

Influence of p53 status on anticancer drug sensitivity and drug resistance

INTRODUCTION

In the search for new drug candidates, multi-parametric cell-based assays can satisfy the enormous demand for high-content information in preclinical evaluation of the therapeutic potential of new compounds. Here, the influence of p53 status on cellular sensitivity to 5-fluorouracil (5-FU), an anticancer drug which is transformed into cytotoxic metabolites to finally inhibit the cells' ability to synthesize DNA, was investigated. A human colon carcinoma cell line expressing the gene active (HCT 116 p53+/+) and a p53 deficient derivate (HCT 116 p53-/-) was tested for up to five days. On-line measurements of the cellular acidification rates, the oxygen consumption and the cell impedance/adhesion provided functional information on the specificity of responses.

MATERIALS & METHODS

Cell culture.

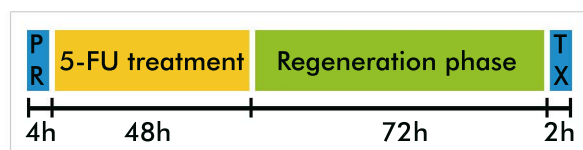
HCT 116 p53+/+ cells and HCT 116 p53-/- cells were cultured overnight on the **Bionas Discovery™ metabolic chip SC 1000** before being inserted into the **Bionas Discovery™ 2500 system**.

Test Compound.

5-fluorouracil (5-FU) was purchased from Sigma Aldrich and applied for 48h at concentrations of 10 μM , 50 μM and 375 μM , respectively.

Implementation of measurement.

The perfusion system supplied the cells with nutrients following a stop and go mode of 4 minutes each.



After the baselines for acidification, oxygen consumption and cell impedance were determined (pre-running phase, PR), 5-FU was applied in the above mentioned concentrations. Cellular responses to 5-FU were measured for 48 hours followed by a regeneration phase with medium without compound for 72 hours. At the end of the experiments cells were killed by adding 0.2% Triton X-100 to get a neutral signal without living cells on the sensor surface (negative control, TX).

Analysis in the Bionas Discovery™ 2500 system.

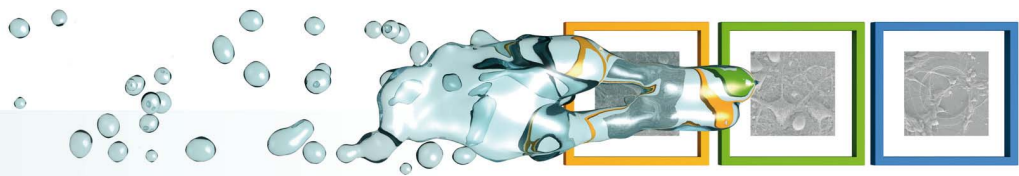
Basically, HCT p53+/+ cells showed higher cellular activity compared to untreated HCT p53 -/- cells. Acidification and respiration rates increased faster in HCT 116 p53+/+ cells than in HCT 116 p53-/- cells, which correlates very well with cell proliferation.

5-FU treatment inhibited metabolic activity, namely acidification and oxygen consumption of both cell lines in a dose-dependent manner. During regeneration, after 5-FU containing medium was replaced by fresh medium without 5-FU, the cells reactivated their metabolism also in a dose-dependent manner. Only the highest concentration of 375 μM further inhibited acidification rates in both cell lines.

Depending on the concentration of 5-FU, cell impedance/adhesion increased at first and then decreased in the presence of 5-FU in HCT 116 p53+/+ cells and showed a dose-dependent variability. At 375 μM 5-FU cell impedance dropped below initial values (Fig. 1). 5-FU treated HCT 116 p53-/- cells did not show any changes of impedance/adhesion during regeneration compared to the 48h treatment, not even at 375 μM (Fig. 2). The values remain unchanged at every dose applied.

The patterns of inhibition or regeneration can be illustrated in more detail by expressing the results as a percentage of control as shown in Figure 3 and Figure 4.





HCT 116+/+ CELLS EXPOSED TO 5-FU

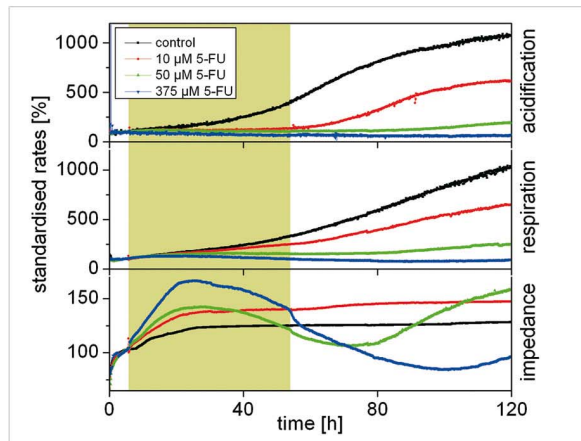


Fig. 1: Standardized rates of 5-FU treatment (yellow) on acidification, oxygen consumption and cell impedance/adhesion of HCT 116 p53+/+.

HCT 116+/+ CELLS EXPRESSED AS POC

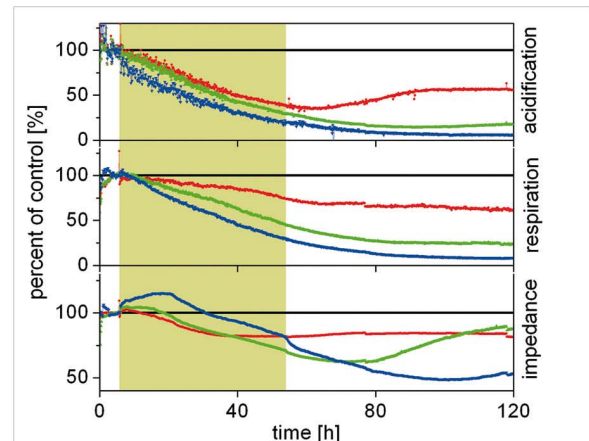


Fig. 3: Percent of control rates of 5-FU treatment (yellow) on acidification, oxygen consumption and cell impedance/adhesion of HCT 116 p53+/+.

HCT 116-/- CELLS EXPOSED TO 5-FU

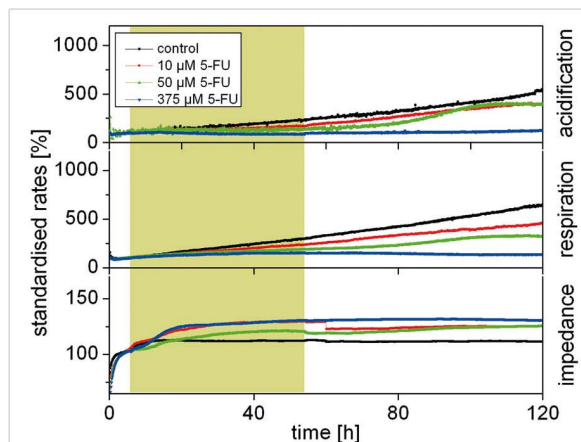


Fig. 2: Standardized rates of 5-FU treatment (yellow) on acidification, oxygen consumption and cell impedance/adhesion of HCT 116 p53-/-.

HCT 116-/- CELLS EXPRESSED AS POC

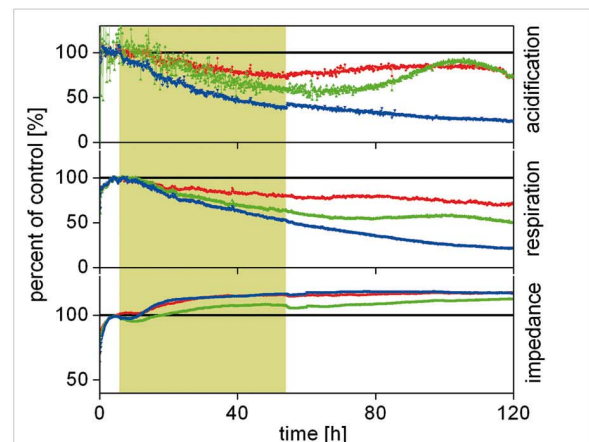


Fig. 4: Percent of control rates of 5-FU treatment (yellow) on acidification, oxygen consumption and cell impedance/adhesion of HCT 116 p53-/-.

CONCLUSION

Cell impedance/adhesion results reflect that 5-FU affects genes which regulate cytoskeleton, induce apoptosis and cause cell detachment in HCT 116 p53+/+ cells. A strong reduction of acidification of HCT 116 p53+/+ after 5FU application indicates a higher effectiveness of 5FU on cells with active p53. In addition, regeneration of cells with activated p53 is attenuated.

Taken together, the **Bionas Discovery™ 2500 system** represents a valuable tool for studying phenotypic changes in response to chemotherapeutic drugs in real time and for the measurement of facet-rich anticancer drug effects.

KEYWORDS

Chemosensitivity, 5-FU, HCT116 cells, p53, cell metabolism, regeneration

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