Understanding chemotherapy-induced replicative stress to identify rational combination therapies

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Abstract

Cells respond to DNA damage by activating complex signaling networks that decide cell fate, promoting not only DNA damage repair and survival but also cell death. We have developed a multi-scale computational model using the U2OS osteosarcoma cancer cell line that quantitatively links chemotherapy-induced DNA damage response signaling to cell fate. The computational model was trained and calibrated based on an extensive data set that comprises cell cycle distribution of the initial cell population, signaling data measured by western blot, and cell fate data in response to chemotherapy treatment measured by time-lapse microscopy. The resulting mechanistic model can predict the cellular responses to chemotherapy alone and in combination with targeted inhibitors of the DNA damage response pathway, which we were able to confirm experimentally. Computational models, like the one presented here, can be used to understand the molecular basis that defines the complex interplay between cell survival and cell death, as well as to rationally identify chemotherapy-potentiating drug combinations.

Construction of a multi-scale computational model to predict cell fate in response to chemotherapy

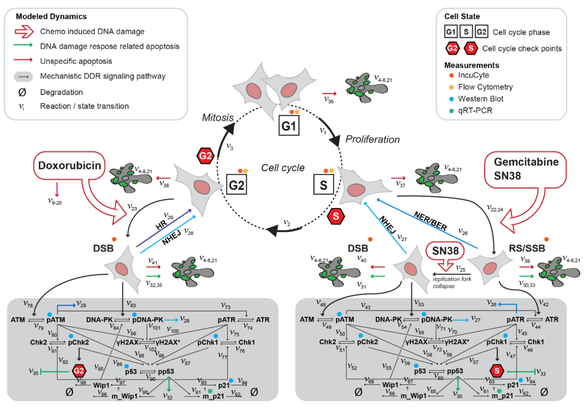

Figure 1. Illustration of the computational model describing the DNA damage response signaling, gene expression changes, cell cycle stages and cellular responses. The model includes a representation of the cell cycle (stages G1, S and G2) and the DNA damage repair signaling pathways (grey boxes). Chemotherapy dose as well as the cell cycle composition prior to treatment are the model inputs. Doxorubicin leads to double strand breaks (DSBs) in G2 phase whereas SN38 and gemcitabine induce single strand breaks (SSBs) in S Phase. DSBs and SSBs trigger distinct individual branches of the signaling model. Stage dependent cell cycle check points (stop signs) and different types of DNA repair pathways (NHEJ, HR, NER/BER) link the signaling model back to the cell cycle model. The model includes p53-dependent apoptosis as well as drug effects on proliferation and survival signaling. Node labels with m_ indicate mRNA. The reactions are labeled with v1-v102.

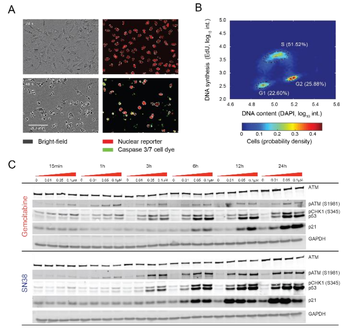

Figure 2. Experimental data-sets used to train the computational model. (A) Proliferation & cell death: exemplary microscopy images of untreated U2OS cells at the 24-hour time point and of cells treated with 1 µM doxorubicin at the 48-hour time point. (B) Cell cycle distribution: distribution of cell cycle stages prior to drug treatment quantified by flow-cytometry measuring DNA content (DAPI) and DNA synthesis rate (EdU). (C) DNA damage response signaling: U2OS cells were exposed to 0, 0.01, 0.05 and 0.1 µM gemcitabine and SN38 (data for doxorubicin not shown) and the abundance of phosphoprotein levels was assessed over a period of 24 hours. pATM, pCHK1, p53, and p21 were measured by Western blot and are exemplarily shown here.

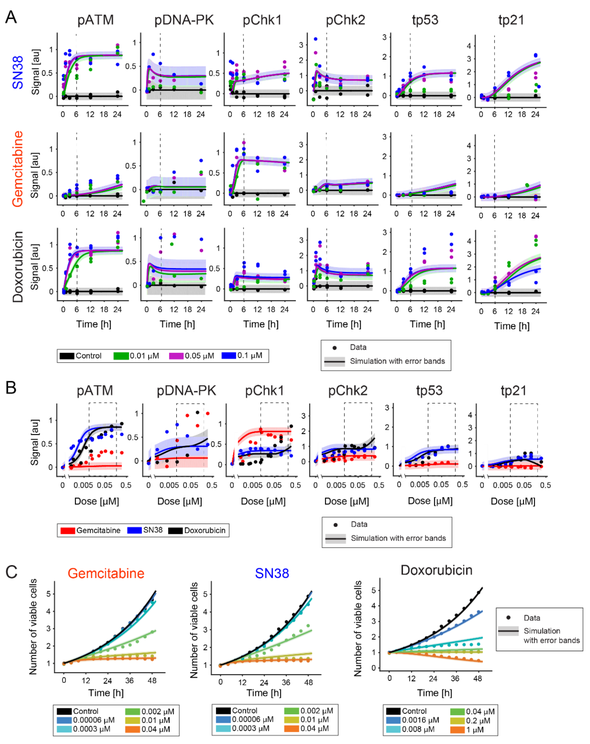

Figure 3. Trained computational model describes signaling as well as proliferation data as a function of time and dose. Dots represent data of separate experiments (N=1); each replicate was individually used to calibrate the computational model; lines indicate the corresponding simulation trajectories. (A, B) Quantification of DNA damage response protein phosphorylation by Western blot data shown in Figure 2. (A) Time courses for 0.01, 0.05, and 0.1 µM of chemotherapy over 24 hours. (B) Dose response curves at the 6h time point. (C) Imaging based quantification of cell proliferation dynamics with and without chemotherapy exposure by counting nuclear reporter positive cells. Data is normalized to time point zero. Dots represent experimental data used to calibrate the computational model and solid lines the simulated model trajectories. Further experimental details are described in the Methods Section.

Identification of chemotherapy-potentiating drug combinations

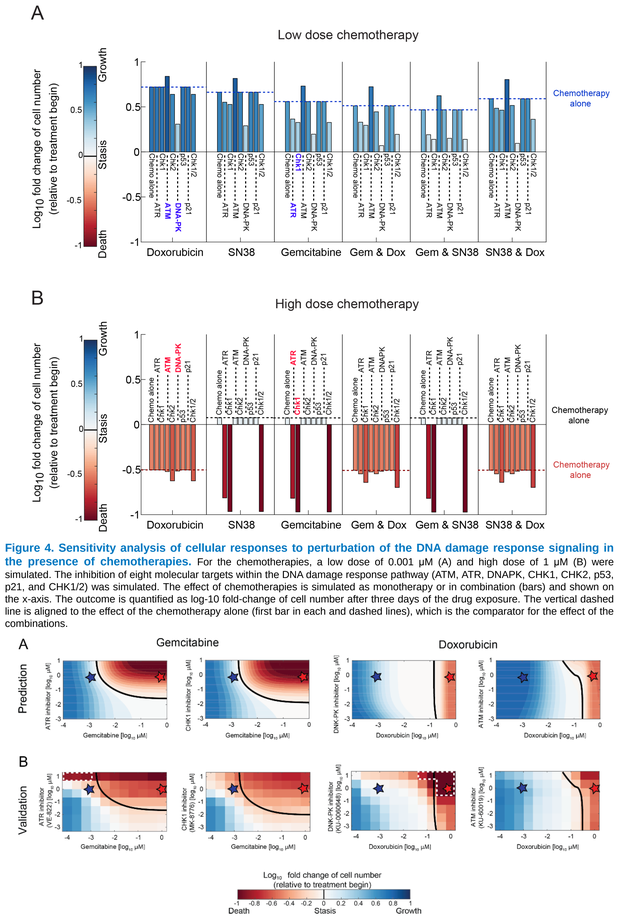

Figure 4. Sensitivity analysis of cellular responses to perturbation of the DNA damage response signaling in the presence of chemotherapies. For the chemotherapies, a low dose of 0.001 µM (A) and high dose of 1 µM (B) were simulated. The inhibition of eight molecular targets within the DNA damage response pathway (ATM, ATR, DNAPK, CHK1, CHK2, p53, p21, and CHK1/2) was simulated. The effect of chemotherapies is simulated as monotherapy or in combination (bars) and shown on the x-axis. The outcome is quantified as log-10 fold-change of cell number after three days of the drug exposure. The vertical dashed line is aligned to the effect of the chemotherapy alone (first bar in each and dashed lines), which is the comparator for the effect of the combinations.

Figure 5. Drug response profiles for gemcitabine and doxorubicin. (A) Predicted by the computational model and (B) experimental validation using small molecule inhibitors of ATR, CHK1, DNAPK, and ATM (B). For the analysis the drug and inhibitor concentrations were varied over a wide range and outcome is quantified after three days. The black line indicates the stasis line as predicted by the computational model. Blue stars represent the low chemotherapy dose and the red stars the high chemotherapy dose in combination with a targeted inhibitor as chosen for the single dose combinations shown in Figure 4. The dashed white boxes indicate areas where the model prediction and experimental data are discordant.

Dynamics of live and apoptotic cells treated with potentiating drug combinations

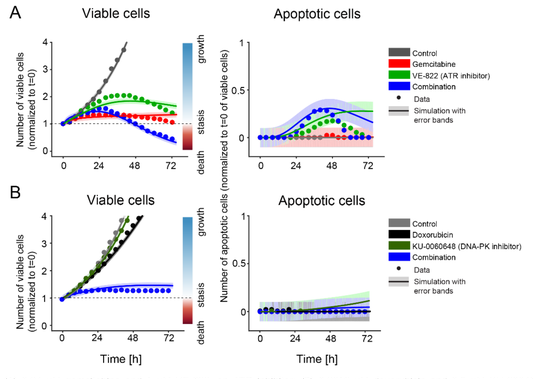

Figure 6. (A) 0.04 µM gemcitabine and 1 µM VE-822, an ATR inhibitor; (B) 0.0016 µM doxorubicin and 0.5 µM KU-60648, a DNAPK inhibitor. The experimental data (dots) are compared to the response simulated by the computational model (solid lines) together with their associated uncertainties (shades).

In vitro and *in vivo* response to SN38 and ATR inhibitor in cervical cancer model

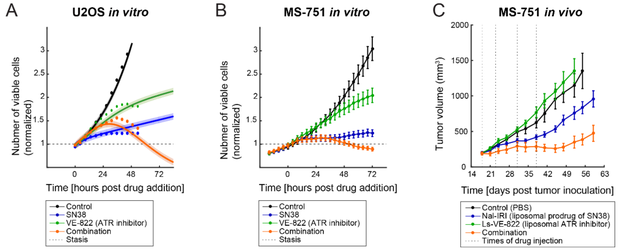

Figure 7. (A) Time course of cell number measurement post treatment with 0.01 µ M SN38 and 1 µ M VE-822 as monotherapies and in combination in U2OS cancer cell line and corresponding model simulation. Drugs were added at the 24-hour time point. (B) Time course of cell number measurement post treatment with 0.01 µM SN38 and 1 µM VE-822 as monotherapies and in combination. Drugs were added at the 24-hour time point. (C) Drugs were injected as monotherapy or in combination on day 17, 24, 31 and 38 post tumor implantations as indicated by the doted lines. A liposomal formulation of the prodrug of SN38 (Nal-IRI) was given at a dose of 5 mg/Kg and liposomal ATR inhibitor Ls-VE-822 at 20 mg/Kg i.v.. For the combination treatment Ls-VE-822 was injected 24h post the nal-IRI injection. Error bars are derived from ten mice per group.

Summary

- We developed a computational model that links the DNA damage response-signaling to cellular responses such as DNA damage repair, cell cycle arrest, and apoptosis and incorporates multiple time scales.
- The model was trained and validated with an extensive signaling data-set and time-lapse microscopy tracking the cell fate.
- Using the computational model, we systematically investigated potentiating drug combinations *in vitro* and *in vivo* between DNA damage inducing chemotherapy and DNA damage signaling modulators.
- These results suggested that the ATR/CHK1 pathway in combination with SSB damage inducing chemotherapy like SN38 or gemcitabine are promising drug combinations.
- Future work will be required to include the characterization and prediction of heterogeneous responses across multiple cell lines.
- The current model can serve as a backbone to understand where cellular heterogeneity will most likely influence the response to drug combinations.

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