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REVIEW OF QUANTITATIVE MECHANISTIC MODELS OF RADIATION-INDUCED NON-TARGETED EFFECTS (NTE)

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Quantitative mechanistic modeling of the biological effects of ionizing radiation has a long rich history. Initially, it was dominated by target theory, which quantifies damage caused by traversal of cellular targets like DNA by ionizing tracks. The discovery that mutagenesis, death and/or altered behavior sometimes occur in cells that were not themselves traversed by any radiation tracks but merely interacted with traversed cells was initially seen as surprising. As more evidence of such 'non-targeted' or 'bystander' effects accumulated, the importance of their contribution to radiation-induced damage became more recognized. Understanding and modeling these processes is important for quantifying and predicting radiation-induced health risks. Here we review the variety of mechanistic mathematical models of nontargeted effects that emerged over the past 2–3 decades. This review is not intended to be exhaustive, but focuses on the main assumptions and approaches shared or distinct between models, and on identifying areas for future research.

INTRODUCTION

Brief history of quantitative mechanistic models of radiation effects

Radiation biology is one of the subfields of biology with a very long and rich history of mathematical modeling since the first half of the 20th century[1,2]. The important role which mathematical modeling continues to play in radiation biology is due to the interdisciplinary nature of this field, where biologists are often well connected with physicists and mathematicians. This connection lead to the development of *target theory*, which postulates that radiation damage in biological systems is caused by energy depositions and ionizations within sensitive cellular targets. Radiation 'hits' (traversals by ionizing tracks) on one or more of these targets lead to cell death (inactivation), mutation or other endpoints.

Initially, the nature of the targets was not well defined, but accumulating evidence led toward genomic DNA as the main target[3] and the double strand break (DSB) as the most important severe radiation-induced DNA lesion[4,5]. The important role of enzymatic repair processes for radiation-induced DSBs in cell survival and formation of chromosomal aberrations was investigated and mathematically modeled[6,7]. Radiation carcinogenesis was also modeled extensively by various approaches that postulated a transition from normal to malignant cells through two or more 'stages', potentially with clonal expansion[2,8,9].

Overview of studies of nontargeted or bystander effects

All of these models were generally based on the target theory concepts that radiation-induced damage such as lethal lesions and mutations are caused by energy deposition events in or very near (e.g. within the diffusion range of short-lived water radiolysis products) to genomic DNA. Implicitly, they also assumed that cells in a multicellular organism are effectively independent from each other with respect to radiation damage. Therefore, no radiation-induced damage was expected to occur in cells that were not 'hit' by radiation tracks.

Some evidence that did not fit into this paradigm gradually accumulated over several decades, reviewed by[10–15]. For example, 'clastogenic factors' were found in blood from irradiated individuals (e.g. cancer radiotherapy patients, Chernobyl accident victims), and 'abscopal effects' (tumor shrinkage) were sometimes observed in tumors that were quite distant from irradiated tumors in the same patient. Since these reports were sporadic and could not be well explained by existing theories, they did not alter mainstream radiobiological thinking for some time.

More attention to such phenomena, which were later called bystander effects (BE) or nontargeted effects (NTE), was drawn after the carefully-designed laboratory study by Nagasawa and Little in 1992[16]. This work showed that in cells exposed to ^{238}Pu alpha particles, the dose response for sister chromatid exchanges rose steeply in the dose range where most

cell nuclei were not expected to be 'hit' by any particle tracks. For example, at a very low dose of 0.31 mGy, Poisson statistics suggested that <1% of cell nuclei were traversed, but 30% of cells showed elevated sister chromatid exchange frequencies.

These findings were initially seen as surprising and controversial. However, similar phenomena were detected by subsequent experiments performed by many laboratories, reviewed by[10,11,15,17–19]. They involved multiple techniques, including medium transfer from irradiated cell cultures to unirradiated cultures, sparse irradiation with charged particles with not all cells being 'hit', and the use of animal models with partial shielding.

In most early NTE experiments, the fraction of cells or cell nuclei 'hit' by radiation tracks was estimated statistically, and it was not known precisely which cells were directly affected by radiation and which were 'bystanders'. This issue was addressed by more advanced experimental designs such as 'striped' cell culture dishes where some 'stripes' of a cell monolayer were exposed, whereas others were protected from radiation by shielding. Even more detailed information was generated by 'microbeam' experiments, where exact numbers of charged particles were delivered to exactly identified cells or subcellular structures[20]. Microbeams were used not only on cultured cells, but also on artificial 3D tissue systems and whole organisms[21,22].

These studies, which were conducted for almost three decades, accumulated a large amount of data on NTE induced by different ionizing radiation types—e.g. high linear energy transfer (LET) particles as well as low-LET photons—in a wide variety of biological systems including cells and whole organisms. NTE were demonstrated not only in laboratory mammals such as mice, but also in fish[23,24], invertebrate animals (e.g. *Caenorhabditis elegans*)[22,25] and in plants (e.g. *Arabidopsis thaliana*)[26,27]. The range of endpoints potentially affected by NTE is also very wide, and includes cell death, mutagenesis, oncogenic transformation, micronucleus formation, genomic instability (GI), gene expression changes, senescence, migration and/or differentiation alterations, and behavioral dysfunction[10,11,15,17–19,28]. Interestingly, NTE could be produced by targeted microbeam irradiation of cell cytoplasm, with the nucleus not being 'hit' at all[10,11,20].

GI which can be defined as persistently elevated rate of mutations and/or genomic rearrangements, is one of the most important deleterious NTE endpoints[17]. It can occur not only in directly irradiated cells, but also in bystander cells that interacted with the irradiated cells. It can be transgenerational, e.g. occurs in unirradiated offspring of irradiated male mice. The likely mechanism of this potentially strongly procarcinogenic phenomenon is epigenetic, for example because irradiated female mice apparently do not transmit it to the next generation unlike males because of 'resetting' of epigenetic signals in the maternal germline.

MECHANISMS AND IMPLICATIONS OF NTE

Molecular mechanisms of NTE

Although the NTE concept where damage and/or altered behavior sometimes occurs in cells that were not themselves traversed by any radiation tracks but merely interacted with cells that were traversed did not fit into the initial target theory-based paradigm of radiation biology, similar concepts were known in other fields. For example, bystander effects and GI can be induced by metals, chemotherapy agents and other toxic chemicals, or by photodynamic stress[10,17,29]. Therefore, radiation is not a unique agent for inducing NTE, and many other stressors can cause similar effects.

The wide variety of NTE causes and outcomes appear to be connected by the following general explanation: stress responses are propagated among cells to cause a group response that can involve a whole organ or even a whole organism. Some studies suggest that stress responses can be communicated even between different individuals[23,30]. In other words, the radiation responses of damaged and undamaged cells are not independent, as previously assumed, but can be connected by many short-range and long-range signaling mechanisms.

This biological phenomenon probably has a very ancient origin, likely predating the appearance of multicellular organisms. For example, unicellular life forms like bacteria communicate and can coordinate their stress responses by quorum sensing and other methods [31,32]. It is plausible to hypothesize that such mechanisms of intercellular communication evolved to respond to natural stressors such as toxic chemicals or infections. Sometimes they can serve a protective function against ionizing radiation exposure as well (e.g. adaptive responses, terminal cell differentiation), but in other cases they can 'overreact' or 'backfire', causing deleterious NTE outcomes such as GI.

The detailed molecular mechanisms that contribute to this multifaceted NTE phenomenon are still not completely understood, although significant progress was made in this field since its inception. Many studies point toward the following generalization about NTE mechanisms[10,11,19,29]. Ionizing radiation causes genotoxic DNA damage and elevates reactive oxygen and nitrogen species (ROS and RNS) concentrations. These reactive oxidants cause oxidative stress that damages DNA and other cellular components (e.g. proteins, lipid membranes) and perturbs multiple redox-sensitive intra- and intercellular signaling pathways (e.g. cyclooxygenase-2 COX-2, mitogen-activated protein kinase MAPK,

nitric oxide synthase NOS, NADPH oxidase, calcium signaling) and gene expression regulation. Positive feedback loops that involve oxidant production, oxidant-induced damage, and responses to this damage and to oxidative stress perpetuate the stressed state for long periods following irradiation. Damaged mitochondria appear to play an important role in initiating and maintaining this process. Since oxidants like ROS and RNS act as signals inside and between cells, persistent elevation of oxidant levels perturbs signaling and causes stress such as chronic inflammation [19].

The types of signals that propagate NTE between cells are very diverse, including small molecules capable of moving through gap junctions (e.g. lipid peroxide products, nucleotides), diffusible long-range signals like proinflammatory cytokines (e.g. tumor necrosis factor-α)[10], and potentially micro RNAs [10] and exosomes[33]. Susceptibility of cells to NTE signals from other cells is also a complex phenomenon, with likely involvement of ATR/ATM- and FA/BRCA-dependent DNA damage response pathways [29].

In summary, current knowledge of radiation-induced NTE mechanisms remains limited, but supports the involvement of the following components: (1) Induction of DNA damage and oxidative stress. (2) Perpetuation of these phenomena by perturbed signaling feedback loops and epigenetic regulation. (3) Propagation of perturbed signaling among cells, sometimes over long distances (e.g. to different organs or even organisms).

Consequences of NTE for understanding and predicting radiation effects

Much controversy in the field of radiation protection is associated with predicting and quantifying radiation risks (e.g. carcinogenesis) at low radiation doses/dose rates, which can occur during occupational exposures (e.g. nuclear industry workers, pilots, astronauts on long-distance space missions such as travel to Mars, some medical personnel), diagnostic medical procedures (e.g. x-rays, computed tomography (CT) scans), or accidental or malicious exposures to radioactive materials (e.g. contamination from nuclear power plant accidents like Chernobyl and Fukushima, potential terrorist attacks using radiological dispersal devices). At low doses, radiation effect sizes are small and statistically very difficult to detect using reasonable sample sizes and resources in experimental or observational studies (e.g. laboratory mouse irradiations or epidemiological studies of human cohorts such as Japanese atomic bomb survivors). Consequently, mechanistic mathematical models of radiation effects are very important in this field for making numerical predictions of risk magnitudes at doses where direct measurements are impractical or impossible.

The concept that all radiation-induced damage is caused by direct energy deposition events, where the sensitive 'target' is an individual cell or its nucleus, provides strong conceptual support for the linear no-threshold (LNT) model of stochastic radiation risks like carcinogenesis. Specifically, at very low doses the radiation exposure at the cellular or subcellular scale is stochastic, so that the target (e.g. cell nucleus) is either 'hit' or not, and reducing the dose simply reduces the average 'hit' probability in a linear manner, but does not reduce the average energy deposited by each 'hit'. NTE phenomena greatly complicate this picture because they expand the relevant target from a single cell or cell nucleus to a group of cells and potentially to an entire organ or organism. Therefore, the dose response involving NTE does not need to be linear even in the low dose range where not all cells are 'hit'. Moreover, even the 'sign' of the response becomes uncertain, because NTE can have either deleterious (e.g. procarcinogenic) or protective (e.g. anticarcinogenic) net effects.

Measuring NTE dose responses and characterizing their shapes is, therefore, an important issue for low-dose radiation biology and risk estimation. Current evidence suggests that NTE dose response shapes tend to be concave functions (with a negative second derivative) that deviate from zero very quickly at low doses and saturate/plateau at higher doses[11,16,17,34]. In contrast, damage associated with direct energy deposition—which can be called targeted effects (TE)—tends to have linear or convex dose responses (with a positive second derivative). These differences are intuitively explainable, considering that NTE are caused by onset and perpetuation of a stressed state by signaling pathways in large groups of cells responding to damage initially induced in a small proportion of 'hit' cells. The stressed state can approximate a binary on/off phenomenon, where the probability of the 'on' switch increases with dose, but the magnitude of the effect of this switch on affected cells is constant. In comparison, TE result from accumulation of energy deposition events from single radiation tracks (linear dose response component) and multiple interacting tracks (approximated by the quadratic component).

The observation of a concave/saturating dose response component at low radiation doses, where statistically not all cell nuclei are expected to be 'hit', can therefore be interpreted as circumstantial evidence for NTE involvement. This conclusion can apply to a variety of data sets, e.g. human and rodent lung cancers induced by radon exposure[34–36] and mouse tumors induced by high-LET radiations [37–39]. An interpretation involving NTE can also be applied to some data on internally incorporated radionuclides, e.g. chromosomal aberration and liver tumor

Table 1. Some early mathematical models of radiation-induced NTE. In this and the following tables, models are arranged in approximately chronological order and grouped by author and/or model type.

Model characteristics and assumptions	Main conclusions	References	Radiation type/source	Studied organisms/systems; endpoints
Brenner *et al*. bystander and direct (BAD) model. The model postulates that the oncogenic bystander response is a binary 'all or nothing' phenomenon in a small sensitive subpopulation of cells, and that cells from this sensitive subpopulation are also very sensitive to direct hits from alpha particles, generally resulting in a directly hit sensitive cell being inactivated.	Bystander effects are important only at small doses—below about 0.2 Gy. At still lower doses, bystander effects may dominate the overall response, possibly leading to an underestimation of low dose risks extrapolated from intermediate doses, where direct effects dominate.	(42)	Alpha particles	*In vitro* cell lines; initiation (oncogenic transformation)
Brenner *et al*. Some model-independent conclusions can be made regarding protraction effects: if the acute dose response is concave (downwardly curving), las occurs in bystander effects, protraction will tend to increase the effect instead of decreasing it.	Bystander effects represent a plausible quantitative and mechanistic explanation of inverse dose-rate effects by high-LET radiation, resulting in nonlinear dose–response relations and a complex interplay between the effects of dose and exposure time. A naive linear extrapolation of radon miner data to low doses without accounting for dose-rate, would result in an underestimation of domestic radon risks by about a factor of 4.	(34,35)	Alpha particles	Humans, lungs; lung cancer
The Brenner *et al*. 'BAD model' is refitted by Little to a slightly updated version of the 11 cohort miner lung cancer data, taking account of the covariance structure, and also exploring the effects of assuming various periods of latency between the development of the first premalignant cell and clinically overt cancer.	The fit of the original model is much improved by assuming a 5- or 6-year period of latency from the first appearance of a premalignant cell to cancer. The fit of this latter model is equivalent to that of a linear relative risk model with adjustment for age at exposure and attained age.	(43)	Alpha particles	Humans, lungs; lung cancer
Little *et al.* construct a novel model of the bystander effect that takes account of spatial location, and also of cell killing and repopulation. A particular feature of the model is the predicted augmentation of effect following fractionated delivery of dose, in a manner dependent on the total dose delivered.	The ionizing radiation dose- and time-responses of this model exhibit pronounced downward curvature in the high dose-rate region, similar to that observed in many experimental systems, reviewed in the paper. It is also shown to predict the augmentation of effect after fractionated delivery of dose.	(44)	Various	Various

Continued

Table 1. Continued

Model characteristics and assumptions	Main conclusions	References	Radiation type/source	Studied organisms/systems; endpoints
Little *et al.* multistage carcinogenesis model. Stochastic carcinogenesis model of Moolgavkar, Venzon and Knudson with two or more mutations was applied to a case-control dataset nested within the cohort and to the full cohort of lung cancer mortality in the Colorado Plateau uranium miners, taking account of exposure to cigarette smoke and to radon daughters. A functional form consistent with the bystander effect was included in the models.	The action of radon daughters and cigarette smoke was markedly nonlinear, particularly in their action on the mutation rates. The overall fit of the two-mutation model is somewhat worse than that of the three-mutation model.	(45)	Alpha particles	Humans, lungs; lung cancer
Nikjoo *et al.* ByStander Diffusion low-dose hyperradiosensitivity Model (BSDM). The model postulates that the oncogenic bystander response observed in nonhit cells originates from specific signals received from inactivated cells. The bystander signals are assumed to be protein-like molecules spreading in the culture media by Brownian motion. The bystander signals are assumed to switch cells into a state of cell death (apoptotic/mitotic/necrosis) or induced oncogenic transformation modes.	The bystander effect cannot be interpreted solely as a low-dose effect phenomenon. It is shown that the bystander component of radiation response can increase with dose and be observed at high doses as well as at low doses.	(46–48)	Various	*In vitro* cell lines; clonogenic cell survival and initiation (oncogenic transformation)
Extension of the Nikjoo *et al.* BSDM model, which assumes that all cells exposed by low-LET radiation send out one or more bystander signals, and that the hit-but-survived cells can accept bystander signal that may be followed by cell death or cell transformation.	A major assumption of the bystander model for high LET irradiation was the source of bystander signals emanated from the inactivated cells. In this paper, a lengthy analysis and discussions were provided on this and other assumptions of the model and how these could be modified in modeling of bystander effect caused by low-LET irradiation.	(49)	Various	*In vitro* cell lines; clonogenic cell survival and initiation (oncogenic transformation)
Jacob *et al.* Possible detrimental and protective bystander effects on mutation and malignant transformation rates were taken into account in the two-stage clonal expansion (TSCE) model.	Data were found to be incompatible with the model including a detrimental bystander effect. The model with a protective bystander effect did not improve the quality of fit over models without a bystander effect.	(50, 51)	Alpha particles	Humans, lungs; lung cancer

Continued

Table 1. Continued

Model characteristics and assumptions	Main conclusions	References	Radiation type/source	Studied organisms/systems; endpoints
Stewart *et al.* microdosimetric model. Generation of medium-borne signals is treated as distinct from the receipt and processing of the signals by other cells. The proposed model assumes that the emission of death signals is a stochastic process that depends on the number of times a cell is hit and the number of cells irradiated, regardless of whether the donor cells eventually live or die.	Our analyses suggest that the emission of death signals is a biexponential function of dose with a distinct plateau in the 5- to 100-mGy range. However, the emission of death signals by HPV-G cells may not become fully saturated until the absorbed dose becomes larger than 0.6 Gy.	(52)	Various	*In vitro* cell lines; clonogenic cell survival
Schollnberger *et al.* State Vector Model (SVM) describes initiation (formation of translocations) and promotion (clonal expansion and loss of contact inhibition of initiated cells). Additional terms either in the initiation model or in the rate of clonal expansion of initiated cells, describe detrimental bystander effects for chromosome aberrations.	The model is based on biological mechanisms relevant for initiation and promotion. The model can be used to describe nonlinear features in dose responses, such as detrimental bystander effects, but also LNT-shaped curves.	(53)	Alpha particles	*In vitro* cell lines; chromosomal aberrations, initiation (oncogenic transformation)
Schollnberger *et al.* State-Vector Model. This work integrates two important cellular responses to low doses, detrimental bystander effects and apoptosis-mediated protective bystander effects, into a multistage model for chromosome aberrations and *in vitro* neoplastic transformation.	An important data set that shows a low-dose detrimental bystander effect for chromosome aberrations was successfully fitted by additional terms within the cell initiation stage. It was found that this approach is equivalent to bystander-induced clonal expansion of initiated cells.	(54)	Various	*In vitro* cell lines; chromosomal aberrations, initiation (oncogenic transformation)
Schollnberger *et al.* Apoptosis induced in nonhit bystander cells is an important biological mechanism which operates after exposure to low doses of low-LET radiation. This process was implemented into a deterministic multistage model for *in vitro* neoplastic transformation.	The calculation of the time-dependent numerical solution of the model also allows to obtain information about the time-dependence of the protective apoptosis-mediated process after low dose exposures.	(55)	Various	*In vitro* cell lines; initiation (oncogenic transformation)

Continued

Table 1. Continued

Model characteristics and assumptions	Main conclusions	References	Radiation type/source	Studied organisms/systems; endpoints
Scott *et al.* NEOTRANS3 model includes DNA damage in cells that can be associated with varying degrees of genomic instability. Cells with persistent problematic instability (PPI) are mutants that arise via misrepair of DNA damage. Progeny of PPI cells also have PPI and can undergo spontaneous neoplastic transformation. Newly induced mutant PPI cells and their neoplastically transformed progeny can be suppressed via a protective apoptosis-mediated (PAM) process.	PAM occurs in a relatively narrow dose-rate dependent, low-LET dose window. The dose window for activating PAM can likely be extended upward by decreasing the low-LET dose rate or extending the exposure period (with dose rate being very low). PAM appears not to be activated by high-LET alpha radiation.	(56–59)	Various	Various; initiation (oncogenic transformation), carcinogenesis
Ballarini *et al.* review several previous NTE modeling approaches and present a new one. Main assumptions of their model, specific for high-LET irradiation of sparsely seeded cells, are the following: (a) each irradiated cell releases signaling molecules; (b) at each time step, the signals move in the extracellular environment according to the diffusion laws; (c) a reaction between a signal and a (bystander) cell occurs when the distance between the signal and the cell centre falls below a reaction radius; (d) whenever a reaction occurs, the signal molecule is ruled out of the simulation, whereas the cell will become damaged; and (e) bystander damaged cells can in turn emit signals.	A fully Monte Carlo approach under development at the University of Pavia was presented. Cells constitute an organized population that responds to external stimuli (such as ionizing radiation) collectively, communicating via different types of molecular signals.	(60–64)	Various	Various

Table 2. Some later mathematical models of radiation-induced NTE.

Model characteristics and assumptions	Main conclusions	References	Radiation type/source	Studied organisms/systems; endpoints
Fleishman *et al.* incorporate new biological concepts to improve the predictive ability of a state-vector model with respect to dose–response data on *in vitro* oncogenic transformation, including mechanisms of DNA damage, DNA repair, cell death, cell proliferation and intercellular communication.	Results suggest a protective, rather than detrimental, bystander cell-killing effect.	(65)	X-rays	*In vitro* cell lines; initiation (oncogenic transformation)
Leonard *et al.* biophysical composite adaptive response (AR) and bystander effect (BE) Microdose Model quantifies the accumulation of hits (Poisson distributed, microdose specific energy depositions) to cell nucleus volumes. This new composite AR and BE model provides predictions of dose response at very low dose BE levels, higher dose AR levels and even higher dose Direct (linear–quadratic) Damage radiation levels.	Bystander factor and AR protection factor are quantified in different data sets: *in vitro* studies and human lung cancers. AR is activated at most by one or two radiation induced charged particle traversals through the cell nucleus.	(66–68)	Alpha particles	*In vitro* cell lines; human lung cancers
Fakir *et al.* triggering-response model describes the bystander component as a sequence of two distinct processes: triggering of signal emission from irradiated cells and response of nonirradiated recipient cells; in principle it can incorporate microdosimetric information as well as the random aspects of signal triggering and recipient response. Late effects are modeled using a one-stage model based on the concepts of inactivation and initiation, which allows for the proliferation of normal and initiated cells; proliferation of initiated cells is analyzed using a stochastic, birth-death approach. The model emphasizes the dependence of bystander effects on dose, which is important for the assessment of low-dose cancer induction by extrapolations of risk from high-dose exposures.	We have argued that bystander killing analyzed is mainly due to signals transferred by the medium and not to gap junction communication. We have also suggested that bystander responses might not be relevant for directly irradiated cells because of triggering of protective mechanisms or because of the much higher direct effects at high doses.	(69)	Ultrasoft x-rays, alpha particles	*In vitro* cell lines; clonogenic cell survival and initiation (oncogenic transformation)

Continued

Table 2. Continued

Model characteristics and assumptions	Main conclusions	References	Radiation type/source	Studied organisms/systems; endpoints
Ebert *et al.* The interaction of radiation with a cell can result in the production of signals, which may be of more than one type. The complete response at any location therefore depends on both the radiation-interaction (RR) and bystander-interaction (RB) effects, assumed to be independent.	The bystander component in cell death was found to be significant. Further experimental evidence is required to determine how these results translate to the *in vivo* situation where tumor control probability (TCP) models that currently assume cellular independence may need to be revised.	(70)	Low-LET	Tumor radiotherapy; clonogenic cell survival
Hattori *et al.* 2D cell automaton simulation model. The model is based on a cellular automaton and consists of four components: (a) irradiation, (b) generation and diffusion of intercellular signals, (c) induction of DNA double-strand breaks (DSBs) and (d) cell-cycle modification or cell death. The intercellular signals are generated in and released from irradiated cells. The signals through the medium-mediated pathway (MDP) and the gap junctional pathway (GJP) are modeled independently based on diffusion equations. The irradiation and both signals raise the number of DSBs, which determines transitions of cellular states, such as cell-cycle arrest or cell death.	The analysis of model dynamics for the bystander cells revealed that the number of arrested cells did not increase linearly with dose. Arrested cells were more efficiently accumulated by the GJP than by the MDP.	(71)	Various	*In vitro* cell lines; dynamics of populations of cells, particularly cell-cycle modification or cell death. DSBs, clonogenic survival
McMahon *et al.* Kinetic-Based Model of Radiation-Induced Intercellular Signaling uses the following key assumptions: irradiated cells generate signal for an extended time period proportional to the delivered dose, regulated to reach some local equilibrium concentration; exposure to this signal above a certain threshold concentration can lead to a damaging response in cells, with a probability related to the time the cell is exposed to the signal above this threshold; this response is binary, with responding cells experiencing a characteristic level of cell damage and nonresponding cells seeing no damage; signaling-induced damage can occur in both hit and non-hit cells, and is additive to other sources of damage, such as that resulting from direct irradiation.	This model suggests that 'bystander' effects play a significant role in determining cellular survival, even in directly irradiated populations, meaning that the inclusion of intercellular communication may be essential to produce robust models of radiobiological outcomes in clinically relevant *in vivo* situations. We assume that the spatiotemporal evolution of the signals is modeled by a reaction–diffusion equation, incorporating the production and decay of the signals from the irradiated cells.	(72)	Various, including nonuniform exposures	*In vitro* cell lines; clonogenic cell survival, mutation frequency

Continued

Table 2. Continued

Model characteristics and assumptions	Main conclusions	References	Radiation type/source	Studied organisms/systems; endpoints
Butterworth and McMahon *et al.* A Monte Carlo model of cellular radiation response incorporated damage from both direct radiation and intercellular communication including bystander signaling. The predictions of this model were compared to previously measured survival curves for a normal human fibroblast line (AGO1522) and prostate tumor cells (DU145) exposed to spatially modulated fields.	The bystander effect is responsible for a significant portion of cell killing in uniformly irradiated cells. This description is a significant departure from accepted radiobiological models and may have a significant impact on optimization of treatment planning approaches if proven to be applicable *in vivo*.	(13, 73–75)		

yields in hamsters injected with Pu isotopes (reviewed in[10]), chromosomal aberrations in snail embryos[40] and embryonic mortality in wild rodents in areas contaminated by fallout from the Chernobyl accident[41].

REVIEW OF MATHEMATICAL MODELS OF NTE

Since NTE can play an important role in radiation responses, particularly at low doses/dose rates, understanding and mathematically modeling these processes is important for quantifying radiation-induced risks in a many types of exposure scenarios including occupational and medical settings, long-distance space exploration and radioactive contamination. Here we review the variety of mechanistic mathematical models of NTE that emerged over the past 2–3 decades. This review is not intended to be exhaustive, but focuses on the main assumptions and approaches shared or distinct between models, and on identifying areas for future research.

To make the comparison more convenient, the models are presented in table form (Tables 1–4) in approximately chronological order, grouped by authors and model types. The main assumptions and findings for each model are presented in the tables, preferably in the author's own wording to minimize misinterpretation. The types of radiation and endpoints investigated by each model are also provided.

DISCUSSION

Over the last few decades NTE phenomena are becoming more accepted and less controversial in radiation biology. It is increasingly clear that stress responses, including responses to ionizing radiation, can involve large cell groupings (organs, whole organisms) rather than individual cells. Such understanding is leading toward incorporation of NTE along with TE into a growing number of mechanistic mathematical models of ionizing radiation effects (Tables 1–4).

Some radiation circumstances where NTE can be particularly important include low dose/dose rate exposures, especially where radiations of different qualities (e.g. sparsely ionizing photons or electrons and densely ionizing charged particles) are involved (e.g. space exploration, radionuclide contamination). How NTE affect radiation-induced health risks in these situations, relative to the LNT assumption, is not completely understood and subject to continuing debate and controversy. Effects in both 'positive' (protective) and 'negative' (harmful) directions can occur. NTE are not limited to low doses and can also be important at high doses (e.g. cancer radiotherapy abscopal effects).

Table 3. Some more recent mathematical models of radiation-induced NTE.

Model characteristics and assumptions	Main conclusions	References	Radiation type/source	Studied organisms/systems; endpoints
Powathil *et al.* The multiscale mathematical model is developed by incorporating intracellular cell-cycle dynamics, an external oxygen concentration field and various effects of irradiation, including bystander effects that occur at multiple spatial and temporal scales. We assume that the spatiotemporal evolution of the signals is modeled by a reaction–diffusion equation, incorporating the production and decay of the signals from the irradiated cells.	We show that bystander responses play a major role in mediating radiation damage to cells at low doses of radiotherapy, doing more damage than that due to direct radiation. The greater cell-kill at higher doses reduces the number of bystander signal producing cells, resulting in lower bystander responses at higher doses.	(76)	Low-LET	Simulated tumor growth; clonogenic cell survival
Olobatuyi *et al.* Reaction–diffusion model allows for a full understanding of the life time of the bystander signal, based on a positive feedback loop. The model lets us quantify how much tissue damage is related to direct radiation damage versus indirect bystander damage.	In an heterogeneous environment, the size of the domain exposed to radiation and the number of radiation exposures can determine whether a signal will persist temporarily or permanently. We use sensitivity analysis to identify those cell parameters that affect the signal's lifespan and the signal-induced cell death the most.	(77)	Various	*In vitro* cell lines; clonogenic cell survival
Peng *et al.* test three assumptions concerning the effective range of bystander signals using both average and local measures of survival. Model 1 assumes short range signaling (e.g. gap-junction mediated) proportional to the local dose gradient, without relying on diffusion across the extracellular medium; Model 2 assumes metabolite diffusion governed by Fick's second law with either negative or both signs of bystander effect; Model 3 assumes that the extent of signal production is dependent on the average of the dose gradient over the field and that the signals have long range distribution.	All models gave better fits than the classical LQ model. Model 2 fitted best with one sign of bystander effect on survival. Model 3 gave the best overall fit of average survival.	(78)	Low-LET	*In vitro* cell lines; clonogenic cell survival
Dobrzynski *et al.* The sigmoidal function is used to model radiation effects, with modifications for bystander effects, adaptive responses, and other nontargeted phenomena.	The nonlinear type of cellular response to ionizing radiation is natural, irrespective of whether dose or dose rate effects are considered. It directly follows the organism's action of defense, an adaptive response that first grows with the dose, saturates or attains a maximum at a certain dose (e.g. 100–200 mGy) and then fades away when the dose is high.	(79)	Various	Various; carcinogenesis

Continued

Table 3. Continued

Model characteristics and assumptions	Main conclusions	References	Radiation type/source	Studied organisms/systems; endpoints
Ruhm *et al.* Biologically based mechanistic models that are used in combining current understanding of human carcinogenesis with epidemiological studies were reviewed.	Bystander effects on outcomes like mutation rates were included in some models. We note that there is of course much experimental evidence that mutations and clonal expansion play a role in carcinogenesis; by contrast the role of bystander effects is less clear.	(80)	Various	Various; carcinogenesis
Chang *et al.* considered two models, one representing targeted effects (TE), which assumes a linear dose response at low doses, and one representing nontargeted effects (NTE), which assumes a nonlinear threshold type response in addition to the linear dose term at low doses. NTE were assumed to be activated by a step function with a threshold dose at 0.001 Gy.	Theoretical modeling of the data show that a nontargeted effect model provides a better fit than the targeted effect model, providing important information at space-relevant doses of heavy ions.	(81)	Galactic cosmic rays	Rodents; carcinogenesis
Cucinotta *et al.* assume the TE contribution is valid with a linear response to the lowest dose or fluence considered, while an additional NTE contribution occurs. The parameters are estimated from low dose radiobiology experiments for mouse Harderian gland tumor induction and chromosomal aberrations. The 'turning on' of NTE at very low doses is estimated to occur at $\sim$1 mGy from alpha-particle experiments.	Prediction of fatal cancer risks for missions to the Martian moon, Phobos of 500-d and the Earth's moon of 365-d for average solar minimum condition show increases of 2- to 4-fold higher in the nontargeted effects (NTE) model compared with the conventional model.	(82, 83)	Galactic cosmic rays	Humans; carcinogenesis

Table 4. Mathematical models of radiation-induced NTE published by our research group and collaborators.

Model characteristics and assumptions	Main conclusions	References	Radiation type/source	Studied organisms/systems; endpoints
Our modeling approach assumes that the bystander phenomenon results from signaling molecules that rapidly propagate from irradiated cells and decrease in concentration (exponentially in the case of planar symmetry) as distance increases. These signals can convert cells to a long-lived activated state (e.g. oxidative stress); cells in this state are more prone to DNA damage and behavior alterations.	Only two parameter combinations are required to describe the shape of the bystander response as a function of distance away from the irradiated cells. Saturation of the fraction of cells expressing any particular bystander effect end point, which is often observed experimentally, is accounted for by our model.	(84)	Alpha particles	Artificial 3D skin system; apoptosis, micronuclei
Here we present a mathematical model-based on the main assumptions from(84), but for spatially homogeneous irradiation, and test it using data on the incidence of dysplastic growths and tumors in the mammary glands of mice exposed to high or low dose rates of gamma-rays and neutrons, either with or without pretreatment with the chemical carcinogen 7,12-dimethylbenz-alpha-anthracene (DMBA).	Our analysis suggests that the neutron-induced risks of mammary carcinogenesis for the selected mouse strain are dominated by radiation-bystander effect-mediated promotion of already premalignant cell clones. Initiation by neutrons of normal cells into the premalignant state could not be ruled out, but was not necessary for explaining the data.	(85)	Gamma rays, neutrons, chemical carcinogen	Mice; carcinogenesis
We used a mechanistically-motivated mathematical model which includes TE and NTE to analyze a large published data set on chromosomal aberrations in pond snail (*Lymnaea stagnalis*) embryos collected over 16 years from water bodies contaminated by Chernobyl fallout, and from control locations.	NTE were very important for describing the nonlinearity of the radiation response: the TE-only model (without NTE) performed dramatically worse than the TE + NTE model. NTE were predicted to contribute > 90% to the radiation response slope at dose rates < 11 μGy/h (0.1 Gy/year).	(40)	Radionuclide contamination from the Chernobyl nuclear power plant accident	Pond snail (*Lymnaea stagnalis*); chromosomal aberrations
A mechanistically-motivated model of targeted effects and nontargeted effects, based on our previous work, was applied to mouse tumorigenesis data induced by simulated galactic cosmic ray exposure.	The data showed clear evidence of nontargeted effects at low doses. Akaike weights for the model without nontargeted effects were 0.881, 0.266, 0.007 and 0.014 for gamma rays, ^{12}C ions, ^{28}Si ions and ^{56}Fe ions, respectively.	(39)	Galactic cosmic rays, gamma rays	$APC^{1638N/-}$ mice; intestinal tumorigenesis
We present a model, which quantifies targeted and nontargeted radiation effects. We fitted it to lung carcinogenesis data in radon-exposed miners and rats, which provide valuable information on carcinogenesis from protracted exposure to densely ionizing radiation.	Persistent activation of NTE signaling during/after irradiation can result in inverse dose-rate effects. This behavior of the NTE model contribution is consistent with earlier modeling approaches.	(36)	Alpha particles	Humans and rats; lung cancer

Importantly, research on NTE mechanisms seems promising for developing clinically useful pharmacological interventions. For example, agents that hinder the propagation of deleterious NTE such as development of GI and chronic inflammation can potentially reduce radiation-induced carcinogenesis. On the other hand, tumor abscopal effects could potentially be pharmacologically enhanced to improve cancer radiotherapy.

An overview of NTE modeling approaches (Tables 1–4) shows that mathematical modeling strategies keep evolving as the biological mechanisms of NTE become increasingly better characterized. Some models are very detailed to specific situations and data sets, whereas others are quite simple and general. Both of these approaches can be useful depending on the context.

So far, comparison of different NTE models with different sets of assumptions on the same data set is not done routinely. Instead, common practice involves fitting a single model or several similar model versions generated from the same conceptual framework. We believe that comparing the performances of different models on the same data sets could improve the NTE modeling field by identifying the most/least plausible models. Development of 'critical tests' for models could also be beneficial by allowing certain model assumptions to be falsified. Such approaches could improve the predictive power of NTE models for estimating the health effects of radiation exposures.

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