Clinicopathological characteristics of KIT and protein kinase C-δ expression in adenoid cystic carcinoma: comparison with chromophobe renal cell carcinoma and gastrointestinal stromal tumour

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Aims: KIT overexpression is frequently observed in adenoid cystic carcinomas (AdCCs), chromophobe renal cell carcinomas (ChRCCs), and gastrointestinal stromal tumours (GISTs). Persistent KIT activation has been reported to be mediated by protein kinase C (PKC)-δ in a subset of colon cancers with wild-type KIT overexpression, and by PKC-θ in GISTs with mutant KIT overexpression. To elucidate the clinical implications of PKC-δ and PKC-θ expression in KIT-expressing tumours, we investigated the expression of KIT, PKC-δ and PKC-θ in AdCCs and ChRCCs in comparison with GISTs.

Methods and results: KIT expression, PKC-δ expression and PKC-θ expression were analysed in whole sections from 41 AdCCs, 40 ChRCCs and 56 GISTs by immunohistochemistry. Membranous expression of KIT was found in 34 AdCCs and all ChRCCs, whereas cytoplasmic expression of KIT was found in 46 GISTs. In AdCCs, PKC-δ expression was associated with histological grade (P = 0.049), lymphovascular invasion (P = 0.004), perineural invasion (P = 0.002), and KIT positivity (P = 0.002). PKC-δ positivity was associated with shorter relapse-free survival (RFS) (P = 0.017) and a tendency for there to be shorter overall survival (OS) (P = 0.090) in patients with AdCCs. No clinicopathological associations were observed between PKC-δ and KIT expression in ChRCCs. In GISTs, PKC-θ expression was associated with higher mitotic count (P = 0.011) and high grade according to the modified National Institutes of Health criteria (P < 0.001). PKC-θ positivity was associated with shorter RFS (P = 0.016) and a tendency for there to be shorter OS (P = 0.051) in patients with GISTs.

Conclusions: PKC-δ expression is associated with KIT expression and the prognosis of patients with AdCCs, suggesting that PKC-δ may be a potential therapeutic target for AdCCs.

Keywords: adenoid cystic carcinoma, chromophobe renal cell carcinoma, gastrointestinal stromal tumour, KIT, protein kinase C-δ, protein kinase C-θ
Introduction

Tumorigenesis is a complex process that involves various signalling pathways. Among these pathways, activation of oncogenic receptor tyrosine kinases (RTKs) plays an important role in the development of many cancers. RTKs are associated with proliferation, migration and survival of non-neoplastic cells. The genetic alterations of RTK genes in cancer cells often result in the persistent overexpression and activation of RTKs, and are associated with the development of cancers.

The representative example of tumorigenesis due to the genetic alteration of an RTK gene is KIT mutation in gastrointestinal stromal tumours (GISTs). Sustained KIT activation as a result of oncogenic mutation plays an important role in GIST tumorigenesis. In addition, KIT overexpression has been reported in acute myeloid leukaemia, systemic mastocytosis, adenoid cystic carcinoma (AdCC), and chromophobe renal cell carcinoma (ChRCC). Various studies have been performed to identify the KIT mutation status in AdCC and ChRCC; however, currently, no identifiable KIT mutation has been reported in ChRCC, and the KIT mutation status of AdCC remains controversial.

Protein kinase C (PKC) is a phospholipid-dependent serine/threonine kinase that plays a role in the signalling pathways mediated by hormones or growth factors. Various PKC isozymes play diverse roles in the development and progression of cancer via numerous mechanisms involving: regulation of proliferation, survival, and apoptosis; invasion of neoplastic cells; angiogenesis; and resistance to chemotherapeutic agents.

In our previous study, we detected sustained KIT activation via PKC-δ-mediated recycling in wild-type KIT-expressing colon cancers. Recently, we also reported that persistent mutant KIT overexpression is mediated by PKC-θ in GISTs. These findings raise the possibility that the expression of PKC-δ or PKC-θ may be associated with the tumorigenesis of KIT-overexpressing tumours, such as AdCC and ChRCC; however, no comparative studies of the expression of PKC-δ and PKC-θ in KIT-overexpressing tumours have been performed, in part because of the unknown clinical implications of PKC-δ and PKC-θ expression. We therefore investigated the expression of PKC-δ and PKC-θ in AdCC and ChRCC in comparison with GIST, and examined the clinical implications of PKC-δ and PKC-θ expression in these carcinomas.

Materials and methods

Patient selection and clinicopathological evaluation

We collected consecutive formalin-fixed paraffin-embedded tissue samples from 41 AdCC patients and 40 ChRCC patients who underwent surgical resection at Severance Hospital in Seoul, Republic of Korea, in 2003–2013 and in 2003–2004, respectively. In addition to 30 cases used in our previous study, 56 consecutive GISTs from patients who underwent surgical resection between 2003 and 2004 were also included. Cases that had received neoadjuvant chemotherapy, chemoradiation therapy or tyrosine kinase inhibitor treatment were excluded.

Various clinical parameters, such as gender, age, and follow-up data, were obtained from the medical record review. Pathological parameters (Table S1) were evaluated for each tumour via slide review by two independent pathologists (C.K.P. and H.K.). Relapse-free survival (RFS) was defined as the time from the date of the first curative operation to the date of the first locoregional or systemic relapse or to the date of death without any type of relapse. Overall survival (OS) was defined as the time from the date of the first curative operation to the date of the last follow-up or to the date of death from any cause. This study was approved by the Institutional Review Board of the Severance Hospital (4-2015-0227).

Immunohistochemistry (IHC) and interpretation

Whole sections with a thickness of 4 μm were used for IHC. IHC for KIT, PKC-δ and PKC-θ was performed with a Ventana Discovery XT automated stainer (Ventana Medical Systems, Tucson, AZ, USA), as previously described. IHC results were evaluated according to the classification system based on the proportion and intensity of staining, as previously described. Proportion category was assigned as the following scores: 0 = 0–4%, 1 = 5–19%, 2 = 20–39%, 3 = 40–59%, 4 = 60–79%, and 5 = 80–100%. Intensity category was assigned as the following scores: 0 = no staining, 1 = weak staining, 2 = intermediate staining, and 3 = strong staining. The Quick-score was defined as the multiplication of proportion and intensity scores. Cases for which the Quickscore was ≥4 were considered to be positive, and the others were regarded as negative.
STATISTICAL ANALYSIS

Data were analysed with SPSS for Windows version 21.0 (IBM, Armonk, NY, USA). Student’s t-test and Fisher’s exact test were used for continuous and categorical variables, respectively. Statistical significance was assumed when $P < 0.05$. Kaplan–Meier survival curves with log-rank statistics were applied to evaluate time to tumour recurrence/metastasis and time to survival. Multivariate regression analysis was performed with a Cox proportional hazards model.

Results

CLINICOPATHOLOGICAL FEATURES OF ADCC, CHRCC AND GIST PATIENTS

The median age of the 41 AdCC patients was 50.0 years. Thirty-four cases occurred in salivary glands, including two cases from minor salivary glands. The remaining seven cases originated from lacrimal glands (two cases), the external auditory canal (two cases), and the lung (three cases). The histological grade was evaluated according to the criteria described by Spiro et al.\textsuperscript{17} Among these cases, 20, 11 and 10 cases were categorized as grade I, II and III, respectively. In addition, 11 cases showed lymphovascular invasion, and 31 cases showed perineural invasion. Extension of AdCC into the surgical resection margin was observed in 25 cases. During a median of 38 months of follow-up (range 5–92 months), seven cases of locoregional recurrence and five cases of distant metastasis were observed. Four patients died during follow-up. As previously described,\textsuperscript{18} KIT mutation status (exons 9, 11, 13, and 17) was analysed in 20 randomly selected AdCC cases, and no mutation was found.

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Figure 1. Representative expression patterns of KIT in adenoid cystic carcinoma (AdCC), chromophobe renal cell carcinoma (ChRCC) and gastrointestinal stromal tumour (GIST) cases. A, Haematoxylin and eosin (H&E) staining of AdCC. B, KIT immunohistochemistry (IHC) indicated strong membranous expression in luminal cells in 34 AdCCs. C, Seven AdCCs showed weak membranous KIT expression in luminal cells, and were considered to be negative (Quickscore of <4). D, H&E staining of ChRCC. E, F, KIT IHC of ChRCC cases showed intense membranous expression in all tumour cells. G, H&E staining of GIST. H, The majority of GISTs showed diffuse and strong cytoplasmic KIT expression. I, Ten GISTs showed weak cytoplasmic expression, and were considered to be negative (Quickscore of <4).
The median age of the 40 ChRCC patients was 56.0 years. Extrarenal extension or renal vein invasion was not observed in any of the cases. Regional lymph nodes were evaluated for three cases, although none of these showed metastasis. Nuclear grade was evaluated according to the chromophobe tumour grade scheme. Of the 40 cases, 32 were grade 1, six were grade 2, and two were grade 3. Pathological T stage was evaluated according to the 7th American Joint Committee on Cancer criteria, and 21 pT1a, 11 pT1b, six pT2a and two pT2b cases were identified. During a median of 61 months of follow-up (range 6–120 months), one case of locoregional recurrence was observed, and two patients died.

The median age of the 56 GIST patients was 62.0 years. The locations of the tumours were distributed from the stomach to the ileum: 33 cases in the stomach, seven cases in the duodenum, and 16 cases in the jejunum or ileum. Considering tumour size and mitotic count, histological grading was evaluated according to the modified National Institutes of Health (NIH) criteria. The distribution of histological grade for these 56 GISTs was as follows: 19 low-grade cases, nine intermediate-grade cases, and 28 high-grade cases. Mutation study results revealed 41 KIT-mutant cases, six platelet-derived growth factor receptor-α (PDGFRα)-mutant cases, and nine cases of wild-type GIST. During the median follow-up period of 50 months (range 2–156 months), 10 cases of locoregional recurrence and five cases of distant metastasis were observed. Thirteen patients died during the follow-up period.

**Expression of KIT in each tumour type**

In 34 of 41 AdCCs, KIT was expressed in luminal cells of tumours with a membranous pattern (Figure 1). Comparison of the various clinicopathological factors according to KIT expression status revealed that KIT overexpression was only associated with the presence of perineural invasion (P < 0.001; Table S2). All 40 ChRCCs showed diffuse and intense KIT expression with a membranous pattern.

Unlike AdCCs and ChRCCs, GISTs showed KIT expression with cytoplasmic localization in 46 of 56 cases (Figure 1).
### Table 1. Clinicopathological characteristics of 41 adenoid cystic carcinomas according to protein kinase C (PKC)-δ and PKC-θ expression status

| Category             | Variables | Case no. (n = 41) | PKC-δ expression | PKC-θ expression |
|----------------------|-----------|-------------------|------------------|------------------|
|                      |           |                   | Positive (%) (n = 22) | Negative (%) (n = 19) | P-value | Positive (%) (n = 8) | Negative (%) (n = 33) | P-value |
| **Age (years)**      |           |                   | 53.6 ± 16.0       | 46.5 ± 13.5       | 0.137   | 59.9 ± 13.3         | 55.2 ± 14.9         | 0.261   |
| Gender               | Male      | 14                | 9 (40.9)          | 5 (26.3)          | 0.326   | 2 (25.0)           | 12 (63.6)           | 0.692   |
|                      | Female    | 27                | 13 (59.1)         | 14 (74.7)         |         | 6 (75.0)           | 21 (66.4)           |         |
| Location             | Salivary gland* | 34             | 16 (72.7)         | 18 (94.7)         | 0.062   | 8 (100.0)          | 26 (78.8)           | 0.310   |
|                      | Other†    | 7                 | 6 (27.3)          | 1 (5.3)           |         | 3 (100.0)          | 7 (21.2)            |         |
| Tumour size (mm)     | ≤20       | 11                | 5 (22.7)          | 6 (31.6)          | 0.597   | 3 (37.5)           | 8 (24.2)            | 0.498   |
|                      | 21–40     | 26                | 14 (63.6)         | 12 (63.2)         |         | 5 (62.5)           | 21 (63.6)           |         |
|                      | >40       | 4                 | 3 (13.6)          | 1 (5.3)           |         | 4 (12.2)           |               |         |
| Grade (by Spiro et al.) | I      | 20                | 7 (31.8)          | 13 (68.4)         | 0.049   | 4 (50.0)           | 16 (48.5)           | 0.606   |
|                      | II       | 11                | 7 (31.8)          | 4 (21.1)          |         | 3 (37.5)           | 8 (24.2)            |         |
|                      | III      | 10                | 8 (36.4)          | 2 (10.5)          |         | 1 (12.5)           | 9 (27.3)            |         |
| Lymphovascular invasion | Absent | 30                | 12 (64.5)         | 18 (94.7)         | 0.004   | 7 (87.5)           | 23 (69.7)           | 0.412   |
|                      | Present  | 11                | 10 (45.5)         | 1 (5.3)           |         | 1 (12.5)           | 10 (30.3)           |         |
| Perineural invasion  | Absent   | 10                | 1 (4.5)           | 9 (47.4)          | 0.002   | 3 (37.5)           | 7 (21.2)            | 0.378   |
|                      | Present  | 31                | 21 (95.5)         | 10 (52.6)         |         | 5 (62.5)           | 26 (78.8)           |         |
| Resection margin     | Clear    | 16                | 9 (40.9)          | 7 (36.8)          | 0.790   | 5 (62.5)           | 11 (33.3)           | 0.225   |
|                      | Involved | 25                | 13 (59.1)         | 12 (63.2)         |         | 3 (37.5)           | 22 (66.7)           |         |
| KIT expression       | Negative | 7                 | 7 (36.8)          |                 | 0.002   | 2 (25.0)           | 6 (18.2)            | 0.642   |
|                      | Positive | 34                | 22 (100.0)        | 12 (63.2)         |         | 6 (75.0)           | 27 (81.8)           |         |
| Pathological T stage† | pT1      | 8                 | 4 (25.0)          | 4 (25.0)          | 0.404   | 3 (37.5)           | 5 (20.8)            | 0.653   |
|                      | pT2      | 17                | 7 (43.8)          | 10 (62.5)         |         | 4 (50.0)           | 17 (54.2)           |         |
|                      | pT3      | 7                 | 5 (31.3)          | 2 (12.5)          |         | 1 (12.5)           | 6 (25.0)            |         |
| Recurrence/metastasis| Negative | 29                | 12 (54.5)         | 17 (89.5)         | 0.014   | 7 (87.5)           | 22 (66.7)           | 0.398   |
|                      | Positive | 12                | 10 (45.5)         | 2 (10.5)          |         | 1 (12.5)           | 11 (33.3)           |         |
clinicopathological factors according to KIT expression status revealed that only KIT-mutant cases were associated with KIT overexpression ($P < 0.001$; Table S3).

**Expression of PKC-δ and PKC-θ in each tumour type**

PKC-δ and PKC-θ were expressed in the cytoplasm in all tumour types (Figure 2). Among AdCCs, PKC-δ and PKC-θ were expressed in 22 (53.7%) and eight (19.5%) cases, respectively (Figure S1). For AdCCs, PKC-δ expression was significantly associated with higher histological grade ($P = 0.049$), the presence of lymphovascular invasion ($P = 0.004$), the presence of perineural invasion ($P = 0.002$), KIT positivity ($P = 0.002$), the presence of recurrence or metastasis ($P = 0.014$), and patient death ($P = 0.049$). No significant correlations between various clinicopathological factors and PKC-θ expression were identified (Table 1). In contrast, all ChRCCs showed PKC-δ and PKC-θ negativity in IHC (Figures 2 and S2).

In GISTs, PKC-δ was expressed in 15 (26.8%) cases, and PKC-θ was expressed in 37 (66.1%) cases (Figures 2 and S3). PKC-θ expression was associated with higher mitotic count ($P = 0.011$), high grade according to the modified NIH criteria ($P < 0.001$), the presence of recurrence or metastasis ($P = 0.011$), and patient death ($P = 0.025$). However, no significant correlations were found between various clinicopathological factors and PKC-δ expression in these tumours (Table 2).

**Prognostic impact of PKC-δ and PKC-θ expression on AdCC and ChRCC patient survival**

Survival analysis indicated that patients who expressed PKC-δ had significantly shorter RFS ($P = 0.017$; Figure 3A) and a tendency to have shorter OS ($P = 0.090$; Figure 3B). In contrast, patients who expressed PKC-θ showed a tendency, albeit insignificant, to have longer RFS and OS ($P = 0.284$ and $P = 0.259$, respectively; Figure 3C, D). Upon univariate analysis, the presence of lymphovascular invasion ($P = 0.039$) and PKC-δ expression ($P = 0.032$) were significantly associated with shorter RFS; however, upon multivariate analysis, only PKC-δ positivity showed a tendency to be associated with shorter RFS ($P = 0.095$). For OS, grade III status ($P = 0.022$) and the presence of lymphovascular invasion ($P = 0.048$) were associated with shorter OS in univariate analysis; however, only the presence of lymphovascular invasion tended, although without

| Category | Variables | Case no. ($n = 41$) | PKC-δ expression | PKC-θ expression |
|----------|-----------|---------------------|-------------------|-------------------|
|          |           | Alive ($n = 37$)    | Positive (%) ($n = 22$) | Negative (%) ($n = 19$) |
|          |           | Expired ($n = 4$)   | Positive (%) ($n = 22$) | Negative (%) ($n = 19$) |

- Includes minor salivary gland (two cases).
- Includes lacrimal gland (two cases), external auditory canal (two cases), and lung (three cases).
- Evaluated in 32 cases originating in major salivary glands.

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Table 2. Clinicopathological characteristics of 56 gastrointestinal stromal tumours (GISTs) according to protein kinase C (PKC)-δ and PKC-θ expression status

| Category          | Variables | PKC-δ expression |              | PKC-θ expression |              |
|-------------------|-----------|------------------|--------------|------------------|--------------|
|                   |           | Case no. (n = 56) | Positive (%) (n = 15) | Negative (%) (n = 41) | P-value |
|                   |           |                  |               |                  |              |
| Age (years)       |           |                  | 62.4 ± 13.4 | 56.3 ± 13.7 | 0.147 |
|                   |           |                  |               |                  |              |
| Gender            | Male      | 28               | 9 (60.0)    | 19 (46.3)    | 0.365 |
|                   |           |                  | 6 (40.0)    | 22 (53.7)    |              |
|                   | Female    | 28               | 6 (40.0)    | 22 (53.7)    | 0.365 |
|                   |           |                  | 15 (40.5)   | 13 (68.4)    |              |
| Location          | Stomach   | 33               | 8 (53.3)    | 25 (61.0)    | 0.140 |
|                   |           |                  | 13.4 ± C6   | 56.3 ± C6    | 0.140 |
|                   | Duodenum  | 7                | 4 (26.7)    | 3 (7.3)      |              |
|                   |           |                  | 13.7 ± C6   | 56.6 ± C6    | 0.607 |
|                   | Jejunum/ileum | 16          | 3 (20.0)    | 13 (31.7)    | 0.140 |
|                   |           |                  | 13.0 ± C6   | 56.6 ± C6    | 0.607 |
|                   |           |                  | 15.6 ± C6   |              |              |
| Tumour size (mm)  | ≤20       | 1                | 1 (2.4)     | 0.605         |              |
|                   |           |                  | 1 (5.3)     |              | 0.134 |
|                   | 21-50     | 22               | 7 (41.7)    | 15 (36.6)    |              |
|                   |           |                  | 12 (32.4)   | 10 (52.6)    |              |
|                   | 51-100    | 24               | 7 (41.7)    | 17 (41.5)    |              |
|                   |           |                  | 17 (45.9)   | 7 (36.8)     |              |
|                   | >100      | 9                | 1 (16.7)    | 8 (19.5)     |              |
|                   |           |                  | 8 (21.6)    | 1 (5.3)      |              |
|                   |           |                  |              |              |              |
| Mitotic count*    | ≤5        | 27               | 7 (46.7)    | 20 (48.8)    | 0.900 |
|                   |           |                  | 13 (35.1)   | 14 (73.7)    | 0.011 |
|                   | 6-10      | 9                | 2 (13.3)    | 7 (17.1)     |              |
|                   |           |                  | 6 (16.2)    | 3 (15.8)     |              |
|                   | >10       | 20               | 6 (40.0)    | 14 (34.1)    |              |
|                   |           |                  | 18 (48.6)   | 2 (10.5)     |              |
| Grade (modified NIH) | Low     | 19               | 6 (40.0)    | 13 (31.7)    | 0.498 |
|                   |           |                  | 10 (27.0)   | 9 (47.4)     | <0.001 |
|                   | Intermediate | 9            | 1 (6.7)     | 8 (19.5)     |              |
|                   |           |                  | 2 (5.4)     | 7 (36.8)     |              |
|                   | High      | 28               | 8 (53.3)    | 20 (48.8)    |              |
|                   |           |                  | 25 (67.6)   | 3 (15.8)     |              |
| KIT mutation      | Absent†   | 15               | 2 (13.3)    | 13 (31.7)    | 0.306 |
|                   |           |                  | 10 (27.0)   | 5 (26.3)     | 0.955 |
|                   | Present   | 41               | 13 (86.7)   | 28 (68.3)    |              |
|                   |           |                  | 27 (73.0)   | 14 (73.7)    |              |
| KIT expression    | Negative  | 10               | 2 (13.3)    | 8 (19.5)     | 0.713 |
|                   |           |                  | 5 (13.5)    | 5 (72.2)     | 0.281 |
|                   | Positive  | 46               | 13 (86.7)   | 33 (81.5)    |              |
|                   |           |                  | 32 (86.5)   | 14 (56.0)    |              |
| Recurrence/metastasis | Negative | 41               | 11 (73.3)   | 30 (73.2)    | >0.999 |
|                   |           |                  | 23 (62.2)   | 18 (94.7)    | 0.011 |
|                   | Positive  | 15               | 4 (26.7)    | 11 (26.8)    |              |
|                   |           |                  | 14 (37.8)   | 1 (5.3)      |              |
| Survival          | Alive     | 43               | 10 (66.7)   | 33 (80.5)    | 0.302 |
|                   |           |                  | 25 (67.6)   | 18 (94.7)    | 0.025 |
|                   | Expired   | 13               | 5 (33.3)    | 8 (19.5)     |              |
|                   |           |                  | 12 (32.4)   | 1 (5.3)      |              |

NIH, National Institutes of Health.
*Counted in 50 high-power fields.
†Consists of nine wild-type GISTs and six PDGFRα-mutant GISTs.
statistical significance, to be associated with shorter OS in multivariate analysis ($P = 0.078$). The results of univariate and multivariate analyses are summarized in Table 3.

Survival analysis and multivariate analysis were not available for ChRCC patients, as only one patient developed locoregional recurrence and two patients died during follow-up.

**PROGNOSTIC IMPACT OF PKC-\( \delta \) AND PKC-\( \theta \) EXPRESSION ON GIST PATIENT SURVIVAL**

Survival analysis failed to reveal any significant association between PKC-\( \delta \) expression and RFS ($P = 0.783$; Figure 4A) or OS ($P = 0.171$; Figure 4B). In contrast, PKC-\( \theta \) positivity was associated with shorter RFS ($P = 0.016$; Figure 4C) and showed a tendency to be associated with shorter OS ($P = 0.090$; Figure 4D). Univariate analysis indicated that tumour size of $>50$ mm ($P = 0.028$), high grade according to the modified NIH criteria ($P = 0.009$), mitotic count of $>5$ per 50 high-power fields ($P = 0.012$) and PKC-\( \theta \) expression ($P = 0.043$) were associated with shorter RFS. Multivariate analysis, however, indicated that only high grade according to the modified NIH criteria ($P = 0.031$) was associated with shorter RFS. In addition, high grade according to the modified NIH criteria ($P = 0.030$) and mitotic count of $>5$ per 50 high-power fields ($P = 0.041$) were significantly associated with OS, whereas PKC-\( \theta \) expression ($P = 0.086$) showed a tendency to be associated with shorter OS ($P = 0.090$).
| Category Variables | RFS | | | OS | | |
|-------------------|-----|---|---|-----|---|
|                   | Univariate | Multivariate | Univariate | Multivariate | |
|                   | HR (95% CI) | P-value | HR (95% CI) | P-value | |
| Age (years)* ≤50  | 1 | - | 1 | - |
| >50               | 2.665 (0.720–9.681) | 0.142 | - | - |
| Gender Female     | 1 | - | 1 | - |
| Male              | 2.156 (0.694–6.698) | 0.184 | - | - |
| Location Salivary gland | 1 | - | 1 | - |
| Other†            | 0.947 (0.443–2.024) | 0.889 | - | - |
| Tumour size (mm) ≤20 | 1 | - | 1 | - |
| >20               | 4.755 (0.612–36.914) | 0.136 | - | - |
| Grade (by Spiro et al.) I and II | 1 | - | 1 | - |
| III               | 1.139 (0.307–4.221) | 0.846 | - | - |
| Lymphovascular invasion Absent | 1 | 1 | 1 | 1 |
| Present           | 3.317 (1.061–10.376) | 0.039 | 1.926 (0.569–6.522) | 0.292 | 9.837 (1.020–94.869) | 0.048 | 8.069 (0.790–82.405) | 0.078 |
| Perineural invasion Absent | 1 | - | 1 | - |
| Present           | 4.179 (0.539–32.413) | 0.171 | - | - | 3.786 (0.099–144.954) | 0.474 | - | - |
| Resection margin Clear | 1 | - | 1 | - |
| Involved          | 1.846 (0.499–6.829) | 0.358 | - | - | 2.329 (0.238–22.778) | 0.467 | - | - |
| Pathological T stage† pT1 | 1 | - | 1 | - |
| pT2               | 2.183 (0.243–19.610) | 0.486 | - | - | 4.907 (0.151–159.333) | 0.370 | - | - |
| pT3               | 5.327 (0.593–47.882) | 0.135 | - | - | 3.158 (0.064–156.282) | 0.564 | - | - |
| KIT IHC Negative  | 1 | - | 1 | - |
| Positive          | 2.453 (0.316–19.031) | 0.391 | - | - | 3.094 (0.019–498.365) | 0.663 | - | - |
| PKC-δ IHC Negative | 1 | 1 | 1 | - |
| Positive          | 5.279 (1.151–24.204) | 0.032 | 4.011 (0.786–20.461) | 0.095 | 5.636 (0.253–125.350) | 0.275 | - | - |
with shorter OS in univariate analysis. The results of univariate and multivariate analyses are summarized in Table 4.

**Discussion**

In this study, we found rare expression of PKC-δ and PKC-θ in ChRCCs, and variable expression of these two enzymes in AdCCs and GISTs. The expression pattern of these two PKC isozymes differed between AdCC and GIST. PKC-δ positivity was much higher than PKC-θ positivity and was associated with various clinicopathological factors and with prognosis in AdCCs. In GISTs, however, PKC-θ expression was higher than PKC-δ expression and was associated with clinicopathological factors and prognosis.

In addition to the different expression patterns of these two PKC isozymes, KIT expression also differed among the tumour types, showing a membranous localization in AdCCs and a cytoplasmic localization in GISTs. Generally, the distribution of KIT protein differs according to KIT mutation status: wild-type KIT localizes to the plasma membrane, and mutant KIT protein localizes to the intracellular compartment. In our study, however, 10 GISTs showed weak and focal cytoplasmic expression of KIT by IHC, and the majority of these cases were devoid of KIT mutations. We recently reported intracellular co-localization of PKC-θ and KIT in GIST cell lines, regardless of KIT mutation status. Considering the exclusive expression of PKC-θ in GISTs, interaction between PKC-θ and KIT may cause cytoplasmic localization of KIT in GISTs in the absence of KIT mutation.

Unlike what was seen in GIST, KIT mutation was not identified in ChRCC, and the genetic status of KIT in AdCC remains controversial. In this study, KIT was expressed in a membranous pattern in all cases of AdCC and ChRCC, and this expression pattern is relevant to the presence of wild-type KIT according to the previous study. Although mutation analysis was performed in only randomly selected 20 AdCCs, it is plausible to assume that AdCC and ChRCC may not harbour KIT mutations, as indicated by the pattern of expression consistent with wild-type KIT. We previously reported that the mechanism that sustains KIT activity differs according to KIT protein type: PKC-δ-mediated recycling in wild-type KIT-expressing colon cancer, and a PKC-θ-mediated mechanism in GIST expressing mutant KIT. Considering the correlation between KIT and different PKC isozymes in each tumour, it is plausible to speculate that the different expression

| Category | Variables | RFS | OS | Univariate | Multivariate | Univariate | Multivariate |
|----------|-----------|-----|----|------------|--------------|------------|--------------|
| PKC-θ IHC | Positive | 0.344 (0.044–2.672) | 0.308 | 0.264 (0.007–10.417) | 0.478 |
| Negative | 1 | 1 | 1 | 1 | 1 |

CI, Confidence interval; HR, Hazard ratio; IHC, Immunohistochemistry; PKC, Protein kinase C.

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patterns of KIT in different tumours may be associated with PKC isoforms that are specific to tumour or KIT mutation status. In addition, this could explain the different impacts of different PKC isoforms on patients’ prognosis for each tumour. However, further studies are required to prove this hypothesis.

The role of KIT protein in the tumorigenesis of AdCC has not yet been fully investigated. MYB protein activation has been reported as a possible mechanism for KIT activation in AdCCs. In one-half of AdCCs, MYB is activated via gene fusion with nuclear factor I/B, and this activated MYB regulates the transcription of KIT. The expression localizations of MYB and KIT differ, however, with MYB expression in myoepithelial cells and KIT expression in luminal cells. Therefore, the difference between MYB and KIT expression localizations raises the possibility of a novel KIT regulation mechanism other than MYB activation. Recently, Phuchareon et al. reported a significant correlation between KIT mRNA and stem cell factor (SCF) mRNA in AdCCs with perineural invasion. Furthermore, a similar distribution of KIT and SCF was observed via IHC, suggesting a possible influence of SCF on KIT activity. In this study, a significant correlation between diffuse KIT expression and perineural invasion was observed in AdCCs, which was in accordance with the results reported by Phuchareon et al.

We previously reported persistent KIT activation via PKC-δ-mediated recycling in wild-type KIT-expressing colon cancers. In AdCCs, PKC-δ expression was associated with patient prognosis.

Figure 4. Relapse-free survival (RFS) and overall survival (OS) of 56 gastrointestinal stromal tumour cases according to protein kinase C (PKC-δ and PKC-θ) expression status. A, B, No significant association between PKC-δ expression and RFS (P = 0.783) or OS (P = 0.171) was observed. C, D, PKC-θ expression was associated with significantly shorter RFS (P = 0.016) and showed a tendency to be associated with shorter OS (P = 0.051).
Table 4. Univariate and multivariate analysis of relapse-free survival (RFS) and overall survival (OS) in 56 gastrointestinal stromal tumours

| Category Variables | RFS | | | OS | | |
|-------------------|-----|-----|-----|-----|-----|-----|
|                   | Univariate | Multivariate | Univariate | Multivariate |
|                   | HR (95% CI) | \(\text{P-value}\) | HR (95% CI) | \(\text{P-value}\) | HR (95% CI) | \(\text{P-value}\) |
| Age (years)*      |       |     |       |     |       |     |
| \(\leq 62\)      | 1     |     | 1     |     | 1     |     |
| \(>62\)          | 1.810 (0.617–5.308) | 0.280 | 2.284 (0.626–8.330) | 0.211 |
| Gender            |       |     |       |     |       |     |
| Female            | 1     |     | 1     |     | 1     |     |
| Male              | 2.005 (0.679–5.920) | 0.208 | 2.358 (0.650–8.554) | 0.192 |
| Location          |       |     |       |     |       |     |
| Gastric           | 1     |     | 1     |     | 1     |     |
| Non-gastric†     | 2.403 (0.795–7.260) | 0.120 | 2.241 (0.622–8.068) | 0.217 |
| Tumour size (mm) |       |     |       |     |       |     |
| \(\leq 50\)      | 1     |     | 1     |     | 1     |     |
| \(>50\)          | 9.724 (1.278–73.996) | 0.028 | 4.712 (0.777–28.570) | 0.092 |
| Mitotic count‡   |       |     |       |     |       |     |
| \(\leq 5\)       | 1     |     | 1     |     | 1     |     |
| \(>5\)           | 13.354 (1.750–101.875) | 0.012 | 8.389 (1.087–64.756) | 0.041 |
| Grade (modified NIH) | Low to intermediate | 1 | 1 | 1 | 1 |
|                   | High | 15.094 (1.981–115.018) | 0.009 | 10.166 (1.232–83.900) | 0.031 | 9.658 (1.252–74.500) | 0.030 |
| KIT mutation      |       |     |       |     |       |     |
| Absent§           | 1     |     | 1     |     | 1     |     |
| Present           | 1.942 (0.545–6.925) | 0.306 | 1.894 (0.503–7.133) | 0.345 |
| KIT IHC           |       |     |       |     |       |     |
| Negative          | 1     |     | 1     |     | 1     |     |
| Positive          | 1.609 (0.362–7.150) | 0.532 | 0.981 (0.211–4.561) | 0.980 |
| PKC-δ IHC         |       |     |       |     |       |     |
| Negative          | 1     |     | 1     |     | 1     |     |
| Positive          | 1.174 (0.373–3.699) | 0.784 | 2.160 (0.699–6.679) | 0.181 |
| PKC-θ IHC         |       |     |       |     |       |     |
| Negative          | 1     |     | 1     |     | 1     |     |
| Positive          | 8.095 (1.063–61.637) | 0.043 | 2.976 (0.361–24.496) | 0.311 | 6.040 (0.775–47.056) | 0.086 |

CI, Confidence interval; HR, Hazard ratio; IHC, Immunohistochemistry; NIH, National Institutes of Health; PKC, Protein kinase C.

*Median age of 56 patients was 62.0 years.
†Includes duodenum, jejunum, and ileum.
‡Counted in 50 high-power fields.
§Contains PDGFRα-mutant and wildtype.
and various clinicopathological factors, especially with KIT protein expression. Considering the correlation between KIT and PKC-δ expression, our findings suggest that KIT may be regulated in AdCCs via a mechanism similar to that observed in wild-type KIT-expressing colon cancers. Therefore, our findings provide evidence for a possible mechanism of KIT regulation other than MYB activation in AdCC; however, further studies are required to more definitively elucidate the relationships among MYB, KIT, and PKC-δ.

Besides the tumour-promoting effect, PKC-δ also acts as a tumour suppressor. PKC-δ negatively regulates several tumorigenesis mechanisms, such as the Wnt signalling pathway, Gli1 activation, the Hedgehog signalling pathway, and extracellular signal-regulated kinase activation, in various types of tumour. In addition, PKC-δ inhibits cell cycle progression and up-regulates apoptosis in prostate cancer. However, in-vitro studies support the role of PKC-δ as a tumour promoter. Therefore, further studies are required to elucidate the role of PKC-δ as a tumour suppressor.

ChRCCs rarely showed PKC-δ and PKC-θ expression in this study. In addition, no correlations between clinicopathological variables and PKC-δ or PKC-θ expression in ChRCCs were identified. Some studies have reported that PKC-δ is associated with invasion and migration in renal cell carcinoma (RCC) cells; however, the majority of studies were conducted on the clear cell RCC or RCC cell lines. Recently, a correlation between increased expression of PKC-ζ and poor prognosis in RCC was reported. In addition, PKC-ζ showed a tendency to be highly expressed in non-conventional RCC, such as papillary RCC or ChRCC. These findings suggest a possible role of PKC-ζ in the development and progression of ChRCC. Further studies are required to investigate possible correlations between clinicopathological factors and PKC-ζ expression in a large cohort of ChRCC cases.

In conclusion, we have identified differences in associations between KIT expression and PKC-δ and PKC-θ expression in patients with AdCC and GIST. In AdCC patients, KIT expression was associated with PKC-δ expression; in contrast, KIT expression was associated with PKC-θ expression in GIST patients. In addition, PKC-δ expression and PKC-θ expression were associated with various clinicopathological factors and patient prognosis for AdCC and GIST, respectively. These findings indicate that PKC-δ and PKC-θ should be investigated as potential therapeutic targets for these tumour types.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

C. K. Park: data acquisition and interpretation, and manuscript preparation. W. K. Kim: study conception and design, and data interpretation. H. Kim: study conception and design, data acquisition and interpretation, and manuscript preparation.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Expression profile of KIT, PKC-δ and PKC-θ in AdCC.

Figure S2. Expression profile of KIT, PKC-δ and PKC-θ in ChRCC.

Figure S3. Expression profile of KIT, PKC-δ and PKC-θ in GIST.

Table S1. Pathological parameters of each tumour obtained by histological evaluation.

Table S2. Clinicopathological characteristics of 41 AdCCs according to KIT expression.

Table S3. Clinicopathological characteristics of 56 GISTs according to KIT expression.