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Review Article

The diverse and complex roles of radiation on cancer treatment: therapeutic target and genome maintenance

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Abstract: Cancer is a genetic disease, grows exponentially with the development of intrinsic and acquired treatment resistance. Past decade has witnessed a considerable progress towards the treatment and understanding of proposed hallmarks of cancer and together with advances in early detection and various treatment modalities. Radiation therapy is an integral part of cancer treatment armamentarium. In developed countries more than half of all cancer patients receive radiation therapy during their course of illness. Although radiation damages both cancer and normal cells, the goal of radiation therapy is to maximize the radiation dose to abnormal cancer cells while minimizing exposure to normal cells, which is adjacent to cancer cells or in the path of radiation. In recent years, life expectancy increases among cancer patients and this increase is due to the results of early diagnosis, screening efforts, improved treatments and with less late effects mostly secondary cancer development. Therefore, cancer survivorship issues have been gaining prominence in the area of radiation oncology research. Understanding the tradeoff between the expected decreases in normal tissue toxicity resulting from an improved radiation dose distribution to the targeted site is an increasingly pertinent, yet needed attention and research in the area of radiation oncology. In recent years, a number of potential molecular targets that involve either with radiation increased tumor cell killing or protecting normal cells have been identified. For clinical benefits, translating these findings to maximize the toxicity of radiation on tumor cells while safeguarding early or late normal cell toxicities using molecular targeted radioprotectors will be useful in radiation treatment.

Keywords: Cancer, radiation therapy, radioprotectors, normal genome maintenance

Introduction

Despite decreases in cancer related death rates in developed countries like USA and in western countries, the number of cancer cases and deaths are projected to be more than double worldwide in the next 20-40 years [1, 2]. The projected increase will be driven largely by growth and aging of populations. Past decade has witnessed a major leap in the understanding of molecular mechanisms involved in tumor pathogenesis, progression and further identified various treatment modalities to control this complex disease. Despite initial high response rates to the various treatment modalities and interventions, a large proportion of cancer patients suffered relapse in years or decades later [3-6], resulting a therapeutic challenge. International Agency for Research on Cancer (IARC) has predicted that by 2030, 27 million new cancer cases and 17 million cancer deaths will occur each year worldwide. That compares to 12.7 million new cancers and 7.6 million cancer death reported by GLOBOCAN 2008 [7].

Cancers are primarily an environmental disease with 90-95% of cases are due to modification in lifestyle and environmental factors, and only 5-10% of cancers occur due to an abnormality inherited from mother or father [8, 9]. Thus, cancer is largely considered a preventable disease. In recent years, many treatment and management options for cancer exist with the primary ones including: surgery, chemotherapy, radiotherapy and palliative care.

Radiation is an invaluable diagnostic and treatment tool used in various clinical applications. Radiation therapy or radiotherapy is a cornerstone of modern cancer management is a highly

effective and widely used to destroy (kill) cancer cells. More than half of all cancer patients mostly in the developed countries receive radiation in the form radiotherapy using various radiation sources [10-12] to cure the disease either alone or in combination with other treatment modalities such us chemotherapy or surgery. Radiotherapy is a highly cost effective single modality treatment, accounting about only 5% of the total cost of cancer care [13]. Radiotherapy (external beam or internal irradiation given as brachytherapy: such as protons, heavy ions, as well as a largely used standard sources –photons) is the most important non-surgical modality for the curative treatment of cancer, but its curative potential is often limited by intrinsic radioresistance of cancer cells/mass and normal cell toxicity.

The first clinical use of radiation for the cancer treatment was recorded in late 19th century [14, 15], soon after Roentgen described X-rays in 1895 and the effectiveness of radiotherapy that has been developed over the years showed an increase in the number of cancer survivors. As more cancer patients undergo radiotherapy and live longer after treatment, the number of cancer survivors in the United States America (USA) has tripled since 1971 and is growing by 2% each year [16, 17], therefore radiotherapy benefit a large number of cancer patients. Though accurate delivery of the radiation dose has been greatly improved, nonuniform dose distributions in the adjacent normal tissues or rests of patient body is inevitable and further this effect also mediated by cellular and tumor microenvironmental signaling [6, 18, 19]. Furthermore, radiation-induced damage of normal tissues surrounding the tumors limits the effectiveness of radiation treatment [20-22]. Long term post radiation exposure survivors particularly children face various health effects and also increased risk of post treatment cancer development in adjacent or at distanced organs, risk of second cancers or late toxicities [23-25]. However, advances in pediatric cancer treatment techniques and care yielded increase in survival during the last four decades. In the USA, approximately 90.4% of children with cancer will survive 5 years or longer in recent years (2000-2005) compared with 83.7% in 1990-1994 [24]. Therefore, there is an increasing concern regarding the radiation related risks or toxicities in long-term radiotherapy survivors [26-29] and counteracting these effects to achieve clinical efficacy.

Radiation and cell's response

Radiotherapy is based on the fact that ionizing radiation (a physical agent) can induce damage in biological tissues and thus efficiently kill tumor cells. In radiation oncology high-energy of radiation (typically 1 MeV or more) is used to eradicate the proliferating cancer cells. Biological effectiveness of radiation depends on the linear energy transfer (LET) that penetrate and deposits energy in tissues, total dose, fractionation rate and radio-sensitivity of the targeted cells or tissues. For cancer treatment, external beam radiotherapy is primarily used for local tumor irradiation. Radiotherapy can also be delivered in the form of targeted radionuclides (radiation is attached to a 'carrier' that selectively kills cancer cells by delivering a lethal dose of radiation) or brachytherapy (placement of radioactive sources in or just next to the tumor). Irradiation is most commonly delivered via photons released from a linear accelerators (X-rays, gamma rays and beta particles; low LET radiations), deposists a relatively small quantity of energy on the targeted tissue. On the other hand, high LET radiations protons (from a cyclotron or synchrotron) deposits more energy on the targeted areas and further causes more biological effects than the low LET radiations. Radiation damages the genetic material (DNA) causing single or double strand breaks, DNA base damage, DNA-protein cross links with chromosomal rearrangements in the cells and thus blocking their ability to divide and proliferate further [30, 31] (**Figure 1**), all these incidences are proportional to the absorbed dose. Although radiation damages both cancer cells as well as normal cells, the advancement in 3D conformal radiotherapy, intensity-modulated radiotherapy and proton beam therapy have all been developed to maximize the radiation dose to abnormal cancer cells while sparing the normal surrounding cells, preventing or reducing normal cells death or late effects has increasingly become a priority in the field of radiation oncology. A complementary approach is to explore mechanisms of protecting or reducing the normal tissue injury while increase the tumor cell killing probability using molecular targeted radioprotectors, which protect the genetic nature of normal cells are evolving. Although we do not have a comprehensive answer about the molecular mechanism of radiation-induced cancer on individual variations in susceptibility, especially of therapy-related cancers, it is well known that cancer is a complex multistep process and ra-

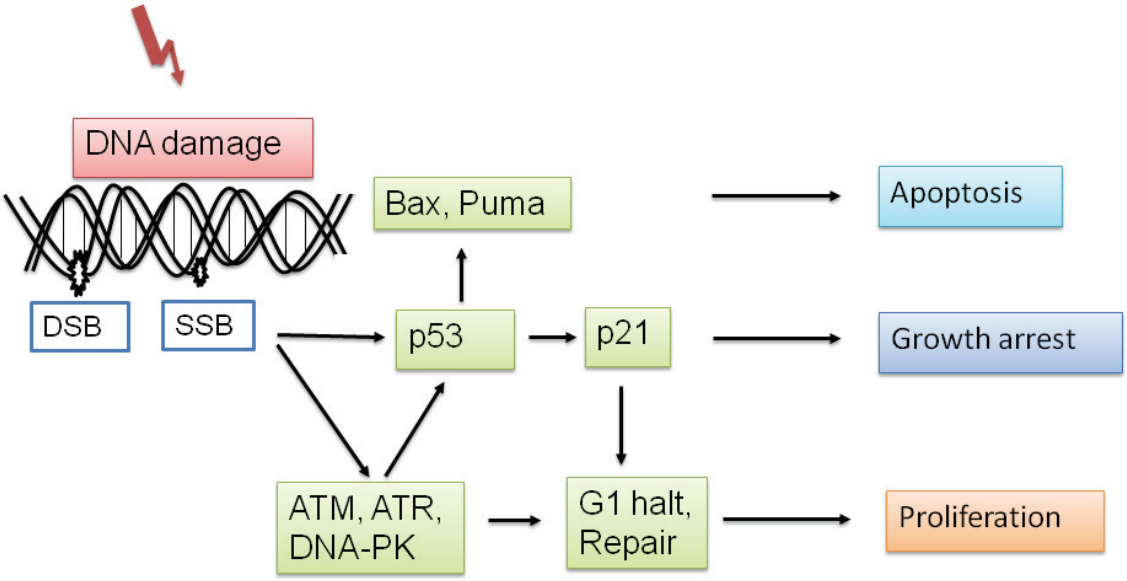

Figure 1. Radiation damages the genetic material (DNA) causing single strand breaks (SSB) or double strand breaks (DSB) in the cells, thus blocking their ability to divide and proliferate further.

diation can influence both initiation and promotion. During the past decade our knowledge of molecular basis of cancer cell resistance and survival has changed our understanding to maximize therapeutic benefits of radiation. Therefore, understanding this problem at a molecular level and any improvement in the efficacy of radiotherapy treatment will benefit a large number of cancer patients.

Radiation is administered single or mostly fractionated with small doses (1.8-2 Gy) of radiation over a period of 5-7 weeks [32]. Furthermore fractionated radiotherapy schedule will allow the recovery of normal cells from sub-lethal damage between radiation schedules, which provides enough time to repopulate the normal cells. Although repopulation also occurs in cancer cells, rapid cell proliferation or accelerated doubling time of cancer cells in which "key signals for growth and cell divisions are always on" than the normal cells [32, 33] showed more radiosensitivity for the better tumor control. In normal and cancer cells, radiation mainly causes accumulation of DNA either directly or by indirectly (through free-radicals) interfering with DNA synthesis is the common lethal mechanisms involved [34]. Cancer cells whose DNA is damaged beyond repair stop dividing and die. X-ray dose of ~1 Gy produces about 1000 single strand breaks (SSB) and about 50-100 double strand breaks (DSB) in a typical mammalian cell [35]. Although both single and double strand DNA breaks are observed, DSBs are the most responsible factor involved in radiation induced cell death [36]. Cells with DNA lesions (for example, incomplete replication and hypomethylation) arrest in the cell cycle at either the G_1-S or the G_2-M transition (G_1 and G_2 checkpoints, respectively). This allows the DNA repair machinery time to attempt repair it's genome and retain the genomic integrity. If the degree of DNA damage is beyond recovery, cells never enter mitosis, stop dividing and undergo cell death (also known as 'mitotic catastrophe'). Radiotherapy does not kill cancer cells right away. It takes hours, days or weeks of treatment before cancer cells start to die. Thus, identifying the importance of radiation induced effective cancer cell deaths and further mechanisms involved in protection of normal cells especially fast growing lymphoid organs, bone marrow, intestinal crypts, testes, and ovaries has potential and clinical implications for improving outcomes with radiotherapy.

Protective nature of cancer cells

Genetic and molecular approaches have fostered remarkable progress in our understanding of cancer development and also control/cure this growing disease. In the process of tumor

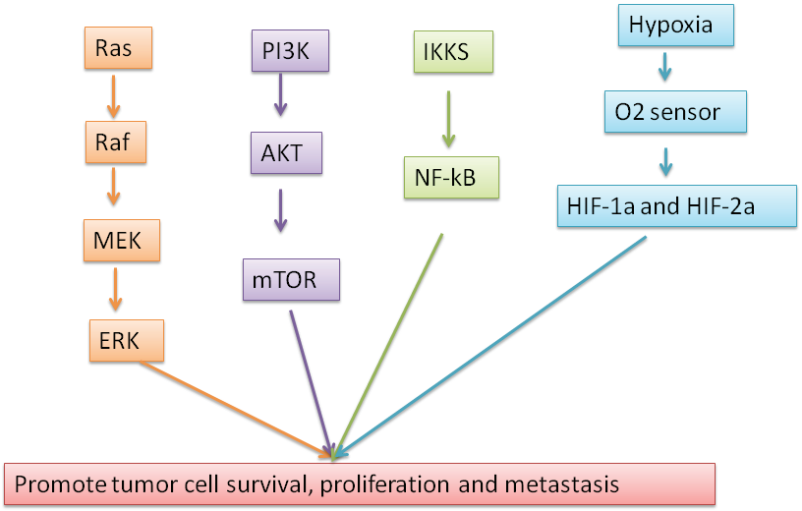

Figure2. Cytoprotective and prosurvival proteins/pathways involved in tumor cell survival, proliferation and metastasis.

formation, cancer cells acquired cytoprotective and prosurvival proteins/pathways (**Figure 2**) during the transformation process, suggesting that these pathways are important for tumor development and growth. Radiotherapy, like most anticancer treatments, achieves its therapeutic effect by interfering cell's cell cycle by halting the cell cycle and ultimately inducing cell death. However, various DNA repair mechanisms within the cancer cells interfere with the radiation induced damage and further increase the radioresistance of cancer cells [37]. Besides these repair pathways, signal transduction pathways are also involved in cellular response to radiation and this cellular responses showed an important implications for fractionated radiotherapy for the cancer cell survival, a protectant mechanisms of cancer cells for any genotoxic insults or radioresistant of the cancer cells is a major clinical problem. Of the many signaling cascades governing the stress response of a cell to various internal and external stimuli, it has been shown that four pathways demonstrated a clear role on the resistance to anti cancer treatment. Three of these PI3K-AKT-mTOR, nuclear factor -kB (NF-kB) and MAPK are involved in cell survival pathways (**Figure 2**) and further DNA damage activates ATM /ATR (ataxia telangiectasia-mutated/ ataxia telangiectasia and Rad3-related) which, in turn, activates proteins involved in cell-cycle control and DNA repair. Depending on the extent of damage and the repair capability of cells, the outcome is either cancer cell death or survival. Among these pathways, PI3K-AKT-mTOR signaling pathway is regarded as most important for the cancer cell survival and proliferation [38, 39], and further involved in the resistance to the anticancer treatment [40].

Oxygen is an essential regulator of cellular metabolism, survival and cell proliferation. Solid cancers are heterogeneous, in addition solid cancers also express hypoxic regions due to high rates of cell proliferation coupled with the formation of structurally and functionally poor vasculature with less nutrition support to divide and grow [41-43]. It has been shown that increased levels of hypoxia-inducible factors (HIF-1a or HIF-2a) in diagnostic tumor biopsies are associated with increased risk of mortality in cancers of bladder, brain, breast, colon, cervix, endometrium, head/neck, lung, ovary, pancreas, prostate, rectum, and stomach [44]. HIFs is a transcription factor which activates various genes that play key role in cancer progression/metastasis [45], including radiation resistance [46], with a potentially promising target for tumor radiosensitisation. Thus counteracting these stress response pathways will be toxic to the cancer cells, prevent further spreading into other part of the body and eventually kill the cancer cells. Defects in these cell proliferation pathways are hallmarks of cancer development [47, 48] and further radiotheraphy alone or with chemotherphy can remove the cancer cells and prevent the cancer spreading.

Normal cell radioprotection and challenges

In recent decades accurate delivery of radiation to the tumor site has greatly improved. However, it is not always feasible to eliminate a tumor without damaging the surrounding normal tissue. Tumor cells heavily rely on the activation of one or two pathways, a phenomenon known as oncogene addiction [49, 50], whereas normal cells use a broader range of molecular signals to overcome various cellular insults [51] to maintain the normal genome. Switching off the

pathways involved in cancer cell survival using chemicals before irradiation, while sparing normal cells will be an effective way to protect the normal cells. In contrast to normal cells, cancer cells mostly fail to activate damage sensor proteins involved in DNA repair pathways are often dysfunctional. DNA repair deficiency in cancer cells stimulates mutagenesis and further leads to tumorigenesis. However, at the same time these tumor cells are prone to the DNA damage by chemotherapy or radiotherapy treatments. From a clinical perspective, a good chemical radioprotectors must target this DNA repair pathways to kill the cancer cells differentially while protecting the normal cells. Therefore, a potential radio-protective agent should have more profound differential radiosensitizing effect on cancer cells include cell cycle arrest, apoptosis, direct and indirect effects on DNA bases, repair proteins and tumor vasculature. Understanding this problem at a molecular level, while reducing any unwanted deposition of radiation doses to the surrounding normal cells/tissues is a central pursuit in radiobiological research.

Repopulation of cells in critical normal tissues between individual dose fractions of either radiotherapy or chemotherapy is an important factor for the recovery or retention of normal organ function, thereby improving tolerance to treatment. Normal cells have a tremendous ability to repair DNA against radiation damage by activating "damage sensor" proteins such as activating ATM, and ATR, checkpoint kinase 1 and 2 (CHK1 and CHK2) or p53 [52-54]. PI3K also plays a role in the integral functions for noncancerous (normal) cells repopulation [55] along with the DNA repair proteins. Once DNA damage detected and sensor proteins activated, each lesion can be repaired by at least one of the six major DNA-repair pathways: BER (base excision repair); NER (nucleotide excision repair); DR (direct repair); MMR (mismatch repair); HR (homologous recombination) or NHEJ (non-homologous end joining) pathways [56, 57], if not, cell death occurs due to residual or misrepaired DNA double-strand breaks [58]. In contrast to normal cells, in cancer cells DNA-repair path-ways are dysfunctional and this may make tumor cells prone to the DNA-damaging agents such as radiotherapy and/or chemotherapy [53, 59, 60]. Furthermore understanding the biological mechanisms involved in signaling pathway(s) for resistance, cell cycle checkpoints, DNA damage and repair, anti-angiogenesis could increase the therapeutic response of tumor microenvironment while sparing surrounding normal tissues. However, work on radioprotective chemicals started several decades ago in the USA, at the inception of the Manhattan Project and the available literature on the topic is enormous, this review focuses only on those relevant and potential agents which are of clinical importance with their mechanism of radioprotection.

In recent years, strategies to improve radiotherapy therefore aim to increase the effect on tumor while limiting the damage to the adjacent normal cells without sensitizing the normal tissues and ultimately without protecting the tumors to the radiation treatment. Despite the availability of various sophisticated technical improvements in radiotherapy [61, 62], treatment planning techniques and stereotactic body radiation therapy (SBRT) modalities, normal tissue toxicity remains a dose-limiting problem in therapeutic programs [19, 63]. The development of radioprotectors or radioprotectants that protect normal tissues surrounding the tumor cells against radiation damage is currently the subject of intense research [64]. Radioprotectors are chemicals and most of the radioprotective compounds are antioxidants, can be given before or at the time of radiation treatment of cancer control. In general, radioprotectors are drugs that are designed with the intent of minimizing the risk of clonogenic death of normal (noncancerous) cells from the damage caused by radiation. These agents also promote the repair of normal cells that are exposed to radiation.

In recent years many chemical agents that have been postulated which protects the normal cells by targeting newly identified molecular and physiological pathways. Therefore, detailed understanding of the pathways influenced by the radioprotectors, as well as identification and characterization of the participating proteins will significantly advance our ability to unravel the complex processes leading to the development of new drugs that protect normal cells/tissues. Despite technical improvements, no radioprotective drug available today shows all the requisite qualities to be an ideal radioprotector and many patients still suffer from recurrent disease after radiotherapy. An ideal radioprotector is relatively non-toxic to normal cells and easy to

administer without compromising the therapeutic effects of radiation treatment for cancer patients. For years, many radioprotective compounds have been developed, a majority of them designed to reduce the levels of radiation-induced free radicals within the cells. Indeed, after several decades of preclinical and clinical research, the first and only approved radioprotective drug by U.S. Food and Drug Administration (FDA) is amifostine, being used in clinical practice.

Amifostine is a prodrug (inactive form) which belongs to a general class of cytoprotective (cell -protecting) agents. In the body, amifostine is converted into an active thiol metabolite WR1065, which helps to protect the cells from DNA damage by scavenging free radicals [65]. The conversion of amifostine to WR1065 is catalyzed by alkaline phosphatase which is a pH dependent and occurring more rapidly in alkaline pH, tumor cells are acidic in nature [66, 67]. Once dephosphorylated, it can freely diffuse mostly into normal cells and can act as a free radical scavenger. Furthermore, the lower concentration of membrane-bound alkaline phosphatase and lower pH (acidic) in tumor microenvironments contributes to a relatively low concentration of active chemoprotectant in malignant tissues and therefore providing a selective cytoprotection of normal tissues [68, 69] around the irradiation field. Since tumors are relatively in hypoxic condition due to the poor vasculature, thus resulting in comparative hypoxia and a low (acidic) interstitial pH than normal tissues [70-73]. Therefore normal tissues can absorb greater level of amifostine than the tumor tissues, and furthermore cancer cells are not protected. The half-life of amifostine is approximately 9 minutes, whereas that of the active metabolite, WR1065, is approximately 15 minutes [74]. Thus, the schedule of amifostine administration is potentially an important factor for optimum efficacy for the radiation treatment to the cancer patients.

In both normal and cancer cells, ionizing radiation has been shown to generate reactive oxygen species (ROS), mitochondrial respiratory chain is a major source of reactive oxygen species under various pathological conditions [75-77]. Furthermore, mutation in mitochondrial DNA (mtDNA) increased the metastatic potential of tumor cells [78]. ROS cause oxidative damage to DNA, proteins, lipids, and other cellular components and therefore pose a significant cellular stress [79] and recent studies suggest that this biochemical property of cancer cells can be exploited for therapeutic benefits. Therefore targeting mitochondria is one of an essential step to therapeutic approach for the cancer control [80]. However, in cancer cells aberrant metabolism and protein translation generate abnormally high levels of ROS [81]. Agents that enhance ROS production, therefore, are expected to cause further stress overload in cancer cells by increase in DNA damage. Since ROS act as a mediator of the cellular damage induced by radiation, compounds that involved in the regulation of ROS may be of great interest in the protection of normal cells against radiation induced damage. For e.g. it has been shown that dichloroacetate, which inhibits pyruvate dehydrogenase kinase (PDK) and therefore stimulates mitochondrial oxidative phosphorylation and ROS production, such mechanisms can selectively increase DNA damage related apoptosis in cancer cells but not in normal cells [82]. Similarly, reducing the cellular ROS buffering capacity through the inhibition of glutamate-cysteine ligase (a rate-limiting enzyme in cellular glutathione synthesis) can markedly increase the radiosensitivity of cancer cells [83].

In humans, as in most animal cells, maintenance and expression of mtDNA are essential for the normal cell survival and metabolism. Manganese superoxide dismutase (MnSOD) exists exclusively in the mitochondria and scavenges toxic superoxide radicals produced by radiation. Therefore it has been proposed that MnSOD play a role in protecting cells against ROS damage during the radiation exposure. Lee et al. [84] showed mitochondrial damage such as altered permeability transition, increase in accumulation of reactive oxygen species, reduction of ATP production and morphological change induced by radiation in cells and mice were protected by the treatment of MnSOD. Preclinical studies in mouse have demonstrated that the expression of human MnSOD transgene confers protection of normal tissues from ionizing irradiation damage and also radiosensitizing the tumor cells [85-87]. Administration of manganese superoxide dismutase plasmid liposomes (MnSOD-PL) carrying DNA damage control genes has been demonstrated to provide local radiation protection to the lung, esophagus, oral cavity, urinary bladder and intestine [88, 89]. Therefore, over expression of MnSOD

has been shown to sensitize the cancer cells to radiation, while differentially over expression in normal cells protect from irradiation [90].

In cancer patients undergoing radiation treatment, highly proliferating organs like intestine and bone marrow limits its beneficial effects. Using mouse model, Burdelya and colleagues recently tested a new approach to protect the intestine from irradiation-induced injury. Burdelya et al. [91] developed an NF-κB-activating CBLB502 a polypeptide drug by modifying a small fragment of a *Salmonella flagella*. CBLB502 is a bioengineered derivative of a microbial protein derived from flagellin. Human immune response to flagellin can be explained by two facts: 1. flagellin is an extremely abundant protein in flagellated bacteria, 2. a specific innate immune receptor that recognizes flagellin is toll like receptor-5 (TLR-5). Most TLRs activates NF-κB, which transcri-ptionally regulates a diverse array of genes. These include cytokines, chemokines and their respective receptors, which are associated with the important role of NF-κB in the inflammatory response, together with genes regulating cell survival, proliferation, cell adhesion in the cellular microenviroment [32, 92-94]. All these events activated by TLR-NF-kB protect normal cells by preventing death of the cell. Although the NF-κB gene targets may be similar between normal and cancer cells, the difference is the 'appropriateness' of the signal and their regulation [95]. For example, in tumor cell NF-κB targets may show sustained induction of their expression, resulting from the loss of negative feedback control mechanisms. Furthermore, NF-κB-transcription dependent gene expressions can either promote growth and survival of cancer cells or contribute towards tumor suppressor mechanisms, depending on the status of tumor cell for e.g loss of key tumor suppressors such as p53 or PTEN can drive NF-κB towards oncogenic and tumor-promoting activity [96]. Therefore, NF-κB pathway can be exploited as a target for normal cell survival from radiation injury.

Burdelya et al. [91] injected CBLB502 into mice before total body irradiation. The treatment prevented radiation induced mortality or completely protected the animals against radiation induced damage. Another interesting application of the drug is that it protects normal cells without diminishing the therapeutic antitumor effect of radiation and further without promoting radiation induced carcinogenicity in the tumor cell injected mice. CBLB502 uniquely showed combined properties of supportive care (radiotherapy adjuvant) and anticancer agent, both mediated via the activation of TLR-5 signaling in normal tissues or in tumor, respectively [96]. Thus, TLR-5 agonists CBLB502 could potentially protect normal cells, while improving the therapeutic index of radiotherapy when large and actively proliferating organs like intestine or bone marrow are considered as a dose limiting factors. MnSOD gene therapy and CBLB502 are still in the early stages of development but in the available radioprotectors it could allow for safer and more effective radiation treatment while protecting the normal cells.

Conclusion

With the improved clinical outcomes of cancer treatment, minimizing radiotherapy related toxicities on normal cells/tissues microenvironment is a priority. Although the overall cancer treatment time allows the repopulation of cells in normal tissues, repopulation of surviving cancer cells also occurs, thereby increasing the number of cancer cells that must be eradicated with the minimal damage to the normal cells. In recent years, development of radioprotective agents that spare healthy tissues during radiotherapy and also to protect against accidental, occupational and terrorist-initiated radiation exposure is the subject of intense research. Though in radiotherapy accurate delivery of radiation dose has been greatly improved over the past 2-3 decades, a major aim in the field of radiotherapy is to identify efficient ways of killing the cancer cells to improve the cancer cure rates, while decreasing the toxicities of normal cells is the greatest challenge in the field of radiation oncology of modern medicine. On the other hand, radiation injuries to the normal cells/tissues represent a significant challenge, because pathophysiology of radiation interactions on cellular communications between cancer and normal cells are not completely understood as yet. The increased nature of cancer cell proliferation with the decrease in cell division time is highly suggestive that the rapid cell proliferation might be sensitive to radiation induced cell killing effect. However, over the past decade, molecular biological approaches applied to radiobiological questions have uncovered several mechanisms by which cancer and normal cells differently respond to ionizing ra-

diation. In recent years, a number of potential molecular targets has been identified and the hope is that translating these basic research findings to enhancing the toxicity of ionizing radiation on the tumor cells for therapeutic exploitation will be able to target only cancer cells with the goal of sharply reducing the number of people suffering and dying from cancer disease.

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