Program title: Targeting activin signalling to restore adult tissue homeostasis

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 proposed:

(1) To use newly developed activin-specific antagonists to reverse activin-induced cachexia

(2) To understand the role of tumour-derived factors in activin-mediated wasting

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

We have recently shown that activins alone, in the absence of tumour-derived factors, are sufficient to promote cachectic-like wasting in mice (Chen et al., 2014). Using an adeno-associated viral (AAV) delivery system, we over-expressed activin in the hind limbs of mice and found that a modest elevation (20-fold) in activin levels could promote a loss in muscle mass of >30%. Moreover, we found that whilst mice injected with control virus gained up to 10% of their starting body mass over a 10-week observation period, mice expressing the activin virus lost up to 12% of their initial body mass. It was demonstrated that activin exerts its catabolic effect in muscle by up-regulating protein degradation pathways and down-regulating protein synthesis pathways. In addition, activin hyperactivity promoted significant fibrosis in the affected tissues. Significantly, we showed that activin-mediated muscle wasting was fully reversible upon removal of activin.

To complement these findings, I recently developed and validated the first activin-specific therapeutic. Activins, like all TGF-beta proteins, are made as larger pro-hormones, comprising a propeptide shield that surrounds the mature 'active' protein. For activin, the affinity of this complex is weak in comparison to other TGF-β proteins (Walton et al., 2009), and the propeptide is readily displaced in the presence of activin receptors. Using extensive structural analysis of TGF-β propeptides (Walton et al., 2009, Walton et al., 2010), we modified activin A and B propeptides to favour high-affinity binding to mature activin proteins. The resultant activin propeptides are specific and potent activin-antagonists – capable of blocking activin actions, and uniquely have no activity

against activin-related proteins such as myostatin (GDF-8) or GDF-11. Importantly, using our AAV delivery model, I have shown that the activin propeptides can ameliorate activin-induced wasting in mice (Walton & Chen et al., 2014).

We are now validating the therapeutic potential of the propeptides in preclinical models of cancer-cachexia. I am using two cancer-cachexia murine models to validate the efficacy of the propeptide; the C26 colon-cancer model, and a CHO cell derived xenograft model (Zhou et al., 2010). Most excitingly, our preliminary results indicate that the activin propeptides can prevent tumour-derived wasting in the CHO cell xenograft model. The outcomes of this study will establish the potential of the activin propeptides to combat activin-mediated wasting in cancer-cachexia.

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

Cancer-cachexia is a multifactorial condition, involving numerous tumour-derived factors. Inflammatory cytokines including tumour necrosis factor -α (TNF-α) (reviewed in Chu, 2013) and interleukins IL-1 and IL-6 (Tisdale, 2009) have been heavily implicated in the aetiology of cachexia. Of interest, several studies have now documented that these cytokines can stimulate activin production (Yoshino et al., 2011, Abe et al., 2013, Trendelenburg et al., 2012). To further these studies, I hypothesised that activin-mediated cachexia may be exacerbated in the presence of proinflammatory cytokines. To address, we have examined the synergistic activities of the proinflammatory cytokines with activins using our established AAV delivery model in mice. Preliminary analysis supports that activin biosynthesis is increased in the presence of the cytokines. The outcomes of this study will allow us to understand the implications of heightened activin activity in the presence of the tumour-derived cytokines. This approach will aid the development of more effective treatments for cancer-cachexia. *This study is intended for publication mid 2015.*

Publications relating to this award:

Walton, K.L.*, Chen, J.L.*, Al-Musawi, S.L., Kelly, E.K., Qian, H., La, M., Lu, L., Lovrecz, G., Ziemann, M., Lazarus, R., El-Osta, A., Gregorevic, P., and Harrison, C.A. (2014). Development of Novel Activin-Targeted Therapeutics. *Molecular Therapy (Nature Publishing Group)*, accepted 17th November 2014. **Authors contributed equally to this study*

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. (2014). Elevated expression of activins promotes muscle wasting and cachexia. *The FASEB Journal.* 28(4):1711-23.

Chen, J. L., **Walton, K. L.,** Gregorevic, P, Harrison, C. A. Targeting activins as a treatment of cancer-cancer. *Intended for submission mid 2015.*

Presentations relating to this award:

1) **Walton, K. L.,** Chen, J. L., Al-Musawi, S.L., Gregorevic, P, Harrison, C. A. Reversal of activin-induced muscle wasting using a modified activin B propeptide. Poster Presentation: ICE-ENDO 2014, Chicago, United States of America.
2) **Walton, K. L**. Genetic engineering of inhibins/activins for the treatment of human disease. Oral Presentation: Northwestern University, Chicago, United States of America. June 2014.
3) **Walton. K. L**., Chen, J. L., Lee, Q., Al-Musawi, S.L., Gregorevic, P. and Harrison, C.A. Increasing muscle mass using novel activin-targeted therapeutics. Poster Presentation: ESA-SRB 2014, Melbourne, Australia.
4) **Walton. K. L**., Chen, J. L., Al-Musawi, S.L., Gregorevic, P. and Harrison, C.A. Generating specific TGF-β superfamily antagonists. Oral Presentation: ESA-SRB 2014 (TGF-β workshop), Melbourne, Australia.
5) **Walton. K. L**., Chen, J. L., Al-Musawi, S.L., Gregorevic, P. and Harrison, C.A. Targeting Activin to Counteract Muscle Wasting and Cachexia. Oral Presentation: MIMR-PHI Staff Retreat, San Remo, Australia.
6) Chen, J. L., **Walton, K. L.,** Al-Musawi, S.L., Gregorevic, P, Harrison, C. A. Targeting Activin to Counteract Muscle Wasting and Cachexia. Poster Presentation: ENDO 2013, San Franciso, United States of America.
7) 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.
8) **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.

Funding relating to award

1) **Walton K.L. (CI)**, Chen J.L., Gregorevic P., and Harrison C.A. (2015-2016). Victorian Cancer Agency Early Career Seed Grant: $198,180.
2) **Walton, K.L.** (2014) Endocrine Society of Australia, ESA_IPSEN Travel award, $3500
3) **Walton K.L.** (2014) Cass foundation travel award, $2750
4) **Walton K.L.** (2014) Endocrine Society of Australia, Postdoctoral Fellowship, $50,000

Student completions

Justin L. Chen awarded PhD for thesis titled "Targeting activins to counteract muscle wasting and cachexia" on 4th November 2014, Monash University, Australia. Justin was recently awarded a

Cancer Council Postdoctoral Fellowship for 2015 (total funding $72,359), which will enable him to continue his studies in our laboratory.

Expenditure summary:

Item	**Pricing**
Production of activin A and B propetide at Recombinant Protein Production and Purification Facility, CSIRO	$10,000
Miscellaneous consumables/Tissue culture (Lipofectamine 2000, FCS, DMEM/OPTI-MEM media, Molecular biology reagents)	$5000
Activin immunoassays/Recombinant proteins/Antibodies	$3000
Immunohistochemistry reagents	$1000

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Walton, K.L., et al (2010) *J Biol Chem* 285, 17029-37

Walton, K.L., Chen, J.L. et al (2014) *Mol Therapy,* accepted 17th Nov

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Zhou, X., et al. (2010) *Cell* 142(4), 531-543