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学位論文名	Interferon-β Is More Potent Than Interferon-α in Inhibition of Human Hepatocellular Carcinoma Cell Growth When Used Alone and in Combination With Anticancer Drugs （インターフェロンαおよびβの単独もしくは抗癌剤との併用におけるヒト肝細胞癌株に対する増殖抑制効果）
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論文内容の要旨

Background：Prognosis of advanced hepatocellular carcinoma (HCC) is extremely poor, and no standard therapy is available yet. Interferon (IFN) is a promising drug for prevention of HCC, and its combinations with chemotherapeutic agents might be effective for advanced HCCs. However, little is known about the underlying mechanism of the response of HCC cells to IFN. Herein, I studied the mechanism of direct antitumor effect of type I IFNs on HCC cell lines and mouse xenograft models.

Materials and methods：The mechanistic study of antitumor effects of type I IFNs (natural IFN-α, IFN-β) was conducted on three HCC cell lines (HuH7, PLC/PRF/5, HLE). Growth-inhibitory assays were performed to study the antiproliferative effect of IFNs and each of their combinations with anticancer drugs (5-fluorouracil, cisplatin, doxorubicin) *in vitro*. Evaluation of drugs interactions was done using isobologram analysis. Expressions of IFN alpha receptors (IFNAR) and base or phosphorylated forms of signal transducer and activator of transcription (STAT) proteins were examined by Western blot. To evaluate the impact of IFNAR2 in the effect of IFNs, neutralizing assay using anti-IFNAR2 antibody was employed. Mouse xenograft models were used to determine the effectiveness of IFN-α or IFN-β treatments on HCC tumor growth.

Results：The cell lines differed in their sensitivities to the IFNs；PLC/PRF/5 was the most sensitive to the IFNs, while HuH7 and HLE were resistant. From IFN signaling components, IFN alpha receptor (IFNAR) 2c was highly expressed only in IFN-sensitive PLC/PRF/5 cells, and the expression levels of STAT1 and 3 (especially those β-isoforms) in these cells were higher than in the other cell lines. Pre-treatment with anti-IFNAR2 antibody dose-dependently blocked the antiproliferative effect of the IFNs. When the cells were assayed with various doses of IFNs, IFN signal transduction (phosphorylated STAT1 and 3, but not STAT2) was high in

PLC/PRF/5 than in other cells.

IFN-β showed a significantly stronger growth-inhibitory effect than IFN-α on HuH7 cells. This effect has resulted from intense and expanded signal transduction induced by IFN-β (1.6-10.6-fold higher depending on IFN doses). By neutralizing assay, a difference in binding of the IFNs to IFNAR2 was found, as well as for suppression of the effects of IFN-β required more antibody than for IFN-α.

Next, I examined the possibility of synergic action of IFN-α or IFN-β with anticancer drugs ; IFN-α showed synergistic effects with 5-fluorouracil or with doxorubicin on PLC/PRF/5 cells only, whereas for IFN-β, cooperative synergistic effects were observed when combined with 5-fluorouracil or cisplatin on HuH7 and PLC/PRF/5 cell lines.

In nude mouse tumor xenograft models, treatment with IFN-β (2×10^4 IU/animal thrice a week) significantly suppressed tumor volume relative to vehicle injection, in mice bearing HuH7 and PLC/PRF/5 tumors (respectively by 47% and 31%), whereas same administration of IFN-α showed weak growth-inhibition.

Conclusion : The results suggest that IFN signaling (expression of IFNAR2c and activation of STAT1 and 3) mediates the antitumor effects of type I IFNs in HCC cells. The spectra of antiproliferative activity of IFN-β and its synergistic effect when combined with anticancer drugs would be more potent than those with IFN-α. Combinations of IFN-β and anticancer drugs may provide a better treatment for HCC when combinations with IFN-α are ineffective.

論文審査の結果の要旨

最近、インターフェロン（IFN）と抗癌剤を併用することにより著効を呈する肝細胞癌症例を経験することがあるが、肝細胞癌に対する IFN の抗腫瘍効果の作用機序は、十分に解明されていない。本研究では、IFN の抗腫瘍効果の作用機序および抗癌剤との併用効果について検討した。3種類のヒト肝細胞癌株を用いた実験では、IFN の抗腫瘍効果は、そのシグナル伝達を担う、IFNAR2c と STAT1、3 の発現量によって制御されていた。IFNAR2c の中和抗体を用いた中和反応試験では、濃度依存性に抗腫瘍効果の抑制が認められ、さらに IFN-β では、IFN-α よりも高濃度の中和抗体を抗腫瘍効果の抑制に必要とした。また、IFN-β は、IFN-α よりも強い増殖抑制効果を示し、抗癌剤との併用においても、多くの組み合わせで相乗効果が認められた。本研究は、肝細胞癌に対する IFN の抗腫瘍効果において、IFN の受容体とそのシグナル伝達が関与することを示し、さらには、IFN-β が IFN-α よりも単剤もしくは抗癌剤との併用においても強い増殖抑制効果を示しすことを明らかにしたものであり、今後臨床応用が期待され、学位の授与に値すると考える。