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研究課題名（和文）計算アプローチによる肝炎の病態・治療に関する分子機構の解明

研究課題名（英文）Elucidation of the molecular mechanisms on the pathology and treatment of hepatitis by computational approach

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研究成果の概要（和文）：第1課題のHCV NS5AのIFN-RBV治療への耐性機序について、多数のNS5Aデータにおいて著効データが少数である場合に有効な準教師付アンサンブル学習手法を開発し、SVRと非SVRの2種の患者群を特徴づけるモチーフを発見した。第2課題のsiRNA抑制効果用に開発した手法では、既知の設計規則に加え2種の新siRNA設計規則を発見し、また他手法では所与のsiRNA配列についてスコアと著効情報をもつ配列と既知の規則で配列の表現力を補強し、新規のテンソル回帰手法にて予測精度を大幅に改善した。第3課題ではエピジェネティク因子と肝炎進行の相互作用に関するヒストン修飾の因果関係網等の中間結果を得た。

研究成果の概要（英文）：For the first task on HCV NS5A resistance mechanisms to interferon/ribavirin therapy, our semi-supervised ensemble method discovered the motifs that well characterizing two class of patients with SVR and non-SVR, especially in case of only a small number labelled NS5A sequences but much unlabelled sequences are available. For the second task on the knockdown efficacy of siRNA, one of our method discovered two siRNA design rules in addition to known design rules, and the other significantly improve the predictive ability for given siRNA sequences. This method employs both scored and labelled sequences as well as known design rules to enrich the poor sequence representation of siRNA by transformed matrices. The prediction is done by a novel tensor regression method on those matrices. For the third task on the interplay between epigenetic factors and hepatitis progression, we reached some intermediate results such as inferring the causual relationship network of histone modifications.

研究分野：データマイニング

科研費の分科・細目：情報学・統計科学

キーワード：医薬生物　統計解析　データマイニング

１．研究開始当初の背景

Viral hepatitis is a disease in which liver tissue is inflamed by the infection of hepatitis viruses. Hepatitis viruses persisting in the liver of most infected people can be treated with medication but the effect of the current hepatitis therapy is still quite limited. The main issue in this study is molecular mechanisms of hepatitis pathogenesis and therapies for hepatic diseases that are poorly understood and remain to be answered. Our joint project focuses on

(1) *HCV NS5A resistance mechanisms to interferon & ribavirin therapy:* HCV is eradicated in only nearly a half of patients treated by current standard therapy of peg-interferon combined with ribavirin (IFN/RBV). Analysis of the virus genomes and their drug resistance mechanisms to this therapy has been pursued for years. Recently, NS5A is known as the protein most reported to be implicated in the interferon resistance, and NS5A inhibitor is a hot topic in HCV research in very recent years (Gao et al., *Nature* 465, 2010). NS5A inhibits IFN activity via its interaction with IFN cellular antiviral pathways and the mutations in NS5A resist IFN therapy (Guillou et al.,*World J. Gastroenterology* 13, 2007). Many questions on this topic remain unanswered such as the enigmatic role of the domains II and III of NS5A or can V3 region in NS5A be a more accurate biomarker than the ISDR region?

(2) *Selection of potent siRNAs for silencing hepatitis viruses*: RNAi is a cellular pathway wherein small RNA molecules, typically siRNAs and miRNAs, control gene expression, viewed as “one of the most exciting discoveries in biology in last couples of decades” (Fire et al., *Nature*, 1998; Nobel prize 2006). RNAi is known as a new therapeutic strategy against hepatitis viruses, especially siRNAs target to HBV, HCV genes to inhibit their replication or host genes required for their replication. Chemically synthesized siRNAs can mimic the native siRNAs produced by RNAi but having different ability and thus selecting appropriate siRNAs before using them in experiments is crucial. It was firstly solved by using guidelines to select siRNAs from designed databases, e.g., siDirect (Naito et al., *BMC Bioinformatics*, 2009), E-RNAi (Arziman et al., *Nucl Acids Res.*, 2005), NEXT-RNAi (Horn, *Genome Bio*, 2010). Recently, there are computational works such as using combined neural network and decision tree (Takasaki, *Computers in Biology and Medicine* 2010) or kernel regression (Qiu and Lane, *IEEE Computational Biology and Bioinformatics*, 2009). However, they mainly focus on either knockdown efficiency or off-target effect of siRNAs. To better solve the problem, we target to computational methods for simultaneously maximum knockdown efficiency and minimum off-target effect of selected siRNAs.

(3) *Interplay between epigenetic factors in hepatitis progression*: RNAi is a cellular pathway wherein small RNA molecules, typically siRNAs and miRNAs, control gene expression, viewed as “one of the most exciting discoveries in biology in last couples of decades” (Fire et al., *Nature*, 1998; Nobel prize 2006). RNAi is known as a new therapeutic strategy against hepatitis viruses, especially siRNAs target to HBV, HCV genes to inhibit their replication or host genes required for their replication. Chemically synthesized siRNAs can mimic the native siRNAs produced by RNAi but having different ability and thus selecting appropriate siRNAs before using them in experiments is crucial. It was firstly solved by using guidelines to select siRNAs from designed databases, e.g., siDirect (Naito et al., *BMC Bioinformatics*,2009), E-RNAi (Arziman et al., *Nucl Acids Res.*, 2005), NEXT-RNAi (Horn, *Genome Bio*, 2010). Recently, there are computational works such as using combined neural network and decision tree (Takasaki, *Computers in Bio. and Med.*, 2010) or kernel regression (Qiu and Lane, *IEEE Comp. Bio. Bioinf.*, 2009). However, they mainly focus on either knockdown efficiency or off-target effect of siRNAs. To better solve the problem, we target to computational methods for simultaneously maximum knockdown efficiency and minimum off-target effect of selected siRNAs.

2．研究の目的

(1) Discover the molecular mechanisms of HCV NS5A resistance to IFN/RBV therapy. We have successfully detected NS5A motifs that characterize well responders and non-respsonders in HCV sub-genotypes 1a-c and 1b. We aim at discovering the partnership of these motifs with IFN cellular antiviral pathways and mutations in NS5A to reveal its unknown IFN/RBV resistance mechanisms.

(2) Uncover the combinatorial effects of various epigenetic factors, especially DNA methylation and PTMs in hepatitis development, which can be divided into three concrete objectives: ①Identify key epigenetic factors in suppressor gene silencing, ② Characterize their combinatorial effects, and ③ Explore deeply the epigenetic regulatory network, which includes the possible correlation, dependency, or causal relationship among those factors.

(3) Develop computational methods for selecting siRNA that simultaneously minimize off-target effect and maximize knockdown efficiency in silencing hepatitis viruses.

(4) Quantitatively analyze the relationships of the above three topics to better understand the hepatitis pathogenesis and therapies and prove that the multiple therapeutic strategy can increase the successful ratio of hepatitis treatment, says, more than 50%.

3．研究の方法

The three problems investigated in our project (Fig. 1) were investigated by computational approach with three main components of data, analysis methods and evaluation methods as illustrated in Figs 2-4, respectively.

For the first problem on NS5A, we used 77 labeled data on SVR (sustained viral response) and non-SVR NS5A sequences from Los Alamos National Database (http://hcv.lanl.gov) plus 25 labeled sequences from Chiba University Hospital (to our knowledge, only such 102 labeled sequences are available in total), and 1424 unlabeled

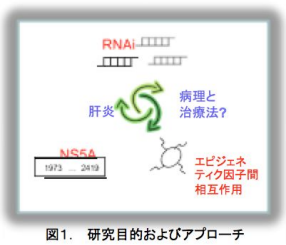

図1． 研究目的およびアプローチ

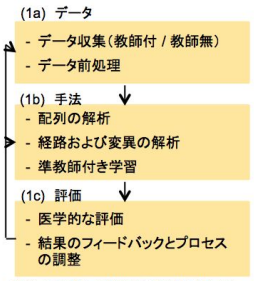

図2. NS5AとIFN/RBV療法に関して

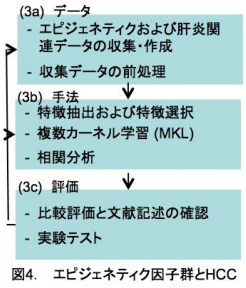

図4． エピジェネティク因子群とHCC

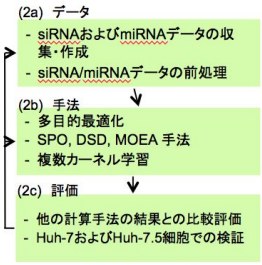

図3. RNAiと肝炎研究

NS5A sequences from Hepatitis Virus Database (http://s2as02.genes.nig.ac.jp) plus 168 sequences from GenBank (http://www.ncbi.nlm.nih.gov/genbank).We developed a semi-supervised ensemble method for sequence analysis that can well discover discriminative motifs (a new direction in motif learning) especially in case of only a small number labelled NS5A sequences but much unlabelled sequences are available. The evaluation is carefully done with 10-fold cross validation on two kind of found motifs. The obtained results are well characterizing two classes of patients with SVR and non-SVR.

For the second problem on siRNAs, we all two kinds of available scored and labeled siRNA sequences obtained by different laboratories. In particular, we employ the following datasets: ① The Huesken dataset of 2431 siRNA sequences targeting 34 human and rodent mRNAs, commonly divided into the training set HU train of 2182 siRNAs and the testing set HU test of 249 siRNAs (Huesken et al., Nature 2005); ② Three independent datasets for evaluation, including the Reynolds set of 240 siRNAs (Reynolds et al., 2004), the Vicker dataset of 76 siRNA sequences targeting two genes (Vicker et al., 2003), and the Harborth dataset of 44 siRNA sequences targeting one gene (Harborth et al., 2003). Our developed method to predict the knowckdown efficacy of a given siRNA based on the following key ideas to overcome the limitations of previous mothods: ① Exploiting both available scored and labeled siRNA datasets as well as the discovered siRNA

design rules to enrich siRNA sequences by converting them into matrices with more integrated information, and ② Learning a tensor regression on those transformed matrices and predict the knockdown efficacy of new siRNA with this tensor regression.

For the third problem on epigenetic factors in hepatitis progression, we have yet reached the final target of understanding the impact of epigenetic factors in hepatitis disease. There are two main reasons of that limitation. One is a good dataset on such relationship has yet available during the research period, and the other is finding such relationship much be based on several well known other processes such as functional linkage between nucleosome dynamics and protein binding profiles or transcriptional relationships. Instead, we focused on investigating the reconstruction of those relation networks from data. In fact, we particularly use different available next-generation sequencing data of the research community for the purpose, such as ChIP-Chip data to learn transcriptional relationships. Different statistical learning methods have been employed with adaptation such as multiple kernel learning, especially methods of probability graphical models to learn structures and parameters of the reconstructed networks.

４．研究成果

For the first task on HCV NS5A resistance mechanisms to interferon/ribavirin therapy, our semi-supervised ensemble method discovered the motifs that well characterizing two class of patients with SVR and non-SVR, especially in case of only a small number labelled NS5A sequences but much unlabelled sequences are available. For the second task on the knockdown efficacy of siRNA, one of our methods discovered two siRNA design rules in addition to known design rules, and the other significantly improved the predictive ability for given siRNA sequences. The latter employs both scored and labelled sequences as well as known design rules to enrich the poor sequence representation of siRNA by transformed matrices, on which a novel tensor regression method makes the prediction task. For the third task on the interplay between epigenetic factors and hepatitis progression, we reached some intermediate results such as inferring the causual relationship network of histone modifications.

５．主な発表論文等

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