Key determinants of DNA replication catastrophe signaling in anti-cancer therapy

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A. Overview/Abstract

Cancer cells experience chronic DNA replication stress due to their hyperproliferative nature and dysregulation of processes to protect and repair stalled DNA replication forks. Accordingly, exacerbating the DNA replication stress of cancer is a viable therapeutic approach being exploited as a form of cytotoxic chemotherapy, replication checkpoint inhibitors (e.g., **ATR** inhibitor), or poly(ADP-ribose) polymerase (**PARP**) inhibitors. Intriguingly, while these therapies are designed to kill cancer cells, they at clinically relevant doses often induce a reversible form of growth arrest known as therapy-induced senescence (**TIS**), which allows cells to persist during the exposure to genotoxic stress while gain stemness to repopulate, thereby leading to drug resistance and unfavorable patient outcomes. Furthermore, transcriptional activation of the senescence-associated secretory phenotype (SASP) program produces proinflammatory cytokines and immune modulators that provide a niche for a more aggressive state. This form of senescence is dynamic and flexible, suggesting that it can be modulated for therapeutic benefit, especially by switching the fate of cells from senescence to apoptosis or other forms of death. **p53** operating under the DNA damage response is known to act as a master regulator of cell cycle and death via induction of CDKN1A/p21 and proapoptotic molecules, respectively; however, the nature of early signaling events originated from DNA replication stress and the regulatory elements that are deployed to dictate the cell death-senescence decision are poorly characterized. In our previous study, we established a degron-based system that triggers acute dysfunction of the replisome and showed that this form of DNA replication damage synergizes with **ceralasertib**, an ATR inhibitor, to trigger **DNA replication catastrophe**, a pathological condition of irreversible fork collapse with massive single-stranded DNA (**ssDNA**) accumulation. We further showed that the catastrophic perturbation of DNA replication forks diverts ATR inhibitor-treated cells from TIS to apoptotic cell death through the novel **DNA-PKcs/CHK1** kinase signaling at stalled replication forks. Importantly, we identified c-Jun N-terminal kinase (**JNK**) as a key effector that triggers cell death under replication catastrophe. These findings implicate to a new regulatory pathway through which distinct cell fates are reached after cancer therapy. Therefore, finding a way to modulate the process of cellular senescence triggered by DNA replication stress will provide a basis for restraining senescence and promoting killing of cancer cells. Herein, we aim to elucidate the principles underlying the decision-making process linked to extensive DNA replication fork instability in response to cancer therapy. *We hypothesize that DNA damage-dependent JNK activation dictates the fate of cells to TIS versus cell death during replication catastrophe by modulating chromatin landscape and augmenting a p53-dependent cell death program.*

To accomplish this goal, we have assembled expertise from the Kim lab on DNA replication stress signaling for JNK activation (**Aim 1**), from the Rahme lab on epigenetics and chromatin modifications (**Aim 2**), and from the Martinez lab on p53 signaling (**Aim 3**). We expect that our study will provide new insight into the relationship between the dynamic modulation of DNA replication stress signaling and the cell fate decision under cancer therapy. Identification of the key determinants of cell fate commitment and a better understanding of the detailed decision network for cell survival versus death will allow us to develop rational drug combinations or design a new strategy to prevent tumor dormancy and render cells more responsive to conventional or targeted anti-cancer therapy.