similar to the reported prevalence of ~66% in resected specimens. They also found that 8 of 61 subjects who had serial duodenal fluid evaluation done, had *GNAS* mutation on baseline assessment, even though baseline radiological images showed no sign of a cyst, which appeared later. Ideno et al. [49] found that the *GNAS* status in duodenal fluid was consistent with that in resected tissue in 21 of 23 (91%) patients, and speculated that a duodenal fluid DNA test could predict the *GNAS* status of IPMNs. As described earlier, IPMNs concomitant with PDACs are frequently of the gastric subtype with wild-type *GNAS* [34], and thus duodenal fluid evaluation for *GNAS* status may be helpful to identify patients at high risk for concomitant PDAC.

Clinical differentiation of pancreatic cystic lesions by the assessment of serum

Circulating cell-free DNA is expected to be a new diagnostic tool for various pancreatic diseases [50]. Berger et al. [51] reported detecting *GNAS* mutation in the circulating cell-free DNA in 15 of 21 (71.4%) patients in whom a benign IPMN was diagnosed and surveyed without resection, while the concordance rate of *GNAS* mutation status between cell-free DNA and resected specimen was only 56.3%. They concluded that although cell-free DNA-based liquid biopsy

can be performed easily and safely, further investigation is needed to improve its diagnostic ability for IPMNs.

Conclusions

GNAS mutational analyses of various samples can provide valuable information for the differential diagnosis, clonality assessment, and identification of high risk for concomitant PDAC during the management of IPMNs.

Acknowledgements This study was supported by a Grant-in-Aid from the Japan Society for the Promotion of Sciences for Scientific Research (B) (Grant no 16H05417).

Compliance with ethical standards

Conflict of interest We have no conflicts of interest to declare.

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