

Project title: Targeting activin signalling to restore adult tissue homeostasis**Project Summary:**

Activins, integral members of the transforming growth factor β (TGF- β) superfamily, are crucial regulators of cell growth and proliferation. Elevated levels of activins promote the development of gonadal tumours and induce cachexia by reducing muscle, liver, stomach, and fat mass (Matzuk et al., 1992; Matzuk et al., 1994; Zhou et al., 2010). A number of studies have also documented elevated serum activin A levels in cancer patients (e.g. in patients with metastatic liver, breast or prostate cancer) (Harada et al., 1996; Leto et al., 2006; Petraglia et al., 1998) and in patients with renal failure, heart failure and rheumatoid arthritis (El-Gendi et al., 2010; Harada et al., 1996; Yndestad, et al., 2004). Consequently, targeted inhibition of activin signalling has received much attention as a means to restore adult tissue homeostasis. However, current strategies to control activin-induced cachexia result in deleterious side effects. In addition, relatively little is known about the tumour-derived factors that promote activin expression in cancer.

To address these limitations in the field, this research project proposes;

- (1) To use newly developed activin-specific antagonists to reverse activin-induced cachexia
- (2) To identify the tumour-derived factors that stimulate activin expression

Study 1: Generation of specific activin antagonists by modification of the native prodomain

In models of cancer cachexia, pharmacological blockade of the activin type II receptor, ActRIIB, not only prevents muscle wasting, but also restores previous muscle loss. Unfortunately, ActRIIB is a receptor for multiple TGF- β ligands, and blockade of this receptor results in significant off target effects. For this reason we engineered modified versions of the activin A and B propeptides to favour high affinity binding with the activins isoforms. Propeptides mediate the biosynthesis of all TGF- β ligands and, for some family members (e.g. TGF- β 1), bind the mature growth factor with high enough affinity to confer latency. Based on the structure of Pro-TGF- β 1 (Shi et al., 2011), we designed specific activin propeptide-based antagonists. The modified activin propeptides demonstrated to be effective agents in modulating activin A and B *in vitro* activity, with potencies comparable to the activin antagonists, follistatin and sActRII/B. Excitingly, the modified activin propeptides did not inhibit the closely related TGF- β ligands, myostatin and GDF-11, making these reagents the first specific activin antagonists.

The next phase in this study is to validate our antagonist's therapeutic potential *in vivo*. We have recently published a study showing that activins drive muscle wasting and cachexia in mice (Chen et al., 2014). We used recombinant serotype 6 adeno-associated virus (rAAV6) vectors to increase circulating activin A levels in C57BL/6 mice. Mice injected with control virus gained 10% of their starting-body mass over a 10 week period, whereas mice injected with activin encoding virus lost

more than 12% of their body mass. Importantly, removal of activins completely restored body mass, highlighting the potential to target activin signalling in the treatment of cachexia.

We are currently using the adeno-associated viral model to assess the *in vivo* potential of the modified activin propeptides. In addition, we will examine the therapeutic potential of the activin propeptides in a physiological model of cachexia.

This study is near completion, and manuscript in preparation.

Study 2: Identification of the tumour derived factors that stimulate activin over-expression

Increasing evidence suggests that activins are elevated in cancer (Harada et al., 1996; Leto et al., 2006, Petraglia et al., 1998), and that up regulation of the activin signalling pathway causes cachexia. In order to reverse the activin-induced wasting in human disease, it is imperative that we identify the factors that drive activin over expression. Toward this aim, in this study we plan to identify the tumour derived factors that upregulate activin expression by activation of the native promoters.

In multiple models of cancer cachexia, administration of the activin type II receptor, ActRIIB, not only reverses the wasting in these mice but also reduces tumour size (Zhou et al., 2010). This finding suggests that activin may serve a pro-tumourigenic role. To address, we are assessing responsiveness of metastatic cell lines to activin signalling, and the pro-tumourigenic capacity of activin.

Publications relating to this award:

Chen, J. L., **Walton, K. L.**, Winbanks, C. E., Murphy, K. T., Thomson, R. E., Makanji, Y., Qian, H., Lynch, G. S., Harrison, C. A., Gregorevic, P. Elevated expression of activins promotes muscle wasting and cachexia. *FASEB J.*, accepted for publication Jan 3.

Presentations relating to this award:

- 1) Chen, J. L., **Walton, K. L.**, Al-Musawi, S.L., Gregorevic, P, Harrison, C. A. Targeting Activin to Counteract Muscle Wasting and Cachexia. Short Oral and Poster Presentation: ENDO 2013, San Francisco, United States of America.
- 2) Chen, J. L., **Walton, K. L.**, Winbanks, C. E., Murphy, K. T., Thomson, R. E., Makanji, Y., Qian, H., Lynch, G. S., Harrison, C. A., Gregorevic, P. Activins are potent negative regulators of muscle mass. Oral Presentation: ESA-SRB 2013, Sydney, Australia.
- 3) **Walton, K. L.**, Chen, J. L., Al-Musawi, S.L., Gregorevic, P, Harrison, C. A. Generation of Fc-fusion proteins: Creation of specific and potent activin antagonists. Oral Presentation: 2013 CSIRO Protein Expression Workshop, Parkville, Australia.
- 4) Chen, J. L., **Walton, K. L.**, Al-Musawi, S.L., Harrison, C. A., Gregorevic, P. Activins are potent negative regulators of muscle mass. Oral presentation: 2013 Australian Society for Medical Search Vic Student Symposium: Melbourne, Australia.

Manuscripts in preparation:

Walton, K. L., Chen, J. L., Al-Musawi, S.L., Winbanks, C. E., Gregorevic, P, Harrison, C. A. Reversal of activin-induced wasting using a modified activin B propeptide. *Intended for submission mid 2014.*

Planned presentations relating to this award in 2014:

- 1) **Walton, K. L.**, Chen, J. L., Al-Musawi, S.L., Winbanks, C. E., Gregorevic, P, Harrison, C. A. Reversal of activin-induced wasting using a modified activin B propeptide. Abstract submitted for ICE-ENDO 2014, Chicago, United States of America.

References

- Chen, J. L., et al. (2014) *FASEB J*, accepted for publication Jan 3
- El-Gendi, S. S., et al. (2010) *Int J Rheum Dis* 13(3), 273-279
- Harada, K., et al. (1996) *J Clin Endocrinol Metab* 81(6), 2125-2130
- Leto, G., et al. (2006) *Clin Exp Metastasis* 23(2), 117-122
- Matzuk, M. M., et al. (1994) *Proc Natl Acad Sci U S A* 91(19), 8817-8821
- Matzuk, M. M., et al. (1992) *Nature* 360(6402), 313-319
- Petraglia, F., et al. (1998) *J Clin Endocrinol Metab* 83(4), 1194-1200
- Shi, M. et al. (2011) *Nature*, 474(7351), 343-9
- Yndestad, A., et al. (2004) *Circulation* 109(11), 1379-1385
- Zhou, X., et al. (2010) *Cell* 142(4), 531-543