Chemotherapeutic Drug-Induced Tumor Neoantigen Discovery

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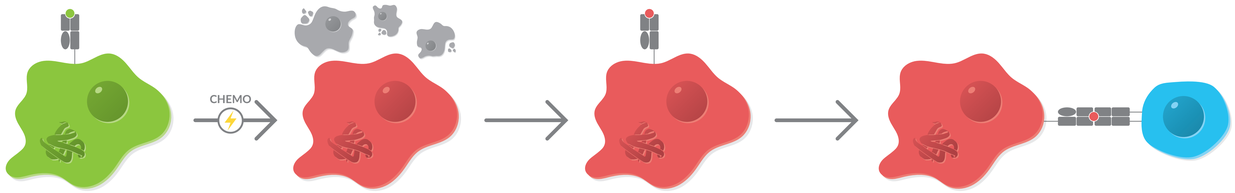
Abstract

Checkpoint inhibitors have shown significant efficacy against a variety of cancers, changing the way many cancers are treated. However, only a subset of patients currently benefits from these therapies. Finding ways to increase the number of patients who can is a primary interest. Tumor mutational burden is one biomarker for checkpoint inhibitor efficacy, which may point to effective strategies for increasing tumor response to therapy. The combination of checkpoint inhibitors with existing chemotherapy is being pursued in several clinical trials and has been shown to have synergistic effects in a mouse tumor model. Chemotherapeutic drugs work in many ways to kill tumor cells, notably by attacking DNA and its replication machinery to prevent proliferation. These DNA-targeting drugs can cause mutations in cells that are not killed outright. We hypothesize that some chemotherapeutics can cause DNA mutations that are translated into proteins and peptides that make their way into the MHC class I peptide presentation pathway. If these neoantigens are seen by CD8+ T cells as foreign, T cells will attack these tumor cells. This could provide one mechanism of action for an increase in therapeutic efficacy in tumors treated with combinations of chemotherapeutics and checkpoint inhibitors. We tested the ability of two drugs, SN-38 (an active metabolite of irinotecan) and oxaliplatin, to induce neoantigens by sequencing peptides from class I molecules in a colon cancer cell line.



Methods and Results

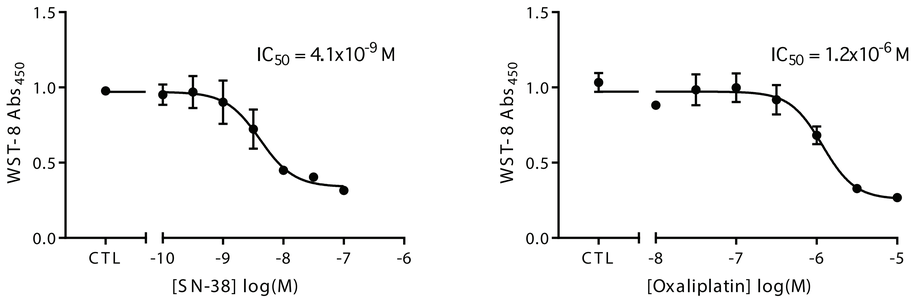
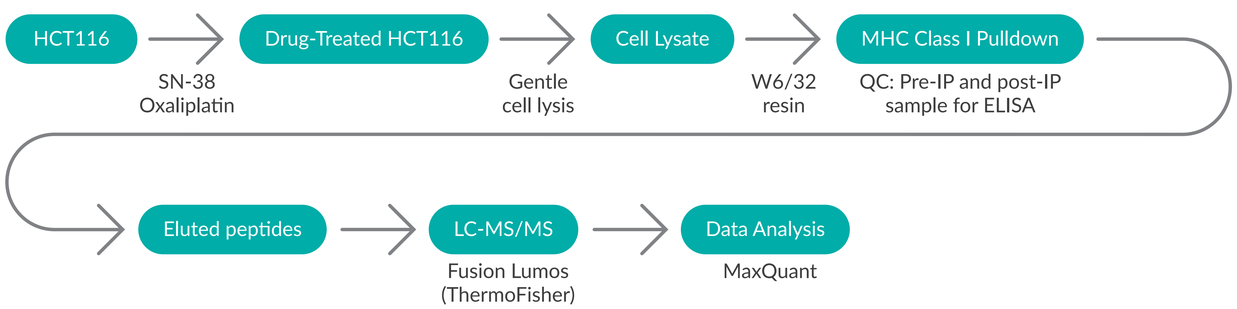


Figure 1. Chemotherapeutic drugs affect the viability of HCT116 cells.
HCT116 cells were plated in 96-well plates and allowed to adhere overnight. Cells were treated with the indicated concentrations of SN-38 (left, Cayman Item No. 15632) or oxaliplatin (right, Cayman Item No. 13106) for three days. At the end of treatment, a WST-8 Cell Proliferation Assay (Cayman Item No. 10010199) was performed to determine the optimal dose of chemotherapeutic. Doses around the IC_{50} and IC_{20} were chosen for the potential to induce mutations in the tumor cells without killing them.

Workflow



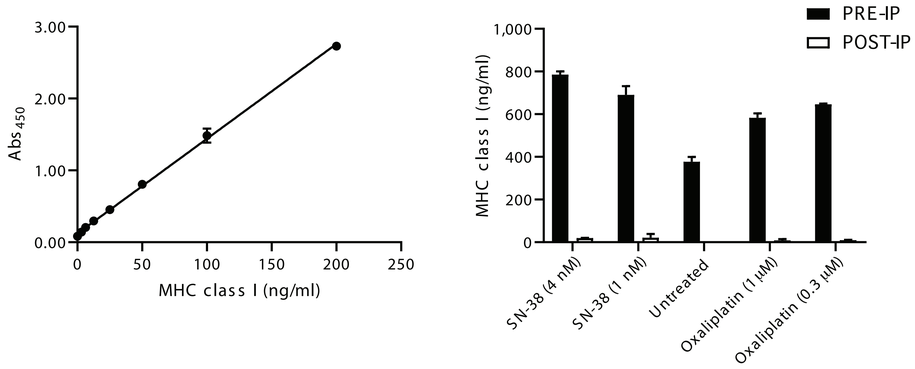


Figure 2. W6/32-conjugated resin captures MHC class I from HCT116 cell lysates.
HCT116 cells were treated for three days with either SN-38 or oxaliplatin, or were left untreated, followed by lysis. Lysates from $60x10^6$ to $120x10^6$ cells were incubated with W6/32 resin to immunoprecipitate MHC class I. To determine the quality of the capture of MHC class I molecules from lysates, PRE-IP lysates (filled bars) and resin flow-through (POST-IP, empty bars) were assessed by ELISA. Plates were coated with 1 µg/well W6/32, the standard curve was generated using a recombinant MHC class I complex, and the captured MHC class I was detected by an HRP-conjugated β2 microglobulin antibody. Data show nearly complete capture of MHC class I from the cell lysates, indicating high quality of the immunoprecipitation.

Table 1: Peptide discovery in eluates immunoprecipitated from MHC class I molecules
The W6/32 resin bound to MHC class I immunoprecipitated from lysates of treated cells was washed thoroughly and peptides were eluted using acetic acid and trifluoroacetic acid. Peptides were analyzed by nano LC-MS/MS, data were processed by MaxQuant software, and searched using Andromeda with the Swiss-Prot human database. The total number of peptides was similar for each of the treatment conditions. In addition, single amino acid substitutions were analyzed for each dataset, and the number of peptides with modifications are presented.

Sample	SN-38 (4 nM)	SN-38 (1 nM)	Untreated	Oxaliplatin (1 µM)	Oxaliplatin (0.3 µM)
Total Peptides	3,119	3,178	2,799	3,038	3,072
Single amino acid substituted peptides	158	176	102	154	141

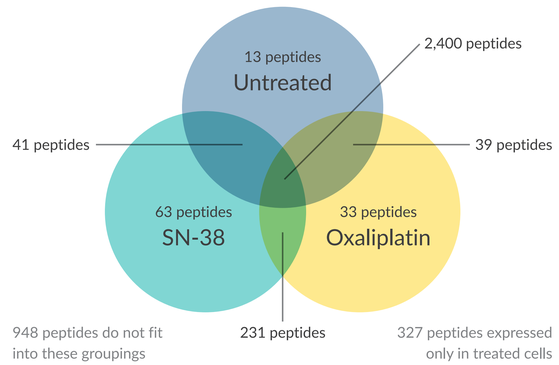


Figure 3. Aberrantly expressed antigens may be found in drug-treated cancer cells.
Peptides sequenced by mass spec were grouped according to treatment, and it was found that the majority of peptides presented by MHC class I are common to all the treatment groups. A substantial portion of peptides identified, however, were unique to the samples that had been treated with chemotherapeutic drugs. As these peptides were all identified using the Swiss-Prot human database, they have the potential to be aberrantly expressed antigens induced by drug treatment.

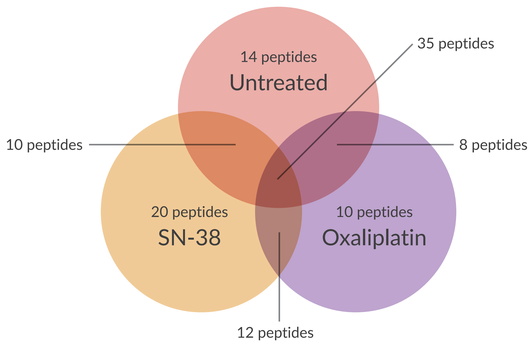


Figure 4. Mutated peptides are found in drug-treated cancer cells.
Single amino acid substitutions were allowed for each peptide dataset, and the peptides discovered were grouped by treatment. Similar numbers of unique mutated peptides were found in each group, while most mutated peptides (108) did not fall along treatment group lines, suggesting that many more replicates would be required to draw significant conclusions.

Conclusions and Further Studies

1. ELISA assessment of the capture of MHC Class I from cell lysates by antibody-coated resin can be used as a quality control measure for immunoprecipitation.
2. Substantial numbers of peptides can be derived from relatively small numbers (60-100 million) of certain cancer cell lines, even without interferon treatment.
3. Treatment of cancer cells with lower doses of chemotherapeutic drugs may result in aberrant expression of peptide antigens or expression of mutated antigens.
4. Induction of aberrantly expressed antigens by drug treatment may explain some of the efficacy of combination therapies with checkpoint inhibitors.
5. Further studies comparing mass spec data with transcriptome sequencing data will help to find relevant tumor/drug treatment-specific antigens for targeting therapy.