GATA2-regulated miR-194 targets Suppressor of Cytokine Signaling 2 to promote prostate cancer metastasis

Rajdeep Das1,2, Philip A. Gregory1,4, Rayzel C. Fernandes1,2, Iza Denis1,2, Qingqing Wang1,5, Scott L. Townley1, Shuang Zhao6, Adrienne R. Hanson1, Marie A. Pickering1, Heather K. Armstrong1,7, Noor A. Lokman8, Elai Davicioni9, Robert B. Jenkins10, R. Jeffrey Karnes11, Ashley E. Ross12, Robert B. Den13, Eric Klein14, Kim N. Chi15,16, Hayley S. Ramshaw1, Elizabeth D. Williams17, Gregory J. Goodall18, Felix Y. Feng19,20, Lisa M. Butler21, Wayne D. Tilley1,2 and Luke A. Selth1,2

1Dame Roma Mitchell Cancer Research Laboratories, School of Medicine, The University of Adelaide, SA 5005, Australia; 2 Freemasons Foundation Centre for Men’s Health, School of Medicine, The University of Adelaide, SA 5005, Australia; 3Centre for Cancer Biology, SA Pathology and University of South Australia, Adelaide, SA 5000, Australia; 4School of Medicine, University of Adelaide, Adelaide, SA 5005, Australia; 5Breast Cancer Genetics Group, Centre for Personalised Cancer Medicine, School of Medicine, The University of Adelaide, SA 5005, Australia; 6Department of Radiation Oncology, University of Michigan, Ann Arbor, MI 48109, USA; 7Cancer Theme, South Australian Health and Medical Research Institute, Adelaide, SA 5005 Australia; 8Research Centre for Reproductive Health, School of Paediatrics and Reproductive Health, Robinson Institute, University of Adelaide, Adelaide, SA 5000, Australia; 9GenomeDx Biosciences Inc., Vancouver, British Columbia, Canada; 10Department of Pathology and Laboratory Medicine, Mayo Clinic, Rochester, MN, USA; 11Department of Urology, Mayo Clinic, Rochester, MN, USA; 12Brady Urological Institute, Department of Urology, Johns Hopkins University, Baltimore, Maryland, USA; 13Sidney Kimmel Medical College, Thomas Jefferson University Hospital, Philadelphia, PA, USA; 14Glickman Urological and Kidney Institute, Cleveland Clinic, Cleveland, OH, USA; 15The Vancouver Prostate Centre, University of British Columbia, Vancouver, BC, Canada; 16Department of Medical Oncology, British Columbia Cancer Agency, Vancouver, British Columbia, Canada; 17Australian Prostate Cancer Research Centre Queensland, Translational Research Institute, Princess Alexander Hospital, Queensland University of Technology, Brisbane, QLD 4102, Australia; 18School of Molecular and Biomedical Science, University of Adelaide, Adelaide, SA 5005, Australia; 19Michigan Center for Translational Pathology, University of Michigan, Ann Arbor, MI; 20Comprehensive Cancer Center, University of Michigan, Ann Arbor, MI 48109, USA.

Background: Dysregulated expression of microRNAs (miRNAs, miRs) is a hallmark of cancer. MiR-194 is elevated in prostate tumors compared to non-malignant tissues and its levels in serum are predictive of post-surgery disease recurrence, but its role in this disease is poorly understood. Here, we demonstrated that miR-194 promotes metastasis of prostate cancer.

Methods: Levels of miR-194 in clinical samples were measured by qRT-PCR and in situ hybridization and by evaluating published transcriptomic datasets. Prostate cancer cell invasion, migration and growth in vitro were measured by specific assays following modulation of miR-194 levels. The effects of over-expressing and inhibiting miR-194 on invasion and metastasis in vivo were assessed by chick chorioallantoic membrane (CAM) assays and murine intravenous and intraprostatic metastasis assays. Levels of miR-194 in clinical samples were measured by qRT-PCR and in situ hybridization. Molecular targets of miR-194 were assessed by qRT-PCR and Western blotting.

Results: Serum levels of miR-194 are higher in men with metastatic versus localized disease, and tissue levels of miR-194 are associated with disease recurrence post-surgery and tumor aggressiveness. Over-expression of miR-194 in prostate cancer cell lines promoted migration, invasion and epithelial-mesenchymal transition in vitro and metastasis of xenografts in vivo. The ubiquitin ligase Suppressor of Cytokine Signaling 2 (SOCS2) was found to be a direct target of miR-194 in prostate cancer and a mediator of its pro-metastatic functions. Low levels of SOCS2 were strongly associated with disease recurrence and metastasis in patients, and its down-regulation augmented metastatic phenotypes. By targeting SOCS2, miR-194 de-repressed key oncogenic kinases including FLT3 and JAK2, leading to enhanced ERK and STAT3 signalling. GATA2 was found to be an upstream transcriptional regulator of miR-194 in vitro, a finding validated by the strong concordance between GATA2 activity and miR-194 levels in patient cohorts.

Conclusions: Collectively, our study has elucidated a novel pro-metastatic pathway in prostate cancer with miR-194 at the nexus, providing further impetus for exploring the potential of this miRNA as a biomarker and therapeutic target.
Conflict of Interest
The authors declare no conflict of interest.

Funding Acknowledgements
This work was supported by funding from the National Health and Medical Research Council of Australia (ID 1083961 to LAS, WDT, GJG and PAG) and a Prostate Cancer Research Programs Transformative Impact Award from the US Department of Defense (W81XWH-13-2-0093 to WDT and LAS). RD is supported by an award from The Hospital Research Foundation. LAS was previously supported by a Young Investigator Award from the Prostate Cancer Foundation (the Foundation 14 award). PAG is supported by a Beat Cancer Project fellowship from the Cancer Council of South Australia. The research programs of LMB, WDT, LAS and EDW are supported by the Movember Foundation and the Prostate Cancer Foundation of Australia through Movember Revolutionary Team Awards.