

## 学位論文の要旨

# EGFR inhibition reverses resistance to lenvatinib in hepatocellular carcinoma cells

(EGFR 阻害は肝細胞癌細胞における lenvatinib に対する耐性を逆転させる)

March, 2023

Xiaoping He

何 小平

Gastroenterology

Yokohama City University Graduate School of Medicine

横浜市立大学 大学院医学研究科 医科学専攻 消化器内科教室

(Doctoral Supervisor Adviser: Shin Maeda, Professor)

(指導教員: 前田 慎 教授)

# EGFR inhibition reverses resistance to lenvatinib in hepatocellular carcinoma cells

(EGFR 阻害は肝細胞癌細胞における lenvatinib に対する耐性を逆転させる)

<https://doi.org/10.1038/s41598-022-12076-w>.

## Introduction

So far, liver cancer remains a global health challenge and its incidence continues to rise globally. Data have reported that liver cancer is the second leading cause of cancer death. In 2018 alone, the disease occurred in about 841000 people, and eventually led to the death of about 782000 people worldwide (Bray et al., 2018)(Zhou et al., 2019). It is predicted that more than 1 million people will be affected by liver cancer every year in 2025 after 3 years(Llovet et al., 2021).

HCC is associated with high mortality partly because initial diagnosis usually occurs in the late stages of the disease. This phenomenon accounts for the majority of cases worldwide, especially in developing countries. As with other cancers, if treatment is started earlier in the course of the disease, the results improve significantly. Because HCC is usually diagnosed in the late stage of the disease, the median survival time after diagnosis is short, about 6 to 12 months(“A new prognostic system for hepatocellular carcinoma,” 1998). Although surgical resection is the preferred treatment, most patients do not meet the conditions of surgical resection due to the limitation of tumor range or potential liver dysfunction.

Lenvatinib affects angiogenesis mainly through VEGFR-1, VEGFR-2, VEGFR-3, fibroblast growth factor receptor ( FGFR ) 1-4, platelet-derived growth factor receptor ( PDGFR )  $\alpha$ , RET, and KIT kinases (Yamamoto et al., 2014). In 2018, as a multi-receptor kinase inhibitor, lenvatinib was approved for the first-line treatment of unresectable HCC in Japan, the United States and the European Union (UpToDate, Inc.).

However, with the widespread use of lenvatinib in clinical treatment, the resistance of targeted therapy cannot be ignored due to primary or adaptive resistance, which hinders the treatment of advanced HCC (Zhu et al., 2017)(Fu et al., 2020). Therefore, exploring the mechanism of Lenvatinib resistance and overcoming advanced HCC through combination therapy or other methods is critical to lenvatinib resistance.

## Methods

To establish lenvatinib-resistant cells (LR cells), Hep3B cells were initially treated with 3 $\mu$ M Lenvatinib. The concentration was gradually increased by 1 $\mu$ M or 0.5 $\mu$ M per week and it reached to 7.5 $\mu$ M 2 months after the initial exposure to lenvatinib. The biological characteristics of these cells were analyzed by ERK activation in the MAPK signaling pathway and a human phospho-receptor tyrosine kinase (RTK) antibody array. Factors possibly related to lenvatinib resistance were analyzed using inhibitors, and cell proliferation was analyzed.

## Results

We established lenvatinib-resistant HCC cells (LR cells) by long-term exposure to lenvatinib. Lenvatinib reduced ERK activation in the parent cells, but not in the LR cells. RTK array analysis showed that the activities of EGFR and insulin-like growth factor 1 receptor (IGF1R)/insulin receptor (INSR) were significantly increased in LR cells, whereas the activities of other RTKs were unchanged. Erlotinib, a widely used EGFR inhibitor, downregulated ERK activation in LR cells. The proliferation of LR cells will also be affected when lenvatinib is combined with erlotinib to treat LR cells. In contrast, inhibition of IGFR/INSR did not affect ERK activation or cell proliferation. Scavenging of reactive oxygen species (ROS) ameliorated the enhanced EGFR activation in LR cells.

## Discussion

Lenvatinib resistance was induced by enhanced EGFR activation, possibly via ROS accumulation, in lenvatinib-resistant cells. These findings may enable the development of lenvatinib combination therapies for HCC

## Keywords

Hepatocellular carcinoma, Hep3B, lenvatinib, erlotinib, resistance, RTK, ERK, EGFR, IGF1R, reactive oxygen species.

## Reference

- A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators, 1998. . *Hepatol. Baltim. Md* 28 3 , 751–755. doi:10.1002/hep.510280322
- Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., Jemal, A., 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA. Cancer J. Clin.* 68 6 , 394–424. doi:10.3322/caac.21492
- Fu, R., Jiang, S., Li, J., Chen, H., Zhang, X., 2020. Activation of the HGF/c-MET axis promotes lenvatinib resistance in hepatocellular carcinoma cells with high c-MET expression. *Med. Oncol.* 37 4 , 24. doi:10.1007/s12032-020-01350-4
- Llovet, J.M., Kelley, R.K., Villanueva, A., Singal, A.G., Pikarsky, E., Roayaie, S., Lencioni, R., Koike, K., Zucman-Rossi, J., Finn, R.S., 2021. Hepatocellular carcinoma. *Nat. Rev. Dis. Primer* 7 1 , 1–28. doi:10.1038/s41572-020-00240-3
- systemic treatment of advanced hepatocellular carcinoma. UptoDate. Search for September 5,2022.
- Yamamoto, Y., Matsui, J., Matsushima, T., Obaishi, H., Miyazaki, K., Nakamura, K., Tohyama, O., Semba, T., Yamaguchi, A., Hoshi, S.S., Mimura, F., Haneda, T., Fukuda, Y., Kamata, J.-I., Takahashi, K., Matsukura, M., Wakabayashi, T., Asada, M., Nomoto, K.-I., Watanabe, T., Dezso, Z., Yoshimatsu, K., Funahashi, Y., Tsuruoka, A., 2014. Lenvatinib, an angiogenesis inhibitor targeting VEGFR/FGFR, shows broad antitumor activity in human tumor xenograft models associated with microvessel density and pericyte coverage. *Vasc. Cell* 6, 18. doi:10.1186/2045-824X-6-18
- Zhou, M., Wang, H., Zeng, X., Yin, P., Zhu, J., Chen, W., Li, X., Wang, Lijun, Wang, Limin, Liu, Y., Liu, J., Zhang, M., Qi, J., Yu, S., Afshin, A., Gakidou, E., Glenn, S., Krish, V.S., Miller-Petrie, M.K., Mountjoy-Venning, W.C., Mullany, E.C., Redford, S.B., Liu, H., Naghavi, M., Hay, S.I., Wang, Linhong, Murray, C.J.L., Liang, X., 2019. Mortality, morbidity, and risk factors in China and its provinces, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Lond. Engl.* 394 10204 , 1145–1158. doi:10.1016/S0140-6736(19)30427-1
- Zhu, Y., Zheng, B., Wang, H., Chen, L., 2017. New knowledge of the mechanisms of sorafenib resistance in liver cancer. *Acta Pharmacol. Sin.* 38 5 , 614–622. doi:10.1038/aps.2017.5

# Publication List

## I. 主 論 文（本人を筆頭とする原著論文）

EGFR inhibition reverses resistance to lenvatinib in hepatocellular carcinoma cells.

Xiaoping He, Yohko Hikiba, Yoshimasa Suzuki, Yoshinori Nakamori, Yushi Kanemaru,  
Makoto Sugimori, Takeshi Sato, Akito Nozaki, Makoto Chuma & Shin Maeda

Scientific Reports volume 12, Article number: 8007 (2022)

<https://doi.org/10.1038/s41598-022-12076-w>.