Effects of Proteasome Inhibitor Drugs on DNA Repair Pathways

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Abstract

Proteasome inhibitors can be utilized as chemotherapeutic agents due to its antitumor effect. In normal conditions, proteins that need to be degraded will be labeled with a ubiquitin tag (Figure 1). Once polyubiquitination occurs, the proteasome recognizes the protein and degrades it into peptides that can be recycled and used by the cell. Proteasomes are useful during the cell cycle to rapidly transition from the different repair pathways. Backup repair genes are also regulated during the cell cycle. The different backup repair mechanisms are not well understood, and this study will help us understand how we can integrate proteasome inhibitors into therapy. For our protocol, we cultured K562-BCL2 cell lines with no treatment, MG132, NSC617145, and MLN4924. Once cells were sufficient and healthy, we utilized High-Throughput Genome-wide Translocation Sequencing to identify double-strand breaks (DSBs) and identify DNA translocation junctions. With this protocol, we studied the effect of proteasome inhibitors on DNA repair mechanisms as well as its correlation with the various types of repair pathways once proteasome inhibitors are involved. Specifically, we studied whether direct joining (NHEJ) or alternative end joining (MMEJ) occurs when proteasome inhibitor drugs are present. Although DNA repair protein levels are regulated throughout the cell cycle, it is unclear what the implications of inhibiting the proteosome would have in quiescent cells. In our experiment, we are studying the impact of proteasome inhibition in human G1/G0 phase leukemia cell line (K562).

Introduction

Proteasomes are essential components of DNA damage response and serve as regulatory factors involved in repair choice. By degrading certain inhibitors or activators, proteasomes can influence whether a cell uses non-homologous end joining (NHEJ) or HR. Two important post-translational modifications are utilized in protein regulation. Polyubiquitination is a crucial tag in the proteasome-ubiquitin cascade, but neddylation is an alternative pathway that utilizes NEDD8 protein and the NEDD-8 activating enzyme. The NEDD-8 activating enzyme will be necessary for Cullin-RING ubiquitin ligase (CRLs), and this ligase is needed for protein degradation in the neddylation pathway. In our experiment, we focused on drugs that inhibited ubiquitination and neddylation. MG132 is a peptide aldehyde that reversibly inhibits proteasome. NSC617145 is a WRN helicase inhibitor. MLN4924 is an inhibitor of NEDD-8 activating enzyme. Proteasome inhibitors increase the sensitivity of tumor cells to DNA-damaging treatments—such as ionizing radiation (IR) and DNA cross-linking agents.

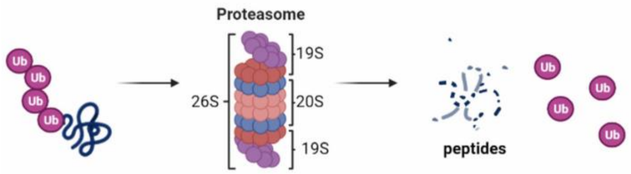


Figure 1. Proteasome–ubiquitination pathway

Repair pathways

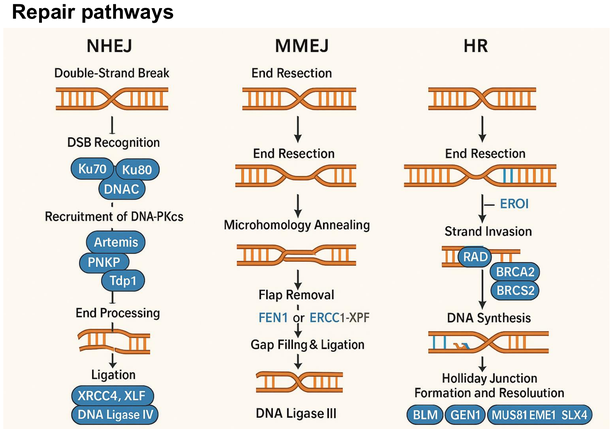


Figure 2. Types of repair pathways
Left is depicting non-homologous end joining (NHEJ), Middle is depicting microhomology mediated end-joining (MMEJ), and Right shows Homologous Recombination(HR)

Cell cycle

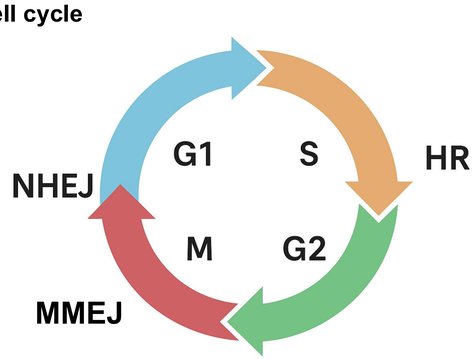
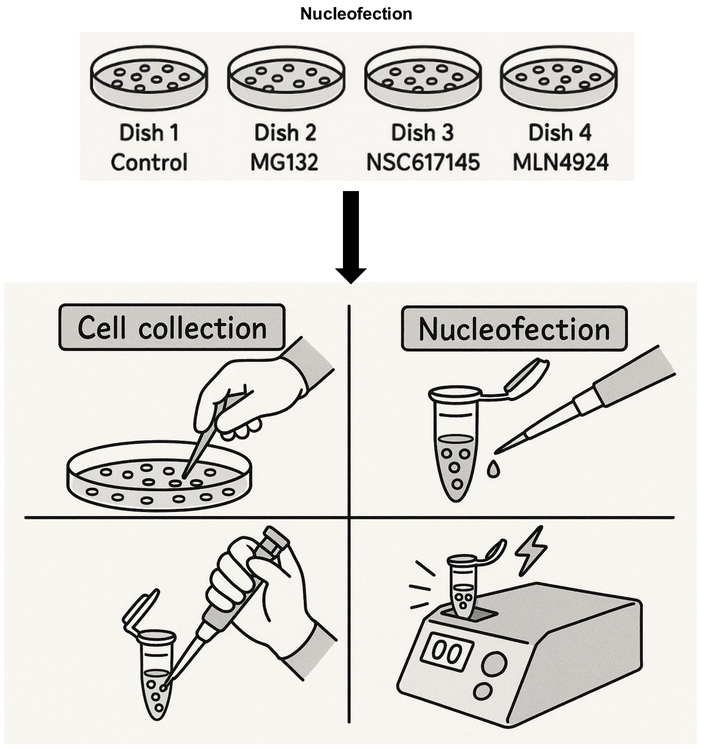


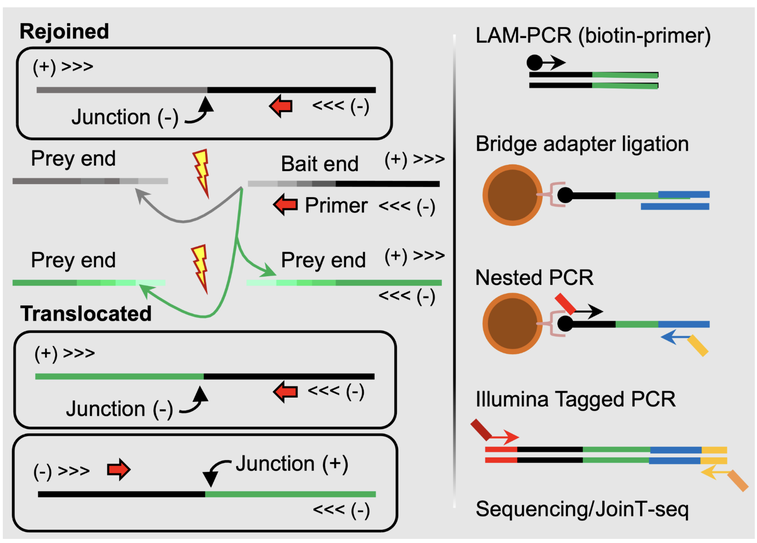
Figure 3. Dominant repair pathways throughout the cell cycle

Methods

Introduced two DSB RAG1D and RAG1L to K562-BCL cells. RAG1D and RAG1L served as prey and bait respectively in HTGTS to better understand its impact in DNA repair and chromosome translocations. Samples will be treated with proteasome inhibitors MG132, NSC617145 (WRN helicase inhibitor), and MLN4924 (inhibitor of NEDD-8 activating enzyme). Nucleofection was used to introduce bait into cells to identify DNA double-strand breaks (DSBs) and identify their translations within the genome

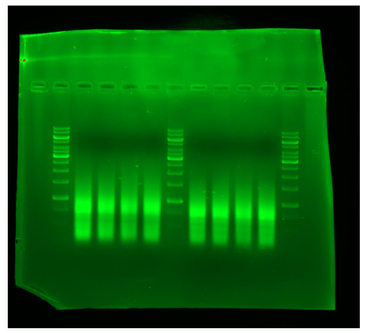


HTGTS Protocol: high-throughput genome-wide sequencing method used to detect genome-wide 'prey' doubles strand break sequences through a translocated bait sequence

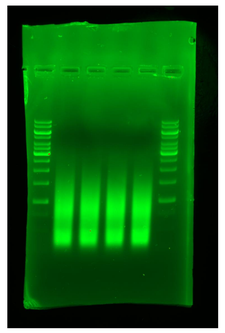


Results (emphasize significance of smears)

Tagged PCR NoTx + MG132

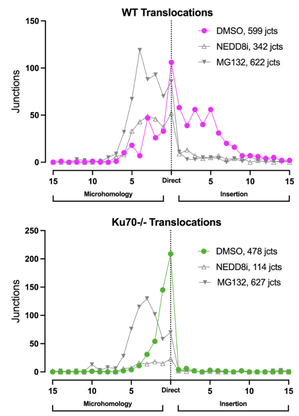


Tagged PCR MLN4924



Mouse Junction Structures

Preliminary: Proteosome inhibition promotes MMEJ only for translocations in G0/G1 cells



Shows HR is latent with proteasome inhibitors present Steers toward microhomologies when proteasome inhibitors are present.

In non-cycling cells, HR is usually not present but with proteasome inhibitors present MMEJ is dominant over HR in translocation.

Discussion

Proteasome inhibitors can be utilized in radiation therapy inhibiting cancer cells' ability to repair DNA damage and by promoting apoptosis (programmed cell death). For example, bortezimib is a proteasome inhibitor that targets cancers such as multiple myeloma. By inhibiting proteasomes, bortezomib prevents the degradation of pro-apoptotic factors, ultimately activating programmed cell death in cancer cells, which depend on suppressing these pathways for survival. Trial is still in progress so a definitive conclusion cannot be made at this time

Acknowledgements

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