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REVIEW PAPER

Models for the Development of Radiation Countermeasures

Indracanti Prem Kumar, Damodar Gupta, Anant Narayan Bhatt, and B.S. Dwarakanath

Institute of Nuclear Medicine and Allied Sciences, Delhi-110 054
Email: prem@inmas.drdo.in; damodar.gupta@gmail.com; anbhatt@yahoo.com; bsd@inmas.drdo.in

ABSTRACT

Appropriate models are essential for making the transition from scientific discoveries to meaningful applications of the knowledge for human use. Acute as well as delayed effects of ionising radiation to the biological systems develop hierarchically starting from damage to the vital macromolecules up to the disturbances caused at the whole organism level. *In vitro* models like bacteria, yeast, various mammalian cells cultured as monolayers (2-D) and spheroids (3-D) as well as cells with specific genetic alterations have provided insight into the complex relationships between damage induction and various signal transduction pathways, allowing identification of molecular and sub-cellular targets vital to the fate of irradiated cells. On the other hand, *in vivo* models (multicellular whole organisms), ranging from simple worms to non-human primates, have been gainfully employed to evaluate efficacy as well as toxicity of potential countermeasure agents (molecules, combinations and formulated preparations) facilitating their deployment in human subjects. This review provides a brief account of the efforts with various *in vitro* and *in vivo* models for understanding the biological basis of radiation damage as well as the development of radiation countermeasures, viz., protectors, mitigators and therapeutics.

Keywords: Radiation countermeasures, ionising radiation, radiation damage, radiation injury

1. INTRODUCTION

Ionising radiation (IR) causes damage at various hierarchical levels of organisation in biological systems, leading to acute effects in terms of morbidity in a dose-dependent manner and delayed manifestations in the form of potentially fatal diseases affecting various organs. With increasing act of terrorism, potential accidents in nuclear installations and ever expanding use in medical applications, damage caused by radiation poses a serious public health problem. However, this problem remains largely impervious to the current practice of medical management. Therefore, there is a compelling need to develop safe and effective radiation countermeasure agents and strategies to prevent, mitigate or treat the harmful consequences of exposure to radiation.

Damage to biological systems by ionising radiation is caused primarily by the macromolecular lesions (particularly the genetic material, DNA) due both to direct interaction of radiation with the DNA as well as indirectly through reactive oxygen species (ROS) and reactive nitrogen species (RNS), amplified by cellular oxygen. At moderate doses, damage to hematopoietic and gastrointestinal systems mainly contributes to the acute effects due to mitotic and interphase deaths of constituent cells, largely related to the residual DNA damage remaining following the action of repair systems. On the other hand, some of the late effects, viz.,cancer can occur in many tissues without any threshold dose and is primarily related to the damage to the DNA[1]. Therefore, development of drugs and/or approaches that modify the induction of radiation damage and/or consequences of damage at any (or many) of the hierarchical levels of damage manifestation forms an important aspect of radiation countermeasure strategies.

Models are central to making the transition from scientific discoveries to applications in human population. One of the greatest challenges in biomedicine is the choice of appropriate preclinical (*in vitro* and *in vivo*) model systems that allow the most reliable and reproducible way of evaluating new agents and treatment strategies in clinical trials before induction in to the clinical practice. The degree of complexity of the model systems is closely related to their usefulness and relevance to the human system[2]. The importance of appropriate model systems in the understanding of biological responses to radiation damage, with particular reference to the development of countermeasure agents cannot be over emphasised. The models should facilitate the understanding of biological effects at different levels of radiation exposure; delineate the mechanisms of radiation injury at different hierarchical levels of biologic organisation, including the role of inflammation, neuro-immune interactions, endothelial injury, and other factors that influence the patho-physiological manifestations of radiation injury. The models should also effectively evaluate the efficacy of potential countermeasure agents, besides providing insight to the mechanisms of protection, mitigation, and treatment afforded by them. While simpler models (*viz., in vitro*)

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have practical advantage in terms of their ease of experimental adoptability and play a major role in the identification of molecular targets for designing interventional agents, relevant *in vivo* models are critical for the evaluation of potential agents and strategies at the systemic level relevant to the human applications. As it is ethically not permissible to carry out clinical trials to demonstrate efficacy of radiation countermeasure agents (unlike in case of clinical trials for particular diseases), regulatory agencies across the globe have seriously considered alternatives and suggested the use of 'Animal Efficacy Rule', where efficacy is demonstrated in higher animal models with comparable mechanisms underlying prevention of patho-physiological effects of radiation injury, thereby ensuring that the efficacy data from animal studies can be extrapolated to humans beings[3].

This review provides a brief account of the development and applications of various *in vitro* and *in vivo* model systems that are currently employed in the area of radiation countermeasures for design and evaluation of potential agents, besides understanding of their mechanisms of action.

2. *IN VITRO* MODELS

2.1 Molecular Model

Damage to biological systems by ionising radiation is caused primarily by the macromolecular lesions due both to direct interaction of radiation with macromolecules as well as indirectly through reactive oxygen species and reactive nitrogen species, amplified by cellular oxygen[1]. Radiation is known to mainly damage nucleic acid, protein, and lipid, and protection of these macromolecules is therefore considered a good countermeasure strategy to protect the cell, and ultimately the organism from the deleterious effects of ionising radiation. There are several classes of compounds, viz.,anti-oxidants, macromolecule binding ligands, etc., known for protecting the macromolecules either by quenching the ROS, which is predominant and applicable to all classes of macromolecules, or by binding to the macromolecule that brings structural change, mainly applicable in case of DNA. Therefore, purified macromolecules in isolation is a simple model system to study the extent of damage induction caused by radiation and its repair using isolated cellular extracts, without confounding effects of multiple concurrent signaling processes seen in cellular models. Isolated macromolecules are widely used in studying the qualitative and quantitative analyses of radiation-induced damage induction, *viz.,* plasmid relaxation assay for DNA, measurement of carbonyl content for proteins, and peroxidation assay for lipids are few examples.

2.2 Cellular Model

In vitro cell cultures are important experimental tools in biomedical research for understanding and analysing the mechanisms underlying various cell functions. These can more easily be manipulated, and hence, help in the systematic studies of multicellular systems. Therefore, these are widely used as important experimental models to evaluate treatment strategies of diseases wrt their efficiency and toxicity, before subjecting these to *in vivo* evaluations[2].

Effect of radiation in terms of damage induction and response in different lineage of tissues and cells in an organism are different due to their varying cellular architecture and lesion-repair proficiency. Moreover, many chemical modifiers, which protect macromolecules in an isolated system, *viz.,* DNA, show varying levels of toxicity in different cellular models[4], indicating the limitation of simpler molecular model and emphasising the need for cellular models for developing radiation countermeasure agents/strategies.

Understanding the mechanisms of radiation response at various levels of organisation of the biological system is essential to generate the knowledge for designing strategies of radiation countermeasure. Cellular systems with evolutionary hierarchy provide models with different degree of complexities. While bacterial systems, which are evolutionary primitive have simpler DNA organisation; mammalian cells with most advanced features have densely packed genetic material involving interactions of DNA with various types of proteins. This wide range of macromolecular organisation in the smallest unit of life (cells) offers excellent model systems to study the effects of radiation and evaluation of countermeasure strategies at the cellular level.

The most radio-resistant bacteria known, *Deinococcus radiodurans,* is a convenient model to study the effects of radiation in unicellular system. All members of this genus are radio-resistant microorganisms[5]. The extreme resistance of *D. radiodurans* to the DNA damaging factors is defined by repair mechanisms which fundamentally differ from those in other prokaryotes and mainly depends on its ability to increase the efficiency of a standard set of the DNA-repairing proteins[6]. However, recent studies suggest that *D. radiodurans* relies not on highly specialised DNA repair machinery, but on a detoxifying mechanism associated with the microbe's unusual intracellular environment, which is rich in manganese contrast to the radio-sensitive microorganisms having iron-rich intracellular milieu[7]. This model of radiation toxicity opens up novel avenues for developing radioprotection agents and strategies in diverse settings.

Microorganism cellular models are unicellular systems and unlike highly developed organisms, they do not have cell-to-cell interaction and communication during a stress condition. Therefore, they cannot mimic the *in situ* cellular milieu constraining the extrapolation of findings to the human applications. However, cells from insects, a more evolved organism, with comparable resistance to the radio-resistant bacteria but cellular organisation similar to the mammalian cells, serves as an excellent model with many attributes of a multicellular radio-resistant system, overcoming the limitations of bacterial model. Among other naturally existing radio-resistant system including yeast and bacteria, insects are evolutionarily closer to mammalian system. Various insect cell lines have been widely studied to understand the mechanisms underlying their high resistance to radiation-induced death, to identify cellular and molecular targets

for the developing radioprotective strategies. Studies with the lepidopteran insect cell line, Sf9 established from the ovarian tissue of fall armyworm, *Spodoptera frugiperda have* suggest that reduced DNA damage, enhanced DNA repair, lack of chromosome breakage and resistance against radiation-induced apoptosis due to reduced nitrosative stress contribute to *Lepidopteran* radio-resistance, besides other mechanisms[8,9].

Mammalian cell systems are relatively complex cellular models than previously described two cell systems but provides closest unit model. The efficacy of radiomodifiers has been widely assessed by evaluating their ability to enhance the degree of survival of irradiated cells using the macro-colony assay. Further, many mechanistic studies on the effects of radiation, extensively utilise various cells grown in culture. This approach has been particularly valuable for the identification of various biologically important forms of DNA damage and cellular responses to the damage, viz., repair and fixation of DNA lesions, cell cycle perturbations, cytogenetic damage, induction of signal transduction pathways, changes in gene expression patterns for the execution of survival and/or death at cellular level. It is pertinent to note that most of the *in vitro* assays are often target-directed, which rely on structurally- or functionally-related proteins obtained from natural or recombinant sources. Extrapolation of findings from *in vitro* models may often fail *in vivo* , either due to non-essentiality in the biological process or because *in vitro* screens do not encompass pharmacokinetic or pharmaco-dynamic properties, including metabolism, protein binding, or physico-chemical properties, such as membrane permeability.

2.3 Cellular Models for High Through-put Screening

Radiation-induced alterations in metabolic, physiological, and intracellular signalling network is regulated at transcriptional (gene expression) and post-translational levels, which collectively determines the survivability and functioning of the cell. Therefore, there has been considerable amount of interest in understanding the role of different cell signalling regulators in radiation response that can be targeted for developing countermeasure agents and/or strategies[10-13]. Since many signalling pathway and their regulators are involved in the cellular responses to radiation damage, systematically dissecting them to identify critical targets in different organs under different circumstances, requires investigations on a large number of cell systems with defined genetic alterations. Creation of isogenic cells with well-defined genetic alterations coupled with high through-put screening (HTS) has greatly facilitated the identification of certain critical molecular targets as well as the evaluation of potential countermeasure agents[14-16].

High through-put screening is a central function in the target identification and drug-discovery process and can be described as the phase from 'target to lead'. Aligning the target choice, assay method, and selection of model (*in vitro* or *in vivo*) in HTS is the most crucial step. Although the radiation-induced changes and their modifications by agent(s) can be analysed by various HTS assays, the cellular platform or background chosen to approach a particular target or radiation-induced ailment or modification of radiation-induced damage have enormous impact, both on the development and the implementation of a HTS for those target(s). The choices could be primary cell(s), immortalised cells and cell lines; both native and engineered[17,18]. The modal selection should be focused and based on the availability, behaviour and reproducibility of the signal(s).

Primary cells of human origin are the most physiologically relevant lead generation platform. Since obtaining primary cells for large scale HTS is difficult, the focus goes toward genetically immortalised cells or cell lines. Immortalised normal cells or transformed cell lines can be further engineered to down regulate or over-express a target of interest. These assays may involve expression of multiple proteins, or expression of reporter proteins, which could be transient or stable.

Employing either fluorescent or luminescent probes and allowing cell-based assays for most of the above mentioned targets, receptors, ion channels, and intracellular enzymes have been found to be very useful in screening of radiation countermeasures using living cells *in vitro.* Moreover, genetically-encoded probes offer the possibility of custom-engineered biological sensors for protein-protein interactions localisation of targets and measuring changes in intracellular signalling (*viz.,* changes in mitochondrial membrane potential, redox status, ATP/ADP ratio, activity of ion channels, detoxification process, etc)[19-22]. Development of HTS assay related to changes in cellular redox status (anti-oxidant, ROS and MMP) in living cells have also played promising role in identification of radiation countermeasures. Isolated lymphocytes, human embryonic kidney cells (HEK), HepG2, Daudi, Raji and many other cells have been used to screen radiation countermeasurers using HTS assay.

To delineate radiation-induced signaling events that activate PKB/AKT signaling and to identify novel compounds that impinge on this pathway, *HEK,* Jurkat T-cell line and many other cells genetically engineered with reporter systems (Luciferase, SEAP, Lac Z, Florescent protein, etc) have been extensively used[15, 23-25]. Similarly, the nuclear factor kB (NFkB) family of transcription factors play crucial roles in the control of many physiological and pathological processes, including host-defence, immune responses, inflammation, cancer, etc. The NFkB activation may occur by the degradation of inhibitor of NFkB (IkB), ligand-receptors interaction, DNA-damage pathway, oligomerisation of cytosolic proteins, radiation exposure, etc. The core event, upon which most of the NFkB activation pathways converge, is activation of inhibitor of kB kinases (IKKs), which allows translocation of Rel subunits into the nucleus where they initiate transcription of various target genes, required for host immunity, proliferation, survival and vital functions of the cells. Identification of molecules, probes, ligands that selectively activate NFkB may be unique research

tools and useful in the development of radiation countermeasures. Cells, co-transfected NFkB reporter have been shown for identification of radiation countermeasures. Burdelya[22], *et al.* have shown the use of NFB reporter system (luciferase, lacZ) in identification of toll like receptor (TLR) agonist as radioprotector[18], and Ahmad and Li have suggested the regulatory role of NFkB following radiation exposure[13]. Urinary bladder cells immortalised using cytomegalovirus or inserting hTERT and HEK cells have also been used in screening radiation countermeasures using NFkB reporter system. Similarly the signalling cascade mediated through G protein-coupled receptors (GPCRs) has also been used in screening of radiation countermeasure using HTS. The ability to conduct HTS based on functional activity of a given GPCR *in vitro* on human epidermoid carcinoma cells (A431) or HEK293-SEAP cell lines, also offers a more direct way of identifying lead agonists or antagonists including toxicity[23,26,27]. Activation of GPCRs can impact signalling cascades, ultimately leading to transcriptional modulation. Cell-based assays relying on transcriptionally-controlled reporter genes are well-suited to monitor the cellular response, induced by GPCRs. GPCRs are the largest known family of trans-membrane receptors that are used in drug-screening strategies. GPCRs are involved in adenylyl cyclase signalling, inositol 1,4,5-trisphosphate (IP3), diacylglycerol (DAG), and calcium ion homeostasis, which, in turn, can regulate protein kinase activities, ultimately leading to phosphorylation, and hence, activation of various transcription factors[23, 28-33].

Changes in gene expression are fundamental to many biological processes, including development, differentiation, and in some instances, progression to disease states. Thus, cells have mechanisms whereby the expression of each gene present in the genome is tightly regulated. Using screening technologies for the existing drugs as potential radioprotectors, interfering RNA (siRNA) library for targeting the genome was used on human glioblastoma T98G cells and over 116 genes were identified. 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. Morevoer Glyburide significantly increased the survival of 32D cl3 murine hematopoietic progenitor cells when administrated before irradiation.

2.4 Three-dimensional Cellular Models

It has long been recognised that the cell-based models used in experimental studies need to recapitulate both the 3-D organisation and multicellular complexity of the organs to translate findings from these studies into clinical applications. Three-dimensional cultures have been utilised in biomedical research since the first-half of the 20th century to gain deeper insight into the mechanisms of organogenesis and expression of malignancy. Using three-dimensional cell culture models, like the multicellular spheroids (MCS), cellular radiation responses can be more realistically examined under physiological conditions comparable to tissues *in vivo,* particularly, the nuclear structure and chromatin organisation, which profoundly influences the repair of DNA damage that critically determines acute (cell survival/ death) as well as late effects, viz., transformation and mutation. Moreover, the MCS can be conveniently employed in the HTS system with cell-based assays, facilitating the drug discovery and evaluation of newer therapies or countermeasure agents. Although utility of 3-D cultures for routine evaluation of drugs is currently speculative, spheroids as a secondary *in vitro* screening system in drug discovery has the potential to enhance predictability of clinical efficacy, and may minimize, if not replace, animal studies to a large extent in the near future[2].

The 2-D and 3-D cultures of hTERT immortalised human cells expressing well-defined loss and gain of function have been recently used to assess the risk of late radiation effects in the form of cancer[34].

Although, MCS offers a great deal of advantage over the monolayer cell cultures mimicking the *in vivo* conditions of the tissue to a very large extent, it does not eliminate the use of animal models as pharmacokinetics, pharmacodynamics, and bioavailability of drugs cannot be studied using spheroids. Further, the influence of immune system on the systemic response of the organism can only be studied using appropriate animal models[2].

3. *IN VIVO* (ANIMAL) MODELS

3.1 How Models Shaped the Success/Failures of Radiatioprotector Development-Historical Perspective

A variety of issues like ethical, religious, social considerations prevent experimental studies with human and therefore, majority of the knowledge regarding human biology is derived from studies carried out in animal models. While analogy is the key for employing appropriate animal models, extrapolations of the results generated strongly depend on the selection of a suitable animal model[35]. A great deal of our understanding on various biological effects of ionising radiation has been obtained from *in vitro* and other model systems. However, these model systems suffer from a major constraint of being devoid of the influence of intact organs, structure, and immune system[36].As a consequence the results obtained with *in vitro* studies often does not corroborate well with *in vivo* studies. Hence it is increasingly being realised that it is crucial to employ model systems that take into account the dynamics and integrity of complex biological networks that exist in humans being[14].

In scenarios, where whole body exposure is inevitable, the organs which are considered to be at high risk include the immune system, the hematopoietic system, gastrointestinal tract, kidney, skin, lung[37].

3.2 Immune System

Owing to the extreme radiosensitivity of peripheral lymphocytes, the immune system is extremely sensitive

and exhibits a rapid decline in their number on exposure to radiation. Loss of granulocyte count and prolonged neutropenia are also excellent markers for evaluating radioprotectors and mitigators, where the former is expected to primarily influence the rate of extent of loss, while the latter may modulate the recovery phase[14, 38]. Mouse strains (C57BL/6 and C3H/HeN) are widely used and are a good measure of the effectiveness of the agents. Owing to the complexity of the response of immune system to ionising radiation, evaluating in more than one murine strain is desirable. However as extrapolation of the effectiveness evaluated in non-human primates is expected to be more reliable, evaluating most promising agents in non-human primate is also required.

3.3 Hematopoietic System

Bone marrow failure and resultant potentially lethal hemorrhage or infections are the important manifestation of biological effects of radiation on hematopoieitc system, which can be linked to the high radio-sensitivity of the progenitor cells[39]. The time course of the bone marrow failure (neutropenia, thrombopenial, anemia, etc) is an excellent marker for evaluating potential countermeasures[40]. Radioprotectors can be expected to modulate the time course of appearance of syndromes and time to recover. The survival, repopulation and lineage formation can all be used as reliable markers for evaluating potential countermeasures. Various strains of mouse (BALB/c, C3H/HeN, B6D2F1/J and C57BL/6), canine (beagle) and non-human primates (*Macaca mulatta*), are among different animal species that have been widely used for evaluating the effects of radiation on hematopoietic system.

3.4 Gastrointestinal Syndrome

Next to the bone marrow, damage to gastrointestinal tract contributes maximally to the lethality and it can be attributed to the radiation-induced death of clonogenic crypt epithelial stem cells, resulting in subsequent loss of formation of enterocytes and vascular damage[41]. Long-term effects are the altered remodelling of the structure, motility and absorption in the gut and fibrosis that renders it more rigid and susceptible to adhesions, stenosis, and perforation[42]. Death due to damage to gastrointestinal (GI) tract and resultant systemic failure and histopathological evaluation of the number of regenerating crypt in murine model are some of the reliable endpoints for evaluating protective and mitigating agents. GI tract motility and permeability, bacterial translocation into the blood stream, and citrulline levels in plasma, and retrograde giant contraction which precedes vomiting and occurs on exposure to low doses are also sensitive end points[43, 44]. Both canine and non-human primates are sensitive models for evaluating drugs to radiation-induced GI tract damage as they respond excellently to radiation-induced vomiting and diarrhea. Murine models are sensitive for evaluating the effects of agents on eneterocyte depletion, perturbations in absorption, secretion of the bowel, and bacterial translocation.

3.4.1 Lung

The sensitivity of a lung to ionising radiation has been well understood from studies carried out following radiation accidents. Radiosensitivtiy of lung is complex, not well understood and the volume effect has been attributed to its regional radiosensitivity[45]. Subacute pneumonitis/alveolitis and late fibrosis are reliable markers for radiation-induced injury and for evaluating drugs for protection[14]. Apart from murine models, pig, dog and the non-human primates are widely used large animal models for studying and evaluating of modulators for effects on lung.

3.4.2 Kidney

Kidney is well understood for being the most radio-sensitive late responding organ and renal failure is often a part of multiple organ system failure. Systolic blood pressure[46], blood urea nitrogen (BUN; an excellent surrogate marker for renal injury), serum creatine and clearance, glomerular filtration rate (GFR)[47] and urine protein are some of important functional end points which have been used widely for evaluating agents for their efficacy. Rat (out-bred CD(SD), WAG/Rij) is more reliable model for radiation-induced kidney damage as mouse exhibit radio-resistance. Canines, pig and non-human primate are most widely studied large animals and usage of canines has advantage as renal physiology has been well documented in literature.

3.4.3 Skin

Unplanned exposures often lead to skin injury and cutaneous syndrome often manifests parallel with systemic injury and determines the extent of multi-organ syndrome. Erythema, pigmentation, dry and moist desquamation, fibrosis and necrosis form battery of end points which, indicate the extent of damage to skin and for evaluation of agents for protection and mitigation[48]. Scar formation (wound contracture), limb shortening, motion and strength are some of the markers for evaluating late effects of ionising radiation on skin. The similarity in onset of patho-physiological manifestation on skin and availability of several genetic variants mouse has been most widely used. Among large animal models porcine has been widely used owing to the similar architecture (sparser hair and the structure of the cutaneous and subcutaneous tissues) and physiology to humans[49].

3.5 Model Organisms for Futuristic Development of Countermeasure Agents

Several decades of research in the field of radiation countermeasures has yielded very few radioprotectors with acceptable side effects. It is now being realised that radiation-induced biological damage is complex and depends upon multiple factors[36]. As the understanding of etiology of radiation-mediated syndromes is widened, novel target are expected to take center stage for evaluation of effective radioprotectors. Several promising approaches are under active investigation, which include unraveling of extreme

radio-resistance of model organisms, chemical genetics, drug repurposing, stem cell biology etc. Since radiation-induced lethality is multidirectional and complex, it offers several windows and targets for intervention, ranging from free-radical scavenging, intra- and intercellular signaling/ communication, bio-molecular (particularly DNA) damage and repair, tissue dysfunction, organs damage, system failure, etc[37]. Ongoing efforts for development of countermeasures require novel animal models along with appropriate techniques depending on the intended benefit. There are several organisms which offer excellent avenues to evaluate both sub-cellular, cellular, tissues, organ damage with greater reliability in comparison to *in vitro* models. These models can function as initial screening workhorses for identifying effective hits, which can be further evaluated in more acceptable animal models like murine, canine, porcine and non-human primate to meet regulatory requirements.

3.5.1 Yeast-Model for Identifying Inhibitors of Signal Transduction Pathways

A great extent of our understanding of mechanisms of basic cellular functions like cell cycle control, membrane trafficking, and signal transduction have been obtained from the simple eukaryote, *Schizosaccharomyces pombe.* Having the smallest genome size among eukaryotes and with its powerful genetics, this organism is also an excellent model system for drug discovery as it is powerfully suited for HTS. The similarities in DNA damage, repair and many other signaling molecules with humans further adds to its advantage as radio-biological model for primary screening of agents for radioprotection[50].

3.5.2 Sponges-Model of Cellular Recognition

Recent studies have clearly suggested that cell-cell interaction and intercellular and tissue communication has an important role in biological manifestation of radiation exposure and have also provided a number of targets for evaluating agents for radiation protection[50]. While intercellular communication studies have been widely carried out in cell lines, structurally more complex organisms are expected to provide reliable results. Sponges, the simplest metazoans, have been traditionally used as models to study cell adhesion, since their abundant extracellular matrix allows mild cell dissociation and the recovery of functionally active macromolecular structures[51]. The specialised cells in sponges can return to an undifferentiated state, which offers valuable system for understanding basis for attaining undifferentiated state (stem cells), and is therefore very useful in the development of radiation countermeasures.

3.5.3 Sea Urchin-Model System for Analysing Cellular Activities during Early Development

Sea urchin embryos, a time tested experimental model, is an important model for understanding the molecular mechanisms of radiation effects for novel targets to develop countermeasure agents. Availability of large amount of homogeneous material, ease of gamete handling, transparency and synchrony of fertilized eggs facilitate investigations on signal transduction and the analysis of morphogenetic movements[52].

3.5.4 Caenorhabditis Elegans-Model for Identifying Novel Therapeutic Targets

The free living nematode, *Caenorhabditis elegans (C. elegans)*, offers a number of distinct advantages like anatomical simplicity, small size, large brood size, short generation time, ease of maintenance, transparence, opportunity to visualise cell migration, apoptosis in the living animal[53]. The ease to develop mutants (more than 8000 genetic strains) and carryout forward genetics has made *C. elegans* a model of choice for medium-to-high throughput screening of potential therapeutic agents using a variety of end points[54]. *C. elegans* is also a powerful model to study complex radio-biological responses like DNA damage processing, induction of cell cycle check points, and apoptosis as these biological functions are evolutionarily conserved[55]. The Abl mutants, which are radio-sensitive to ionising radiation, offer a reliable whole organism to screen compounds[56].

3.5.5 Honey Bee-Model for Behavioural Genomics

Post traumatic stress disorder (PTSD) is an important long term effect in case of survivours of radiation accidents. Recent studies revealed that traumatic stress experienced by mice early in life has epigenetic repercussions that reverberate across multiple generations. There is a great deal of efforts in trying to develop effective drugs to reduce these inherited behavioural alterations, testing gene-regulating drugs on mouse models to identify the relevant genes with altered methylation. Apart from mice, honey bee is an important model system that provides insight into many areas of biomedical science like phenotypic plasticity, development and aging, circadian rhythms, muscle metabolism and behavioural genomics[57].

3.5.6 Zebrafish-Model for Development and Small Molecule Screening

Zebrafish (*Danio rerio)* are small freshwater teleosts that have emerged as powerful models for developmental biology studies, bridging the genetic power of invertebrates and the relevance of rodents. Inexpensive housing, maintenance, quicker sexual maturity, high fecundity, external fertilization, transparent embryos and accessibility of internal organs for imaging, medium throughput ability are some of the key features which have valuable implications in screening of large number of agents for development of radiation countermeasures[58, 59]. The developing embryos offer an excellent source for fast evaluation of agents for radioprotective action and also for their ability to modulate radiation-induced developmental abnormalities[37, 58]. The amenability to forward genetics makes it a valuable model for identifying gene targets for a given phenotype in a totally unbiased manner. Another important contribution of zebrafish is its feasibility to carry out chemical genetics for identification of signaling molecules important for radio-

biological consequences through use of small molecule libraries[58]. The ability to study hematopoiesis and amenability to end points like *in situ* hybridisation, visual inspection of stem cell markers, it is expected to function as a valuable vertebrate model for screening large libraries of small molecules and repurposing of clinical compounds for radioprotective action[60].

3.5.7 Guinea Pigs-Model for Cholesterol and Lipoprotein Metabolism

One of the late effects of radiation over exposure is perturbations in cholesterol and lipoprotein metabolism which can lead to cardiovascular complications in survivors of radiation exposure. Lipoprotein anabolism and catabolism are highly complex when considering the multifaceted aspects that regulate the synthesis and removal of lipoproteins from plasma[61]. Gunie pig is an important pre-clinical model for lipoprotein anabolism as majority reverse-cholesterol transport and delipidation cascades are similar to the human being[61]. It offers an excellent resource for validating agents for protection against radiation of the tissue and organ damage, leading to complication in lipid metabolism.

4. NOVEL ANIMAL MODELS FOR RADIO-BIOLOGICAL STUDIES

4.1 Rat Knockout and Mutant Models

Rat is a better predictable model for some of the important aspects of radio-biological consequences and the lack of knockout and mutant rat models due to failure of genetic manipulation lead to use less valuable mouse knockouts for studying human diseases. However, recent development in rat genetic models have promised renewed interest in using knockout rat models for developments of therapeutics for human diseases. Recently SAGE laboratories (*www.sageresearchmodels.com*) using CompoZr™ targeted genomic editing technology, created the world's first targeted knockout rats and presently a number of knockout rat models for a number of targets of radio-biological relevance (p53, prkdc, Foxn1, Rag1, Rag2 etc) which can be employed to evaluate agents and unravel targets for radiation countermeasures.

4.2 Transgenic Mouse Models

Murine models have been widely used in studying radio-biological consequences and validating agents for radioprotection. Most of the end points that have been evaluated require culmination of experiment and sacrifice of the animals hence the resulting data represents a snap shot of the physiological events. Understanding the radiation effects *in vivo* and in real time has great implications in pre-clinical evaluation and development of radioprotectors. Reporter mouse models expressing fluorescent or luminescent reporter gene downstream of promoter of gene of radio-biological importance offers an excellent resource to understand changes in radiation-induced gene expression in real time and in a tissue-specific manner[62]. A number of reporter mouse models of radio-biological relevance (inflammation-GADD45α, Cox2 or Ptgs2, NFB -RE, TNFα; Angiogenesis-VEGFr2, VEGFr2-KI, VEGF, E1-luc/E1-Tag; Drug metabolism and toxicity- Cyp3A4, Cyp3a11, SOD1, GADD45α, γGcs-h or Gclc, GADD153 etc) are commercially available and form an important workhorses for validating acute as well as late effects of ionising radiation exposure.

4.3 Humanised Mouse Models

In spite of critical role played by murine in our understanding of human biology, the results obtained often cannot be extrapolated to human beings[14]. In view of the inability to study radio-biology and efficacy studies *in vivo* in humans, a more acceptable alternate is carrying out such studies in humanized mice. Transplantation of human cells or tissues and transgenic expression of human molecules fall under the umbrella of the generic term 'humanized' mice[63]. However, humanized mice of radio-biological relevance include engraftment of immuno-deficient mice with human hematopoietic stem cells or human peripheral blood mononuclear cells permits investigation of the development and function of a human immune system *in vivo*[63]. Humanized mice have been widely used in acquired immunodeficiency syndrome research and it continues to provide important insight into the human immunodeficiency virus infection. However its use in radiation biology and development of radiation countermeasures is in its infancy and it offers valuable source for studying radio-biology and for evaluating agents for radiation-induced systemic syndromes. A number of mice with organs of human origin can easily be generated and used for studying effects of radiation on different organs (human liver, hematopoietic system, etc).

5. DIRECTIONS TO CARRY OUT EFFICACY STUDIES IN HUMANS

1. One of the confounding factors in applicability of an agent as radioprotector is lack of efficacy data in humans. As per the drug regulatory authorities, exposing volunteers to ionising radiation to test the efficacy of an agent is unethical. However it is now increasingly being realised that variation occurs at individual level and optimal utilisation of a therapeutic agents in human beings depends upon complex interaction of the genotype. In view of the paradigm shift in preventive and supportive care towards individualisation, it is very unlikely that the efficacy data obtained from animal models will completely address the safety issues. It is also being realised that at some point of time, human efficacy data may not be totally avoidable. Although evaluation of efficacy in human beings may not be feasible in the classical mode of trials, carefully designed small-scale studies, exploiting some of the following scenarios may be considered keeping in view the enormous gain that will accrue when evaluation is successfully completed: Responders carrying out rescue operations can also function as subjects for evaluation of prophylactics, mitigators and therapeutics. However such studies suffer from uncertainty and ability to

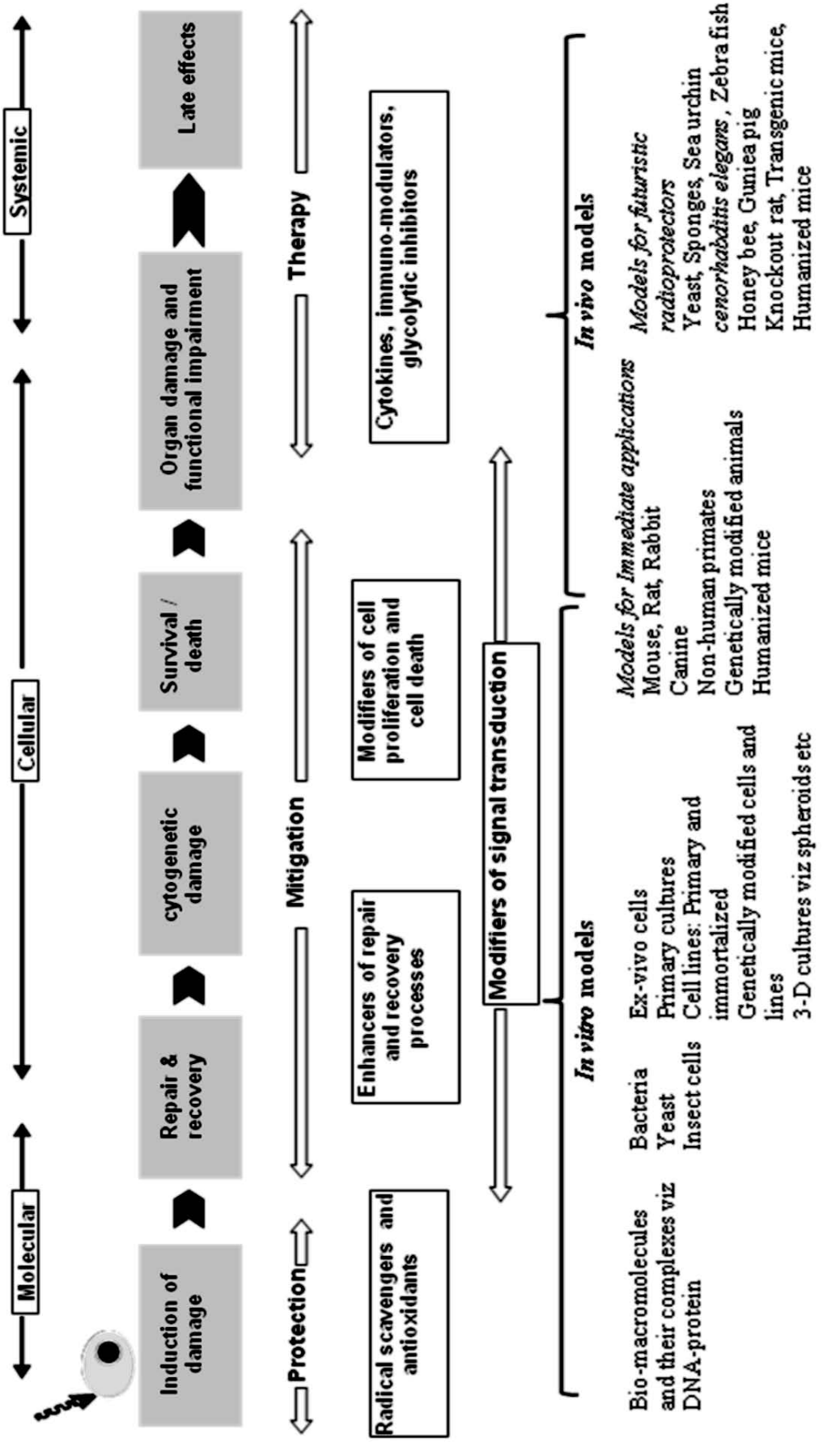

Figure 1. Models for studying biological responses to radiation damage and development of countermeasure agents and strategies.

prepare for such studies.

2. Studies with partial body exposures and organ damage may not be impractical when coupled with effective alternative care (organ transplantation, xenografting, and effective therapeutics).
3. The victims of radiation overexposure with informed consent can also be used as subjects for evaluation of mitigators, therapeutic, and supportive care.

6. CONCLUSIONS

Biological effects of ionising radiation develop hierarchically, starting from damage to the vital macromolecules in cells to ultimately culminating in disturbances to whole organism in the form of morbidity or late development of diseases. *In vitro* models, ranging from bacteria to various mammalian cells cultured as 2-D and 3-D systems including defined genetic manipulations, have been very helpful in identifying cellular targets vital to the fate of irradiated cells (Fig. 1). On the other hand, *in vivo* models ranging from simple worms to non-human primates have been gainfully employed to evaluate efficacy as well as toxicity of potential countermeasure agents (Fig. 1). Recent developments of novel animal models with genetic manipulation is expected to facilitate studies on understanding of complex biological processes underlying radiation response in whole organism, while the humanized mouse model is expected to accelerate the development of countermeasure agents for human use.

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Contributors

Dr Indracanti Prem Kumar obtained his PhD from Chaudhary Charan Singh University, Meerut, in 2003. Presently, he is working as scientist 'D' in the Department of Radiation Biosciences, Institute of Nuclear Medicine and Allied Sciences (INMAS), Delhi and his areas of research include: Drug repurposing, zebrafish biology, small animal non-invasive imaging, and radioprotection.

Dr Damodar Gupta obtained his PhD in Microbiology from Choudhary Charan Singh University, and is presently working as Scientist 'D' at INMAS, Delhi. His area of interest is development of *in vitro* models for identification of radiation countermeasures, and radiation biology.

Dr Anant Narayan Bhatt obtained his PhD in Biochemistry from Central Drug Research Institute, Lucknow, and joined Division of Radiation Biosciences, at INMAS as Scientist. He is presently working on metabolic cell signaling approaches for radioprotection and individualisation of therapy.

Dr B.S. Dwarakanath obtained his PhD in Biophysics from the Bangalore University, Bengaluru. He served at National Institute of Mental Health and Neurosciences before joining INMAS, where he is currently the Additional Director and Head, Division of Radiation Biosciences. His areas of interest include: Glucose metabolism and cell signaling, radioprotection, and radio-sensitisation.