Proceedings of the Nutrition Society (2011), **70 (OCE3)**, E56 doi:10.1017/S0029665111000966

Joint Irish Section and American Society for Nutrition Meeting, 15–17 June 2011, 70th anniversary: 'Vitamins in early development and healthy ageing: impact on infectious and chronic disease'

Development of a high throughput, quantitative assay of the effects of fungal-derived griseofulvin and cordycepin, and butyrate on the cytoskeleton

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Keratins are intermediate filaments (IF) found in abundance in epithelial cells. Depolymerisation of these filaments causes the cell to collapse and become more plastic. We have previously shown that SCFC may trigger depolymerisation of keratins through altered protein acetylation[1,2]. The aim of this study was to develop a high-throughput method to quantify IF polymerisation and to apply as a screen for IF-perturbing nutrients and drugs. Three treatments were used in a proof-of-principle study: the anti-fungal drugs griseofulvin and cordycepin (the former a c-mitotic drug known to suppress microtubule growth[3], the latter inducing abnormal mitosis by suppressing microtubule dynamics[4]) and sodium butyrate, a histone deacetylase inhibitor that disrupts IF formation in cancer cells via post-translational modification of keratins[1].

A high content analysis (HCA) approach was developed. HCA is a tool allowing quantification of staining intensities by use of an Arrayscan II platform linked to a fluorescent microscope that can read ninety-six-well plates[2]. Sixty wells of a ninety-six-well plate were seeded with 2.5×10^3 MCF-7 cells in 100 µl RPMI media. Plates were incubated for 24 h at 37°C, after which, media were replaced with either griseofulvin treatment (2–200 µM, 48 h), cordycepin (0–60 µM, 15 min) or sodium butyrate (0–20 mM, 16 h). Treatment was removed and plates were fixed with ice-cold methanol for 5 min.

Immuno cytochemistry was used to visualise K8. An anti-K8 antibody and Hoechst stain were used to stain cells. Indicators of depolymerisation include K8 fluorescence, texture measurement, spot fibre count and spot fibre total area (arbitrary units). Filamentousness was measured using an algorithm based on co-occurrence of adjacent pixel intensity[2]. *Z prime values between 0.5 and 1 indicate suitability as a high-throughput assay. A paired student's *t* test was used to compare control and test values. †$P<0.05$ is statistically significant.

Treatment	Conc.	Statistical test	Cell count	K8 fluorescence	Texture measurement	Spot fibre count	Spot fibre total area
Griseofulvin	100 µM	Z Prime	− 1.42	− 1.38	0.32	0.95*	0.73*
Griseofulvin	100 µM	*t* test	0.024†	0.046†	0.015†	0.012†	0.0005†
Cordycepin	60 µM	*t* test	0.57	0.04†	0.025†	0.64	0.16
Sodium butyrate	20 mM	*t* test	0.2	0.8	0.0000006†	0.016†	0.003†

An HCA assay for intermediate filament integrity has been demonstrated, establishing a good proof of principle with griseofulvin. We are currently producing a rat monoclonal K8 antibody to be used in conjunction with a mouse tubulin antibody and phalloidin stain for actin, allowing development of a novel triple cytoskeletal stain assay with commercial potential.

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