Sequence Variation In Hepatitis C Virus Nonstructural Protein 5A Predicts Clinical Outcome Of Pegylated Interferon/Ribavirin Combination Therapy

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【 学位論文題目 】

Sequence Variation In Hepatitis C Virus Nonstructural Protein 5A Predicts Clinical Outcome Of Pegylated Interferon/Ribavirin Combination Therapy(ペグインターフェロン?リバビリン併用療法における C 型肝 炎ウイルス非構造タンパク質 NS5A の遺伝子多様性の検討)

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Background and purpose: Hepatitis C virus (HCV) infection is the major cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma in industrialized countries. However, HCV infection is curable and its complications can be prevented by antiviral therapy. Currently, the most effective treatment of chronic HCV infection is based on a combination of pegylated interferon (PEG-IFN) and ribavirin (RBV). Even with this treatment regimen, however, sustained virological response (SVR) rates for those infected with the most resistant genotypes, HCV-1a and -1b, still hover at ~50%. Considering the high cost and the significant side effects associated with this combination therapy, it is worthy to identify patients most likely to benefit from therapy. As the HCV genotype is one of the major factors affecting IFN-based therapy response, IFN resistance is, at least partly, genetically encoded by HCV itself. In this context, HCV-nonstructural protein 5A (NS5A) has been widely discussed for its correlation with IFN responsiveness. Therefore, in this study we aimed to explore a predictive marker within NS5A protein by which the hepatologistes can predict the ultimate virological response of HCV-1b-infected patients treated with PEG-IFN/RBV combination therapy.

Methods: Pretreatment sequences of NS5A of HCV were analyzed for 45 HCV-1b infected patients who were treated in Kobe Asahi Hospital with PEG-IFN/RBV combination therapy for 48 weeks followed by follow-up observation for 24 weeks. Quantitative estimation of HCV-RNA in patients' sera was also performed before the treatment and at 4 weeks intervals during the whole observation period (72 weeks). As well as, HCV-core antigen titers were assessed before the treatment and at 24 hours, 1 week, 2 and 4 weeks after the initiation of the treatment.

Results: Of 45 patients enrolled in this study 21 (47%) achieved sustained virological response (SVR), patients who had undetectable level of serum HCV-RNA 72 weeks after the initiation of PEG-IFN/RBV therapy, whereas the remaining 24 (53%) patients were non-SVR. When the pretreated sequences of HCV-NS5A obtained from SVR and non-SVR were aligned and compared to the consensus sequence, the mean number of amino acids (aa) mutations in the variable region 3 (V3) plus its upstream flanking region of NS5A (aa 2334–2379), referred to as IFN/RBV resistance-determining region (IRRDR), was significantly higher for HCV isolates obtained from SVR patients than for those obtained from non-SVR patients. Subsequently, we carried out the receiver operating characteristic (ROC) curve analysis to estimate a cutoff number of mutations in IRRDR predicting SVR. The result revealed that six mutations were an optimal number of mutations to predict SVR; because it achieved the highest sensitivity (76%) combined with the highest specificity (92%) and yielded an area under the curve of 0.81. Indeed, sixteen (76%) of 21 SVR, but only 2 (8%) of 24 non-SVR, had HCV with ≥6 mutations in IRRDR (IRRDR≥6) ($P < 0.0001$).

Furthermore, when the IRRDR sequences obtained from all 45 patients were aligned along with the consensus sequence, we also noticed that 10 (48%) of 21 patients with SVR had alanine at position 2360 (Ala^{2360}) whereas only 3 (13%) of 24 patients with non-SVR did ($P = 0.02$).

In the purpose of analyzing the impact of IRRDR sequence variation on HCV kinetics during PEG-IFN/RBV treatment, firstly, by using Kaplan-Meier analysis, we analyzed the viral clearance rates of patients infected with HCV of IRRDR≥6 and those with IRRDR≤5 at 4-week intervals during the treatment period (48 weeks). This

analysis clearly demonstrated that, after the initiation of the IFN/RBV treatment, HCV clearance was achieved significantly more rapidly in patients infected with HCV isolates with IRRDR≥6 than those with IRRDR≤5, with the difference between the two groups being statistically significant ($P < 0.0001$, log-rank test).

Secondly, we analyzed the correlation between the degree of sequence variation in IRRDR and the proportion of patients who could achieve a certain reduction rate of HCV-core antigen titre during the early stages of treatment period. The result obtained clearly revealed a significant correlation between IRRDR≥6 and the proportion of patients who could achieve the very rapid reduction of HCV core antigen titers 24 hours and 1, 2 and 4 weeks after the initiation of treatment. Most notably, all 18 patients infected with HCV isolates of IRRDR≥6 achieved significant (≥1 log) reduction and/or disappearance of serum HCV core antigen titers 24 hours after the first dose of PEG-IFN/RBV whereas 10 (37%) of 27 patients with HCV of IRRDR≤5 did ($P < 0.0001$). This, in particular, suggests a possible influence of IRRDR≥6 on HCV replication kinetics during IFN-based therapy since the direct effect of IFN begins a few hours after the first dose.

Consequently, by using multivariate logistic regression analysis, we aimed to identify significant independent SVR predictors. This analysis yielded IRRDR mutations dichotomized at 6 (odds ratio = 16.0; CI, 2.4 – 104.3; $P = 0.004$) and Ala^{2360} (odds ratio = 9.3; CI, 1.1 – 78.8; $P = 0.04$) as independent predictors of SVR.

Finally, in the term of positive and negative predictive values, we assessed the predictability of SVR and non-SVR using IRRDR≥6 and Ala^{2360}. Interestingly, the positive predictive value of IRRDR≥6 for SVR was 89% (16/18; $P= 0.0007$) with its negative predictive value for non-SVR being 81% (22/27; $P = 0.0008$). Similarly, Ala^{2360} could also predict SVR with positive predictive value of 77% (10/13; $P = 0.046$).

Conclusion: Our present results suggest that a high degree of sequence variation in IRRDR (IRRDR≥6), and a particular aa mutation (Ala^{2360}) to a lesser extent, would be a useful marker(s) to predict SVR.

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論文審査の結果の要旨

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論文題目 Title of Dissertation	Sequence variation in hepatitis C virus nonstructural protein 5A predicts clinical outcome of pegylated interferon/ribavirin combination therapy ペグインターフェロン/リバビリン併用療法におけるC型肝炎ウイルス非構造タンパク質NS5Aの遺伝子多様性の検討		
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（要旨は１，０００字～２，０００字程度）

要旨

C型肝炎ウイルス（HCV）による慢性肝炎の治療にはペグインターフェロン（PEG-IFN）／リバビリン（RBV）併用療法が用いられている。HCV-1b高ウイルス量の患者の約半数が治療によりウイルスを完全に排除しsustained virological responder（SVR）になるが、残る半数はウイルスが残存しNon-SVRになる。SVR、Non-SVRを規定する因子は患者側とウイルス側の双方にあると考えられるが、その具体的な指標は明らかではない。本研究では、HCV NS5AのIFN/RBV resistance-determining region（IRRDR）高変異がPEG-IFN/RBV併用療法後のSVRの予測因子として有用であるかどうかを検討した。

PEG-IFN/RBV併用療法（48週間）を行い、6ヶ月経過観察したHCV-1b慢性肝炎患者45名のうちSVRは21/45（47%）、Non-SVRは24/45（53%）で、Non-SVRの11/24（46%）はcomplete non-responder（CNR）、13/24（54%）がrelapserであった。患者血清中のHCV全長NS5A塩基配列と推定アミノ酸配列を求め、コンセンサス配列と比較し、その変異を調べた。その結果、HCV NS5A V3領域を含むaa.2334-2379の領域（IRRDRと略記）の変異数はNon-SVRに比べSVRで有意に高かった（P<0.001）。Non-SVRの2/24（8%）、SVRの16/21（75%）にIRRDR高変異（≥6）が認められ、IRRDR高変異（≥6）とPEG-IFN/RBV感受性に相関が認められた（P<0.0001）。CNRにおいてはIRRDR高変異（≥6）は認められず（P<0.0001）、relapserでは2/13（15%）のみであった（P<0.001）。SVRの10/21（48%）においてAla^{2360}が認められたが、Non-SVRでは3/24（13%）のみであった（P<0.05）。また、SVRの9/21（43%）においてThr^{2378}が認められたがNon-SVRでは3/24（13%）のみであった（P<0.05）。また、解析の結果明らかとなった変異とHCV RNA量、core抗原量との相関について検討した。IRRDR高変異（≥6）のSVR陽性的中率は89%（16/18; P<0.001）、陰性的中率は82%（22/27; P<0.001）であった。SVRでIRRDR高変異（≥6）が認められた16名全てにおいて、PEG-IFN/RBV投与24時間以内にcore抗原量の顕著な減

少(≥1 log) が認められた (P<0.0001)。

以上，本研究は，HCV NS5A の IFN/RBV resistance-determining region (IRRDR) 高変異が PEG-IFN/RBV 併用療法後の SVR の予測因子として有用であるかどうかを検討した研究であるが、従来明らかではなかった SVR、Non-SVR を規定する因子について HCV NS5A の IRRDR 高変異(≥6) 及び IRRDR 低変異(≤5) が有用であるとことを示し、さらに、Ala^{2360} と Thr^{2378} のアミノ酸置換も SVR の二次予測因子として有用であることを示した研究であり、重要な知見を得たものとして価値ある集積であると認める。よって、本研究者は博士（医学）の学位を得る資格があると認める。