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研究課題名（和文）Transcriptome-wide identification of key regulatory networks in drug resistant cancer cells

研究課題名（英文）Transcriptome-wide identification of key regulatory networks in drug resistant cancer cells

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研究成果の概要（和文）：本研究では、がん細胞の薬物耐性を克服するための代替的または組合わせ治療戦略を明らかにすることを目的とした。我々はまず、副作用をもたらす、または新規用途になる得る薬剤の効果を分析可能な解析フレームワークの開発に取り組んだ。3種類の異なる細胞種に対して、4種類のスタチンを用いた薬剤応答を対象とし、開発した解析フレームワークを適用することで、転写因子と応答経路の有意に関連するペアを同定し、オン/オフ標的経路の調節を解明する制御ネットワークが示唆された。本研究で開発した解析フレームワークは、研究対象とする薬物の影響についての新しい知見を提供し、新規用途や潜在的な副作用の同定に有用であることを示した。

研究成果の概要（英文）：In order to identify alternative or combinatorial therapeutic strategies to overcome drug resistance of cancer cells, we firstly developed an analytic framework that can dissect the effects of drugs, which could be adverse effects or new usages. We selected Statins as an example to evaluate our framework. Several studies have suggested that Statins may have potential as cancer therapy in human malignancies. In contrast, some studies revealed that Statins increase cancer risk of prostate cancer. However, the detail of mechanisms of these unexpected effects are still not clear. Applying our framework to treatment of three different cell types with four widely used Statins, we identified significantly associated pairs of transcriptional factors and pathways, and infer regulatory networks to elucidate the regulation of on/off target pathways. We show that our framework provides new insights about the drug effects under study, and helps identification of new usages or potential side effects

研究分野：　Systems Biology

キーワード：　Cancer　Drug　CAGE　FANTOM　Adverse Effects　Drug Repositioning　Statin

１．研究開始当初の背景

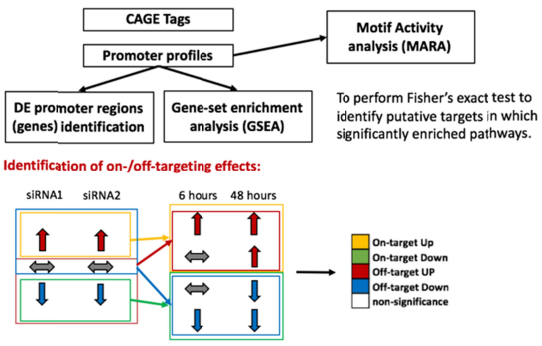
Precision medicine is the major challenge for cancer therapeutics. Using preclinical data can not only reduce the cost and time for drug development but also prevent patients from serious adverse effects (AEs) in the early stages of drug treatment. However, a method that systematically uses preclinical data to elucidate drug-related pathways and identify putative AEs is still lacking. In this study, we established an integrative framework that can elucidate putative on- and off-target effects of drugs by combing the expression profiles after drug treatment with gene perturbation of the primary drug target.

２．研究の目的

Our ultimate goal of this proposed study is to establish an integrative framework to identify alternative or combinatorial therapeutic strategy to overcome drug resistance of cancer cells. We attempt to identify the key regulatory networks that are associated to drug response by integrating the atlas of promoters/enhancers in FANTOM5 with the genomic features, mRNA expression levels and bio-activities in Cancer Cell Line Encyclopedia (CCLE) Then, discovering the inverse expression signatures of drug resistance regulatory networks, which induced by the small molecules in Connectivity Map (cMap). Finally, the new combinatorial therapeutic strategies will be validated on primary cultured cancer cells.

３．研究の方法

An overview of our pipeline is shown below.

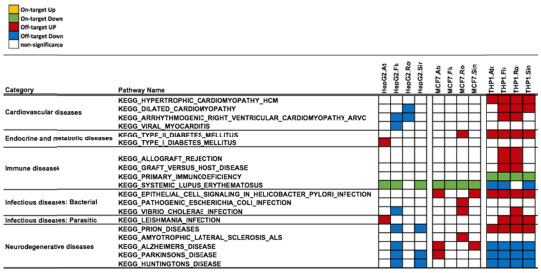


We used statins, which are conventionally used for cholesterol lowering and have several pleiotropic off-target effects, such as potential as anti-cancer drugs, to demonstrate utility of the framework. In order to characterize the regulatory mechanism of on/off target effects of statins, we use Cap Analysis of Gene Expression (CAGE) profiles to perform gene-sets enrichment analysis (GSEA), and Motif Activity Response Analysis (MARA) to identify significantly enriched motif-pathways pairs contributing to the identified on- and off-target effects.

４．研究成果

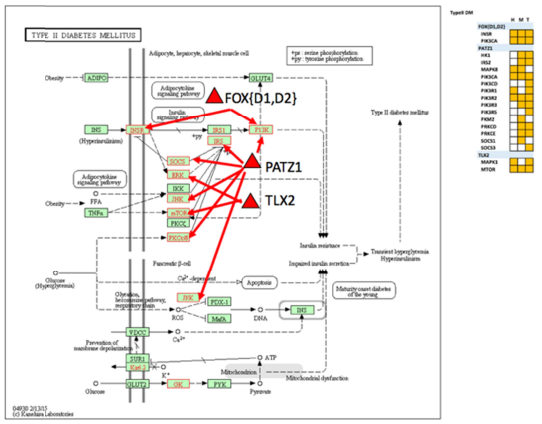
For promoters of genes, we rarely identified common on- or off-target responders, neither across statins nor cell lines. Apart from a set of key genes in cholesterol biosynthesis, (ACLY, MSMO1 and FDFT1), there were no other differentially expressed promoters shared in at least two cell lines. In contrast, there were many pathways shared among different statins and cell lines, such as the on-target effects up-regulation of “Steroid biosynthesis” and “Terpenoid backbone biosynthesis pathway” for negative feedback of statins, and off-target effects, systemic lupus erythematosus (SLE) pathway which is down-regulated under statin treatment. In addition, the most well-known AE, the “Type II diabetes mellitus” pathway, was identified as an up-regulated off-target. Our finding suggests that statin treatment targets key pathways similar in different cell types rather than specific genes. Comparing our statin-induced gene-signatures with pre-compiled signatures from the LINCS database further validated our putatively identified on-/off-targets of statins. Signatures from drugs (RS-17053 and Niguldipine) that can decrease LDL and triglycerides and increase HDL were significantly enriched. In addition, several anti-cancer drugs signatures in the LINCS database were significantly associated as well. Furthermore, Type II DM therapies and PARP inhibitors showed inverse correlation with statin signatures.

These results not only support our enrichment results but also show that our framework can dissect on- and off-target effects of drugs, and may be used for repositioning to other usages.



The CAGE method generates expression profiles at the promoter level, which also allowed us to identify putative regulators of on- and off-target effects. Our framework not only identified key regulators which have been previously reported for known off-target effect of statins such as systemic lupus erythematosus and anti-cancer-related pathways but also novel regulators (FOXD1/2, PATZ1, and TLX2) that may play important roles in statin-increased risk for Type II diabetes mellitus (T2DM). Some FOX (forkhead) transcription factors are associated with diabetes-related vascular disease 54 where they have roles in the regulation of immune responses and inflammation. FoxD1 has been reported to play a role in the induction of plasminogen activator inhibitor-1 (PAI-1) to regulate the vascular function and modulate thrombosis, inflammation, and the extracellular matrix. PATZ1 binds to DNA and plays an important role in chromatin modeling and since there are few genetic variants associated with T2DM (such as PPARG, KCNJ11 and TCF7L2), epigenetic regulation has been proposed to play significant role in T2DM instead. Considering our results showing statin effects on histone acetylation, we speculate that statin treatment may increase the risk of T2DM through an epigenetic mechanism.

Figure 5. Significantly activated motifs in Type II DM pathways



Off-target effects generally occur in any perturbation experiment. Therefore, we chose a conservative approach and created the on-target effective gene sets in this study by using negative control siRNA and two HMGCR-specific siRNAs, which minimizes non-specific effects of siRNA treatment as well as siRNA sequence-specific off-target effects. We observed that a very small amount of genes could be defined as on-target effect genes using these strict criteria. This suggests that HMGCR is an ideal drug target for cholesterol lowering, with minimum effect on other cellular functions. On the other hand, we observed that statin treatment resulted in significant changes in a large amount of off-target genes. This is unlikely to be due to incomplete siRNA KD efficiency, since we rarely observed "off-target" genes common to all statin treatments. Thus, those off-target effects are likely to reflect differences in molecular structure of the four statins used here, where each statin may weakly associate with additional other cellular molecules or proteins. Because expression of those molecules may be variable between different cell-types, it is beneficial to use multiple cell-types for detection of off-target effects in this approach.

Based on a similar experimental design, applying this framework in early stage of drug development may filter out the drug candidates with potential AEs to reduce time and costs.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計 0 件）

〔学会発表〕（計 0 件）

〔図書〕（計 0 件）

〔産業財産権〕
なし

〔その他〕
なし

６．研究組織

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