

Recent Advances in Radiation Therapy of Cancer Cells: A Step towards an Experimental and Systems Biology Framework

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Abstract: Due to rapid emergence of recombinant and antibody-based reagents targeting specifically biomarkers of disease, radiolabeling of antibodies has enabled the imaging and therapy of various reactive oxygen species (ROS)-mediated pathological conditions, such as cancer. Key contributions to this topic have been dissected through two main standpoints: (1) immunotherapeutics for advanced cancer care, including radiolabeling for cancer imaging and therapy, design and testing of antibodies, and radioimmunotherapy innovations for treating malignancies and (2) search for a more efficient drug-targeted delivery method for cancer therapy. Because tremendous progress has been made in recent years, the future of cancer radioimmunotherapy is suggested to be bright. The question, whether measurement of oxidative damage to DNA has clinical relevance, is addressed. To make biomarkers of oxidatively damaged DNA useful clinical tools, further validation of biomarkers, followed by further elucidation of the role of damage in disease, is suggested. To understand the role of oxidative damage by focusing on cellular processes under oxidative stress conditions, the complementarities of mechanistic cell biology studies and systems biology strategies in identifying new therapeutic targets are demonstrated for liver cancer cells. Since most morphological, physiological and molecular studies on death of cells in tissues have been carried out on isolated cell populations, systems biology is suggested to be a means of overcoming known difficulties manifested by interference and interaction with surrounding cells. The elucidation of fundamental background of the ability of cells to interpret the same signal action in distinct fashions - survival vs. death signal transduction is suggested to facilitate more localized and efficient treatments of various ROS-mediated pathologies.

Keywords: Cancer radiotherapy, imaging, biomarkers, cell death, systems biology.

1. INTRODUCTION

Various agents and processes such as drugs [1], ionizing radiation [2], and autoxidation [3] induce damage to DNA *in vitro* and *in vivo* [4]. Up to 10^3 oxidative damaging events upon the DNA of each cell in the human body are estimated to occur every day [5]. Among reactive oxygen species, such as singlet oxygen (1O_2), hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($\bullet OH$), and hydroperoxyl radical ($HOO\bullet$), hydroxyl radical is believed to be the most damaging [5,6]. ROS are able to disturb the oxidant-antioxidant balance in the human body in favor of the former, thereby influencing many pathological conditions. Such pathologies, including cancer, Alzheimer's disease, Parkinson's disease, sepsis, osteoporosis, diabetes, multiple sclerosis, rheumatoid arthritis, and inflammatory bowel diseases are essentially associated with genetically programmed cell death (PCD), a phenomenon that plays an important role in normal development, morphogenesis, tissue remodeling, and immune regulation [7]. To understand signaling pathways underlying PCD, it is important to note that most morphological, physiological and molecular studies on death of cells in tissues have been carried out on isolated cell populations due to known difficulties manifested by interference and interaction with surrounding cells. The key puzzle is thus to elucidate fundamental background of the ability of cells to interpret the same signal action in distinct fashions, e.g., survival vs. death signal transduction [8]. To facilitate more localized and efficient treatments of various ROS-mediated pathologies, resolving the puzzle could make the identity of particular therapeutic targets more knowable.

Cancer as one of the most threatening human diseases is most commonly treated by chemotherapy. The key problem associated with such cancer treatment is that chemotherapies are relatively non-specific. Besides targeting tumor cells, the therapeutic drugs acting intravenously may cause general systemic distribution with deleterious side effects on surrounding healthy cells. There are some pitfalls in the search for a novel, more generally applicable drug-targeted delivery method for cancer therapy. The key objectives are (i) to reduce both the extent of systemic distribution

of the cytotoxic drug and unwanted side effects and (ii) to reduce the dosage influenced by more localized targeting of the drug [9]. In this light, recent advances in radioimmunotherapeutics for improved cancer care are herein reviewed. In the context of an advanced stage in cancer progression such as peritoneal carcinomatosis characterized by the spread and implantation of cancer cells throughout the peritoneal cavity, special emphasis is placed on development of an ideal vehicle to carry therapeutic agents selectively to the target [10]. More efficient magnetically controlled chemotherapy [9,11] with its clinical applications is also discussed. In the context of liver cancer cells, the complementarities of mechanistic cell biology [12] and systems biology approaches [13] towards both understanding signaling pathways underlying the pathology and identifying relevant therapeutic targets are presented.

2. IMMUNOTHERAPEUTICS FOR ADVANCED CANCER CARE

Promising radioimmunotherapy based on a team approach to patient management is associated with building and encouraging relationships between oncologists and nuclear medicine physicians [14,15]. The contemporary trend was conspicuously promoted at the 52nd Annual Meeting of the Society of Nuclear Medicine, which was an important event held in Toronto, Canada, in June 2005.

Radiolabeling for Cancer Imaging and Therapy

The potential of radiolabeled proteins, particularly antibodies, to act as diagnostic and therapeutic reagents has been the subject of evaluation for many years [16,17]. Such reagents are imagined to be particularly useful as cancer therapeutics. Therefore, the importance of identifying tumor-specific antigens and cognate ligands or antibodies binding to such antigens is quite conceivable. By administering a radiolabeled ligand or antibody with binding specificity for a tumor-specific antigen, coupled to a radioisotope that has a short range, high energy and abundant particle emission, it may be possible to deliver a lethal dose of radiation directly to the tumor cell. Based upon the applicable particle range of the particular isotope, labels may be chosen according to their suitability for targeting a particular type of cell. Gamma emitters generally serve for diagnostic purposes, i.e., visualizing tumors, but are found ineffective as killing agents. In contrast, alpha and beta

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emitters may be employed to effect cell killing [18]. Even though one-particle emission in some cases may be quite suitable to effect cell killing, alpha emitters may be particularly useful for blood-borne diseases or vascular tumors where good penetration is needed [19,20]. Evaluation of relative biologic effectiveness of an alpha-emitter *in vivo* [21] for the ^{211}At radioimmunotherapy of subcutaneous human ovarian cancer has been recently undertaken [22]. The chemistry of targeted radiotherapeutics has indicated that the radiolytic effects of ^{211}At alpha-particles influence the N-succinimidyl 3- ^{211}At -astato benzoate synthesis [23]. Some promising results with targeted alpha-particle therapy [24,25] have been observed in the context of melanoma therapy via peptide-targeted alpha-radiation [26]. The first clinical experience with alpha-emitting radium-223 in the treatment of skeletal metastases has been reported [27]. Applications of engineered liposomes for potential alpha-particle therapy of metastatic cancer have been hypothesized [28]. Even though there is evidence for the use of alpha-particle immunotherapy for a variety of malignancies, particularly those in which small-volume, minimal residual, or micrometastatic disease is present [19], antitumor effects of this therapy may be further enhanced by the use of alternative radioisotopes [29-36]. For instance, tumor-specific ^{225}Ac radioimmunoconjugates could kill a variety of tumor cell lines *in vitro* at doses 1000 times lower than ^{213}Bi -containing constructs [37]. Also in xenograft models of disseminated human lymphoma and solid prostate carcinoma, single doses at kBq (nanocurie) levels are able to prolong survival, and consequently cure a substantial fraction of animals without toxicity [38]. While alpha emitters need to be located right at the cell surface [39,40] as indicated for the targeting of breast cancer cells [41], beta emitters (such as ^{90}Y) with a longer emission range are applicable for bulkier, more localized disease.

There are several reasons elucidating why ^{90}Y is particularly suited for radioimmunotherapy and radioligand therapy. The 64-hour half-life of ^{90}Y is sufficiently long to allow antibody accumulation by the tumor and, unlike e.g., ^{131}I , it is a pure beta emitter of high energy (a maximum amount of 2.27 MeV) with no accompanying gamma irradiation in its decay. The ^{90}Y particle emission range is 100 to 1000 cell diameters, providing a sufficiently minimal amount of penetrating radiation that outpatient administration would be possible. In addition, internalization of labeled antibodies is not required for cell killing, and the local emission of ionizing radiation should be lethal for adjacent tumor cells lacking sometimes the target antigen. The radiolytic nature of ^{90}Y and similar radioisotopes has been extensively discussed for many years [42,43]. Radionuclides like ^{90}Y deliver a large amount of radiation to the antibody both during the labeling process and during storage, leading to instances of significant antibody damage, which can eliminate preferential targeting of tumor cells and expose non-target tissues to significant levels of toxicity. The mechanism for radiation damage has been attributed to the generation of free radicals [44]. Energy of 2.2 MeV of the ^{90}Y -emitted beta particles [42] could easily break most chemical bonds including the disulfide bridges of an antibody, which have bond strength of only 4.4 eV [45]. Thus, the shorter the amount of time that the protein to be labeled is exposed to destructive radioisotopes such as ^{90}Y , the greater the probability will be that the protein will possess the structural integrity and binding specificity needed to interact with the target antigen up until the time it is administered and reaches the target site. This kind of skepticism on the applicability of yttrium radiolabeling kits in hospital and outpatient settings has been recently dissolved [46]. This invention for radiolabeling proteins, peptides and ligands with radiolytic isotopes, particularly yttrium-90, has provided a substantial progress in the field, whereby sufficient purity, specific activity and binding affinity are achieved such that the radiolabeled protein may be directly administered to a patient without further column purification. The long felt need that has been recognized by many cancer patients and doctors with

regard to the commercial applicability and accessibility of protein-based, radiolabeled cancer therapeutics is now satisfied by the fact that labeling with ^{90}Y may be achieved in as little as one-two minutes or even as quickly as 30 seconds [46]. There is a family of proteins - integrins, which are generally expressed at low levels on epithelial cells and mature endothelial cells, but they are highly expressed on the surface of both endothelial cells in tumor neovasculature and some tumor cells, including osteosarcomas, neuroblastomas, glioblastomas, melanomas, and carcinomas of the lung, the breast, the prostate, and the bladder. There is a recent evidence that the expression of some ^{90}Y -labeled integrins is well-correlated with the tumor progression in melanoma, glioma, and ovarian and breast cancers [47]. Note a recent work on the cell uptake and radiotoxicity of an nuclear localization signal peptide-intercalator conjugate labeled with $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ [48]. The studies have established that a dose-dependent significant radiotoxicity of the nucleus-targeting radiopharmaceutical is clearly related to the low energy decay of $^{99\text{m}}\text{Tc}$. The principal result may imply a possible therapeutic strategy based on the use of the low-energy Auger electron-emitting $^{99\text{m}}\text{Tc}$ radionuclide attached to nucleus-targeting molecules and comprising an intercalator [48].

Radiolabeling of antibodies has influenced a recent approval of rituximab, gemtuzumab ozogamicin, alemtuzumab, and ibritumomab tiuxetan for cancer radioimmunotherapy by the US Food and Drug Administration (FDA). Most extensively investigated the monoclonal antibodies, such as (mAb) and anti-CD20 mAbs, have been proven to possess definitive clinical efficacy. Rituximab is a genetically engineered chimeric anti-CD20 mAb, having mouse variable and human constant regions [49]. Based upon clinical trials, rituximab is shown to be a highly effective agent (either as a single agent or in combination with chemotherapy) with acceptable toxicities against indolent and aggressive B-cell non-Hodgkin's lymphomas. Minimum toxicity to normal cell is associated with radioimmunotherapy that uses the mAb to target radiation to lymphoma tissue [50-57]. Beta-emitting ^{90}Y ibritumomab tiuxetan (*Zevalin*; IDEC Pharmaceuticals Corporation, San Diego, California) and I-131 tositumomab (*Bexxar*; GlaxoSmithKline, Philadelphia, Pennsylvania) have shown clinical efficacy in relapsed B-cell non-Hodgkin's lymphomas with acceptable toxicities [58-63].

Design and Testing of Various Antibodies

Immunodiagnosis is the topic of vital importance dedicated to delineating cancer. There have been several recent contributions to this topic that need to be highlighted. The development of a pretargeting strategy for carcinoembryonic antigen (CEA)-expressing tumors with a new bispecific mAb has been reported [64]. The very high tumor-to-background ratios have been accomplished with this agent due to its high specificity and rapid background clearance. The researchers have also proposed a similar strategy with bispecific antibodies for pretargeting of renal cell carcinoma [65]. The radiolabeled bispecific pretargeting system with dynamic imaging has been evaluated, and a bispecific pretargeting system has been shown to be superior to directly radiolabeling antibody targeting methods because of an increase in the signal-to-background ratio [66]. The indications have been examined using a radiolabeled anti-P-glycoprotein (Pgp) antibody, where Pgp is a membrane efflux pump protein that is upregulated in some tumors [67]. Pgp is associated with multidrug resistance and poor response to several chemotherapeutics. *In vivo* targeting and visualization of Pgp have been argued to provide knowledge on multidrug resistance prior to treatment. The studies on nude mice bearing human uterus sarcoma cells with either high- or low-Pgp expression have indicated an average of 7.8% higher radioactivity concentration in the high-Pgp-expressing tumors [67].

Micropositron emission tomography (PET) is a practical technique that is usually employed to obtain *in vivo* biological

information quantitatively with a wide variety of positron emitter labeled radiopharmaceuticals. The radiochemical purity of the radiopharmaceuticals is important to the quality of a PET study. An ancient work, dealing with the stability of compounds labeled with ^3H and ^{14}C in the long-term storage, has suggested that the degree of radiolysis of labeled compounds depends on the level of radioactivity, the level of specific activity, the characteristics of the radiation from the radioisotopes, the structure of the labeled compound, and the position labeled [68]. There have however been successful studies dealing with the radiolysis of short-lived PET radiopharmaceuticals [69-73]. The development of [^{11}C]methyl triflate [74], a useful [^{11}C]methylation precursor, has influenced the possibility of producing ^{11}C -labeled PET radiopharmaceuticals with a large amount of radioactivity [75-79]. Some radiopharmaceuticals were labeled with extremely high specific activity (B4.7 TBq/mmol), and their effectiveness has been demonstrated in receptor imaging studies [80,81]. These novel developments have been associated with the increasing risk of decomposition by radiolysis. This is in line with the fact that many ^{11}C -radiopharmaceuticals, produced with a high dose of radioactivity and high level of specific activity, have low radiochemical purity due to radiolytic decomposition accompanying the synthesis. The problem has recently been solved by investigating the stability of ^{11}C radiopharmaceuticals with factors affecting the decomposition using a practical method of preparing ^{11}C -radiopharmaceuticals with high radiochemical purity [82]. The copper-64-labeled antibody fragments in HER-2 and CEA tumor antigen systems have been evaluated using PET. The CEA antibody fragment demonstrated excellent targeting. In the HER-2 system, an increase of the minibody size has been followed by enhancing targeting, suggesting that microPET imaging may be handy for tailoring the antibody fragment-targeting properties [83].

The spread and implantation of cancer cells throughout the peritoneal cavity, arising from malignancies that originate primarily in the peritoneum [84,85], as well as from gastrointestinal and gynecological tumors [86] among others, are associated with peritoneal carcinomatosis, an advanced stage in cancer progression that can hardly be controlled by a single treatment strategy or a combination of traditional treatment strategies. There are indications that selective targeting of therapeutic agents offers a promising therapeutic modality for these disseminated lesions [87-89]. The avidin (Av)-dendrimer-chelate complex, which can be labeled with indium-111, emitting Auger and conversion electrons, with very high specific activity, has been developed for internal radiation therapy of intraperitoneally disseminated tumors [10].

Radioimmunotherapy Innovations for Treating Malignancies

There have been several reports dealing with the emerging role of immunotherapy. Re-188-labeled 3E8 antibody (an affinity-improved anti-TAG-72 antibody against TAG-72 antigen, expressed by many human adenocarcinomas) has shown ability to suppress tumor growth temporarily [90]. Radiolabeling the antibody with the alpha emitter At-211 has shown to enhance the antitumor effect of trastuzumab (*Herceptin*) for animal-implanted ovarian tumors with high HER-2/*neu* antigen expression [91]. Initial biokinetic data on a new promising immunotherapy for neuroblastoma have been based on the radiolabeling of an antibody directed against the neural crest adhesion molecule, which is expressed on almost all neuroblastoma cells to a great extent [92]. Further improvements of the radioimmunotherapy may be accomplished by pretargeting. A possible strategy has been probed for the radioimmunotherapy of colon cancer, indicating that pretargeting with a trivalent bispecific mAb improves antitumor responses when compared with a directly radiolabeled antibody administered at equitoxic doses [93]. In a mouse model of medullary thyroid cancer, pretreatment with an antiangiogenic drug (thalidomide or cyclopeptidic vascular endothelial growth inhibitor)

has been associated with improved efficacy of the anti-CEA 131I-F6 mAb radioimmunotherapy with an acceptable toxicity [94].

Several encouraging results for the radioimmunotherapy of lymphoma have been reported. The safety and efficacy of an ongoing phase 1-2, multicenter, dose-escalation trial on humanized anti-CD22 epratuzumab radiolabeled with Y-90 have been raised to a satisfactory level [95]. The use of Y-90-ibritumomab tiuxetan in a stem cell transplantation-conditioning regimen has been shown to be relatively safe and may improve outcome in severe refractory non-Hodgkin's lymphoma [96]. The I-131 rituximab radioimmunotherapy of non-Hodgkin's lymphoma has indicated the potential for efficacious repeat treatments upon relapse [97]. The phase 1-2 data of Lu-177 rituximab (anti-CD20) for the treatment of relapsed lymphoma have demonstrated that this regimen is quite effective [98]. Noteworthy is the study on the Zevalin Image Registry done on patients in clinical trials prior to market launch [99]. Of more than 600 patients, only 1 patient has not received Y-90 ibritumomab tiuxetan because of altered biodistribution. Of the 953 patients treated within the 1-year time framework of market launch, less than .7% has been precluded from radioimmunotherapy because of altered biodistribution. The first In-111 ibritumomab tiuxetan scan has detected all the rare cases of altered biodistribution, suggesting that the typical second scan and potentially a third scan may be unnecessary [99].

3. RADIOLABELING OF NANOPARTICLES FOR MAGNETICALLY TARGETED THERAPY

In the search for a more efficient drug-targeted delivery method for cancer therapy, magnetically controlled targeted chemotherapy has been proposed [11]. A cytotoxic drug is attached to a biocompatible magnetic nanoparticle carrier before the particles are administered intravenously. The particle complex is focused at a particular target by applying external, high-gradient magnetic fields. The process of drug localization is essentially the interplay between forces exerted on the particles by the blood compartment and magnetic forces generated from the magnet. A drug is more localized if the magnetic forces exceed the linear blood flow rates in arteries ($\sim 10\text{ cm/s}$) or capillaries ($\sim 0.05\text{ cm/s}$) [11]. The tumor cells take up the drug released from drug/carrier using either enzymatic activity or changes in physiological conditions such as pH, osmolality, or temperature [100].

Successful cytotoxic drug deliveries have been achieved by using various animals models including swine [101,102], rabbits [100] and rats [103]. This strategy has also been employed to target cytotoxic drugs to brain tumors [104]. The major practical difficulty is that once the drug is released, no attraction of the drug by magnetic field is longer observed. The idea tested to solve the problem is that the therapeutic agent needs to remain attached to the magnetic carrier during the treatment. Targeting of a magnetic carrier coupled to beta emitters (Y90 and 188Re) has thus been shown to be quite effective for focusing radiation on the desired site in both animal and cell culture studies [105-108]. The 188Re-labeled albumin microsphere has been shown to be a promising agent for radiotherapy [109]. For the purpose of magnetically targeted therapy, the preparation of human serum albumin-coated magnetic particles of about 200 nm in diameter with narrow size distribution radiolabeled with 188Re has been reported [9].

Due to exposure to a variety of physical or chemical perturbations, cellular levels of damage may increase under oxidative stress conditions. Such consequences associated with modified DNA can give rise to alterations in cellular processes leading to mutations, cytotoxicity (apoptosis/necrosis), cytostasis, or proliferation [110]. In this context, whether measurement of oxidative damage to DNA has some clinical relevance or not is the question of vital importance.

4. DOES MEASUREMENT OF OXIDATIVE DAMAGE TO DNA HAVE CLINICAL RELEVANCE?

All reactive oxygen species do not cause direct damage to DNA [111]. For instance, H_2O_2 and $O_2^{\bullet-}$ initiate DNA damage by interacting with transition metal ion chelates, while the most damaging hydroxyl radical can form over twenty different products by attacking DNA bases [112,113]. Effects of oxidatively damaged DNA (ODD) upon cellular processes have been extensively reviewed [114-120]. Factors demonstrating that ODD is important for a particular disease are: (1) the formation of ODD is observed at the site of injury, (2) the formation of ODD is not detected well after disease is established, (3) prevention or removal of ODD formation has beneficial effects, and (4) induction of ODD in model systems at levels found *in vivo* corresponds to most of the symptoms of disease. Well-established methods for investigating the role of ODD in disease include high performance liquid chromatography (HPLC) [121-124], mass spectrometric methods [125-134], ^{32}P -post-labeling [135-139], immuno-detection [140-150], and comet assay [151-159]. Elevated levels of oxidatively damaged DNA have been measured and reported for numerous diseases [160]. Based upon these data, a hypothesis that ODD plays an integral role in the aetiology of a specific disease has been made. This standpoint has been thoroughly discussed [161].

Biomarkers of oxidative DNA damage can be used for monitoring oxidative stress, but they may have the potential both to act as markers of disease development risk (intermediate biomarkers of a disease endpoint) and to assess efficacy of therapy. Reference ranges for DNA damage markers need to be established before determining pathological levels of oxidative stress. European Standards Committee on DNA Damage (ESCODD) has made some progress towards defining an acceptable range of values obtained from a given tissue. However, several questions clearly show how much work remains before markers of ODD may be used clinically. First, does the range of damage levels alter substantially from one cell type to another? Second, do levels of damage vary between differentiated and undifferentiated (stem) cells? Third, do the levels of damage in DNA isolated from surrogate tissues correlate with those from other tissues? Finally, why do various methods of analysis generate different reference ranges? For example, levels of ODD measured by the Comet assay are significantly lower than those determined by HPLC-EC, as ESCODD recently reported. Despite these questions, ODD has been proposed to play an important role in disease, as there are multiple pathways for its repair [161]. Simply the cell does not tolerate this damage to persist. Therefore, further validation of biomarkers of ODD, followed by further elucidation of the role of damage in disease, is needed to make these biomarkers become viable clinical tools.

5. CELLULAR PROCESSES UNDER OXIDATIVE STRESS CONDITIONS: FROM EXPERIMENT TO SYSTEMS BIOLOGY

To understand the role of oxidative damage, it is important to focus on principal mechanisms that are responsible for cellular functions under oxidative stress conditions. A remarkable work [162], dating back to early seventies, has identified two pathways of PCD, apoptosis and necrosis, as well as a less likely variant of necrotic death. While apoptosis was characterized by condensation of nucleus and cytoplasm [163], necrosis was rationalized by abundant autophagic vacuoles with minimal or effectively no nuclear changes [164]. The unique features of an apoptotically dying cell have remained unchanged for different cell types and genotypes, and none of them has been observed in cells dying by necrosis. Noteworthy is that apoptosis and necrosis can be observed in the same cell. Therefore, a subtle balance between the two pathways, based upon the relative expression level of several genes, may be suggested [163].

Over the past decade, there has been a considerable progress towards identifying constituent components of the molecular mechanisms that trigger PCD. Molecular details of the overall machinery directing apoptotic pathways have been established in various types of cells [7,163]. An intrinsic component or a modulator of a particular pathway is capable of interacting with other signaling pathways within a cell. For instance, transcription factors acting on gene promoters are able to trigger several possible mechanisms, each of which could lead towards different outcomes in various biological contexts. The key issue is to elucidate fundamental background of the ability of cells to interpret the same signal action in distinct fashions, e.g., survival vs. death signal transduction [8]. As most morphological, physiological and molecular studies on death of cells in tissues have been carried out on isolated cell populations due to well established difficulties associated with interference and interaction with surrounding cells, it has been believed that cellular functions, such as signal transmission, may be discovered by transition from molecular to modular cell biology [165]. To achieve the goal, applications of the state-of-the-art systems biology methods, derived from engineering and computer science, may be quite relevant.

Due to the explosive progress in systems biology, biology has arguably established itself as one of the most exciting scientific fields [166]. The principal challenge facing systems biology is complexity in terms of investigating the interrelationships of all of the components in a functioning system in order to elucidate how the system works. With more than 30000 genes in the human genome the study of all relationships simultaneously becomes an incredibly difficult task [167]. Bearing in mind that biological phenomena cannot be predicted with the level of numerical accuracy as in classical physics, computational model building and signaling pathway analysis are anticipated to play a major role in the search for organizing principles [168]. Systems-level kinetic models are thus expected to explain dynamic behavior and go far beyond the static pictures of the topologies of signaling pathways [169]. In this light, the stronger interplay between an experimental mechanistic study done on liver cancer cells [12] and system biology strategy [13] in identifying new therapeutic targets and elucidating relevant signaling pathways is herein discussed.

An attractive pathway (Fig. (1)) for ROS-promoted cell death has been proposed [12]. It is shown that $TNF\alpha$ -induced ROS generation can either be suppressed by NF- κ B targets or lead to PCD by inactivating JNK phosphatase from the mitogen-activated protein kinase (MAPK) phosphatase family (MKP). NF- κ B therefore acts as a modulator of the $TNF\alpha$ -induced death response by controlling both ROS accumulation and MKP activity. This means that transient JNK activation is observed in cells with a negative regulator of PCD such as NF- κ B (solid line in Fig. (1)), while sustained JNK activation is observed in NF- κ B-deficient cells (dashed line in Fig. (1)). From a more biochemical perspective, ROS inhibit the JNK-inactivating phosphatase by both oxidizing and converting its catalytic cysteine to sulfenic acid. The induction of mitochondrial superoxide dismutase (MnSOD) has some impact on the prevention of ROS accumulation by NF- κ B. A possible

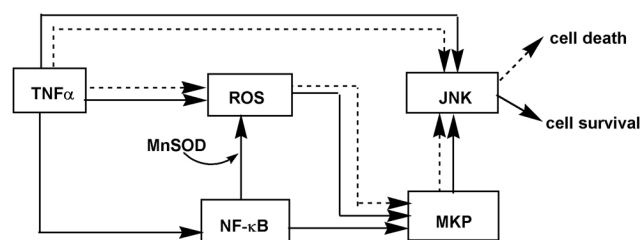


Fig. (1). $TNF\alpha$ -induced NF- κ B/JNK cross-talk [12]. Transient JNK activation in NF- κ B-rich cells (solid line) and sustained JNK activation in NF- κ B-deficient cells (dashed line).

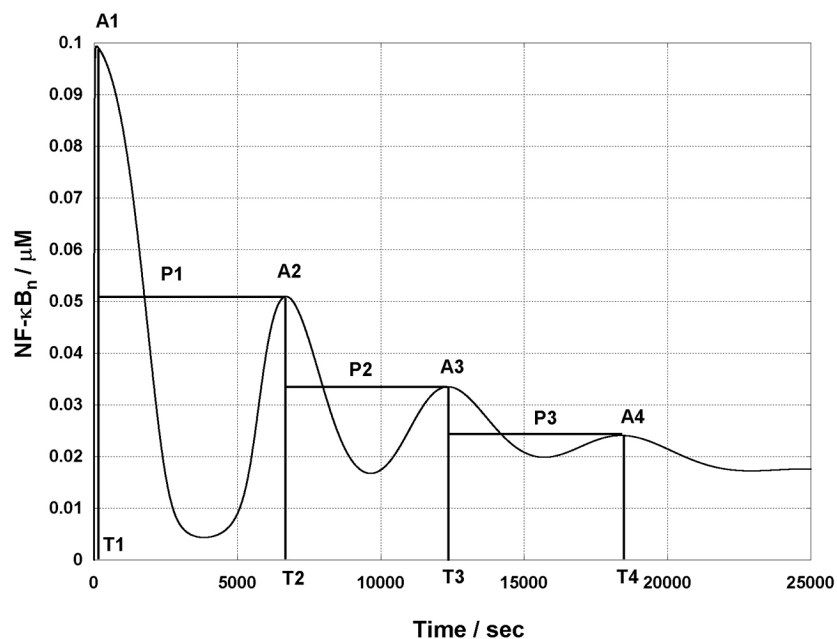


Fig. (2). Nuclear NF-κB (NF-κB_n) vs. time [13]. The definitions of the amplitudes (A), times (T) and periods (P) characterize nuclear NF-κB as a function of time.

mechanism for the TNFα-induced ROS generation is unknown [12]. To rationalize two different outcomes such as cell survival and cell death corresponding to transient JNK activation and sustained JNK activation respectively, a systems biology model taking into account a simultaneous interplay of the TNFα-induced NF-κB and JNK pathways has been developed [13]. Since MKP has been proposed as a possible therapeutic target [12], the kinetic model [13] has been employed to establish the correlation of MKP activity with JNK activation.

The ROS-mediated NF-κB/JNK cross-talk has been modeled using 36 species and 77 unidirectional reactions. The NF-κB pathway is based on the model of Ihekweba *et al.* [170], while the JNK pathway is formally the MAPK cascade of Kholodenko [171]. The two signaling routes are linked by three additional reactions being in between NF-κB and ROS, NF-κB and MKP, and ROS and MKP, respectively [13]. The ultimate goal has been to determine the “JNK concentration vs. time” plots underlying different outcomes (cell death and cell survival). As the NF-κB pathway of

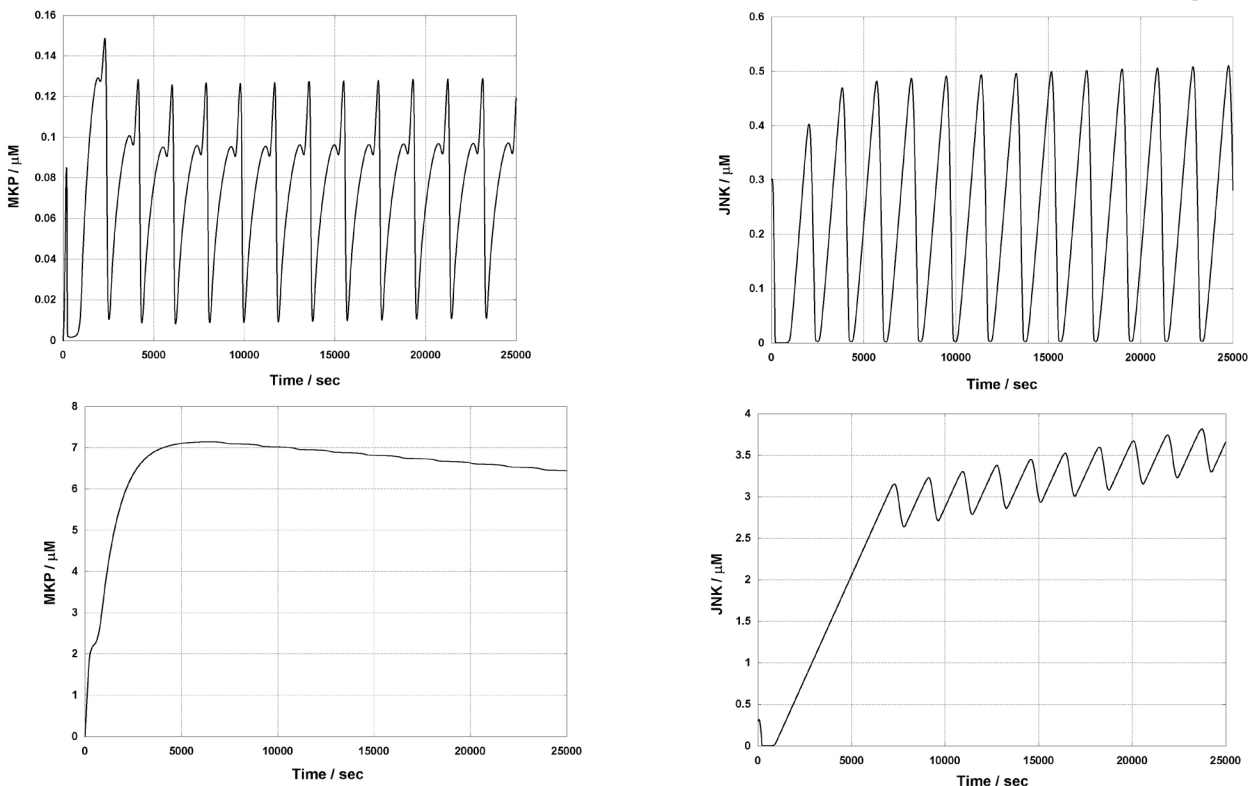


Fig. (3). MKP vs. time (left) and JNK vs. time (right) for initial ROS concentrations of 0.3 μM (top) and 10 μM (bottom). Note that the dynamic picture of JNK corresponds to a transient response associated with cell survival [13].

Ihekwa *et al.* [170] has been based on the $\text{I}\kappa\text{B}$ -NF- κB signaling pathway of Hoffmann *et al.* [172] modeling the experimental data reasonably well, the oscillatory concentration of NF- κB in the nucleus (NF- κB_n) for all initial levels of ROS being in the 0.3-10 μM concentration range has been observed (Fig. (2)).

Besides controlling ROS accumulation, NF- κB controls the TNF α -induced death response by influencing MKP activity [12]. Hence, the time courses of MKP associated with the time-dependent responses of JNK for ROS initial concentrations of 0.3 and 10 μM are given in Fig. (3). For an initial ROS concentration of 0.3 (Fig. (3top)), both MKP and JNK display a clear oscillatory behavior, that of JNK being quite similar to the time course of MAPK that has been reported by Kholodenko [171]. This means that the negative feedback, coming from JNK-PP and influencing the $\text{MKKK} \rightarrow \text{MKKK-P}$ reaction within the cascade [13], likely brings oscillations in the MAPK cascade [171]. This may indicate that the MAPK cascade has the prevailing impact on JNK as a function of time relative to the NF- κB signaling pathway. The dynamic plot of JNK is in line with a transient response to a stimulus [173]. By raising the initial concentration of ROS, from 0.3 to 10 μM , the presence of oscillations in the dynamic response of JNK is preserved, while the preserved MKP activity without oscillations becomes more visible (Fig. (3bottom)). Noteworthy is a more pronounced interplay between the NF- κB and JNK signaling pathways.

Based upon an extensive sensitivity analysis of parameters controlling oscillatory signaling, Ihekwa *et al.* [170] have shown that the NF- κB signaling pathway consisting of 26 species linked by 64 unidirectional reactions can be reduced to 9 most important reactions/parameters. Interestingly, these 9 parameters directly affect the concentrations of only 2 reactants other than NF- κB_n , IKK and $\text{I}\kappa\text{B}\alpha$. The three stated variables, IKK, $\text{I}\kappa\text{B}\alpha$ and JNK plotted against each other, reveal an intimate involvement of the mediators in the oscillatory behavior of JNK (Fig. (4)). The extreme non-linearity of the entire system indicates the broad range of possible instabilities, which could have therapeutically undesirable consequences for reducing ROS generation by mitochondrially-targeted antioxidants. The phase plane interpretation of the model (Fig. (4)) indicates that a much lower-dimensional representation of the system of 77 kinetic equations could narrow down the range of possible instabilities that the oscillatory signaling can exhibit.

In contrast to the negative feedback for transient JNK activation, MAPK cascade with a strong positive feedback can show a dynamics corresponding to one of the three different steady states: stable "off" and stable "on" states with low and high phosphorylation levels, respectively, separated by an unstable state determining a threshold level [174]. When the kinase activity is larger than the threshold, the cascade switches to its "on" state. The time course of the concentration of JNK, when the cascade activation exists and the negative feedback is not present, is shown

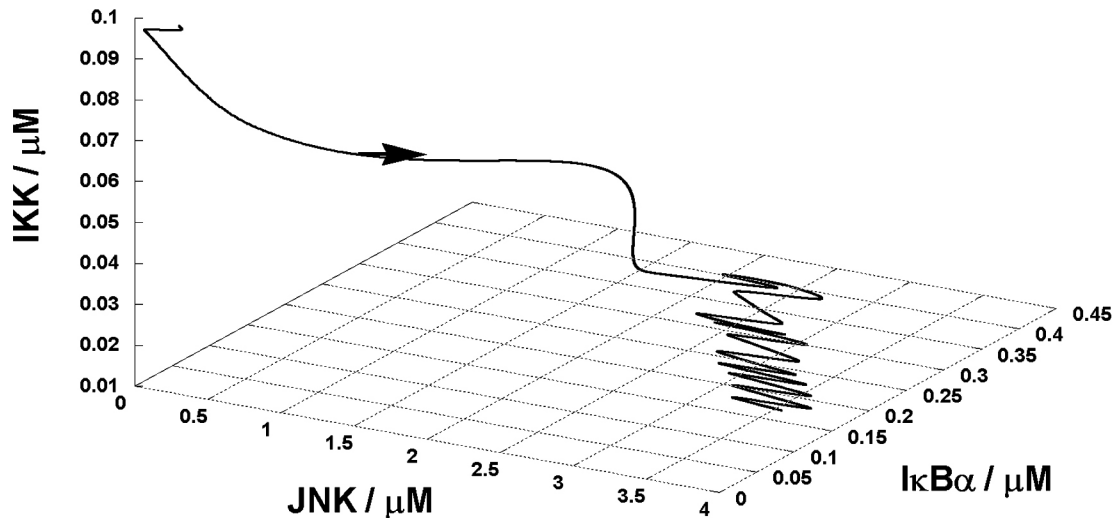


Fig. (4). Phase plane plot between the concentrations of IKK, $\text{I}\kappa\text{B}\alpha$ and JNK. Initial concentration of ROS is 10 μM [13].

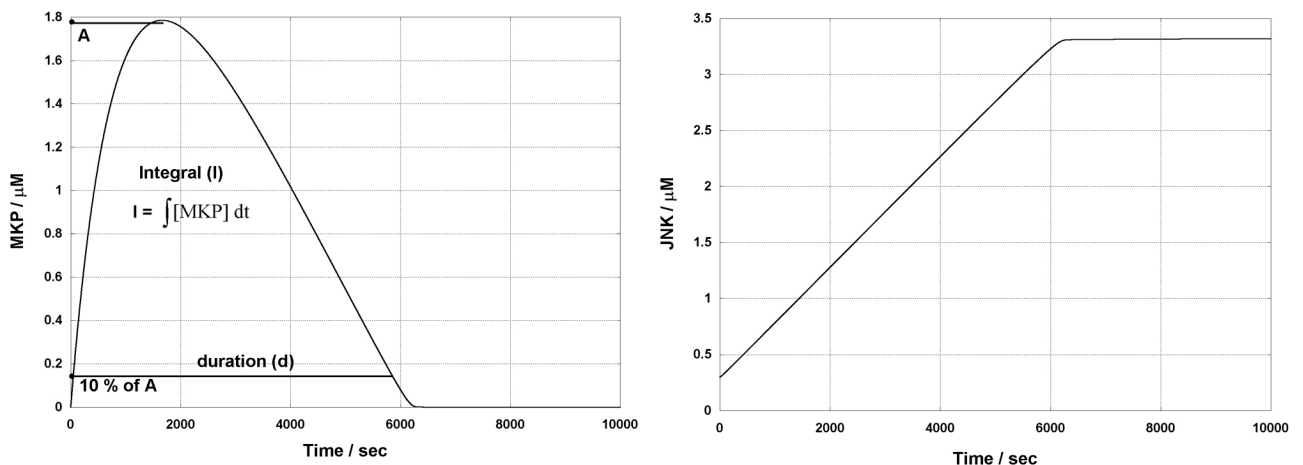


Fig. (5). MKP vs. time (left). Amplitude (A), duration (d) and integrated response (I) depict the transient profile of MKP. Sustained activation of JNK (right) corresponds to cell death. Initial [ROS] is 3 μM [13].

schematically in Fig. (5). This dynamic picture corresponds to a sustained JNK activation [173].

6. SUMMARY

The future of radioimmunotherapy appears to be bright. We continue to witness the development of new, radiolabeled, immune-based agents for the treatment of cancer. The long felt need that has been recognized by many cancer patients and doctors with regard to the commercial applicability and accessibility of protein-based, radiolabeled cancer therapeutics has recently been satisfied by the fact that labeling with ^{90}Y may be achieved in as little as one-two minutes or even as quickly as 30 seconds. The design and testing of various antibodies make immunodiagnostics more capable of delineating cancer before disease is really cancer. Some radioimmunotherapy innovations for treating malignancies have provided tools for further improvements of the radioimmunotherapy of various cancers. Substantial progress has also been made in the search for a more efficient drug-targeted delivery method for cancer therapy, such as magnetically controlled targeted chemotherapy. Possible validation of biomarkers of oxidatively damaged DNA and further elucidation of the role of damage in disease will make biomarkers viable clinical tools soon.

Most morphological, physiological and molecular studies on death of cells in tissues have been carried out on isolated cell populations due to known difficulties manifested by interference and interactions with surrounding cells. Hence, a novel means of investigating general principles governing cellular functions under oxidative stress conditions is needed in order to shed more light on the role of radiation damage in a specific disease. It is believed that, for example, signal transmission may be discovered by transition from molecular to modular cell biology. Systems-level kinetic models are thus expected to explain dynamic behavior and go far beyond the static pictures of the topologies of signaling pathways.

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