Frequent GNAS mutations in low-grade appendiceal mucinous neoplasms

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Background: The molecular basis for the development of appendiceal mucinous tumours, which can be a cause of pseudomyxoma peritonei, remains largely unknown.

Methods: Thirty-five appendiceal mucinous neoplasms were analysed for GNAS and KRAS mutations. A functional analysis of mutant GNAS was performed using a colorectal cancer cell line.

Results: A mutational analysis identified activating GNAS mutations in 16 of 32 low-grade appendiceal mucinous neoplasms (LAMNs) but in none of three mucinous adenocarcinomas (MACs). KRAS mutations were found in 30 LAMNs and in all MACs. We additionally analysed a total of 186 extra-appendiceal mucinous tumours and found that GNAS mutations were highly prevalent in intraductal papillary mucinous tumours of the pancreas (88%) but were rare or absent in mucinous tumours of the colorectum, ovary, lung and breast (0–9%). The prevalence of KRAS mutations was quite variable among the tumours. The introduction of the mutant GNAS into a colorectal cancer cell line markedly induced MUC2 and MUC5AC expression, but did not promote cell growth either in vitro or in vivo.

Conclusion: Activating GNAS mutations are a frequent and characteristic genetic abnormality of LAMN. Mutant GNAS might play a direct role in the prominent mucin production that is a hallmark of LAMN.

Keywords: low-grade mucinous appendiceal neoplasm; GNAS; KRAS

Primary appendiceal adenocarcinomas are estimated to occur in 1 to 2 per 1 000 000 persons per year (Nielsen et al., 1991; Thomas and Sobin, 1995; Smeenk et al., 2008); among them, mucinous tumours constitute the most common histological type (Carr et al., 1995). The histological classification of appendiceal mucinous neoplasms is controversial because of the frequent discrepancies between the histological findings and clinical behaviour. Previously, appendiceal mucinous neoplasms were classified as either an adenoma or an adenocarcinoma based on histological evidence of invasive growth (Carr et al., 2000). However, unlike ordinary-type adenocarcinomas, appendiceal mucinous tumours may show intraperitoneal spreading even in the absence of high-grade cytological atypia or apparent invasive features (Panarelli and Yantiss, 2011).

Misdraji et al. (2003) reviewed 107 cases of appendiceal mucinous neoplasms, and classified them into low-grade appendiceal mucinous neoplasms (LAMNs) and mucinous adenocarcinoma (MAC) based on their architectural complexity and degree of cytological atypia. Low-grade appendiceal mucinous neoplasms lack histological evidence of invasion and exhibit a villous or flat proliferation of mucinous epithelium with low-grade atypia. Meanwhile, MAC is characterised by high-grade cytological atypia and complex epithelial proliferation and often exhibits lymphatic and hematogenous invasion (Misdraji et al., 2003; Carr and Sobin, 2010). Their study showed that while LAMNs may spread outside the appendix, LAMNs were associated with a better prognosis than MACs, indicating the prognostic relevance of this classification (Misdraji et al., 2003). Currently, this classification has been adopted by the World Health Organization classification (Carr and Sobin, 2010).

Appendiceal mucinous tumours may spread throughout the peritoneal cavity, causing the slow but relentless accumulation of...
mucin, a condition known as pseudomyxoma peritonei (Carr et al., 2012). Over time, mucin accumulation in the peritoneal cavity gradually causes massive symptomatic distension and functional gastrointestinal obstruction. The overall 10-year survival rates of pseudomyxoma peritonei were reported to be 21–45% (Misdraji et al., 2003; Miner et al., 2005), but a recent study reported a rate of 63% for patients treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (Chua et al., 2012).

The molecular basis for the development of appendiceal mucinous tumours remains largely unknown. KRAS mutation is present in the majority of LAMNs and MACs (Szych et al., 2002; Kabbani et al., 2002; Zauber et al., 2011) and is virtually the only recurrent genetic abnormality identified so far. Microsatellite instability and p53 overexpression are reported to be infrequent (Carr et al., 2003; Miner et al., 2005), but a recent study reported a rate of 63% for patients treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (Chua et al., 2012).

In tumours concurrently harbouring two nucleotide substitutions in GNAS, the PCR products were subcloned using the TOPO TA cloning kit (Invitrogen, San Diego, CA, USA) and each clone was sequenced as described above.

**Materials and methods**

**Study group.** This study was approved by the Ethics Committee of the National Cancer Center, Tokyo, Japan. A total of 35 appendiceal mucinous tumours were identified in our case files between 1974 and 2012. We also retrieved a total of 186 mucinous tumours of extra-appendiceal origin, including those from the colorectum, ovary, pancreas, lung and breast. Tissue samples were provided by the National Cancer Center Biobank, Japan.

**Histological analysis.** All the tissue samples were obtained by surgical resection and were fixed in 10% formalin and embedded in paraffin. Appendiceal mucinous neoplasms were histologically re-evaluated and classified into LAMN and MAC based on the definitions proposed by Misdraji et al., 2003. Tumour samples of the primary site were not available for four LAMNs, and specimens obtained from peritoneal or omental deposits were analysed in these cases.

Immunohistochemistry was performed for all the appendiceal mucinous tumours. Deparaffinised 4-µm-thick sections from each paraffin block were exposed to 0.3% hydrogen peroxide for 15 min to block endogenous peroxidase activity. Antigen retrieval was performed by autoclaving in a 10-mM citrate buffer (pH 6.0) for 10 min. Anti-MUC2 (Ccp58; 1:200 dilution; Novocastra, Newcastle upon Tyne, England) and anti-MUC5AC (CLH2; 1:200 dilution; Novocastra) were used as the primary antibodies. For staining, we used an automated stainer (Dako, Glostrup, Denmark) according to the vendor’s protocol. ChemMate EnVision (Dako) methods were used for detection. The immunohistochemistry for MUC2 and MUC5AC were scored as: 0, <10% positive cells; +1, 11–50% positive cells; +2, >50% positive cells.

**Mutation analysis.** In all, 10-µm sections of the tumour specimens were stained briefly with haematoxylin and used for DNA extraction. The tumour epithelium was dissected using sterilised toothpicks under a microscope. The dissected samples were incubated in 50 µl of DNA extraction buffer (50 mM Tris–HCl, pH 8.0, 1 mM ethylenediaminetetraacetic acid, 0.5% (v/v) Tween 20, 200 µg ml−1 proteinase K) at 30°C overnight. Proteinase K was inactivated by heating at 100°C for 10 min. The samples were subjected to a polymerase chain reaction (PCR) using pairs of primers encompassing exons 8 and 9 of GNAS and exon 2 of KRAS, which contain frequently mutated regions (Table 1). The PCR products were electrophoresed in a 2% (w/v) agarose gel and were recovered using the QIAquick Gel Extraction Kit (Qiagen, Germany). Isolated PCR products were sequenced using an Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems Inc., Foster, CA, USA). When GNAS mutations were detected, the corresponding non-neoplastic tissues were additionally analysed to confirm their somatic nature.

In tumours concurrently harbouring two nucleotide substitutions in GNAS, the PCR products were subcloned using the TOPO TA cloning kit (Invitrogen, San Diego, CA, USA) and each clone was sequenced as described above.

**Cell culture.** The colorectal cancer cell line HT29, which has wild-type GNAS and KRAS and mutant BRAF alleles (http://www.sanger.ac.uk/genetics/CGP/CellLines/), was obtained from the National Cancer Institute tumour repository (Frederick, MD, USA) and was maintained in RPMI 1640 medium supplemented with 10% foetal bovine serum. HA-tagged rat GNAS<sup>201H</sup> cDNA (Bastepe et al., 2004) was subcloned into the EF1a-IRES-Zeo plasmid to generate an EF1a-GNAS<sup>201H</sup>-IRES-Zeo plasmid that expresses GNAS<sup>201H</sup> and the Zeocin-resistant gene as a single transcript under the control of the EF1a promoter. The HT29 cells were transfected with an EF1a-GNAS<sup>201H</sup>-IRES-Zeo or control EF1a-IRES-Zeo plasmid and cultured in the presence of 20 µg ml<sup>−1</sup> of Zeocin for 3 weeks to obtain stable transfectants. The Zeocin-resistant cells were expanded in bulk culture, to avoid seed contamination.
any biases resulting from cloning and were then subjected to further analysis.

For the cell proliferation assay, $1 \times 10^5$ cells were seeded into 12-cell culture plates in triplicate and counted after 1–3 days. To inhibit PKA activity, 20 nM of H-89 (Sigma, St Louis, MO, USA) was added to the culture medium.

Reverse transcription–PCR. RNA extraction, reverse transcription and conventional PCR were performed using standard protocols. For conventional reverse transcription–PCR (RT–PCR), the PCR products were electrophoresed in an agarose gel and visualised under UV light with ethidium bromide staining. Quantitative RT–PCR (qRT–PCR) reactions were performed in triplicate using FastStart Universal Probe Master (Roche Applied Science, Penzberg, Germany). The expression level of each gene was determined using GUSB as a standard, as previously described (Sekine et al., 2011). The primer sequences and probes used are listed in Table 1.

cAMP assay. Two thousand cells were seeded into 96-well cell culture plates and cultured overnight. After incubation in serum-free media containing 500 μM IBMX and 100 μM Ro20-1724 for 15 min, cAMP levels were measured using the cAMP-Glo Max Assay (Promega, Madison, WI, USA). The assays were done in triplicate.

Animal experiments. The mice used in the present study were maintained in barrier facilities according to the protocols approved by the Committee for Ethics in Animal Experimentation at the National Cancer Center, Japan. Five-week-old nu/nu athymic BALB/c mice were inoculated subcutaneously with $3 \times 10^6$ tumour cells. Three weeks later, the mice were sacrificed and the tumours were weighed and analysed histologically. Ten xenografts were analysed for each group.

Statistical analysis. The Fisher exact test was used to analyse each two-by-two table. Comparisons of continuous variables were using
The present study identified GNAS mutations in half of the LAMNs that were examined. All the mutations that were identified have been previously reported in other tumours and have been shown to be activating mutations (Landis et al, 1989; Lyons et al, 1990; Furukawa et al, 2011; Wu et al, 2011; Yamada et al, 2012). Additionally, in agreement with previous studies, a large majority of LAMNs and MACs harboured activating GNAS mutations (Szych et al, 1999; Kabbani et al, 2002; Zauber et al, 2011). Remarkably, GNAS mutations often co-existed with KRAS mutations also in various gastrointestinal tumours (Furukawa et al, 2011; Wu et al, 2011; Matsubara et al, 2012; Yamada et al, 2012). The concurrent occurrence of GNAS and KRAS mutations in LAMNs is an additional example of the co-existence of these two genetic alterations and implies a functional interaction of these two oncogenes during tumourigenesis.

Among the tumours of digestive organs, GNAS mutations frequently occur in IPMT of the pancreas, villous adenomas of the colorectum, and pyloric gland adenomas of the stomach and duodenum (Furukawa et al, 2011; Wu et al, 2011; Matsubara et al, 2012; Yamada et al, 2012). On the other hand, GNAS mutations are rare or absent in ordinary-type adenocarcinomas of these organs (Lee et al, 2008; Matsubara et al, 2012; Yamada et al, 2012). These observations imply that GNAS mutations might be preferentially associated with tumours with a benign or indolent biological behaviour. In this context, it is intriguing that MACs, which represent high-grade appendiceal mucinous tumours, lacked GNAS mutations; however, since only three MACs were included in our case series, further analyses are needed to confirm whether GNAS mutations are exclusively present in LAMNs among mucinous appendiceal tumours.

Because prominent mucin production is a common feature of LAMN and IPMN, both of which have frequent GNAS and KRAS mutations (Furukawa et al, 2011; Wu et al, 2011), we additionally analysed mucinous tumours of diverse organs for these mutations. The results showed that GNAS mutations were present in a rather organ-specific manner, whereas the prevalence of KRAS mutations was highly variable among each type of tumours. The distinct mutational profiles of GNAS and KRAS mutations might be potentially helpful in determining the origins of metastatic mucinous tumours in clinical situations.

To probe the functional significance of the GNAS mutation, we established a colorectal cancer cell line stably expressing GNASR201H (Figure 3A, hereafter referred to as HT29-GNASR201H cells). The expression of GNASR201H protein was confirmed using western blotting (data not shown). As expected, the introduction of GNASR201H led to an elevated level of cAMP but did not alter cell proliferation in vitro (Figure 3B and C). However, morphologically, the HT29-GNASR201H cells showed the prominent formation of cytoplasmic vacuoles (Figure 3D). Furthermore, a quantitative RT–PCR analysis showed remarkable increases in MUC2 and MUC5AC expression (Figure 3E). The expressions of MUC2 and MUC5AC were partly downregulated by the addition of the PKA inhibitor H89, supporting the role of the cAMP-PKA pathway in the regulation of mucin production.

Next, we transplanted the HT29-GNASR201H cells and the controls subcutaneously into nude athymic BALB/c mice. No significant difference in tumour growth was seen as determined by the tumour weight at 3 weeks after implantation (Figure 3F). A histological analysis showed that the transplanted HT29-GNASR201H cells exhibited more pronounced luminal formation compared with the controls (Figure 3G). Overall, the introduction of GNASR201H did not alter cell growth but significantly promoted mucin production in HT29 cells.

**DISCUSSION**

The present study identified GNAS mutations in half of the LAMNs that were examined. All the mutations that were identified have been previously reported in other tumours and have been shown to be activating mutations (Landis et al, 1989; Lyons et al, 1990; Furukawa et al, 2011; Wu et al, 2011; Yamada et al, 2012). Additionally, in agreement with previous studies, a large majority of LAMNs and MACs harboured activating GNAS mutations (Szych et al, 1999; Kabbani et al, 2002; Zauber et al, 2011). Remarkably, GNAS mutations often co-existed with KRAS mutations also in various gastrointestinal tumours (Furukawa et al, 2011; Wu et al, 2011; Matsubara et al, 2012; Yamada et al, 2012). The concurrent occurrence of GNAS and KRAS mutations in LAMNs is an additional example of the co-existence of these two genetic alterations and implies a functional interaction of these two oncogenes during tumourigenesis.

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Because prominent mucin production is a common feature of LAMN and IPMN, both of which have frequent GNAS and KRAS mutations (Furukawa et al, 2011; Wu et al, 2011), we additionally analysed mucinous tumours of diverse organs for these mutations. The results showed that GNAS mutations were present in a rather organ-specific manner, whereas the prevalence of KRAS mutations was highly variable among each type of tumours. The distinct mutational profiles of GNAS and KRAS mutations might be potentially helpful in determining the origins of metastatic mucinous tumours in clinical situations.
cAMP-generating enzyme adenylyl cyclase, which transduces signals from seven-transmembrane receptors to agonists (Landis et al, 1989; Lyons et al, 1990). We used a colorectal cancer cell line HT29 for a functional analysis of mutant GNAS because this cell line is reportedly capable of differentiating into mucin-secreting cells upon appropriate stimuli (Velcich and Augenlicht, 1993). As expected, the introduction of mutant GNAS into mucin-secreting cells upon appropriate stimuli (Velcich and Augenlicht, 1993). As expected, the introduction of mutant GNAS into mucin-secreting cells upon appropriate stimuli (Velcich and Augenlicht, 1993).

| Age/sex | Histology     | Serosal exposure | Peritoneal implant | LN | MUC2* | MUC5AC* | GNAS     | KRAS     |
|---------|---------------|------------------|--------------------|----|-------|---------|----------|----------|
| 1       | 30/M          | LAMN             | —                  | —  | 2+    | —       | —        | c.35G>A  |
| 2       | 59/F          | LAMN             | —                  | —  | 2+    | 2+      | c.602G>A | c.35G>A  |
| 3       | 72/M          | LAMN             | —                  | —  | 2+    | —       | —        | c.34G>T  |
| 4       | 63/F          | LAMN             | —                  | —  | 2+    | 2+      | c.601C>T | c.35G>T  |
| 5       | 62/F          | LAMN             | —                  | —  | 2+    | —       | —        | —        |
| 6       | 53/M          | LAMN             | —                  | —  | 2+    | 1+      | —        | c.35G>A  |
| 7       | 71/F          | LAMN             | —                  | —  | 2+    | 1+      | —        | —        |
| 8       | 77/F          | LAMN             | —                  | —  | 2+    | 2+      | c.602G>A | —        |
| 9       | 82/M          | LAMN             | —                  | —  | 2+    | 2+      | c.602G>A | c.35G>A  |
| 10      | 72/M          | LAMN             | —                  | —  | 2+    | 1+      | c.602G>A | c.35G>T  |
| 11      | 55/F          | LAMN             | —                  | —  | 2+    | 1+      | —        | c.35G>A  |
| 12      | 55/M          | LAMN             | —                  | —  | 2+    | —       | —        | c.35G>A  |
| 13      | 72/M          | LAMN             | —                  | —  | 2+    | —       | —        | c.35G>A  |
| 14      | 40/M          | LAMN             | —                  | —  | 2+    | —       | —        | c.35G>A  |
| 15      | 84/F          | LAMN             | —                  | —  | 2+    | 2+      | c.602G>A | c.35G>T  |
| 16      | 70/M          | LAMN             | —                  | —  | 2+    | 1+      | —        | c.35G>A  |
| 17      | 66/F          | LAMN             | +                  | —  | 2+    | 2+      | —        | c.35G>C  |
| 18      | 56/F          | LAMN             | +                  | —  | 2+    | —       | —        | c.35G>A  |
| 19      | 52/F          | LAMN             | +                  | —  | 2+    | 2+      | c.601C>T | c.35G>T  |
| 20      | 82/F          | LAMN             | +                  | —  | 2+    | 1+      | —        | c.38G>A  |
| 21      | 47/M          | LAMN             | +                  | —  | 2+    | 2+      | —        | c.35G>A  |
| 22      | 52/F          | LAMN             | +                  | —  | 2+    | —       | c.601C>T | c.602G>A |
| 23      | 59/M          | LAMN             | +                  | —  | 2+    | 2+      | c.601C>T | c.35G>T  |
| 24      | 69/F          | LAMN             | +                  | —  | 2+    | 2+      | —        | c.35G>T  |
| 25      | 51/F          | LAMN             | +                  | —  | 2+    | 1+      | —        | c.35G>T  |
| 26      | 49/F          | LAMN             | +                  | —  | 2+    | 1+      | —        | c.35G>T  |
| 27      | 69/F          | LAMN             | +                  | —  | 2+    | 2+      | c.602G>A | c.35G>T  |
| 28      | 64/F          | LAMN             | +                  | —  | 2+    | 2+      | c.601C>T | c.602G>A |
| 29      | 56/M          | LAMN             | N/A                | —  | 2+    | 2+      | c.601C>T | c.35G>A  |
| 30      | 57/F          | LAMN             | N/A                | —  | 2+    | 2+      | c.601C>T | c.35G>A  |
| 31      | 67/F          | LAMN             | N/A                | —  | 2+    | 2+      | c.601C>A | c.35G>A  |
| 32      | 68/M          | LAMN             | N/A                | —  | 2+    | 2+      | c.601C>T | c.35G>T  |
| 33      | 58/F          | MAC              | —                  | —  | 2+    | 1+      | —        | c.34G>A  |
| 34      | 57/M          | MAC              | +                  | —  | 2+    | 1+      | —        | c.35G>A  |
| 35      | 51/M          | MAC              | +                  | —  | 2+    | 1+      | —        | c.35G>A  |

Abbreviations: LN = lymph node metastasis; M = male; F = female; LAMN = low-grade appendiceal mucinous neoplasm; MAC = mucinous adenocarcinoma; N/A = not assessed.

aThe immunohistochemistry for MUC2 and MUC5AC were scored as: 0, < 10% positive cells; +1, 11–50% positive cells; +2, >50% positive cells.

bTumour specimens of primary appendiceal tumours were unavailable and thus peritoneal (cases 29–31) or omental (case 32) deposits were analysed.

GNAS encodes the α-subunit of a stimulatory G-protein (Gsα), which transduces signals from seven-transmembrane receptors to the cAMP-generating enzyme adenyl cyclase. GNAS mutations cause the constitutive activation of adenyl cyclase and an elevated cAMP level, regardless of the presence or absence of receptor agonists (Landis et al, 1989; Lyons et al, 1990). We used a colorectal cancer cell line HT29 for a functional analysis of mutant GNAS because this cell line is reportedly capable of differentiating into mucin-secreting cells upon appropriate stimuli (Velcich and Augenlicht, 1993). As expected, the introduction of mutant GNAS resulted in an elevated level of cAMP, but did not promote cell growth either in vitro or in vivo. While mutant GNAS likely promotes tumourigenesis, our observation might be consistent with the indolent biological behaviour of LAMN.

On the other hand, the expressions of MUC2 and MUC5AC were markedly induced by mutant GNAS and were partly inhibited by the PKA inhibitor, H89, implying a regulatory role of the GsαAMP-PKA pathway in mucin gene expression. Indeed, previous studies have reported that cAMP-dependent signalling induces mucin production in various cell types, including colorectal cancers (Laburthe et al, 1989; Hokari et al, 2005; Song et al, 2009). These findings suggest that mutant GNAS might play a role in maintaining the mucin production and the indolent biological behaviour of LAMN.
direct role in prominent mucin production, which is a hallmark of LAMN. On the other hand, considering the fact that LAMNs consistently express mucins (particularly MUC2) regardless of the presence or absence of GNAS mutations, additional mechanisms might be responsible for the mucin production in LAMN.

Even though mutant GNAS upregulated mucin production in HT29 cells, the mouse xenografts did not form mucin pools, a histological determinant of mucinous tumours. We also performed intraperitoneal injections of HT29-GNASR201H cells, but this procedure did not reproduce the phenotypes of pseudomyxoma peritonei and instead resulted in the formation of solid tumours (data not shown). This outcome is probably a limitation related to the use of established cancer cell lines, which have a higher proliferative activity than LAMN. While challenging, the modulation of non-transformed appendiceal or colon epithelium might be required to establish a model of pseudomyxoma peritonei.

Table 3. GNAS and KRAS mutations in mucinous tumours of diverse organs

| Site                  | Histology                     | N     | Total mutated | n   | Nucleotide | Amino acid | Total mutated | n   | Nucleotide | Amino acid |
|-----------------------|-------------------------------|-------|---------------|-----|------------|------------|---------------|-----|------------|------------|
| Appendix              | Low-grade appendiceal mucinous neoplasm | 32    | 16 (50%)      | 1   | c.601C>A   | p.R201S    | 30 (94%)      | 1   | c.34G>T    | p.G12C     |
|                       |                               |       |               | 6   | c.601C>T   | p.R201C    |               | 13  | c.35G>A    | p.G12D     |
|                       |                               |       |               | 7   | c.602G>A   | p.R201H    |               | 1   | c.35G>C    | p.G12A     |
|                       |                               |       |               | 2   | c.601C>T, c.602G>A | p.R201C, p.R201H | 11  | c.35G>T    | p.G12V     |
|                       | Mucinous adenocarcinoma       | 3     | 0             | 3   | c.34G>A    | p.G12S     |               | 2   | c.35G>A    | p.G12D     |
|                       |                               |       |               |     |            |            |               | 2   | c.35G>A    | p.G12D     |
| Colorectum            | Mucinous adenocarcinoma       | 33    | 3 (9%)        | 1   | c.601C>T   | p.R201C    | 9 (27%)       | 4   | c.35G>A    | p.G12D     |
|                       |                               |       |               | 2   | c.602G>A   | p.R201H    |               | 4   | c.35G>T    | p.G12V     |
|                       |                               |       |               |     |            |            |               | 1   | c.38G>A    | p.G13D     |
| Ovary                 | Mucinous cystadenoma          | 23    | 2 (9%)        | 1   | c.602G>A   | p.R201H    | 7 (30%)       | 2   | c.35G>A    | p.G12D     |
|                       |                               |       |               | 1   | c.601C>T, c.602G>A | p.R201C, p.R201H | 5   | c.35G>T    | p.G12V     |
|                       | Mucinous borderline tumour    | 24    | 0             | 21  | (88%)      | c.34G>C    | p.G12R        | 11  | c.35G>A    | p.G12D     |
|                       |                               |       |               |     |            |            |               | 8   | c.35G>T    | p.G12V     |
|                       |                               |       |               |     |            |            |               | 1   | c.37G>T    | p.G13C     |
|                       | Mucinous cystadenocarcinoma   | 15    | 0             | 8   | (53%)      | c.34G>C    | p.G12R        | 2   | c.35G>A    | p.G12D     |
|                       |                               |       |               |     |            |            |               | 5   | c.35G>T    | p.G12V     |
| Pancreas              | Intraductal papillary mucinous neoplasm | 37    | 30 (81%)      | 1   | c.601C>A   | p.R201S    | 25 (68%)      | 1   | c.34G>A    | p.G12S     |
|                       |                               |       |               | 15  | c.601C>T   | p.R201C    |               | 3   | c.34G>C    | p.G12R     |
|                       |                               |       |               | 12  | c.602G>A   | p.R201H    |               | 7   | c.35G>A    | p.G12D     |
|                       |                               |       |               | 2   | c.601C>T, c.602G>A | p.R201C, p.R201H | 13  | c.35G>T    | p.G12V     |
|                       |                               |       |               |     |            |            |               | 1   | c.38G>A    | p.G13D     |
| Lung                  | Mucinous adenocarcinoma*      | 18    | 0             | 14  | (78%)      | c.34G>T    | p.G12C        | 2   | c.34G>T    | p.G12C     |
|                       |                               |       |               |     |            |            |               | 7   | c.35G>A    | p.G12D     |
|                       |                               |       |               |     |            |            |               | 5   | c.35G>T    | p.G12V     |
| Breast                | Mucinous adenocarcinoma       | 36    | 0             | 0   |            |            |               |     |            |            |

N, total number of examined lesions; n, lesions with each mutation.
*
Formerly mucinous-bronchioloalveolar carcinoma.

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The present study revealed the frequent presence of activating GNAS mutations in LAMN. GNAS mutations are also common in IPMT of the pancreas, but are rare or absent among mucinous tumours of other organs. Our analysis also suggested a direct role of mutant GNAS in mucin production in LAMN. Since exaggerated mucin production is responsible for the major complications in pseudomyxoma peritonei, the cAMP pathway might be a potential therapeutic target for this disease.

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