Abstract of Presentation

Note: This paper should be typed in "Times New Roman" of 12pt.

Identification of cellular mechanisms required for the recovery from DNA replication fork arrest

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The mechanisms that control DNA damage repair and genome stability are highly conserved. They require components of the checkpoint control pathway, including mammalian ATR-ATRIP / *S. cerevisiae* Mec1-Ddc2, a member of the PI3 kinase family. ATR/Mec1 activates both S and G2/M checkpoints. A specific Mec1 allele, *mec1-100,* retains the ability to activate the G2/M checkpoint, but is deficient for the S-phase checkpoint response. Stalled replication forks are partially destabilized in the *mec1*-100 strain. A parallel pathway that ensures replication fork stability requires Sgs1 *in S. cerevisiae*, which is a homologue of conserved RecQ helicase. Cells bearing both *mec1-100* and *sgs1Δ* mutations grow normally but have high rates of gross chromosomal rearrangements. They show enhanced sensitivity to hydroxyurea (HU), which inhibits ribonucleotide reductase, and other inhibitors of DNA replication. Recently it has been shown that evolutionarily conserved TOR (target of rapamycin) pathway is also involved in the recovery from the replication fork associated damage. Since the fork associated damage is a hallmark of many cancer cells, we are interested in identifying the pathways that accentuate the lethality of current cancer drugs that interfere in fork progression. We screened for and identified a set of compounds that exhibit hypersensitivity to HU in either *mec1-100* or *sgs1Δ* single mutant but not in the wild-type cells. Here we show the effect of these compounds on the stability of stalled replication fork and the recovery from the fork arrest. We also examined the effect of these compounds in combination with other reagents that generate DNA alkylation (MMS) or double strand breaks; DSBs (Camptothecin, Zeocin). Two compounds exhibited a selective toxicity to mec1-100 only when combined with HU and retarded the fork elongation. Interestingly we observed that another compound, PI3 kinsae -TOR pathway inhibitor, exhibited a strong synergistic toxicity not only with HU but also with Zeocin. This suggests that TOR pathway is also required for the efficient recovery from DSBs. We are further elucidating the mechanism of action of those compounds in mammalian cells and in *S. cerevisiae*.