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Biological evaluation and molecular docking of Rhein as a multi-targeted radiotherapy sensitization agent of nasopharyngeal carcinoma

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ABSTRACT

Radiation resistance of nasopharyngeal carcinoma (NPC) is a joint effect caused by complex molecular mechanisms. The development of multi-target radiotherapy sensitization agents offered a promising method for the treatment of NPC. In this work, the probability of Rhein to be a multi-target radiotherapy sensitization agent was explored through computer aid virtual screening by inverse docking study. In order to validate the accuracy of the computational results, radiotherapy sensitization of Rhein to NPC cells and its effects on the expression of target proteins were evaluated separately by CCK8 assay and Western blotting analysis. Our result demonstrated that Rhein possessed strong binding affinity with RAC1 and HSP90. No cytotoxic concentration of Rhein had radiosensitization effect on nasopharyngeal carcinoma CNE1 cells. After treatment with Rhein and 2Gy radiation, the expression of RAC1 upregulated and the expression of HSP90 down-regulated in cells. Based on the above data, Rhein is likely to become an attractive lead compound for the future design of multi-target radiotherapy sensitization agents.

1. Introduction

Nasopharyngeal carcinoma (NPC) is an epithelial malignancy tumor with high incidence in northern Africa, Alaska, Southeast Asia [1] and southern China. At present, radiotherapy together with surgery and chemotherapy as first-line therapy for NPC are widely used in clinic [2]. However, as other human cancers, evasion of apoptosis of the NPC cells leads to the resistance of cancers to radiotherapy. Therefore, it is really an imminent need to seek new ways to improve efficiency of radiotherapy.

Anthraquinone compounds, a class of biological reduced agent, including Rhein, Emodin, Aloe-emodin and the derivatives of Emodin, are found in rhubarb, multiflorum, Polygonum and other Chinese herbs. It has been reported that anthraquinone compounds have wide pharmacological activities such as anti-inflammatory, antioxidant, antimicrobial activities and especially significant antitumor bioactivities [3]. Recent studies have reported the radiosensitization activity of anthraquinone compound on tumor cells. For example, Yaqin Qu demonstrated that the anthraquinone compound Aloe-emodin could effectively inhibited the proliferation of HeLa cells, meanwhile induced cell cycle arrest in G2/M and S phase thereby enhanced the radiosensitivity of HeLa cells [4]. In addition, Kwangmo Yang confirmed that Emodin is a potential radiosensitization agent for the HCC cells by attenuated radioresistance in the HepG2 cells via up-regulation of poly (ADP-ribose) and polymerase (PARP)-1 signals and down-regulation of JMJD1A andJMJD2B proliferative signals [5]. Our early findings, a series of anthraquinone compounds including Emodin, and the derivative of Emodin (1, 8-dihydroxy-3-acetyl-6-methyl-9, 10 anthraquinone), could decrease not only the expression level of HIF-1α but also the double-stranded DNA break repair genes KU70/KU80 in NPC CNE1 cells after radiation therapy, and exhibit effective radiosensitization activity with low toxicity.

To investigate the potential radiotherapy sensitization targets

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directly regulated by 1, 8-dihydroxy-3-acetyl-6-methyl-9, 10 anthraquinone, the proteomic approach with an isobaric tag for relative and absolute quantitation (iTRAQ) labelling was performed in CNE1 cells. As a result, five candidate proteins for potential radiotherapy sensitization was identified including epidermal growth factor receptor (EGFR), ras-related C3 botulinum toxin substrate 1 (RAC1), cadherin 1(CDH1), COP9 signalosome complex subunit 2(COPS2) and Heat shock proteins HSP90 [6]. The above results suggested that the redox-active compounds which contain anthraquinone structure could regulate multiple targets and play important roles in radiosensitization.

Rhein (1,8-Dihydroxy-3-Carboxy anthraquinone) is a kind of anthraquinone compound. There were also numbers articles reported its pharmacology activities. Antitumor effect of Rhein on cancer cells has been particularly reported in the previous literature [7]. Jung-Hyun Shim reported that Rhein was able to mediate cell proliferation, cell cycle progression, tumorigenesis and apoptosis directly by inhibiting the tumor-promoting effects of Pin1 and further suppressing the promoter activities of AP-1 and NF-κB [8]. Rhein can down-regulate the expression of EGF and reduce the activity of Hsp90α under the normoxic or hypoxic conditions in breast cancer cells [9]. However the radiosensitization activity of Rhein has not been found, therefore, we believed that it might be a new pointcut to study and evaluate the radiotherapy sensitization activity on Nasopharyngeal Carcinoma.

So far, molecular modeling has wide spread utility in the field of drug development. Various molecular simulation softwares have been extensively employed to reveal the structure of proteins and identify new targeted drugs, for example MOE (Molecular Operating Environment), Autodock and Discovery Studio. They have the ability to screen large compound database in silico and select a limited number of candidate compounds to identify novel compounds with desirable biological activities. In addition, another important use of these computational screening softwares is to effectively identify promising therapeutic targets [10]. Inverse docking is an effectively prominent method in identification of multiple anti-cancer targets of small molecules and drug discovery. L. Hui-fang et al. have testified multiple targets of eight compounds from Drug Bank through five inverse docking strategies, namely, GOLD, FlexX, TarFis Dock, TarSearch-X and TarSearch-M [11]. Sam Z. Grinter et al. used in silico inverse-docking approach to identify oxidosqualene cyclase (OSC) as potential targets of PRIMA-1, which was considered to be a promising target for developing new therapies for human breast cancer [12].

There has been considered that one drug should be one single target to avoid side effects for a long time, however, more and more researchers are beginning to realize that multifactorial diseases are better treated with multi-target drugs relatively to single-target drugs [13]. The development of multi-target drugs has inspired new therapeutic strategies in cancer researches. Through docking methodologies, it was found that the macro biological activities of Traditional Chinese Medicine against cancers [14].

The current study aimed to validate whether these 5 proteins are the potentially key targets of radiotherapy sensitization mediated by Rhein, inverse docking studies were performed with 5 proteins as receptors and Rhein as ligand using molecular docking software of MOE. The inhibitory activity of Rhein on NPC cells and its effects on the expression of target proteins were evaluated by CCK8 assay and Western blotting analysis, separately. Our study will be conducive to reveal the targets of radiotherapy sensitization and identified attractive lead compounds for multi-target radiotherapy sensitization agents.

2. Materials and methods

2.1. Computational software

The software package MOE 2008 [15] is an integrated software system developed by Chemical Computing Group lnc (CCG Montreal, Canada) [16] for the research of pharmaceutical and life sciences. In a unified molecule operating environment, MOE support multiple complex applications, like cheminformatics analysis, molecular modeling, Structure-based virtual screening, and is widely used in the research and development of new drugs. In this study, all of the computer docking simulations were carried out in MOE 2008.

Chem Office Ultra 12.0 was developed by Cambridge Soft Corporation in the United States. It is composed by Chem Bio Draw Ultra, Chem3D Ultra and Chem Finder Ultra which employed in developing modeling structure [17].

2.2. Preparation of receptors

Our research group has identified five candidate proteins using a proteomic approach with iTRAQ labelling, those of which were relevant with the radiotherapy sensitization. The receptors were EGFR, RAC1, CDH1, COPS2 and HSP90, respectively. Structures of five proteins were obtained from the Protein Data Bank (http://www.pdb.org). Several principles must be followed when downloaded the three-dimension crystal structures of the proteins from the Protein Data Bank. Firstly, if the structure of the protein has more than one entries, one with high resolution has the priority to be taken into consideration. Secondly, if a protein was in a complex, and its binding pocket information could be extracted directly from the specific position of ligand, this PDB entry would be preferentially selected [18]. The basic information of the receptors for this docking study including its name, UniProtKB, PDB code, length and resolution was listed in Table 1. The crystal structure coordinates of these receptors were loaded into MOE in a default form of linear structure models and docked with Rhein respectively. Partial charges were added to the receptors. Hydrogen atoms were optimized and protonated automatically using protonation 3D function under the Compute module of MOE [19]. The energy of the proteins were minimized using the energy minimization algorithm of MOE. The energy optimization was operated under the following parameters settings. The gradient value was set to 0.05. The Chiral Constraint was set as Current Geometry, and the energy optimization was carried out under the Amber89 force field. The energy minimization was terminated once the root mean square gradient (RMSG) fell below 0.05 [20]. The energy minimized structures were then reserved for docking.

2.3. Preparation for ligand molecule

The structure of Rhein (1, 8-dihydroxy-3-carboxylic-9, 10-anthraquinone), is shown as Fig. 1. The dimensional structure of Rhein was written and saved as PDB format by ChemBio Draw Ultra

Table 1
The basic information of the receptors.

Name of receptors	UniProtKB	PDB ID	Length [28]	Resolution
CDH1	P12830	3FF8	101	2.0 Å
COPS2	P61201	4D18	403	4.08 Å
EGFR	P00533	3LZB	259	2.7 Å
HSP90	P07900	5FNC	236	2.2 Å
RAC1	P63000	1G4U	180	2.3 Å