Modelling DNA Damage-Induced Apoptosis

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Introduction

Cancer develops following an initial oncogenic transformation event that ultimately creates an imbalance between cell proliferation and cell death. This results in an inappropriate proliferation of cells and the development of a tumour. Various therapeutic strategies are designed to address this balance by intervening at distinct points in either the cell proliferation, cell death or associated regulatory pathways. Physiomics is developing mathematical models of these processes in order to aid mechanistic understanding and assist in the identification and validation of novel therapeutic targets.

The intrinsic pathway of apoptosis is activated in response to DNA damage caused by agents such as radiation, reactive oxygen species and compounds such as cisplatin and mitoxantrone. The signalling pathways activated by DNA damage converge on the regulation of the bcl2 family of proteins that critically determine mitochondrial permeability and the irreversible commitment to apoptosis [1]. Mutations in key proteins involved in these pathways often lead to oncogenic transformation and they are consequently of key interest in cancer therapy. It is clear that a systems understanding of these pathways will underpin the development of more efficacious anti-cancer therapies.

Here we present some results obtained with our model of the intrinsic pathways of apoptosis, activated by DNA damage. The model has been created through a collaboration with ValiRx plc to aid in their research and development of novel therapeutics against the bcl2 family. Our model is reproducing published experimental results.

Methods

We have created a large model of DNA damage-induced apoptosis and calibrated it for the prostate cancer cell line, LNCaP. The model includes over 120 reactions, over 80 species, more than 200 parameters and has been created with information carefully curated from the available scientific literature. Once apoptosis is initiated, the response is rapid, occurring within minutes. However, within a population of cells, considerable variation occurs between the time of stimulus and the time of onset of apoptosis. Our completed model therefore contains ordinary differential equations that reflect the average behaviour of a population of cells. An outline of the apoptosis model is shown in Figure 1.

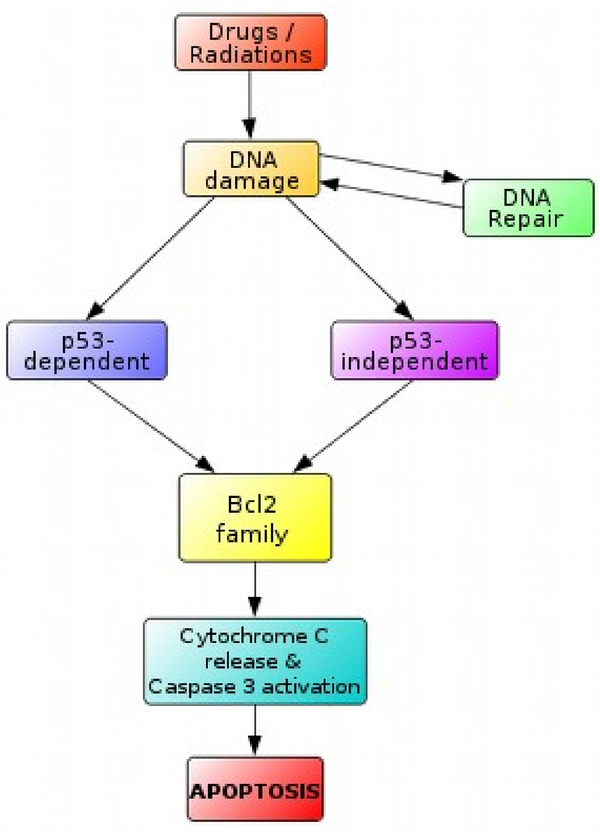

Figure 1: DNA damage-apoptosis model structure. The diagram depicts the general structure of the model.

In order to calibrate the model we parameterised it to fit with published cisplatin concentration-response and time course experiments in LNCaP cells [2]. We also compared model simulation results with those of other published experiments [3] and further refined the model as necessary. We simulated caspase 3 activation and related this to cell apoptosis as these events have been shown to be temporally separated by only a few minutes compared with the time-course for apoptosis of tens of hours [4].

Results

Figures 2 and 3 below compare model simulations (solid line) of caspase 3 activity as a measure of cell death compared with published data for growth inhibition of LNCaP cells after exposure to cisplatin (ref. [2], shown by crosses). Figure 2 shows cell death at 48 hours for different concentrations of cisplatin; Figure 3 shows cell death as a function of time after exposure to 10μM cisplatin.

Although a full simulation would involve a multi-cellular simulation of a growing plate of cells (i.e. proliferation and death processes), there is nevertheless good agreement between the model results (cell death) and experimental data (growth inhibition) at moderate-high concentrations and time points.

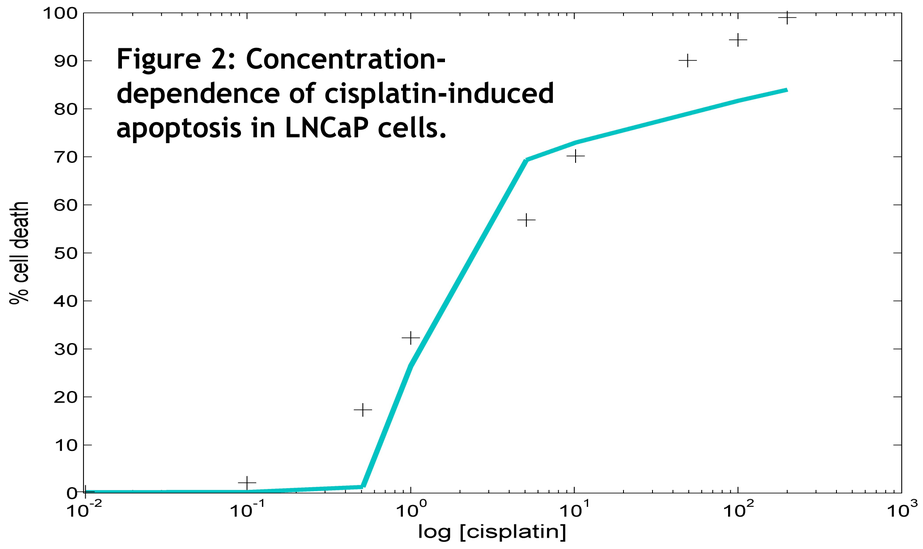

Figure 2: Concentration-dependence of cisplatin-induced apoptosis in LNCaP cells.

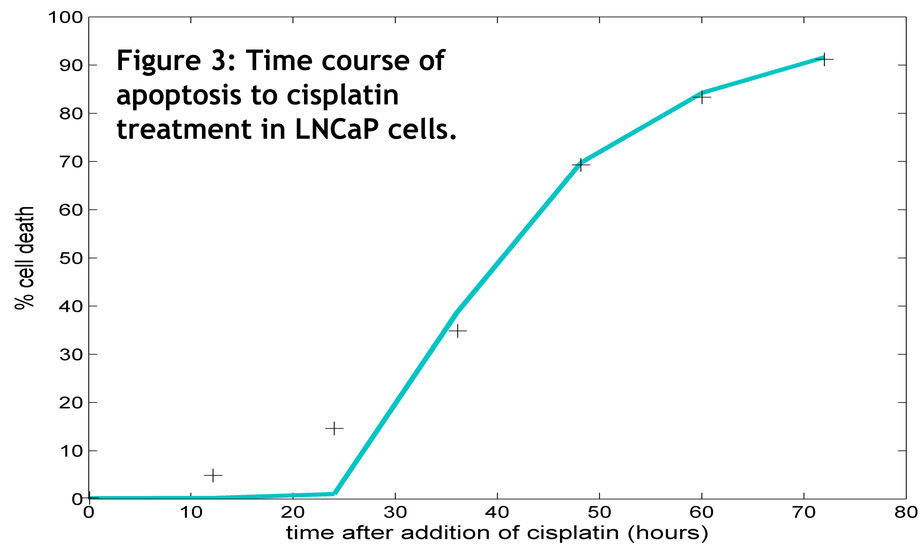

Figure 3: Time course of apoptosis to cisplatin treatment in LNCaP cells.

Figure 4 shows the effect on LNCaP cell apoptosis of over or under-expressing bcl-xL. In the original experiment [3], mitoxantrone was added together with an antisense oligonucleotide shown to inhibit bcl-xL mRNA translation, or it was added to cells overexpressing bcl-xL by 8.6 fold. Cell death was assessed after 72 hours. Simulation results show good agreement for under-expression, but the overexpression results are consistent with a fold increase of only 2.25. A possible explanation is that not all of the over-expressed protein is functional in the experiments.

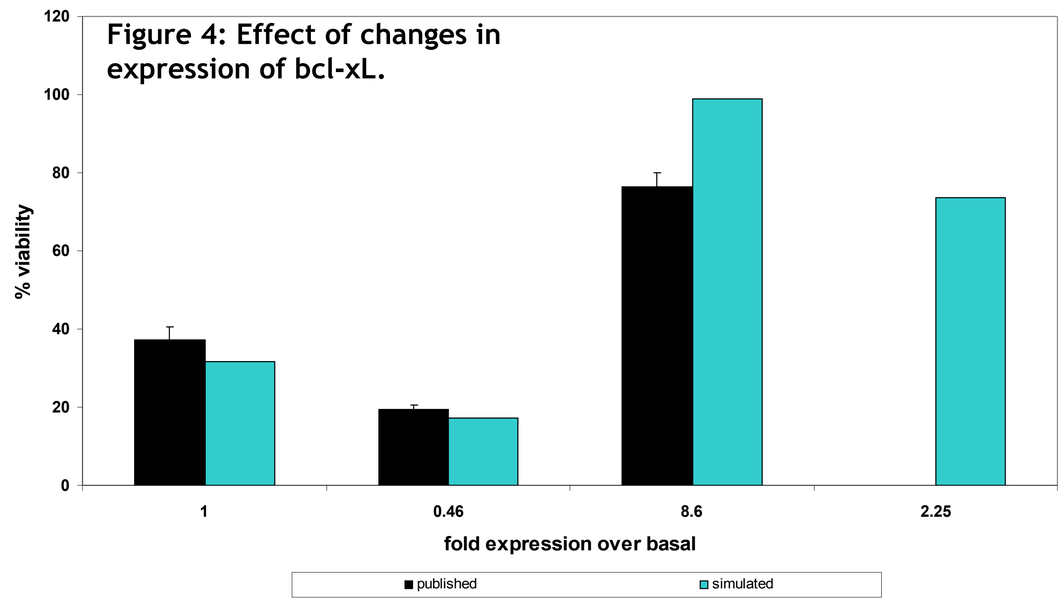

Figure 4: Effect of changes in expression of bcl-xL.

Conclusions

Our model is clearly paralleling the published observations both for the response of LNCaP cells to cisplatin treatment and for the effects of a reduction in bcl-xL expression on mitoxantrone-induced LNCaP apoptosis. Although the results of model simulations do not perfectly match published observations, there are tolerances both in the model and original experimental results and the fit is generally good. Moreover, they suggest that our model is capable of providing a mechanistic insight into the results of experimental observations and may be used to identify and validate novel therapeutic targets.

[1] Norbury & Zhivotovsky, Oncogene (2004) 23:2797-2808
[2] Nomura *et al.*, Urologic Oncology (2004) 22:453-460
[3] Lebedeva I *et al.*, Cancer Res (2000) 60:6052-6060
[4] Tyas *et al.*, EMBO Reports (2000) 1(3):266-270