

Assessing ZFAND6 as a Dual Regulator of DNA Repair and Innate Immune Signaling

DNA damage can cause dramatic cellular effects and drive species diversity and evolution; however, DNA damage must be well-controlled for normal growth and development. Cells have evolved to respond to DNA damage and protect their genomes through many checkpoint and repair mechanisms, collectively referred to as DNA damage responses (DDR). When a cell cannot effectively mitigate harmful DNA damage, a variety of cellular processes can be induced such as stalled cell proliferation or programmed cell death, termed apoptosis.¹ While several cellular DDR pathways have been well described, there is an incomplete analysis of the roles that the immune system may play in regulating organismal responses to DNA damage.

Recent studies have connected the immune system and DDR by showing that DNA damage activates canonical immune-system signaling pathways: damage can result in DNA leakage into the cytoplasm, activating the cytosolic DNA sensor cGAS, which induces STING and interferon (IFN) regulatory factor (IRF) signaling.² These factors contribute to innate immunity mechanisms that induce the type I IFN class of cytokines, most widely known for their role in communication and response to pathogen invasion. STING activation is a well-known response to DNA-virus infection. Induction and secretion of type I IFN early during infection induce IFN-stimulated genes (ISGs). ISGs are involved in many cellular processes that dictate how a cell responds to stress.³ In addition, type I IFN and ISG induction has been shown to occur as a result of inhibition of topoisomerase II, an enzyme that repairs double strand DNA breaks.⁴ This evidence suggests that DNA damage may activate immune pathways as a communication tool and response mechanism to stress on cellular DNA. However, the effects of immune signaling on the DDR remain unknown. Furthermore, the molecular underpinnings linking these pathways are largely unexplored.

New discoveries by [REDACTED] at [REDACTED] have identified a link between the DDR and immune signaling through the immune modulating protein Zinc Finger AN1-Type Containing 6 (ZFAND6). A novel interaction was found between ZFAND6 and a protein involved in the nucleotide excision repair (NER) pathway of the DDR. NER excises and repairs bulky DNA lesions induced by damaging agents such as ultraviolet (UV) light. In the absence of ZFAND6, ISG upregulation was observed despite no increase in type I IFN expression, suggesting that ZFAND6 may function to prevent unwarranted ISG induction.^{5,6} Assessing ZFAND6 effects on NER and ISG expression will provide insights into how immunity affects DNA repair processes.

I will work with [REDACTED] to examine this novel molecular connection between immune signaling and DDR through ZFAND6 and ISG induction. We hypothesize that ZFAND6 may participate in the NER DNA damage recognition complex; DNA damage may accumulate in the absence of ZFAND6, leading to the leakage of DNA into the cytoplasm where it would be sensed by the cGAS-STING pathway; and IFN regulatory factors may induce ISGs independent of IFN in ZFAND6 deficient cells (Figure 1). To test our hypotheses, two aims are proposed:

Aim I: examine the role of ZFAND6 in DNA damage regulation

A yeast two-hybrid screen revealed an interaction between ZFAND6 and an NER protein.⁵ A plasmid construct containing ZFAND6 will be generated and purified for overexpression and

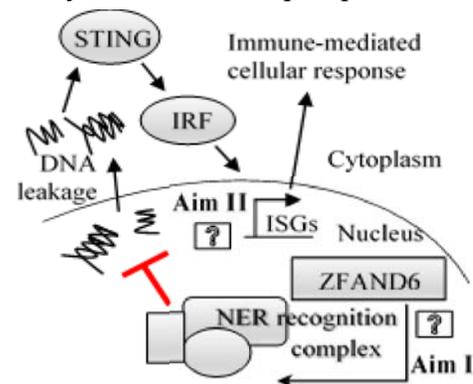


Figure 1. Schematic of proposed ZFAND6 activity

immunoprecipitation experiments to confirm this interaction. To determine if ZFAND6 plays a role in regulating NER, we will quantify levels of DNA damage, apoptosis, and proliferation in various murine ZFAND6 knockout (KO) cell types (e.g. fibroblasts, macrophages) either unstimulated or UV-stimulated. Results will be confirmed in a human monocytic cell line (THP-1) knocked out for ZFAND6 using CRISPR/Cas9 technology. To determine if there is an intrinsic defect in NER, we will measure UV-induced NER by oligonucleotide retrieval. DNA damage will be assessed by western blot analysis for phospho-gamma H2AX, whereas apoptosis and proliferation will be examined by flow cytometry based assays (annexin and CFSE) to verify if DNA damage induces apoptosis or decreases cellular proliferation.

Aim II: elucidate ISG induction mechanisms in the absence of ZFAND6

To determine if the upregulated ISGs in ZFAND6 KO cells are due to cGAS-STING activation, we will cross *Zfand6*^{-/-} mice with *cgas*^{-/-} and *Sting*^{-/-} mice. Bone marrow-derived macrophages and tissues will be isolated from the KO mice and examined for ISG expression (MX-1, IRF7, STAT1, ISG15) by qRT-PCR. To determine if loss of ZFAND6 leads to DNA accumulation in the cytoplasm, cytoplasmic extracts will be examined for DNA by agarose gel electrophoresis. Lysates will be digested with S1 nuclease which degrades ssDNA or DNASE1 which degrades dsDNA. ZFAND6 KO cells, unstimulated and UV-stimulated, will also be stained with anti-ssDNA or anti-dsDNA and subjected to confocal microscopy and flow cytometry to examine DNA leakage in the cytoplasm. Finally, we will examine IRF1 and IRF7 expression and activation, which are immune pathways known to induce ISGs in an IFN-independent manner.⁷

Intellectual Merit

There is evidence to support the hypothesis that ZFAND6 may mitigate DNA damage through NER, thereby repressing ISG expression.³⁻⁶ Examining the role of ZFAND6 in the NER pathway and ISG induction will elucidate one mechanism that connects immune signaling and DNA damage regulation, enhancing our understanding of the cellular and molecular responses to DNA stressors. The methods we proposed in mouse and human cells can be applied to various cell types from any model system with IFN signaling. Understanding how cells respond to stress is important on an evolutionary and ecological level, as it affects the cell survival and death responses in many organisms. The nature of this project in connecting the historically separate fields of innate immunology and DNA damage would lead to advancements in knowledge across interdisciplinary scientific fields. Our findings may yield insights into mitigating the effects of DNA damage on associated diseases, such as cancer, through ZFAND6 targeting.

Broader Impacts

I will serve as a mentor for summer and rotation students at the undergraduate and graduate levels and teach cell biology techniques, theories behind experiments, and lab safety. I am looking forward to providing encouragement and information on attaining advanced scientific training. Our work will be communicated through oral and poster presentations at [REDACTED], scientific meetings, and local high schools through events organized by [REDACTED]. We will publish in peer-reviewed scientific journals to ensure others can build on our exciting discoveries.

References

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