Cell cycle specific effects and associated DNA damage of selective inhibitors of nuclear export (SINE)

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Background and Retionology
Anti-cancer response to small molecule drugs or natural products are determined by the molecular and cellular scale. Understanding cell responses and their treatment following use of population samples are (e.g., immunolocalization, mass, and cell viability) and differences in timing, and drug concentrations treat various responses. To more completely understand the complexity of drug response we must track molecular responses and cell fate choice simultaneously in individual cells. In this study, we used a long-term orthogonal approach to follow a single cell or a population of cells in a time span to evaluate the effects of each drug on cell survival within a population, and how these factors contribute to population response dynamics. Anti-cancer inhibitors have been developed and are being evaluated in preclinical trials, but the cellular and molecular mechanisms mediating cell cycle arrest and cell fate choice are incompletely understood. In this study, we have addressed these issues

Abstract
Nuclear export protein is fundamental for cell growth and function. Selinexor is a SINE compound that is in clinical development for the treatment of multiple myeloma. SINEs act by selectively targeting the nuclear export protein (XPO1) and inhibiting its ability to shuttle non-covalently bound to Exportin-1 (XPO1), preventing its association with protein cargos, thereby resulting in their nuclear retention. XPO1 cargo includes the majority of tumor suppressor proteins (TP53 and cell cycle regulators such as p53, p16, and p21) that have key roles in cancer progression and response to treatment. It is unclear how Selinexor affects cell cycle progression in individual cells and the overall phenotype and fate of those cells. To elucidate Selinexor action, we developed cell lines that stably express fluorescein ubiquitinated XPO1 cargo (FUSC), and followed individual cells longitudinally using continuous-time lapse microscopy for 72 hours. We report that the presence or absence of XPO1 cells that express either wild-type p53 and p21. 10% of the initial population became arrested with 80-90% in G0/G1 or S-phase almost immediately or by 72 hours after the cell cycle delay or arrest. We also found that 40% of cells divided, but the phenotype was arrested in G1/S phase of the cell cycle cycle - often with a single cell amind or slow cell cycle progression. Using FUSC, we tracked the response of cells treated acutely in specific cell cycle phases. Cells treated in G0/G1 phase most often arrested in G0/G1, whereas, cells treated in G2/M phase, appeared programmed to divide. A delay of 24 hours in cell death during Taxol induced 5 phase progression defect and associated cell death, we further characterized this phenotype. Using nucleic acid isolation and fluorescence detection, we find that as soon as 2 hours after Selinexor treatment cells are undergoing DNA replication and those that are, are doing so at significantly higher rates. At 24 hours, the G2/M phase was fully arrested, whereas, the S-phase was significantly reduced. These data suggest that Selinexor may exert anti-cancer effects even on slow growing tumors where the bulk of the cell cycle population is in the G0/G1 phase. However, these cells are more likely to respond to cell cycle progression inhibitors.

Summary of HT080 FUSC response to Selinexor

Monitoring cell cycle progression using the FUSC system

Drug response experiment with SELN: Cycles 2 and 3

Does DNA damage correlate with active DNA synthesis?

Summary
Selinexor treatment results in cell cycle arrest and S-phase progression defects associated with striking XPO1 loci. DNA damage leads to multiple cell cycle defects, including arrest and death. Cells that contain DNA damage are more likely to accumulate in late S-phase and to be arrested at the G2/M boundary. This study will provide further evidence for the use of SINE compounds in cancer therapy.