(Revised 07/09)

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| *(please insert ref. above)* |

# NSFC/RGC Joint Research Scheme
## Joint Completion Report

*(Please attach a copy of the completion report submitted to the NSFC by the Mainland researcher)*

## Part A: The Project and Investigator(s)

### 1. Project Title

Mechanism and novel therapy for Aristolochic Acid Nephropathy: role of Smad7

Smad7在馬兜鈴酸腎病發病機制中的作用及治療

### 2. Investigator(s) and Academic Department/Units Involved

| | Hong Kong Team | Mainland Team |
|---|---|---|
| Name of Principal Investigator *(with title)* | Professor Lan Hui Yao<br>藍輝耀教授 | Professor Fu Ping<br>付平教授 |
| Post | Professor 教授 | Professor 教授 |
| Unit / Department / Institution | Department of Medicine & Therapeutics, and LiHS, CUHK 香港中文大學內科及藥物治療學系 | Department of Medicine /Sichuan University<br>四川大學華西醫院腎臟內科系 |
| Co-investigator(s) *(with title)* | Dr Lan-Hunag Xiao Ru藍曉茹博士;<br>Dr Chung Arthur Chi-Kong 鍾志剛 助理教授, Li Ka Shing Institute of Health Sciences, CUHK, 香港中文大學李嘉誠健康科學研究所 | Dr Zhou Li 周莉博士,<br>Dr Qin Wei 秦偉副教授<br>Dr Liu Fang 劉芳副教授,<br>Dr Liu Fei 柳飛博士, 四川大學華西醫院腎臟內科系 |

### 3. Project Duration

| | Original | Revised | Date of RGC/ Institution Approval *(must be quoted)* |
|---|---|---|---|
| Project Start date | 01/01/2011 | | 04/11/2010 |
| Project Completion date | 31/12/2013 | | |
| Duration *(in month)* | 36 | | |

## Part B: The Completion Report

### 5. Project Objectives

5.1 Objectives as per original application

1. To explore mechanisms of Smad7 in protection against Aristolochic Acid-induced renal fibrosis and inflammation in vitro in renal tubular cells that lack or over-express Smurf2 or Smad7.

2. To determine the protective role and mechanisms of Smad7 in vivo in Aristolochic Acid-induced chronic nephropathy in Smad7 wild-type and knockout mice.

3. To develop a specific therapeutic strategy to prevent and treat chronic Aristolochic Acid nephropathy by ultrasound-microbubble-mediated Smad7 gene therapy.

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____

*1.*
*2.*
*3. ....*

## 6. Research Outcome

Major findings and research outcome
*(maximum 1 page; please make reference to Part C where necessary)*

There are three major findings obtained from this joint Research Scheme.

First, we found that TGF-b/Smad7 is protective in Aristolochic Acid (AA)-induced nephropathy (AAN). This is supported by the finding that mice lacking Smad7 developed

much more severe AAN including a significant increase in levels of 24-hour urinary protein, serum creatinine, renal fibrosis (collagen I and α-SMA) and inflammation (TNF-α, MCP-1, CD3+ T cells and macrophages). ). In contrast, restored renal Smad7 to the normal level largely inhibited the development of AAN in vivo and in vitro.

Second, we also identified that enhanced renal fibrosis and inflammation in Smad7 KO mice with AAN were associated with a marked activation of TGF-β/Smad3 and NF-κB signaling, which was inhibited by overexpression of renal Smad7 in vivo and in vitro.

Finally, we demonstrated a therapeutic potential of Smad7 in the established mouse model of AAN by Smad7 gene therapy. Treatment with Smad7 for 4 weeks from day 14 of AAN was capable of improving renal dysfunction and inhibiting progressive renal fibrosis and inflammation.

In addition, we also extended this study to investigate the protective role of Smad7 in angiotensin II-induced hypertensive nephropathy since angiotensin II has been shown to be unregulated in AAN. Similar to the findings in AAN, deletion of Smad7 largely promotes angiotensin II-induced kidney injury, which is prevented by overexpressing renal Smad7 (Refs 1 and 2).

Furthermore, based on the role of inflammation, particularly inflammatory macropahges, in the development of AAN, we have collaborated with an international drug company J&J to test a new compound c-fms kinase inhibitor, BC-1601013, as a therapeutic strategy to prevent or reverse AAN in mice. This study is currently ongoing in Professor Lan's Lab and is conducting by Dr Dai Xiao-yu after completion of his PhD.

In summary, this study has resulted in 5 peer-reviewed paper published （Refs 1-5） and one major paper is currently under submission (Ref 6). One PhD student (Dr Dai Xiao-yu) has been successfully trained. Outcomes from this study reveal that Smad7 plays a protective role and has therapeutic potential for chronic AAN. Blockade of TGF-β/Smad3-mediated renal fibrosis and NF-κB-driven renal inflammation may be the central mechanisms by which Smad7 protects against AAN. Thus, findings from this study are novel and add new information for our better understanding of the pathogenesis and prevention or treatment of AAN.

## 7. The Layman's Summary

*(describe in layman's language the nature, significance and value of the research project, in no more than 200 words)*

Chinese herbal therapy is widely used. However, some of these herbal medicines have devastating severe side-effects on kidney disease such as aristolochic acid (AA). Clinically, patients with aristolochic acid-induced nephropathy (AAN) exhibit a rapidly progressive renal function deterioration and kidney scarring, resulting in end-stage renal disease. However, the mechanisms of AAN remain unclear and there is no cure for AAN. Thus, the present study investigate the mechanisms and therapeutic potential for AAN. By using gene deficient mouse model of AAN, we found that mice lacking Smad7 are promoted AAN. In contrast, overexpression of this molecule in the kidney can prevent the development of AAN and importantly has therapeutic effect on AAN. We also identified that blockade of TGF-β/Smad3-mediated renal fibrosis and NF-κB-driven renal inflammation may be the central mechanisms by which Smad7 protects against AAN. Thus, we conclude that Smad7 plays a protective role and has therapeutic potential for chronic AAN.

## Part C: Research Output

**8. Peer-reviewed journal publication(s) arising directly from this research project**
*(Please attach a copy of each publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.))*

| The Latest Status of Publications | | | | Author(s) *(**bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)* | Title and Journal/Book *(with the volume, pages and other necessary publishing details specified)* | Submitted to RGC *(indicate the year ending of the relevant progress report)* | Attached to this report *(Yes or No)* | Acknowledge the support of this Joint Research Scheme *(Yes or No)* |
|---|---|---|---|---|---|---|---|---|
| Year of publication | Year of Acceptance *(For paper accepted but not yet published)* | Under Review | Under Preparation *(optional)* | | | | | |
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|---|---|---|---|---|---|---|---|---|
| 2013 | | | | Liu Gx, Li Yq, **Huang Xr**, Wei L, Chen Hy, Shi Y, Heuchel Rl, **Lan HY*** | Disruption of Smad7 promotes ANG II-mediated renal inflammation and fibrosis via Sp1-TGF-beta/Smad3-NF.kappaB-dependent mechanisms in mice. ***Plos One.*** 2013;8(1):e53573 | Yes | Yes | Yes |
| 2013 | | | | Lv J, **Huang XR**, Klug J, Fröhlich S, Lacher P, Xu A, Meinhardt A, **Lan HY*** | Ribosomal Protein S19 is a novel therapeutic agent in inflammatory kidney disease. ***Clin Sci*** (Lond). 2013 May;124(10):627-37. | Yes | Yes | Yes |
| 2014 | | | | Chen HY, Zhong X, **Huang XR**, Meng XM, You YK, **Chung ACK, Lan HY*** | MicroRNA-29b Inhibits Diabetic Nephropathy in db/db mice. ***Mol Ther.*** 2014 Apr;22(4):842-53. | No | Yes | Yes |
| 2013 | | | | Meng XM, **Chung AC, Lan HY.*** | Role of the TGF-β/BMP-7/Smad pathways in renal diseases. ***Clin Sci (Lond)***. 2013 Feb;124(4):243-54 | No | Yes | Yes |
| 2014 | | | | Liu GX, Li YQ, **Huang XR**, Wei LH, Zhang Y, Feng M, Meng XM, Chen HY, Shi YJ, **Lan HY.** | Smad7 inhibits AngII-mediated hypertensive nephropathy in a mouse model of hypertension. ***Clin Sci (Lond)***. 2014 Aug;127(3):195-208 | No | Yes | Yes |
| | | | 2014 | Xiao Yu Dai, **Li Zhou, Xiao Ru Huang, Ping Fu,* Hui Yao Lan*** | Protective role and therapeutic potential of Smad7 in chronic aristolochic acid nephropathy | No | No | Yes |

## 9. Recognized International conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each delivered paper)*

| Month/Year/ Place | Title | Conference Name | Submitted to RGC *(indicate the year ending of the relevant progress report)* | Attached to this report *(Yes or No)* | Acknowledged the support of this Joint Research Scheme *(Yes or No)* |
|---|---|---|---|---|---|
| 18-21/05/2013 Istanbul | Conditional deletion of macrophages protects against aristolochic acid nephropathy in mice | The 50th Congress of European Dialysis and transplant Association 2013 | | Yes | Yes |
| 31/05/2013-04/06/2013, Hong Kong | Disruption of Smad7 enhances Aristolochic Acid Nephropathy | World Congress of Nephrology 2013 | | Yes | Yes |
| 31/05/2013-04/06/2013, Hong Kong | Smad3 inhibitor SIS3 is a novel therapeutic agent for aristolochic acid nephropathy | World Congress of Nephrology 2013 | | Yes | Yes |

## 10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

| Name | Degree registered for | Date of registration | Date of thesis submission/ graduation |
|---|---|---|---|
| Dai Xiao-yu | PhD (Sichuan University) | 9/2011 | 4/2014 |

**11. Other impact** *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

Based on the novel finding from this study that AA can induce both renal inflammation and fibrosis. We further hypothesized that renal inflammatory response may promote renal injury in chronic AAN and that blockade of renal inflammation by targeting macrophages may represent a novel therapy for AAN. Thus, Dr Zhou Li (Co-I in this project) has extended this study by successfully obtain a NFSC grant for further study by targeting macrophages using conditional macrophage knockout mice. She presented this study in the 50th Congress of European Dialysis and Transplant Association (18-21/05/2013, Istanbul) received a Best Paper Award (see attachment).

In addition, the findings from this joint research scheme also enabled us to successfully obtain a 973 grant (Mechanisms of chronic kidney diseases慢性肾脏病进展的机制研究 **2012CB5177000;**课题5：肾脏纤维化的细胞内信号分子及基因调控**2012CB5177005)**to further investigate the molecular mechanisms of TGF- /Smad3-mediated AAN via the microRNA-dependent pathway.

Furthermore, based on the role of inflammation, particularly inflammatory macrophages, in the development of AAN. We are currently collaborating with Johnson & Johnson to To determine whether a drug-based inhibitor of c-fms kinase activity (BC-1601013) can prevent or halt aristolochic acid-induced nephropathy. This study is currently ongoing in Professor Lan's Lab and is conducting by Dr Dai Xiao-yu after completion of his PhD.

Thus, this NFSC-RGC Join Research Scheme has largely promoted the collaborative research and postgraduate student training programs between CUHK and Sichuan University.