Abstract: 158P

Metabolic regulation of skeletal muscle regeneration after injury

John H. Nguyen[1], Kristy Swiderski[1], Kristin K. Brown[2,3], Craig A. Goodman[1], David D. Souza[4], René Koopman[1], and Gordon S. Lynch[1],

[1]Centre for Muscle Research, Department of Anatomy and Physiology, The University of Melbourne, VIC 3010, Australia [2]Peter MacCallum Cancer Centre, VIC 3010, Australia, [3]Department of Biochemistry and Pharmacology, The University of Melbourne, VIC 3010, Australia and [4]Metabolomics Australia, The University of Melbourne, VIC 3010, Australia

Skeletal muscle regeneration is a highly complex and coordinated process involving a variety of cell populations. Muscle stem cells (MuSCs) undergo several stages of proliferation and differentiation to facilitate tissue repair. Fibroadipogenic progenitors (FAPs) are required to support the MuSC response to muscle injury (Fiore *et al*., 2016). Previous studies identified a role for metabolism as a key determinant in cell state and lineage progression (Lunt & Vander Heiden, 2011; Ryall et al., 2015; Pala et al., 2018). We sought to characterise the metabolic milieu after muscle injury and identify key metabolites that regulate MuSC and FAP proliferation and differentiation.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code for the care and use of animals for scientific purposes (8th ed. Canberra: NHMRC). All procedures involving mice were conducted under anaesthesia (2-5% isoflurane gas). The right tibialis anterior (TA) muscles of C57BL/6 male mice were injured by either intramuscular injection of the myotoxin, 1.2% barium chloride ($BaCl_2$, n=35), or reperfusion after ischaemia from temporary occlusion of blood flow (IR n=49). Mice were administered buprenorphine (0.05 mg/kg) to minimise post-operative pain. After induction of injury, mice were killed via rapid cervical dislocation and uninjured (day 0) and injured muscles (at day 3, 5, 7, 10, 14, and 28 post-injury) were excised and prepared for untargeted steady-state metabolomic GC-MS analyses to identify metabolites of interest. The immortalised C2C12 myoblast cell line and primary MuSCs and FAPs were isolated from C57BL/6 mice and used to assess the effects of key metabolites on proliferation and differentiation, based on analyses of raw cell counts and immunofluorescence and western immunoblotting.

A metabolic signature of skeletal muscle was generated for uninjured and injured muscles after $BaCl_2$ and IR injury, with distinct profiles for both models of injury. Following annotation and normalisation, over 200 unique metabolites were identified as differentially expressed. Pathway enrichment analysis across the timepoints revealed 'Pantothenate and CoA Biosynthesis' and 'Spermidine and Spermine Biosynthesis' were consistently among the top enriched pathways for regenerating muscles after $BaCl_2$ and IR, respectively. We selected two metabolites that were among those with the highest fold change (post-injury), for further analyses *in vitro*. Pantothenic acid increased 49.5-fold at 7 days post-injury compared to control, after $BaCl_2$ injury. Putrescine increased 19.6-fold at 3 days post-injury, compared to control, after IR.

Metabolomic signatures of regenerating skeletal muscles after $BaCl_2$ and IR injury revealed differential responses depending on the mode of injury. Two key metabolic pathways and metabolites 'Pantothenic Acid' and 'Putrescine' were identified for further investigation. These results have important implications for the development of targeted treatments for specific muscle injuries. Future studies should determine the role of these metabolites during muscle regeneration.

Lunt SY & Vander Heiden MG. (2011). *Annual Review of Cell and Developmental Biology* **27**; 441- 464.
Pala F, Di Girolamo D, Mella S, Yennek S, Chatre L, Ricchetti M & Tajbakhsh S. (2018). *Journal of Cell Science* **131**; jcs212977.
Ryall JG, Dell'Orso S, Derfoul A, Juan A, Zare H, Feng X, Clermont D, Koulnis M, Gutierrez-Cruz G, Fulco M & Sartorelli V. (2015). *Cell Stem Cell* **16**; 171-183.
Fiore, D., Judson, R.N., Low, M., Lee, S., Zhang, E., Hopkins, C., Xu, P., Lenzi, A., Rossi, F.M.V., and Lemos, D.R. (2016). *Stem Cell Research* **17**; 161-169.