microRNAs as biomarkers of oxidant/antioxidant status

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The discovery of small non-coding RNA (sncRNA) has generated enormous interest in their use as potential biomarkers and as new drugs targeting genes. These molecules are involved in gene expression regulation: they are able to regulate gene expression mainly by base-pairing to the 3'-UTR of specific target mRNAs so controlling, at epigenetic level, a wide range of biological processes. MicroRNAs (miRNAs), a class of sncRNAs, are single stranded non-coding RNA ~19-25 nt in length, that are transcribed from intergenic and intronic sequences and are released also in extracellular compartment carried by various proteins, lipids and by exosomes, spreading in this way, molecular signals in biological fluids from one district to others. Therefore, miRNAs may represent a fine-tuning of signaling able to reach different body districts and to integrate multiple inputs and outputs. This makes them suitable to be used both as markers of disease state and as new drugs for the control of protein expression (i.e. oncogenes). The discovery that circulating miRNAs are measurable in serum and plasma and in other biological fluids and that their expression varies in the presence of pathologies makes them of great potential in diagnostic applications.
Many epidemiological studies correlate the beneficial effects on population health to food containing polyphenols with the antioxidant property of these molecules, but there are still unclear points regarding the useful concentrations. For example the influence on gene transcription and gene regulation is far to be elucidated. Some recent studies have shown that polyphenols are able to influence gene expression at the epigenetic level inducing the transcription of sncRNAs, in particular miRNAs, that are able to inhibit the expression of target genes by interfering with the translation of their RNA messengers (Bartel, 2004).
In our study we aimed to investigate, in the human intestinal Caco-2 cell line, if different concentrations of the polyphenol Gallic Acid (GA) can modulate differently mithocondrial antioxidant activities by inducing different levels of miRNA which down-regulate Mn SOD, Catalase and Peroxidase enzimes. In addition, cell proliferation (mitotic index) and death (MTT test) were evaluated. Total RNA was obtained both from cells and culture media of untreated and treated cultures and quantitative real-time PCR (qRT-PCR) of several miRNAs (mir-17-3p, mir-21-5p, mir-421) was performed using cDNA obtained following the reverse transcription reaction with the miRCURY LNA™ Universal RT micro RNA PCR kit. From our results GA treatment induces a modulation of different miRNAs that, in turn, influences a number of different biological processes. The modulation of miRNA synthesis was confirmed in culture medium. Our results demonstrated a dose-dependent miRNA expression with a progressive increasing in miRNA levels that down regulate mitochondrial antioxidant activity (mir-17-3p), cell proliferation (mir-21) and damage repair (mir-421). The progressive increase in miRNA levels correlate with a progressive increase in toxic effects of GA. Our results are in agreement with other studies showing that polyphenols are able to modulate miRNA synthesis (Bartel, 2004) and suggest the need of further research for a safe use of antioxidant supplements in the light of the complex miRNA network interactions to avoid possible adverse effects.

Bartel (2004). *Cell* 116: 281-97.