Transport Phenomena in Biological Systems
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Lecture - 73
Pseudo-Steady State Approximation Applied to Cancer Treatment

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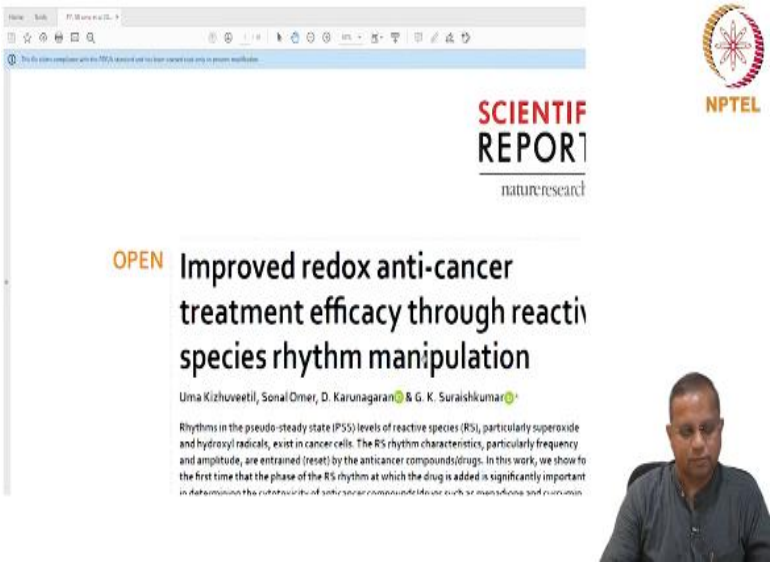

Welcome back, today let us look at the application of the concept of Pseudo-Steady State when we are looking at some aspects related to cancer, this case it is our research for cancer and reactive oxygen species some very interesting aspects related to the rhythms of reactive oxygen species. So we are going to discuss this paper improved redox anti-cancer treatment efficacy, through reactive species rhythm manipulation.

The authors are Uma, Sonal, Mr. Karunagaran and myself. Uma is my PhD student who has just submitted a thesis, Sonal is my current PhD student, Mr. Karunagaran is a colleague of mine my collaborator and myself this was published in scientific reports it is a reputed Journal.

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Let us go to the introduction again abstract, I am not too sure whether we need to get to such level of detail in this lecture. Reactive oxygen species or reactive species RS such as superoxide and hydroxyl radicals seem to be an important set of molecular mediators of the effectiveness of many

anti-cancer therapies. They are also important determinants of the cellular redox status and conditions such as hypoxia even.

Reactive species are also known to regulate cellular rhythms and the components of the cellular redox system such as glutathione, glutathione reductase NAD^+ have been shown to be rhythmic, such rhythms along with the crosstalk with the timekeeping mechanisms control the metabolic transcriptional and translational machinery in the cells, further their rhythms and their alterations have been linked to immune getting responses a lot of detail do not worry about it.

Let me go to this directly; however the rhythms in these Pseudo Steady State levels of the more fundamental molecules the reactive species themselves have not been reported in the above context probably due to an incorrect perception of their utility given their high reaction rates. The cellular antioxidant levels have been used as an indirect measure of the oxidative stress in the cells and oxidative stress is caused by an imbalance in the rates of production and consumption of reactive species.

The indirect measure seems to arise from an expectation based on the molecular interaction between enzymatic antioxidants and the relevant reactive species. For example, superoxide dismutase which quills, or which scavenges superoxide, however the dynamic aspects of the cell system do not seem to be considered in that expectation. For example, the rate constants of reactive species reaction are many orders of magnitude higher than the synthesis rates of the antioxidant enzymes through transcription translation.

Time constants for transcription translation is at least a few minutes long whereas these rate constants signal the rate constants at 10^{10}, 10^9 and so on so forth very high so there is a mismatch here. In other words if the cell needs to wait to sense reactive species and then start the process of making antioxidants it is just not going to work, there is certainly going to be some basic level of antioxidants presented to take care of exigencies.

But it has to respond to the node of reactive species and the mechanism seems to be different which we are not very sure of at the stage, but anyway let us get back to this the also the presence of a certain that is what I meant also the presence of a certain basic level of antioxidants does not explain the dynamic relationship between reactive species and antioxidants, here we show that no correlation exists between the temporal inter-cellular Pseudo Steady State.

Specific levels of superoxide dismutase and superoxide in untreated in these cells and so on, it is a reasonably common practice to use the easily measurable antioxidant enzyme levels as a surrogate measure of the oxidative stress caused by the increased RS in cells. The lack of a relationship between RS and the relevant antioxidant in the cancer cell line a mammalian cell line a bacterium and a microalga a close across different domain.

Even you are talking mammalian cells which is the eukaryote, you are talking of bacterium which is a prokaryote rate and microalga is of course a eukaryote. In both domains this relationship seems to hold that is what we found, it is what hit in the face actually, the Pseudo Steady State levels of reactive species can be measured using cell-permeable fluorescent dyes the common approach.

However is to measure total oxidative capacity of cells using this fluorescence which we already talked about this approach is inaccurate due to contributions to measure fluorescence by many molecules is already well documented. But still the practice continues unfortunately however the use of other dyes to obtain the; Pseudo Steady State levels of reactive species have been shown to be valuable.

They also provide the variations in the individual Pseudo Steady State RS levels, which we sure to be important in terms of drug activity. So we have found and in essence what we have found in this paper first, then I will tell you the Pseudo Steady State aspects or this is related to the Pseudo Steady State aspects you take the Pseudo Steady State levels which we actually measure and we find that it follows a certain rhythm in the cell even under normal times and that rhythm gets altered when it encounters stressful conditions one.

Two, the cancer cells have a certain rhythm and when you treat those cancer cells that rhythm changes and the time of treatment seems to determine the way the rhythm changes or the extent to with the rhythm changes. Once we have found this we did further experiments, we also found that by appropriately tuning the time at which you take the anti-cancer drug you could increase the effectiveness of the drug by as high as 27% that was a big contribution of this work.

So all that comes down to Pseudo Steady State levels that is what I am trying to tell you here right a couple of things here Menadione and Curcumin differently altered the superoxide and hydroxyl radical levels graphs are given here. The temporal levels of antioxidants and reactive species are not correlated which is an important aspect this has very many significances. So if you are using antioxidants to infer the state of the cell and then the level of antioxidant if it is high it just shows that the damage has been done gone with and the antioxidant level is high.

It does not provide you a means by which to make a decision as to how you need to treat it just indicates the state that is already passed, it does not give you information on what to do if you just follow the antioxidants. Whereas if you use the Pseudo Steady State levels of reactive species instead of the antioxidants, antioxidants give you past information Pseudo Steady State levels give you a present information and we need the present information to decide on how to treat the cell.

So that the human being who is made up of these cells and many more cells recovers from the infliction from the disease. So in that sense the aspect of using the Pseudo Steady State levels of reactive species becomes crucial in very many different disease conditions, this is what we had trying to say in this paper and temporal levels of antioxidants and reactive species are not correlated and so on and so forth.

And so the entrainment of redox rhythms in menadione and Curcumin these are two model compounds which act against cancer cells by inducing reactive species. By the way most treatments all chemotherapy or radiotherapy act by increasing the reactive oxygen species levels in the cell, to us to a level that can kill the cancer cells that is the way they act. So, this is fundamental for that and let me see whether we have something more interesting here.

This is the lack of correlation that in cancer cells at least this is the superoxide dismutase level the antioxidant level versus the superoxide levels, they do not seem to be related in any useful way at all. So this is what we meant, we also showed this in microalga, Chlorella vulgaris we also showed this in Bacillus subtilis which is a bacterium and when I first saw this I said something must be wrong.

Go back and check and then we started collecting data after a few times, this kept occurring in every single time and I could not ignore it anymore and then we checked one system the other system everywhere it worked out and then it shows something very significant which has a lot of therapeutic importance and so on so forth, treatment strategies it has importance for development of treatment strategies and things like that.

So that is important here I just thought I will let you know I think conclusions may not be very relevant here fine. So in this series of lectures we saw the applications of the principles that we learned in this course on transport phenomena directly applied to even cutting-edge research to make significant contributions that is a take-home message, here we have spent a good enough time getting that across when we meet next we will get back to another case of multiple driving forces resulting in fluxes see you in the next class bye.