

SUMMARY STATEMENT
(Privileged Communication)

Release Date: 11/09/2015

PROGRAM CONTACT:
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Application Number: 1 R01 AI123162-01

Principal Investigator

JIANG, MENGXI PHD

Applicant Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Review Group: VIRB
Virology - B Study Section

Meeting Date: 10/29/2015
Council: JAN 2016
Requested Start: 04/01/2016

RFA/PA: PA13-302
PCC: M34A

Project Title: Intersection of polyomavirus infection and host cellular responses

SRG Action: Impact Score: [REDACTED] Percentile: [REDACTED]

Next Steps: Visit http://grants.nih.gov/grants/next_steps.htm

Human Subjects: 10-No human subjects involved

Animal Subjects: 10-No live vertebrate animals involved for competing appl.

Project Year	Direct Costs Requested	Estimated Total Cost
1	[REDACTED]	[REDACTED]
2	[REDACTED]	[REDACTED]
3	[REDACTED]	[REDACTED]
4	[REDACTED]	[REDACTED]
5	[REDACTED]	[REDACTED]
TOTAL	[REDACTED]	[REDACTED]

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

ADMINISTRATIVE NOTE, EARLY STAGE INVESTIGATOR, NEW INVESTIGATOR

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1R01AI123162-01 JIANG, MENGXI

**EARLY STAGE INVESTIGATOR
NEW INVESTIGATOR
SCIENTIFIC REVIEW OFFICER'S NOTES**

RESUME AND SUMMARY OF DISCUSSION: The application will examine how polyoma viruses control and destabilize normal host cellular processes to facilitate viral replication, and how these interactions may result in polyomavirus-induced oncogenesis. The investigator hypothesizes that “an activated host cellular DNA damage Response (DDR) is crucial in facilitating viral replication and maintaining host genome stability during polyomavirus infection”. More precisely, the investigators want to determine how host mismatch repair (MMR) proteins are required for polyoma virus replication and DDR activation, the viral DNA structures that are recognized by the host, and how T Ag affects host cell DNA replication. The proposed work is considered highly significant and likely to advance the field and uncover novel molecular mechanisms underlying polyomavirus replication and viral oncogenesis. Additional strengths include: preliminary data indicating that viral replication rather than T Ag may be the key trigger for DDR activation; cutting edge approaches, such as the primary human renal proximal tubule epithelial cell culture infection model; the accomplished investigative team; thorough consideration of alternative approaches. In sum, there is considerable enthusiasm in support of the investigator, the important topic, the highly compelling preliminary data, and the innovative experimental plan.

DESCRIPTION (provided by applicant): Polyomaviruses cause a variety of severe human diseases particularly in immunocompromised individuals. No specific anti-viral treatments or prophylactic approaches exist to target this family of viruses. There are several critical gaps in our current knowledge of the molecular mechanism of viral replication and tumorigenesis. Our long-term goals are to identify how these viruses subvert normal host cellular processes to facilitate viral replication, and how these interactions may result in oncogenesis. Our previous studies revealed an intricate balanced relationship between viral replication and virus-induced host genomic instability. These results lead to our central hypothesis that an activated cellular DNA damage response (DDR) is important for facilitating viral replication and maintaining host genome stability during polyomavirus infection. Towards this hypothesis, we have identified host mismatch repair system and replicating viral DNA as novel factors contributing to DDR activation. We have also discovered that the ability of polyomavirus to cause host genomic DNA damage is linked to its ability to replicate viral DNA. Guided by strong preliminary data, we propose to pursue three Specific Aims to characterize DDR activation mechanism and how the DDR ties together viral replication and host genomic stability: (1) To define the role of host mismatch repair proteins in polyomavirus replication and polyomavirus-induced DDR activation. (2) To determine the viral DNA triggers that activate the DDR upon polyomavirus infection. (3) To elucidate the molecular mechanism by which polyomavirus induces host genome instability. Collectively, our proposed research will broadly impact the field by characterizing the essential roles that the DDR plays in promoting viral replication and maintaining host genome stability. These studies will have the potential to uncover novel molecular mechanisms underlying polyomavirus replication as well as viral oncogenesis. These findings may be extrapolated to other DNA viruses and to our understanding of normal cellular processes.

PUBLIC HEALTH RELEVANCE: Polyomaviruses are a family of viruses associated with severe human diseases and a subset of them can also cause cancers. Our proposed studies aim to understand the interactions between polyomaviruses and the host DNA damage response, a cellular pathway important for both viral replication and host genome maintenance. This research will have the potential to reveal novel therapeutic host targets to treat polyomavirus-related diseases.

CRITIQUE 1:

Significance: 2
Investigator(s): 2
Innovation: 2
Approach: 2
Environment: 2

Overall Impact: The enthusiasm for this application is based on the significance, innovation, and approach. The research proposed in this application should fill gaps in knowledge about the connection between polyomavirus-induced DNA damage response (DDR), viral replication, and host genome stability. This is significant, because a number of polyomavirus and other DNA viruses modulate the DDR pathway, with implications for viral replication and oncogenesis. Dr. Mengxi Jiang is a new investigator, holding an assistant professor position at UAB. She has made and published important findings about the human polyomavirus BKPyV induction of the DDR pathway, and in particular, she has new preliminary data that identify host mismatch repair proteins upregulated and involved in the DDR activation. She also has evidence that is paradigm-shifting that indicates that viral replication rather than T Ag may be the key trigger for DDR activation. The strengths of the proposal are the significance, because the investigator is in a strong position to make important findings that will be applicable to the DNA virus field. There are clearly designed experiments to test logical hypotheses. In addition to the preliminary data, feasibility of the approaches is evident because the investigator and co-investigator have experience in the techniques. Experimental design is thoroughly described, and expected outcomes are discussed. Even the possibility of results that disprove the hypotheses are discussed with alternative experiments and strong experimental design to test new hypotheses. There are a few minor weaknesses that do not significantly affect the overall impact and are outweighed by the significance, innovation, and hypothesis-driven comprehensive approach.

1. Significance:

Strengths

- A tractable model system is being used to study a human pathogen; findings can likely be extended to other human polyomaviruses and potentially other viruses: other DNA viruses modulate the DDR pathway, promoting viral replication and/or oncogenesis.
- Builds on important findings newly implicating the host mismatch repair proteins in activating the DNA damage response during infection.
- May shift paradigms about the viral triggers important for DDR activation, which could inform future studies to prevent host DNA damage caused by polyomaviruses, with implications for oncogenesis
- Determination of how polyomavirus (and likely T Ag specifically) induces host genome instability (that is overcome if the virus replicates or if DDR is activated) might lead to approaches to selectively induce host DNA damage to help eliminated infected cells.

Weaknesses

- None noted.

2. Investigator(s):

Strengths

- Dr. Mengxi Jiang, investigator, is an Assistant Professor at the University of Alabama, Birmingham. She did her postdoctoral training with Dr. Michael Imperiale at the University of Michigan, where she focused on molecular aspects of host-BK polyomavirus interactions (viral entry and replication, and host nuclear architecture rearrangements). She discovered that polyomavirus replication and host genome stability rely on activated host DDR pathways, leading to the basis for this proposal. She continued this work in her own independent laboratory, making the key discoveries underpinning this proposal, i.e., that mismatch repair proteins are required for polyomavirus replication and that viral DNA replication drives the activation of the DDR. She has a strong publication record and is well positioned to carry out the proposed research.
- [REDACTED]
[REDACTED] Her research focus is replicative stress in yeast, and chemotherapeutic drug action and cellular pathways regulating drug responses in human cell and mouse models. Apropos of the proposed project, she has extensive experience in analysis of DNA damage response pathways, including 2-D gel and DNA fiber assays. She is well qualified for the proposed role in this project.

Weaknesses

- None noted.

3. Innovation:

Strengths

- Conceptual innovation is high: paradigm-shifting model that polyoma activates the host DDR response leading to both productive replication and prevention of host DNA damage, especially with the novel concepts of connection between viral DNA replication, DDR activation and virus-induced host genome instability. Model that viral DNA replication triggers DDR is innovative.
- Some cutting-edge approaches are proposed, which alone are not innovative, but together comprise innovative application to polyomavirus and the host DNA damage response. These include 2-D gels and DNA fiber assays to examine replication intermediates, metaphase spreads, FISH and single-cell immunofluorescence staining, and iPOND (involving click chemistry and immunoblotting to identify proteins on nascent DNA).
- Use of the RPTE primary cell culture model that is biologically relevant because the cells have intact cell cycle checkpoint regulation (contrast to transformed cell lines).

Weaknesses

- None noted

4. Approach:

Strengths

- Testable model is proposed that is addressed in three independent but related aims.
- Strong preliminary data indicating that BKPyV activates and requires the DDR for productive infection and that DDR is required for host DNA stability.
- Use of powerful SILAC and 2-D LC-MS/MS to identify host mismatch repair protein upregulation of MSH6 and MSH2 by BKPyV, and good confirming evidence of the importance of MSH6 and MSH2 in productive infection and DDR activation.

- Good evidence of requirement of viral replication for DDR activation using both ectopic T Ag expression and viral infection. This is key evidence for the paradigm-shifting hypothesis that T Ag presence is not the sole (or even major) driver of DDR activation.
- Aims are clearly described, with strong rationale and testable hypotheses.
- Appropriate controls are included, and experiments are feasible. Expected outcomes are described. Outcomes that disprove the hypotheses are discussed, with additional experiments proposed to test the alternative outcomes.
- Aim 1 is a logical set of experiments that thoroughly examine the role of host mismatch repair proteins in BKPyV replication and DDR activation.
- Building on the preliminary findings that viral DNA replication is necessary for DDR activation, the investigator will test whether it is sufficient. She will use a strong genetic approach that should be informative, transfecting RPTE cells with a plasmid with a wt or nonreplicable BKPyV ori (or no-ori control). She will introduce various forms of T Ag using lentivirus constructs: either a wild-type T Ag or a mutant that abolishes viral replication (in hand) or one she will construct that will lack an ori-binding domain.
- The examination of specific DNA lesions on viral DNA (Aim 2.2) on a single-cell level is exciting and may be the first comprehensive look at such lesions for a DNA virus genome.
- Aim 3.1, examining whether forcing cells into mitosis produces host DNA damage, tests an intriguing hypothesis that T Ag induced replication stress plus mitosis causes chromosome damage; it uses approaches that are in hand and should be successful.
- Aim 3.2, is exciting because it tests directly whether T Ag induces *host* DNA replication stress using powerful cutting-edge techniques. The findings could be related to reports of other oncogenes causing replication stress.

Weaknesses

- The reduction in viral titer is only ~ 1 log unit in some assays (Fig. 3C, 7C). This requires biological replicates in order to claim statistical significance; it's not clear if the statistics shown are based on technical or biological replicates.
- The rationale for using the siRNA-resistant form of MSH6 or MSH2 in Aim 1.1 is not clear.

5. Environment:

Strengths

- The physical and scientific environment as described are outstanding. In addition, Dr. Jiang's department chair provides a strong letter of support describing the institutional commitment to her success as an independent investigator.

Weaknesses

- None noted.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Not Applicable (No Biohazards)

Select Agents:

Not Applicable (No Select Agents)

Resource Sharing Plans:

Not Applicable (No Relevant Resources)

Budget and Period of Support:

Recommend as Requested

CRITIQUE 2:

Significance: 2

Investigator(s): 1

Innovation: 2

Approach: 3

Environment: 1

Overall Impact: This is a new application to determine the interplay between polyomavirus genome replication and the double strand DNA break (DDB) response. Dr. Jiang is a well trained new investigator and the application is based on her discovery that BKV genome replication and not simply TAg expression, is necessary to fully induce the DDB response. The established co-investigator is an expert in DNA damage, particularly replication stress. The overall hypothesis that drives the application is that “an activated cellular DNA damage response (DDR) is important for facilitating viral replication and maintaining host genome stability during polyomavirus infection.” There are many strengths to this proposal. These include the well qualified principal investigator and co-investigator, an interesting and innovative hypothesis and an extraordinarily well crafted, logical and compelling research plan that includes the following aims: (1) To define the role of host mismatch repair proteins in polyomavirus replication and polyomavirus-induced DDR activation; (2) To determine the viral DNA triggers that activate the DDR upon polyomavirus infection; and (3) To elucidate the molecular mechanism by which polyomavirus induces host genome instability. The hypotheses driving individual aims are generally well supported by strong preliminary data. Concerns are that some of the proposed state-of-the art experimental approaches that are proposed lack feasibility data. These concerns, however, are mitigated by the fact that in most cases more standard alternative methods are proposed and they do not detract from the generally very high enthusiasm for this application.

1. Significance:

Strengths

- Human polyomaviruses, including BK virus cause significant disease particularly in immunosuppressed patients. The incidence is likely to increase given the more frequent use of immunosuppressive drugs in organ transplant patients and for the treatment of other diseases.

- These studies may yield data that are significant for other viruses, including other polyomaviruses, papillomaviruses and potentially other viruses with small double stranded DNA genomes.

Weaknesses

- None noted

2. Investigator(s):

Strengths

- Mengxi Jiang is a well-trained new investigator. She presents an impressive set of published and unpublished preliminary data that support this application, and she is well suited to direct these studies.
- [REDACTED] is an established senior investigator with a strong record of accomplishments in the area of DNA damage. She has also developed and implemented a novel DNA fiber assay, which will be used in aim 3 to address the hypothesis that BKV TAg induces DNA replication stress. She is an asset to this proposal.

Weaknesses

- None noted

3. Innovation:

Strengths

- While the overall concept that some DNA viruses induce double strand DNA breaks and harness some of these pathways to support their replication cycles is not novel, the investigator's concept that the viral replication process induces the DNA damage response to stabilize stalled viral replication forks and prevent host genome destabilization is novel and potentially paradigm shifting.
- Technical approaches are cutting edge.

Weaknesses

- None noted

4. Approach:

Strengths

- The research plan is well conceived and follows a logical plan.
- The overarching hypothesis of the proposal is supported by strong preliminary data.
- Expected and unexpected outcomes are carefully considered and discussed, and appropriate follow-up experiments are proposed even those that would be performed if the results do not support their hypotheses.
- Appropriate alternative approaches are considered and proposed.
- Experiments will be performed in primary human renal proximal tubule epithelial (RPTE) cells.

Weaknesses

- Feasibility data for some of the proposed approaches (single-cell FISH, iPOND) are missing. This is a minor weakness as in most cases more standard alternative approaches are proposed.

5. Environment:

Strengths

- The research environment at UAB is excellent

Weaknesses

- None noted.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Not Applicable (No Biohazards)

Select Agents:

Not Applicable (No Select Agents)

Resource Sharing Plans:

Not Applicable (No Relevant Resources)

Budget and Period of Support:

Recommend as Requested

CRITIQUE 3:

Significance: 3

Investigator(s): 4

Innovation: 5

Approach: 4

Environment: 3

Overall Impact: Polyomaviruses cause a variety of severe human diseases particularly in immunocompromised individuals. The numbers of individuals affected are relatively modest compared to other viral diseases. However, specific anti-viral treatments are lacking for this family of viruses. The proposed studies will examine the interactions between the BKPyV and the DDR. These studies are likely to be successful and may suggest potential targets for the development of antiviral therapies. More likely, these studies will reveal additional details regarding the mechanisms of activation of the

cellular DDR. The proposed analyses are clearly articulated and are likely to be successful. Technical issues have generally been well considered and appropriate collaborations are in place which makes it likely that useful information can be developed as a result of these studies. A greater focus of the role of the DDR in cellular transformation/cancer development using BKPyV as a probe of these processes may have increased the medical relevance of these studies.

1. Significance:

Strengths

- BKPyV appears like an excellent probe to study the DDR. Understanding the relationships between the DDR and BKPyV may permit the development of novel antiviral agents.

Weaknesses

- BKPyV is not a major human pathogen (when compared to other viral pathogens).

2. Investigator(s):

Strengths

- Dr. Jiang, Assistant Professor, UAB, is well qualified to perform the proposed studies. [REDACTED], [REDACTED] will provide the required support for the proposed 2-D gel and DNA fiber assays of BK polyomavirus DNA replication intermediates.

Weaknesses

- None noted.

3. Innovation:

Strengths

- The central hypothesis that polyomavirus modulates the host DDR to facilitate productive viral replication and to prevent host DNA damage is testable.
- The investigator has teamed up with experts in the DNA damage and repair field to adapt a series of approaches including two-dimensional agarose gels and DNA fiber assays to examine viral DNA replication intermediates and the effects of viral infection on host DNA replication.
- In RPTE cells, TAg expression alone cannot fully activate the ATR response as demonstrated by a minimum induction of Chk1-pS317 compared with infection conditions that have similar levels of TAg. This suggests the DDR is induced, in part, by BKPyV replication in these cells (which is distinct from transformed cells where TAg alone is sufficient for the induction of the DDR).

Weaknesses

- None noted.

4. Approach:

Strengths

- In Aim 1, the investigator plans to define the role of host mismatch repair (MMR) proteins in polyomavirus replication and polyomavirus-induced DDR activation. The analysis is well described and reasonable. The only caveat is the possibility that the majority of MMR proteins

do not play a major role in polyomavirus replication (with the exception MSH6 which has been shown to have a role). This seems unlikely and the approach is well justified.

- In Aim 2, the investigator plans to determine the viral DNA triggers that activate the DDR upon polyomavirus infection. These studies are designed to challenge the current paradigm that TAg is the main driver of DDR activation during polyomavirus infection. Although this may be true in RPTE cells, it is not clear if this is unique to this particular cell type, something the investigator should consider. It is probably worth repeating some the proposed analysis in another cell type which also demonstrates cell cycle checkpoint control.

Weaknesses

- In Aim 2, the hypothesis that both ssDNA lesions and DSBs occur on replicating viral DNA which serve as DDR activation triggers is proposed. Is it possible that BKPyV replication, either directly or indirectly, cause DNA lesions in the cellular DNA which are ultimately responsible for DDR activation?
- In Aim 3, the investigator plans to elucidate the molecular mechanism by which polyomavirus induces host genome instability. The model suggests TAg-induced aberrant host DNA replication stress followed by mitosis causes chromosome damage. If true, viral DNA triggers that activate the DDR upon polyomavirus infection may not be required for the DDR i.e. it may be caused by TAg affecting host DNA replication rather than BKPyV replication (although why BKPyV replication appears to enhance the DDR then becomes less obvious unless there are additional viral proteins/RNAs rather than viral DNA replication intermediates involved). This Aim is not described in sufficient detail to fully appreciate the expected goals and potential outcomes of the analysis.

5. Environment:

Strengths

- The environment is appropriate for these studies.

Weaknesses

- None noted.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Acceptable

- All of the BSL-2 work proposed in the studies will be performed in the Jiang laboratory. There are designated and approved areas in the laboratory for handling infectious agents including BK polyomavirus and lentivirus. Approval from the UAB Occupational Health and Safety Office will be obtained for all the studies involving infectious agents. An annual laboratory audit will be performed by UAB to ensure compliance with the safety requirements. All members of the lab have been trained to safely handle infectious agents and are re-trained on a yearly basis.

Select Agents:

Not Applicable (No Select Agents)

Resource Sharing Plans:

Unacceptable

- No resource sharing plan is presented.

Budget and Period of Support:

Recommend as Requested

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

SCIENTIFIC REVIEW OFFICER'S NOTES: The VIRB Study Section has been recalibrated with the October 2015 meeting (January, 2016 Council Round). This process involves guiding the reviewers to address progressive score inflation and reset the percentile base to establish a benchmark for future VIRB meetings.

The NIH special practice for new investigator R01 applications reviewed in the Center for Scientific Review study sections applies to this application. Resubmission (amended -A1) R01 applications from new investigators may be submitted on a special receipt date for review in the very next review cycle. See this notice in the NIH Guide for Grants and Contracts for more details:
<http://grants.nih.gov/grants/guide/notice-files/NOT-OD-11-057.html>.

You should contact the NIH program officer whose name is shown in the upper left hand corner of page one of this Summary Statement for information about whether this application may be fundable or whether you will need to submit an amended application. The program officer can also help you decide whether the changes and improvements necessary to address the weaknesses noted in the reviewers' critiques could be accomplished in the relatively short time available. You are also strongly advised to seek input from mentors, your Department chair, etc.

If you choose to submit a resubmission application for the next review cycle under this policy for new investigators, your amended application must be received at NIH no later than Thursday, December 10, 2015.

You may, of course, choose to take more time to resubmit your application. If so, you should prepare the resubmission for the normal dates for amended applications as specified in this table:
<http://grants1.nih.gov/grants/funding/submissionschedule.htm>.

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html>. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.