主論文の要旨

Role for Daple in non-canonical Wnt signaling during gastric cancer invasion and metastasis

（胃がんの浸潤と転移に関与する非古典的Wntシグナル経路におけるDapleの役割）

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Introduction

In gastric cancer, the non-canonical Wnt signaling pathway is activated by Wnt5a, which has a critical role in disease outcome. High levels of expression of Wnt5a have been reported to promote invasion in advanced gastric cancer by inducing the expression of laminin γ2, through the activation of Rac, JNK, and the transcription factor jun D. Importantly, cytoplasmic staining for laminin γ2 has been observed at the invasive front of gastric cancer and correlated with Wnt5a expression, indicating the relevance of the Wnt5a/laminin γ2 pathway in gastric cancer progression. However, the mechanisms by which Wnt5a induces this process remain unclear.

In the present study, we investigated the involvement of Dvl-associating protein with a high frequency of leucine residues (Daple) in the Wnt5a／laminin γ 2 pathway in gastric cancer. We previously reported that Daple mediates the Wnt5a-induced interaction of Dvl with atypical protein kinase C (aPKC), which promotes Rac activation and lamellipodia formation in migrating fibroblasts. Here, using tissue sections from patients with gastric cancer, we demonstrated the relevance of Daple expression to gastric cancer progression. We also clarified, using cultured cancer cells and a xenograft mouse tumor model, that Daple mediates Wnt5a-induced laminin γ2 expression and regulates gastric cancer invasion and metastasis.

Materials and Methods

We obtained 130 tissue samples from patients with gastric cancer who underwent surgical treatment at Nagoya University Hospital. Tissue sections were stained with anti-Daple, anti-Wnt5a, anti-laminin γ2, and anti-β-catenin antibodies. Human gastric cancer cell lines MKN45 and KKLS cells were transfected with Daple siRNA or transduced with Daple shRNA expressing retrovirus. Cells were lysed and analyzed by Western blot or Quantitative RT-PCR. GTP loading of Rac was determined by pull-down assay using GST-PAK-PBD. Cell migration and invasion was examined with transwell assays using 8-μm pore polyethylene terephthalate membranes or Biocoat Matrigel invasion chambers. In metastasis assays, Daple stably knockdown KKLS cells were injected into the spleen of nude mice. After 5 weeks, the numbers of metastatic nodules were counted in the liver.

Results

Immunohistochemical analysis showed no or weak staining for Daple in the epithelia of normal stomach or in cancer cells from the early stage of gastric cancer, whereas Daple expression was clearly observed in cancer cells at more advanced stages (Fig. 1a). Daple staining was graded by intensity score (Fig. 1b) and proportion score (Fig. 1c). A total score >3 was defined as Daple positive; these constituted 85／130 (65.38%) cases. Daple expression was statistically correlated with the depth of gastric wall invasion ($P = 0.001$), the frequency of lymph node metastasis ($P = 0.0162$) and clinical stage ($P = 0.0037$) (Table 1). Furthermore, the Kaplan–Meier survival curve showed that the postoperative survival rate was significantly lower for patients who were

Daple-positive rather than Daple negative (P = 0.0166 by log-rank test) (Fig. 1d).

We found that Daple expression was significantly correlated with Wnt5a/b positivity (P < 0.001) but not with β-catenin nuclear staining in our cohort (P = 0.3194) (Table 2), suggesting a role for Daple in the non-canonical Wnt signaling pathway. Both Wnt5a/b and cytoplasmic laminin γ2 expression significantly correlated with Daple positivity when limited to the diffusescattered type (P < 0.001 and P < 0.01, respectively) (Table 2, Fig. 1e). In other types, although Daple and Wnt5a/b expression were significantly correlated (P < 0.001), significant correlation was not observed between Daple and cytoplasmic laminin γ2 expression (P = 0.54) (Table 2, Fig. 1f). These data, together with the association of Daple expression with clinicopathological features, suggest that Daple preferentially coexpresses with Wnt5a/b and laminin γ2 to regulate the noncanonical Wnt signaling pathway in invasive gastric cancer.

In the MKN45 gastric cancer cell line, Daple depletion led to the suppression of Wnt5a-induced Rac and JNK activation, laminin γ2 expression (Fig. 2a), and cell migration (Fig. 2b) and invasion (Fig. 2c). In KKLS cells, which expresses high levels of Wnt5a (Fig. 3a), Daple depletion significantly decreased laminin γ2 expression (Fig. 3b), migration (Fig. 3c) and invasion (Fig. 3d). However, cell proliferation was not affected, showing the specific role of Daple for cell motility (Fig. 3e).

Given that Daple expression was correlated with lymph node metastasis (Table 1), we examined the effect of Daple knockdown on metastasis in a xenograft tumor mouse model (Fig. 4). Control KKLS cells transplanted intrasplenically exhibited the propensity for liver metastasis in immunocompromised mice (Fig. 4a). In contrast, Daple knockdown KKLS cells rarely metastasized to the liver, and the number of metastatic nodules was significantly decreased compared with control cells (P = 0.0397) (Fig. 4a,b). However, metastatic nodule size was not significantly affected by Daple knockdown (Fig. 4c). Immunohistochemical analysis of metastatic tissues showed coexpression of Daple and Wnt5a/b in control but not Daple-depleted tumors (Fig. 4d). These findings suggest that Daple mediates Wnt5a-expressing gastric cancer cell metastasis.

Discussion

Here, we showed that Daple is highly expressed in advanced gastric cancer, where its expression significantly correlated with the depth of gastric wall invasion, frequency of lymph node metastasis and poor prognosis. We also demonstrated that Daple mediates the non-canonical Wnt signaling pathway to regulate laminin γ2 expression, previously shown to be critical for gastric cancer progression. These findings offer an opportunity for the development of new therapeutics for advanced gastric cancer

Our data showed that Daple expression significantly correlates with laminin γ2 only in diffuse-scattered type gastric cancer (Table 2), which notably was also shown to exclusively exhibit correlation of Wnt5a with laminin γ2. One plausible hypothesis to explain such

histologically-specific functioning is that, because laminin γ2 is a major component of laminin 5, which constitutes the cancer stroma that supports cancer cell invasion, laminin γ2 expression might be specifically important for the invasion of small cancer cell nests that accompany the desmoplastic reaction and fibrosis of cancer stroma as occurs in the diffuse-scattered type of gastric cancer. Furthermore, Daple expression correlated with Wnt5a/b expression independently of histological type (Table 2), suggesting that the Wnt5a / Daple pathway has multifaceted, laminin γ2-independent roles in cancer progression (Fig. 4e). Overall, these disparate possibilities indicate that further studies are required to reveal the biochemical mode of Daple function downstream of non-canonical Wnt stimulation in various types of gastric cancer.

Conclusion

Our results suggest that the non-canonical Wnt signaling pathway contributes to gastric cancer progression at least in part via Daple, which provides a new therapeutic opportunity for the treatment of the disease.