News Release

Title

Connective tissue growth factor-specific monoclonal antibody inhibits growth of malignant mesothelioma in an orthotopic mouse model

Key Points

- Mesothelioma is one of the most aggressive tumors and its median survival is expected as 4-18 months for pleural forms. It is thus necessary to develop molecularly targeted drugs to improve the patients' prognosis.
- *CTGF* overexpression is reported in several distinct human diseases, including mesothelioma, idiopathic pulmonary fibrosis, nephropathy/glomerulosclerosis, pancreatic ductal adenocarcinoma, malignant melanoma and ovarian cancer in association with progression of the disease and/or poor survival
- In orthotopic nude mice model, a human monoclonal antibody that antagonizes CTGF (FG-3019, pamrevlumab) significantly inhibited mesothelioma growth.

Summary

Malignant mesothelioma is an aggressive neoplasm with no particularly effective treatments. We previously reported that overexpression of connective tissue growth factor (CTGF/CCN2) promotes mesothelioma growth, thus suggesting it as a novel molecular target. A human monoclonal antibody that antagonizes CTGF (FG-3019, pamrevlumab) attenuates malignant characteristics of various human cancers and is currently under clinical trial for the treatment of pancreatic cancer. This study reports the effects of FG-3019 on human mesothelioma *in vitro* and *in vivo*. We analyzed the effects of FG-3019 on the proliferation, apoptosis, migration/invasion, adhesion and anchorage-independent growth in three human mesothelioma cell lines, among which ACC-MESO-4 was most efficiently blocked with FG-3019 and was chosen for *in vivo* experiments. In orthotopic nude mice model, FG-3019 significantly inhibited mesothelioma growth. Histological analyses revealed that FG-3019 not only inhibited the proliferation but also induced apoptosis in both mesothelioma cells and fibroblasts. Our data suggest that FG-3019 antibody therapy could be a novel additional choice for the treatment of mesothelioma.

Research Background

Mesothelioma is a neoplasm caused primarily by asbestos exposure. Asbestos is now recognized as a carcinogen and its use has been legally prohibited in most developed

countries. However, because it takes 30-40 years to develop mesothelioma after asbestos inhalation, the incidence of mesothelioma is expected to rise in the coming decades. In Europe, its peak incidence is predicted to be in 2015-2020 whereas there is a delay in the peak in Japan and in other non-Western countries. Mesothelioma is one of the most aggressive tumors at the time of diagnosis and its median survival is expected as 4-18 months for pleural forms. It is thus necessary to understand the molecular mechanisms that regulate mesothelial carcinogenesis and mesothelioma progression, and to develop molecularly targeted drugs to improve the patients' prognosis.

Research Results

We first performed western blot analysis to confirm which human mesothelioma cell lines express high levels of CTGF. All the cell lines examined expressed CTGF, but several cell lines expressed low levels of CTGF (Figure 1A and 1B). We chose the cell lines which expressed higher CTGF levels, including ACC-MESO-4. We evaluated the effects of FG-3019 on human mesothelioma cells *in vitro* and *in vivo*. We evaluated the proliferation, apoptosis, migration, invasion and anchorage-independent cell growth of the three mesothelioma cell lines under the agents (IgG/FG-3019: 100 μg/ml, Pemetrexed (PEM): 1 μM). FG-3019 (+ PEM) inhibited migration and adhesion of mesothelioma cells (Figure 1C and 1D).

In vivo model, ACC-MESO-4 cells were implanted orthotopically (intrapleurally) into *BALB/c nu/nu* mice. IgG or FG-3019 was administered at 40 mg/kg intrapleurally twice a week; PEM was administered at 50 mg/kg intraperitoneally once a week. At 9 weeks after transplantation (day 56-57), the mice were euthanized and the pleural cavity was examined. As a result, the average tumor weight was significantly lower in the FG- 3019 group than in the IgG group ($P < 0.05$; Figure 2A and 2B). The average tumor weight was also significantly lower in FG- 3019 + PEM group than in the IgG group ($P < 0.001$) or in the IgG + PEM group ($P < 0.05$), demonstrating an enhanced effect. Blocking CTGF with FG-3019 inhibited proliferation and induced apoptosis not only in mesothelioma cells but also in stromal cells (Figure 2C-2F). We summarized the scheme of this article (Figure 3).

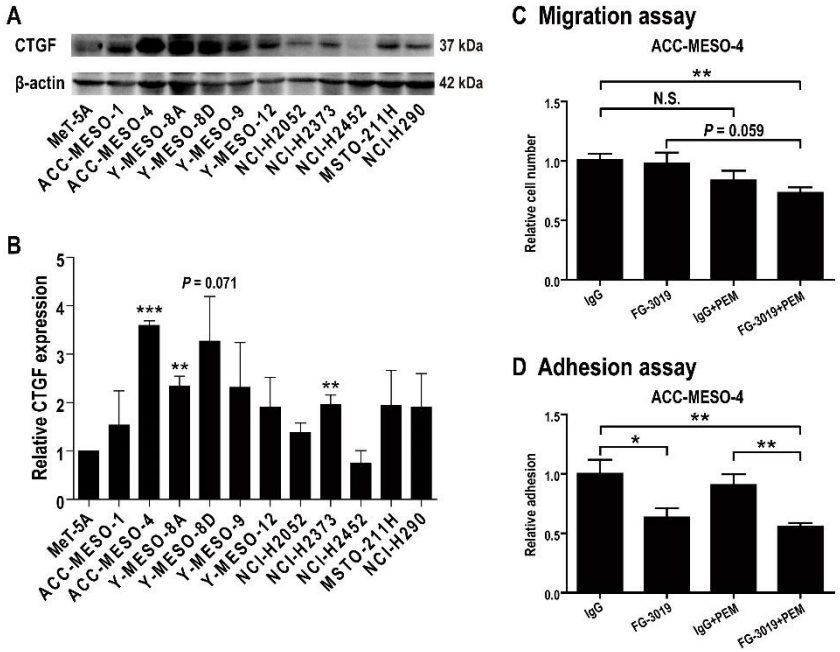

Figure 1: ***In vitro*** **experiments. (A)** Western blot analysis. All the cell lines examined expressed CTGF, but several cell lines expressed low levels of CTGF. **(B)** Semiquantitative analysis of western blot analysis. Relative CTGF expression in comparison to MeT-5A was calculated. **(C)** Migration assay. As compared with IgG group, migration was inhibited only in FG-3019 + PEM group, indicating that FG-3019 enhanced the effect of PEM. **(D)** Adhesion assay. Attached cells on Matrigel matrix-coated plate were measured with MTT assay.

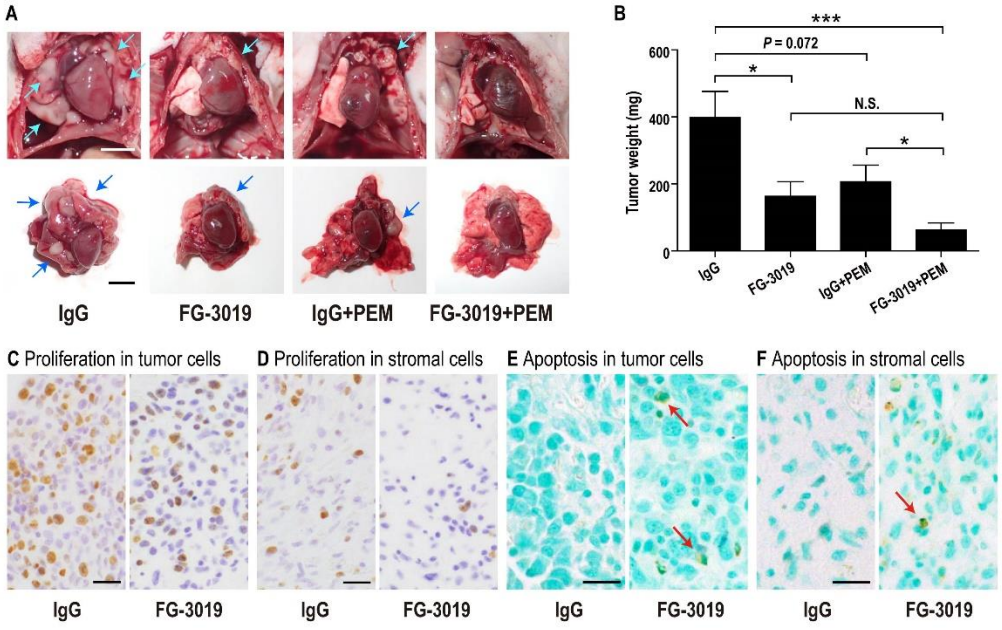

Figure 2: *In vivo* experiments. (A) Macroscopic findings. The tumors (arrows) grew in the pleural space (upper panel). The tumor including thoracic organs was dissected (lower panel). **(B)** Weight of tumors. Tumor weights of FG-3019-treated group were significantly lower than those of IgG control group. **(C, D)** Quantitation of proliferation using Ki-67 antibody. **(E, F)** Quantitation of apoptosis using TUNEL assay (arrows).

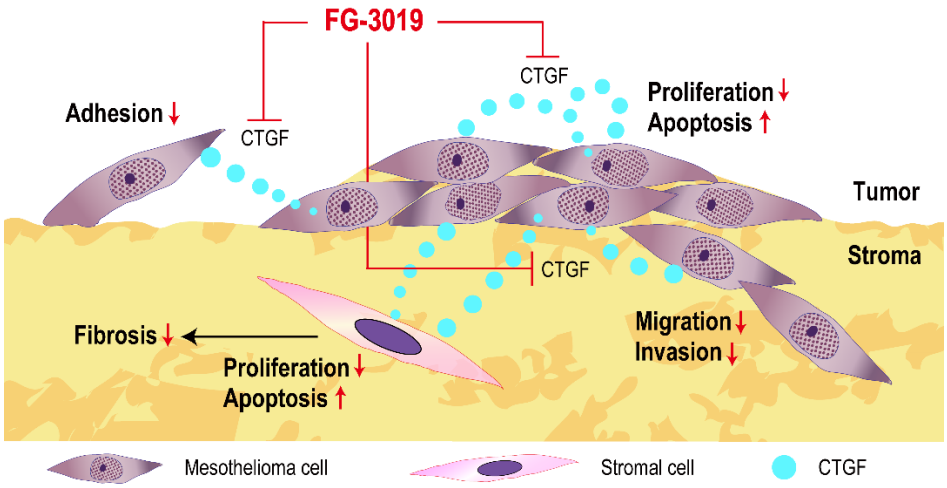

Figure 3: Summary scheme. FG-3019 attenuated proliferation and induced apoptosis both in mesothelioma cells and stromal cells. FG-3019 may target both autocrine and paracrine effects of CTGF on mesothelioma and stromal cells. Light blue circles indicate CTGF.

Research Summary and Future Perspective

In conclusion, the human monoclonal antibody, FG-3019, was effective as mesothelioma treatment in a murine orthotopic implant model. Our results suggest that FG-3019 would be beneficial to human mesothelioma patients. There is a strong medical need for more effective treatment of mesothelioma, and the data reported here provide a rationale to consider testing FG-3019 in clinical trials for mesothelioma patients as an addition to conventional therapies.

Publication

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" Connective tissue growth factor-specific monoclonal antibody inhibits growth of malignant mesothelioma in an orthotopic mouse model ", *Oncotarget*, published online on April 6, 2018. DOI: 10.18632/oncotarget.24892

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