Data Mining for Drug Repurposing and New Targets Identification for Radioprotection

Yogesh Kumar Verma and Gangenahalli Ugraiah Gurudutta[*]

Division of Stem Cell and Gene Therapy Research, Institute of Nuclear Medicine & Allied Sciences, Delhi - 110 054, India
[*]E-mail: gugdutta@rediffmail.com

ABSTRACT

Ionising radiation (IR) is responsible for various types of tissue injury leading to morbidity at low doses and mortality at high radiation exposure. Although many radioprotective and pharmacological agents are being tested for decreasing radiation injury, however, the availability of Amifostine as the only clinically used radioprotector with limited indication has prompted us to find out new potential molecules through drugs repurposing for protecting or decreasing radiation damage by data mining. In this work we have used text-mining based network generation approach to find out the gene targets of radioprotectors under evaluation by Agilent Literature Search app in Cytoscape. Extracted genes were evaluated for their association with radiation in Radiation Genes database. These genes were searched against therapeutic drugs and molecules under clinical trial in the Drug Gene Interaction database. We found that most of the radiation target genes were involved in cell death, proliferation, homeostasis, cell cycle and cancer pathways. Many of these genes were druggable and could be targeted by the drugs under clinical research, whereas there were few genes (new targets), which were never considered for radioprotective drug development. This study would likely help in repurposing of identified drugs for use in the event of radiation fallout, keeping in mind that no radiation medical countermeasure for acute radiation syndrome has been approved by the US FDA for use in humans. Results also revealed new target genes for drug targeting and indicates use of similar pipeline in other pathologies for drug repurposing and development.

Keywords: Radioprotective agents; Network based drug discovery; Gene-drug mapping; Radiation induced genes; Amifostine; Microarray

NOMENCLATURE

IR	Ionising radiation
US FDA	United States food and drug administration
cAMP	Cyclic adenosine monophosphate
NOS	Nitric oxide synthase
ATP	Adenosine triphosphate
GO	Gene Ontology
STITCH	Search tool for interactions of chemicals
EMBL	European molecular biology laboratory
CTD	Comparative toxicogenomics database
EGF	Epidermal growth factor
FGF	Fibroblast growth factor
HDAC	Histone deacetylase
IGF-1	Insulin growth factor
TGF	Tumor growth factor
AED	5-Androstenediol
G-CSF	Granulocyte-colony stimulating factor
GM-CSF	Granulocyte monocyte-colony stimulating factor
IL	Interleukin
PEG	Polyethylene glycol
ACE	Angiotensin converting enzyme
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Fluorescence recovery after photobleaching

1. INTRODUCTION

Recently, there has been spurt in research on radioprotective agents due to incidents like leakage of radioactivity in Fukushima, Japan[1] and Mayapuri incident in Delhi[2]. Radiation causes ionisation of atoms in cells and tissues, resulting in damaging effects on the whole body. This leads to induction of mutations, chromosomal aberrations and cell death (or apoptosis). To decrease the radiation induced cellular injury, various drugs, chemical and biological compounds were tested, for example; thiol group (-SH) containing compounds (e.g., Aminofostine), Melatonin (N-acetyl-5-methoxytryptamine), NOS inhibitors etc[3]. Among these, sulfhydryl compounds have been the most extensively used. These agents are very good radioprotectors when given before X-ray exposure, but are very toxic in humans and do not provide any differential protection to normal tissues[4]. Studies have suggested that certain physiological substances such as cAMP[5] and some vitamins (A, C and E) may be of radioprotective value for normal cells[6-7]. Calcium antagonists are also considered as good radioprotective agents with minimal toxicity[8], nonetheless, the specificity of its effect for normal cells is unknown. Similarly, other compounds have been shown to have radioprotective ability in lab testing (Table 1)[3]. However, Amifostine is the only clinically used drug for decreasing radiation injury. It is used for a narrow medical indication i.e., for reduction of xerostomia (dry mouth) that results from salivary gland injury

Table 1.   Classification of radioprotective agents and their mechanism of action. Agents evaluated in labs across the world for radioprotection, yet, none is used clinically for radiation protection except Amifostine

Group	Radioprotective agents	Mechanism of action
Thiols (-SH)	Cysteines, mercaptoethylamine, cystamine, amino ethyliso- thiourea dihydrobromide, 2-mercaptoethylguanidine	Free radical scavenger; production of hypoxia; formation of mixed disulfide bonds; reversible DNA inhibition
Other Sulfur compounds	Thiourea, thiouracil, dithiocarbamates, dithioxamide, thiazo- lines, sulfoxides, sulfones, dimethylsulfoxide	Free radical scavenger; production of hypoxia; formation of mixed disulfide bonds; reversible DNA inhibition
Pharmacological agents	Ethyl alcohol, morphine, heroine, sodium salicylate, reserpine, acetylcholine, metacholine, carbaminoylcholine, epinephrine, methoxamine, dopamine, histamine, serotonin, estradiol, estro- gen, colchicines, melatonin, melanocyte-stimulating hormone	Anesthetics; analgesics; tranquilizers; cholinergics; free radical scavengers; hormones
Other radioprotec- tive agents	Cyanide, 5-hydroxy-4-methyluracil, 5-amino-4-methyluracil, 5-amino-4-mehtyl cytosine, AMP/ATP, pyridoxal-5-phosphate, sodium fluoroacetate, para-aminopropiophenone, melittin, ty- phoid-paratyphoid vaccine, imidazole, cAMP	Miscellaneous

in patients undergoing radiotherapy for the treatment of head and neck cancer[9].

Drug repurposing/ drug re-profiling/ therapeutic switching is the application of known drugs and compounds to new diseases. An advantage of drug repositioning over routine drug development is that since the repositioned drug has already passed a number of pre-clinical and clinical phase, its safety is known and the risk of failure due to adverse effects are reduced. In addition, repurposed drugs can bypass much of the initial cost and time needed to bring a drug to market[10]. Repurposing of drugs utilises the relationship among genes, drugs, diseases and pathways, existing in databases. The relationship is displayed in the form of network for discovering potential drug targets. This strategy is likely to miss the information available in literature only and if no inter-relationship among molecules/ drugs has been established. Unavailability of this critical information in databases has detrimental effect on drug discovery and repurposing. Semantic-based biological networks generation integrates drugs, genes diseases, pathways and SNPs into one system for discovering potential drug targets. This technique extracts functional relationship between genes and generate network if two genes are frequently mentioned in the same sentence. Also, literature association networks are useful as a general search tool, since each link is referred to the supporting publication[11]. However, this may require some refinement by removing unrelated hits.

Network based discovery can be used to identify on-target, off-target and indirect effects, thus helping in the identification of novel therapeutic opportunities for drug repositioning[12]. In this work, we have used literature mining and networking tools to identify gene targets of radioprotectors. The identified genes were screened against already available drugs/molecules for decreasing radiation injury. Agilent literature search (ALS)[11], one of the Cytoscape[13] app was used to find out the genes and their interaction on the basis of text-mining. The input terms were related to radiation and radioprotection in radiation genes database[14] to find out their involvement in radiation induced pathways. The function of each gene in the interaction was found by using ClueGO app[15]. Further, the clinical/ experimental drugs/ molecules available against these genes were identified from DGIdb[16]. The drug-gene network

was relayed in Cytoscape to display all the drugs/chemicals targeting identified genes. This network helped us in predicting the drugs, which are being used in some other pathology but could be tested as radiation medical counter measure (MCM) in humans exposed to undesired radiation.

2.   MATERIALS AND METHODS
2.1   Agilent Literature Search for Candidate Genes

ALS is a meta-search tool for automatically querying multiple text-based search engines (both public and proprietary) to aid manual searching and extracting associations among genes/proteins of interest[11]. ALS was used in conjunction with Cytoscape, which generated a network view of gene/protein associations. The query terms related to radiation were entered; the retrieved results were fetched from their respective sources. Each resulting document was then parsed into sentences and analysed for protein-protein associations. ALS app used a set of 'context' files (lexicons) for defining protein names (and aliases) and association terms (verbs) of interest. Lexical parser was able to tell the subject and direct object from a sentence. Associations extracted from the documents were collected into a Cytoscape network. The sentences and source hyperlinks for each association were further stored as attributes of the corresponding Cytoscape edges. The coding genes for detected proteins were further searched in Radiation Genes database to find out their role with reference to radiation[9]. The Radiation Genes database collects microarrays data on transcriptional effects of ionising radiation obtained from public repositories or from published papers and supplementary materials. The database classifies it in terms of radiation quality, dose, dose rate and sample timing, so as to facilitate data integration and comparison.

2.2   ClueGO Functional Annotation

ClueGO app was used to visualise the non-redundant biological terms for large clusters of genes in a functionally grouped network constructed from ALS results. The radiation related genes symbols were uploaded in the ClueGO app. From the used ontology sources, the terms were selected by different filter criteria. The related terms which share similar and associated genes were used to reduce redundancy. The

ClueGO network was created with kappa statistics (this links the terms in the network) and reflected the relationships between the terms based on the similarity of their associated genes. Following parameters were selected for analysis; Analysis mode: Function; Ontologies/pathways: Biological pathways; Pathways selection with pV≤0.05; Enrichment/depletion: By two sided hypogeometric test; pV correction: Bonferroni step down; GO term interval: 3-8; Pathways selection: number of genes/per cent genes; Selection of cluster consisting of: Minimum 3 genes and 8 per cent genes; Kappa score for pathway network connectivity: 0.4.

2.3 DGIdb Search for Genes to Drugs Mapping

To find out the drugs available for the genes targeted in radiation or radiation protection, the candidate gene list was analysed in DGIdb. It searched list of genes against a compendium of drug-gene interactions and potentially druggable genes. DGIdb organised genes of the druggable genome into two main classes. First, included genes with known drug interactions obtained by literature mining or by parsing publically available database. Second, included genes that may not currently be targeted therapeutically but are potentially druggable according to their membership in gene categories associated with druggability[16]. The gene list with associated drugs, interaction type and source of interaction was downloaded as .tsv file.

2.4 Network Generation in Cytoscape

The network of genes with associated drugs and interaction type was generated in Cytoscape. The DGIdb .tsv file was used for this purpose. In order to visualise individual connected components, the network analyser was used for sub-network creation in 'Tools' menu of Cytoscape.

3. RESULTS

3.1 Agilent Literature Search

Two searches were performed, i.e. Search 1 and Search 2, with different search terms (Table 2). In Search 1, the search terms were categorised into 04 categories viz. radioprotective agent, pharmacological agents, mechanism of action and other. In Search 2, individual radioprotective agents, oncogenes, pharmacological agents and antioxidants were input as query terms.

The general term like 'Facilitate DNA and cellular repair' has been included in Search1 because irradiation (>1000 rads) of DNA solution *in vitro* has been shown to cause breakage of hydrogen bonds, chain breaks, cross-linkages, disruption of the sugar-phosphate backbone of DNA, impairment of the transforming ability of DNA, DNA base damage and inability of the DNA to act as a template for the synthesis of new DNA[3]. In mammalian cells, direct interaction of IR and DNA leads to single- and double strand breaks in DNA. Indirectly, IR induces formation of reactive oxygen species (ROS) through radiolysis of water, which causes DNA damage in cells. Therefore, a good radioprotector is attributed to have following characteristics; free radical scavenger, protect radio-oxidative damage, facilitates DNA and cellular repair, immune-modulatory and facilitates repopulation of damaged and affected organs[22].

Table 2. **Search terms queried in ALS to find out associated genes through text mining. The query terms in Search 1 and Search 2 were input in ALS and option was set to use a *Homo sapiens* concept lexicon to resolve aliases and to limit search engine 'hits' to 5/search engine/query line. Entries in the query matched panel corresponded to the sentences found in the literature when that search was executed. An association was extracted for every sentence containing at least two concept names and one verb. Associations were then converted into interactions, which were further grouped into a network in Cytsoscape**

Category	Terms	Reference
Radioprotective agents	ExRad, *Hippophae rhamnoidea, Podophyllum hexandrum*	17-19
Pharmacological agents	Amifostine, Melatonin, 2-deoxyglucose	20, 21
Mechanism of action	Free radical scavenging, radio-oxidative damage, facilitates DNA and cellular repair, immune-modulation, population of damaged and affected organs	3
Other	Drugs for radiation injury, cell death and gamma radiation, radioprotectors, radiomitigators, ionising radiation	3
Radioprotective agents	Sulphydryl compounds and irradiation	3
Radioprotective agents	Cyclic nucleotides and irradiation	3
Radioprotective agents	Eicosanoids and irradiation cytokines and irradiation	3
Oncogenes	Oncogenes and irradiation	3
Pharmacological agent	Pharmacological agents and irradiation	3
Radioprotective agents	AS101 and irradiation	3
Radioprotective agents	Calcium antagonists and irradiation	3
Antioxidants	Antioxidant vitamins and irradiation	3
Antioxidants	Vitamin and beta carotene and irradiation	3
Antioxidants	Vitamin E and irradiation	3
Radioprotective agents	Radioprotective agents	3

Similarly in Search 2, 'cAMP' semantic was used, which is better known to have more general role in other cellular pathways. However the basic observation on radioprotective role of cAMP has been confirmed and extended by several investigators[23-25]. It was also suggested that a number of radioprotective agents, for e.g., sulfhydryl compounds, mediate their effect via cAMP[26]. The omission of this and similar terms, which have well established role in radiation protection, would have led to the missing of important leads in this study.

Twenty references were found related to Search 1 and 38 references were found corresponding to Search 2. On the basis of these references a network was generated in the Cytoscape (Figs. 1 and 2). The network consisted of genes (as nodes) and their interactions (as edges/ directed arrow). In case of Search 1, fourteen independent networks were detected, among these the biggest network contained well known radiation associated genes such as p53, CDKN1A, ROS1, NOS2, ATM, and AKT1[3]. Among the detected genes, except BCL-2, BCRA1, GLUTATHIONE, HOMOX1, PTPNSLC17A5, MPILC3B, H2AFX, NROB2, MIR25 and ALP1, other genes have been shown to be induced upon radiation exposure in microarray studies as detected in radiation genes database. However, exceptional genes are reported to be associated with radiation in scientific literature.

For Search 2, BCL-2 was again found to have betweenness centrality for the biggest network among 09 independent networks. Among detected genes, the expression of BCL-2, PSML6, MCL-1, PTPN6, IF127 and VCP3 is not affected by radiation exposure as found out from Radiation Genes database results.

3.2 Functional Annotation of Genes

In order to find the meaningful interpretation of the genes, their gene ontology was determined using ClueGO v2.1.6 (Figs. 3(a), 3(b). GO describes gene products in terms of their associated biological processes, cellular components and molecular functions. Gene ID was used to input as identifier in ClueGO. A total number of 17 clusters were identified with 18 GO term connections for Search 1. Most of the genes were found to be associated with apoptotic pathways, cell ageing and cell homeostasis. Detection of these genes indicates their involvement in radiation sensitivity or radiation protection. For Search 2, a total number of 86 clusters were identified with 312 GO term connections. The target genes were detected for cell death, proliferation, homeostasis, cell cycle and cancer pathways. Detected genes were also involved in amytrophic lateral sclerosis and Chagas disease. These results indicate that most of the genes targeted for radiation damage and protection are involved in cell death pathway, suggesting importance of cell death related genes. In both searches, 08 common genes were detected viz. BCL-2, ATM, CDKN2A, FAS, NOS2, AKT1, PTPN6 and BAX.

3.3 Repurposed Drugs

The genes identified in Search 1 and Search 2, were input in DGI database for their druggability. DGIdb integrates data from 13 primary sources covering disease relevant human genes, drugs, drug-gene interactions and potential druggability. The sources of drug includes Entrez genes[27], Ensembl[28], Pubchem[29], dGene, Russ and Lampel druggable genes, Hopkins and Groom druggable genes, GO, My Cencer Genome, TALC (targeted agents in lung cancer), TEND (trends in exploration
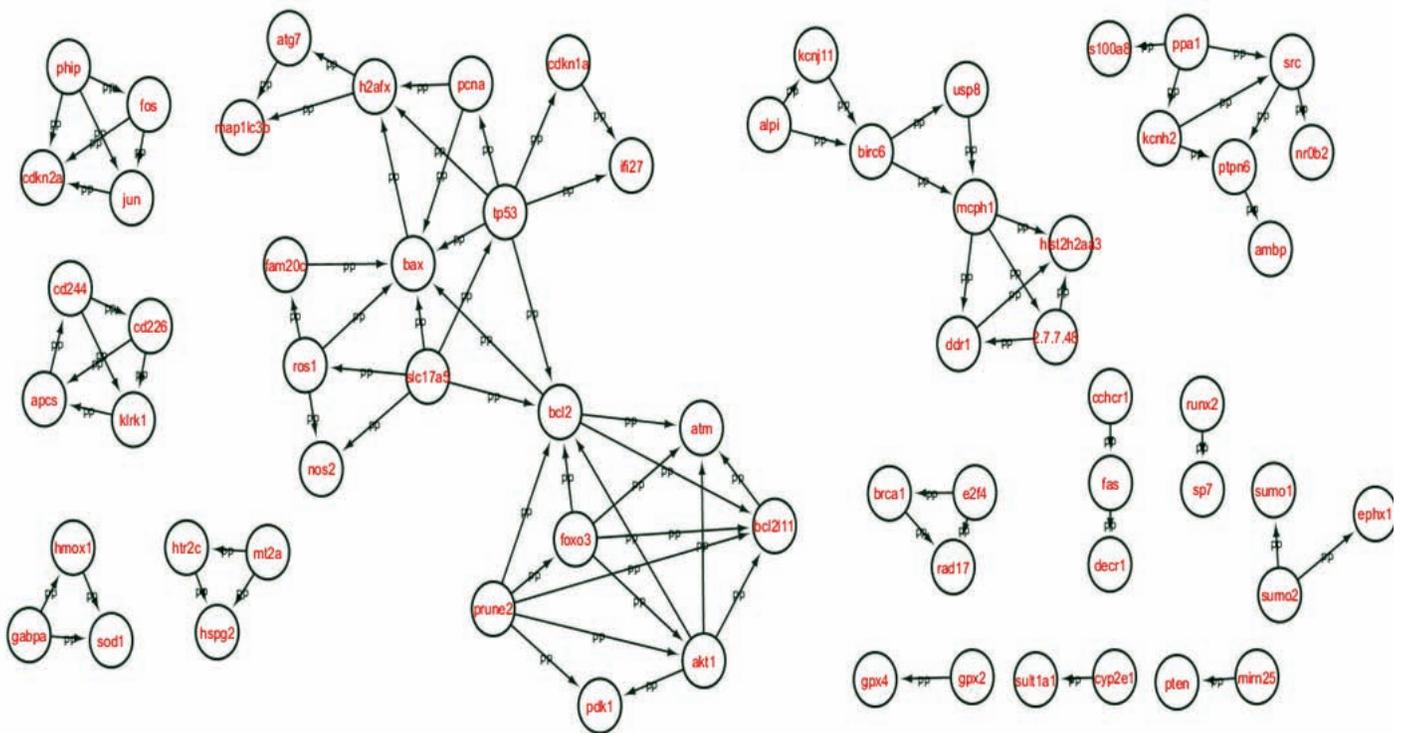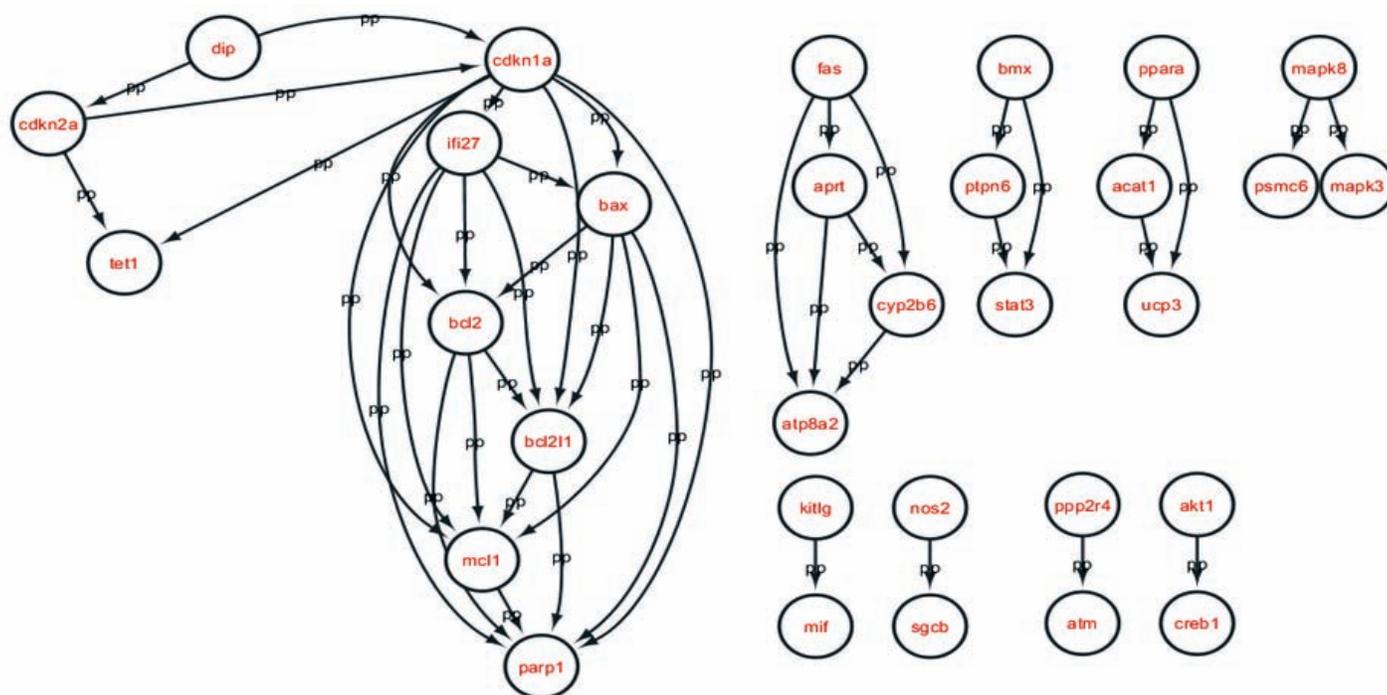

Figure 1. The orthogonal layout of Cytoscape network for Search 1 showing 14 individual components related to radiation terms. BCL-2 node had betweenness and closeness centrality[37] in this network, indicating its importance. Directed arrows indicating flow of signal to and from a gene. pp: Extracted relationship on the basis of protein-protein interaction.

Figure 2. **09 individual components were detected for Search 2, displayed in hierarchical layout in Cytoscape. Directed arrows indicating flow of signal to and from a gene. pp: Extracted relationship on the basis of protein-protein interaction.**

of novel drug targets), PharmGKB, TTD (therapeutic target database), DrugBank, CancerCommons, Clearityfoundation, STITCH, Supertarget, ChEMBL, Promiscuous and CTD.

Search 1 resulted in 481 entries for drug interactions for the input genes (Figs. 4(a), (b)). It also found search terms matching one gene, but no interactions were present for them, which included MAPILC3B, ATG7, H2AFX, NROB2, KLRK1, CD226, CD244, BAX, SLC17A5, MIR25, BCL2L11, FOXO3, PRUNE2, RAD17, E2F4, MCPH1, USP8, ALPI, HIST2H2AA3, SUMO2, SUMO1, FAM20C, MT2A, PHIP, SI00A8, GABPA, PCNA, IFI27, SP7 and RUNX2. No matches were found for search terms BCRA1, Glutathione, HOMOX1, MPIL, C3B, SLC17A5 and NROB2. It was observed that SRC, Genes KCNH2, NOS1, PTEN, AKT-1, HTRC2, BCL-2 and HMOX1 are most druggable genes whereas ≤5 drugs are available against AMBP, PPA1, APCS, ROS1, ATM, CDKN2A, JUN, FOS, FAS, CCHR1, DECR1, TP53, GPX4, GPX2 and SOD1. KCNH2, which is activated in response to drug and negatively regulate ion transport, was targeted by most of molecules in PharmGKB. In addition, highest number of drugs was found to target this gene.

Search 2 resulted in 326 entries for drug interactions for the input genes (Fig. 5(a), (b)). It also found search terms matching one gene, but no interactions were present for them, which included KITLG, SGCB, DIP, TET1, IF127, VCP3, ATP8A2, ATP8A2, BAX, BMX, PPP2R4. No match was found for search terms PSML6, IFI27 and VCP3. In Search 2 also most of the genes were targeted by molecules in DrugBank as in Search 1. Maximum number of drugs in all the databases was found to target AKT1, which is a protein kinase B involved in myelin maintenance and spongiotrophoblast

layer development. PPRA, CYP2BG, BCL-2, MAPK3/8, PAPR1, MIF and AKT1 were found to have more targeting drugs as compared to ACAT1, APART, FAS, PTPN6, MCL1, BCL2L11, CDKN1A, CDKN2A, PSMC6 and CREB1.

In total, 32 genes were detected to be druggable in Search 1 and 21 genes in Search 2 (Table 3). Druggable genes are those thought to be potentially druggable by various methods of prediction. These genes may/ may not have existing drugs that target them[16]. Of the two searches, 07 genes were found to be common, viz. BCL-2, ATM, CDKN2A, FAS, NOS2, AKT1 and PTPN6. If we look at the non-druggable genes, 30 were from Search 1 and 10 were from Search 2. These are potential new target genes which were never explored for drug targeting. These genes are likely to have role in decreasing radiation induced cellular injury. BAX gene was found to be common between two searches, indicating its importance in radiation and radiation protection.

On the basis of drug-gene interaction. We have shortlisted few drugs which are likely to decrease injury upon radiation and may have repercussions in radiation MCM (Table 4). The drugs/ clinical trial drugs are available against genes viz. BCL-2, NOS2, PDK1 and KCNJ11 (for Search 1). These genes, except KCNJ11, are shown to modulate cell death. Similarly, drugs for genes ACAT1, BCL-2, PARP and NOS2 (for Search 2) are available, which regulate cell survival[16]. We removed target genes (PDK1 and PARP1) corresponding to investigational/ clinical trial drugs for further analysis.

Among the shortlisted drugs, the druggable genes common in both 'Searches', i.e. Bcl-2 and NOS2, are targeted by rasagiline and doxycycline, dexamethasone respectively. Rasagiline is an activator of anti-apoptotic protein Bcl-2,

(a)

(b)
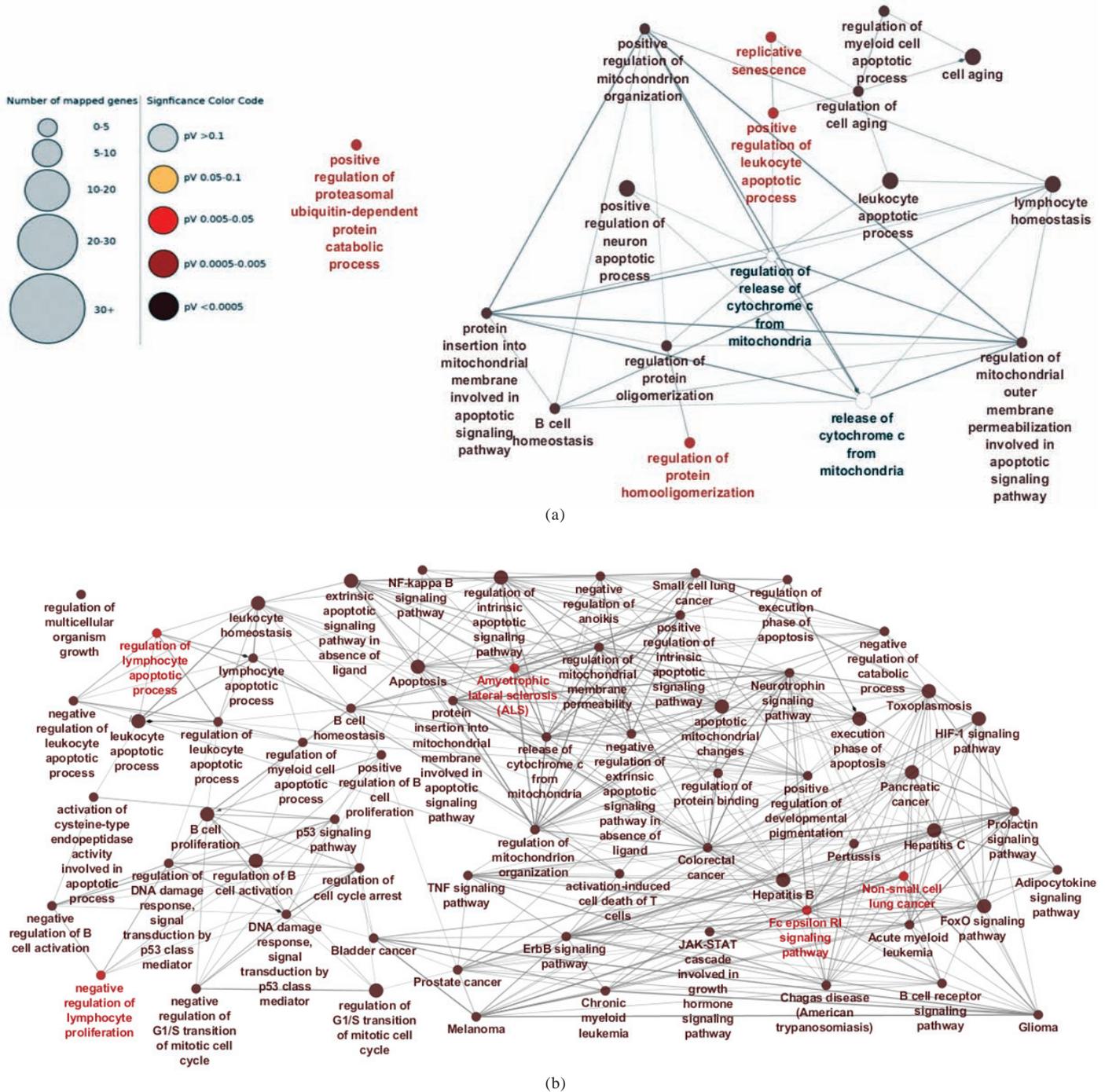
Figure 3. GO terms specific for detected genes: Functionally grouped annotation network (organic layout) of the genes reflecting the relationship between the terms based on the similarity of the associated genes. The size of the nodes reflecting the enrichment significance of the terms. The degree of connectivity between terms (edges) was calculated using kappa statistics. Ungrouped terms are shown in white color. Group significance indicating the number of unique genes found from the uploaded genes as associated with the terms included in the group and the total number of unique genes associated with those terms. (a) Search 1. (b) Search 2.

which improves cell survival. It is more commonly known as monoamine oxidase inhibitor. Members of its class, clorgyline has been shown to protect nonmalignant human cells from IR and chemotherapy toxicity. The protective effect of clorgyline involves activation of anti-apoptotic pathways within the normal cell, which is supported by reduced level of apoptosis in HaCAT and in normal bladder explants cultures[30].

NOS2 (nitric oxide synthase) gene is expressed in liver and is inducible by a combination of polysaccharide and certain cytokines. Its transcriptional activation to produce nitric oxide is associated with cerebro-vascular endothelium cell death[31]. Inducible nitric oxide synthase has also been shown to regulate T cell death and immune memory[32]. Dexamethsone and doxycycline are inhibitor of this gene. Dexamethasone is
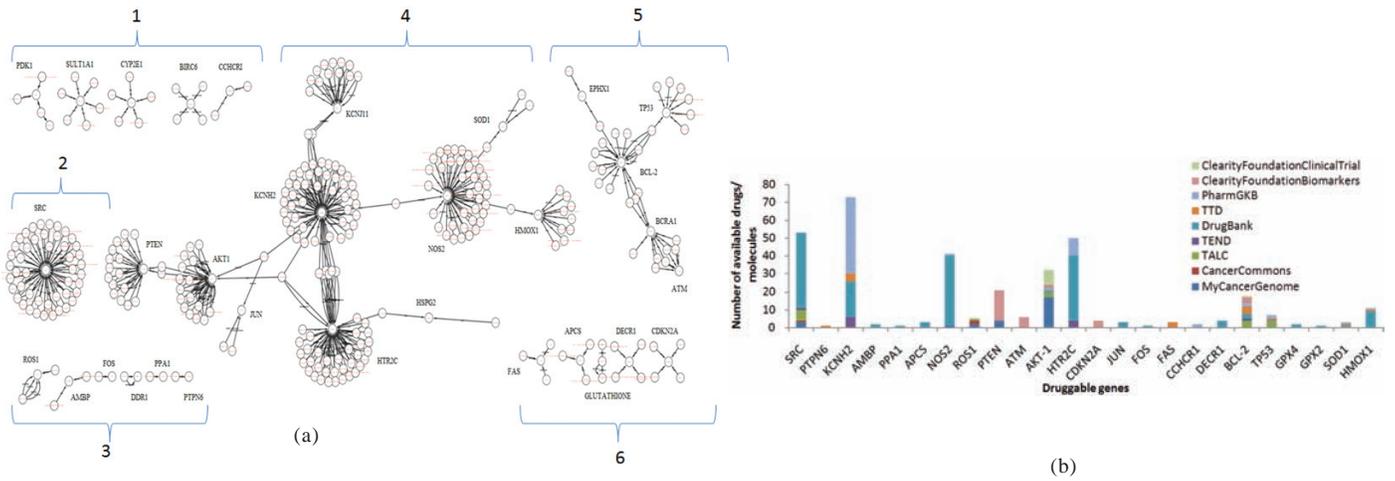
Figure 4. **(a) Drug-gene interaction network for Search 1. Individual networks were extracted using Network Analyzer in Cytoscape. Networks are clustered arbitrarily from 1-6. Of the 65 search terms entered, only 54 were matched definitely, 01 has ambiguous matching and 01 did not have any match in the database. Number of interactions for definite matches were found to be 481. The central node in each network indicates radiation related gene. The peripheral nodes connected to central node are drug molecules extracted from DGIdb. Directed edges representing the mode of action of each drug on respective target gene. Few drugs, like Arsenic Trioxide, were observed to be commonly targeting multiple genes viz. KCNH2, JUN and AKT1. This drug likely to show side-effects/ off-target effects *in vivo* when used for therapeutic purpose due to simultaneous genes interaction. (b). Number of drugs available for each gene in databases for Search 1.**
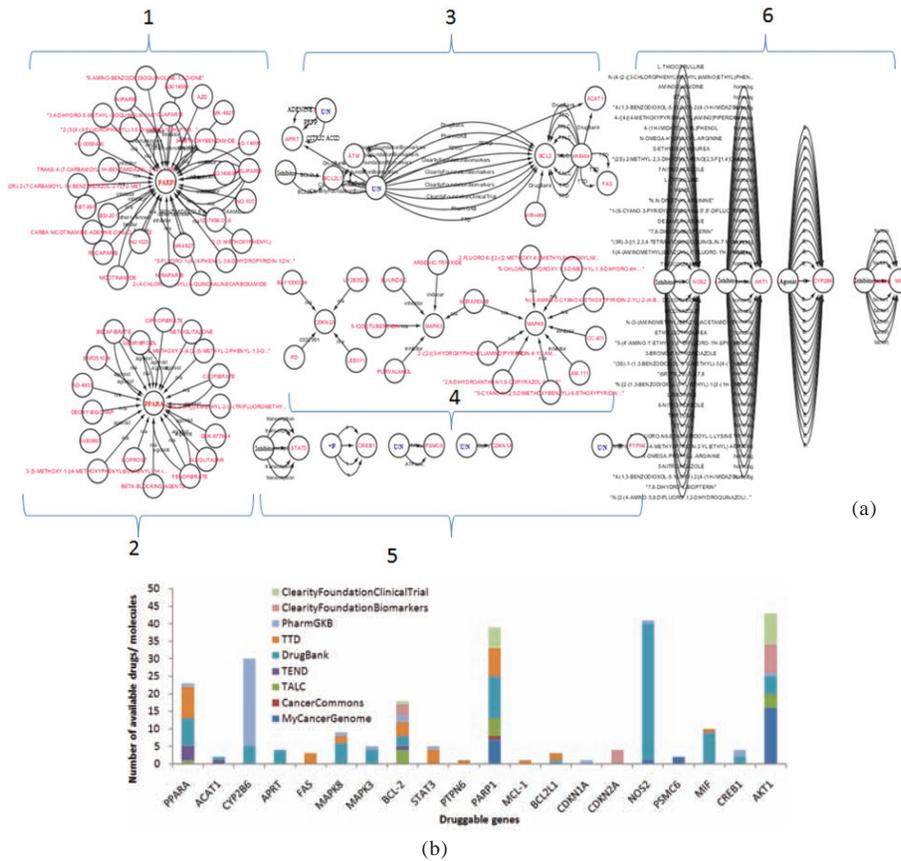
Figure 5. **(a) Drug-gene interaction network for Search 2. Individual networks were extracted using Network Analyser in Cytoscape. In total 31 search terms were entered, all terms matched definitely in the database. A total of 326 interactions showed definite matches. Networks are clustered arbitrarily from 1-6. The nodes in cluster 3 and 6 represents gene and mode of action of drugs. Directed arrows representing targeting drugs/ database to genes viz. NOS2, AKT1, CYP2B6 and MIF. In cluster 5, although drugs were found to target PSMC6 and CDKN17 genes but their effect on respective gene is still unknown. In cluster 1, 2 and 4, central node represents gene, surrounded by targeting drugs (peripheral nodes). U/N: unknown interaction. (b). Number of drugs available for each gene in databases for Search 2.**

Table 3. Druggable/ Non-druggable genes (new targets) in Search 1 and Search 2. Genes identified in 'searches', which are druggable and new targets. In druggable gene category, 07 genes were found to be common whereas 01 gene was common in new targets category.

Genes for which drugs available (Druggable)		
Search 1	Search 2	Common genes
PDK1, SULT1A1, CYP2E1, BIRC6, CCHCR1, KCNH2, KCNJ11, EPHX1, TP53, BCL-2, BCRA1, ATM, CDKN2A, DECR1, GLUTATHIONE, APCS, FAS, HOMOX1, SOD1, NOS2, HSPG2, HTR2C, JUN, AKT1, PTEN, SRC, ROS1, AMBP, FOS, DDR1, PPA1, PTPN6	PARP1, PPARA, APRT, BCL2L1, ATM, BCL-2, ACAT1, FAS, CDKN2A, MAPK3, MAPK8, NOS2, AKT1, CYP2B6, MIF, STAT3, CREB1, PSML6, CDKN1A, MCL-1, PTPN6	7 common elements in 'SEARCH 1' and 'SEARCH 2': BCL-2, ATM, CDKN2A, FAS, NOS2, AKT1, PTPN6
Genes for which no drug available (New targets)		
Search 1	Search 2	Common genes
SLC17A5, MPILC3B, ATG7, BAX, H2AFX, NROB2, CD244, KLRK1, CD226, MIR25, BCL2L11, FOXO3, PRUNE2, RAD17, E2F4, MCPH1, USP8, ALP1, HIST2H2AA3, SUMO2, SUMO1, FAM20C, MT2A, PHIP, S100A8, GABPA PCNA, IFI27, SP7, RUNX2	KITLG, SGCB, DIP, TET1, IF127, VCP3, ATP8A2, BAX, BMX, PPP2R4	1 common element in 'SEARCH 1' and 'SEARCH 2': BAX

Table 4. Gene-drug association on the basis of their interaction and sourced file. Candidate genes, playing role in radiation signaling, against which drugs are available. Search 1 shortlisted KJNJ11 gene and Search 2 indicated ACAT1 as a potential target of drug. Bcl-2 and NOS2 genes were found to be common in both the 'Searchs' against which drugs are available; these drugs could be considered for repurposing

Search 1				
Genes	Drugs (Trade name: therapeutic dose range)	Interaction	Source	Drug indication
KCNJ11	Levosimendan (Daxim: 0.01-10µM)	Inducer	Drug Bank	Calcium sensitizer for congestive heart failure
KCNJ11	Diazoxide (Proteoglycem: 200 µM)	Inducer	Drug Bank	Anti-hypoglycemic
Search 2				
ACAT1	Sulfasalazine (Azulfidine: 25-100 µM)	Inhibitor	Drug Bank	Rheumatoid arthritis, psoriatic arthritis, reactive arthritis and arthritis associated with bowel inflammation
Common in Search 1 and Search 2				
BCL-2	Rasagiline (Azilect: 1-10 µM)	Activator	Drug Bank	Anti-parkinsons; Type A monoamine oxidase, induces neuroprotective Bcl-2
NOS2	Doxycycline (Doryx: 1 µg/ml) / Miconazole (10^{-9} to 10^{-5} M)	Inhibitor	Drug Bank	Antibiotic/ Antimycotic
NOS2	Dexamethsone (Baycadrone: 10-1000 µM)	Negative Modulator	Drug Bank	Glucocorticoid agonist

used to treat inflammatory conditions including allergies, skin conditions, ulcerative colitis and breathing disorders. It has been shown to modify radiation response *in vitro* cultured cells. Treatment of V-79 cells with dexamethasone results in 1.3 fold decrease in D_0[33].

The doxycycline class member, tetracycline hydrochloride, effectively scavenges free radical in ABTS, DPPH and FRAP assays[34]. This demonstrated its ability to scavenge γ-radiation induced free radicals by formation of malondialdehyde. It confers dose modification facto (DMF) of 2 and 4 at 100 µM and 250 µM respectively, and offers 20 per cent protection at a lethal radiation dose of 9 Gy. The role

of other drugs, levosimendan and diazoxide (both targeting KCNJ11 gene) and sulfasalazine (targeting ACAT1 gene), has not been established in radioprotection. But, there is strong possibility of their involvement as supported by our prediction on rasagiline, doxycycline and dexamethasone, which has confirmatory role in published literature.

However, it will be interesting to see whether shortlisted drugs against genes (which are differentially regulated in microarray studies upon IR exposure) would have positive or negative effect on radiation injury mitigation. Further research on these drugs with respect to cell survival improvement upon IR is likely to answer these questions.

4. DISCUSSION

Attempting to translate research from animals to humans is not as efficient as studying humans directly as 92 per cent of drugs that passed preclinical testing failed in clinical trials[35]. Presently there are various candidate molecules being evaluated for radiation counter measure for ARS in mouse model namely 17DMAC, ALXN4100TPO, anticeramide antibody, AVX-470M, captopril, CBLB612, CBLB613, Delta-tocotrienol, EGF, Ex-Rad, FGF-P, Gamma-tocotrienol, Genistein, GRI977143, HDAC inhibitors, IGF-1, Leridistim, LY294002, R-spondin 1, Somatostatin analog (SOM230), superoxide dismutase, Tempol, TGF-Beta and Tocopherol succinate. Others like 5-AED, AEDL, 10150, Amifostine, CBLB502, G-CSF, GM-CSF, IL12 and PEGylated G-CSF are being tested in non-humans primates for radiation syndrome as MCM. Chances of these candidate molecules clearing all phases of drug development are bleak as in the last 16 years only 05 molecules have been approved by US FDA to be used as drug under animal rule. Among these molecules 'none' has utility for ARS[36].

Despite the fact that drug development remains a long and arduous journey, the prospect of genome based therapy remains an extremely exciting one. The possibility of treatment based on the genomic readout is now becoming a reality. In this study, by establishing relationship among heterogeneous dataset on parsing literature terms in ALS, biological network related to radiation is generated. This method is most appropriate when a subject related information is not integrated in databases and scarcity of this information is inconclusive for the investigation. In such a situation, text-mining based networks identify more general association types and offer an alternative network source when interaction data is limited.

On the basis of drug-gene interaction, we shortlisted few drugs against genes, which are likely to decrease cell death upon irradiation? The drugs/ clinically tried drugs are available against genes viz. PTPN6, PTEN, Bcl-2, NOS, PDK1 and KCNJ11 (in Search 1), which modulate cell death. Similarly, drugs for genes ACAT1, Bcl-2, PARP1 and NOS2 (in Search 2) are available, which regulate cell survival. These drugs are potential molecules, likely to show good efficacy in MCM for radiation injury. These shortlisted drugs may be stockpiled, after thorough validation, to be used in case of medical emergency arising due to radiation injury, such as Chernobyl nuclear accident in which 29 people died among 203 hospitalised.

The repurposing of these drugs for MCM could remove the initial 1-1.5 years of preclinical and Phase I development time in drug development. However, the later stages of the regulatory review process for these repositioned drugs would remain same as with new chemical entities – because research would be conducted on a different target population with a correspondingly different set of efficacy criteria.

5. CONCLUSIONS

Radioprotective drug development has suffered with 100 per cent failure rate before reaching to culmination point. This subsumes dearth of radiation genes-drug association, which makes the task of drug repurposing very difficult. The text-mining based identification of gene-drug association in this study would help in repurposing of under trial and clinical drugs to be tested in radiation exposed individuals. However, it should be confirmed that a valid association exists in between detected gene-drug pair with pre-clinical validation of results before delving into the clinical phase. The success of this strategy also depends upon the careful selection of search terms, the inclusion of general terms or unrelated terms likely to fetch unreliable results and makes the interpretation doubtful. Nevertheless, this strategy has potential to find out drugs and new targets for other pathologies, whose information is lacking in interaction databases and exists only in literature.

REFERENCES

1. Buesseler, K.O. Fishing for answers off Fukushima. *Science*, 2012, **338**, 480-82.
   doi: 10.1126/science.1228250
2. Arora, R.; Chawla, R.; Marwah, R.; Kumar, V.; Goel, R.; Arora, P.; Jaiswal, S. & Sharma, R.K. Medical radiation countermeasures for nuclear and radiological emergencies: Current status and future perspectives. *J. Pharm. Bioall. Sci.*, 2010, 2, 202-12.
   doi: 10.4103/0975-7406.68502
3. Prasad, K.N. Handbook of Radiobiology. 2nd Ed.. CRC Press Inc, 1995.
4. Anne, P.R. Phase II trial of subcutaneous amifostine in patients undergoing radiation therapy for head and neck cancer. *Semin. Oncol.*, 2002, **29**, 80-3.
   doi: 10.1053/sonc.2002.37346
5. Lehnert, S. Modification of post-irradiation survival of mammalian cells by intracellular cyclic AMP. *Radiat. Res.*, 1975, **62**, 107-116.
   doi: 10.2307/3574188
6. Tannock, L.D.; Suit, D.H. & Marshall, N. Vitamin A and the radiation response of experimental tumors. An immune mediated effect. *J. Natl. Cancer Inst.*, 1972, **48**, 731-41.
7. Konopacka, M.; Widel, M. & Rzeszowska-Wolny, J. Modifying effect of vitamins C, E and beta-carotene against gamma-ray induced DNA damage in mouse cells. *Mutat. Res.*, 1998, **417**, 85-94.
   doi: 10.1016/S1383-5718(98)00095-3
8. Floersheim, G.L. Calcium antagonists protect mice against lethal doses of ionizing radiation. *Br. J. Radiol.*, 1992, **65**, 1025-29. doi: 10.1259/0007-1285-65-779-1025
9. Culy, C.R. & Spencer, C.M. Amifostine: an update on its clinical status as a cytoprotectant in patients with cancer receiving chemotherapy or radiotherapy and its potential therapeutic application in myelodysplastic syndrome. *Drugs*, 2001, **61**, 641-84
   doi: 10.2165/00003495-200161050-00012
10. Sleigh, S.H. & Barton, C.L. Repurposing strategies for therapeutics. *Pharmaceutical Medicine*, 2010, **24**, 151-59.
    doi: 10.1007/BF03256811
11. Vailaya, A.; Bluvas, P.; Kincaid, R.; Kuchinsky, A.; Creech, M. & Adler, A. An architecture for biological information extraction and representation. *Bioinformatics*, 2005, **21**, 430-38.

doi: 10.1093/bioinformatics/bti187

12. Iorio, F.; Rodriguez, J.S. & di Bernardo, D. Network based elucidation of drug response: from modulators to targets. *BMC Syst. Biol.*, 2013, **7**, 139-147.
doi: 10.1186/1752-0509-7-139

13. Shannon, P.; Markiel, A.; Ozier, O.; Baliga, N.S.; Wang, J.T.; Ramage, D.; Amin, N.; Schwikowski, B. & Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.*, 2003, **13**, 2498-2504.
doi: 10.1101/gr.1239303

14. Chiani, F.; Iannone, C.; Negri, R.; Paoletti, D.; D'Antonio, M.; De Meo, P.D. & Castrignanò T. Radiation Genes: a database devoted to microarrays screenings revealing transcriptome alterations induced by ionizing radiation in mammalian cells. *Database (Oxford)* 2009:bap007.
doi: 10.1093/database/bap007

15. Bindea, G.; Mlecnik, B.; Hackl, H.; Charoentong, P.; Tosolini, M.; Kirilovsky, A.; Wolf-Herman, F.; Pagès, F.; Trajanoski, Z. & Galon, J. ClueGO: A Cytoscape plug-in to decipher functionally grouped gene ontology annotation networks. *Bioinformatics*, 2009, **25**, 1091-93.
doi: 10.1093/bioinformatics/btp101

16. Griffith, M.; Griffith, O.L.; Coffman, A.C.; Weible, J.V.; McMichael, J.F.; Spies, N.C.; Koval, J.; Das, I.; Callaway, M.B.; Eldred, J.M.; Miller, C.A.; Subramanian, J.; Govindan, R.; Kumar, R.D.; Bose, R.; Ding, L.; Walker, J.R.; Larson, D.E.; Dooling, D.J.; Smith, S.M.; Ley, T.J.; Mardis, E.R. & Wilson, R.K. DGIdb: Mining the druggable genome. *Nature Methods*, 2013, **10**, 1-14.
doi: 10.1038/nmeth.2689

17. Ghosh, S.P.; Perkins, M.W.; Hieber, K; Kulkarni, S.; Kao, T.; Reddy, E.P.; Reddy, M.V.; Maniar, M.; Seed, T. & Kumar KS. Radiation protection by a new chemical entity, Ex-Rad™: Efficacy and mechanisms. *Radiat. Res.*, 2009, **171**, 173-79.
doi: 10.1667/RR1367.1

18. Goel, H.C.; Gupta, D.; Gupta, S.; Garg, A.P. & Bala, M. Protection of mitochondrial system by Hippophae rhamnoides L. against radiation-induced oxidative damage in mice. *J. Pharm. Pharmacol.*, 2005, **57**, 135-43.
doi: 10.1211/0022357055218

19. Sagar, R.K.; Chawla, R.; Arora, R.; Singh, S.; Krishna, B.; Sharma, R.K.; Puri, S.C.; Singh, P.; Kumar, R.; Sharma, A.K.; Singh, S.; Prasad, J.; Gupta, V.; Ahmed, B.; Dhar, K.L.; Khan, H.A.; Gupta, M.L. & Qazi GN. Protection of the hemopoietic system by Podophyllum hexandrum against gamma radiation-induced damage. *Planta Med.*, 2006, **72**, 114-20.
doi: 10.1055/s-2005-873148

20. Karbownik, M. & Reiter, R.J. Antioxidative effects of melatonin in protection against cellular damage caused by ionizing radiation. *Proc. Soc. Exp. Biol. Med.*, 2000, **25**, 9-22.
doi: 10.1046/j.1525-1373.2000.22502.x

21. Heminger, K.; Jain, V.; Kadakia, M.; Dwarakanath, B. & Berberich, S.J. Altered gene expression induced by ionizing radiation and glycolytic inhibitor 2-deoxy-glucose in a human glioma cell line: implications for radio sensitization. *Cancer Biol. Ther.*, 2006, **5**, 815-23.
doi: 10.4161/cbt.5.7.2812

22. Yamini, K. & Gopal V. Natural radioprotective agents against ionizing radiation – An overview. *Int. J. Biochem. Mol. Biol.*, 2011, **2**, 295-302.

23. Jacobson, E.A.; Kock, C.J.; Inch, W.R.; Tustanoff, E.R. & McCredie, J.A. Radiation induced 3'-5'-adenosine monophosphate (cAMP) changes in aerobic and hypoxic mammalian cells. *Proc. Am. Assoc. Cancer Res.*, 1980, **21**, 30a.

24. Pazdernik, T.L. & Uyeki, E.M. Enhancement of the radioprotective effects of 2-mercaptoethylamine on colony forming cells by agents which alter cyclic nucleotide levels. *Int. J. Radiat. Biol.*, 1974, **26**, 331-40.
doi: 10.1080/09553007414551311

25. O'Connor, M.K.; Malone, J.F.; Moriararity, M. & Mulgrew, S.A. Radioprotective effect of vitamin C observed in Chinese hamster ovary cells. *Br. J. Radiol.*, 1977, **50**, 587-91.
doi: 10.1259/0007-1285-50-596-587

26. Mitzengg, P. On the mechanism of radioprotection by cysteamine. II. The significance of 3'-5'-AMP for the cysteamine induced radioprotection effects in white mice. *Int. J. Radiat. Biol.*, 1973, **24**, 339-344.

27. Maglott, D.; Ostell, J.; Pruitt, K.D. & Tatusova, T. Entrez Gene: gene-centered information at NCBI. *Nucleic Acids Res.*, 2005, **33**(Database issue), D54-8.
doi: 10.1093/nar/gki031

28. Hubbard, T.; Barker, D.; Birney, E.; Cameron, G.; Chen, Y.; Clark, L.; Cox, T.; Cuff, J.; Curwen, V.; Down, T.; Durbin, R.; Eyras, E.; Gilbert, J.; Hammond, M.; Huminiecki, L.; Kasprzyk, A.; Lehvaslaiho, H.; Lijnzaad, P.; Melsopp, C.; Mongin, E.; Pettett, R.; Pocock, M.; Potter, S.; Rust, A.; Schmidt, E.; Searle, S.; Slater, G.; Smith, J.; Spooner, W.; Stabenau, A.; Stalker, J.; Stupka, E.; Ureta-Vidal, A.; Vastrik, I. & Clamp, M. The Ensembl genome database project. *Nucleic Acids Res.*, 2002, **30**, 38-41.
doi: 10.1093/nar/30.1.38

29. Wang, Y.; Xiao, J.; Suzek, T.O.; Zhang, J.; Wang, J.; Zhou, Z.; Lianyi, Han.; Karapetyan, K.; Dracheva, S.; Shoemaker, B.A.; Bolton, E.; Gindulyte, A. & Bryant, S.H. PubChem's bioassay database. *Nucleic Acids Res.*, 2012, **40**, D400-12.
doi: 10.1093/nar/gkr1132

30. Seymour, C.B.; Mothersill, C.; Mooney, R.; Moriarty, M. & Tipton, K.F. Monoamine oxidase inhibitors l-deprenyl and clorgyline protect nonmalignant human cells from ionising radiation and chemotherapy toxicity. *Br. J. Cancer,* 2003, **89**, 1979-86.
doi: 10.1038/sj.bjc.6601361

31. Constantin, D.; AlaAldeent, D. & Murphy, S. Transcription activation of nitric oxide synthase 2, and NO-induced cell death, in mouse cerebrovascular endothelium exposed to Niesseria meningitides. *J. Neurochem.,* 2002, **81**, 270-76.
doi: 10.1046/j.1471-4159.2002.00816.x

32. Vig, M.; Srivastava, S.; Kandpal, U.; Sade, A.; Lewis, V.; Sarin, A.; George, A.; Bal, V.; Durdik, J.M. & Rath S. Inducible nitric oxide synthase in T cell regulate T cell death and immune memory. *J. Clin. Invest.*, 2004, **113**, 1734-42.
doi: 10.1172/JCI20225

33. Brock, W.A.; Williams, M.; McNaney, D.; Milas, L.; Peters, L.J. & Weichselbaum, R.R. Modification by dexamethasone of radiation response of in vitro cultured cells. *Int. J. Radiat. Oncol. Biol. Phys.*, 1984, **10**, 2113-17.
doi: 10.1016/0360-3016(84)90210-4

34. Alok, A. & Chaudhury, N.K. 2016. Tetracycline hydrochloride: A potential clinical drug for radioprotection. *Chem. Biol. Interact.*, 2016, **245**, 90-9.
doi: 10.1016/j.cbi.2016.01.001

35. Harding, A. More compounds failing phase I. The Scientist, 2004, Sept 13: available at http://www.the-scientist.com/?articles.view/articleNo/23003/title/More-compounds-failing-Phase-I/ (Accessed on 12 March 2017).

36. Singh, V.K.; Newman, V.L.; Berg, A.N. & MacVittie, T.J. Animal models for acute radiation syndrome drug discovery. *Expert Opin. Drug Discov.*, 2015, **10**, 497-517.
doi: 10.1517/17460441.2015.1023290

37. Choi, S. Introduction to Systems Biology. Basic explanation of how to use computational methods to explain underlying biological meanings. Springer Publication, 2007.

CONFLICT OF INTEREST

The Authors declare no conflict of interest.

CONTRIBUTORS

Dr Yogesh Kumar Verma, completed his MSc and PhD in Biomedical Science from Delhi University. Presently working as Scientist 'D' in Institute of Nuclear Medicine and Allied Sciences, Delhi. He has 21 publications and 03 patents (filed) to his credit. He is presently working in the area of stem cell research, microencapsulation, tissue engineering and omics data mining and analysis for decreasing various types of injuries. Contribution in the current study: Conception, writing and data analysis of the manuscript.

Dr Gurudutta Gangenahalli, presently an Additional Director and Head of the Division of Stem Cell Research at Institute of Nuclear Medicine and Allied Sciences, Delhi. He worked in the area of therapeutic potential human stem cell fate response signaling, such as apoptosis, adherence, osteogenesis, differentiation, homing, by using genetic-engineering of human stem cell genes (of CD34, BCL-2, CXCR4, PU.1, SCFr/c-Kit, APC, OSx, Wnt etc) and by High-throughput gene-expression analysis. He is also working on developing the human stem cell shielding formulations and NMR stem cell tracking methods. Contribution in the current study: Research guidance and editing of the manuscript.