RADIATION-INDUCED SIGNALING: EXPLORATIONS OF PAST, PRESENT AND THE FUTURE

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Abstract

This book chapter delves into the birth and evolution of radiation signaling research at Bio-Science Group (BSG) of Bhabha Atomic Research Centre (BARC), tracing its origins from the discovery of X-rays by Wilhelm Roentgen in 1895 to the intricate molecular studies of radiation-induced pathways, as known today.

The chapter emphasizes the contributions of BSG scientists in elucidating the role of signal transducers like PI-3K and PKC in radiation response, the differential responses of these kinases to varying doses of radiation, the role of MAP kinases in determining cell fate, and the exploration of natural compounds like curcumin in modulating radiation-induced signaling.

The chapter also explores the impact of fractionated irradiation on cellular signaling, particularly in cancer cells, revealing mechanisms behind radioresistance and potential strategies for radiosensitization. Additionally, it covers the research on charged particle-induced signaling, including the differential effects of heavy ions, carbon ions, protons, and oxygen beams on cellular pathways and DNA damage responses.

The chapter underscores the transition from basic radiation biology to the complex study of molecular signaling, highlighting the evolution of research

at BARC and its contributions to the field. The discoveries made by the Bio-Science Group at BARC continue to influence the understanding and application of radiation therapy in oncology.

1. Introduction

Radiosensitivity of tumors and normal tissues varies considerably among individuals; for treatment with same radiation dose to tumors in patients, some experience more severe reactions than others, while few will experience unacceptable late reactions. Using simple laboratory tests for identifying radiosensitive individuals in advance who are particularly sensitive to radiation would be of immense benefit for clinical treatment.

Irradiating cells under experimental controlled conditions and being able not just to predict fate of the cells such as growth, death, resistance or mutation but also to correlate these biological end points to the molecular and cellular alterations is the ultimate goal of radiation biologists. However, many times these correlations are often difficult to make because of the cell specific pathways which are activated leading to different end points in different cells. Understanding effects of radiation on signaling networks in search of molecular targets which could predict or alter the sensitivity of the cells to radiation would be an advantage.

Possibility for the future may be to screen patients who are to undergo radiation therapy for the presence of genes or proteins that may confer radiosensitivity or radioresistance and then decide the course of treatment to avoid post radiotherapy complications in radiosensitive patients.

2. Importance of Radiation-Induced Signaling

Radiation-induced signaling studies are essential for understanding the complex biological responses to ionizing radiation (IR), which has significant implications for medicine, environmental safety, and radiation protection. These studies focus on how cells detect and respond to radiation exposure, leading to various cellular outcomes such as DNA repair, apoptosis (programmed cell death), senescence (cell aging), and carcinogenesis (cancer development). Here are some key aspects highlighting the importance of these studies:

2.1. Understanding DNA Damage and Repair Mechanisms

Radiation can cause direct damage to DNA or produce reactive oxygen species (ROS) that indirectly damage cellular components. In addition to ensuing repair pathways activation, DNA damage sensors and other survival pathways are also activated which affect the final outcome of cellular repair or death. Understanding these signaling pathways activated in response to DNA damage is crucial for developing strategies to protect normal cells during radiation therapy and to enhance the repair of radiation-induced damage in various medical and industrial applications.

2.2. Enhancing Cancer Treatment

Radiation therapy is the cornerstone of cancer treatment. By studying radiation-induced signaling, researchers can identify targets to improve the efficacy of radiation therapy. For instance, inhibiting certain signaling pathways that promote cancer cell survival can enhance the sensitivity of tumors to radiation, making the treatment more effective. Additionally, understanding these pathways can help in minimizing the side effects on normal tissues, thereby improving the overall therapeutic index.

2.3. Radioprotection and Mitigation

Understanding the signaling pathways involved in radiation response can lead to the development of radioprotective agents that can be used to protect individuals from the harmful effects of radiation exposure. This is particularly important for people working in high-radiation environments, such as healthcare workers, nuclear power plant employees, and astronauts. These studies can also aid in developing strategies to mitigate the effects of accidental or intentional radiation exposure.

2.4. Biomarker Development

Research in radiation-induced signaling can lead to the identification of biomarkers for radiation exposure and response. These biomarkers can be used for early detection of radiation-induced damage, monitoring the effectiveness of radioprotective measures, and assessing individual susceptibility to radiation. This has important applications in personalized medicine, where treatments can be tailored based on a patient's specific biological response to radiation.

2.5. Advancing Basic Science

These studies contribute to the broader field of cell biology by providing insights into fundamental processes such as signal transduction, gene regulation, and cellular stress responses. The knowledge gained can have far-reaching implications beyond radiation biology, informing research in areas like cancer biology, immunology, and developmental biology.

In summary, radiation-induced signaling studies are critical for advancing our understanding of how living organisms respond to radiation at the cellular and molecular levels. The insights gained from these studies have wide-ranging applications in improving cancer treatment, enhancing radioprotection, safeguarding public health, and advancing basic biological research.

3. Purpose and Scope of the Chapter

The purpose of this chapter is to provide a comprehensive overview of the pioneering research conducted at the Bhabha Atomic Research Centre (BARC) in Mumbai on radiation-induced signaling. This chapter aims to chronicle the evolution of scientific understanding in this field, highlighting key discoveries and experimental breakthroughs from the early years to the present day. It explores the various mechanisms by which different forms of ionizing radiation, including gamma rays, protons, and heavy ions, interact with cellular components to initiate complex signaling pathways. The scope of

this chapter encompasses detailed discussions on the contributions of notable researchers, the methodologies employed in their investigations, and the implications of their findings for both fundamental biology and clinical applications. By examining past achievements, current advancements, and future directions, this chapter seeks to provide readers with an in-depth appreciation of the significance of radiation-induced signaling research at BARC and its impact on the broader scientific and medical communities.

4. Birth and Evolution of Radiation Signaling

In 1895, Wilhelm Roentgen named the rays which blackened photographic film at the end of the tube as 'X' rays, with 'X' representing the unknown. Soon after, in 1898, first biological effects of X-rays were known, when Henry Becquerel observed skin damage and ulceration around the area of his vest pocket where he kept the radium. These results were further confirmed by Pierre Cardin in 1901 and thus, the field of Radiation Biology; study of action of ionizing radiation on living beings, was born. Within few years, X-rays were used to treat cancer and other diseases.

Early studies on radiation and living systems primarily focused on radiation-induced cell death. These studies were mainly conducted on microorganisms because there was no simple, effective technique for large-scale colony production from single mammalian cells, unlike the plating and colony-forming techniques available for bacteria. In the 1950s, Marcus et al. developed a similar plating-based assay to estimate the reproductive potential of mammalian cells in a petridish. This breakthrough provided ample scope for *in vitro* studies on mammalian systems and is still considered the gold standard for assessing radiation-induced cell killing. This development generated lot of enthusiasm among radiation biologists, who could now study the effects of radiation on mammalian cells grown in plates and elucidate mechanisms such as damage to DNA, membranes, and other biomolecules. Evidences then started accumulating towards DNA being principal target for the biological effects of radiation, including cell killing, carcinogenesis, and mutation. Studies on DNA damage and repair therefore, took the center stage; however, cells from IR-sensitive ataxia telangiectasia patients showed that double strand break (DSB) repair was not sufficient to prevent IR hypersensitivity. Subsequently, upregulation of genes and ensuing signal transduction process after radiation were explored. In the late 1980s, it was discovered that radiation increased the expression of early response genes, which were previously known to be activated by physiological inducers such as growth factors.

These early response genes, being transcription factors, could induce the expression of many other proteins responsible for cell division and growth. Investigations were then carried out to identify the kinases involved in early gene expression from damaged DNA. In the early 1990s, it was reported that Mitogen-Activated Protein Kinases (MAPK) and tyrosine kinases, which are directly activated by growth factors or mitogens, were also activated by free radicals and radiation.

The discovery of kinase activation and early response gene induction by radiation transformed the field of radiation signaling, which had previously focused on understanding the mechanisms of radiation-induced killing. This led to a new era of research on cytoplasmic and proliferative signaling after radiation, making it plausible to study differential cell sensitivity and the development of radioresistance in cancer cells. Many radiation biologists worldwide began investigating the effects of radiation on cellular signaling networks and how these networks influenced the final outcomes. The scientists at the Bio-Science Group of BARC were also inspired by this enthusiasm and sought to uncover the key to the cell's radiation response.

5. Early Years of Radiation Signaling Work at Bhabha Atomic Research Centre

Early work on two main signal transducers known at that time, phosphoinositide kinase (PI-3K) and protein kinase C (PKC) activity in response to whole-body irradiation laid the foundation for understanding the molecular intricacies of radiation's impact on living tissues. The study published in 1999, revealed that alterations in PI3K pathway could be detected as early as 15 minutes post-irradiation. This was a significant finding, as it indicated that the initial changes in diacylglycerol (DAG) levels and PKC activation were crucial early events that could potentially trigger tumorigenesis. These early alterations highlighted the potential for manipulating tumor responses to radiotherapy.

In 2001, deeper insights into activation of signaling molecules following whole-body gamma irradiation in mice model were attempted. The work showcased the differential responses of tyrosine kinase and PKC to varying doses of radiation. It was shown that while tyrosine kinase responded sharply to lower doses (10 cGy), PKC required higher doses (3 Gy) for activation. This nuanced understanding underscored the complexity of the whole organ's response compared to single-cell studies, emphasizing the importance of considering whole-animal responses in radiobiological research.

In subsequent years, dose-dependent expression of PKC isozymes in mouse lymphocytes after gamma irradiation was investigated. In an early work conducted in 2004, the activities of three main cytoprotective kinases, tyrosine kinase, PKC, and MAP kinase, in *ex vivo* and *in vivo* settings were compared at different doses. Another study revealed a dose-dependent differential response in PKC activity and highlighted the importance of MAP kinase activation at higher doses *in vivo*. These works highlighted significant differences in isozyme responses to *in vivo* and *ex vivo* irradiation. These insights were crucial for developing strategies to manipulate tumor responses to radiotherapy, considering the whole animal's response rather than relying solely on single-cell data.

With observed *in vivo* and *in vitro* differences in activation of kinases and various reports on radiation- induced activation of both cytoprotective and cytotoxic kinases it was becoming evident that activation of the kinase *per se* was not the deciding factor of the fate of the cell and that there was fine tuning of networks. Elegant studies done in 2004 showed that duration of activation of kinases and their chronology of activation in the cells could play an important role. These studies done in radioresistant liver cells showed early and transient activation of cytotoxic kinases which probably was in response to

stress and for the immediate requirements of the cell and did not further feed into cytotoxic pathways; however, the cytoprotective kinases such as ERK which showed prolonged activation led to final outcome of liver cell survival.

Similar studies were performed to understand the relative roles of two major DNA damage sensors known at that time, Poly (ADP-ribose) polymerase (PARP) and DNA-dependent protein kinase (DNA-PK). Another study published in 2004 provided clues into how these enzymes, both vital for DNA repair, shared their responsibilities for recognizing DNA damage after radiation. It was observed that at high doses, when probability of DNA DSBs is high, DNA-PK is more active while PARP is switched off, which could be safe bet for cells as high activation of PARP leads to cell death. At high doses therefore, early sensing and transducing DNA damage was assigned to DNA-PK so that repair prevails.

Modulation of Signaling Factors

As radiation-induced cytoprotective/proliferative pathways were being discovered, the hunt was on to find natural compounds which could modulate their activation so as to achieve radio-sensitization for desired clinical outcome of radiotherapy. Scientists at BSG also delved into this area of testing effect of natural phenolic compounds on radiation-induced kinases. Their work demonstrated that compounds like curcumin, ellagic acid, and quercetin could inhibit PKC activity, with curcumin and ellagic acid being particularly effective which could thereby prevent the development of radioresistance in tumor cells. Most of the radiation-induced studies done till then were on mouse lymphocytes as both *ex vivo* and *in vivo* irradiation studies could be easily done with these cells. However, there was need to study radiation signaling in tumor cells for which fibrosarcoma cells were chosen to generate tumors in mice which could be later excised and made in to single cell suspension and reinjected if the need be. This tumor model therefore, supported studies on both *ex vivo* and *in vivo* irradiation of tumor cells.

Nuclear factor-kappaB(NF-kB), an important transcription factor with pleiotropic role was also found to be induced by radiation during early 90s. The BSG team thus, looked at the modulation of NF-kB in addition to other cell survival factors like PKC, and ERK in fibrosarcoma induced in mice. Their results were promising: while PKC isoforms were unaffected, both ERK and NF-kB were significantly inhibited by curcumin and nicotinamide. This suggested potential new strategies to enhance tumor cell killing and prevent radioresistance.

Another modulator which was very intriguing was Nitric Oxide (NO). NO was initially identified as a vasodilator generated by endothelial cells and later was found to be produced by macrophages during anti-pathogenic and anti-tumor response. These important initial studies led to explosion of NO research which had started revealing the importance of this diatomic molecule in biological systems as a signaling molecule. Just like reactive oxygen species which were known by then to be important signaling mediators in living systems, reactive nitrogen species were also researched if they could act in a similar way. Studies were therefore done to explore modulation of radiation-induced signaling in presence of nitric oxide**,** as ROS generated by radiation could lead to

RNS in presence of NO which could then modify proteins differently. Tyrosine moieties of MAP kinases, one of which is phosphorylated for the kinase to be active was looked for their nitration under NO rich environment after irradiation. The study revealed that NO donor could inhibit radiation-induced tyrosine phosphorylation of kinases; however, it did not have any effect on cellular function as assessed by phagocytic efficiency of these phagocytic cells. These studies were intriguing and supported the idea that nitro group could probably do the job of phosphor group on tyrosine. Estimating activation of signaling kinases via phosphorylation in a pathway thus needed a revisit in context of high nitric oxide rich environment inside the tumors which contained many different cells including phagocytes.

The transition from basic principles of radiation biology to intricate studies of cellular signaling pathways underscores the evolution of research at the institution. The early focus on understanding radiation's biological effects expanded into a detailed exploration of molecular mechanisms, driven by the relentless curiosity and dedication of BSG scientists.

The above-mentioned studies were done on single doses used in the therapeutic range (2Gy - 4Gy). However, during radiation therapy, radiation is delivered not in single but in multiple repeated doses to kill tumor cells while limiting the dose to normal cells. Studies were therefore, designed to simulate this mode of radiation delivery akin to the regimen used in cancer radiotherapy and then look at the activation or upregulation of signaling components which could answer the development of radioresistance in tumors due to repeated multiple doses.

6. Navigating Radioresistance: The Role of Fractionated Irradiation Signaling

In a pivotal study published in 2007, scientists at BSG explored the effect of fractionated doses of Co-60 gamma-irradiation on a murine fibrosarcoma model. Role of the three major MAP kinases: p44 MAP kinase, p38 MAP kinase, and stress-activated protein (SAP) kinase was probed. These kinases are crucial in determining whether a cell will survive or undergo apoptosis after radiation exposure. The study showed that fractionated irradiation elicited an adaptive response, characterized by sustained activation of the prosurvival p44 MAP kinase over seven days. This was balanced by increased activation of the proapoptotic p54 SAP kinase shortly after irradiation. Interestingly, the dual specificity phosphatase PAC1 was also induced, potentially acting as a feedback regulator of p44 MAP kinase. These findings highlighted the potential of targeting p44 MAP kinase to enhance the efficacy of radiotherapy.

Building on this foundation, the role of the Rad52 gene in fractionated irradiation-induced signaling in A549 lung adenocarcinoma cells was explored. The study compared the effects of fractionated doses of gamma-irradiation with acute doses and found that A549 cells exhibited increased radioresistance when the 10 Gy dose was delivered fractionally. Microarray analysis revealed the upregulation of DNA repair and cell cycle arrest genes in cells exposed to fractionated irradiation. Key DNA repair pathway-associated genes, including DNA-PK, ATM, Rad52, MLH1, and BRCA1, were intensely

activated. Moreover, phospho-p53 translocated to the nucleus, indicating an active DNA damage response. Remarkably, silencing the Rad52 gene in fractionated A549 cells rendered them radiosensitive, underscoring the crucial role of Rad52 in mediating radioresistance.

Comprehensive studies on fractionated radiation-induced radioresistance and the subsequent activation of signaling pathways were also conducted utilizing an isogenic cell line developed through repeated irradiation of the lung cancer cell line A549 and a pivotal role of the transcription factor Nrf2 in promoting radioresistance was uncovered. These findings highlighted the Nrf2 pathway as a potential target for radiosensitization, presenting a promising avenue in the fight against resistant lung cancer subpopulations.

The cumulative efforts of the Bio-Science Group at BARC underscore a profound understanding of fractionated irradiation-induced signaling. These studies not only unravel the molecular intricacies of radiation response but also pave the way for potential therapeutic strategies. By targeting specific signaling pathways and genes, such as p44 MAP kinase, Rad52 and Nrf2 researchers aim to enhance the sensitivity of cancer cells to radiotherapy, thereby improving treatment outcomes.

7. Chronicles of Charged Particle Induced Radiation Signaling Work at BARC

The Bhabha Atomic Research Centre (BARC) has long been at the forefront of radiation research, pioneering advancements that bridge physics and biology. Within this hallowed institution, the Bio-Science Group embarked on a journey to unravel the mysteries of charged particle-induced radiation signaling, a field teeming with promise and challenges. The team's research has significantly advanced our understanding of how different forms of ionizing radiation, such as heavy ions, carbon ions, protons, and oxygen beams, influence cellular signaling pathways and DNA damage responses. Through meticulous research and relentless pursuit of knowledge, they illuminated pathways that hold the potential to revolutionize cancer therapy and enhance our understanding of cellular responses to high-linear energy transfer (LET) radiation.

7.1. Early Explorations: NF-kappaB and ERK Pathways

In 2004, a pivotal study examining the effects of heavy ion irradiation on the expression of NF-kappaB and extracellular signal-regulated kinase (ERK) in cells was published. Heavy ion irradiation was found to be more biologically effective than gamma radiation due to its propensity to cause clustered DNA damage. The study revealed that both NF-kappaB and ERK, which are critical for cell survival and act as anti-apoptotic factors, exhibited fluctuating levels post-irradiation. This suggested that the enhanced biological effectiveness of heavy ions might be due to altered signaling patterns, potentially leading to increased mutagenicity and inhibition of apoptosis.

7.2. Delving Deeper: Carbon Ion Irradiation

Further expanding on the above work, another study published in 2005 investigated the effects of carbon ion irradiation on signaling pathways. The work focused on the expression of p44/42 MAPK and NF-kappaB, which are crucial for cell survival. It was

found that the responses to high and low doses of carbon ion irradiation were markedly different. Specifically, the expression of p44/42 MAPK varied with the dose, and its inhibition by wortmannin, a DNA-PK inhibitor, indicated a complex interplay between DNA repair mechanisms and signaling pathways. Notably, NF-kappaB expression was higher at 1Gy compared to 0.1Gy and was not affected by DNA-PK inhibition. These findings underscored the need for further investigation into the time-dependent nature of cellular responses to high LET radiation.

7.3. Cytoprotective Pathways after Proton, Gamma and ROS

In 2009, differential activation of mitogen-activated protein kinases (MAPKs) in murine macrophage cells following exposure to gamma and proton radiation was explored. This study demonstrated that the activation patterns of ERK, JNK, and p38 kinases varied significantly between proton ions and X-rays. High LET radiation resulted in a prolonged but marginal activation of the prosurvival ERK pathway and a significant activation of the proapoptotic p38 pathway. This research highlighted the distinct cellular responses elicited by different types of radiation and emphasized the importance of the MAPK signaling cascade in determining cell fate post-irradiation.

7.4. Proton Beam and Lung Adenocarcinoma Cells

The quest to differentiate the biological effects of proton beams from gamma radiation continued with a study published in Cancer Investigation in the year 2010. It was found that proton beams were more cytotoxic to A549 lung adenocarcinoma cells than gamma radiation, leading to distinct signaling outcomes. The proton beam-induced cell death was linked to the upregulation of pro-apoptotic Bax and downregulation of anti-apoptotic Bcl-2, revealing crucial insights into the mechanisms driving proton beam therapy's effectiveness.

7.5. Carbon Beam and DNA Damage Response

A 2011 study further investigated the differential signaling responses to gamma and carbon beam irradiation in A549 cells. Despite both radiation types activating the same repair molecules, such as ATM and BRCA1, the study found significant differences in the nature and extent of DNA damage responses. Carbon beams, characterized by high LET, induced early-phase apoptosis and did not activate the prosurvival ERK pathway, unlike gamma radiation. This suggested that the distinct macromolecular complexes formed by high and low LET radiation could explain the differential cellular responses observed.

7.6. Oxygen Beam Induced Signaling Differences

In the same year 2011, another study compared the effects of oxygen ion and gamma irradiation on A549 cells. Oxygen beams were found to be significantly more cytotoxic, inducing efficient DNA repair only in cells exposed to gamma radiation but not in those exposed to oxygen beams. The study highlighted the significant activation of sensor proteins ATM and ATR, as well as Chk1, Chk2, and p53, in oxygen beam-irradiated cells. This comprehensive analysis provided valuable insights into the unique signaling pathways activated by different types of high LET radiation

7.7. Comprehensive Analysis: Proton vs. Gamma Irradiation

In 2015, an extensive study was conducted on the biological effects of proton and gamma irradiation on human non-small cell lung carcinoma cells (A549). Various biological endpoints, including gene expression, cell cycle, cell death, epithelial-mesenchymal transition (EMT), and cancer stem cell traits were investigated. Proton beams were found to be twice as cytotoxic as gamma radiation, inducing higher and longer cell cycle arrest, and affecting a broader range of genes. Proton irradiation also reduced cell adhesion, migration ability, and the population of cancer stem cell-like cells, indicating its potential biological advantages in cancer therapy. Their studies corroborated the radiation biologist's apprehension of use of 1.1 RBE for protons which were found be more cytotoxic to cells due to activation of various pathways that were triggered. Protons therefore, did not just have a physical dose deposition advantage but also a biological advantage in terms of lower metastatic potential and stemness of the irradiated cells. Hence, there use in treatment of pediatric tumors could all the more be justified and preferred and that considering their higher toxicity, the RBE of 1.1 could be revisited so as to reduce the dose to pediatric patients while achieving the desired tumor cell kill.

The Bio-Science Group at BARC has thus, made monumental strides in understanding charged particle-induced radiation signaling. Their work has unveiled the intricate signaling pathways activated by different types of radiation, providing a foundation for developing more effective cancer therapies. Through their dedication and pioneering research, they have illuminated the path toward a future where radiation therapy can be tailored to maximize its therapeutic potential while minimizing adverse effects. The legacy of their discoveries continues to inspire and guide the scientific community in the quest for breakthroughs in radiation biology and oncology.

While the impact of radiation-induced signaling within cells was being meticulously studied to understand its effects on cellular fate, intriguing discoveries were made regarding the communication between irradiated and nearby unirradiated cells. A dedicated team in the Bio-Science Group was trying to uncover the mysteries of how cells not directly exposed to radiation could still experience profound biological changes, thanks to signals from their irradiated neighbors.

8. The Unseen Messengers: Unraveling the Bystander Effect

The journey began in the year 2006 with studies on bystander effects of gamma radiation on murine lymphocytes. Irradiated conditioned medium (ICM) from gamma-irradiated lymphocytes was used to observe its effects on unirradiated lymphocytes. The results were startling. Unirradiated lymphocytes exposed to ICM showed increased proliferation, elevated levels of reactive oxygen species (ROS), and enhanced expression of proliferation markers such as CD25 and cyclin D. This finding suggested that soluble factors released from irradiated cells initiated a cascade of signaling events in non-irradiated cells, potentially leading to increased radioresistance.

Building on this foundation, in the following year, gap junction-independent signaling was found to be responsible for the observed bystander effects in human erythroleukemia cells (K562). The medium from irradiated cells, rich in signaling molecules and stable free radicals, activated critical proteins like NF-kappaB and p21 in bystander cells, leading to apoptosis and other stress responses.

These studies were further extended to explore intercellular communication between different cell types. It was found that irradiated lymphoma cells (EL-4) and macrophages (RAW 264.7) could also induce bystander effects. Interestingly, this signaling was highly dependent on the activation of inducible nitric oxide synthase (iNOS) and the subsequent production of nitric oxide (NO), emphasizing the role of specific signaling pathways in mediating these effects.

Meanwhile, the differential responses of normal and cancer cells to bystander signaling was being investigated by separate teams in BSG. A study published in the year 2011 highlighted that low-dose and high-dose gamma-irradiated conditioned medium (ICM-L and ICM-H, respectively) from human leukemic cells induced varying levels of apoptosis in both normal lymphocytes and cancer cells. The dose and dose rate of radiation played crucial roles in determining the extent of the bystander response, with high-dose ICM inducing more significant apoptosis in both cell types.

The complexity of these interactions was further elucidated and cytokine profiles of various tumor cell lines following acute and fractionated doses of gamma radiation were analyzed. It was revealed that the radiation-induced cytokine response varied significantly across different tumor types, influencing the survival and growth of bystander cells. This nuanced understanding suggested that radiation therapy's effectiveness could be modulated by targeting specific cytokines involved in bystander signaling.

Study conducted in 2015 focused on the role of ataxia-telangiectasia mutated (ATM) protein in bystander signaling between human monocytes and lung adenocarcinoma cells. The study demonstrated that ATM activation was crucial for the bystander effect, especially in maintaining cell survival and DNA repair mechanisms in both directly irradiated and bystander cells. Suppressing ATM with siRNA significantly reduced the bystander effect, highlighting its potential as a therapeutic target.

The culmination of these studies led to a profound understanding of how bystander effects could be harnessed in cancer therapy. In a groundbreaking *in vivo* study using a mouse fibrosarcoma tumor model, it was demonstrated that irradiated tumor cells could inhibit the growth of unirradiated bystander tumor cells. This effect was mediated by soluble factors such as cytokines and proteins like VEGF, Rantes, and PDGF, which were differentially expressed in the supernatants of irradiated cells. These findings provided a deeper insight into the damaging and protective aspects of bystander effects, offering new avenues for enhancing the efficacy of radiotherapy.

Through meticulous research and collaboration, the Bio-Science Group at BARC has significantly advanced our understanding of radiation-induced bystander effects. Their work underscores the intricate web of intercellular communication and the potential for

manipulating these signals to improve cancer treatment outcomes. The unseen messengers within cells continue to reveal their secrets, paving the way for novel therapeutic strategies in the fight against cancer.

9. Modern Era: Innovation and Future Directions

Navigating early technological limitations and safety concerns, research at BARC has evolved significantly in both knowledge and techniques. Initially, studies focused on estimating the activation of kinases or receptors were limited to enzyme assays, gel electrophoresis and Western blotting, where only individual proteins could be analyzed using specific antibodies and needed elaborate procedures and dedicated rooms. However, with advancements in omics technologies, it became possible to simultaneously examine the activation of multiple genes and proteins within a cell. DNA damage and repair studies, once reliant on lengthy methods like PFGE and comet assays, have been streamlined with quicker techniques such as gamma H2AX estimation using confocal microscopy or flow cytometry. The roles of signaling proteins can now be directly assessed through specific inhibition using si/shRNAs, which effectively reduce their levels in situ. Additionally, the availability of high-throughput systems enables the simultaneous study of the radioprotective or sensitizing effects of various inhibitors within a single assay. Furthermore, live-cell imaging systems at Bio-Science Group allow for the automatic monitoring of cell growth, freeing up researchers' time to stay updated with the latest developments in radiation signaling.

The modern era of radiation signaling research at the BARC has been marked by significant strides in innovation and the exploration of future directions in radiobiology. As the foundational principles of radiation-induced cellular damage and signaling pathways have been established, current research has shifted towards more complex and integrative approaches. These advancements aim to enhance our understanding of radiation's effects on biological systems and to develop novel strategies for improving therapeutic outcomes, particularly in cancer treatment.

One of the most promising areas of research has been the exploration of charged particle therapy, such as proton and carbon ion therapy, which offers superior precision in targeting tumors while sparing surrounding healthy tissues. Studies at BARC have demonstrated that high LET radiation, including heavy ions and protons, induces distinct cellular responses compared to traditional low LET radiation like X-rays. These findings have led to a deeper understanding of how different types of radiation affect signaling pathways, including those involved in DNA repair, apoptosis, and cell cycle regulation. The research has revealed that proton beams, for example, are more cytotoxic than gamma rays, potentially offering a biological advantage in reducing metastatic potential and cancer stem cell traits in tumors.

In addition to exploring new types of radiation, BARC scientists have also focused on the modulation of radiation-induced signaling pathways to enhance radiotherapy's effectiveness. This includes investigating the role of natural compounds, such as

curcumin and quercetin, in sensitizing tumor cells to radiation while protecting normal tissues. By targeting specific signaling pathways, such as the mitogen-activated protein kinase (MAPK) pathway, researchers aim to overcome radioresistance in cancer cells, a major challenge in current cancer therapy.

Another innovative direction in BARC's research has been the study of fractionated radiation doses, which mimic the clinical delivery of radiation in multiple sessions rather than a single high dose. This approach has provided insights into how cells adapt to repeated radiation exposure, leading to the activation of survival pathways that contribute to radioresistance. The identification of key genes and proteins involved in this adaptive response, such as Rad52 and Nrf2, has opened up new avenues for developing targeted therapies that can disrupt these pathways and improve the efficacy of fractionated radiotherapy.

Looking to the future, the integration of advanced technologies, such as genomics, proteomics, and bioinformatics, is expected to play a crucial role in further unraveling the complexities of radiation signaling. These tools will enable a more comprehensive analysis of how radiation affects cellular networks and may lead to the discovery of new biomarkers for predicting treatment responses and outcomes. Additionally, the development of personalized radiotherapy approaches, tailored to the unique genetic and molecular profiles of individual patients, holds great promise for improving the precision and effectiveness of cancer treatment.

The modern era of radiation signaling research at BARC is characterized by a commitment to innovation and a forward-looking approach to addressing the challenges of cancer therapy, mainly radioresistance.