Metformin regulates the differentiation of murine mesenchymal stem cells via AMPK-independent suppression of p70s6-kinase

SC Chen1, R Brooks1, SF Ahmed1, SJ Yarwood2
1Developmental Endocrinology Research Group, Royal Hospital for Sick Children, School of Medicine, University of Glasgow, UK
2Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK

Background and Objective

Metformin is widely used as oral anti-hyperglycaemic agent to treat Type 2 diabetes, with increasing reports of an additional, potential bone protective role. On a cellular level, mesenchymal stem cells (MSCs) have been reported to have reciprocal differentiation into bone at the expense of fat, and vice versa. We set out to investigate the effects of metformin on the multipotent mesenchymal stem cell differentiation and the underlying molecular mechanism(s) involved.

Methods

Confluent murine MSCs (C3H10T1/2) were treated with metformin (500μM), a known AMPK activator (A769662;100μM) or the p70S6K inhibitor (rapamycin;10μM), in both control and adiopogenic-inducing environments (using pioglitazone;10μM) for 5 days. Nuclear extracts were separated by SDS-PAGE and immunoblotted with primary antibodies to p38 mitogen-activated protein kinase (MAPK), phospho-p38 (ser180) and p70S6K (ser389). Immunoblots were scanned using a Licor fluorescent reader. PPARγ and Runx2 activities were determined using Luciferase reporter assays and adipogenesis was quantified histochemically by staining neutral lipids with Oil Red O.

Results

MSCs treated with pioglitazone demonstrated marked adiopogenic phenotype staining positively with Oil Red O. In contrast, treatment with both metformin and A769662 impaired adiopogenesis (Figure 1). Pioglitazone induced an (p<0.01) increase in PPARγ expression, whilst metformin and A769662 suppressed PPARγ expression to basal levels, p<0.05 and p<0.01 respectively. Runx2 activity was significantly increased by metformin (p<0.001) and A769662 (p<0.001) but not Runx2 protein levels (Figure 2 and 3). As expected, A769662 promotes phosphorylation of ACC, but not so with metformin (Figure 4). Instead, metformin suppressed (p<0.05) the phosphorylation of p70s6k, as did A769662 (p<0.05) and rapamycin (p<0.001) (Figure 5). Luciferase reporter assays confirmed the reciprocal action of metformin on adipogenesis and osteogenesis, namely suppression of PPARγ activity (p<0.001) and induction of Runx2 activity (p<0.001).

Conclusion

• Metformin inhibits adipogenesis and promotes osteogenesis
• Acting through AMPK-independent pathway, involving the suppression of p70s6k in the mTOR signalling (novel mechanism of action)
• Rationale for the reported bone protective role of metformin