Targeting the DNA Repair Enzyme TDG to Enhance Sensitivity of Cancer Cells to Anti-Leukemic Agents

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Disclosure: I have no conflict of interest concerning this presentation.
Genomic instability is a hallmark of tumorigenesis and it is defined as an increased propensity for genomic alterations. DNA repair pathways have a central role in maintaining genomic stability by repairing DNA damages or replication errors. DNA repair may be an important determinant of drug sensitivity. We focus on DNA repair as a molecular target to improve cancer therapy.

- Mismatch Repair (MMR)
- Nucleotide Excision Repair (NER)
- Base Excision Repair (BER)

Hanahan and Weinberg, Cell 2011
Base Excision Repair Machinery

- BER is responsible for repairing damage induced by endogenous metabolic processes (methylation, deamination, ROS and hydrolysis) (Wood et al, Science 2001)

- BER-specific enzymes are DNA N-glycosylases and AP endonuclease

- Our laboratory studies Thymine DNA Glycosylase (TDG)
Thymine DNA Glycosylase (TDG)

- TDG recognizes G·T mispairs at CpG sites
- CpG sites are hotspots for mutations due to the “spontaneous” deamination of 5-MeC to T, causing a G/T mismatch
- **TDG is involved in restoration of C instead of 5-MeC, leading to overall demethylation in key regions of the epigenome**

Adapted from Fitzgerald and Drohat, JBC 2008
Mechanisms of active DNA demethylation

Dalton and Bellacosa, Epigenomics, 2012
Disruption of the balanced epigenetic network may have significant impact on chromatin structure and transcriptional activity.

Cortellino et al, Cell, 2011
Leukemia as an epigenetic disease
Leukemia as an epigenetic disease

✓ **KNOWN:** DNMT inhibitor (Decitabine) yields promising results in hematopoietic malignancies (*Galm et al, Blood 2006*)

✓ **QUESTION:** Can we target TDG for therapeutic purposes?
Cytotoxic enhancement to BDM by TDG inactivation

TDG wt VS. TDG −/− MEFs

Bendamustine (BDM)
- Nitrogen mustard
- Used for:
  - Chronic lymphocytic leukemia
  - Non-Hodgkin’s lymphoma
  - Multiple myeloma

Cortellino et al, Cell, 2011

Beeharry et al, PLoS ONE, 2012

BDM

48h: DNA damage repair
TDG is involved in cytotoxicity induced by BDM
TDG is involved in cytotoxicity induced by BDM

HOW?
TDG is involved in cytotoxicity induced by BDM

**HOW?**

Repair of DNA damage?
TDG is involved in cytotoxicity induced by BDM

**HOW?**

Repair of DNA damage?

Epigenetic effect?
TDG is involved in cytotoxicity induced by BDM

**HOW?**

Repair of DNA damage?

Epigenetic effect?

Both?
The concept of TDG inhibition
Development of a high-throughput TDG repair assay

Optimization of a high-throughput TDG repair assay

- TDG is a single turnover enzyme ($K_{off}$, dissociation E from P, is rate limiting)
- APE increases TDG turnover by 42- and 26-fold for G:T and G:U substrates
- TDG and APE are in vast excess over substrate, therefore conditions approximate single turnover kinetics
- Equilibration of TDG with potential inhibitors will allow screening in pre-steady state conditions
Optimization of a high-throughput TDG repair assay: Determination of the Z’ factor
Optimization of a high-throughput TDG repair assay: Determination of the Z’ factor

Z’ factor is a characteristic parameter for the quality of the screening itself, without intervention of test compounds (Zhang et al, J Biomol Screen 1999)
Optimization of a high-throughput TDG repair assay: determination of the Z’ factor

Z’ factor = 0.79

1 > Z ≥ 0.5  Separation band is large and the assay is excellent
Conclusions

- TDG has a dual role in both DNA repair (*genomic stability*) and active DNA demethylation & transcriptional regulation (*epigenomic stability*)

- TDG loss / activity inhibition enhances tumor killing

- We have developed and optimized a homogeneous fluorescence-based high-throughput assay to evaluate inhibition of TDG

- Inhibitor concentrations in the same range of substrate concentration should give a drastic reduction of fluorescence generation, facilitating inhibitor discovery

- Pharmacological inhibition of TDG could lead to significant sensitization to known anti-leukemic agents, including bendamustine (BDM)

- This study could lead to the development of investigator-initiated preclinical and clinical trials
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