RADIOBIOLOGY RESEARCH WITH FOCUS TO HUMAN HEALTH AND CANCER RADIOTHERAPY

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Abstract

This Chapter gives a brief account of research contributions of 'Radiobiology Research Group' at Bhabha Atomic Research Centre (BARC) since early years of Indian atomic energy program. The major focus of our research consists in understanding the mechanisms of ionizing radiation damage at various levels of biological organizations such as cellular, molecular, membrane, and at tissue levels with relevance to radiation-mediated cell death for cancer radiotherapy and protection of normal cells/tissues from radiation damage. Research contributions have made significant contributions in advancing knowledge on the formation, characterization and involvement of primary free radicals from water radiolysis, reactive oxygen species, and their reactions with DNA and components of plasma membrane in triggering the cascade of structural and signalling mechanisms for determining the radio responses of normal and tumour cells. These results are published in reputed journals and mechanisms of radiation oxidative damage, role of apoptotic sensitivity,

involvements of ROS in radiation damage in cells enabled us to predict the tumour radiosensitivity. In our efforts to selectively kill tumour cells without affecting normal cells, we developed liposome-based nanosized vesicles to envelope anticancer drugs into surface engineered formulation and demonstrated targeting and enhanced toxicity of loaded cargo by many folds to the experimental tumour and succeeded in overcoming radioresistant tumors. To further improve upon therapeutic outcome and overcome radioresistance, we experimented electro-radio-chemotherapy technology for more efficient drug delivery to intended target with considerable success in *in vitro* and in animal tumour models. Taking note of emerging new knowledge in radiobiology, we turned to study the mechanism of 'Non-targeted Effects' of low (gamma, X-rays, electron) and high-LET radiations (viz. alpha particles and proton) such as bystander effect, abscopal effect, adaptive responses, radiation hormesis and low dose radiation effects in cell and animal models, which have received wide recognition among peers. These lines of research are actively continuing and newer research is initiated to stay ahead in the field. To aid and support DAE's three-stage nuclear power program, 'Nuclear Radiobiology Research' was initiated and developed to evaluate radiological and chemical toxic effects of Thorium and Uranium in cells and animal models and develop technologies/approaches for decontamination of internalized radionuclides. In summary, research of our Group has made sustained progress in basic radiobiology research and applications earning the credit of some patents. It firmly foreseen that active low dose radiobiology research may open new vistas for advancing knowledge and staying competitive among professional groups and exploring newer health applications for better serving the society.

1. Preamble

The atomic energy program of our country has been founded on the premise of peaceful applications including electricity generation by nuclear method and health improvement programs such as cancer treatment. The program was started under the Chairmanship of Dr Homi J. Bhabha, a famous nuclear physicist, with the formation of Atomic Energy Commission (AEC) by the Government of India in January 1953. It is pertinent to recall that revolutionary discoveries of X-ray by W. R. Rontgen of Germany in 1895 and radioactivity by Henry Becquerel of France in 1896 had generated enormous excitements often claiming that new discoveries may prove panacea for all existing problems in the world such as shortage of electricity for industrialization, daunting problem of hunger and poverty and treatment of diseases to save life. The promises made by scientists and policy makers seemed sound and achievable but a few adverse health effects came to notice demanding urgent attention. Within a few years of the new discoveries, the discoverers and researchers encountered some ill effects of atomic radiations on their health e.g., skin burns, rashness, inflammation and blisters raising alarms on the harmful

effects of radiation necessitating the urge to understand the biological effects of radiation giving birth to a new field of research called radiation biology. Over the years, intensive research on mechanisms of radiation action on living and non-living matters revealed that X-rays and the radiations emitted from radioactive substances were either charged particles such as alpha, beta particles or electromagnetic waves such as gamma rays. Both these types of radiations produce ionizations and excitations in the medium through which they travel and therefore they were called ionizing radiations. In later years, studies on biological effects of radiation became a front-line research project in most of the advanced institutes and atomic energy establishments. In early 20th century, it became known mainly from the results on fruit flies reported by US and German Scientists that atomic radiations were capable to cause mutations in the living organisms. Further, during WWII, dropping of atomic bomb on Hiroshima and Nagasaki in 1945 caused enormous loss of human life and destruction of property created enormous horror and negative image in the mind of public. In the following years, there was unusual push to intensify and accelerate radiobiological research to assess radiation effects on health and environment. In this backdrop of observed harmful effects of atomic radiation, especially induction of cancer in the bomb exposed Japanese population, radiobiology research found re-focus by the scientists and policy makers of atomic energy to ensure safety standards and protection of health of researchers, nuclear workers as well as general public. Indian atomic energy program was no exception and Dr. Bhabha invited Indian scientists with expertise and experience from advanced countries to build laboratories for studies on biological effects of radiation on microbial, mammalian and plant systems at the laboratories in Bombay then called Atomic Energy Establishment (AEE), Trombay. The scientists were apt and quick to foresee the urgency and need for radiation biology research in nuclear program for power production. Dr. Bhabha foresaw the need for building research and development laboratories in physics, chemistry and biology at AEE. Dr. A. R. Gopal-Iyengar who obtained his Ph.D. Degree from University of Toronto, Canada and was the Chief Cytologist at Tata Memorial Hospital, Parel, Bombay, was invited to head the then Biology Division at AEE. Radiobiology program was aimed to investigate the basic mechanisms of radiation effects and emerging applications. The major focus of radiobiology program was to understand the mechanism of ionizing radiation-induced damage to biomolecules, model membrane systems, cells and animal tissues with relevance to radiation risk assessment and improvement of cancer radiotherapy. Under the dynamic leadership of Dr. Iyengar close to 2 decades, the radiation research programs at Biology Division progressed continuously and made significant contributions matching with the advances made from western countries and in alignment with the guidance of International Atomic Energy Agency (IAEA), Vienna. The radiobiology research and technological developments at this Centre received appreciation and recognition from international scientific community. Professional expertise and laboratory facilities have grown over the years and research based applications have culminated into identified technology groups dedicated to serve the nation and benefit to society, namely, radiation induced mutant development in agriculture (now Nuclear Agriculture & Biotechnology Division), food sterilization by

radiation for avoiding spoilage and prolongation of shelf life (Food Technology Div.), radiation insect sterilization for insect control, soil chemical analysis for detection and quantification of radionuclide in soils, biological effects of radiation on living systems to advance knowledge on radiation damage mechanisms and their modifications (radiosensitization and radioprotection) applied to cancer radiotherapy and radioprotection including free radical mediated damage to cellular biomolecules e.g. DNA, lipids and others (Radiation Biology & Health Sciences Div.) and radioisotopes applications in nuclear medicine for diagnosis and therapy (Radiation Medicine Centre) of patients. It was in 1970s that deep interest grew to understand the health effects on population especially for cancer risk assessment and other ailments to people residing in high background radiation areas (HBRA) in Kerala and a research laboratory was established in Kollam. Dr. Gopal Iyengar directed the biology and radiobiology research program for almost two decades until his superannuation in 1970. More recently, the radiobiology research program has been further expanded and diversified in frontline research areas such as high LET radiation effects on cellular systems, non-targeted radiation damage, evaluations of effects of radionuclides after internal contamination with actinides (Thorium, Uranium, etc.) and tumor-targeted nano formulations. Some of the current radiobiology research programs such as dose-response assessment in low dose ranges, thorium research and technology are aimed to advance knowledge and develop expertise matching with the progress and developments in advanced laboratories of the world.

2. Biophysical, Molecular Radiobiology and Free Radical Research

In the early 20th century, it was widely known that nuclear radiations and X-rays cause ionizations and excitations in the molecules of the medium in which they travel. Further, extensive research suggested that ionizing radiations produce free radicals either after directly interacting with the cellular molecules such as DNA, proteins, lipids or cellular molecules or they can cause indirect damage by depositing the energy in water leading to formation of short lived radicals namely, $^{\cdot}OH$, e^{-}, O_2^{-}, H atoms (called primary radicals produced after water radiolysis). The central concept of radiobiology was evolving with the fact that IR-induced free radical-mediated events were primarily responsible for radiation injury. In 1940s, it was mostly agreed that the free radicals formed following radiation action were either chemically scavenged (or repaired) by free radical scavengers (such as GSH or cysteine) to be restored to their original structure (RH or H_2O), or if O_2 was present, primary as well as secondary free radicals formed of critical biomolecules could react to form peroxyl radicals ($ROO^{\cdot}$), which was thought to fix the damage and enhance radiobiological damage. This line of argument helped to explain the dramatic effect of O_2 on the observed enhancement of IR-induced tissue injury that was universally noted and commonly called "Oxygen Effect" in radiobiology. Noting the developing scientific discussions on the roles of IR induced free radicals in biological damage, the then Director, Biological Research Group, Dr. Iyengar, was prompt to build a team of researchers in 1960s to investigate radiation generated formation of free

radicals and their reactions with biological macromolecules such as DNA. The Research Group was named Physical Radiobiology Section within Biology Division of AEE. Dr. Bam Bahadur Singh, a physicist from 3rd batch of Training School was made the Head of this research program. He built a very active research team consisting of biophysicists, chemists and biologists, who were trained for research in nuclear science and technology. This team was dedicated to detect, identify and characterize the radiation induced free radicals and understand the molecular mechanisms in radiobiological damage to cells and tissues (**Fig. 1**). Molecular radiobiology was considered frontline and hot subject the world over and this research group earned the reputation of leader in India. The research publications on identification and characterization of free radical mediated damage to DNA, adenine, thymine, guanine, cytosine in aqueous alkaline-frozen systems following gamma irradiation using electron spin resonance (ESR) technique drew due attention of international scientific researchers. The group grew from strength to strength and made seminal contributions on understanding the mechanisms of ionizing radiation produced electrons and hydroxyl radical reactions with DNA and its constituents. This research program further evolved and explained the chemical mechanisms of radiosensitizers and radioprotectors. Taking note of active research and publications, this research team was invited by IAEA for expert consultations and participation in coordinated research programs (CRP) in chosen topics for many years. The early research and subsequent sustained progress in radiation biophysics laid firm foundation for the detailed understanding of radiation generated free radical reactions with cellular biomolecules, molecular mechanisms of radiation damage in biological systems with relevance to cellular injury and modification of radiation damage with special emphasis on relevance to radiotherapy and radioprotection.

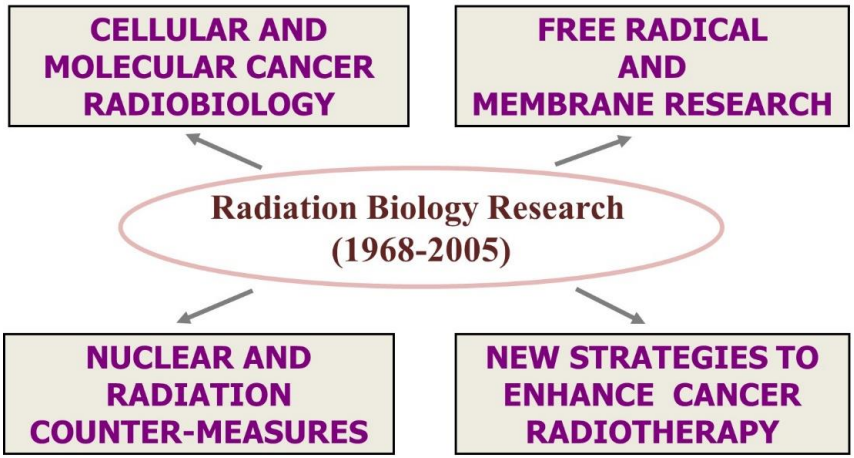

Fig. 1: Early radiation biology research during 1970s-2005

Extensive radiobiology research in 1920s showed that X-rays induced mutations in fruit flies (Drosophila). Prof. H. J. Muller (1927) provided the first evidence of genetic effects of radiation. In following years, enormous interest was generated in radiobiological

research concerning paradoxical findings that radiation caused diseases mainly cancer (carcinogenesis) as well as it can be used to kill cancer cells (cancer radiotherapy) opening up many new directions of research in radiobiology with regard to radiation safety and risk assessment. Studies on basic mechanisms in radiobiological research on bacterial and mammalian cells with relevance to improvement of cancer radiotherapy, search for drugs and molecules which could be effective radiosensitizers, developing new strategies for overcoming radioresistance of tumor have been the main thrust of our group in the years that followed. After superannuation of Dr. B. B. Singh in 1998, Dr. (Mrs) A. M. Samual, a reputed Nuclear Medicine physician and Head, RMC, took over as Director, Biomedical Group, BARC who galvanised radiobiology research activities in terms of human resources, equipment and laboratory modernization and upgradation. It is satisfying to note that our continued original research publications have been widely cited by peer research groups and our research Group at BARC is considered lead radiobiology research group of India by the global scientific community. It may be noted that after the death of Dr. Bhabha, AEET has been named Bhabha Atomic Research Centre as a mark of honour to him for his leadership role in Indian Atomic Energy Program.

By 2000s, our group diversified and expanded the research programs in relevant and frontline areas of radiobiology in sync with the contemporary research in other countries. Research programs were redesigned and refocused to accept new challenges in advancing the knowledge on assessment of molecular mechanisms of radiation induced oxidative stress in normal cells, tumour cells and tissues involving free radicals and reactive oxygen species (ROS) as mediators of radiation damage by employing fluorescent probes and electron spin resonance spin labelling techniques to address fundamental questions in emerging radiobiology using cells in culture, tissues and animal models. Apart from studies on DNA as a target of radiation effects, our research team began focusing attention to exploring the mechanisms and consequences of membrane oxidative damage following irradiation of normal and tumour cells *in vitro*. To understand the role and relevance of membrane damage in the radiation mediated cell death following gamma radiation, research program was undertaken using phospholipid vesicles (liposomes) as model membrane system to investigate the effect of ionizing radiation on biophysical properties of membrane such as membrane fluidity, permeability, lipid damage, etc. Gamma radiation-induced changes in the liposomal membrane permeability were monitored by measuring the leakage of pre-encapsulated 6-carboxyfluorescein fluorescent probe, and alterations in lipid bilayer fluidity were determined by 1,6-diphenyl-1,3,5-hexatriene fluorescence polarization. The changes in permeability and fluidity in the bilayer were found to be dependent on the radiation dose in a biphasic manner. These results were interpreted in terms of lipid bilayer fluidization after exposure to doses up to 1 kGy, however, rigidization was observed in the lipid bilayer at higher doses probably due to cross reactions of lipid radicals in the bilayer model. Notably, it was for the first time that our group reported these findings in radiation research field establishing a relationship between alterations in membrane permeability and fluidity after irradiation. Radiation-induced changes in the permeability of the

liposomes after exposure to gamma radiation and their modification by antioxidants postulated the role of free radical mechanism in the membrane damage, which offered new insights for the modification of cellular radiosensitivity for radioprotection of normal cells and cancer radiotherapy. In view of the seminal research findings and its relevance in radiobiology research, scientists from Germany came forward to develop Indo-German Collaborative research in this field with Prof H. Diehl from Germany.

During 1990s, it was considered a challenging task to search for new compounds and probable drugs, which could sensitize tumour cells to ionizing radiation and develop strategies to overcome radio resistance of tumour cells because many drugs under investigation as radiosensitizers had failed in clinical trials. Our Research Team took up the challenge and started exploring the possibility of antioxidants (AO) as radiosensitizers and use of pulsed electric field to overcome radioresistance of tumour cells by permeabilization of plasma membrane of these cells. During 1980s, electroporation, a membrane-associated biophysical technique was used. Electroporation is a membrane-associated bio-physical technique, which involves transient increase in the permeability of the plasma membrane by the application of external electric field of high voltage and of short duration. Electroporation causes non-selective plasma membrane permeabilization due to structural modification of the membrane (formation of micropores), which allows entry of impermeable molecules into the cell interior and offers a controlled method for incorporation of drugs/antibodies (**Fig. 2**). This technique was found to be highly useful in the area of cancer therapy, where the main challenge was to overcome resistance of tumor cells toward uptake of anticancer drugs and/or radiation. Combination of electroporation with anticancer drugs led to the emergence of a new field called ***electro-chemotherapy***.

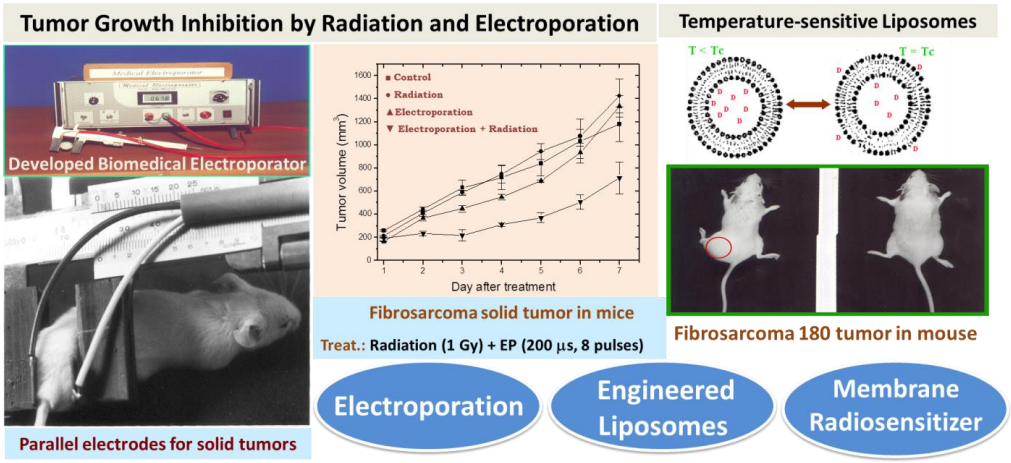

Fig. 2: A synergy was discovered between radiation action and electroporation with the objective to enhance anticancer drug delivery and cancer radiotherapy. Right panel shows the application of temperature sensitive liposomes for drug delivery in fibrosarcoma tumor.

Electroporator Technology Transferred

A biphasic electroporator (2005) was designed and developed and its technology was transferred to M/s D. S. Electronics, Mumbai. Using this technology, a synergy between electro-chemotherapy and radiation was explored for cancer treatment. Radioiodine is a highly effective therapy applied at RMC for treatment of thyroid cancer owing to the presence of membrane transporter for iodine in thyroid cancer cells. However, in clinic it was observed that a subset of thyroid patients was refractory to radioiodine uptake. Therefore, during 2000-2003, we have explored the application of electroporation to enhance the radioiodine uptake in thyroid cancer cells. It was one of the highly cited research contributions from our laboratory. Further, the synergistic cytotoxic effects of anticancer drug (doxorubicin) and γ-radiation in combination with electroporation was successfully demonstrated in the fibrosarcoma tumor model of Swiss mice. Electroporation being a tumor-targeted modality was found to be significantly useful for reducing the dose of chemotherapeutic drugs and radiation for treatment of solid cancer in patients.

3. Radiation Damage to Membranes and Cells

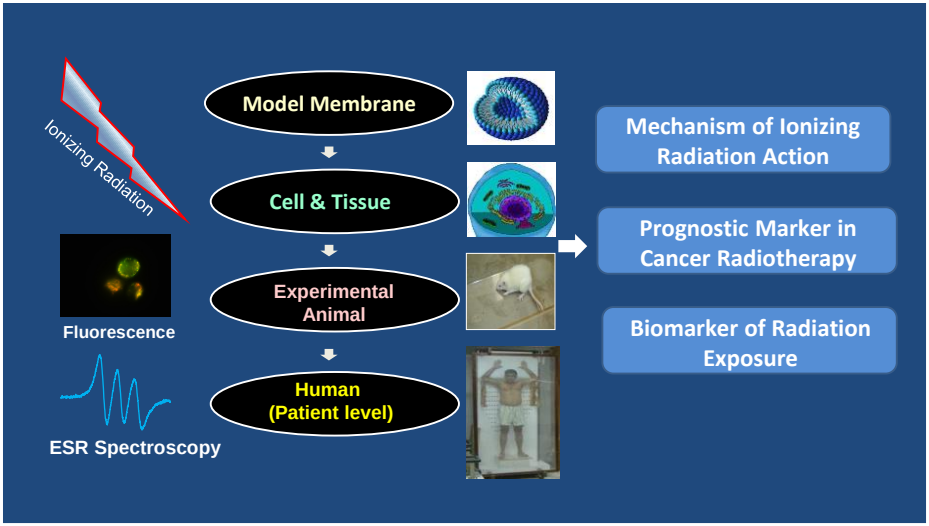

Fig. 3: Ionizing radiation triggers a cascade of free radical-mediated reactions leading to membrane oxidative damage, which was investigated in various membrane systems (liposomes, cells, tissues, tumor and cancer patient's samples) with implications to (i) understand the mechanism of radiation action and (ii) membrane damage as a predictive/prognostic marker of cancer radiotherapy and biomarker of radiation exposure.

Radio-oxidative damage to the plasma membrane of cells and its consequences in the mechanism of cell death has been investigated, which received significant attention of radiation scientists during 2000-2007 (**Fig. 3**). Various fluorescence probes (cis-parinaric acid, DPH, DCH-FDA, etc.) were employed to determine changes in the permeability

and fluidity of the plasma membrane, intracellular level of reactive oxygen species and lipid peroxidation in mouse thymocytes following gamma-irradiation within a low to moderate radiation dose range (a few cGy to 10 Gy). These studies have shown a correlation between radiation-induced membrane changes, ROS generation and apoptotic cell death in moderate dose range. These observations highlighted the distinct mechanisms of low dose radiation effects at cellular and membrane levels.

Further research was taken up to modulate radiation-induced membrane oxidative damage, generation of ROS, and apoptosis by natural antioxidants. The inhibition of apoptosis by membrane-localized antioxidants such as eugenol, isoeugenol, and alpha-tocopherol was more effective than the cytosolic antioxidant such as ascorbic acid. It was inferred that damage to membrane played a significant role in radiation-induced apoptotic death, which was markedly modified by membrane-localized antioxidants. Thus, it has been demonstrated that membrane oxidative damage was initiated by radiation-induced ROS, which could be modified by structure modulating factors such as cholesterol or membrane-specific antioxidants viz. tocopherol. Post-irradiation permeability changes in thymocyte membrane suggested propagation of initial events with the passage of time. Results have shown that fluorescence probes give a good account of chemical and structural alterations in membranes. Furthermore, it was suggested that measurement of membrane injury may provide an indicator of radiation exposure. These studies provided the evidence that membrane damage may initiate or contribute to processes leading to induction of cell death. Further extension of these studies was found to have considerable implications in basic radiobiology and in clinical cancer radiotherapy.

4. Classical Radiobiology Research: Mechanisms and Perspectives

Gamma radiation-induced tumor induction in thymus and its suppression by pre-exposure to low dose irradiation (10-30 cGy) has been investigated in Swiss albino mice. These studies showed that a single dose of whole-body γ-irradiation (3 Gy) induced thymic lymphoma (TL) after 3-4 months followed by shortening of the life span of tumor-bearing animals. These findings have been extended to detailed investigations on the mechanisms of radiation-induced occurrence of tumor and its modification by antioxidants and low dose exposures prior to tumor-causing radiation dose. Further studies have confirmed that pre-exposure of animals to low doses of radiation significantly suppressed the growth of the lymphoma tumor. Radiation-induced tumor induction was found to be dependent on the age of animal at the time of irradiation. The younger age mice showed greater sensitivity to radiation-induced TL. In addition, radiation-mediated tumor induction was found to be gender-dependent. In irradiated female mice, the TL incidence was significantly higher and the growth of tumor in terms of weight and size was more aggressive than in males of the same age. Moreover, mice with higher age groups at the time of irradiation showed substantial decrease in TL incidence and its aggressiveness. Interestingly, these effects were more conspicuous in males than in females. It was further observed that the post-irradiation feeding of animals with antioxidants resulted in a significant decrease in TL incidence, and the prevention in

TL incidence was more in animals fed with curcumin (55%) than with ascorbic acid and eugenol (20%). These results have provided a significant insight about radiation-induced TL incidence and its modification by antioxidants. These studies and in-house developed radiation tumor models have great potential to develop low dose radiation research program with relevance to cancer treatment by low dose therapy or radon exposure at BARC. Whole body exposure of animals to sub-lethal doses (1-5 Gy) was observed to cause a dose-dependent increase in ROS and consequent apoptosis in thymocytes of irradiated animals, which was found to be inhibited by antioxidants such as vit-E, curcumin, ascorbic acid and eugenol. This project revealed the role of gamma-radiation generated ROS in cell/membrane oxidative damage and also the role of apoptosis in the mechanism of radiation-induced lymphoma tumor in mice. These studies further revealed a correlation between the magnitude/kinetics of DNA damage in peripheral blood leukocytes of mice exposed to whole body gamma irradiation (3 Gy) and aggressiveness of thymic lymphoma.

5. Basic Radiobiology Studies on Tumor Cells for Cancer Radiotherapy

The discoveries of X-rays and radioactivity at the end of 20^{th} century brought revolution in diagnosis and treatment of many diseases including cancer. Early radiobiological studies showed that ionizing radiations could kill the rapidly proliferating cells. In fact, physicians have quickly applied radiations such as X-rays and gamma rays for treatment of cancer patients. However, further studies revealed that radiation does not distinguish between normal and tumor cells thereby imposing a limitation on the dose of radiation for therapy. Most often, due to unacceptable adverse effects of therapeutic radiation on normal tissues, treatment of cancer by single large dose of radiation has to be discontinued. Based on the observations on radiation cellular effects, it was suggested to employ the strategy of fractionated doses in the clinical settings taking into account the repair of DNA damage. Extensive studies on the mechanism of cellular damage by radiation revealed the intracellular generation of ROS. These radiobiological results allowed the utilization of observed higher oxidative stress status of tumor cells compared to corresponding normal cells in causing selective radiotoxicity in tumor cells. Therefore, we have actively pursued exploring higher radiation killing of tumor cells in presence of herbal pro-oxidative flavonoids. Research from our group has shown the promise of several plant-based compounds in combination with radiation to selectively kill tumor cells while sparing the normal cells. It is emphasized that future research challenges lie in gaining the deeper insight in the mechanisms of radiation-induced damage on normal and tumor cells for developing novel protocols for effective treatment of cancer patients.

There exists enormous prospect for screening and evaluation of herbal/plant products for developing effective radiosensitization and radioprotection relevant to nuclear research program. Research was focused on the mechanism of activity of variety of anticancer and antioxidative agents, viz. Betulinic acid, Diospyrin, Eugenol, (EU), Ellagic acid (EA), Plumbagin, Triphala (TPL), Tocopherol Succinate (TOS), Thymoquinone, Silibin and Arachidonic acid on normal and cancer cells with view to design effective protocols in

practical radioprotection and cancer radiotherapy. This project was mainly focused on studies on the mechanism of cytotoxic effects in cancer cell lines and their mouse tumor models. Results have shown that these agents produced radio-sensitizing action involving oxidative damage, membrane alteration, damage to DNA and apoptosis induction. It has been found that cytotoxic effect was induced by initiating membrane oxidative damage and by triggering intracellular generation of ROS by γ-radiation in combination with phytochemicals like TPL, EA, diospyrin, and TOS in tumor cell lines viz. Ehrlich Ascites (EAC), Human cervical (HeLa), breast (MCF-7, T47D), human and mouse fibrosarcoma (HT1080 and WEHI164) and lung cancer cells. It was concluded that modulation of membrane peroxidative damage and intracellular ROS may help in achieving efficient killing of cancer cells vis-à-vis normal cells, which may provide a new approach for developing effective cancer treatment.

A research project was initiated in collaboration with Radiation Oncology and Hyperthermia Division of Nanavati Cancer Hospital, Mumbai to test whether membrane oxidative damage and associated apoptosis could be a predictive marker of radiotherapy in cervical cancer patients. The plasma membrane fluidity and intracellular SOD with relation to apoptotic death in cervical carcinoma cells of cancer patients after radiation therapy was evaluated. Cells from biopsies of cancer patients (stage IIIB) prior to and 24 h after radiation dose of 2 Gy were examined. Plasma membrane fluidity and SOD activity showed significant decrease but percentage apoptotic cells, as determined by Annexin-V/PI and TUNEL assays, were found to be increased by two folds after radiotherapy. This project validated our findings of radio-oxidative membrane damage and consequent induction of apoptosis in cancer patients. Hence it was suggested that decrease in DPH polarization in membrane, reduction in SOD activity and increased apoptosis in cervical cells of cancer patients treated with radiation may be consequent to oxidative damage induced by reactive oxygen species, which may have potential implications in developing predictive protocol in cancer radiotherapy. An inverse correlation was observed between membrane fluidity/SOD level and apoptosis in cervical carcinoma cells. On the other hand, a positive correlation was observed between intracellular calcium level and apoptosis. These results suggested that changes in membrane fluidity, SOD and calcium level were involved in the mechanism of radiation-induced cervical cell apoptosis. Moreover, apoptotic sensitivity of these cells after the first dose of radiation treatment showed a direct correlation with the radiation treatment outcome in patients after completion of radiotherapy (50-70 Gy), suggesting that apoptotic index may serve as a basis for prognosis in radiotherapy of stage-III cervical carcinoma patients.

In another collaborative research with Department of Radiation Oncology, Tata Memorial Hospital, Mumbai, radiobiological studies were performed in the relapsed/refractory Non-Hodgkin's Lymphoma (NHL) cancer patients undergoing low dose total body irradiation (LDTBI). These patients were treated to a total 200 cGy at 10-20 cGy per fraction with five fractions a week. LDTBI has shown a good efficacy in NHL patients; however, it was important to determine the haematological effects following LDTBI to establish its efficacy and safety. This study demonstrated that

LDTBI is a well-tolerated treatment in patients with good clinical response. However, in non-responding patients with progressive disease, LDTBI exacerbates the haematological parameters. The crab's jaw shaped pattern was observed in polymorph and lymphocyte count, which indicated that LDTBI works by altering immune mechanisms and apoptosis. Radiobiological analysis was performed by measuring Superoxide dismutase, Catalase, ROS and apoptosis in the lymphocytes of NHL. These initial results were highly encouraging, which initiated further evaluation of LDTBI by assessing haematological profile, clinical response, survival and patient's quality of life in combination with radiobiological studies to understand the cellular and molecular basis of 'crab's jaw patterns in LDTBI radiotherapy of NHL cancer patients. Developments of biomarkers for prognosis of metastasis and radiotherapy outcome are warranted for better management of cancer patients. Transcriptomics studies of radio-resistant lung cancer cells and its correlation with lung cancer patients TCGA data base identified cancer stem cell gene signatures as prognostic marker for responders to radiotherapy. In collaborative study with Tata Memorial Hospital, Mumbai, serum biomarkers (VEGF, IL-8 and MMP-9) in metastatic lung cancer patients showed their ability to predict metastasis occurrence. In another study with Nanavati Hospital, Mumbai, prognostic efficacy of serum HSP90 beta was studied in head and neck cancer patients subjected to hyperthermia therapy, which showed complete response in patients with lower HSP90 beta.

6. Paradigm Shift in Radiation Biology: Non-targeted Radiation Effects

DNA is considered as primary target of the cellular response to radiation and presumably no radiation effect would be expected in cells that receive no direct radiation exposure through nucleus. The dogma dominated in classical radiobiology was challenged by several radiobiologists, which showed that not only DNA but cytoplasm, membrane and mitochondria act as targets of radiation. Seminal discoveries showed that plasma membrane, which was otherwise considered merely as cell boundary, act as radiation signalling hub. These discoveries made paradigm shift in radiobiology. Joining the global radiobiologists, studies were initiated to investigate the role of membrane as target of radiation effects. Owing to simulation to cellular structure and ease to prepare a desired composition, liposomes, a bilayered phospholipid membrane with aqueous core, were used as a model membrane. The dose dependent and oxidative stress associated damage was found in liposomes prepared with unsaturated fatty acids sensitive to ionizing radiation. Cholesterol is one of the major ingredients of cellular membrane (~30 %) and is known to vary in different cell organelles. While mitochondria, peroxisome and endoplasmic reticulum membranes are cholesterol poor, plasma membrane is enriched with cholesterol. Lipid rafts and caveolae (the signaling hubs harboring receptors) are highly enriched with cholesterol. Moreover, due to structure cholesterol is known to affect the rigidity of membrane. Hence, liposomes with varying concentrations of cholesterol were prepared to understand how cholesterol content can modify the membrane and thus cellular radiosensitivity. Enrichment of cholesterol resulted in increased rigidity of liposomal membrane, which was correlated with formation of lipid

oxidative products after radiation exposure (1999). Moreover, liposomes prepared with membrane localized antioxidants like eugenol resulted in prevention of membrane damage (2004). These results were further extended in immature mouse thymocytes, which are sensitive to oxidative damage and apoptosis after radiation treatment.

Experimental evidence generated in many laboratories including ours reveal the fact that radiation effects also occur in cells or populations, where cells do not encounter direct radiation exposure. Such non-targeted effects of radiation can be either localized to neighbouring cells, known as "radiation-induced bystander effect (RIBE)" or extended to distant tissues/organs [radiation-induced abscopal effects (RIAE)]. Even though, radiation-induced bystander effect (RIBE) was discovered in 1970s and radiation-induced abscopal effect (RIAE) in 1930s, the studies pertaining to cancer radiotherapy are limited in literature. The non-targeted radiation effects (NTRE), encompassing RIBE and RIAE, are highly relevant during cancer radiotherapy due to several reasons. (i) The tumor mass is surrounded by normal tissues and thus their mixed boundaries provide an excellent platform for bystander interaction, when the tumor is targeted by radiotherapy. (ii) In some situations, tumors are located very close to critically important organs like lungs, brain, thus partial tumor irradiation is performed which provide possibility of bystander interaction between irradiated and non-irradiated regions of the same tumor. (iii) Majority of the cancer patients are diagnosed with metastasis at critical sites or spread at multiple locations in the body. Generally, radiation oncologists are able to treat only the primary site of tumor, which in-turn might affect the distant metastatic/micro-metastatic tumors. Hence, the magnitude, nature (damaging/protective) and direction (unidirectional/bidirectional) of interaction of irradiated and bystander cells/distant tissues and subsequent fate of these cells would govern the clinical outcome of cancer radiotherapy.

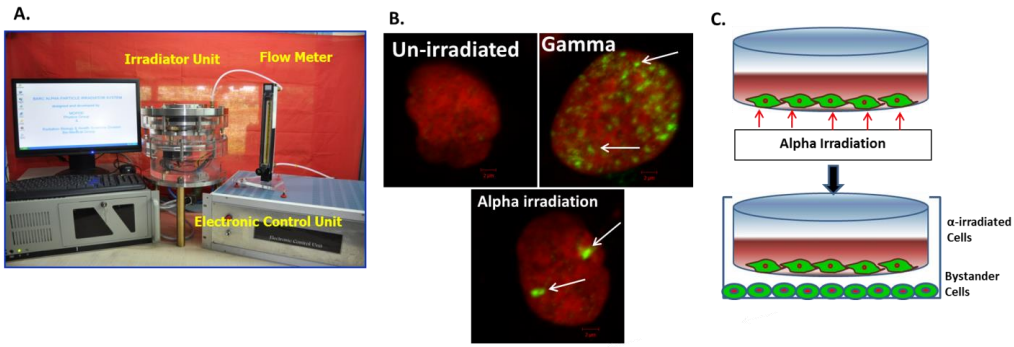

Fig. 4: (A) In-house developed alpha-particle irradiator (BARC Bio-alpha) in collaboration with TPD, BARC to develop 'Alpha Radiobiology Research Program' at BARC. Its technology has been transferred to M/s Ande Mechatronics, Mumbai through TTCD, BARC. Technology incubation for new compact Bio-alpha is done to M/s General datum Product Design Pvt. Ltd., Hyderabad. (B) Alpha particle-induced DNA damage (gamma-H2Ax foci) in comparison to gamma radiation in human lung carcinoma cells (A549). (C) Scheme depicts co-culture experiments to investigate the mechanism of alpha-radiation-induced bystander effect in normal and cancer cells.

To understand the mechanism of radiobiological response of normal and tumor cells following alpha particle exposure, an alpha-particle irradiator was designed and developed in collaboration with Technical Physics Division, BARC (**Fig. 4**). This led us to develop alpha radiobiology research in India, which has relevance to address key questions for targeted alpha radiation therapy program of BARC for cancer treatment. In addition, it also helps in investigation of bystander effect and radiological effects of alpha-emitting radionuclides in normal cells following internal contamination. The development of this research facility has generated potential research opportunities especially the characterization of alpha-particle-induced clustered DNA damage and the mechanism of consequent cellular response in a variety of cell and tissue models. Depending on the experimental settings, cell types and exposure doses, 'BARC Bio-alpha' will enable us to contribute towards space radiobiology, high-LET radiobiology for cancer therapy and scientific basis for radiation protection models, bystander effects as well as nuclear radiobiology research.

Multiple experimental models/strategies were employed to develop the non-targeted radiation research. In one of the seminal works simulating cancer and normal cell proximity during charged particle therapy, human lung cancer and normal fibroblast cells were co-cultured, where nuclei of either caner or normal cells were selectively irradiated with 500 protons using proton microbeam (3.4 MeV) facility at National Institute of Radiological Sciences (NIRS), Japan followed by measurement of DNA double-strand break in irradiated/bystander cells. Transmission of DNA damaging signal was observed from the proton irradiated lung cancer cell to bystander lung cancer cell. It was interesting to observe that the magnitude of DNA damage in the irradiated lung cancer cells was attenuated, when human normal fibroblasts were placed neighbouring to these irradiated cells. The damaging bystander effect was abrogated, when gap junction between irradiated and bystander cells was blocked. These findings for the first time established the bidirectional and rescuing bystander effect between lung cancer and normal cells after proton microbeam irradiation. The factors/cytokines released from the irradiated-cancer cells are also known to contribute to the bystander effect. In this direction, cancer cells of different tissue origins (breast, lung, fibrosarcoma, colon and brain) showed variation in secretion of cytokine profile when irradiated either with acute or fractionated doses of gamma radiation. Furthermore, the conditioned medium from the irradiated lung cancer cells showed toxicity to bystander lung cancer cells.

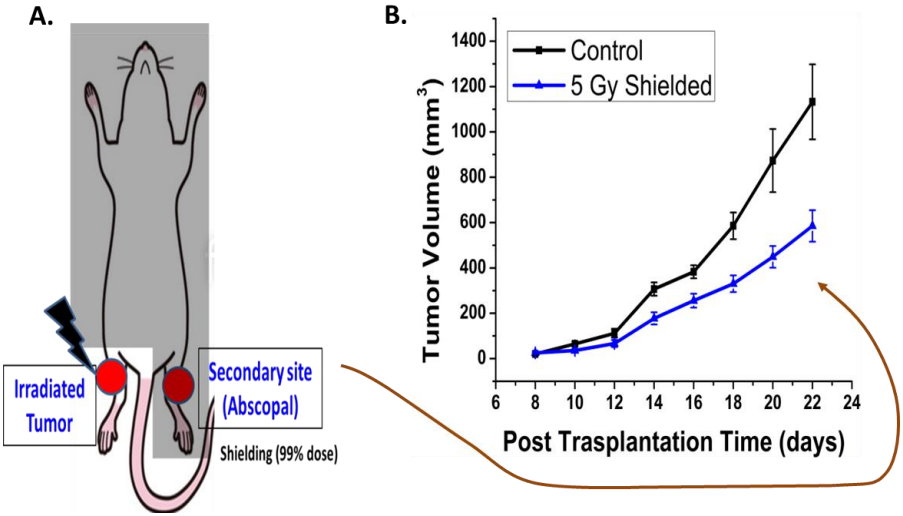

Fig. 5: A. Development of mouse tumor model to investigate the tumor suppressive effect on second un-irradiated tumor by gamma-radiation-induced abscopal effect from primary irradiated tumor. B. Tumor growth kinetics of radiation shielded (un-irradiated) fibrosarcoma tumors of control and 5 Gy irradiated tumor

Studies were extended at animal level to demonstrate and understand the NTRE results in mouse fibrosarcoma tumor models (**Fig. 5**). For this, following strategies were employed. **(i) Co-implantation of lethally-irradiated tumor cells with bystander cells:** In this approach, only a fraction of tumor cells was irradiated with lethal high doses of gamma radiation (15 Gy), mixed with bystander cells followed by implantation of the cell mixture in mice for the measurement of tumor growth. It was interesting to observe that a fraction of high dose irradiated tumor cells inhibited the growth of bystander tumor cells and thus developed into smaller tumors. The inhibition of growth of tumor was found to be associated with secretion of anti-tumor proteins/factors from the irradiated cells, which resulted in cell death of bystander cancer cells as well as decrease in the process of angiogenesis during tumor progression. **(ii) Partial tumor irradiation:** For this a cone irradiator (designed by DRHR, BARC and dosimetry by RPAD, BARC) for Cobalt-60 teletherapy irradiator, was used to irradiate only part of mouse tumor. The study showed that compared to control, significant tumor inhibition was observed, when only ~10 % volume of tumor was irradiated. **(iii) Non-targeted radiation effects at distant tumors:** In this, we have studied the possibility to control the distant tumors, when only primary tumor was irradiated. Such studies have gained attention of researchers as well as clinicians as they can be exploited to enhance radio-immunotherapy of metastatic tumors and prevent post-irradiation tumor recurrence. To simulate NTRE at distant tumors, mouse fibrosarcoma tumors were developed in both the hind limbs. While one of the tumors was irradiated with gamma radiation, the other tumor and animal body parts were shielded. Decrease in tumor growth in non-irradiated shielded tumor was observed when tumor in another leg was irradiated, which was more prominent at higher doses than

conventional therapeutic dose (2 Gy). The directly irradiated tumors showed expression of immunogenic cell death markers. To enhance the NTRE in distant tumors, radiation in combination with CpG-ODN (cytidine phosphate guanosine-oligonucleotides), an immunomodulatory oligo, was administered in the irradiated tumor after delivering radiation dose to the tumor. CpG-ODN in conjunction with radiation resulted in better tumor control. The tumor growth-inhibitory effects were mediated through increase in immunomodulatory markers and induction of apoptosis in the shielded tumors. It was interesting to observe that in these animals freshly transplanted tumor cells didn't produce tumors suggesting long lasting non-targeted radiation effects.

Gap junction or cellular synapses are the major direct cell-to-cell contact modality for intercellular communication in normal and tumor microenvironment (TME) conditions. In addition to these modalities of communication, intercellular bridges widely referred to as tunneling nanotubes (TNTs) or shedding of independently migrating viable cell fragments (VCFs) referred to as microplasts or cytoplasts in cancer cells/tissues have gained attention of researchers as emerging modes of cellular interaction. TNTs are actin-based membrane bound cytoplasmic bridges formed between donor and recipient cell, through which wide variety of cellular cargos and organelles can be directly transported to distantly connected cells (up to few hundred microns). Cellular communication through TNTs have known to govern several processes during cancer pathogenesis and chemo-resistance. For instance, the drug resistant phenotypes were found to spread through TNTs in the cancer population by intercellular transfer of ABC transporter P-gp, mitochondria or miRNA (2023). In our laboratory, TNTs and microplast formation were studied in human breast cancer cells treated with macrophage conditioned medium. Mitochondria, vesicles and cytoplasm could be transferred from parent cell body to microplasts through connecting TNTs. Microplast formation was inhibited in the presence of cell migration inhibitor, cytochalasin-B. Metalloproteinases (MMP) activity localized in vesicles in the cell body as well as in microplasts suggests their potential role in the process of invasion.

7. Research on Thorium Toxicity and Radiotoxicity: Cell and Animal Models

Dr. Bhabha has envisioned Thorium-based nuclear research programme for DAE. Therefore, it was imperative for radiation biologists to carry out fundamental research towards understanding the radiobiological effects of Thorium and other relevant radionuclides in human cells and experimental animals. By 2002, Atomic Energy Research Institutes of several nations (Japan, France, US and UK) have conceived such research programs and developed dedicated Departments/Institutes for development of *Nuclear Radiobiology Research* (**Fig. 6**). India has the largest Thorium reserve and operating sand beach mineral separation plants under Indian Rare Earth Limited (IREL, DAE) at Manavalakurichi, Aluva, Udyogmondal and Chavara for separation of various minerals including monazite (Th ore) and Orissa Sand Complex (OSCOM) is a dedicated facility of IREL for extraction and purification of Thorium from monazite. In addition, DAE has developed the long-term plan for advanced nuclear reactor technologies for

Thorium utilization. Therefore, a need for establishing '***Thorium-centric nuclear radiobiology research***' program at BARC was recognized. The potential implications of Thorium biology research are **i)** fundamental high-LET radiobiology, **ii)** targeted alpha cancer therapy and **iii)** development of novel decorporation strategies for management of internal nuclear contamination.

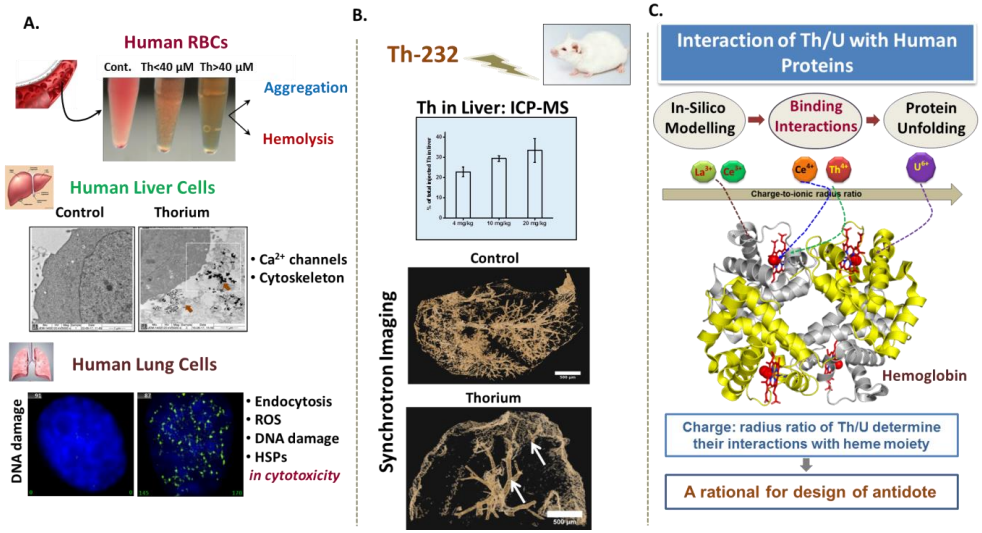

Fig. 6: An overview of nuclear radiobiology research highlighting the radiobiological responses of Th-232 in (A) human cells and (B) target organ (mice liver) as well as (C) the mechanism of interaction of Thorium with human blood protein (hemoglobin)

7.1. Understanding the mechanism of radiobiological response to Thorium

In RB&HSD, research has been pursued to understand the basic mechanisms of Thorium interaction with human cells and proteins and its consequences. A decade-long research activities revealed crucial answers for **i**) the mechanism of effects of Thorium in human cells; **ii**) the mechanism of cellular internalization of Thorium; **iii**) fundamental aspects of the binding of Thorium to protein and their functional consequences; and **iv**) major target organs of Thorium and their early and late chronic effects. Understanding these aspects of Thorium at organ, cell and molecular levels using biophysical, biochemical, microscopic, spectroscopic and computational approaches led to the rational design and development of antidotes for removal of Thorium and mitigation of its associated radiological and chemical effects (**Fig. 6**). Thus, the Bio-Thorium Research Program at BARC has significant implications for developing India's capability for efficient utilization of Thorium with adequate human health and environmental protection.

Research was primarily focused on understanding the mechanism of toxic effects of Thorium in human cells viz. red blood cells, lung cells, liver cells, and bone, which represent the target organ/sites of Thorium accumulation/toxicity in human/animal models (**Fig. 6**). Our experimental data revealed that radiobiological response to Thorium depends on the cell type, chemical forms, concentration and exposure time of Thorium.

Briefly, in human lung cells, Th-dioxide (colloidal) was found to be more toxic than the Th-nitrate at equivalent metallic concentration of Thorium. This was found to be due to higher uptake of Th in cells exposed to its dioxide form as compared to the Thorium uptake from its nitrate form. Moreover, transmission electron microscopy in combination with confocal microscopy revealed the mechanism of Thorium internalization, which was found to be via clathrin/caveolin-mediated endocytosis following Th-dioxide exposure as compared to membrane perforation in cells exposed to Th-nitrate. Following internalization, Thorium induces oxidative stress, DNA damage response and proteotoxic stress, which play major roles in determining cytotoxicity. Following transmigration through air-blood barrier in lung, Th gets circulated via blood and finally accumulates in liver and skeleton. Our studies on human liver cells have identified the role of cytoplasmic calcium in Thorium uptake, suggesting the possible application of calcium modulators for minimizing Th internalization. Using ultrasensitive analytical techniques, we have identified cytoskeleton as the major intracellular target of Thorium. Interestingly, effect of Thorium on human red blood cells (RBCs) was found to be determined by Th:RBCs ratio. Lower Th-to-RBCs ratio caused aggregation due to neutralization of surface negative charge. However, at higher Th-to-RBCs ratio, RBCs undergoes cell lysis (hemolysis) through colloid-osmotic mechanism. The mechanistic understanding of Th-induced cytotoxicity has significant implications to develop rational approaches for mitigation of Th-toxicity.

During circulation in blood, Thorium ions can also interact (in addition to RBCs) with soluble proteins such as albumin/globulin in blood plasma as well as haemoglobin localized on RBC's membrane. Using biophysical tools, the binding sites of Thorium ions in human serum albumin and haemoglobin proteins were investigated. Interestingly, Th ions were found to perturb the structural and functional integrity of Fe-containing heme of haemoglobin. This was due to the similar charge-to-ionic-radii ratio of Th with Iron. This led to understand the binding of Th at Fe-binding sites in iron transport/storage proteins (e.g. transferrin, ferritin, catalase, etc.). Thorium interaction with haemoglobin was further investigated in the environmentally-important aquatic midge, *Chironomus*, which may serve as bioindicator of Th contamination in aquatic systems. Importantly, our spectroscopic data determined the ability of actinide ions including Thorium to unfold the proteins with significant alteration in their functionally-important conformations. In this direction, further research is being pursued using computational modelling and biochemical approaches to characterize the bio-coordination of Thorium and other actinide ions with relevant proteins, which have been identified as a molecular target of toxicity.

Extensive studies in experimental mice/rat models were carried out to identify the major sites of Th accumulation and underlying mechanism of toxicity at cellular and molecular levels. Liver, skeleton and spleen were found to be the major target organs of Thorium following its administration. We have determined the biodistribution, histological and functional changes as well as alterations in gene expression in organs/tissues after 6 to 12 months of Thorium exposure. Our recent analysis of gene expression and system biology

approaches highlighted the involvement of β-catenin/Myc driven signalling pathways in Thorium-induced oncogenesis in mice.

Since inhalation can be a potential route of radionuclide exposure in occupational and accidental scenarios, a facility dedicated to simulate inhalation route of exposure in animals (nose-only inhalation exposure facility) was developed, which led to inhalation nuclear toxicology research at BARC for understanding the response of lung tissues to chronic and acute doses of Thorium and other radiometals/toxicants/materials. Recently, using this facility, effect of Th aerosols exposure was investigated in Swiss mice, which revealed alterations in the expression of functionally-essential lung surfactant proteins. This facility will be used for evaluation of newly designed and synthesized chelating agents for development of rational decorporation therapy for various radionuclides relevant to Indian Nuclear Fuel Cycle.

7.2. Technology Development for Decorporation of Internalized Thorium

Design and development of effective mitigation approaches for internal contamination with Thorium and other radiometals in human and environment is the need of the hour. Presently, there is no FDA-approved antidote for treatment of human subjects in case of Thorium contamination. DTPA (Ca/Zn salt of diethylenetriamminepentacetate) is the only available agent recommended for Plutonium, Americium and Curium contamination. Our experience from animal experiments suggested inability of DTPA to remove Thorium from liver and skeleton. Therefore, further research was pursued to design Th-specific rational antidote. Our learning experience for the mechanism of Th interaction at cell/protein levels has provided significant clues about the biological ligands (proteins/DNA, etc.) with which antidote (chelator) need to potentially compete for Th decorporation. In this direction, multidisciplinary approaches were adopted such as **i)** rationally-selected existing molecules; **ii)** rationally-designed new agents and **iii)** improvement of organ distribution profile of chelators using drug delivery systems. Our efforts on screening of rationally-selected existing molecules have identified L52S (a hepatoprotective formulation) for its potential of Th decorporation in comparison to DTPA as a reference agent (**Fig. 7**).

Further research has been extended to gain deeper insights about the cellular and molecular mechanisms of radiobiological responses of cells/tissues to Thorium and other heavy metal radionuclides. These biological investigations would lead to the identification of sensitive and specific biomarkers of Thorium exposure/effects. Extensive efforts are underway to develop rational and novel decorporation agents/therapy for removal of internalized Th/other radiometals from lungs, surface wounds and other vital organs based on the understanding of mechanisms of their toxicity and interaction. Fundamental understanding of mechanisms of Th interaction/toxicity would help in optimizing the combination of chelating agent and pharmacological inhibitor of Th-specific toxicological processes for enhanced decorporation and mitigation of toxicity.

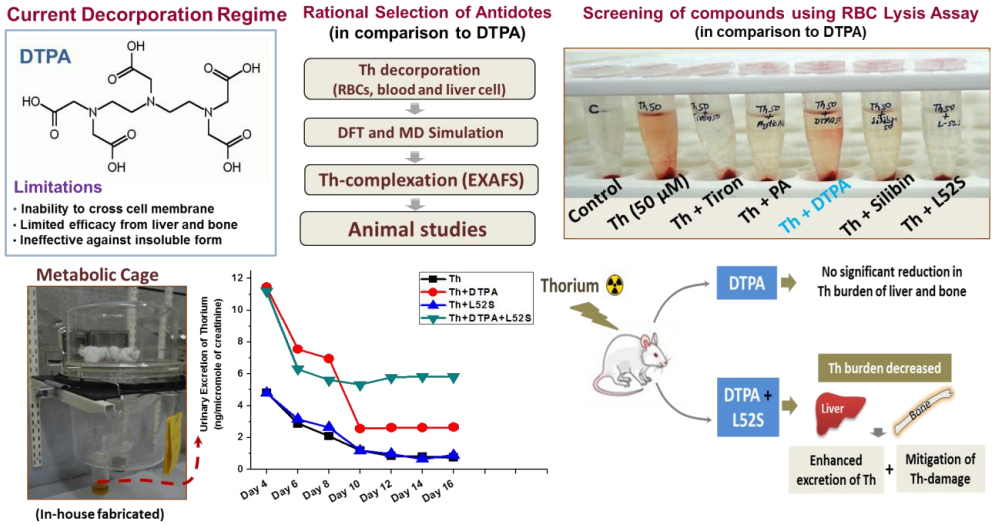

Fig. 7: Screening of rationally-selected agents for Thorium decorporation and mitigation. Top right panel shows the relative efficacy of selected agents for preventing hemolysis as a measure of Thorium chelation in comparison to DTPA. Lower left panel shows in-house fabricated metabolic cage for determination of Thorium decorporation efficacy in mice. L52S, a lead candidate was found to significantly enhance Th excretion efficacy of DTPA through urine and mitigated Th-toxicity in liver.

8. New Approach to Improve Cancer Radiotherapy: Nanomedicine

The most challenging part for any modality of cancer treatment is the tumor-specific delivery of anti-cancer drug or radiation dose for maximizing the damage to tumor cells, while sparring the surrounding normal tissue. Although, around 60 % of solid tumors are treated with Chemo-Radio Therapy (CRT), its non-tumor-specificity severely hampers its therapeutic efficacy, resulting in dose-limiting toxicities and subsequent development of treatment resistance. Thus, a holistic approach that can simultaneously improve the selectivity of anti-cancer drugs to the tumor, minimize their adverse health effects and augment the efficacy of radiotherapy would be desirable by clinicians for achieving better therapeutic outcomes. In this direction, nanotechnology has great potential to contribute by facilitating targeted delivery of the anti-cancer drugs to the tumor site, as well as by, augmenting and facilitating the application of multi-modal therapies including hyperthermia and targeted CRT. Hyperthermia therapy (HT) has been used in clinics as an adjuvant to CRT to enhance its therapeutic efficacy. HT involves heating the tumor in the range of 40-43°C, which can either kill the cancer cells directly or sensitize them for subsequent CRT. In clinics, conventional HT is applied using infra-red, microwaves, high intensity focused ultrasound, sauna bath or water bath. However, conventional HT suffers the limitations of in-sufficient and non-homogenous heating of tumors often leading to development of thermo-tolerance during subsequent HT sessions.

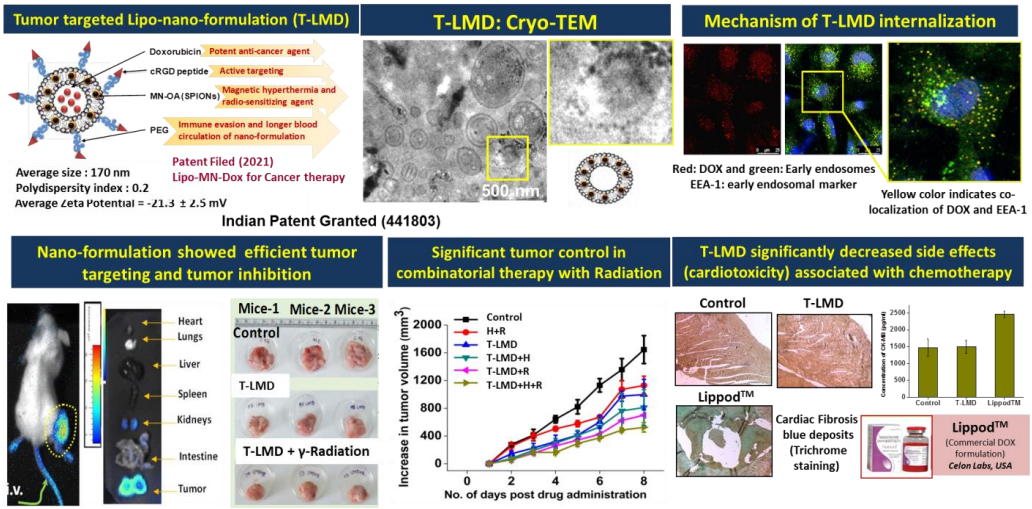

Fig. 8: Top left panel shows the patented design of tumor-targeted liposomes encapsulated with magnetic nanoparticles and anticancer drug (T-LMD) for multimodal chemotherapy, radiotherapy and hyperthermia therapy of cancer. Lower left panel shows specific targeting of tumor by T-LMD, which enhances the effect of gamma radiotherapy. Lower right panel shows minimal-to-no side effects of T-LMD in cardiac tissues in comparison to commercially available liposomal-dox formulation (Lippod^{TM}).

Thus, development of alternate and more efficient hyperthermia modalities with ability to induce nano-heating effects at cellular/molecular level becomes vital. Photothermal therapy (PTT) and magnetic hyperthermia therapy (MHT) are two such modalities with superior hyperthermia efficacies and better therapeutic abilities as compared to conventional HT. In this direction, our laboratory has been working since the year 2008 towards development of rationally designed nanoparticles for improvement of therapeutic efficacy of CRT. One of the first designs of nanoparticles developed in our laboratory comprised of super-paramagnetic iron-oxide nanoparticles (SPIONs) coated with oleic acid for delivering MHT to cancer cells. In MHT, SPIONs generate heat under the influence of an alternating current (AC) magnetic field (AMH) predominantly by Néel or Brownian relaxation. Unlike CHT, in MHT, SPIONs can be functionalized using tumor-targeting surface ligands to specifically internalize inside the tumor cells and distribute to cellular compartments sensitive to heat, such as plasma membrane. Thus, we developed oleic acid coated SPIONs termed as 'MN-OA' which can specifically target the cell membrane owing to its hydrophobic nature. We have demonstrated the membrane localization and significant anti-cancer efficacy of MN-OA in combination with MHT in cancer cells and animal tumor model. Moreover, MN-OA induced hyperthermia also resulted in significant enhancement of radiation induced DNA damage in cancer cells and animal tumor models. For the first time, our laboratory reported the role of HSP90 modulation in the mechanism of radio-sensitization and tumor growth inhibition after treatment with MN-OA, wherein interaction of MN-OA with intra-cellular HSP90,

probably via hydrophobic interactions, resulted in down-regulation of its down-stream client proteins playing important role in cell survival, cell cycle progression (AKT, CDC2, CyclinB1) and DNA repair (RAD51, CHK1 and BRCA1). Moreover, the role of mitotic catastrophe as an alternate cell death mechanism induced by MN-OA in combination with γ-radiation was also demonstrated.

For further improving the tumor targeting of MN-OA and to impart multi-modal tumor therapy, we designed a liposomal nano-formulation termed as 'T-LMD'. These liposomes were co-encapsulated with MN-OA and doxorubicin (DOX) in the bilayer and core of the liposomes, respectively (**Fig. 8**). Further, to impart tumor-specificity, they were functionalized with cyclic RGD (cRGD) peptide, which enables the targeting of αvβ3 integrin receptor over-expressing tumor cells (including triple negative breast carcinoma, melanoma, glioblastoma, ovarian and cervical cancers, etc.) as well as tumor neo-vasculature. In vitro evaluation showed significantly higher cyto-toxicity of T-LMD (average size of ~170 nm) as compared to commercial nano-formulation of liposomal-DOX (Lippod^{TM}), in cancer cells of skin, breast, lung and brain origin. Importantly, the cyto-toxicity of T-LMD was significantly lower in normal lung epithelial cells as compared to cancer cells. Moreover, T-LMD showed significant radio-sensitization of murine fibrosarcoma cells predominantly via activation of JNK mediated pro-apoptotic pathway. Furthermore, recent findings in our laboratory established ferroptosis induction by T-LMD in triple negative breast carcinoma cells and its tumor model in mice. T-LMD was found to be internalize in MDAMB-231 cells by clathrin and caveolin mediated endocytosis, followed by induction of lipid and cytosolic reactive oxygen species (ROS), damaging the cell's plasma membrane and mitochondria, ultimately culminating in increased DNA double strand breaks and cell death by ferroptosis. Release of immunogenic damage associated molecular patterns (DAMPs: HMGB-1) was also observed in culture supernatant of MDAMB-231 cells treated with T-LMD, suggesting the ability of T-LMD to activate anti-tumor immunity, a desired phenomenon reported to improve the therapeutic efficacy of CRT.

Most importantly, bio-distribution studies of T-LMD in live mice showed ~2-9 folds higher accumulation in fibrosarcoma tumors as compared to other off-target organs (Liver, spleen, kidney, heart and lungs), suggesting their superior tumor targeting ability. Moreover, in combination with radiation (R) or MHT (H) or both, T-LMD showed ~ 3-fold higher tumor growth inhibition compared to single treatments alone. Another important limitation for most of the clinically used anti-cancer drug is their systemic toxicity which severely hampers their therapeutic efficacy and negatively affects the quality of life in cancer patients. However, the nano-drug (T-LMD) developed in our laboratory showed significant inhibition of DOX induced cardio-toxicity in mice as suggested by in-significant induction of cardiac fibrosis studied by trichrome staining and immuno-fluorescence detection of phospho-Smad-3, which is one of the mediators of TGFβ-induced fibrosis in heart tissue. Moreover, serum levels of early cardiac damage marker, CK-MB were also found to be unaffected by T-LMD treatment as against Lippod^{TM} which showed significant increase in serum CK-MB levels as compared to

control (2022). These results suggest in-significant toxicity of T-LMD in healthy mice re-emphasizing its clinical potential as a targeted and multi-modal anti-tumor agent. Currently, toxicological studies in higher rodents and pharmaco-kinetic studies are being pursued to further facilitate the clinical translation of T-LMD.

9. Future Research in Radiation Biology

Radiobiology is a rapidly progressing field, and it opens new areas of research and brings new challenges to researchers. Intensive research in the past years has brought new knowledge to the forefront. Undisputedly, radiation damage to DNA continues to occupy central space in the basic research as well as applications but radiation damage to cell membrane and its role in the cell death remains inadequately investigated. Using microbeam technology, it may be important future task to determine the relative or exclusive role of radiation membrane oxidative damage or specific radiation targeted damage in cytosol in cell death process. It is important to understand and quantify the effects of High Linear Energy Transfer (HLET) radiation on human health with relevance to space exploration (Space Radiobiology). Further, the discovery of transmitting radiation effects from irradiated cells to unirradiated cells called bystander phenomenon has generated many new questions to address the implications to cancer therapeutic as well as risk as estimation task. Intensified research in future would allow assessment of impact on health. A new emerging area in radiobiology is the multifaceted role of Reactive Oxygen Species (ROS) in the oxidative mechanisms of survival and death of tumour cells. It is an active area of current radiobiology research how tumour cells form their strategy to survive anticancer therapies by cleverly manipulating intracellular balance of ROS and antioxidative capacity (AOC). It will be important to identify and control radiation sensitive genes and their implications in cellular and individual radiosensitivity. From the point of developing new technologies based on low dose radiation, it would be warranted and may prove highly rewarding to advance knowledge in low dose radiobiology with relevance to low dose health benefits and contributions to acceptance or denial of Linear-No-Threshold (LNT) model of radioprotection and radiation risk assessment goals for public health.

10. Acknowledgement

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