

Identification of polyphenol regulators of the insulin-sensitive transcription factor FOXO1a

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Introduction

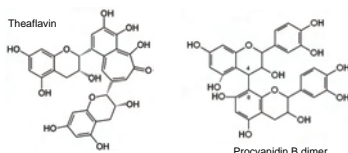
The aim of this project is to identify food compounds which induce insulin-like effects on cells.

Compounds with these properties may help to alleviate some of the problems caused by the insulin insensitivity of type 2 diabetes mellitus (T2DM). Normally, when blood glucose levels are elevated, insulin is released from the pancreas, stimulating glucose uptake and storage. This occurs via the insulin signalling pathway, a intracellular phosphorylation cascade, ultimately regulating various effectors involved in glucose regulation, including phosphorylation and inactivation of the transcription factor, FOXO1a.

Oligoproanthocyanidins

Previous work found that black tea polyphenols called theaflavins stimulated the insulin signalling pathway, and FOXO1a, at an as yet undetermined point (Cameron *et al.*, 2008). This project aims to determine what other dietary phenolic compounds can also cause this effect and to examine the mechanism of action.

Oligoproanthocyanidins (OPCs) are a class of polyphenols commonly found in a wide variety of foods. OPCs consist of polymerised catechins and are enriched in grape seed, pine park and berries.



Results

Cell culture and Western blotting

Human Embryonic Kidney 293 (HEK293) cells were treated with increasing concentrations of grape seed extract (GSE) and Western blotting was employed to detect phosphorylated FOXO1a. This confirmed that a concentration of 100µg/ml induced FOXO1a phosphorylation (data not shown). Similar effects were found with lingonberry (LB) and fractions from pine bark (PBE), and cranberry (CB) (Fig 1). Chromatography on Supelco Discovery polyamide columns was used to separate bound OPCs from other polyphenols.

When 100µg/ml amounts of each fraction were applied to the HEK293 cells, western blotting confirmed that the bound OPC-rich fractions of each sample stimulated phosphorylation of FOXO1a whereas the unbound fractions did not.

Liquid chromatography /mass spectrometry

To determine the composition of each sample, analysis was carried out using normal phase HPLC on a LCQ Deca system.

Oligoproanthocyanidins (OPCs) were confirmed to be present in the bound fractions and enriched in fractions which stimulated phosphorylation of FOXO1a (Fig 2, 3).

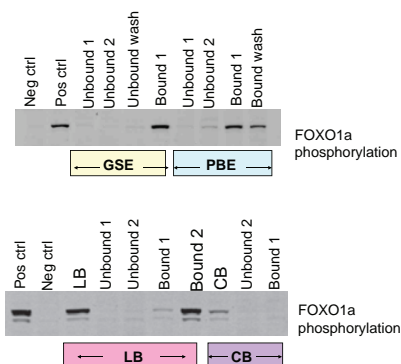


Fig 1: Western blot analysis of hydrophobic and hydrophilic fractions suggests hydrophobic fractions contain the active compound(s).

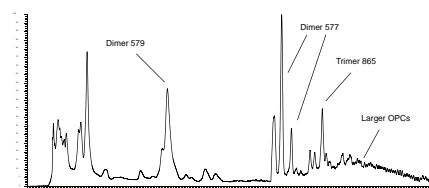


Fig 2: LC-MS of active OPC-rich fraction of PBE.

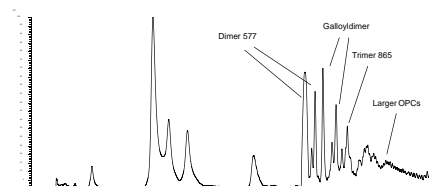


Fig 3: LC-MS trace of active OPC-rich fraction of GSE

Current work

Peaks collected from the normal phase LC runs have been confirmed to contain only one or two OPCs by direct MS analysis. These purified fractions are currently being tested for their ability to stimulate phosphorylation of FOXO1a by Western blot analysis to identify the most active components.

Future work

- To investigate structure/activity relationships for OPCs on this pathway
- To determine the mechanism of action of the compounds, using specific kinase inhibitors to delineate the pathways involved

References

Cameron, AR *et al.* Black tea polyphenols mimic insulin/ insulin-like Growth factor-1 signalling to the longevity factor FOXO1a. *Aging Cell.* (2008). 7. 69- 77.

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