Impaired Insulin and IGF-2 signalling in the primordial growth disorder 3-M Syndrome

Jins Kallampallil, Ksenija Acimovic, Daniel Hanson, Andrew Whatmore, Peter Clayton
Paediatric Endocrinology, Centre for Paediatrics and Child Health, Institute of Human Development, Faculty of Medical and Human Sciences, The University of Manchester

Introduction

3-M Syndrome is a rare autosomal recessive primordial growth disorder associated with a distinct facial appearance, prominent fleshy heels and minor skeletal abnormalities1 (Fig. 1).

Caused by mutations in CUL7, OBSL1 and CCDC83,4.

In cells derived from patients with 3-M syndrome, Growth hormone and IGF-1 signalling and expression of IGF-2 and insulin receptor isoforms are all altered3,5.

In view of this altered growth factor signalling, we have investigated the activation of AKT by IGF-2 and insulin.

Results

Relative pAKT was reduced in 3-M fibroblasts compared to normal cells when stimulated with either insulin (p = 0.048) or IGF-2 (p = 0.033) (Fig. 2).

For both treatments, CUL7+/− and OBSL1+/− cells had a markedly lower activation of pAKT than did CCDC8−/− cells. CUL7+/− cells had an earlier activation in response to insulin (p = 0.016) than other cell types.

After 60 mins Insulin stimulation, pAKT was 65-70% of normal in CUL7+/− and OBSL1+/− and 90% in CCDC8−/−.

With IGF-2, pAKT at 60 mins was 70-80% of normal in all 3-M cells.

Method

Fibroblasts were cultured from skin biopsies taken from 3 3-M patients (1 each with a CUL7, OBSL1 or CCDC8 mutation) and from normal controls.

Sub-confluent cells were stimulated with 500ng/ml IGF-2 or 100ng/ml insulin for 0, 5, 15 and 60 minutes.

Relative phosphorylation of AKT was assessed by western blotting and comparisons made both within and between cell lines.

Conclusion

Reduced AKT activation in response to IGF-2 and insulin stimulation mirrors that previously seen with IGF-1 although to a lesser extent.

CCDC8−/− cells were least affected, consistent with the clinical phenotype observed in these children.

Diminished growth factor signalling could account for the growth failure in 3-M Syndrome, and may contribute to insulin resistance observed in children born small for gestational age.

Fig. 1 - Phenotypic features of 3-M syndrome
A) Fleshy tipped nose, prominent full lips, triangular shaped face and prominent forehead. B) Prominent fleshy heels (Images courtesy of Professor Peter Scambler, UCL Institute of Child Health, London)

Fig. 2 AKT activation in response to Insulin and IGF-2 treatment. Bars represent mean fold activation relative to untreated +/- standard errors. Representative experimental blots are shown for Insulin.