Published in final edited form as:

Radiat Res. 2009 October ; 172(4): 414–422. doi:10.1667/RR1674.1.

Synthetic Protection Short Interfering RNA Screen Reveals Glyburide as a Novel Radioprotector

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Abstract

To assist in screening existing drugs for use as potential radioprotectors, we used a human unbiased 16,560 short interfering RNA (siRNA) library targeting the druggable genome. We performed a synthetic protection screen that was designed to identify genes that, when silenced, protected human glioblastoma T98G cells from γ -radiation-induced cell death. We identified 116 candidate protective genes, then identified 10 small molecule inhibitors of 13 of these candidate gene products and tested their radioprotective effects. Glyburide, a clinically used second-generation hypoglycemic drug, effectively decreased radiation-induced cell death in several cell lines including T98G, glioblastoma U-87 MG, and normal lung epithelial BEAS-2B and in primary cultures of astrocytes. Glyburide significantly increased the survival of 32D cl3 murine hematopoietic progenitor cells when administered before irradiation. Glyburide was radioprotective *in vivo* (90% of C57BL/6NHsd female mice pretreated with 10 mg/kg glyburide survived 9.5 Gy total-body irradiation compared to 42% of irradiated controls, $P = 0.0249$). These results demonstrate the power of unbiased siRNA synthetic protection screening with a druggable genome library to identify new radioprotectors.

INTRODUCTION

The identification and validation of novel drug targets continues to be a major bottleneck in drug development. The advent of high-throughput analysis of gene function using short interfering RNA (siRNA)-based screens provides an efficient means to predict novel functions of gene products in cellular signaling pathways and to identify potential drug targets (1,2).

A portion of the human genome has been identified that encodes proteins with functions that are predicted to be or are targets for drugs used for human diseases; this so-called druggable genome comprises between 3,000-10,000 genes (3,4). The products of these genes include protein classes such as kinases, G-protein coupled receptors (GPCRs), phosphatases, proteases, and ion channels (3). It has been hypothesized that by focusing on the druggable genome, the likelihood of finding useful drug targets for human diseases could be increased.

The identification and development of radioprotectors (administered prior to irradiation) and mitigators (administered after irradiation but before clinical syndromes are detected) is of considerable importance because of the possible applications in clinical radiotherapy, after accidental exposure to radiation, and in radiation counterterrorism (5).

In the present study, we tested the hypothesis that well-characterized drugs might have radioprotective effects. Using a synthetic protection assay, we screened siRNAs that were nontoxic *in vitro* for protective effects against a cytotoxic dose of ionizing radiation. We used a siRNA library comprising 16,560 unique siRNA sequences targeting 5,520 genes (Supplementary Table 1) that are known to encode gene transcripts considered to be actual or potential drug targets or to modify disease. We discovered that the commonly used hypoglycemic agent glyburide protected mice against total-body irradiation. These results illustrate the power of a combined approach of high-throughput siRNA screening and conventional cell-based assay to identify new radioprotectors.

MATERIALS AND METHODS

Reagents

The DharmaFECT 2 transfection reagent and 5× siRNA resuspension buffer were from Dharmacon (Lafayette, CO). The cell Titer-Blue Cell Viability Assay was from Promega (Madison, WI). The 384-well tissue-culture treated microtiter plates were from Greiner Bio-One (GmbH, Frickenhausen, Germany). OptiMEM, MEM and FBS were from Invitrogen (Carlsbad, CA). The Silencer Druggable Genome siRNA Library (Version 1.1) was from Ambion (Austin, TX). The Annexin V kit was from Biovision (Mountain View, CA). The lactate dehydrogenase (LDH) viability kit was from Sigma (St. Louis, MO). Rabbit polyclonal anti-ABCC8 antibody was from Abcam (Cambridge, MA). Mouse monoclonal anti-actin antibody was purchased from Sigma (A3853).

Cell Culture

Human glioblastoma T98G and U-87 MG cells (American Type Culture Collection, Manassas, VA) were maintained in MEM supplemented with 2 mM glutamine, 10% FBS and penicillin-streptomycin. Human primary astrocytes were purchased from ScienCell (Carlsbad, CA) and maintained according to the manufacturer's instructions. Normal human lung epithelial BEAS-2B cells were from American Type Culture Collection and were cultured in a serum-free bronchial epithelial growth medium (Lonza, Walkersville, MD). The 32D cl3 mouse hematopoietic progenitor cell line, dependent for growth upon interleukin 3 (IL-3), has been described previously (6). 32D cl3 cells were passaged in fresh RMPI 1640 medium containing 10% FBS, 1% glutamine, penicillin-streptomycin and 15% WEHI-3 conditioned medium as a source of IL-3.

High-Throughput siRNA Delivery by Reverse Transfection

Human glioblastoma T98G cells were reverse transfected (7) with the siRNA library in 384-well plate at a final concentration of 20 nM/target in a one-gene, one-well format. The siRNA complexes were prepared by mixing DharmaFECT 2 transfection reagent with a pool of three unique siRNA duplexes (each designed against a different region of the target transcript) per well in OptiMEM serum-free medium. T98G cell suspensions in culture medium were added (350 cells/well) directly onto the siRNA complexes. The siRNA-containing medium was replaced with fresh complete culture medium 5 h later. Cells were incubated for 48 h to allow for gene silencing (8) before irradiation (25 Gy) or mock treatment. Cell viability was measured with the CellTiter-Blue cell viability assay according to the manufacturer's protocol after 72 h postirradiation incubation.

Threshold for Selecting Primary Radioprotective siRNA

The radiation siRNA screen was performed three times over three separate weeks. A candidate radioprotective siRNA was identified if the siRNA protected T98G cells $\geq 15\%$ from radiation-mediated cell death in each of three independent screens.

Western Blot Analysis

T98G cells (2×10^5 /well) were reverse transfected in six-well plates using an ABCC8 duplex siRNA cocktail (10, 20 and 40 nM final concentration) or scrambled control siRNA (Ambion Silencer Negative Control no. 3 siRNA, 20 nM final concentration) using DharmaFECT 2 transfection reagent according to the manufacturer's instructions. After 48 h incubation, harvested cells were lysed on ice for 30 min in RIPA buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% IGEPa, 0.5% sodium deoxycholate, 0.1% SDS in the presence of proteinase inhibitors) and then centrifuged at 10,000g for 5 min. The resulting supernatants were subjected to 8% SDS-PAGE and then transferred to a nitrocellulose membrane. The membrane was cut into two sections according to the position of molecular markers and then probed with anti-ABCC8 and anti-actin (loading control), respectively, followed by horseradish peroxidase-coupled detection.

Validation of siRNA Detected Targets with a Cell-Based Assay

For target validation experiments, cells were seeded in 35-mm dishes at a density of 1×10^5 /dish and allowed to attach overnight. Cells were incubated with drugs in fresh culture medium at the indicated concentrations 1 h before or 30 min after irradiation. Cells were γ -irradiated (10 Gy) with a Shepherd model 143-45A irradiator (J. L. Shepherd & Associates, San Fernando, CA) at a dose rate of ~ 4 Gy/min. The viabilities of cells were determined using the phosphatidylserine (PS) externalization/propidium iodide (PI) permeabilization assay or the LDH assay after 72 h postirradiation incubation.

PS Externalization/PI Permeabilization Assay

After 72 h postirradiation incubation, attached cells were harvested by trypsinization, pooled with detached cells, and stained with Annexin V-FITC and PI for 5 min in the dark prior to flow cytometry analysis (FACscan, Becton-Dickinson, Rutherford, NJ). Cell debris was gated out by forward and side scatter under the same conditions, and a minimum of 10,000 gated cells were acquired. Percentages of Annexin V-positive cells were calculated by combining Annexin V⁺/PI⁻ (early apoptotic) and Annexin V⁺/PI⁺ (late apoptotic or necrotic) cells.

LDH assay

For human primary astrocytes, cell viability was determined using an LDH viability kit activity according to the manufacturer's instructions (Sigma). The enzyme activity from the culture medium and floating cells were used as an index of cell death. The adherent cells were lysed in 0.5% Triton X-100 and assayed for LDH activity. This cell-associated LDH activity was then added to the LDH activity in the culture medium. The amount of LDH present in the medium was calculated as a percentage of the total, which was then used to estimate the percentage of cell death in the corresponding samples.

In Vitro Radiation Survival Curves

Murine hematopoietic progenitor cells 32D cl3 cells were incubated with 10 μ M glyburide 1 h before or after irradiation by adding glyburide to the IL-3-supplemented semisolid methycellulose-containing medium in which the cells were incubated for colony assay. The cells were irradiated with 0-8 Gy and incubated at 37°C for 1 week, at which time colonies of

greater than 50 cells were counted (9,10). The data were fitted using the single-hit, multitarget model (9,10).

Animal Welfare

All animal protocols were approved by the University of Pittsburgh Institutional Animal Care and Use Committee and were performed under the supervision of the Division of Laboratory Animal Research of the University of Pittsburgh. Veterinary care was provided by the Division of Laboratory Animal Research of the University of Pittsburgh.

Survival of Irradiated Mice

To determine the effect of glyburide in a model of radiation-induced hematopoietic syndrome, we carried out total-body irradiation of mice as described previously (10). Glyburide was dissolved in a 50:50 solution of cremophor EL and ethanol that was then diluted 1:4 in water. Female C57BL/6NHsd mice (15 per group) were injected intraperitoneally with 5 or 10 mg/kg glyburide (in two independent experiments) either 10 min before or 10 min after the LD_{50/30} dose (9.5 Gy) of irradiation. Other groups of mice were irradiated control mice and vehicle-only treated, irradiated mice. Mice were followed for the development of the radiation-induced hematopoietic syndrome as demonstrated by a loss of weight and decreased activity, at which time they were euthanized according to Institutional Animal Care and Use Committee protocols.

Statistics

All data were expressed as the means $\pm$ SD of at least three independent experiments. Data from the *in vitro* radiation survival curves were analyzed with Student's *t* test, while the mouse survival data were analyzed by a log rank test (9-11). Statistical comparisons between different groups were performed with Student's *t* test. $P < 0.05$ was considered significant.

RESULTS

An Automated Druggable Genome Radioprotective siRNA Library Screen

We used a high-density 384-well plate format and large-scale siRNA screen to rapidly and robustly identify genes that modulate the response of human glioblastoma T98G cells to ionizing radiation. Employment of relatively radioresistant T98G cells (12) provided the advantage of screening to identify both radiosensitizing and radiation resistance-inducing genes.

The 6-day workflow of the siRNA screening is illustrated in Supplementary Fig. 1. siRNA molecules (final concentration 20 nM) were added to the wells, targeting one gene per well. In-plate scrambled siRNA control wells were included on every plate. For each of the 5,520 genes, we pooled three unique siRNA duplexes to reduce the cost and improve throughput. Transfection complexes were prepared using automated liquid handling instruments, and after a 15-20-min incubation they were divided between two new 384-well plates followed by the addition of T98G cells in suspension. Plate pairs were incubated for 48 h before irradiation (25 Gy) or mock treatment. Seventy-two hours later, cell viability was measured by the CellTiter-Blue assay. The dose of 25 Gy was chosen to reduce the control cell viability by approximately 50%. Based on data from three independent siRNA screens, we identified 116 genes that, when silenced, protect T98G cells from γ radiation (Table 1). Among these genes were transporters, kinases, transcription regulators, transmembrane receptors, G-protein coupled receptors, phosphatases, ion channels and peptidases (Table 1 and Fig. 1). Figure 2 illustrates a protective siRNA that silenced ATP-binding cassette, subfamily C, member 8 (ABCC8). The down-regulation of ABCC8 protein levels was confirmed by Western blotting. Silencing of ABCC8

with siRNA duplexes exerted no detectable effect on the viability of T98G cells, while 25 Gy decreased cell viability by 55%. Silencing of ABCC8 48 h prior to irradiation reproducibly protected cells by 35%.

Target Validation using Small Molecule Inhibitors

From the 116 identified siRNAs, we focused on 13 candidate siRNA gene transcripts for further study because of the ready availability of drugs that targeted the gene product functionality (Table 2). Two of the candidate siRNA gene transcripts, PDE4D and PDE4D, shared a common inhibitor, rolipram, and three candidates, ABCA4, ABCA8 and ABCC8, shared glyburide as an inhibitor. The radioprotective effects of these 10 identified compounds were tested against control and irradiated T98G cells, which were initially used in the siRNA screen. Radiation-induced cell death was determined by PS externalization and PI permeability using flow cytometry. Around 30% of cells displayed externalized PS and/or increased membrane permeability after 72 h postirradiation incubation. Glyburide (Fig. 3), a second-generation sulfonylurea used clinically for type II diabetes, attenuated radiation-induced cell death in a concentration-dependent manner (12.5-200 μM) when administered 1 h prior to irradiation (Fig. 3). Moreover, glyburide protected cells from radiation-induced apoptosis to a similar extent when administered 30 min after γ irradiation (Fig. 4A). Treatment of cells with 200 μM glyburide effectively protected cells over a range of radiation doses (Fig. 4B). The other nine chemicals tested showed no protective effect against radiation-induced cell death (Fig. 3).

In Vitro Radioprotection in Different Cell Phenotypes

To further explore the radioprotective effect of glyburide, we extended the screening test to six other histologically distinct human cell lines: glioblastoma U-87 MG, cervical adenocarcinoma HeLa, lung carcinoma H1299, permanent bone marrow stromal cell line KM101, bronchial epithelial cells BEAS-2B, and human primary astrocytes. Cells were exposed to 10 or 25 Gy based on their radiosensitivity as determined by the Annexin V assay. Glyburide protected U-87 MG and BEAS-2B cells and human primary astrocytes (Fig. 4C, D). Glyburide had no detectable effect on γ -irradiated H1299, HeLa or KM101 cells (data not shown).

In Vitro Clonogenic Radiation Survival Curves

The radioprotective effect of glyburide was next tested in a clonogenic survival assay. Cells of the murine hematopoietic progenitor cell line 32D cl3 were incubated in liquid culture with 10 μM glyburide for 1 h before irradiation (0-8 Gy) or by transferring the cells to semisolid methycellulose medium containing 10 μM glyburide after irradiation. Cells incubated in glyburide before irradiation were protected from radiation as seen by the increased shoulder on the radiation survival curve ($n = 34.9 \pm 0.5$ compared to 3.1 ± 1.3 or 2.5 ± 0.9 for control irradiated cells or cells incubated with glyburide after irradiation) (Fig. 5). There was no significant difference in the D_0 (1.14 ± 0.20 Gy) compared to cells incubated in glyburide before or after irradiation (1.18 ± 0.13 and 1.10 ± 0.09 Gy, respectively).

In Vivo Total-Body Radioprotection against the Hematopoietic Syndrome

Mice were injected intraperitoneally with 5 or 10 mg/kg glyburide in two independent experiments either 10 min before or 10 min after 9.5 Gy total-body irradiation. The mice were followed for the development of bone marrow failure (Fig. 6). Mice injected with glyburide before irradiation were protected, seen as a significant increase in survival compared to irradiated control mice (90% of mice pretreated with 10 mg/kg glyburide survived compared to 42% of irradiated controls, $P = 0.02$). Mice injected with glyburide after irradiation or with vehicle were not protected from radiation.

DISCUSSION

The present data show that siRNA library screening affords an unbiased method for large-scale and rapid target identification. It has been employed successfully to identify novel targets for cancer therapeutics in transformed human cells (13,14), especially in a synthetic lethal format (15). There are no previous reports exploiting this technique to discover new radiation protectors. In the present study, we exploited the synthetic protective siRNA screening concept to identify genes that, when silenced, protected human glioblastoma cells from radiation-induced cell death. We identified 116 potential radioprotective genes by screening a human druggable genome library. Ten compounds with binding capacities to 13 candidate gene products were tested. One of the 10, glyburide, demonstrated *in vitro* and *in vivo* radioprotective effects.

Several of the 10 selected compounds are known to have multiple pharmacological targets, and disruption of these additional targets could have mitigated the cellular response to radiation. We found that several compounds increased the toxicity of radiation. Moreover, siRNA causes a loss of the entire protein while pharmacological agents generally target only the enzymatic activity of a translated protein. For this reason, it is often difficult to fully replicate results of siRNA with small molecules.

The specific mechanisms by which glyburide provides radioprotection are unknown. Sulfonylureas, including glyburide, are widely used in the treatment of diabetes mellitus and act to block ATP-sensitive potassium (K_{ATP}) channels, such as ABCC8 (16,17). The hypoglycemic actions of the sulfonylureas are due at least in part to their high affinity for binding the ABCC8, the sulfonylurea receptor 1 and subsequent inhibition of the pancreatic K_{ATP} channel, which is essential for triggering insulin secretion via membrane depolarization (18). Interactions of glyburide with sulfonylurea receptor 1 may explain its role in regulating radiation-induced apoptosis (19-21). Apoptosis may be modulated by the regulatory effects of glyburide on K_{ATP} channels, which control intracellular Ca^{2+} levels and may affect the mitochondrial permeability transition pore (21,22). Other drugs have been shown to act as regulators of this channel and to alter the cellular apoptosis program (21,23). Further studies will be required to evaluate the molecular mechanisms of radiation protection of glyburide and other potassium channel inhibitors.

The radioprotective effect of glyburide may have occurred through indirect interaction with other molecular targets. We demonstrated here that siRNA knockdown of ABCA4 and ABCA8 (ATP-binding cassette members, subfamily A, member 4 and 8) protected T98G cells from radiation. Little information exists about the potential involvement of ABCA4 and ABCA8 in the apoptosis signaling pathway. Furthermore, their tissue expression is distinct from ABCC8. Since glyburide showed less protection of irradiated non-neuronal (central nervous system) H1299, HeLa and KM101 cells, the data may indicate that the radioprotective effect is cell phenotype specific. Molecular intermediate steps in this process are not yet identified. Since glyburide protected non-tumorigenic 32D cl3 cells *in vitro* as well as total-body-irradiated mice, the data are consistent with the hypothesis that the drug may provide normal tissue radioprotection.

Glyburide is an orally active, safe, clinically available drug used to treat type 2 diabetes. The maximal tolerated dose of glyburide has been established as 10 mg, twice daily, in patients <65 years old or half the dose, 10 mg, daily, in patients ≥65 years old (24). Since glyburide can produce severe hypoglycemia (25,26) at high doses, it is encouraging that the doses of glyburide used in our study did not cause hypoglycemia in C57BL/6NHsd mice (data not shown).

While preirradiation treatment with glyburide effectively blocked radiation-induced apoptosis and improved survival, it lacked efficacy as a mitigator in mice after irradiation. We are currently developing different screening strategies using more radiosensitive human cells transfected with siRNA after irradiation to identify radiation mitigators with significant activity after irradiation.

The potential targets of action of glyburide may or may not be independent of DNA repair mechanisms associated with ionizing radiation. Gene products directly associated with DNA repair mechanisms have been shown to be targets for developing radioprotectors (27-29); however, other non-DNA repair targets have also been proposed (30). Ionizing radiation initiates a variety of cellular signal transduction pathways distal to DNA damage that lead to cell death. Gene products associated with disruption of the mitochondrial electron transport chain, mitochondrial membrane oxidation, permeabilization of the mitochondrial outer membrane, and release of pro-apoptosis factors including cytochrome c may be targets for developing radioprotectors (9). The present drug discovery process led to identification of some targets that appeared to be independent of DNA repair; however, indirect effects through DNA repair cannot be ruled out. Some DNA repair pathway-related gene products were identified in our list of positives (such as VAV3-guanine nucleotide exchange factor). However, inhibition of mRNA transcripts of DNA repair molecules by synthetic siRNA would be expected to confer radiosensitization rather than radiation resistance. In other experiments in which radiation-induced apoptosis increased above control levels, we would expect to find more siRNAs targeting mRNA encoded by DNA repair genes. In addition, mRNA and/or protein levels of many DNA repair gene products may not have been decreased under the current experimental conditions. We pursued further validation of the gene products identified by the positive siRNA results. Finally, we used criteria for radiation protection that may have been so stringent that we missed detecting some DNA repair genes.

The present results demonstrate the power of unbiased siRNA screening with a druggable genome library and suggest that glyburide might be a clinically useful radioprotector.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work is supported by NIH NIAID grants U19 AI 068021 and CA78039.

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FIG. 1.
Functional categories of the 116 primary radioprotective siRNAs.

FIG. 2.

Panel A: Silencing of ABCC8 with siRNA duplexes protects T98G cells from ionizing radiation. Cells were exposed to 25 Gy radiation 48 h after siRNA transfection. Cell viability is normalized to the viability of cells transfected with control siRNA duplexes. Data are plotted as means $\pm$ SD ($n = 3$). * $P < 0.01$ compared to siRNA-transfected cells without radiation, # $P < 0.05$ compared to irradiated cells without siRNA transfection. Panel B: Sequences for the three unique siRNA duplexes targeting ABCC8. Panel C: Western blot staining of ABCC8 in siRNA duplex-transfected T98G cells. The expected ABCC8 band based on the calculated molecular weight (~115-120 kDa) is indicated by the arrow.

FIG. 3.

Target validation using small molecular inhibitors in T98G cells. Panel A: Effect of inhibitors on γ -radiation-induced cell death in T98G cells. Data are plotted as means $\pm$ SD ($n = 3$). * $P < 0.01$ compared to irradiated T98G cells in the absence of glyburide. Panel B: Chemical structure of glyburide.

FIG. 4.

Radioprotective effect of glyburide in T98G, U-87 MG and BEAS-2B cells and primary astrocytes. Panel A: Postirradiation protective effect of glyburide in T98G cells. Glyburide was added to cell culture 30 min after irradiation (IR) of T98G cells (25 Gy). Panel B: Cells were incubated with 200 μM glyburide 1 h prior to γ irradiation (5-25 Gy). Panel C: Cells were incubated with glyburide at indicated concentrations 1 h prior to irradiation of U-87 MG and BEAS-2B cells (10 Gy). Panel D: Human primary astrocytes were treated with glyburide (50 and 100 μM) 1 h prior to irradiation (25 Gy). Cell viability was determined with either flow cytometry (panels A, B, C) or LDH release assay (panel D). *, # $P < 0.01, 0.05$ compared to irradiated cells without glyburide treatment.

FIG. 5.

Protection of 32D cl3 cells from radiation by glyburide: clonogenic assay. The data were fitted by the single-hit, multitarget model. Data points are means $\pm$ SE ($n = 3$). Cells incubated in glyburide showed an increased shoulder on the survival curve. There were no significant changes in the D_0 .

FIG. 6.

Radioprotective effect of glyburide in total-body-irradiated mice. Mice were injected with glyburide 10 min before or 10 min after exposure to 9.5 Gy. Mice injected with glyburide before irradiation had a significantly increased survival compared to the control irradiated mice. Injection of glyburide after irradiation had no significant effect.

TABLE 1
Protective Genes Detected by Radiation siRNA Library Screening

Symbol	Gene name	Symbol	Gene name
AADAT	aminoadipate aminotransferase	NME6	non-metastatic cells 6, protein expressed in
ABCA4	ATP-binding cassette, subfamily A (ABC1), member 4	OAS3	2'-5'-oligoadenylate synthetase 3, 100 kDa
ABCA8	ATP-binding cassette, subfamily A (ABC1), member 8	OFD1	oral-facial-digital syndrome 1
ABCC8	ATP-binding cassette, subfamily C (CFTR/MRP), member 8	PAK1	p21/Cdc42/Rac1-activated kinase 1(STE20 homolog, yeast)
ACCN2	amiloride-sensitive cation channel 2, neuronal	PAK4	p21(CDKN1A)-activated kinase 4
ACOXL	acyl-Coenzyme A oxidase-like	PCCB	propionyl Coenzyme A carboxylase, beta polypeptide
ADAM10	ADAM metalloproteinase domain 10		
ADAMTS17	ADAM metalloproteinase with thrombospondin type 1 motif, 17	PDE11A	phosphodiesterase 11A
		PDE2A	phosphodiesterase 2A, cGMP-stimulated
ALDH1A3	aldehyde dehydrogenase 1 family, member A3	PDE4B	phosphodiesterase 4B, cAMP-specific
ALDH1B1	aldehyde dehydrogenase 1 family, member B1	PDE4D	Phosphodiesterase 4D, cAMP-specific
ALDH4A1	aldehyde dehydrogenase 4 family, member A1	PDE6C	Phosphodiesterase 6C, cGMP-specific, cone, alpha prime
ARHGEF6	Rac/Cdc42 guanine nucleotide exchange factor (GEF) 6		
ATP8A2	ATPase, aminophospholipid transporter-like, class I, type 8A, 2	PIGH	phosphatidylinositol glycan anchor biosynthesis, class H
CACNA1B	calcium channel, voltage-dependent, N type, alpha 1B subunit	PIP5K1B	phosphatidylinositol -4-phosphate 5-kinase, type I, beta
CAMK2B	calcium/calmodulin-dependent protein kinase (CaM kinase) II beta	PKIA	protein kinase (cAMP-dependent, catalytic) inhibitor alpha
CAPN13	calpain 13	PRPS1L1	phosphoribosyl pyrophosphate synthetase 1-like 1
CAPN2	calpain 2, (m/II) large subunit	PRSS1	protease, serine, 1 (trypsin 1)
CAPNS2	calpain , small subunit 2	PTPN9	protein tyrosine phosphatase, non-receptor type 9
CARD9	caspase recruitment domain family, member 9	PTPRA	protein tyrosine phosphatase, receptor type, A
CATSPER2	cation channel, sperm associated 2	REV1	REV1 homolog (S. cerevisiae)
CHRNA1	cholinergic receptor, nicotinic, alpha 1(muscle)	RFPL3	ret finger protein-like 3
CHRNA2	cholinergic receptor, nicotinic, beta 2(neuronal)	ROR2	receptor tyrosine kinase-like orphan receptor 2
COX8A	cytochrome c oxidase subunit 8A (ubiquitous)	SKP2	S-phase kinase-associated protein 2 (p45)
CSNK1G2	casein kinase 1, gamma 2	SLC10A1	solute carrier family 10, member 1
CTSA	cathepsin A	SLC15A1	solute carrier family 15, (oligopeptide transporter), member 1
CYP3A5	cytochrome P450, family 3, subfamily A, polypeptide 5		
DGKQ	diacylglycerol kinase, theta 110 kDa	SLC16A3	solute carrier family 16, member 3
DMPK	dystrophin myotonic-protein kinase	SLC17A5	solute carrier family 17 (anion/sugar transporter), member 5
DUSP22	dual specificity phosphatases 22		
DYRK1B	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1B	SLC18A2	solute carrier family 18 (vesicular monoamine), member 2
ECE1	endothelin converting enzyme 1	SLC19A1	solute carrier family 19 (folate transporter), member 1
ENPP3	ectonucleotide pyrophosphatase/phosphodiesterase 3	SLC1A6	solute carrier family 1, member 6

Symbol	Gene name	Symbol	Gene name
EPHA1	EPH receptor A1	SLC25A22	solute carrier family 25, member 22
EPHB2	EPH receptor B2	SLC27A3	solute carrier family 27 (fatty acid transporter), member 3
FOLR1	Folate receptor 1 (adult)		
GABRD	gamma-aminobutyric acid (GABA) A receptor, delta	SLC44A5	solute carrier family 44, member 5
GALK2	galactokinase 2	SLC4A9	solute carrier family 4, member 9
GCDH	glutaryl-Coenzyme A dehydrogenase	SLC6A1	solute carrier family 6, member 1
GRM8	glutamate receptor, metabotropic 8	SLC6A2	solute carrier family 6, member 2
H6PD	hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase)	SLC6A3	solute carrier family 6, member 3
		SLCO4C1	solute carrier organic anion transporter family, member 4C1
HARS2	his-dyl-tRNA synthetase 2, mitochondrial (putative)		
IBSP	integrin-binding sialoprotein (bone sialoprotein, bone sialoprotein II)	SRM	spermidine synthase
		STK24	serine/threonine kinase 24 (STE20 homolog, yeast)
KCNE2	potassium voltage-gated channel, Isk-related family, member 2	STK39	serine threonine kinase 39 (STE20/SPS1 homolog, yeast)
KCNK1	potassium voltage-gated channel, subfamily G, member 1	STX11	syntaxin 11
		STX4	syntaxin 4
KCNK4	potassium voltage-gated channel, subfamily G, member 1	TAP1	transporter 1, ATP-binding cassette, subfamily B (MDR/TAP)
KCNK9	Potassium channel, subfamily K, member 9		
KLK5	kallikrein-related peptidase 5	TGFB1/1	transforming growth factor beta 1 induced transcript 1
LMLN	leishmanolysin-like (metallopeptidase M8 family)		
MAPKAPK2	mitogen-activated protein kinase-activated protein kinase 2	TLL2	tolloid-like 2
MC2R	melanocortin 2 receptor (adrenocorticotrophic hormone)	TMEM 11	transmembrane protein 11
MED16	mediator complex subunit 16	TMPRSS9	transmembrane protease, serine 9
MLKL	mixed lineage kinase domain-like	TRIB3	tribbles homolog 3 (Drosophila)
MMP27	matrix metallopeptidase 27	TRMT1	TRM1 tRNA methyltransferase 1 homolog (S. cerevisiae)
MMP3	matrix metallopeptidase 3 (stromelysin 1, progelatinase)		
MMP9	matrix metallopeptidase 9	TRPC1	transient receptor potential cation channel, subfamily C, memb 1
MST1R	macrophage stimulating 1 receptor (c-met-related tyrosine kinase)		
		TRPC3	transient receptor potential cation channel, subfamily C, memb 3
MTHFD2	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2		
		TRPC4	transient receptor potential cation channel, subfamily C, memb 4
NME4	non-metastatic cells 4, protein expressed in		
		TSSK6	testis-specific serine kinase 6
		TTL	tubulin tyrosine ligase
		UCK1	uridine - cytidine kinase 1

Symbol	Gene name	Symbol	Gene name
		VAV3	vav 3 guanine nucleotide exchange factor
		WDR31	WD repeat domain 31
		WNK1	WNK lysine deficient protein kinase 1

TABLE 2
Chemicals Selected for Validation of Radiation Protection in Cell-Based Model

Drug/inhibitor	Target gene symbol	Gene description	Location	Family
SB 203580	MAPKAPK2	Mitogen-activated protein kinase-activated protein kinase 2	Nucleus	Kinase
Tyrphostin AG 112	MST1R	Macrophage stimulating 1 receptor (c-met-related tyrosine kinase)	Plasma membrane	Kinase
LY294002	PAK4	p21 (CDKN1A)-activated kinase 4	Cytoplasm	Kinase
IMBX	PDE11A	Phosphodiesterase 11A	Cytoplasm	Enzyme
Rolipram	PDE4B	Phosphodiesterase 4B, cAMP-specific (phosphodiesterase E4 dunce homolog, Drosophila)	Cytoplasm	Enzyme
	PDE4D	Phosphodiesterase 4D, cAMP-specific (phosphodiesterase E3 dunce homolog, Drosophila)	Cytoplasm	Enzyme
Disulfiram	ALDH1A3	Aldehyde dehydrogenase 1 family, member A3	Cytoplasm	Enzyme
Glyburide	ABCA4	ATP-binding cassette, sub-family A (ABC1), member 4	Plasma membrane	Transporter
	ABCA8	ATP-binding cassette, sub-family A (ABC1), member 8	Plasma membrane	Transporter
	ABCC8	ATP-binding cassette, sub-family C (CFTR/MRP), member 8	Plasma membrane	Transporter
Hexamethonium chloride	CHRNA1	Cholinergic receptor, nicotinic, alpha 1 (muscle)	Plasma membrane	Transmembrane receptor
Doxycycline hyclate	MMP9	Matrix metalloproteinase 9 (gelatinase B, 92-kDa gelatinase, 92-kDa type IV collagenase)	Extracellular space	Peptidase
Aprotinin	KLK5	Kallikrein 5	Cytoplasm	peptidase