

Rad18: A Mutagenic Driver in Cancer ?

Introduction: The mechanisms by which DNA damage is sensed, processed and repaired impact all aspects of cancer including susceptibility to environmental carcinogens, tumor initiation and progression, and responsiveness to therapy. However, there are major gaps in our understanding of how pre-neoplastic cells tolerate intrinsic replication stresses and environmental genotoxic exposures while acquiring 'mutator phenotypes' that drive carcinogenesis. Importantly, DNA damage and replication stress tolerance mechanisms selected for during carcinogenesis are also likely to endow cancer cells with chemo/radio-resistance. The current gaps in our knowledge of DNA damage tolerance limit our understanding of carcinogenesis and preclude effective prevention and treatment of cancer.

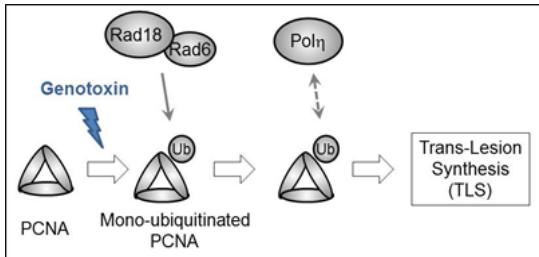


Fig. 1 Mechanism of Rad18-mediated TLS

Trans-Lesion Synthesis (TLS) is a DNA damage tolerance mechanism that allows DNA replication to proceed in the face of DNA damage from environmental exposures (e.g. solar radiation), endogenous genotoxins (e.g. reactive oxygen species), and chemotherapies (e.g. cisplatinum). In response to DNA damage, the DNA polymerase processivity factor PCNA is mono-ubiquitinated by the E3 ubiquitin ligase 'Rad18'. Mono-ubiquitinated PCNA selectively recruits error-prone 'Y-family' TLS polymerases such as Pol eta (Pol η) to stalled replication forks (**Fig. 1**). Collectively, Rad18-dependent TLS polymerases allow replication of damaged genomes and confer tolerance of many environmental,

endogenous, and therapy-induced DNA lesions.

In Rad18- or TLS-deficient cells, replication forks encountering DNA lesions (represented by '*' in **Fig. 2**) stall persistently and eventually 'collapse' giving rise to DNA Double Strand Breaks (DSB) (**Fig. 2, left**). Unrepaired DSB are lethal, and if

repaired inaccurately (e.g. via indiscriminate end-joining) can generate translocations and genome rearrangements. However, TLS can also cause adverse outcomes: TLS polymerases are inherently error-prone, particularly on undamaged templates and can cause mutations (**Fig. 2, right**). Thus TLS is a 'double-edged sword' that must be regulated stringently and used sparingly to avoid mutagenesis. Remarkably, although the biochemistry of the TLS pathway is well-understood in vertebrates, absolutely nothing is known about how Rad18 influences genome stability or carcinogenesis *in vivo*.

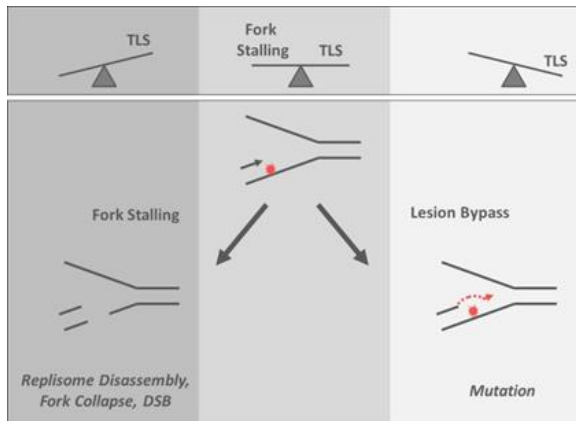


Fig. 2 Potential impact of TLS on DNA replication and genome stability.

Objective: The *objective* is to determine how Rad18 influences genome maintenance and carcinogenesis *in vivo*. We will test the *hypothesis* that genome instability and tumorigenesis are driven by error-prone DNA replication when Rad18 is present and via translocations and GCR in the absence of Rad18.

Deliverables:

The overall goal to test our model and the first ever information on how Rad18 and its effector TLS polymerases affect carcinogenesis can be achieved through the following points:

- Map complex genomic data to reference genome, taking into account the non standard nature of the studied genome, ie aneuploidy.
- Identify somatic events, point mutations, Insertion-Deletion, and large Structural Variants in this complex context.
- Compare the two groups to identify how Rad18 mutant affect carcinogenesis via the above.

Potential outcome:

Regulation of Rad18 and TLS have been studied extensively in tissue culture models. It is well established that this pathway can cause mutations however, those few investigators that do study mutagenic outcomes use very artificial

systems such as the SupF assay or transiently transfected extrachromosomal reporter plasmids harboring site-specific DNA lesions. There has not been a study in mammalian cells that has examined how Rad18/TLS influences mutagenesis/genome stability in the biologically-relevant context of chromosomal genomic DNA. No matter the result of the analysis, this experiment will yield important new information that will further the fields of DNA replication, mutagenesis and cancer.

Available data: The DNA-damaging agent 7, 12-Dimethyl benzanthracene (DMBA, a known environmental mutagen and carcinogen) was used to induce basal cell carcinomas (BCC) in cohorts of *Rad18*^{+/+} and *Rad18*^{-/-} mice. The resulting tumors were large (~1g) and homogeneous (based on histopathology). Genomic DNA from the BCC was subject to both exome sequencing and aCGH and those data are now ready for analysis.

Potential extension to PhD:

This work can easily extend into a PhD with the inclusion of data from Rad18 knock-in mice that express phosphorylation site mutants of Rad18. So it would be interesting to determine how these mutant alleles affect genome stability.

Furthermore, several other avenues can be followed for a PhD depending on the results of this first analysis:

- i) How other carcinogens (that are processed via the TLS pathway) affect genome stability as a function of Rad18 (e.g. ultraviolet radiation),
- ii) How therapies such as cisplatin (which causes therapy-induced neoplasia in human cancer patients) affects genome stability via Rad18.
- iii) These studies could also be done with other tumor models eg. lung or other tissues, and in response to both carcinogens (as we have done here) or using oncogenes.
- iv) More sophisticated experiments could include looking at genetic interactions with other DNA repair pathways (given the double-strand breaks that are formed in Rad18-null cells, DSB repair-deficient mice would be interesting).