Role of Different Enzymes in H_2O_2 Neutralization and Cellular Radioresistance Estimated by Mathematical Modeling

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Article

Role of Different Enzymes in H_2O_2 Neutralization and Cellular Radioresistance Estimated by Mathematical Modeling

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Abstract

Reactive oxygen species (ROS) are fundamental components found in cells that exist in an oxygen environment. While they are often viewed as detrimental metabolic byproducts that can harm cells, leading to aging and cell death, they can also play a role in cellular regulatory processes and have beneficial effects. One of the main ROS present in all cells is hydrogen peroxide (H_2O_2), which can function as a signaling molecule in extra- and intracellular signaling. To enhance our understanding of how various enzymes regulate cellular H_2O_2 level we created a mathematical model of H_2O_2 neutralization and performed computer simulations to estimate the neutralization efficiency in various types of cells. Data on gene expression for genes participating in this process was incorporated into the calculations, along with enzymes' regulation of oxidation and reduction processes. The conducted simulations demonstrate that cells originating from different tissues differently utilize systems neutralizing H_2O_2 which results in differences in H_2O_2 cellular levels. The simulation findings suggest that the differences in radiosensitivity seen in various cancer cell types may be linked to their effectiveness in neutralizing H_2O_2. Analysis of results from model simulations for colorectal, lung and breast cancer cell lines indicated that radiosensitive cell lines exhibit elevated levels of H_2O_2, attributed to the reduced efficiency of neutralizing enzymes.

Keywords: reactive oxygen species; H_2O_2 neutralization; ROS neutralization mathematical modelling

1. Introduction

Reactive oxygen species (ROS) are generally regarded as harmful byproducts resulting from life in an oxygen environment [1]. They are recognized as secondary messengers in the overall cellular signaling [1,2]. For a long time, these species were thought to be damaging to cells, involved in aging and cell death [1,3,4]. Today, they are seen as crucial players in the regulation of cellular processes such as proliferation, senescence, and apoptosis [3,5]. Hydrogen peroxide (H_2O_2) is non-radical member of reactive oxygen species group, which occurs in large amounts in cell of different organisms including plants [6,7]. H_2O_2 concentration in cells was shown to vary and is assumed to be 1-700nM [8,9] with cytotoxic values inducing apoptosis over 700nM [8,9]. It should be noted the actual intracellular concentration of H_2O_2 reached in the steady state incubation is lower than the extracellular value that leads to cell death [8]. Thus, the levels of H_2O_2 found outside the cell in vitro, which are generally cytotoxic, are significantly elevated compared to the intracellular concentrations of H_2O_2 that are assumed to be harmful [4]. Concentrations up to 15μM have been found to stimulate cell proliferation, whereas those over 1mM were shown to lead to necrotic cell death. H_2O_2 is found

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to be stable in comparison to other members of ROS group, with half-life about milliseconds [6]. Due to its long half-life, it is considered a signaling molecule [10] which is able to influence cellular processes like apoptosis or cell proliferation [6,7]. Hydrogen peroxide is formed by dismutation of superoxide ($O_2^{\cdot-}$), it is also generated by enzymes such as amino acid oxidase, xanthine oxidase and NOX [11,12]. H_2O_2 can freely pass through cell membranes and directly induce the breaking of phosphodiester bonds [1,13]. H_2O_2 can react with metals, producing $^{\cdot}OH$ (Fenton reaction) [14]. It is believed that the greatest toxicity of H_2O_2 and $O_2^{\cdot-}$ is achieved during the transition to hydroxyl radicals [14-16].

Neutralization of hydrogen peroxide and thus protection against damage is carried out mainly by catalase (CAT) [1,13,17], an antioxidant present in almost all aerobic organisms, through the interaction with glutathione peroxidase (GPX), in which glutathione is converted to its oxidized form GSSG [18-20], and by enzymes from the peroxiredoxin family (PRDX), which were used for oxidation by the H_2O_2 (Figure 1) [21-23].

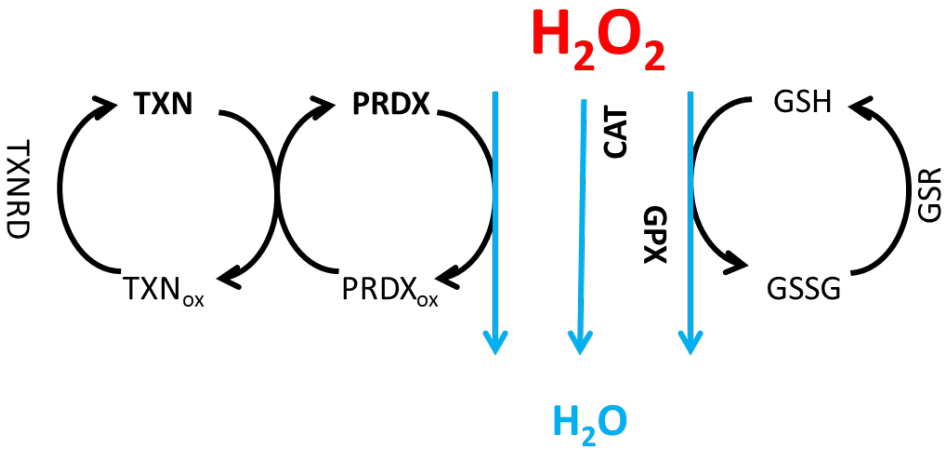

Figure 1. H_2O_2 neutralization to water by enzymes: peroxiredoxin (PRDX) with thioredoxin (TXN), catalase (CAT) and glutathione peroxidase (GPX) with glutathione (GSH). PRDX in reduced form is oxidized (PRDXox), TXN is used for re-reduction, which is then oxidized (TXNox) and then reduced again by thioredoxin reductase (TXNRD). In the case of GPX, GSH is used for reduction, which is oxidized to GSSG and then reduced by glutathione reductase (GSR).

Neutralizing enzymes targeting H_2O_2 are evolutionarily conserved from sponges to mammals, with the glutathione-linked GPX system being the youngest neutralizing family. CAT and PRDX are old families, well conserved between species, their essential antioxidant functions having led to the conservation of genes throughout the animal kingdom [24]. Catalase exists in three main types: typical catalases (in aerobic organisms), catalase-peroxidases (in fungi, archaea, and bacteria), and manganese catalases (bacteria-specific). Typical catalases are categorized into three subgroups, the third of which encompasses animals, including humans [25,26]. Catalase is commonly found in cells, particularly in peroxisomes (liver cells) and in the cytoplasm (erythrocytes). Besides neutralizing hydrogen peroxide, it also decomposes peroxynitrite and oxidizes nitric oxide to nitrite [20,21]. Glutathione peroxidase functions by using glutathione (GSH) to neutralize ROS. This process relies on the redox cycling of GSH, which is regulated by glutathione reductases and peroxidases. GPX1 is found in the cytosol, nucleus, and mitochondria; GPX2 is located in the cytosol and nucleus; GPX3 is present in the cytosol; and GPX4 is distributed in the nucleus, cytosol, mitochondria, and cell membranes [18,27].

Cells require balanced redox conditions to proper function, maintained by strictly controlled oxidation and reduction processes. For the antioxidant system to function correctly, it is necessary to convert oxidized enzymes back to their reduced state. This function is carried out by glutathione

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reductase (GSR) which catalyzes the reduction of GSSG to GSH [18] and thioredoxins (TXN) which reduce peroxiredoxins [28]. Peroxiredoxins, thioredoxins and glutathione contain cysteines with thiol groups, which occur in reduced (S-H) or oxidized (S-S) forms. During the reduction of H_2O_2, peroxiredoxins are oxidized and later reduced by thioredoxins, which are in turn oxidized. To ensure the system works correctly, oxidized thioredoxins must be reduced by thioredoxin reductases (TXNRD), the basic elements of the thioredoxin system, which restore their enzymatic activity [29]. The electrons essential for the reduction of enzymes originate from the constant reduction reactions occur during metabolism [23,29].

Antioxidant action of H_2O_2 neutralizing enzymes vary among organelles. Melo et al. showed that neutralization systems do not function equally in different cellular locations, e.g., in erythrocytes CAT is more vital for cytoplasmic antioxidant protection than for that of membrane components, when its activity is impaired, PRDX and GPX are transported to the cell membrane, probably to protect against lipid peroxidation [30]. Catalytic activity of main H_2O_2 antioxidant enzymes also differ. PRDX catalytic activity was proven to compete with GPX in H_2O_2 neutralization and PRDX reaction rate constant is in range of $10^7 M^{-1}s^{-1}$, comparable to catalase which reaction rate constant is in range $10^7 M^{-1}s^{-1}$, whereas for GPX1 it was shown to be ~$10^8 M^{-1}s^{-1}$ [31].

For a better understanding the processes observed in cells, mathematical models are used. They in a simplified way represent the reactions occurring in cells. Computer simulations make possible to track many different variants of cell behavior. Regulatory processes of ROS ensure the proper functioning of cells, and a disturbed balance, called oxidative stress, can lead to cell death or its pathology. Differences in the expression of neutralizing enzymes observed in our previous studies and analyzes [32,33] suggested that it would be important to be able to predict differences in ROS levels for different cell types of different tissue origin. There are multiple models in the literature describing systems related to reactive oxygen and nitrogen species [34-37]. The existing mathematical models related to the neutralization of ROS focused mainly on the oxidative processes taking place in the mitochondria (see Kembro et.al [28]). Therefore, we created model describing the process of H_2O_2 neutralization regarding oxidation/reduction of antioxidant enzymes, focused on the differences in neutralization between different types of cells. We conducted series of computer simulations using publicly available expression data of neutralizing enzymes to compare differences of various cell types with special attention to colorectal, lung and breast cancer. Using the created model, we also tried to check whether there are differences in H_2O_2 neutralization between cell lines of the same origin but differing in expression of neutralizing enzymes. We considered influence of radiation and explored relationships of ROS neutralization and radiosensitivity and radioresistance. In the conducted simulations we focused on neutralization systems with enzymes such as: catalase, peroxiredoxin with thioredoxin and glutathione peroxidase with glutathione and we show that these neutralization systems can differentiate radiosensitive and radioresistant cells among colorectal, lung and breast cancer cell lines.

2. Model Formulation

In our previous research, we found that different cell types exhibit distinct levels of antioxidant enzymes. We concluded that the neutralization process can occur via different leading pathways in those cells, which explains the observed differences in various kinds of ROS levels among these cells. The aim of introduced model is to analyze how the levels of H_2O_2 are affected by different neutralization systems. We simulated neutralization process and checked whether there are differences, between the H_2O_2 neutralization systems using the following enzymes: peroxiredoxin (PRDX) with thioredoxin (TXN), glutathione peroxidase (GPX) using glutathione, and catalase (CAT). The first two systems are complex and require several oxidation and reduction reactions, which is why we included these dependencies in the model. To better understand neutralization process we analyzed multiple cell lines from different tissue origin available in Cancer Cell Line Encyclopedia (CCLE) database based on RNA-seq data [38]. The analysis was performed for 1025 cancer cell lines from various tissues and simulated their neutralization of H_2O_2. Figure 1 presents

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process of H_2O_2 neutralization through different pathways: first pathway is PRDX/TXN, second is CAT and third is GPX-GSH pathway.

We aimed to determine if the three neutralization pathways (PRDX/TXN, CAT, and GPX/GSH) are engaged in the neutralization process in all cell types with comparable effectiveness, or if there are specific preferences among different cell lines. We examined this by disabling certain pathways, and one of them remained operational. It is crucial to understand that in living cells, the levels of H_2O_2 and other ROS fluctuate, and our model simplifies this by representing an average ROS level at a given moment. The dynamic fluctuations of ROS, which are closely related to their behavior, are not accounted for. Instead, we implemented oxidation and reduction processes, which are crucial component of maintaining redox balance in cells. The equations for the mathematical model were implemented in the Matlab Simulink environment, where all simulations of model equations were performed. The version of MATLAB used was R2021a. The created model was used to conduct series of computer simulations.

Values of enzymes concentration in multiple cell lines are hard to obtain due to practical issues therefore the data for the model are sourced from existing literature and represent values obtained from various cell lines. These data were adapted from [34,35,39-42] and presented in Table 1. Model parameters were obtained from [34] and [35] through linearization of equations presented in those papers in operating point to quantify proper values for equations' coefficients used in model presented in Table 2. Rate constants (k) show the speed of processes and shows how quickly the process happens. Our model assumes that the reaction parameters remain the same for different cell types, but the expression of neutralizing enzymes varies between cells. The levels of enzymes were estimated on their transcript levels given in publicly available databases (CCLE) [38]. These values were normalized as the ratio of mRNA expression levels in a particular line to those in the HCT116 line. For HCT116, the proportion parameter is 1, indicating that this cell line corresponds to the concentration values found in Table 1, whereas other cell lines exhibit concentrations that vary proportionally according to their expression levels. Due to the fact that we used available expression data of antioxidant genes (which generally but not always correlates with protein levels) not actual protein amount our results of H_2O_2 level in steady state after neutralization are presented in arbitrary units. The original data presented in the study obtained from the mathematical model are openly available in RepOD repository at [10.18150/ZDFI94/https://doi.org/10.18150/ZDFI94].

Table 1. Initial concentration of enzymes used in the model of H_2O_2 neutralization.

Description	Symbol	Value[1] [mM]
CAT concentration	CAT	0.001
PRDX concentration	PRDX	0.15
TXN concentration	TXN	0.025
TXNRD concentration	TXNRD	0.025
GSH concentration	GSH	3.0
GPX concentration	GPX	0.05
GSR concentration	GSR	0.05

[1] A set of parameters to which the remaining calculated values of expression are referred.

Table 2. Parameters used in the model of H_2O_2 neutralization.

Description	Symbol	Value [unit]
Rate constant of CAT	kCAT	0.034 [$mM^{-1}ms^{-1}$]
Rate constant of PRDX	kPRDX	0.26 [$mM^{-1}ms^{-1}$]
Rate constant of TXN	$kTXN_{ox}$	0.23 [$mM^{-1}ms^{-1}$]
Rate constant of TXNRD	kTXNRD	0.31 [$mM^{-1}ms^{-1}$]
Rate constant of GSR	kGSR	0.08 [$mM^{-1}ms^{-1}$]
Rate constant of GPX[1]	kGPX	67 [$mM^{-2}ms^{-1}$]
H_2O_2 influx to the system[1]	H_2O_{2IN}	10^{-6}[$mMms^{-1}$]

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[1] Adjusted.

2.1. Model Equations

Here we present set of mathematical model equations (1-7) used in article. The model contains the following components: (a) Main equation (equation 1) of H_2O_2 neutralization by neutralizing enzymes through 3 different neutralization pathways with H_2O_2 as state variable, (b) the GSH system, encompassing the GPX and GSR, with GSH/GSSG as state variables (equations 2 and 3) and (c) PRDX/TXN system which considers the reduced/oxidized species PRDX and TXN as state variables (both couples linked through relationships) (equations 4-7). In the present model, catalase was also taken into account and appears in equation 1 as additional ROS scavenger.

$$\frac{dH_2O_2}{dt} = H_2O_{2\,IN} - k_{CAT}H_2O_2{*}CAT - k_{GPX}H_2O_2{*}GSH{*}GPX - k_{PRDX}H_2O_2{*}PRDX \quad (1)$$

$$\frac{dGSH}{dt} = -k_{GPX}H_2O_2{*}GSH{*}GPX + k_{GSR}GSSG{*}GSR \quad (2)$$

$$\frac{dGSSG}{dt} = k_{GPX}H_2O_2{*}GSH{*}GPX - k_{GSR}GSSG{*}GSR \quad (3)$$

$$\frac{dPRDX}{dt} = -k_{PRDX}H_2O_2{*}PRDX + k_{TXN}TXN{*}PRDX_{ox} \quad (4)$$

$$\frac{dPRDXox}{dt} = k_{PRDX}H_2O_2{*}PRDX - k_{TXN}TXN{*}PRDX_{ox} \quad (5)$$

$$\frac{dTXN}{dt} = -k_{TXN}TXN{*}PRDX_{ox} + k_{TXNRD}TXN_{ox}{*}TXNRD \quad (6)$$

$$\frac{dTXNox}{dt} = k_{TXN}TXN{*}PRDX_{ox} - k_{TXNRD}TXN_{ox}{*}TXNRD \quad (7)$$

In set of equations (1)-(7): [PRDX], [$PRDX_{ox}$], [TXN], [TXN_{ox}], [GSH], [GSSG] represent the concentrations of peroxiredoxin, oxidized peroxiredoxin, thioredoxin, oxidized thioredoxin, glutathione and oxidized glutathione, respectively. We assumed that the total amount of reduced and oxidized forms is constant and typical for the cell. It should be noted that $PRDX_{TOTAL}$=PRDX+$PRDX_{ox}$, GSH_{TOTAL}= GSH+GSSG, TXN_{TOTAL}=TXN+TXN_{ox}. In the implementation, we also assumed that the concentrations of GSR, GPX, TXNRD, CAT are constant values, dependent on the cell type. Moreover, we assumed that the H_2O_2 influx in cells is approximately constant and is influenced by many factors, e.g., constant influx from mitochondria or peroxisomes. In equation (1) H_2O_{2IN} is to reflect this constant H_2O_2 influx therefore for the implementation we used a step function which was designated as H_2O_{2IN}.

3. Results and Discussion

One of the major reactive oxygen species present in all cells is H_2O_2, which can act as a signaling molecule in intracellular and extracellular signaling. Using the created model, we tried to check whether there are differences in H_2O_2 neutralization between cell lines in which the expression of neutralizing enzymes is different. Considering the connection between ROS and radiation, we also explored how H_2O_2 levels might relate to radiosensitivity and radioresistance. Our objective was to assess whether all three neutralization pathways (PRDX/TXN, CAT and GPX/GSH) are equally effective in every cell type or if certain preferences exist. In computer simulations, we used a constant supply of H_2O_2 to the system, which in a real system can be considered as a constant supply from mitochondria, and then we checked how H_2O_2 neutralization proceeded by switching off different neutralization systems one by one (such a situation is possible, e.g., in the case of mutation of the enzymes) and for all systems operating correctly. We observed how this mutation influences the level of H_2O_2 in different types of cells.

We investigated kidney, thyroid, ovary, bone, haematopoietic, breast, large intestine and lung cancer cell lines. The results of H_2O_2 neutralization mathematical model are demonstrated in Figure

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2 which shows that when all systems are working there are slight differences in H_2O_2 level and we are not able to distinguish any particular pattern, the process of neutralization remains similar in different tissues. When all systems are working neutralization of H_2O_2 is the least efficient is in haematopoietic and bone tissue and the most efficient in breast and kidney tissue.

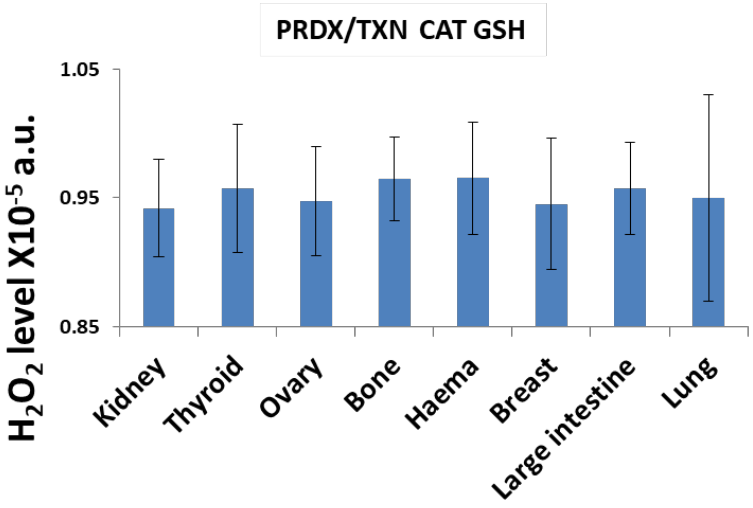

Figure 2. Computer simulations of H_2O_2 levels in cell lines of different tissue origin obtained with assumptions that all systems of neutralization are active. The results present average values obtained for different cell lines of the same origin ±SD.

Because there are not particular differences in neutralization process when all neutralization pathways are switched on, we wanted to check if there are any differences when some of pathway/s are switched off. Turning off one of the neutralization systems indicated differences among cell lines originating from various tissues, but these differences were only significant for the PRDX/TXN and CAT pathways. The combination of these two systems effectively neutralizes H_2O_2, particularly in breast and large intestine tissues, while exhibiting the least efficiency in kidney cells (Figure 3). The results for CAT+GSH and PRDX/TXN+GSH were comparable to those of GSH alone, demonstrating that this system is the most effective neutralization pathway in our model, and this is why only this one was included to the Figure 3. Such results are observed due to the high concentration of GSH assumed in our model. GSH is extensively used also in other cellular processes, suggesting that the actual GSH available for GPX/GSH neutralization is probably much lower.

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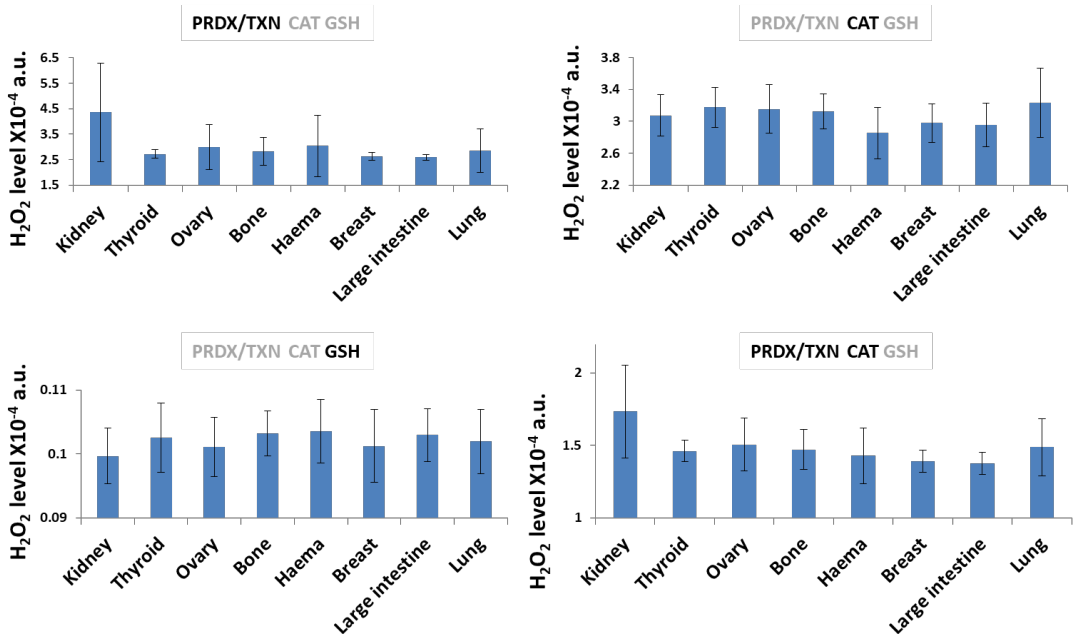

Figure 3. Computer simulations of H_2O_2 levels in cell lines of different tissue origin obtained with assumptions that only some of the systems of neutralization are active. Active system of neutralization was marked as black, the grey is the one switched off. The results present average values obtained for different cell lines of the same origin ±SD.

Analysis of cell lines from different tissue types reveals that system with only PRDX/TXN system working is the least efficient in kidney tissue and the most efficient in large intestine and breast tissue. Neutralization pathway of catalase is the least efficient in lung tissue and the most efficient in haematopoietic tissue. The glutathione system is the least efficient in haematopoietic tissue and the most efficient in kidney tissue. There are differences among tissues in neutralizing H_2O_2 observed for different pathways of neutralization.

3.1. H_2O_2 Neutralization Pathways and Their Connection to Radioresistance

Analyses show the differences among tissues according to neutralization pathway. Consequently, we aimed to investigate whether there are variations among cell lines derived from the same tissue origin. The analyses were performed on various cell lines from a single tissue type, based on the characterizations documented in the literature. We conduced series of analyses and selected lung, breast, and colorectal cancer cell lines based on their radiosensitivity. Indeed, there is difference in radiosensitivity depending on neutralization systems observed in cell lines of these tissues. In colorectal cancer there is difference between radiosensitive and radioresistant cell lines; cell could be distinguished due to GPX-GSH neutralization system, as radioresistant cell lines are more efficient in neutralizing H_2O_2. We also observed major differences among kidney cell lines (data not shown), but this type of cancer is not well characterized in literature. Some of analyzed cell lines with less effective peroxiredoxin system are radioresistant, but due to low data availability the analyzes could not be done. Figure 4 shows the results with all three systems of neutralization working.

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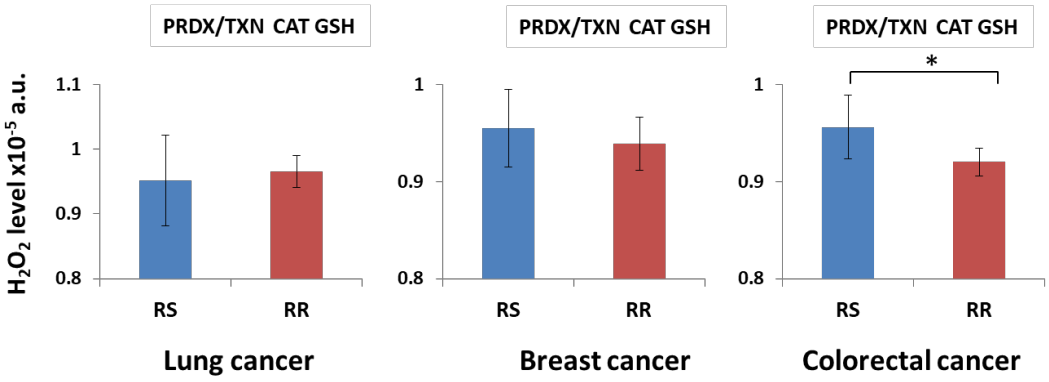

Figure 4. Average H_2O_2 level for radiosensitive (RS) and radioresistant (RR) lung, breast and colorectal cancer cell lines with all neutralization systems active obtained in computer simulations. The results are presented as the mean ±SD. Asterisk denote statistical significance with p-value <0.05 comparing radioresistant and radiosensitive cell line groups.

Lung, breast and colorectal cancers are the most frequently diagnosed cancers. Here we show simulation results of H_2O_2 neutralization for cell lines of this tissue origins. They vary due to its potential to neutralize H_2O_2 by H_2O_2 neutralization enzymes and show differences between radioresistant and radiosensitive cell lines presented in Table 3.

Table 3. Radioresistant and radiosensitive lung, breast and colorectal cancer cell lines used in mathematical model of H_2O_2 neutralization.

Type of cancer	Cell line	Radiosensitive (RS)/ Radioresistant (RR)	Reference
Lung	A549	RR	[43]
	H1703	RR	[44]
	H661	RR	[45]
	H1299	RR	[46]
	H1339	RR	[47]
	H292	RR	[48]
	H358	RR	[48]
	H23	RS	[48]
	H441	RS	[49]
	H1650	RS	[50]
	H522	RS	[50]
	HCC827	RS	[44]
	H69	RS	[51]
	H460	RS	[52]
Breast	MCF-7	RR	[53], [54], [55]
	SK-BR-3	RR	[56]
	ZR-751	RR	[57]
	HCC1428	RR	[58]
	T47D	RR	[59], [60]
	HS578T	RR	[54]
	UACC-812	RR	[61]
	MDA-MB-175VII	RR	[58]
	MDA-MB-361	RS	[59]

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	HCC70	RS	[62]
	MDA-MB-231	RS	[53], [60], [55]
	BT474	RS	[54], [63]
	JIMT-1	RS	[55]
	CAL-51	RS	[64]
	HCC1395	RS	[65]
Colorectal	HT115	RR	[66]
	DLD-1	RR	[66]
	Lovo	RR	[66]
	HT29	RR	[66]
	Caco-2	RR	[67], [68]
	SW480	RR	[69], [70]
	MDST8	RR	[71]
	Colo-201	RS	[66]
	Colo-205	RS	[66]
	Colo-320	RS	[66]
	HCT116	RS	[66]
	SW48	RS	[69], [70]

Fourteen lung cell lines were classified as radiosensitive (RS) and radioresistant (RR) according to literature [43-52]. Sixteen breast cancer cell lines were classified as RS and RR according to literature [53-65]. Twelve radioresistant colorectal cancer cell lines were classified as RS and RR according to literature [66-70]. Contrary to other radioresistant colorectal cancer cell lines SW48 [69,70] seems to be radiosensitive with efficient neutralization of H_2O_2 by GPX/GSH system. The results of H_2O_2 neutralization in these cells are shown on Figure 5.

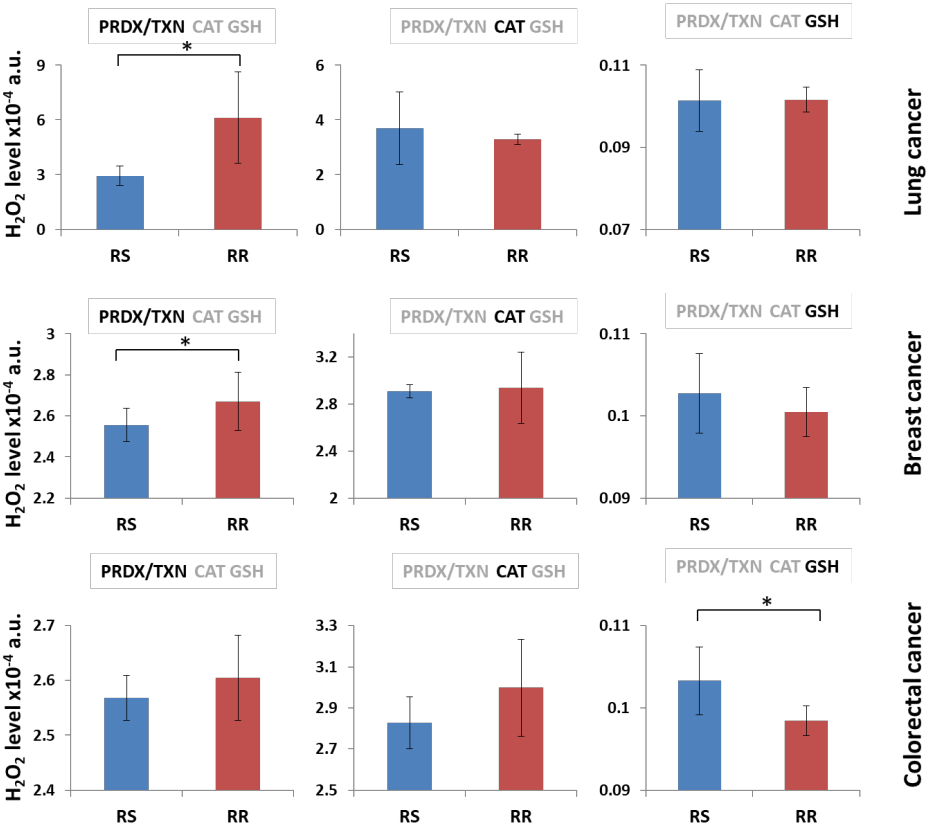

Figure 5. Average H_2O_2 level for radiosensitive (RS) and radioresistant (RR) lung cancer cell lines with some of neutralization systems active obtained by computer simulations. First row shows neutralization of H_2O_2 in lung

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cancer, second row in breast cancer and the third in colorectal cancer. Active system of neutralization was marked as black, the grey is the one switched off. The results are presented as the mean ±SD. Asterisk denote statistical significance with p-value <0.05 comparing radioresistant and radiosensitive cell line groups.

Computer simulation results suggest significant role of PRDX/TXN and PGX/GSH systems in functioning of the cells. In our simulations cell lines with the less effective H_2O_2 neutralization through PRDX/TXN system (the highest H_2O_2 amount in steady state after neutralization) correlated with radioresistance of these cells. Significant differences were observed for lung and breast cancer cell lines, in colorectal cancer cell lines PRDX/TXN system is also less efficient in radioresistant cells, but this observation is not statistically significant.

Peroxiredoxins (thioredoxin peroxidases), are major thiol-targeting enzymes that represent up to 1% of the protein content in some organisms. Their primary role is to serve as antioxidants for H_2O_2 and ONOO-, effectively reducing cellular peroxides by nearly 90% [21]. Six peroxiredoxins can be distinguished: PRDX1, PRDX2 and PRDX6 located in the cytosol, PRDX3 located in mitochondria, PRDX4 located in the extracellular space and PRDX5 located in mitochondria and peroxisomes [23]. Results from [65] indicated that higher PRDX1 and PRDX2 mRNA levels were linked to better survival in lung cancer, while PRDX5 and PRDX6 were associated with worse outcomes [72]. H_2O_2 added to cells can block antioxidant enzymes through microRNA action for specific window of H_2O_2 doses [73]. Such response to oxidants exposure is rather counterintuitive. However, H_2O_2 acts as a signaling molecule, and the observed changes may be elements of the establishment of H_2O_2 levels that are specific and optimal for a given cell type. Indeed, H_2O_2 acts as a promoter for cell cycle progression by oxidizing specific thiol proteins [74]. This group includes PRDXs that serve as signaling mediators, enabling the local accumulation of H_2O_2 through the inactivation of their peroxidase function [74]. Rising H_2O_2 levels can overoxidize PRDXs, limiting their scavenging ability [80]. The concentrations of intracellular H_2O_2 increase as the cell cycle progresses from G1 to mitosis [74]. Cells are the most radiosensitive in G2/M phase of cell cycle, however G2/M cell cycle arrest were shown to correlate with radioresistance of cells [75,76]. Our findings of increased H_2O_2 after PRDX/TXN neutralization need further study, as PRDXs may prevent premature cell cycle progression under oxidative stress from UV or IR during interphase [74] and knockdown of PRDX2 sensitized glioma cells to IR and its decrease lowered GSH and GSR activity, increased cell cycle doubling time and reduced clonogenic cell survival after IR and H_2O_2 [77].

Neutralization by catalase is comparable in lung and breast cancer cells and less effective in radioresistant colorectal cancer cell lines, however it is not statistically significant. D-penicillamine (DPEN) with copper can generate H_2O_2 in cancer cells and induce clonogenic cell killing while catalase inhibited this effect [78]. H_2O_2 induced apoptosis in leukemia KG1 cells, likely via catalase deactivation, ROS accumulation, ATP depletion, caspase-3 activation, and altered Bcl-2 family expression [79]. According to the findings in [80], different concentrations of H_2O_2 can affect both cell growth and programmed cell death. At 50–200 μM, growth of MCF-7 breast cancer cells was inhibited; 1–10 μM stimulated hepatoma 7721 cell growth, and 10 μM increased HT-29 colon cancer cell proliferation. A high dose (1000 μM) induced apoptosis, while 100 μM H_2O_2 reduced migration of H460 lung cancer cells [80].

We also observed the difference for GPX/GSH system of neutralization (similar pattern like for all enzymes active, Figure 4). In colorectal cancer radioresistant cells are significantly more effective in H_2O_2 neutralization than radiosensitive cells, similarly on average in breast cancer cells, however this difference is not statistically significant. In lung cancer neutralization by GPX/GSH pathway is comparable. In research of Zhang et al. they showed that radioresistant cell lines has lower level of ROS and sensitization of LS180 cells with GNP-PEG and GNP-PEG-R8 increased ROS and induced apoptosis. In this studies ROS were marked with dye DCFH-DA which detects several radicals, however it was first used for detection of H_2O_2 [81,82] so observed changes may reflect mainly H_2O_2 levels what corresponds with our findings. In [83] similarly to our simulation results, they showed that lower ROS levels in colorectal cancer are connected to radioresistance and higher levels are

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connected to radiosensitivity [83]. In [84] researchers demonstrated that radioresistant cell lines, developed through repeated irradiation, had lower ROS levels and increased expression of cell viability genes compared to wild-type cells [84]. GSH significantly influences cell cycle progression, with its levels changing during the cycle; the highest concentrations of GSH are detected in the G2 and M phases, intermediate levels are noted in the S phase, and the lowest levels are seen in the G1 phase of the cell cycle. [85]. Analogous to colorectal cancer, cancer stem cells (CSCs) overexpress genes involved in GSH synthesis. Depletion of these genes resulted in elevation of ROS levels and reduced the colony formation in CSCs [86]. Depletion of GSH itself enhanced radiosensitivity of CSCs. Emmink et al. found that GPX2 downregulation increased ROS, increased sensitivity to H_2O_2-induced apoptosis, and reduced colon cancer cell growth and metastasis, suggesting ROS elevation could improve chemotherapy response [87]. The same pattern regarding ROS was observed in head and neck cancers, MitoTam treatment raised ROS levels and increased cell death, even in radioresistant UT-SCC-5 cells [88]. Park et al. showed that H_2O_2 inhibits lung cancer cell growth by inducing cell death and G1-phase arrest in Calu-6 and A549 cells, but not in HeLa cells; the effect varied by cell type and H_2O_2 concentration [89]. It appears that H_2O_2 and its neutralization enzymes levels fluctuate regularly and are directly related to the cell cycle, which may impact the radioresistance of particular cells.

Cancer cells produce higher levels of reactive oxygen species (ROS) due to an increased metabolic rate, genetic mutations, and hypoxia. Studies demonstrated that cancer cells can adapt to elevated ROS levels by activating antioxidant pathways, which enhances their ability to neutralize ROS [90]. A reduced level of ROS in tumor cells correlates with enhanced radioresistance [86]. However, H_2O_2 is used in therapy to sensitize radioresistant cells. Good sensitization effects were observed for melanoma [91] and for cervical, liver and breast cancer [92]. Panieri et al. showed that resistant NSCLC cells can be sensitized by high H_2O_2 levels (48 μM), causing DNA damage and ATP depletion via a caspase-independent pathway, or by lower levels (6.5 μM), which inhibit glycolysis and ATP recovery [80]. Antioxidant enzyme expression differs across various cell types, and the neutralization process does not contribute equally in all cell types. As a result, different levels of reactive oxygen species (ROS) are observed in cells, which may relate to the differing effects of H_2O_2 at various concentrations, particularly on apoptosis and cell proliferation.

Data from our model analyzing cell lines of various tissue origins demonstrate that neutralization systems can differentiate cells both within the same tissue origin and across different origins. This capability is especially relevant due to the significant role of ROS generated during anticancer therapies involving ionizing radiation which induces ROS, leading to changes in cells. In irradiated cells there are observed changes in gene expression, inhibition of proliferation, and death rate. However, some cells do not respond to treatment and those that avoid death are called radioresistant [93]. The effectiveness of radiation therapy in curing human tumors varies greatly. This variability in treatment has been linked to several factors, including the presence of hypoxic cells, inadequate reoxygenation during therapy, low intrinsic radiosensitivity, and the potential for repairing lethal damage [94]. Cells that are mature, differentiated, and not actively dividing (e.g., neurons) are more radioresistant. A cell that is radiosensitive would be more prone to die after exposure to ionizing radiation than a radioresistant one [95].

Findings from the simulations imply that the disparities in radiosensitivity across various cancer cell types could be related to their capacity to neutralize H_2O_2. Analysis of colorectal, lung and breast cancer cell lines showed that radiosensitive cells have different levels of H_2O_2 than radioresistant cell lines, which may be due to the decreased effectiveness of neutralizing enzymes. Previous analyzes [32,33] together with data collected from the model confirm importance of H_2O_2 and suggest that it might be key molecule to understand radioresistance in cancer cells. However, the exact role of H_2O_2 in this process remain to be elucidated.

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