A strategy to block the dilemma from hepatoma cells under doxorubicin-resistant surroundings via systems pharmacology concept

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Introduction

Hepatocellular carcinoma (HCC) is represented the most significant type of liver cancer, which is one of malignant cancer types occurred from hepatocytes. Hepatitis B virus (HBV) is a primary causative to develop HCC under its infection, replicating in the hepatocytes and interconnecting with some cellular protein-coding genes. The molecular investigation of certain relationships between HBV and Hepatitis C virus (HCV) is scrutinized, however, is still under study, and it is a nuanced result due to continuous altered signaling pathways, modifying the cellular environment and induce host DNA damage. Also, anti-cancer treatment alters the tumor microenvironment (TME) of HCC, and the changed TME can attune its efficacy and influence drug resistance (DR). Likewise, DR alters TME, to be specific, synchronizes therapeutic mark(s), implying that the harsh condition might be made by protein-protein interaction (PPI) to modulate the upregulated or downregulated genes. Commonly, DR is classified into two categories: either intrinsic drug resistance (IDR; before therapy) or acquired drug resistance (ADR; after therapy). This study was directed to analyzing doxorubicin-resistant conditions (DRCs) on HCC. Doxorubicin (DOX) is an antibiotic isolated from *Streptomyces peucetius* bacterium and achieves as an inhibitor topoisomerase Ⅱ to interfere with DNA replication via various cancer treatment, including HCC. DOX is an anti-cancer agent to enable to alleviate HCC, but its efficacy has been faded away due to DR. Accordingly, we challenged to discover key target(s) and mediator (s) against DOX from hepatoma cells obtained by clinical data (GSE125180).

Indeed, ADR develops after exerting at least one favorable response to a certain disease. As exposed to longer time on anti-cancer agent(s), genetic mutations or insensitivity have been accelerated via aberrant mechanisms. A therapeutic strategy against ADR is of importance approach to harness anti-cancer drug(s) in dilemma under developing the proliferation and survival of cancer cells despite treatment. Nonetheless, the conundrum has yet to be veiled in ADR, especially, cancer therapeutics. Herein, the investigation of cancer PPI interactome might be a decrypt to unravel the foggy target(s), and ligand(s) as adjuvant with the first line medication. Currently, bioinformatics, cheminformatics, and computer screening tools have advanced rapidly and opened up a wealth of public online webservers and powerful programs to dissect the complicated PPI networks. In a plethora of data available, a systemic concept is required to comprehensively verify and scientifically identify the most appropriate information to evade ADR [1,2]. In spite of that, a crucial gap finds in accessible methods devised to employ these tools to mine key target(s), and biomolecule(s) against ADR. The aims of this study was to lessen the gap by adopting PPI networks with upregulated or downregulated genes in transcriptomic dataset, realizing with blue (upregulated genes) and red color (downregulated genes). In particular, a methodology to visualize the dataset was devised by database webservers, and R program. The workflow of this study is as below.

1) The employment of STRING, and R program: The DEGs from GSE125180 were identified by STRING databases, and the scattered DEGs were assembled via R program (Step1-2).

2) The identification of key target(s), and its affinity between ligand(s): Kaplan-Meier Plotter, AutoDock 1.5.6, and GaussView6 were adopted to investigate the therapeutic value. All processing was unfolded in **Figure 1**.

Abstract

Background: The aim of this study was to investigate key target(s), mechanism(s) from hepatoma cells in harsh doxorubicin-resistant conditions (DRCs) from GSE125180 datasets in GEO (Gene Expression Omnibus).

Methods: The protein-protein interaction (PPI) networks were assembled to identify the relationships between upregulated and downregulated genes via STRING, and R program. The potential molecule(s) counteracted to DRCs were identified by a proof of concept, then, molecular docking test (MDT), Kaplan-Meier Plotter, and density functional theory (DFT) were adopted to identify its therapeutic potentiality.

Results: In the |log2 FC| > 1, and |log2 FC| > 2 subgroups, the uppermost target was a non-receptor tyrosine kinase (SRC) downregulated in DRCs, indicating that the dampened SRC is a therapeutic mark by doxorubicin (DOX). In contrast, in the |log2 FC| > 3 subgroup, the most significant target was Cluster of Differentiation 93 (CD93) upregulated in DRCs, suggesting that the overexpressed CD93 is a resistant mark in DRCs.

Conclusions: Metoclopramide (MET) @ CD93+DOX conformer is a highlighted capture to illuminate as combination therapy to overcome DRCs because MET is an inhibitor against CD93 as well as a non-competitive inhibitor on (MET @ CD93+DOX) conformer.

Key words: GSE125180, SRC, CD93, metoclopramide

Results

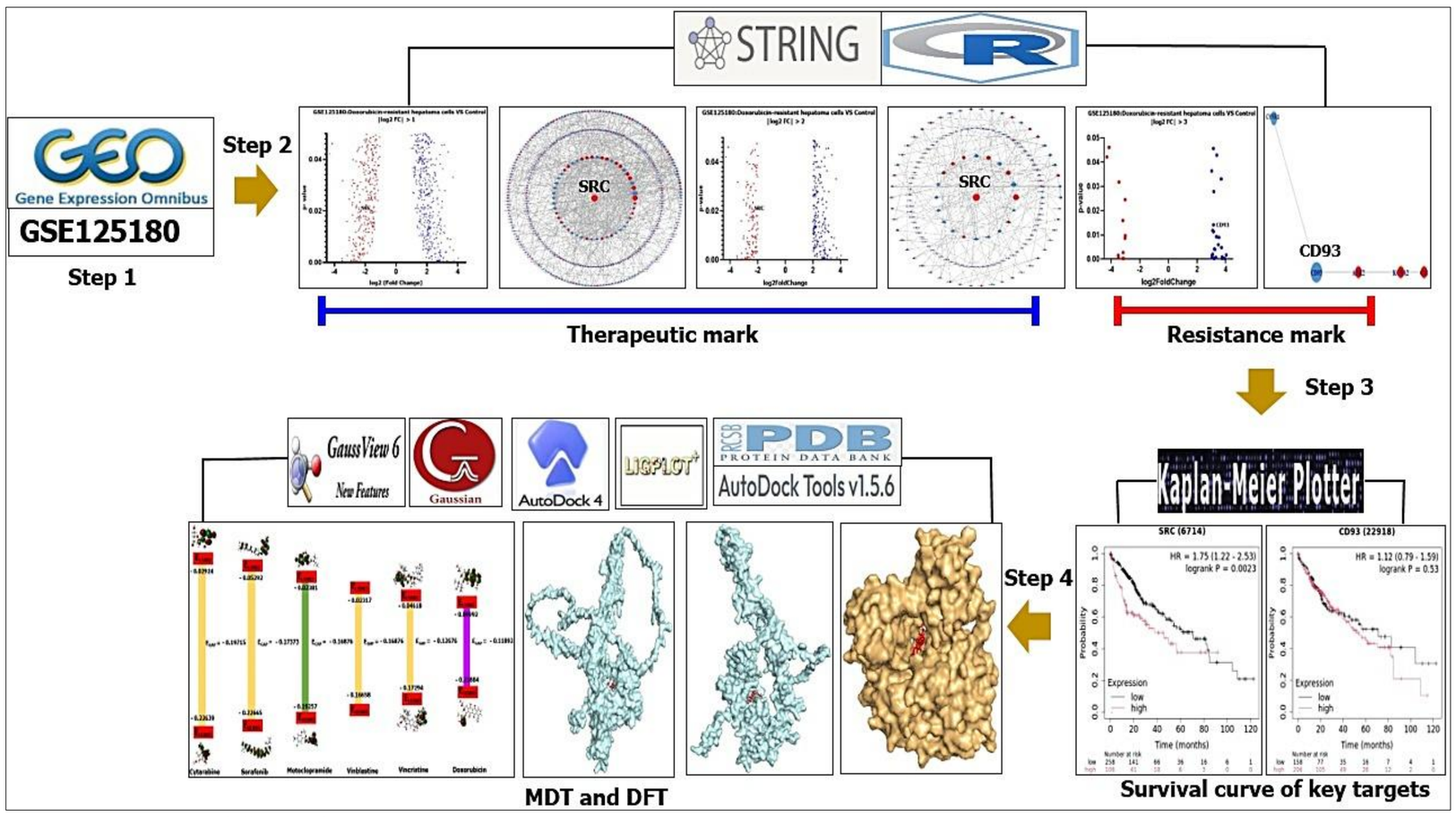

Figure 1. The workflow of this study.

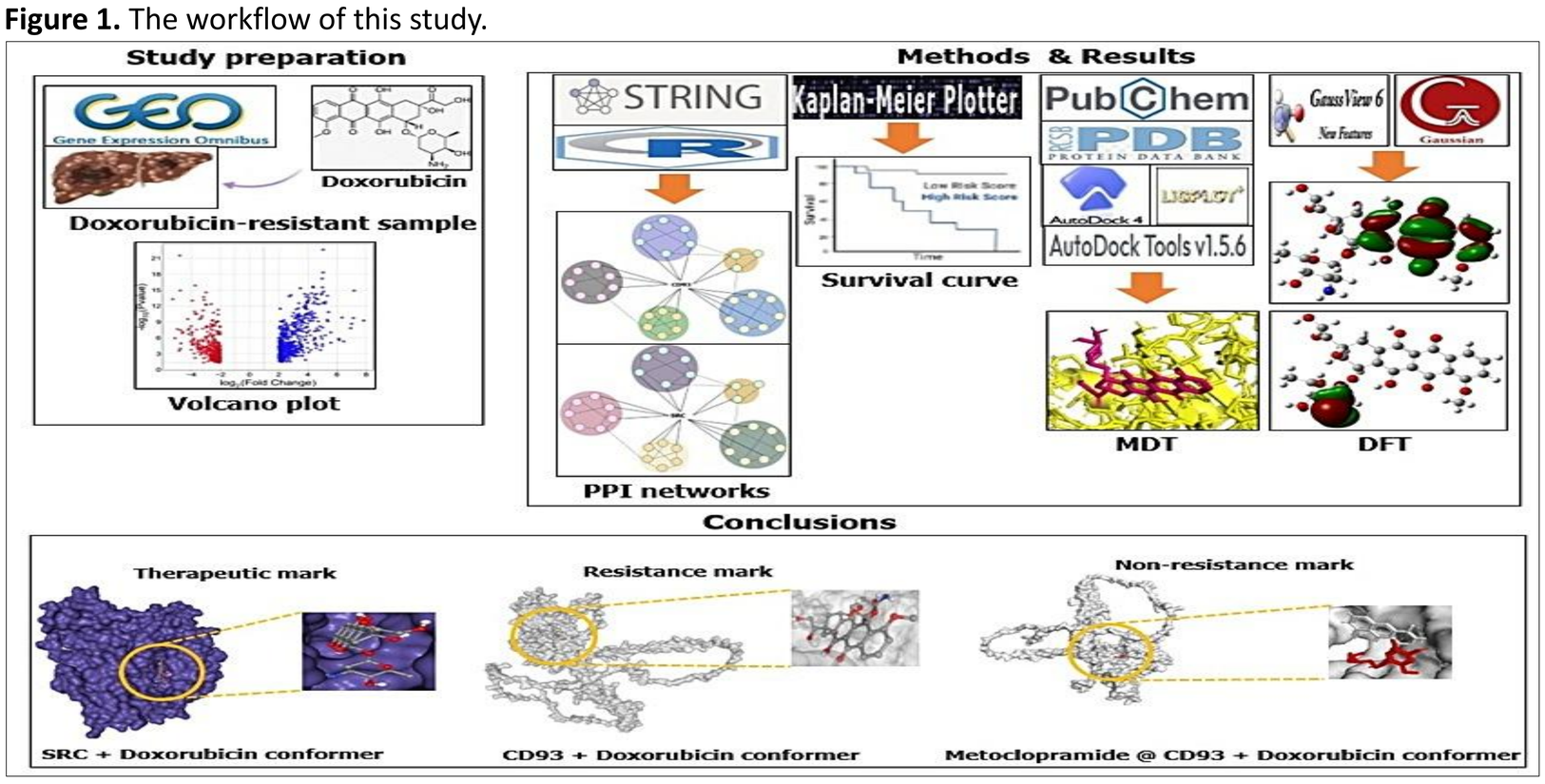

Figure 2. The key findings of this study.

Materials and methods

Methods

The differentially expressed genes (DEGs) from GSE94550

The DEGs from the GSE94550 dataset in DRCs were obtained to scrutinize the therapeutic strategy, which was comprised by three filtering, and its standard was followed by below.

1) |log2 Fold Change| > 1 or p-value ≤ 0.05

2) |log2 Fold Change| > 2 or p-value ≤ 0.05

3) |log2 Fold Change| > 3 or p-value ≤ 0.05, indicating how responds to the DRCs.

DEGs trimming

The DEGs were divided into downregulated or upregulated genes between experimental and control groups via volcano plot (red circle: downregulated gene, blue circle: upregulated gene) with GraphPad Prism software, the separated DEGs were visualized as PPI networks with R program. The relationships between downregulated (red circle) and upregulated genes (blue circle) were identified by the PPI networks, key target(s) on the most center were considered as a therapeutic mark or a resistance mark in DRCs. The clues were significant information to unravel the DRCs. In detail, DEGs trimming was a crucial method to discover bona fide target(s) to modulate DRCs.

Investigating of Kaplan-Meier Plotter (KMP) on key target(s)

The key target(s) responded potently to DRCs were probed for associations with overall survival via Kaplan-Meier Plotter (KMP) webserver [18]. The KMP is an accessible web-based platform to screen the survival rate according to gene species. Thus, KMP analysis was conducted to unveil the therapeutic value concerning the key gene(s) in DRCs. The interrelationship between survival rate and target(s) was determined by Logrank P-value (≤0.05).

The investigation of MDT

The sdf file of DOX (PubChem ID: 31703) bound to a key target related to therapeutic mark was browsed through by PubChem (https://pubchem.ncbi.nlm.nih.gov/) (accessed on 20th March 2025). The sdf file was transformed into pdb file via PyMOL software and converted into pdbqt file to dock with AutoDock 1.5.6. The target was identified by STRING database, its PDB file was obtained by RCSB PDB data bank (accessed on 22th March 2025), (https://www.rcsb.org/), and reformatted into pdbqt file via AutoDock 1.5.6. The confirmed pdbqt files (DOX, another adjuvant ligand, and a key agent) were docked with the molecular docking tool. An adjuvant ligand was retrieved by literature mining with a proof of concept, likewise, the creation of pdbqt files were followed the same process as DOX. Through molecular docking test (MDT), a key target + DOX (conformer A) for therapeutic mark, another key target + DOX (conformer B) for resistance mark, and conformer B + an adjuvant ligand (conformer C) against DRCs. The file of another key target was identified by AlphaFold Protein Structure Database (accessed on 22th March 2025) (https://alphafold.ebi.ac.uk/). The cutoff of docking score was set up at –6.0 kcal/mol to decide either a stable or an unstable conformer [19]. It means that certain conformer(s) with less negative value enable more stable complex to exert the pharmacological effects.

Conclusion

In summary, this study provides a therapeutic mark (SRC inhibition), and a resistance mark (CD93 hyperactivation) under DRCs. Importantly, MET as CD93 inhibitor might be a prioritized medication to evade DRCs. Alternatively, MET could form stably a di-conformer on CD93 + DOX complex. In the context of pharmacological theory, MET might function as uncompetitive inhibitor to arrest the resistance on CD93 + DOX conformer. In conclusion, MET treatment is a promising adjuvant drug to overcome DRCs in terms of combination therapy.

Reference

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