A Mechanistic Approach to Fruit Phytochemicals and Cancer Chemoprotection

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The correlation between a diet high in fruit and vegetable and a reduced incidence of a range of cancers is now well accepted. However the basis for this beneficial effect if still unclear. Some phytochemicals and their breakdown products (metabolites) have been shown to increase the levels of detoxication enzymes (aldo-keto reductase, NAD(P)H:quinone oxidoreductase 1 (NQO1), and glutathione *S*-transferase (GST)), and antioxidant proteins in human cell line and animal studies. Elevation of the levels of these enzymes and proteins should bolster protection against free radical damage and by association reduce the incidence of the radical derived degenerative diseases such as cancer. It is proposed that key to the regulation of many genes encoding detoxication and antioxidant proteins is the nuclear factor-erythroid 2 related factor 2 (*nrf2*)*(Fig. 1)*.

Model studies have shown that the absence of *nrf2* results in an inability to respond, or have a blunted response, to the model cancer chemopreventive agents. It is unknown whether dietary-derived compounds like those abundant in soft fruit (e.g. anthocyanins, flavanols, catechins, procyanidins, hydroxy-benzoic acids) exert their anecdotal beneficial effect via the same *nrf2*-dependent mechanism.

Approach

1) State-of-the-art chemical purification methods will be used to isolate and characterise individual polyphenols from soft fruit. These will include anthocyanins, flavanols, catechins, procyanidins, hydroxy-benzoic acids etc (Fig 2)

2) Identify polyphenols that activate *nrf2* by treating cells that contain artificial Nrf2-driven reporter systems. These are used to probe the impact of fruit phytochemicals on various antioxidant and xenobiotic related genes (Fig 3).

3) The ability of polyphenols to increase the intracellular levels of antioxidant and detoxification enzymes will be studied in model experimental systems that either possess *nrf2* or lack the factor

4) The ability of polyphenols to prevent oxidative and DNA damage in model systems in an *nrf2* -dependent fashion will be studied.

5) Follow the fate of the applied phytochemicals and changes in cellular metabolites using LC & GC-MS based metabolomics approaches.

Currently artificial NQO1 and *nrf2*-reporter gene systems have been constructed and are being validated (Fig 3). A host of pure polyphenlic compounds have been prepared and will be tested against these systems to establish whether activity is confined to polyphenolic chemical classes and if within a class there is a quality activity structure relationship evident.

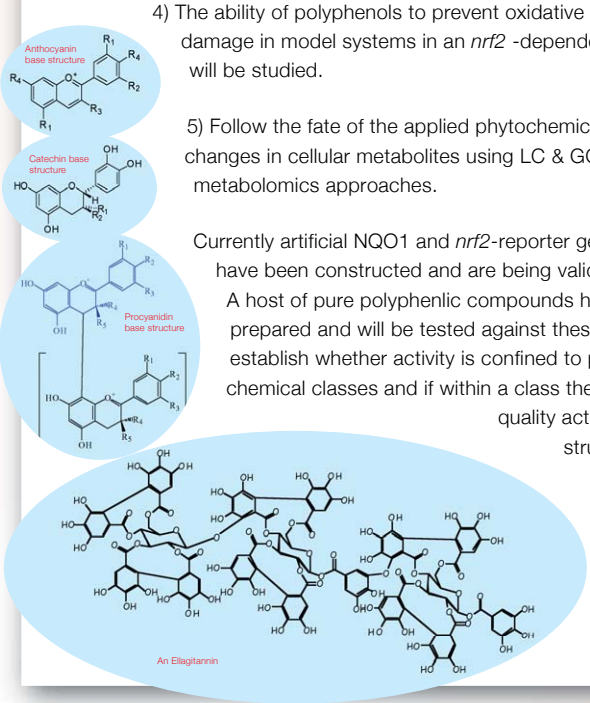

Fig 2 General structures for some common fruit polyphenolics

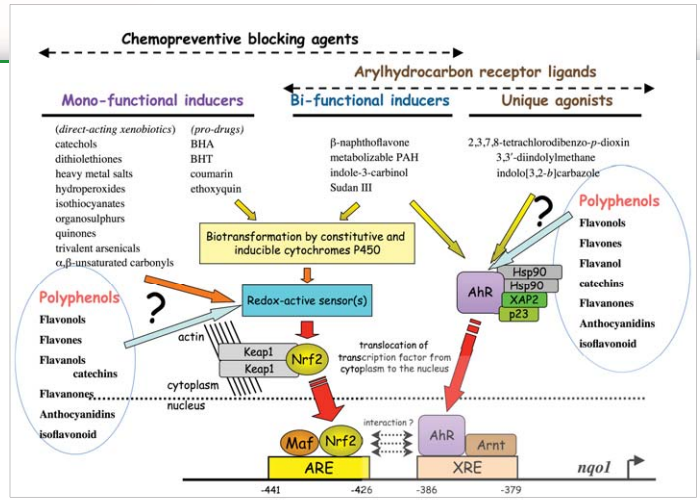

Figure 1 The proposed mechanisms whereby established chemopreventive blocking agents act and the points at which it is thought that dietary polyphenols may interact
ARE - Antioxidant responsive element
AhR - aryl hydrocarbon receptor
XAP2 - X-associated protein 2
ARNT - AhR nuclear translocator

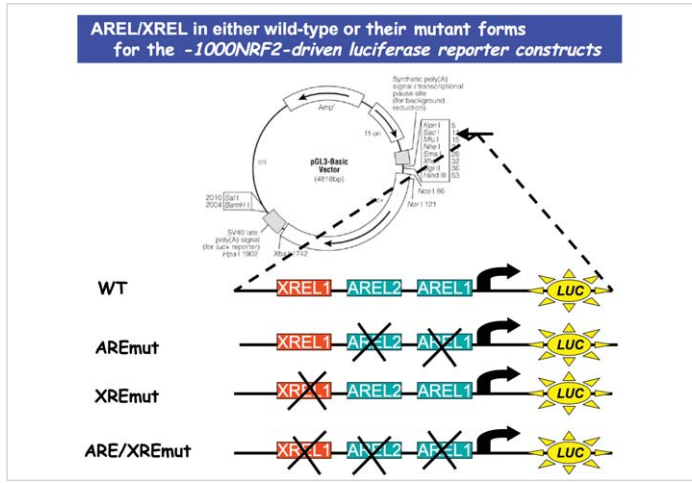

Figure 3. Schematic diagram of the reporter assays to study how fruit phytochemicals interact with the Nrf2-Antioxidant Response Element (ARE) and/or Xenobiotic Response Element (XRE) related pathways

Acknowlegement
HX acknowledges funding from SCRI, Dundee University Medical School and support from the TENOVUS and the Blackcurrant Foundation.

References
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