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SYSTEMS CANCER BIOLOGY AND THE CONTROLLING MECHANISMS FOR THE J-SHAPED CANCER DOSE RESPONSE: TOWARDS RELAXING THE LNT HYPOTHESIS

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□ The hormesis phenomena or J-shaped dose response have been accepted as a common phenomenon regardless of the involved biological model, endpoint measured and chemical class/physical stressor. This paper first introduced a mathematical dose response model based on systems biology approach. It links molecular-level cell cycle checkpoint control information to clonal growth cancer model to predict the possible shapes of the dose response curves of Ionizing Radiation (IR) induced tumor transformation frequency. J-shaped dose response curves have been captured with consideration of cell cycle checkpoint control mechanisms. The simulation results indicate the shape of the dose response curve relates to the behavior of the saddle-node points of the model in the bifurcation diagram. A simplified version of the model in previous work of the authors was used mathematically to analyze behaviors relating to the saddle-node points for the *J*-shaped dose response curve. It indicates that low-linear energy transfer (LET) is more likely to have a J-shaped dose response curve. This result emphasizes the significance of systems biology approach, which encourages collaboration of multidiscipline of biologists, toxicologists and mathematicians, to illustrate complex cancer-related events, and confirm the biphasic dose-response at low doses.

Key words: hormesis, bi-phasic behavior, systems biology approach, ionizing radiation, cell cycle control

1. INTRODUCTION

This paper contributes to the understanding of the effect of low dose exposures, focusing on cancer. The dose-response policy context of risk reduction depends on causal determinations in which dose-response models are pivotal. The coupling of legally mandated acceptable risks to a dose-response model provides doses (or exposures) safe for society (Ricci, 2006). In the US, for cancer, those exposures are regulated at either acceptable or tolerable public additional risk over background

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between $1*10^{-6}$ and $1*10^{-4}$ individual lifetime probabilities. Policy choices not based on sound dose-response models do not increase protection; paradoxically, less protection is likely despite the large sums spent to reduce what turns out to be a phantom hazard, created by conservative (erring on the side of precaution) assumptions that may be incorrect. Specifically, the issue we discuss is exemplified by the differences in the choices of the evidence and the models of dose-response, between the US and the French Academies (i.e., the US National Academies of Science, the French Academy of Sciences, and the French National Academy of Medicine) regarding the effects of ionizing radiations (IR) at low doses. Although the US Biological Effects of Ionizing Radiation (BEIR) (BEIR, 2006) supported the LNT, the French Academies raised doubts about its validity. The US used epidemiological studies; France instead included in vitro cell line results. Although these alternative choices of data and dose-response model are scientifically unambiguous, their different results for regulation are ambiguous. Alternative and essentially diametrically opposite views affect clean-ups, energy development, and consequently has many economic and social effects.

For cancer risk assessments, regulatory agencies (e.g., US EPA, 2005) default to linearity at low doses (LNT):

> … A default approach for linearity extends a straight line from the Point of Departure (POD) to zero dose/zero response. The linear approach is used when: (1) there is an absence of sufficient information on modes of action or (2) the mode of action information indicates that the dose- response curve at low dose is or is expected to be linear. Where alternative approaches have significant biological support, and no scientific consensus favors a single approach, an assessment may present results using alternative approaches.

In Europe, its laws address concepts such as the maximum residue limit (MRL) regulation (via a number of Regulations and Directives) based on the principle of "zero tolerance", which means that no concentration is safe unless that substance has not been given a MRL (for Annex 1 substances). In this case, knowing the shape of the dose-response function is seemingly irrelevant.

In the EU, the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (REACH regulation, 2006) aims for a high protection of human health and the environment. This law requires manufacturers and importers "to gather information on the properties of their chemical substances and to register the information in a central database". The question that REACH does not answer is: Does this requirement include alternative choices of dose-response models, given the alternatives that are currently available and demonstrably superior to the

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default to linearity? The wording of REACH is explicit and allows those superior choices—when available:

> This Regulation should ensure a high level of protection of human health and the environment as well as the free movement of substances, on their own, in preparations and in articles, while enhancing competitiveness and innovation. This Regulation should also promote the development of alternative methods for the assessment of hazards of substances. The dilemma that REACH introduces is that it also states that it:
>> … is based on the principle that it is for manufacturers, importers and downstream users to ensure that they manufacture, place on the market or use such substances that do not adversely affect human health or the environment.

REACH's provisions are based on the EU version of the precautionary principle, which trumps the effect of *scarce information* on potential public exposure to agents that could cause serious or irreversible harm by favoring protection, even when scientific certainty about cause and effect is low. Yet, if regulations do not consider either threshold or beneficial effects, when those are demonstrated to exist, they contravene the very reason for the precautionary principle. Specifically, the precautionary principle either causes actual damage (even when it is supposedly protective) or results in unwarranted costs. In either of these two cases society suffers unduly under an ostensible protection that does not in fact exists. That is, if the EU were to use the LNT because lack of certainty about mechanisms of action of an agent under the precautionary principle, the choice would appear to be protective: zero mass implies zero risk. Yet, at levels of risk below *0.01*, the LNT is unknowable, while biphasic or hormetic models, as we will shown, are knowable (provided that the experimental data is not unwittingly biased towards linearity). This bias, for example, affects the US National Cancer Institute-National Toxicological Program cancer animal bioassay experiments that use Maximum Tolerated Dose, and fractions of the MTD plus a control group, and thus generally do not provide the sufficient information (about seven data points) about a nonlinear dose-response relationship.

To set the stage, we consider radon decay products and lung cancer risks from low levels of cumulative exposure (in this example measured as working levels-month, WLM), developed by Wakeford (2004). A summary epidemiological analysis of several epidemiological studies (Wakeford, 2004) suggests that at low exposures there is a protective (or, at least, a threshold) effect when the relative risk, RR, < 1.0. The results up to but not including 100 WLM are statistically insignificant, perhaps due to small sample size relative to the size of the effect at those low dose

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rates. Adverse effects, the RR > 1.0 become statistically significant thereafter.

US agency rulemaking for drinking water encountered uncertain causation regarding the effect of radon. The regulatory process began in 1974. The EPA based its risk calculations on an unpublished study, estimating that approximately *200* excess cancer deaths would occur in the US per year, and the cost of each death averted (by the regulation, even though the deaths are not averted but are postponed to later years) would be approximately *3* million dollars. However, the EPA Science Advisor asked the agency to consider the "enormous uncertainty" about the risk, staing that the "maximum contamination level" might be in the range of *1500* to *2000* pCi/liter, rather than the agency's *300* pCi/liter. The EPA Science Advisory Board (SAB) had concluded that although "there is no direct epidemiologic or laboratory animal evidence of cancer being caused by ingestion of radon in drinking water," and that "it is not possible to exclude the possibility of zero risk from ingested radon", also suggesting *3000* pCi/liter. A review of the EPA found that the U. S. Environmental Protection Agency's (EPA) "policy and regulations are frequently perceived as lacking strong scientific foundation." In 1999, it was concluded that radon in the drinking water would results in cause "almost negligible" cancer risks. However, exposures to inhaled radon (as well as smoking) has been stated to cause approximately 21,000 cancer deaths (due to lung cancer from *inhalation*) per year by the US EPA (U.S. EPA, 2010)). Regulatory risk assessment of exposure to Radon has combined policy with science. Specifically, in the context of drinking water, causation involves an assumption of both linearity and no threshold, unless the evidence is clearly contrary to this default. The US EPA has maintained the use of the LNT. Specifically:

> " … radionuclides emit ionizing radiation and, absent data indicating that there is a threshold level at which exposure does not present a risk, EPA uses a linear, non-threshold model … (such that) risk associated with the exposure increases proportionally to the concentration of the radionuclide (from zero exposure) (Federal Register, Vol. 65, p. 21,579, 2000 (parentheses added)).

IR can create public and occupational cancer risks, as well as adverse effects such as burns. Yet, "it is the dose that makes the poison" that clearly matters for our work, as we do not deal with acute effects. Current US and European regulations and international organizations assume that a liner-no-threshold (LNT) model of cancer applies at low doses. For example, the BEIR VII report concludes the LNT risk model for IR regulation (BEIR, 2006) is appropriate. The LNT model assumes that the primary mode of action is linearly related to low doses, and must go through the

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0, 0 intersection of the dose-response axes. However, recent research suggests damage induced by IR (as a process that includes repairs of that damage) could be nonlinear. These repair process include DNA repair, intracellular metabolic oxidation/reduction (redox) reactions; cell cycle checkpoint controls; intra - and intercellular signaling cascades and in certain instances, senescence (cell aging), apoptosis, and/or mitotic linked cell death (Dauer *et al.* 2010).

The more general dose-response relationship is hormetic (or bi-phasic): it describes biological phenomena broadly characterized by a low - dose stimulation and a high - dose inhibition (Calabrese and Baldwin, 2002; Calabrese, 2010). In our work we continue to use a system biology approach that places emphasis on the interaction of cell signaling pathways and networks, the distribution effects and responses throughout the networks and redundancy within and between the networks to develop the correct dose-response patterns (Dauer *et al*, 2010). Given these advantages, we developed a model linking cell cycle checkpoint control mechanisms to IR induced toxicological perturbation signal and cancer disease model to predict the dose response relationship of IR induced tumor transformation frequency. The model captured a monotonically increasing to J-shaped dose response under different parameter values. In this article, we will further investigate how the biological and mathematical mechanisms of the model influence the predicted shape of the dose response of IR induced tumor transformation. Specifically for IR, Upton (2001) found enhancements to immunological responses such as antibodies, lymphocytes, apoptosis in cells of rabbits, sheep, mice, and humans, from exposure to gamma and *X*-rays, including *in vitro* test (human lymphocytes, mouse spleen cells). UNSCEAR (1994) and later studies document the stimulatory effect of low-LET background radiation (Pollycove and Feinedegen, 2001).

The ambiguity between the LNT and hormesis continues to motivates our work (Zhao and Ricci, 2010). Fundamentally, we seek to find the biological basis for the beneficial (or at least threshold-like) behaviors of chemicals and IR. Specifically, we clarify the mechanisms of cancer induction as well as cast some new insight is the correct choice of cancer dose-response. Our scope is to aid these findings via a common analytical framework that causally accounts for critical behaviors at the cellular level.

2. METHODOLOGY AND REVIEW OF PREVIOUS MODEL DEVELOPMENT: NON-LINEAR ORDINARY DIFFERENTIAL EQUATIONS AND BIFURCATION THEORY

Our first generation of cell cycle-tumor transformation model based upon systems biology approaches is a model consisting of a set of non-linear ordinary differential equations (ODEs) as shown in equation (1):

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$$\frac{dx_i}{dt} = f_i(x_i,\ldots,x_n;\, p_1,\ldots,p_m),\ i = 1,\ldots,n \tag{1}$$

where,

x_i = concentration or activity of the ith protein in the reaction network
f_i = the ODE that describes the rate of change in the ith protein's activity
p_j = value of the jth parameter (e.g. rate constant)
n = number of proteins in the network

Bifurcation theory is a technique that contributes to understanding unexpected results when using non-linear ODEs. It describes and explains how generic properties of a dynamical system depend on its (generally initial) parameter values. The attractor of its vector field in a multidimensional state space that cannot be easily visualized characterizes the behavior of a regulatory network. There is only a limited number of ways in which these attractors might change when moving continuously through the parameter space: bifurcation diagrams depict the dynamic behavior of one or more state variables according to the change of one or more specific parameter (Tyson *et al*, 2001). Matcont software is used to draw the bifurcation diagram.

The first generation of IR induced cell cycle checkpoint control model

The cell cycle consists of the series of events that takes place in a cell leading to its division and duplication, which is a fundamental biological phenomenon. Cell cycle time is the inverse of cell division rate, which tends to be shorter in tumor cell than that in normal cell. Checkpoint control, such as G1/S or G2/M checkpoint control, are protective mechanisms in cell cycle regulation mainly to ensure that: (1) cell sizes are large enough to warrant the next step (e.g. G1/S checkpoint control is to ensure that cell is large enough to warrant a new round of DNA synthesis while G2/M checkpoint control is to ensure that cell is large enough to divide); (2) any damage suffered by DNA has been repaired (Tyson *et al*, 2002). A series of mathematical models describing the checkpoint control mechanisms have been developed based on systems biology approaches, in the past two decades (Tyson and Novak, 2001; Qu *et al*, 2003a, b, 2004; Jeffries et al, 2012). These authors found that the switch-like behavior of key regulating proteins is the central biological aspect for checkpoint control. To reflect this aspect, Tyson's group summarized the essential factors of checkpoint control mechanism at the systems level as including: (1) positive/double negative feedback between regulating proteins; (2) non-linear relationship in the feedback; (3) and cell growth (Tyson and Novak, 2001; Zhao and Ricci, 2010).

Adopting these mechanisms, we developed our first generation IR-induced cancer dose response relationship model based on systems biology approach (Zhao and Ricci, 2010; Zhao *et al*, 2012). The signaling

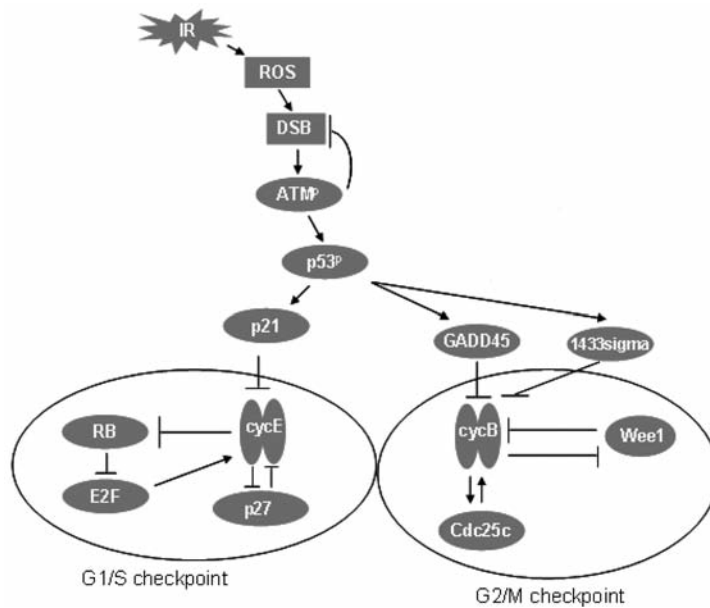*Systems Cancer Biology and the Controlling Mechanisms for the J-shaped Cancer Dose Response*

FIGURE 1. Signaling pathway of checkpoint control and IR-induced perturbation

pathway that the model is based on is indicated in Figure 1. The G1/S and G2/M checkpoint control modules are indicated in the circles, where direct or indirect positive feedbacks occur. Phosphorylation reactions exist between CycE (Cyclin E with its cyclin dependent kinase) and Rb in G1/S checkpoint as well as between CycB (Cyclin B with its cyclin dependent kinase) and Weel in G2/M checkpoint. The ordinary differential equation for phosphorylation follows Michalies-Menten kinetics, which characterizes the nonlinearity constraint for the checkpoint control mechanisms. Because the key checkpoint control regulators CycE and CycB accumulate in the nuclear cytoplasm, the effective concentrations of CycE and CycB equal to their concentrations (multiplying by cell mass, m). In this way, spontaneous cell growth is a promoting factor in the positive/double negative feedbacks. Therefore, the model qualifies the three essential conditions that generate the switch-like behavior of the key regulators at the checkpoint transition. Cell cycle time can be identified as a function of when the switch-like behavior happens from bifurcation diagram. When the upper toxicological signals interact with the key regulating proteins of the checkpoint arrest, the switch-like behavior of the key regulating proteins was delayed and cell cycle time increases.

The antagonistic reaction governing G1/S checkpoint in the model takes place between CycE and Rb and antagonism reaction governing G2/M checkpoint takes place between CycB and Wee1. P27 and Cdc25 contribute to the two checkpoint transitions, respectively. To describe the
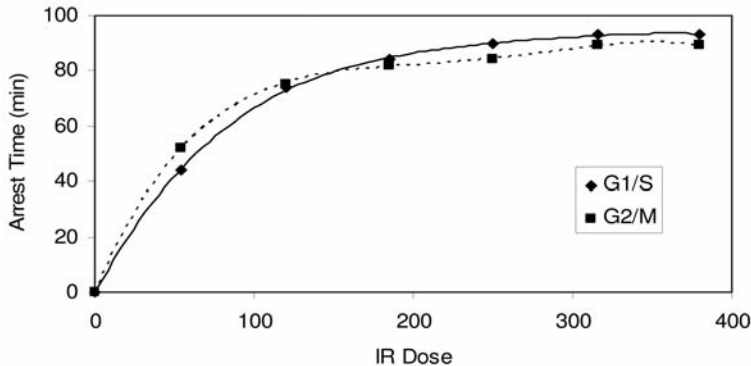
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FIGURE 2. Dose response curve of IR induced G1/S and G2/M checkpoint arrest time

perturbation caused by IR, IR first induces double strand break (DSB), then DSB activates ATM by autophosphorylation. Phosphorylated ATM (ATM^P) has repair function to DSB and it can also phosphorylate and activate the transcriptional factor p53. Activated p53^P then transcriptionally activate p21, GADD45 and 1433 sigma. These are the genes that repress G1/S and G2/M transitions. P53 dependent signaling also mediates the entry into apoptotic pathway through regulation of Bax and Bcl-2. Bax is pro-apoptotic and is transcriptionally activated by p53. Bcl-2 is anti-apoptotic and is transcriptionally repressed by p53. Box Bax and Bcl-2 signal to caspases, which are proteases at the core of the apoptotic machinery.

The model consists of three modules. The systems module represents the signaling pathway/interactions in the two checkpoint controls. The perturbation module represents the upper signaling pathway showing how IR induces cell cycle checkpoint arrest as shown in Figure 1. The disease module uses the two-stage clonal growth cancer model; cell proliferation rate is one of its parameters. The systems/perturbation module is linked with the disease module through the inverse relationship between cell proliferation rate and cell cycle time, (Zhao and Ricci, 2010).

Identified biological mechanisms: the coexistence of the LNT with biphasic J-shaped dose-response relationship

The endpoint of the simulation in this model, the transformation frequency, uses the product of cell proliferation rate alpha (unit: time^{-1}) and mutational rate mu (unit: fraction) in the clonal growth model as its surrogate. Recent research suggests damage induced by IR appears to increase linearly with dose, thus mutation rate is a linear function of IR dose. The simulation results show a monotonically increasing to J-shaped dose response relationship for IR induced transformation frequency.

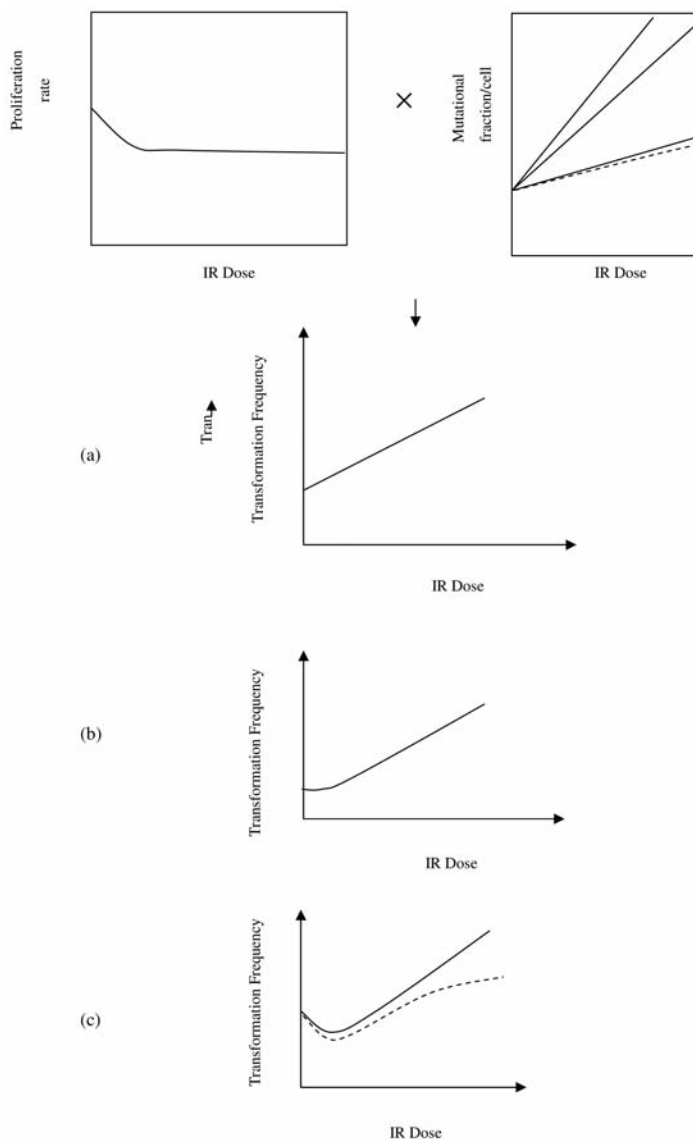*Systems Cancer Biology and the Controlling Mechanisms for the J-shaped Cancer Dose Response*

FIGURE 3. Illustration of the theoretical shapes of dose response curves for different combination of parameter values: LNT, threshold, and bi-phasic J-shaped curves

Simulation results show that when cell mutational rate is sensitive to IR dose, a monotonically increasing dose response curve is more likely to happen; when cell mutational rate is not sensitive to IR dose, a J-shaped dose response curve is more likely to happen. This outcome matches experimental findings, such as those for high linear energy transfer (LET) particles (e.g. alpha particles, emitted in its decay process by radon, among other species) with higher sensitivity of mutational rate
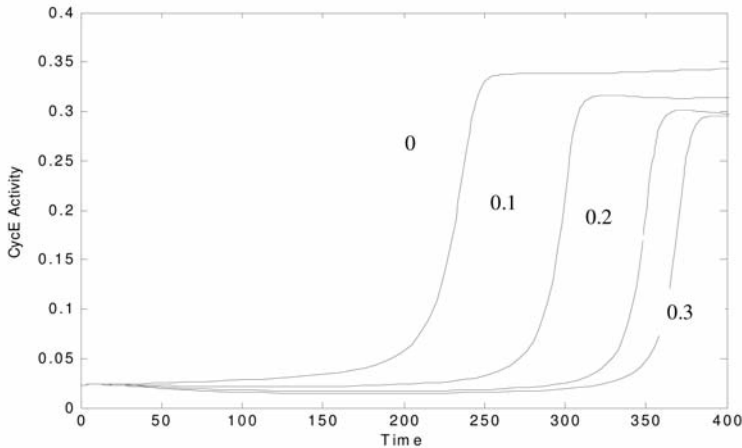
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FIGURE 4. Time course activity for CycE under different IR exposure

tend to have a monotonically increasing dose response relationship while low-LET particles (e.g. gamma particle or X ray) are more likely to result in the J-shaped dose response (Bettega *et al*, 1992; Azzam *et al*, 1996; Redpath *et al*, 2001).

When IR increases, the checkpoint arrest time saturates and thus cell proliferation rate alpha saturates as well. Figure 2 shows how the simulated checkpoint arrest time saturates with time. Figure 3 is a conceptual explanation for the J-shaped dose response curve. The mutation rate mu is linearly increasing with IR dose with three different slopes, as shown in Cases 1, 2 and 3. In Case 1, when the slope of mu is very large, it dominates the product of alpha and mu, the transformation frequency, so it leads to a monotonically increasing dose-response: the LNT. In Case 3, the slope of mu is very small: the alpha part dominates the results of transformation frequency, and then with the saturation of alpha, the mu part dominates the results. Therefore, the product of these two first decreases then increases, the result is the biphasic J shaped dose-response. In Case 2, when the slope of mu has an intermediate value, the transformation frequency results in the threshold dose response relationship. These findings are the fundamental biological reasons for the J-shaped dose response curve as well as for the possibility of either the LNT or a threshold for response (Zhao and Ricci, 2010).

In this paper, we use a linear function to describe the relationship between mutational rate and IR while did not consider the adaptive repair process. The adaptive repair process will more likely to make the mutational fraction/cell and transformation frequency toward a plateau form, as the dashed line shown in Figure 3.
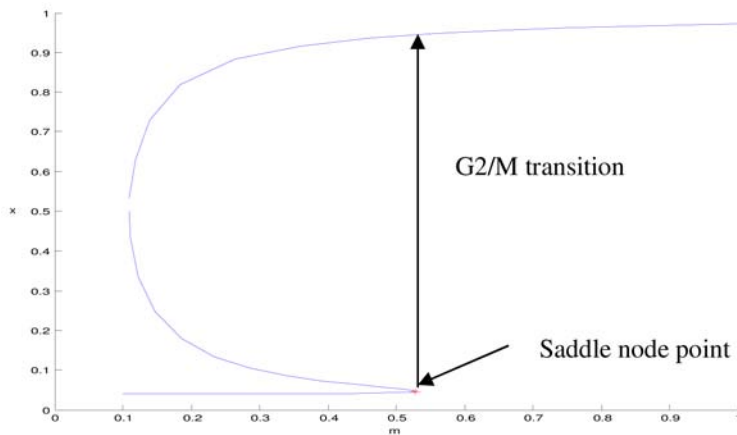
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FIGURE 5. Bifurcation diagram indication of the saddle-node behavior in the simulation

The biological mechanism leading to the J-shaped dose response was identified as saturation of the checkpoint arrest time: The induced checkpoint arrest time is longer per unit of IR dose in the low dose region than that in the high dose region. It is consistent with how toxicologists intuitively perceive bi-phasic hormetic phenomena (detailed review of previous work of model development can be found in Zhao and Ricci, 2010 and Zhao *et al*, 2012).

Fundamental mathematical mechanisms for the J-shaped dose response relationship

The timing of the switch-like behavior of key cellular regulators identifies cell cycle time and checkpoint arrest time. It can be identified by time course chart or bifurcation diagram of cell mass. Figure 4 shows the time course chart when the G1/S switch behavior (checkpoint) happen for IR = 0, 0.1, 0.2, 0.3. Here we define that CycE activity jumps to 0.1 corresponding to when G1/S transition happen. We can see that with IR increasing, there is checkpoint arrest and the arrest time tends to saturate, which is the fundamental biological mechanisms for the J-shaped dose response curve.

As cell mass m is a logistic function of time and the increase of m is one of the essential conditions for the switch-like behavior to happen, we analyze the relationship between IR dose and cell mass at the switch transition. This yields deeper insights regarding the fundamental systems control for the J-shaped dose response curve. The behavior of cell mass at the switch-like transition can be indicated in the bifurcation diagram. It depicts how the steady state value of CycB concentration changes with cell
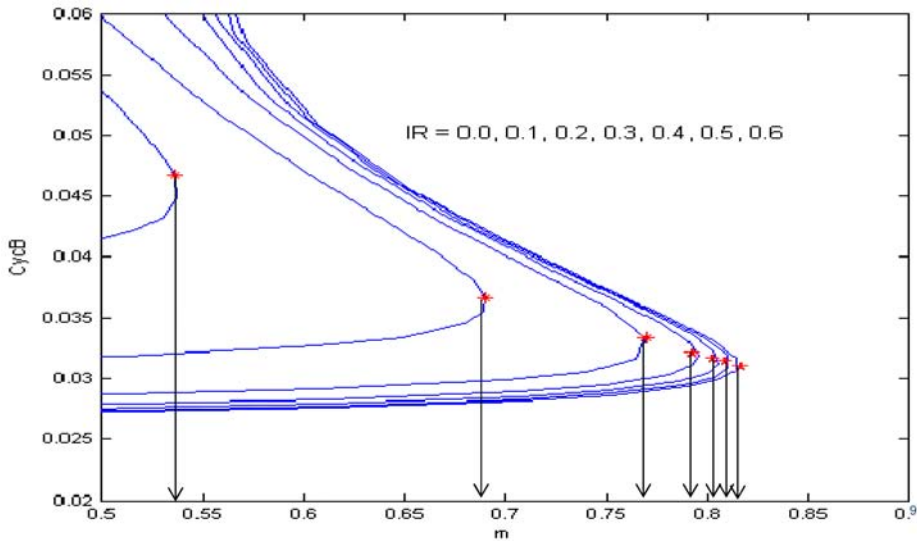
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FIGURE 6. Saddle node points in the bifurcation diagram of various IR doses from the original model

mass m that, at the switch-like transition, corresponds to the saddle node point of the bifurcation diagram.

Figure 5 shows the bifurcation diagram and the saddle node point for G2/M check point control. The saddle node point indicates at what cell size the G2/M transition (switch-like behavior) happens. Here, we define m at the saddle node to be the *critical m*. Figure 6 is the bifurcation diagram of G2/M checkpoint from the original model with various IR doses. It indicates when IR dose increases linearly, the corresponding critical m increases superlinearly: the *critical m* saturates with IR increasing. This matches the saturating behavior in the time course.

In the earlier modeling (Zhao and Ricci, 2010; Zhao *et al.*, 2012), m is introduced through a logistic function of time t. The relationship between the two propositions A and B (A: the saturation of critical m according to linear increase of IR; B: the saturation of time t according to linear increase of IR) is that A satisfies B. Therefore, if A is true, a J-shaped dose response can occur. Both numerical and analytical methods are used to investigate mechanisms of the saturation of critical m with IR increasing in the Results section of this paper.

Model simplification to only incorporate the key "systems" elements

The original model contains 19 ODEs. In order to investigate the behavior for the saddle node points, the model is simplified to only incorporate the essential elements for the system. According to Tyson (Tyson and Novak, 2001), the fundamental equations that describe cell cycle

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TABLE 1. Parameter values of equation (2) and (3)

k1	k2′	k2″	k3′	J3	k4	J4
0.04	0.04	1	1	0.04	35	0.04

checkpoint control are listed as:

$$dx/dt = k1 - (k2' + k2''y)x \tag{2}$$

$$dy/dt = \frac{k3'(1-y)}{J3+1-y} - \frac{k4mxy}{J4+y} \tag{3}$$

The parameter values of equations (2) and (3) are listed in Table 1 (Tyson and Novak, 2001).

The x and y in (2) and (3) identify the key regulating proteins of the checkpoint control and satisfy the three constrains required to generate the switch-like behavior of these key regulating proteins. Assuming the perturbation signal influences the synthesis process, the perturbation signals linking IR to cell cycle checkpoint control can be written as a Michaelis-Menten function of IR, as follows [personal communication, Fangting Li, Academy of Advanced Interdisciplinary Studies, Peking University]:

$$Signal = kr1 * IR / (kr2 + IR).$$

Adding the toxicological perturbation signal, equation (2) can be rewritten as:

$$dx/dt = k1 - (k2' + k2''y)x - kr1 * IR/(kr2 + IR) \tag{4}$$

3. RESULTS

Numerical results

To find the steady state, equations (3) and (4) were set to zero. The following equation was obtained for the steady state:

$$m_{critical} = \frac{(k3'k2''x + k3'k2'x - k3'k1new)(J4k2''x - k2'x + k1new)}{\{[(J3+1)k2''x + k2'x - k1new](k1newk4 - k2'k4x)x\}} \tag{5}$$

In which $k1new = k1 - kr1*IR/(kr2+IR)$, with $kr1$ was set to 0.01 and $kr2$ was set to 0.07. The saddle node point behavior can be directly
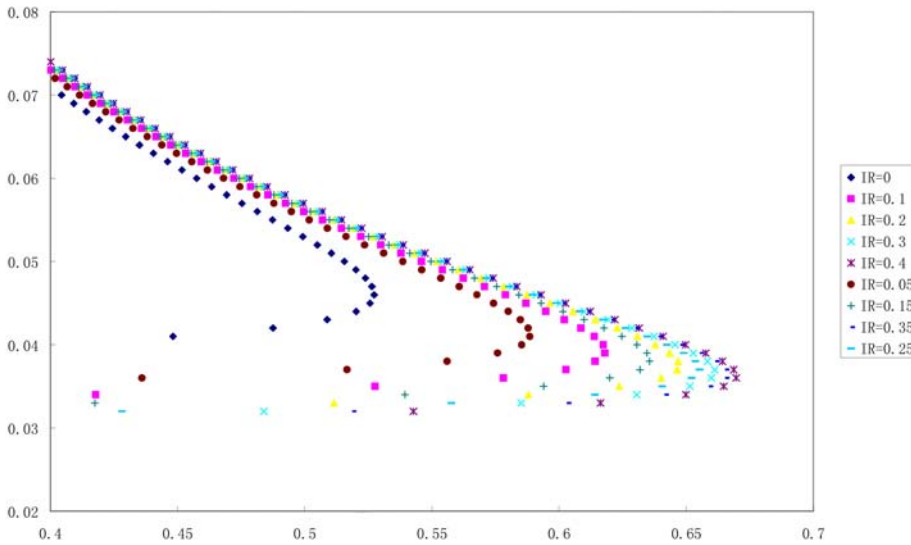
FIGURE 7. Saddle node points in the bifurcation diagram of various IR doses from the simplified model

obtained from equation (5) as shown in Figure 7. It is seen that after simplification, the *critical m*, corresponding to the linear increase of IR, grows in the same superlinear way as that from the original model in Figure 6. Mathematically, the behavior of $m_{critical}$ in Figure 7 can be expressed as the second derivative of $m_{critical}$ relative to IR ($m_{critical}''$) is less than zero.

Analytical results

Equation (5) can be rewritten as a three power distances for variable *x*. The saddle node point corresponds to the root which leads to the of the three power distances to zero. The analytical solution of critical m is obtained as equation (6) with parameter values set Table 1.

$$m_{critical} = \frac{2}{4 - \dfrac{100kr/{*}IR}{kr2 + IR}} \tag{6}$$

Using Matlab® we obtain:

$$\begin{aligned} m_{critical}'' = &-(4{*}((100{*}kr1)/(IR + kr2) - (100{*}IR{*}kr1)/ \\ &(IR + kr2)^2)^2)/((100{*}IR{*}kr1)/(IR + kr2) - 4)^3 - (2{*}((200{*}kr1)/ \\ &(IR + kr2)^2 - (200{*}IR{*}kr1)/(IR + kr2)^3))/ \\ &((100{*}IR{*}kr1)/(IR + kr2) - 4)^2 \end{aligned} \tag{7}$$

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TABLE 2. Number of positive and negative values of $m_{critical}''$

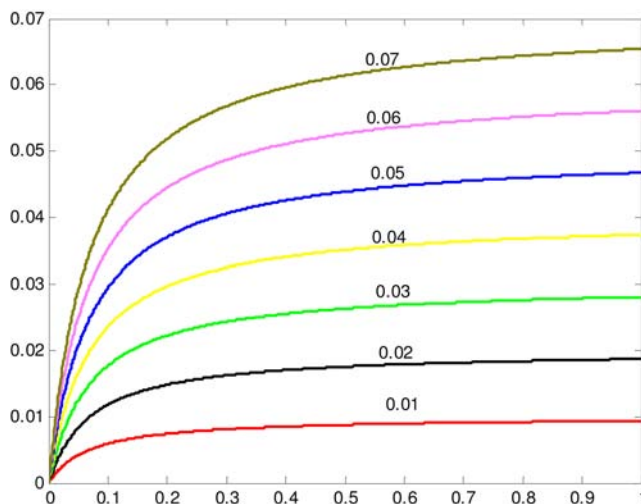| B	0.01	0.02	0.03	0.04	0.041	0.045	0.05	0.06	0.07
number of positive values of $m_{critical}''$	0	0	0	0	234	1082	1911	2917	3517
number of negative values of $m_{critical}''$	10000	10000	10000	10000	9766	8918	8089	7083	6483

FIGURE 8. The change of IR perturbation signals vs. IR dose corresponding to various values of kr1 (0.01~0.07)

We also identify when $m_{critical}''$ is less than zero. When $IR > 0$, $kr2 > 0$ and $kr1$ is less than 0.04, $m_{critical}''$ is less than zero for all 10,000 random values. When kr1 is larger than 0.04, positive values of $m_{critical}''$ appear. Table 2 indicates the number of positive values for $m_{critical}''$ when running the program once. In the program, $kr1$ is drawn from 10,000 random values, which are uniformly distributed from 0.001 to B while IR and $kr2$ are kept positive. We also find that the number of positive values of $m_{critical}''$ increases and negative values decreases when B increases from 0.04. Therefore, it is concluded that when $kr1$ is less than 0.04, it is more likely to lead to a J-shaped dose response curve. Figure 8 shows how toxicological signal (IR_{signal}) induced by IR changes with IR when $kr1$ value increases from 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 and 0.07. From Table 2 and Figure 8, when $kr1$ is small, it is more likely to lead to a J-shaped dose response curve. This finding is again consistent with the fact that low-LET particles tend to have a J-shaped dose response curve, and that high-LET particles do not.

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DISCUSSION

Mathematical methods, such as bifurcation theory, play an important role in understanding biological behaviors and thus in their analysis: the bifurcation diagram allows the demonstration and understanding of the biological mechanism of J-shaped dose response relationship in term from the switch-like behavior of regulating proteins to transfer to the mathematical dynamical control issue—the behavior of the saddle-node points.

In this paper, mathematical methods are developed based on systems biology approach to identify the controlling mechanisms for the J-shape dose response relationship of IR induced tumor transformation frequency. The results indicate that the behavior of the saddle node points in the bifurcation diagram is related to the resulting J-shaped dose response. Specifically, a J-shaped dose-response curve can occur if $m_{critical}''$ is less than zero. To assess saddle node points, the model is first simplified to only include the key elements of the system; the parameter of the systems module is directly taken from the original model developed by systems biologists (details in Zhao and Ricci, 2010; Zhao *et al.*, 2012). Both numerical and analytical solutions show that, under certain range of parameter values that $m_{critical}''$ is less than zero is definite, which means a J-shaped dose response is very likely to occur for low LET particles.

The simulation in this paper is at low dose. At high dose, the cell is more likely to go to apoptosis. When going to apoptosis, there is supplementary regeneration. Future work can focus on modeling apoptosis process.

Simplification of the original model is a key step in this work. Therefore, it is important to determine the essential elements of the systems used as a function of available knowledge. For cell cycle checkpoint control, systems biologists have defined the key factors in the system. With the continuing relevance of systems biology, a set of mathematical models that explicitly account for a very large number of biological processes (e.g. apoptosis) have been developed. To apply such models in dose response modeling, the toxicologists' role is first to identify the key elements in the "systems control". This step requires toxicologists to have a deep understanding of the feedbacks and interaction of mathematical model. The more expansive step, of course, is to increasingly require the collaboration of biologists, toxicologists and mathematician to increase their collaboration and use mathematical and probabilistic methods to further clarify when J-shaped dose-response models should normatively be used, and when either a threshold model or a LNT should be demonstrable—as opposed to assumed—alternatives.

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