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財団法人　日中医学協会　御中

貴財団より助成金を受領して行った研究テーマについて報告いたします。

添付資料：**研究報告書**

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２．研究テーマ

宿主由来HGFによる変異型EGFR陽性肺癌のゲフィチニブ耐性機構解析とその克服

３．成果の概要

ヒト肺癌株として、EGFR活性型変異(Exon 19 の deletion)を有しているが T790M second mutation や MET 増幅を有しておらず、ゲフィチニブやエルロチニブに高感受性を示す PC-9 および HCC827 を用いた。ヒト線維芽細胞株として MRC-5 と IMR-90 を使用した。これらヒト細胞株の HGF 産生を ELISA で測定したところ、肺がん細胞株や血管内皮細胞株（HUVEC と HMVEC）ではなく線維芽細胞株が HGF を高発現していた。切除肺癌組織を用いた蛍光染色でも、症例によっては間質の線維芽細胞が HGF を過剰発現していた。また共培養することにより、HGF を高発現する線維芽細胞株および肺癌組織から得た初代培養線維芽細胞は、肺癌細胞株（PC-9 や HCC827）のゲフィチニブ耐性を誘導した。線維芽細胞由来 HGF はその受容体である MET をリン酸化し、EGFR や ERBB3 とは無関係に PI3K/Akt 経路を活性化することにより、ゲフィチニブ耐性を誘導していた。さらに、SCID マウス皮下に PC-9 とヒト線維芽細胞株 MRC-5 を混合接種するモデルにおいて、腫瘍はゲブィチニブに耐性化したが、抗 HGF 抗体や HGF-MET 阻害薬である NK4 併用することで線維芽細胞によるゲフィチニブ耐性を克服したえ。以上の結果より、腫瘍微小環境を形成する線維芽細胞が産生する HGF が EGFR 活性型変異を有する肺癌のゲフィチニブニ対する自然耐性および獲得耐性の原因の一つになっている可能性が示唆された。また、HGF-MET 阻害薬併用により耐性が克服できる可能性が示されたため、現在最適の耐性克服治療法を確立すべくさらに検討を進めて

いる。

４．研究業績

（１）学会における発表　　無・有（学会名・演題）

1. Stromal fibroblasts induce resistance of lung cancer to EGFR tyrosine kinase inhibitors. 13th Japanese Association for Molecular Target Therapy of Cancer. 第１３回日本がん分子標的治療学会. June 24-26, 2009. Tokushima.
2. Stromal fibroblasts induce resistance of lung cancer to EGFR tyrosine kinase inhibitors. *18th Annual Meeting of the Japanese Association for Metastasis Research.* 第１８回日本がん転移学会. July 23-24, 2009. Asahigawa, Hokaido.
3. Stromal fibroblasts induce resistance of lung cancer to EGFR tyrosine kinase inhibitors. *2009 Asia-Pacific Conference of Tumor Biology and Medicine and Fourth Forum of Chinese Young and Middle-aged Oncologists.* Oct 24-27, 2009. Xi'An, China.

（２）発表した論文　　無・有（雑誌名・題名）

Wang W, Li Q, Yamada T, Matsumoto K, Matsumoto I, Oda M, Watanabe G, Kayano Y, Nishioka Y, Sone S, Yano S. Crosstalk to stromal fibroblasts induces resistance of lung cancer to EGFR tyrosine kinase inhibitors. ***Clin Cancer Res.*** 2009 Nov 1;15(21):6630-8.

-日中医学協会助成事業-

宿主由来HGFによる変異型EGFR陽性肺癌のゲフィチニブ耐性機構解析とその克服

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要旨

Most EGFR mutant NSCLCs initially respond to EGFR tyrosine kinase inhibitors, gefitinib and erlotinib, but these tumors invariably develop drug resistance. Wang W, et al, found that lung cancer cells recruited human fibroblasts whereas fibroblasts induced resistance of lung cancer to EGFR-TKIs by secreting HGF. Co-localization of fibroblasts and HGF was detected in both xenograft tumors and lung cancer patient specimens. Anti-HGF antibody, HGF antagonist and MET-TKI could overcome the resistance to EGFR-TKIs of lung cancer. Therefore, crosstalk to stromal fibroblasts plays a critical role in lung cancer resistance to EGFR-TKIs and maybe an ideal therapeutic target for lung cancer.

Key Words　EGFR-TKI, HGF, Tumor microenviroment, Fibroblast, Drug Resistance

緒　言：

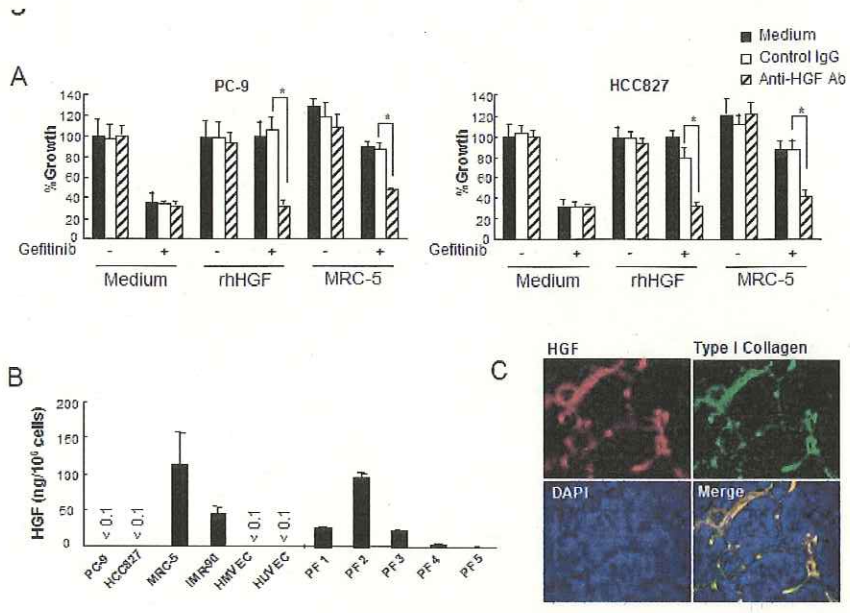
Lung cancers with *EGFR* activating mutations show good clinical response to gefitinib and erlotinib, selective TKIs to epidermal growth factor receptor (EGFR), but these tumors invariably develop drug resistance. Host stromal cells have been found to have a considerable effect on the behavior of cancer cells. Little is known, however, about the role of host cells on the sensitivity of cancer cells to receptor tyrosine kinase inhibitors (TKIs). We have therefore assessed the effect of crosstalk between stromal cells and lung cancer cells harboring *EGFR* mutations on susceptibility to EGFR-TKIs.

対象と方法：

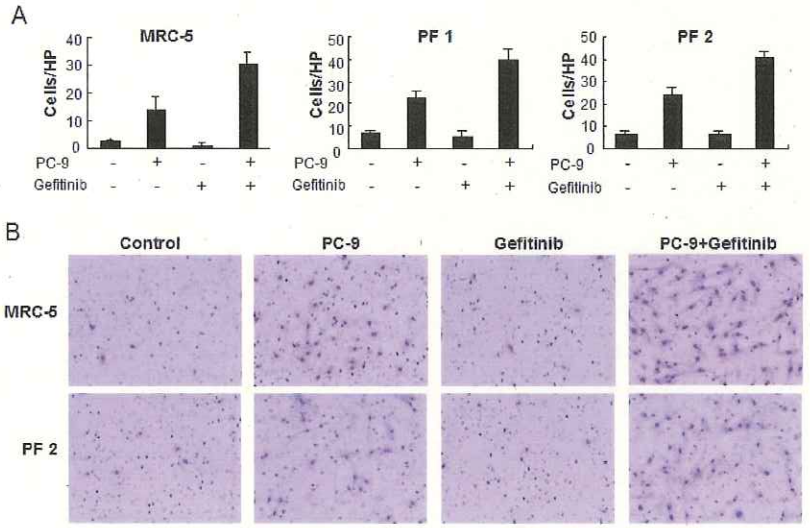
We evaluated the gefitinib sensitivity of lung cancer cells with *EGFR* activating muations, PC-9 and HCC827, when co-cultured with fibroblasts and co-injected into SCID mice. We also examined the effect of lung cancer cells to fibroblasts recruitment.

結　果：

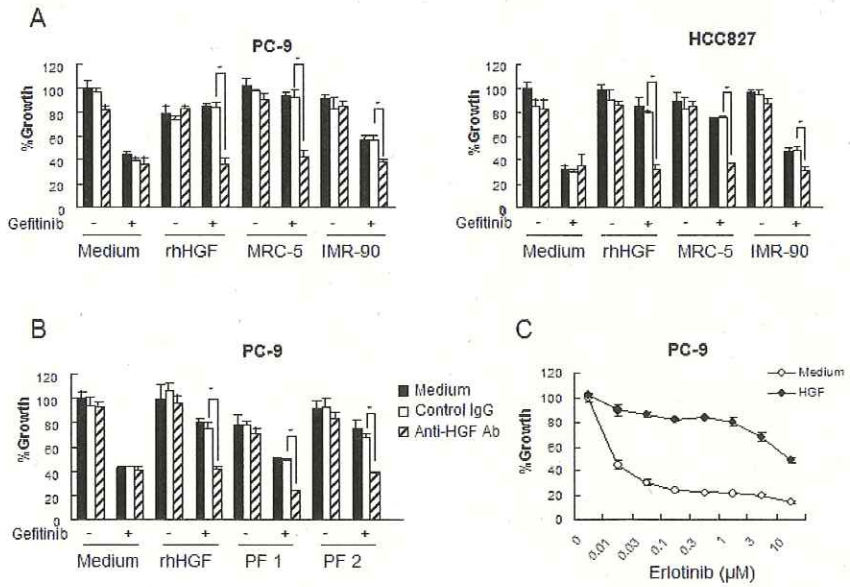
1. Fibroblast-derived HGF induces gefitinib resistance in lung cancer cells.



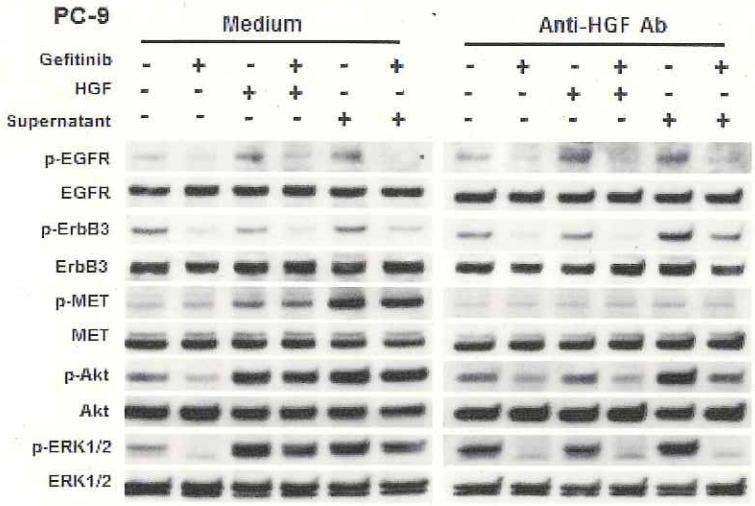
2. Lung cancer cells induce migration of fibroblasts.



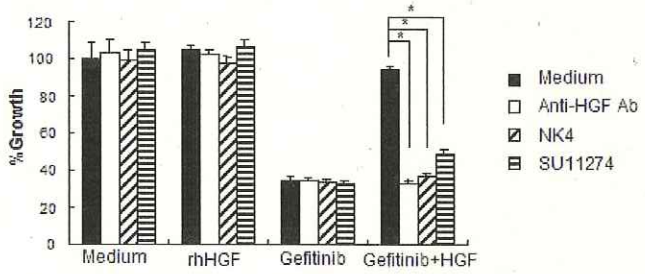
3. Fibroblast supernatants induce gefitinib resistance in lung cancer cells.



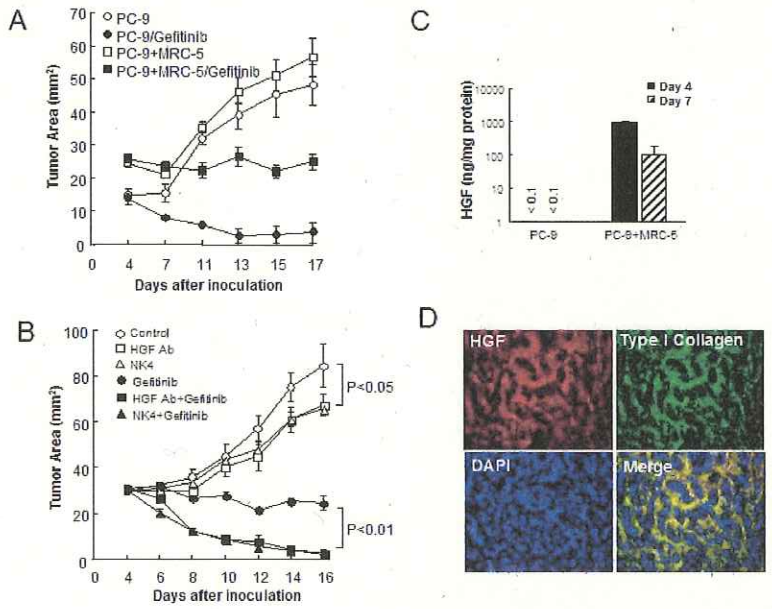
4. Fibroblast-derived HGF restores PI3K/Akt pathway via MET but not EGFR or ErbB3.



5. Anti-HGF antibody, NK4, or SU11274 abrogate HGF induced gefitinib resistance in lung cancer cells.



6. Fibroblast-derived HGF induces gefitinib resistance in PC-9 tumors in SCID mice.



考　察：

These findings indicate that crosstalk to stromal fibroblasts plays a critical role in lung cancer resistance to EGFR-TKIs and may be an ideal therapeutic target in lung cancer with *EGFR* activating mutations.

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注：本研究は、2009 年 6 月第１３回日本がん分子標的治療学会と 2009 年 7 月第１８回日本がん転移学会と 2009 年 10 月 Asia-Pacific Conference of Tumor Biology and Medicine and Fourth Forum of Chinese Young and Middle-aged Oncologists 口演発表、**Clin Cancer Res** 2009 Nov 1;15(21) に掲載。

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