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様式１０７

学位論文審査の結果の要旨

令和　4年　2月　9日

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論文題目	Evaluating the Effect of Lenvatinib on Sorafenib-Resistant Hepatocellular Carcinoma Cells			
学位論文の審査結果	（合格）・　不合格　（該当するものを○で囲むこと。）			

〔要旨〕

Hepatocellular carcinoma (HCC) is one of the major causes of cancer-related deaths worldwide. Sorafenib has been used as a first-line systemic treatment for over a decade. However, resistance to sorafenib limits patient response and presents a major hurdle during HCC treatment. Lenvatinib has been approved as a first-line systemic treatment for advanced HCC and is the first agent to achieve non-inferiority against sorafenib. Therefore, in the present study, we evaluated the inhibition efficacy of lenvatinib in sorafenib-resistant HCC cells. Only a few studies have been conducted on this topic. Two human HCC cell lines, Huh-7 and Hep-3B, were used to establish sorafenib resistance, and *in vitro* and *in vivo* studies were employed. Lenvatinib suppressed sorafenib-resistant HCC cell proliferation mainly by inducing G1 cell cycle arrest through ERK signaling. Hep-3B sorafenib-resistant cells showed partial cross-resistance to lenvatinib, possibly due to the contribution of poor autophagic responsiveness. Overall, the findings suggest that the underlying mechanism of lenvatinib in overcoming sorafenib resistance in HCC involves FGFR4-ERK signaling. Lenvatinib may be a suitable second-line therapy for unresectable HCC patients who have developed sorafenib resistance and express FGFR4.

《Q&A》

Q1: Which domain of FGFR4 does lenvatinib bind to or inhibit?
A: Lenvatinib binds to the intracellular domain of FGFR4, which functions as a tyrosine kinase domain.

Q2: How does lenvatinib change the expression levels of miRNAs?
A: Lenvatinib regulates the signaling pathways involved in gene expression, leading to the changes of miRNA expression.

Q3: Is lenvatinib used for other cancers, or specific for HCC?
A: Lenvatinib was also approved in the treatment of thyroid cancer and renal cell carcinoma.

Q4： Is sorafenib resistance in HCC related to gene mutation in the cells?
A: Vascular endothelial growth factor (VEGF)-A amplification may be sensitive to sorafenib response. The activation of beta-catenin is associated with sorafenib resistance.

Q5: Do Huh-7 and Hep-3B cell lines have the mutations of some genes such as cyclin and p70S6 kinase?
A: Huh-7 and Hep-3B cells have a P53 point mutation [at codon 220 (A:T-->G:C)] and a non-sense P53 mutation, respectively. In addition, Hep-3B cells, but not Huh-7 cells, carry HBV DNA.

Q6: Did you observe the same changes of miRNA expression pattern in the mouse model tissues?
A: No, I didn't find the same changes. Instead, I used the TCGA-liver hepatocellular carcinoma database to analyze human sample data.

Q7: Can miRNAs be biomarkers to identify sorafenib resistance or defense?
A: Yes, miR-181, 216a, and 494 were previously suggested to be associated with sorafenib resistance.

Q8: Why was the *in vivo* study stopped in 10 days?
A: The study was stopped in 10 days when animals kept good physical conditions.

Q9: Why did Huh-7SR and Hep-3BSR cells show different responses to lenvatinib?
A: These two cell lines showed different autophagic responses and only Hep-3B cells were hepatitis B virus DNA carriers.

Q10: Did you examine further changes about EGFR and FGFR downstream signaling such as the formation of autophagosome?
A: No, I didn't examine the formation of autophagosome.

Q11: What are the clinical doses of sorafenib and lenvatinib?
A: The clinically recommended blood concentrations for sorafenib and lenvatinib are 10 μM and 0.1 μM, respectively.

Q12: Do you have the data of FGFR4 inhibitor for these cell lines?
A: No, I do not.

Q13: Do lenvatinib and sorafenib affect endothelial cells in *in vivo* study?
A: Yes, lenvatinib inhibited angiogenesis. VEGF receptors are also the target molecules of sorafenib, and sorafenib could inhibit angiogenesis in the mice model.

Q14: When you established sorafenib-resistant cells by culturing the cells in the presence of increasing doses of sorafenib, did you clone the survival cells before use, or use the cells without cloning?
A: The survival cells were used without cloning.

Q15: Did you confirm statistical significances in Western blot analyses (Figures 4 and 5)?
A: The assays were repeated at least three times and densitometric analyses were performed to quantify the intensity of the immunopositive bands.

学位論文公開審査会において申請者が行った発表並びに質疑応答は申し分のないものであり、審査委員会は一致して本論文が博士（医学）の学位論文に相応しいものであると判断した。

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（備考）要旨は，１，５００字以内にまとめてください。