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Radiation protective effects of baclofen predicted by a computational drug repurposing strategy

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ABSTRACT

Exposure to ionizing radiation causes damage to living tissues; however, only a small number of agents have been approved for use in radiation injuries. Radioprotector is the primary countermeasure to radiation injury and none radioprotector has indeed reached the drug development stage. Repurposing the long list of approved, non-radioprotective drugs is an attractive strategy to find new radioprotective agents. Here, we applied a computational approach to discover new radioprotectors *in silico* by comparing publicly available gene expression data of ionizing radiation-treated samples from the Gene Expression Omnibus (GEO) database with gene expression signatures of more than 1309 small-molecule compounds from the Connectivity Map (cmap) dataset. Among the best compounds predicted to be therapeutic for ionizing radiation damage by this approach were some previously reported radioprotectors and baclofen ($P < 0.01$), a chemical that was not previously used as radioprotector. Validation using a cell-based model and a rodent *in vivo* model demonstrated that treatment with baclofen reduced radiation-induced cytotoxicity *in vitro* ($P < 0.01$), attenuated bone marrow damage and increased survival *in vivo* ($P < 0.05$). These findings suggest that baclofen might serve as a radioprotector. The drug repurposing strategy by connecting the GEO data and cmap can be used to identify known drugs as potential radioprotective agents.

1. Introduction

The health of humans is increasingly threatened by ionizing radiation (IR), which has been found more frequently in the environment since the first atom bomb was exploded in the 1940s. With the widespread use of nuclear power, the possibility of nuclear industry disasters (*e.g.*, Fukushima Nuclear Power Plant disaster, March 2011) and medical accidents intensifies the need to develop appropriate and functional radiation countermeasure agents, including prophylactic, mitigating, and therapeutic agents. The administration of radioprotectors before radiation exposure is expected to diminish excess free radicals in cells [1]. Radioprotectors could also be functional in radiotherapy for shielding tissues surrounding a targeted focus area from the effects of radiation, and also in radiation emergencies for first responders undertaking rescue operations. Conversely, radiomitigators are administered after exposure for reducing radiation injuries in accidental situations. Because radiation-induced injuries cause lowering of immune function and spreading of infections, a number of supportive therapies will be essential for the management of radiation victims.

At present, only a few chemicals have been approved for use in radiation accidents/incidents: Prussian blue, calcium-diethylenetriaminepentaacetic acid (DTPA), zinc-DTPA, potassium iodide (KI), and S-2-(3-aminopropylamino) ethyl phosphorothioic acid (WR2721) [2,3]. Several potential radioprotectors have been found to be effective, but none have reached the drug development stage. WR2721 has shown significant radioprotection in animal models against lethal radiation doses and was approved by the US Food and Drug Administration (FDA) as a cytoprotectant for reducing the xerostomia in patients with head and neck cancer who are undergoing postoperative radiation. However, because of its systemic toxicity in humans, WR2721 was found unsuitable as a radioprotector [4].

In the past half century, the discovery of radiation protectors has mainly focused on chemical syntheses of thiol and amine derivatives [5]; protein and cytokine purification [6–8]; and high-

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throughput screening of small-molecule libraries [9]. Alternatively, a chemogenomics strategy has also been used to find radiation migitators by relating chemicals to their gene targets. For example, mitochondrial targeting of small molecule decreased irradiation-induced cell death *in vitro* and prolonged survival of lethally irradiated mice [10]. Furthermore, Jiang et al. used siRNA technology-based screening to identify glyburide as a radioprotector [11]. As an extension of chemogenomics, instead of focusing on a single drug target, using a gene signature (a suite of genes) to query the Connectivity Map (cmap, http://www.broad.mit.edu/cmap/) may foster identification of potential new drugs[12]. The cmap database consists of 6100 drug signatures derived from five different cell types treated with 1309 bioactive molecules of various concentrations and experimental duration. Of the 1309 drugs included in the cmap database, most are currently used in clinical treatment or are well-developed drugs. Thus, by providing users' own signatures, potential drugs can be rapidly identified and advanced to clinical trials. To date, the cmap database has been successfully used in drug repurposing for Alzheimer's disease[13], bronchial asthma[14], and cystic fibrosis [15], et al.

In the past few decades, microarray technology has been widely used in all biological research areas including the biological effects of ionizing radiation. Fifty-two radiation-related gene expression profile datasets were found by querying the Gene Expression Omnibus (GEO) website (http://www.ncbi.nlm.nih.gov/geo/). Comprehensive lists of radiation-induced gene expression profiles, by different types and doses of radiation, are included in these datasets.

In this study, we purposed to identify and validate new radiation protector by using a systematic computational approach for drug repurposing, based on an integration of the gene expression signatures of known drugs and radiation damage. We systematically evaluated ionizing radiation-induced gene expression signatures from publicly available experiments obtained from the GEO datasets [16] against a compendium of gene expression signatures, comprising 6100 drug signatures derived from five types of cells treated with 1309 drugs. Among the highest-scoring therapies predicted by our approach, some has previously been reported as radiation countermeasure agents, such as atropine, diclofenac, piperine, and simvastatin [17–20]. A derivative of γ-aminobutyric acid (GABA), baclofen, which emerged in the examination of radioprotective gene-related drugs, also ranked highly. This chemical is primarily used to treat spasticity and has not previously been described to have a therapeutic application in ionizing radiation-induced injuries. We therefore evaluated the efficacy of baclofen and other predicted drugs against ionizing radiation using gamma-irradiated cell-based models and an *in vivo* mouse model.

2. Materials and methods

2.1. Radiation damage gene expression signature generation and assessment of the potential radioprotector

A cmap query gene signature was obtained from the publicly available gene expression database (GEO) (http://www.ncbi.nlm.nih.gov/geo/) [21] using data from a published experiment measuring the expression levels of genes in irradiated immortalized B-lymphoblastoid cells. A radiation damage gene expression signature (radiation signature) was generated from microarray data, GSE26835, using the significance analysis with microarrays (SAM version 4.0) software [22], which was a list of significantly upregulated and downregulated genes between the irradiated and sham-irradiated control samples. Computational repurposing and assessment of the potential radioprotectors were performed with a method referred to as Connectivity Map (http://www.broadinstitute.org/cmap/) using *in silico* screening of publicly available gene expression data. The current cmap version, build 02, was utilized in this research. A false discovery rate threshold of 0.05 was accepted for the q values.

Table 1
Radioprotective drugs computationally predicted by the cmap method.

Cmap name	Mean	N	Enrichment	P
Negative result				
biotin	−0.68	3	−0.86	0.00559
atropine*	−0.649	4	−0.819	0.00199
AG-012559	−0.621	3	−0.868	0.00451
5182598	−0.594	2	−0.874	0.03175
simvastatin*	−0.549	4	−0.812	0.00243
diclofenac*	−0.541	5	−0.649	0.01302
Prestwick-1085	−0.523	4	−0.67	0.02662
baclofen	−0.388	5	−0.718	0.00389
hydralazine	−0.375	6	−0.544	0.03558
heliotrine	−0.367	6	−0.536	0.03971
piperine*	−0.348	4	−0.665	0.02851
flutamide	−0.345	5	−0.775	0.00104
lithocholic acid	−0.338	6	−0.528	0.04465
geldanamycin	−0.33	15	−0.508	0.0004
ciclacillin	−0.319	4	−0.718	0.01281
thiamazole	−0.299	6	−0.587	0.01712
Positive result				
acenocoumarol	0.092	5	0.575	0.04418
lasalocid	0.196	4	0.652	0.03445
pioglitazone	0.203	11	0.415	0.03174
chlorphenesin	0.21	4	0.644	0.03873
tetraethylenepentamine	0.212	6	0.629	0.00806
anisomycin	0.22	4	0.648	0.0364
puromycin	0.224	4	0.652	0.03438
ethosuximide	0.229	4	0.728	0.01092
monobenzone	0.23	4	0.786	0.00408
SB-202190	0.265	5	0.598	0.03212
tiratricol	0.316	4	0.731	0.01044
diloxanide	0.331	4	0.633	0.04482
clonidine	0.344	4	0.84	0.00101
quinpirole	0.348	4	0.664	0.02875
PHA-00767505E	0.353	4	0.648	0.0365
cephaeline	0.358	5	0.689	0.00721
SC-19220	0.372	4	0.635	0.04351
hycanthone	0.38	4	0.858	0.00054
bacampicillin	0.401	4	0.705	0.0157
NU-1025	0.405	2	0.886	0.0268
enalapril	0.409	4	0.711	0.0142
irinotecan	0.426	3	0.85	0.00641

The mean was the computed score; drugs with a negative score showed a potential effect against the radiation signature. A positive score indicates that the drug exhibits an expression pattern that is synergistic with the disease. A negative score indicates that the drug exhibits an expression pattern that is opposite to the disease. N is the number of times the previously described experiments were repeated; $P < 0.05$ was accepted as significant. The drugs marked with "*" are drugs reported previously to be radioprotectors. The drug baclofen, which was predicted to be a radioprotector, was selected for experimental validation.

2.2. Animal husbandry

Male C57BL/6 mice (8–10 weeks age and weighing 18–22 g) were used in this study. The mice were purchased from the Animal Center of the Academy of Military Medicine Science (AMMS) and were caged and routinely shipped. After shipping, mice were allowed a minimum of six days to acclimate to laboratory conditions before experimental or control manipulation. The mice were maintained in a specific pathogen-free environment in an animal center with air-conditioning. All protocols involving animals were reviewed and were approved by the Animal Ethics Committee of the Beijing Institute of Radiation Medicine in comply with the regulations of the Beijing Administration Office of Laboratory Animals, Beijing, China.

Typically, mice were exposed to 7.5 Gy or 3 Gy gamma irradiation in cages during a single dosing session. The irradiation source