Original Article

Cell Survival Entropy and Cellular Resistance Activation Dose: Effect of Calprotectin on Gastric Adenocarcinoma Cell Line

Mostafa Rezaei-Tavirani[1], Mohammad Rahmati-Roodsari[2], Mehdi Mirzaie[3], Pooneh Amini Geram[1], Sara Sobhi[5]

Abstract

Background: The survival curves generally have been used for analysis of cell survival under stress conditions. They depict the relationship between the fraction of cells retaining their reproductive integrity and the absorbed dose. The median lethal dose, Lethal Concentration, 50% (LC50) of a toxin, radiation, or pathogen is the dose required to kill half the members of a tested population after specified test duration. LC50 frequently are used as a general indicator of a substance's acute toxicity. In this study a novel thermodynamic model is introduced to examine cell survival under stress condition. The survival function describes the surviving fraction of cell survival in different doses and contains information about cell survival. However, for interpretation of cells behavior under stress condition, thermodynamic formalism is utilized.

Methods: Gastric adenocarcinoma cells line were seeded, in the 96 well plates and incubated at 37 °C under 5% CO2 atmosphere for 48 hours. After that, the various concentrations of calprotectin were induced into cells for 48hours for MTT assay proposes. Viability data were analyzed by entropy function and correspond results was plotted. The statistical analysis applied for data validation.

Results: The entropy of survival function has a maximum value at LC50 and is asymmetric around LC50 value, and the rate of change of entropy function is different below and above LC50 value which indicates that cells have different behavior. The entropy function has a more gentle slope above LC50 so we can assume that living cells above LC50 are more resistant to poison used.

Conclusion: It can be concluded that regard entropy function and its derivations provides more possibility for revealing mechanism of cell behavior in stress conditions.

Keywords: Entropy; Cell survival; Lethal concentration; Lethal dose

Please cite this article as: Rezaei-Tavirani M, Rahmati-Roodsari M, Mirzaie M, Amini Geram P, Sobhi S. Cell Survival Entropy and Cellular Resistance Activation Dose: Effect of Calprotectin on Gastric Adenocarcinoma Cell Line. Iran J Cancer Prev. 2013; 6(Suppl):12-16.

1. Proteomics Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Skin Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Proteomics Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
5. Cancer research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Corresponding Author: Sara Sobhi, MD
Tel: (+98) 21 2274 80 01-2
Email: mds.sobhi@gmail.com

Received: 20 Dec. 2012
Accepted: 18 Jan. 2013
Iran J Cancer Prev 2013; Suppl: 12-16

Introduction

One common method for analysis the cell survival under stress is the cell survival curves as a function of applied stress [1]. In this context, several models for accurate interpretation of the different responses of cellular systems such as radiotherapy to kill cancer cells and cancer growth rate after irradiation have been presented [2]. For example single target-single hit model proposes that a single hit on a single sensitive target within the cell leads to cell death. This generates an exponential cell survival curve which appears as a straight line on a semi-logarithmic scale. This model is useful for highly sensitive human tissues, if high Linear Energy Transfer (LET) radiation is used, or if a low dose rate is used [1]. Several mathematical methods, based on the concept of the random nature of energy deposition by radiation such as Multi-Target Single Hit Inactivation model, two component model and linear quadratic models have

been developed to define the shape of cell survival curves [3].

The median lethal dose, LC50 of a toxin, radiation, or pathogen is the dose required to kill half the members of a tested population after specified test duration. LC50 frequently are used as a general indicator of a substance's acute toxicity [4]. The test was created by J.W. Trevan in 1927. The lower value of LC50 is regarded as more toxic, as it means a smaller amount of the toxin is required to cause death [5].

The interpretation of entropy in statistical mechanics is the measure of uncertainty [6], which remains about a system after its observable macroscopic properties, such as temperature, pressure and volume. The equilibrium state of a system maximizes the entropy because we have lost all information about the initial conditions except for the conserved variables. Maximizing the entropy maximizes our ignorance about the details of the system. Although the concept of entropy is derived from thermodynamics and statistical mechanics, however, it has numerous applications in information theory, economics, linguistics, music and biology [7, 8]. Alfano [9] hypothesized that a cell's phenotypic entropy is determined as a function of the survival fraction or proliferation rate of a tumor and additionally, the number of transformed and differentiated states that arise within a particular cell population. The mathematical relations that they have formulated can quantitatively determine how ablation of an oncogene's protein activity can result in apoptosis and/or a decrease in proliferation within a population of tumor cells. The formalism is then used to determine which oncogene or set of oncogenes contribute(s) maximally to a tumor cell's survival and thereby to predict which oncogene(s) is (are) the most appropriate target(s) for maximizing tumor cell destruction [9,10].

In this study a novel thermodynamic model is introduced to examine cell survival under stress condition. The survival function describes the surviving fraction of cell survival in different doses and contains information about cell survival. However, for interpretation of cells behavior under stress condition, thermodynamic formalism is utilized. The entropy of survival function has a maximum value at LC50. At concentrations below as well as above LC50 this function is ascending and descending, respectively. Our hypothesis is that the rate of change of entropy function has considerable information about cells behavior under stress condition. Therefore, derivation of entropy function is studied. The positive, zero and negative values of derivation of entropy function have considerable information about LC50 and resistant cells. In fact, cells have different internal resistance and in our hypothesis, we can show the relationship between dose and resistant cell. As the entropy function has a more gentle slope above LC50, we can assume that living cells above LC50 are more resistant to poison used. Here we show results for "single-hit, single-target" model, but results for other models are similar.

Materials and Methods

Cell Culture

Gastric adenocarcinoma cell line (AGS, NCBI: C-131) was obtained from National Cell Bank of Iran, Pasteur Institute of Iran. The cell culture medium (RPMI), Fetal Bovine Serum (FBS), penicillin and streptomycin were provided by Gibco BRL (Life Technologies, Paisley, Scotland). MKN45 cell line was obtained from cell bank (Pastuer Institute, Tehran, Iran), 3-(4, 5-dimethyl-thiazol-2-yl)-2, 5 diphenyltetrazolium bromide (MTT), Annexin V-FLOUS staining kit (Cat. No. 11 988 549 001) were provided from Roche Diagnostics GmbH (Germany). The adenocarcinoma gastric cell line (MKN45) was cultured in the RPMI medium, that had been treated with FBS (10%, v/v), streptomycin (100 □g/mL), and penicillin (100 U/mL). Cultures were maintained at 37°C in 5% CO2 and 95% air, and the medium changed two times for each week.

MTT Assay

The cells were seeded, in the 96 well plates and incubated at 37 °C under 5% CO2 atmosphere for 48 hours. After that, the various concentrations of calprotectin (0, 1.25, 2.05, 4.1, 8.2, and 16.4μM) were induced into cells for 48hours for MTT assay proposes. The inhibition of cellular proliferation was determined by the modified MTT 3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay, based on the ability of live cells to convert thiazolyl blue to dark blue formazan . After treatment of MKN45 cells with essential oil of Rosa Damascene for 48hours, 20 ml MTT (5 g/l) was added into the wells and incubation continued at 37°C for 4 hrs, and 100 ml DMSO was pipetted to solubilize the formazan product for 30 min at room temperature. The absorbency at 570 nm was measured using ELISA reader. Percent of Cytotoxicity = (1 - mean absorbance of toxicant - treated cells) × 100 Mean absorbance of negative control Percent Viability = 100 – percent of Cytotoxicity

Thermodynamic Analysis

In order to investigate the behavior of cell survival, we use the entropy function [9]. This function is defined as follows:

$$Nc\ Ent(\text{cell survival}) = c\ \ln(Nc!/ns!(Nc-ns)!) \quad (1)$$

Where Nc is the number of cells tested, and ns the number of living cells. Using Stirling's approximation and taking fs = ns / Nc, the following equation is obtained:

$$Ent(\text{cell survival}) = -c[(1-fs)\ \ln(1-fs) + fs\ \ln fs] \text{ for } 0<fs<1 \quad (2)$$

In addition, we have:

Ent(cell survival) = 0 for fs= 0 or .

In the case of "single-hit, single-target" model the entropy function is as follows:

$$Ent(S) = -c\ [(1 - \exp(-KD))\ \ln(1 - \exp(-KD)) - KD \exp(-KD)] \quad (3)$$

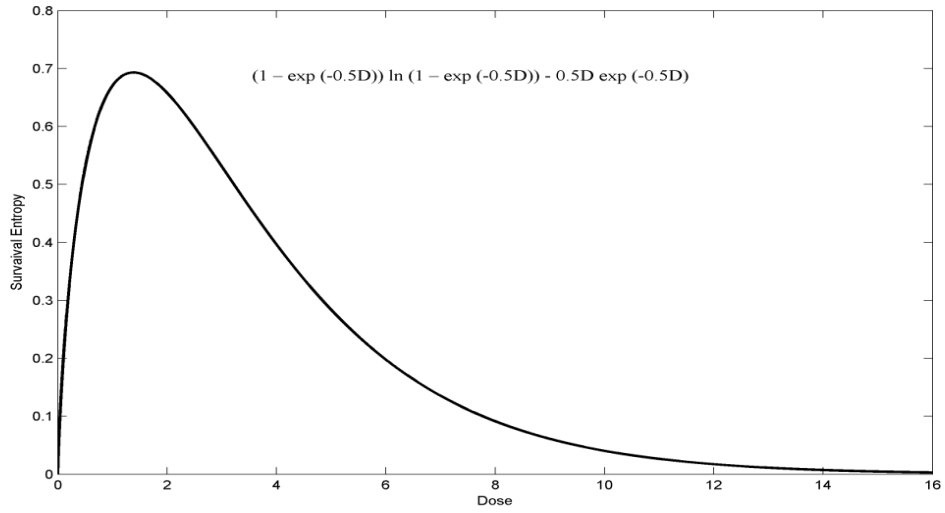

Figure 1. The entropy function in "single-hit, single-target" model with k=0.5

Results

As shown in Figure, this function has the following properties:

1. Function at LC50 dose, takes its maximum.
2. At concentrations below as well as above LC50 dose, the entropy is rapidly increasing and very gently decreasing, respectively.
3. There is a dose above LC50, where the concavity of graph changes.

For more resolution, the derivation of entropy function can be calculated as follows:

$$[Ent(S(D))]' = -K \exp(-KD)\,[\ln(1-\exp(-KD)/\exp(-KD))] \quad (4)$$

In other words:

$$[Ent(\text{cell survival})]' = K(\text{cell survival})\,[\ln(\text{cell survival}/(1-\text{cell survival}))]$$

Where, 1-cell survival=cell death, so

$$[Ent(\text{cell survival})]' = K(\text{cell survival})\,[\ln(\text{cell survival}/\text{cell death})] \quad (5)$$

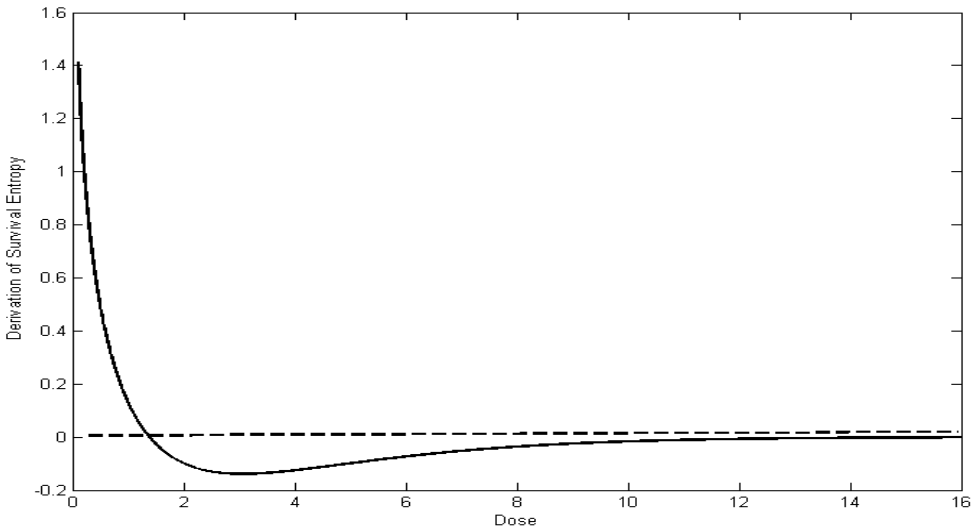

Figure 2. The derivation of entropy function

This function is product of two functions (– K exp (–KD)) and ln (cell death /cell survival) relating derivation of survival function and the ratio of cell death to cell survival, respectively. As shown in Figure 3 rate of change of entropy function below and above LC50 is different. Below LC50, the derivation of entropy function is descending and rapidly decreasing with positive values. At LC50, the

derivation of entropy is zero and above LC50 the values of function are negative. This function has a minimum value above LC50 and after the minimum point increases very gently so that in high dosage values converges to zero. The gentle slope of entropy function indicates existence of cells that are resistant to poison used. This could be due to cell adaptation to poison or activation of cell resistance system. In fact, after getting the minimum of this function, the cells have different behavior. Therefore, our model introduces a dose that is different from LC50 and we call it "cellular resistance dose activation". Here, we explain how to calculate this dose.
At this point the derivative of entropy function minimized, so the second derivative of it is equal zero. Second derivative of entropy function is as follows:
$[Ent(S(D))]'' = -K2 \exp(-KD)[1/(1-\exp(-KD)) - \ln(1-\exp(-KD)/(\exp(-KD)))]$

Now, by $[Ent(S(D))]'' = 0$, the following equations are obtained:

$[Ent(S(D))]'' = 0 \Rightarrow 1/(1-\exp(-KD)) = \ln((1-\exp(-KD))/\exp(-KD))$ (6)

$(1-\exp(-KD))\ln(1-\exp(-KD)) - KD\exp(-KD) + KD = 1$

Consequently, according to equation 3, we have:
$-Ent(D) + KD = 1$
So, the second derivative of entropy function is equal to zero where:
$Ent(D) = KD - 1.$ (7)

This means that the cellular resistance dose activation is where the entropy function and the line KD-1 coincide.

The relationship between LC50 and cellular resistance dose activation:

According to equation 7 at cellular resistance dose activation (Dr) the following equation is hold:
$Ent(Dr) = KDr - 1$

Therefore, $[Ent(Dr)]' = K$.

Since at LC50 derivation of entropy function is zero, by above equation, we imply that cellular resistance dose activation is different from LC50. Additionally, by equation 6 the following equations are obtained:

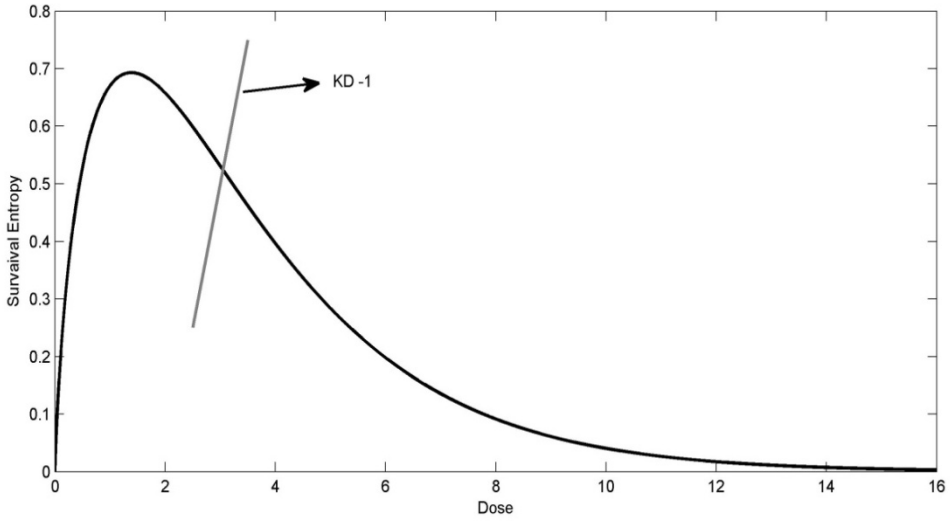

Figure 3. The cellular resistance dose activation is where the entropy function and the line KD-1 coincide.

$(1-\exp(-KDr))\ln((1-\exp(-KDr))/\exp(-KDr)) = 1$
(Cell death) ln(cell death/cell survival) = 1 (8)

On the other hand, below LC50, the expression ln(cell death/cell survival) is negative value and (Cell death) is positive value. So for doses below LC50, the expression "(Cell death) ln(cell death/cell survival)" is negative, while the equation 8 is hold at "cellular resistance dose activation dose". This means that "cellular resistance dose activation dose" is greater than LC50.

Discussion

The curves in Figures 1 and 2 imply that there is an intrinsic difference between the two figures. The curve in Figure 1 is always descending, while Figure 2 shows a significant phase change corresponding to maximum of entropy at LC50 value. It seems that the descending phase of entropy function has considerable information for interpretation of the cell behavior against the used poison. Therefore, derivation of entropy function was studied. The

positive values of this function decrease rapidly, but as depicted in Figure 3, this procedure is stopped at LC50 value. Above LC50, the negative values increases very gently, so in the high dosage values converge to zero. Figure 3 implies a nonsymmetrical situation for cell survival before and after LC50 value. It is reported that cell responses to used doses is different in a dose depended manor. The gentle slope of graph in the negative values can be probably interpreted as activation of cell resistance system against the used poison. There are many evidences about occurrence of cell resistance against stress in high values of stress [11-12]. As it is shown in Figure 3 this perspective opens new insight about interpretation of cell response in the various conditions of stress. It can be concluded that regard entropy function and its derivations provides more possibility for revealing mechanism of cell behavior in stress conditions.

Conclusion

Thermodynamic analysis showed that entropy function and its derivations can be powerful tools for cell survival interpretation.

Acknowledgment

This study was supported by Proteomics Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Conflict of Interest

The authors have no conflict of interest in this study.

Authors' Contribution

Mostafa Rezaei-Tavirani, Mohammad Rahmati-Roodsari, Mehdi Mirzaie, Sara Sobhi designed the study, gathered and analyzed the data and wrote the paper. Pooneh amini geram contributed to study design and sample collection.

References

1. Zali H, Marashi SA, Rezaei-Tavirani M, Toossi P, Rahmati-Roodsari M, Shokrgozar MA. On the mechanism of apoptosis-inducing activity of human calprotectin: zinc sequestration, induction of a signaling pathway, or something else? Medical hypotheses. 2007; 68(5):1012-5.

2. Marashi SA, Rezaei-Tavirani M, Zali H, Shokrgozar MA. Mitochondrial DNA might be influenced in calprotectin-induced cell death. EXCLI Journal. 2008; 7:163-8.

3. Rezaei-Tavirani M, Zali AR, Rostami A, Moghaddamnia SH, Ranjbar B, Rohandeh M, et al. Biophysics, Shahid Beheshti University of Medical Sciences Publisher, Tehran, Iran; 2009.

4. Ardeshiry A, Rezaei-Tavirani M, Mortazavi SA, Barzegar M, Moghadamnia SH, Rezaee MB. Study of Anti Cancer Property of Scrophularia striata Extract on the Human Astrocytoma Cell Line (1321). Iranian Journal of Pharmaceutical Research.2010; 9(4): 403-10.

5. Trevan JW. The error of determination of toxicity. Proceedings of the Royal Society (London).1927; Series B 101: 483-514.

6. Shannon CE. A Mathematical Theory of Communication. Bell Syst Tech J. 1948; 27:379-423.

7. Konopka AK. Information theories in molecular biology and genomics. Nat Encyclopedia Hum Genome. 2005; 3:464-9.

8. Udgaonkar JB. Entropy in Biology. Resonance. 2001; 6: 61-6.

9. Alfano FD. A stochastic model of oncogene expression and the relevance of this Model to cancer therapy. Theor Biol Med Model. 2006; 3:5.

10. Alfano FD. A stochastic model of cellular transformation and its relevance to chemical carcinogenesis. Math Biosci. 1998; 149:95-106.

11. Salmon AB, Murakami S, Bartke A, Kopchick J, Yasumura K, Miller RA. Fibroblast cell lines from young adult mice of long-lived mutant strains are resistant to multiple forms of stress. Am J Physiol Endocrinol Metab. 2005;289:E23-9.

12. Kapahi P, Boulton ME, Kirkwood TBL. Positive correlation between mammalian life span and cellular resistance to stress. Free Radical Biology & Medicine. 1999; 26: 495-500.