The biological basis and function of GNAS mutation in pseudomyxoma peritonei: a review

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Abstract
Purpose Pseudomyxoma peritonei (PMP) is a rare clinical malignancy syndrome characterized by the uncontrollable accumulation of copious mucinous ascites in the peritoneal cavity, resulting in “jelly belly”. The mechanism of tumor progression and mucin hypersecretion remains largely unknown, but GNAS mutation is a promising contributor. This review is to systemically summarize the biological background and variant features of GNAS, as well as the impacts of GNAS mutations on mucin expression, tumor cell proliferation, clinical-pathological characteristics, and prognosis of PMP.

Methods NCBI PubMed database (in English) and WAN FANG DATA (in Chinese) were used for literature search. And NCBI Gene and Protein databases, Ensembl Genome Browser, COSMIC, UniProt, and RCSB PDB database were used for gene and protein review.

Results GNAS encodes guanine nucleotide-binding protein α subunit (Gsα). The mutation sites of GNAS mutation in PMP are relatively stable, usually at Chr20: 57,484,420 (base pair: C-G) and Chr20: 57,484,421 (base pair: G-C). Typical GNAS mutation results in the reduction of GTP enzyme activity in Gsα, causing failure to hydrolyze GTP and release phosphoric acid, and eventually the continuous binding of GTP to Gsα. The activated Gsα could thus continuously promote mucin secretion through stimulating the cAMP-PKA signaling pathway, which is a possible mechanism leading to elevated mucin secretion in PMP.

Conclusion GNAS mutation is one of the most important molecular biological features in PMP, with major functions to promote mucin hypersecretion.

Keywords Pseudomyxoma peritonei · GNAS · Gene mutation · Signaling pathway · Mucin

Introduction
Pseudomyxoma peritonei (PMP) is a rare clinical malignancy syndrome usually caused by the perforation of appendiceal mucinous tumor and the “redistribution phenomenon” of mucus and tumor cells, with an incidence of 1–2/million (Mittal et al. 2017; Smeenk et al. 2008). PMP is characterized by a large volume of mucinous ascites, multiple peritoneal implantations, omental cake, and ovarian involvement in women macroscopically, and abundant mucus pools microscopically. The chronic and uncontrollable mucus accumulation is one of the major clinical features of PMP (O’Connell et al. 2002a, b), which gradually leads to intraperitoneal organ adhesion, bowel obstruction, malnutrition, and eventually cachexia and death. Aggressive cytoreductive surgery (CRS) combined with hyperthermic intraperitoneal chemotherapy (HIPEC) could bring significant survival benefit to PMP (Chua et al. 2012; Li et al. 2018), and has been recommended by Peritoneal Surface Oncology Group International (PSOGI) as the standard treatment of PMP (Li et al. 2014, 2019).

Although treated with CRS plus HIPEC, patients frequently suffered from relapse, presenting aggravated “jelly belly”. One of the difficulties in studying PMP is the scarcity of knowledge in the fundamental molecular mechanisms underlying mucin hypersecretion. It has been reported that Kirsten rat sarcoma viral oncogene homolog (KRAS) and guanine nucleotide-binding protein alpha subunit (GNAS) are two of the most frequently detected variants in PMP, and
GNAS mutation plays an important role in the regulation of mucin expression (Bradbury 2000; Jarry et al. 1994; Nishikawa et al. 2013). To have a better insight into the role of GNAS gene in PMP, we systemically reviewed the biological background of GNAS, current studies concerning the variant feature of GNAS, the impacts of GNAS mutations on mucin expression, tumor cell proliferation, and clinical–pathological characteristics and prognosis.

The biological background of GNAS gene

Basic structure and function

The GNAS gene is located at chromosome 20q13.32 (chromosome 20: 57,414,773–57,486,247), which also names GNAS complex locus (Fig. 1a), consisting of 13 exons and 12 introns. GNAS is responsible for the encoding of stimulatory guanine nucleotide-binding protein (G protein) α subunit (Gsα), which transduces signals from G protein-coupled receptors (GPCR) to adenyl cyclase (AC), and finally regulates the expression of cyclic adenosine monophosphate (cAMP).

DNA transcription and translation

The promoter region of Gsα is located at the CpG island upstream of exon 1, which is usually unmethylated in alleles of both parental origins (Bird 1986; Gardiner-Garden and Frommer 1987). It was reported by Mantovani et al. (2002) and Germain-Lee et al. (2005) that Gsα imprinted with tissue-specific pattern in kidney cortex, thyroid gland, pituitary gland, and ovary, which is mainly maternally expressed. There are four kinds of alternative promoter regions upstream of Gsα exon 1 (Weinstein et al. 2001): (1) promoter 1, about 49 kb upstream of Gsα exon 1, encodes neuroendocrine secretory protein 55 (NESP55). The coding sequence is within the upstream of Gsα exon 1, leaving exon 2–13 untranslated region; (2) promoter 2, about 2–3 kb upstream of XL exon, initiates NESP55 exon transcription from the opposite direction; (3) promoter 3, about 35 kb upstream of Gsα exon 1, encodes extra-large alphas protein (XLαs),

![Fig. 1](image_url)
whose coding sequence is composed of XL exon and Gsα exon 1; (4) promoter 4 locates at about 2.5 kb upstream of Gsα exon 1. The resulted exon 1A transcripts were presumed to be untranslated mRNAs. The imprinted expression patterns of the aforementioned promoters are highly complicated. NESP55 is maternally expressed, while NESP antisense, XLαs, and exon 1A are paternally expressed (Fig. 1b) (Crane et al. 2009).

The UniProt database (https://www.uniprot.org/) was used to search for proteins encoded by GNAS, with the searching term as "gene: GNAS AND reviewed: yes AND organism: “Homo sapiens (Human) [9606]”". The result showed four kinds of proteins encoded by GNAS: (1) Gsα, with a length of 394 amino acid residues, is encoded by GNAS exon 1–13; (2) XLαs, with a length of 1037 amino acid residues, is paternally expressed and responsible for the stimulation of AC-cAMP–PKA signaling pathway. XLαs is one of the isoforms of Gsα, with similar downstream receptor to Gsα. But there is no evidence showing that seven-transmembrane receptors activating Gsα can also activate XLαs; (3) protein ALEX, with a length of 626 amino acid residues, is the product of paternal expression of XL exon and possibly contributes to the inhibition of AC activity in XLαs subunit (Abramowitz et al. 2004); (4) NESP55, with a length of 245 amino acids, is maternally expressed and encoded by NESP55 exon. NESP55 forms LHAL tetrapeptide and GPIPIRRH peptide after modification and shear.

The structure and function of Gsα

Among the four reviewed proteins, Gsα is the main product of GNAS gene, which includes two domains (Rose et al. 2018) (Fig. 2). The first is guanosine triphosphate (GTPase) domain, which is formed after the fold of 39–394th amino acid residues. GTPase domain functions as the guanois-biding and interaction site for receptors and effectors. There are four guanosine triphosphate/guanosine diphosphate (GTP/GDP)-binding sites, located at 47–55th, 197–204th, 223–227th, and 292–295th amino acid residues respectively; and two magnesium ion-binding sites, located at 54th and 204th amino acid residues, respectively. Two of the four GTP/GDP-binding sites are highly conserved [arginine201 (Arg201) and glutamine227 (Gln227)], which play a vital role on the hydrolysis of the bound GTP. The second is helical domain, with a possible function of maintaining the binding status between GTP/GDP and Gsα (Weinstein et al. 2001). Besides the four domains, there are five motif structures in Gsα, including G1 (42–55th amino acids), G2 (196–204th amino acids), G3 (219–228th amino acids), G4 (288–295th amino acids), and G5 (364–369th amino acids).

The signaling from GPCR to the downstream molecules is carried out through G protein cycle (Fig. 2, red-dotted box): (1) Gsα releases GDP and combines with GTP due to the affinity reduction between Gsα and GDP caused by activation from ligand-binding GPCR to Gsα; (2) GTP-binding Gsα separates with β and γ subunits and turns into an activated status, which is able to stimulate downstream molecules; (3) as reacting with the downstream molecules,
the GTPase activity of Gsα is activated and then GTP is hydrolyzed. Eventually, Gsα returns to the primary structure and reforms trimer with β and γ subunits.

The molecular changes of GNAS mutation

A thorough literature research identified 13 papers reporting the genetic variants and corresponding gene mutation rates in PMP. Only variants reported in ≥5 papers were listed in Table 1. As listed in Table 1, the two most frequent variants in PMP are KRAS and GNAS mutations, with a median mutation rates of 77.8% (range 40.0–100%) and 45.7% (range 25.7–100%) respectively. By reviewing papers describing the detailed variant form of GNAS, we found that the most frequently detected GNAS mutation forms were c.602G>A (p.R201H) and c.601C>T (p.R201C) (Table 2). Despite the different variant forms reported by Pengelly et al. (2018) and Saarinen et al. (2017), the variant sites were relatively stable, both located at Chr20: 57,484,420 and Chr20: 57,484,421, which was identical to c.602G>A (p.R201H) and c.601C>T (p.R201C). Various transcripts chosen after sequencing might have resulted in the different expression patterns of mutation sites. Thus, it can be concluded that Chr20: 57,484,420 C>T (c.601C>T: p.R201C) and Chr20: 57,484,421 G>A (c.602G>A: p.R201H) are the two most significant variant forms in PMP GNAS mutations.

Taking the encoding of Gsα for example, once c.601C>T and c.602G>A mutation occur, the 201th amino acid residue, Arg, changes into cysteine (Cys) and histidine (His) respectively. The variants significantly alter the structure of GTPase domain in Gsα, and vastly decrease GTPase activity. As a consequence, Gsα fails to hydrolyze GTP and release phosphoric acid, remaining in activated status, which continuously stimulates downstream molecules (Fig. 3, blue dotted box).

Influences of GNAS mutation to mucin secretion and cell proliferation

Mucin expression in PMP

There are two major types of mucins, gel-forming mucins and transmembrane mucins (Johansson and Hansson 2016).
Gel-forming mucins mainly include MUC2, MUC5AC, MUC5B, and MUC6. Transmembrane mucins mainly consist of MUC1, MUC3, MUC4, MUC12, MUC13, MUC16, and MUC17. A thorough review of the published literatures on mucin expression in PMP identified some distinctive features (Table 3). First, most researches focus on the expression status of gel-forming mucins, while little attention has been paid to transmembrane mucins. Second, MUC2 and MUC5AC are the most frequently expressed gel-forming mucins in PMP, with positive rates being 99.1% (314/317) and 96.5% (193/200), respectively, among the detected samples. MUC6 is rarely detected in PMP compared with MUC2 and MUC5AC, with positive rate of 12.5% (2/16). Third, the transmembrane MUC1 expresses variably in PMP, with positive rate being 41.3% (33/80). The expression status of MUC4 is currently unclear due to the limitation of sample number. Based on the available data from published literatures, it is advisable to focus more attention on in-depth study on MUC2 and MUC5AC.  

Table 1 Summary of the top 5 mutations in pseudomyxoma peritonei

| References       | Cases | Gene panel                                                                 |
|------------------|-------|-----------------------------------------------------------------------------|
| Tokunaga et al.  | 183   | KRAS GNAS TKP53 SMAD4 APC PI3CA                                             |
| Pengelly et al.  | 5     | 100.0 100.0 100.0 NA NA NA NA                                             |
| Gleeson et al.   | 19–31 | 80.6 73.7 87.0 5.0 10.0 NA NA                                               |
| Saarinen et al.  | 9     | 100.0 55.6 55.6 NA NA NA NA                                               |
| Borazanci et al. | 116–396 | 57.3 28.2 NA 23.4 16.2 10.7 5.3                                           |
| Pietrantonio et al. | 40   | 72.0 52.5 NA 12.5 2.5 NA NA                                               |
| Nummela et al.   | 19    | 100.0 63.2 NA 5.3 15.3 0.0 5.3                                            |
| Noguchi et al.   | 18    | 77.8 44.4 NA 22.2 16.7 NA 11.1                                            |
| Sio et al.       | 10    | 70.0 40.0 40.0 NA NA NA                                                |
| Liu et al.       | 35    | 42.9 25.7 NA 20.0 14.3 22.9 5.7                                           |
| Alakus et al.    | 29    | 89.7 70.0 NA 0.0 NA NA                                                   |
| Singh et al.     | 55    | 40.0 31.0 NA 2.5 15.3 NA NA                                               |
| Nishikawa et al. | 35    | 94.3 45.7 NA 24.9 NA NA NA                                              |
| Range            | NA    | 40.0–100.0 25.7–100.0 40.0–100.0 0–40.0 2.5–16.7 0–22.9 0–11.1           |
| Median           | NA    | 77.8 45.7 55.6 16.3 15.7 10.4 5.9                                       |

NA not available

*aNumber of patients varied by different genes detected

*bPatients with neuroendocrine tumors of appendix were excluded

GNAS functions on the regulation of mucin secretion

GNAS mutation is frequently detected in mucinous neoplasms of appendix (50%) and intraductal papillary mucinous neoplasm (IPMN) of pancreas (81%) (Furukawa et al. 2011; Wu et al. 2011), while the mutation rate in mucinous adenocarcinoma of colorectum, ovary, lung, and breast are relatively lower, even being 0% (Nishikawa et al. 2013). In addition, both PMP and IPMN share similar inertia biological behavior as well as hypersecretion of mucus. Therefore, it is inferred that GNAS might play some role in the regulation of mucin secretion (Alakus et al. 2014; Noguchi et al. 2015; Tokunaga et al. 2019).

The effect of GNAS mutation to mucin secretion has been proved by Nishikawa et al. (2013). The author transfected HT29 cells with an EF1a-GNASR201H-IRES-Zeo plasmid. The result showed that cAMP, MUC2, and MUC5AC level elevated after the expression of GNASR201H. While the application of PKA inhibitor downregulated the expression of MUC2 and MUC5AC genes. Nishikawa’s study demonstrates that GNAS mutation might regulate mucin production through cAMP–PKA signaling pathway (Bradbury 2000; Jarry et al. 1994). The potential regulation method of cAMP–PKA signaling pathway might be stimulating cAMP-response element-binding protein (CREB) and activating transcription factor (ATF) family (Velcich and Augenlicht 1993). After entering nucleus, the activated CREB/ATF combines to the upstream cis-acting element of mucin genes and thus regulate mucin expression. Other studies have also proved that inhibitors of both PKA and heterotrimer G protein complex could also significantly downregulate mucin expression. Although GNAS mutation is proved to be an important promoter in mucin secretion of PMP, the current experiment was performed in colorectal cancer cell lines due to the difficulties in the culture of PMP tumor cells (Nishikawa et al. 2013). Besides, the influence of GNAS mutation to different types of mucin still needs further exploration.
Table 2 The variant forms of GNAS mutation

| References                  | Cases | Gene panel | GNAS mutation, N | GNAS mutation rate | GNAS variant form (%) | c.602G>A (p.R201H) | c.601C>T (p.R201C) | c.601C>A (p.R201S) | p.Q227STOP | p.Q227H (p.R186C) | c.G557T (p.R186H) | c.G557A | c.G560A (p.R187H) |
|-----------------------------|-------|------------|------------------|-------------------|-----------------------|-------------------|-------------------|-------------------|------------|------------------|-------------------|--------|-------------------|
| Tokunaga et al. (2019)      | 183   | 592        | 57               | 31.0              | NA                    | NA                | NA                | NA                | NA         | NA                | NA                | NA     | NA                |
| Pengelly et al. (2018)      | 5     | 54         | 5                | 100.0             | 0                     | 0                 | 0                 | 0                 | 0          | 20.0 (1/5)        | 30.0 (3/5)        | 20.0 (1/5) |                    |
| Gleeson et al. (2018)       | 19    | 47         | 14               | 73.7              | 54                    | 46                | 0                 | 0                 | 0          | 0                 | 0                 | 0      |                    |
| Saarinen et al. (2017)      | 9     | Whole exome| 5                | 55.6              | NA                    | NA                | NA                | NA                | NA         | NA                | NA                | NA     | NA                |
| Borazanci et al. (2017)     | 124   | 47         | 35               | 28.2              | NA                    | NA                | NA                | NA                | NA         | NA                | NA                | NA     | NA                |
| Pietrantonio et al. (2016a, b) | 40  | 50         | 21               | 52.5              | 71.4 (15/21)          | 23.8 (5/21)       | 0                 | 4.8 (1/21)        | 0          | 0                 | 0                 | 0      |                    |
| Nummela et al. (2015)       | 19    | 48         | 12               | 63.2              | 58.3 (7/12)           | 41.7 (5/12)       | 0                 | 0                 | 0          | 0                 | 0                 | 0      |                    |
| Noguchi et al. (2015)       | 18    | 50         | 8                | 44.4              | 75.0 (6/8)            | 25.0 (2/8)        | 0                 | 0                 | 0          | 0                 | 0                 | 0      |                    |
| Sio et al. (2014)           | 10    | 236        | 4                | 40.0              | 50.0 (2/4)            | 50.0 (2/4)        | 0                 | 0                 | 0          | 0                 | 0                 | 0      |                    |
| Liu et al. (2014)           | 35    | 50         | 9                | 25.7              | NA                    | NA                | NA                | NA                | NA         | NA                | NA                | NA     | NA                |
| Alakus et al. (2014)        | 29    | NA         | 20               | 69.0              | 11.1 (1/9)            | 77.8 (7/9)        | 0                 | 0                 | 11.1 (1/9) | 0              | 0                 | 0      |                    |
| Singhi et al. (2014)        | 55    | 2          | 17               | 30.9              | 58.8 (10/17)          | 41.2 (7/17)       | 0                 | 0                 | 0          | 0                 | 0                 | 0      |                    |
| Nishikawa et al. (2013)     | 35    | 2          | 16               | 45.7              | 50.0 (9/18)           | 44.4 (8/18)       | 5.6 (1/18)        | 0                 | 0          | 0                 | 0                 | 0      |                    |
| Range                       | N     | NA         | NA               | 25.7–100.0         | 0–75.0               | 0–77.8            | 0–5.6             | 0–4.8             | 0–11.1     | 0–20.0            | 0–30.0            | 0–20.0 |                    |
| Median                      | NA    | NA         | NA               | 45.7              | 54.0                  | 41.7              | 0                 | 0                 | 0          | 0                 | 0                 | 0      |                    |

NA not available

*a*20 patients were reported to harbor GNAS mutation and variant forms of nine patients were described in detail by the author

*b*Eighteen variant forms were found in 16 patients
The existed pathways which have cross reaction with cAMP–PKA pathway also participate in the regulation of mucin expression indirectly (Fig. 3): (1) MAPK signaling pathway. The activated cAMP influences MAPK signaling pathway via activating Ras or inhibiting Raf-1 by PKA. In pulmonary cystic fibrosis, it has been illustrated that hyperexpression of MUC2 was mainly regulated through Src/Ras/MAPK/pp90 rsk signaling pathway (Li et al. 1998). However, the function of Src/Ras/MAPK/pp90 rsk in PMP is not proven currently; (2) Ras–PI3K–Akt signaling pathway. PDE4B activated by this pathway functions as an antagonist against cAMP–PKA signaling pathway by clearing cAMP (Alakus et al. 2014); (3) PKC signaling pathway. Activated PKC has synergistic effect on cAMP–PKA pathway through activating Raf-1. Besides, Ca²⁺-dependent PKC-epsilon could also upregulate MUC2 and MUC5AC expression (Hong et al. 1999).

**GNAS functions on the regulation of tumor cell proliferation**

Generally, the current studies support the notion that PMP and colorectal cancer share similar gene mutation profiles, but vary vastly in mutation rate. PMP possesses higher mutation rates in GNAS and KRAS, while lower mutation rates in TP53, APC, and PIK3CA (Alakus et al. 2014; Tokunaga et al. 2019). Nishikawa et al. transfected HT29 cells with an EF1a-GNASR201H-IRES-Zeo plasmid. The cell proliferation remained the same, but accompanied with elevated mucin secretion. The result indicated that GNAS mutation mainly affect the expression level of mucin instead of tumor cell proliferation. KRAS is another important variant in PMP, and has been reported to promote tumor cell proliferation through the activation of MAPK signaling pathway (Alakus et al. 2014; Pylayeva-Gupta et al. 2011).

**Impacts of GNAS mutation to clinical–pathological characteristics and prognosis**

**Correlation of GNAS mutation and clinical–pathological characteristics**

In a study cohort of 55 patients, Singhi et al. (2014) demonstrated no significant association between GNAS mutation and gender, age, and adverse histological features (including cytologic grade, destructive invasion, tumor cellularity, angiolymphatic invasion, perineural invasion, and signet ring cells) ($P > 0.05$). However, the author found that GNAS-mutated PMP was prone to harbor concurrent KRAS mutation compared with GNAS-wild-type PMP (65% vs. 29%, $P = 0.018$).

### Table 3 Mucin expression status in pseudomyxoma peritonei

| References                  | Cases | Method | Gel-forming mucins (%) | Transmembrane mucins (%) |
|-----------------------------|-------|--------|------------------------|--------------------------|
|                             |       |        | MUC2       | MUC5AC | MUC6 | MUC1 | MUC4 |
| Yan et al. (2019)           | 21    | IHC    | 100 100 NA   | NA     | NA   | NA   |
| Yan et al. (2020)           | 5     | IHC    | 100 NA       | NA     | 60   | NA   |
| Li et al. (2017a, b)        | 9     | IHC    | 100 NA       | NA     | NA   | NA   |
| Li et al. (2017a, b)        | 8     | IHC    | 100 NA       | NA     | NA   | NA   |
| Guo et al. (2011)           | 35    | IHC    | 97.1 NA      | NA     | 0    | NA   |
| Flatmark et al. (2010)      | 5     | IHC    | 100 60.0 NA  | NA     | 0    | 100  |
| Ferreira et al. (2008)      | 7     | IHC    | 100 28.6     | 28.6   | NA   | NA   |
| Semino-Mora et al. (2008)   | 16    | FISH   | 100 NA       | NA     | NA   | NA   |
| McKenney and Longacre (2008)| 1     | IHC    | 100 NA       | NA     | NA   | NA   |
| Nonaka et al. (2006)        | 42    | IHC    | 100 NA       | NA     | NA   | NA   |
| Heiskala et al. (2006)      | 9     | IHC    | 100 100 0    | NA     | NA   | NA   |
| Bibi et al. (2006)          | 26    | IHC    | 100 NA       | NA     | NA   | NA   |
| Mohamed et al. (2004)       | 33    | IHC    | 100 NA       | NA     | 84.8 | NA   |
| O’Connell et al. (2002a, b)| 100   | IHC    | 98.0 95      | NA     | NA   | NA   |
| Total                       | 317   | NA     | 99.1 96.5    | 12.5   | 41.3 | 100  |
| Range                       | NA    | NA     | 97.1–100     | 60.0–100| 0–28.6| 0–84.8| 100–100|
| Median                      | NA    | NA     | 100 100 14.3 | 28.6   | 100  |

*MUC2 mucin 2, MUC5AC mucin 5AC, IHC immunohistochemistry, FISH fluorescence in situ hybridization, NA not available*
Pietrantonio et al. (2016a, b) analyzed 15 patients with relapsed PMP, and revealed no association between GNAS mutation and gender, age, Eastern Cooperative Oncology Group performance status, histological grade, time elapsed from surgery to relapse, peritoneal cancer index (PCI), and completeness of cytoreduction. In another study of 40 PMP patients, Pietrantonio et al. (2016a, b) found that GNAS mutation was correlated to incomplete cytoreduction ($P = 0.05$) and KRAS mutation ($P = 0.002$). Besides, neither GNAS nor KRAS mutation were associated with pathological grade ($P = 0.338$ and 0.427, respectively).

From the studies by Pietrantonio et al. (2016a, b) and Singhi et al. (2014), it could be inferred that the presence of GNAS mutation is related to KRAS mutation. Considering the high incidence of these two variants in PMP and the statistically close relationship, the independent and synergistic effect as well as the crosslink between GNAS and KRAS could be important issues to be explored in the mechanical studies of PMP.

Despite of the application of different criteria in histopathological classification, most of the studies showed that GNAS mutational status had no association with histopathological grade (Gleeson et al. 2018; Nummela et al. 2015; Pietrantonio et al. 2016a, b; Singhi et al. 2014). However, opposite opinions existed concerning the relation between GNAS mutation and histopathological grade. Noguchi et al. (2015) investigated mutation profiles of 18 PMP patients, revealing GNAS mutation in five low-grade PMP and three high-grade PMP. Noguchi hold the view that GNAS mutation might play a key role in both low-grade and high-grade PMP. On the contrast, in a study performed by Alakus et al. (Alakus et al. 2014), the result revealed that GNAS mutation rate is lower in high-grade PMP (21/23 vs. 1/6, $P = 0.005$). For the only patient with high-grade PMP presenting GNAS mutation, it was observed that the histopathology of the intraperitoneal implantation was a mixture of partly low-grade and partly high-grade PMP. Considering the existence of low-grade loci, Alakus et al. made a conclusion that high-grade PMP might not evolve from low-grade PMP.

### Impacts of GNAS mutation on PMP prognosis

Few studies were performed to investigate the association between GNAS mutation and prognosis of PMP. The results varied among different studies. Singhi et al. (2014) found that GNAS mutation did not affect the overall survival (OS) or time to disease progression. High tumor grade (AJCC G2 and G3) ($P = 0.002$) and lymph node involvement ($P = 0.025$) were associated with poorer OS. While HIPEC was associated with improved OS. Cox proportional hazard model identified that only lymph node involvement was the independent prognostic factor of PMP. In a study performed by Pietrantonio et al. (2016a, b), it was found that patients with GNAS mutation had significantly shorter median progression-free survival (PFS) than GNAS-wild type patients (5.3 months vs. not reached, $P < 0.007$). Later, in a study cohort of 40 patients, Pietrantonio et al. again demonstrated that GNAS mutation was associated with PFS. The other variables correlated to PFS were completeness of cytoreduction score, PCI score, and KRAS mutation status. However, multiple variate analysis revealed only PCI $> 20$ and KRAS mutation were the independent predictors of PFS.

### Summary

To sum up, GNAS mutation is one of the most important molecular biological features in PMP, which might function as promoting the secretion of mucin. The mutation sites of GNAS mutation is relatively stable, usually at Chr20: 57,484,420 (base pair: C-G) and Chr20: 57,484,421 (base pair: G-C). The presence of GNAS mutation results in the reduction of GTPase activity in Gsα, causing failure to hydrolyze GTP and release phosphoric acid, and eventually the continuous combining status of Gsα and GTP. The activated Gsα could thus continuously stimulate mucin secretion through the stimulation of cAMP–PKA signaling pathway. As presented above, there were already several studies proving that GNAS could elevates secretion level of mucin, but the experiments were limited in the cell lines of colorectal cancer. A more reliable evidence provided by experiments of genetic and protein level in PMP cell line is in urgent requirement.

The high mutation rate of GNAS in PMP patients has been observed about 10 years ago, when fresh tumor tissue or formalin-fixed, paraffin-embedded tissue was used for variant detection. However, the number of patients was limited, and most of the sequencings were non-whole-exome sequencing, which indicated the deficiency on the comprehensive view of PMP mutation profile. Generally speaking, the establishment of stable PMP cell line combined with comprehensive mutation profile would vastly help to improve the understanding of PMP genetically, and uncover the mechanism of PMP, especially the influence of GNAS mutation to mucin hypersecretion, which might eventually facilitate the innovation of new drugs targeting the molecules in the GNAS-related signaling pathways.

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Conflict of interest The authors declare that they have no competing interests.

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