Effects of edible plant microRNAs on cancer cell proliferation: a beneficial cross-kingdom interaction

NETTAB & IB 2015, October 14-16
Joint NETTAB and Integrative Bioinformatics Meetings

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Introduction

Diet in human health is no longer simple nutrition but, in the light of recent findings, it might play a pivotal role on cell health status by modulating apoptosis, detoxification, and appropriate gene response to environmental stresses. Epidemiological studies suggest a role of fruits and vegetables in protection against several diseases, and nutrients have been demonstrated to alter gene expression by DNA methylation and histone modifications [1]. Diet has also been found to modulate micro RNA (miRNA) expression, leading to a subsequent regulation of the effectors genes. Furthermore, recent studies demonstrate that some plant/food-derived microRNAs (miRNAs) regulate gene expression in a sequence specific manner [2].

Aim

We have carried out a pilot study, using a combined *"in-silico and wet"* approach, to investigate the potential effects, and elucidate the molecular mechanisms of edible plant miRNAs on the expression of human genes involved in cancer onset and progression. This poster illustrates our approach and results obtained by transfecting 2 colon cancer cell lines, p53 wild type and p53 knock-out, with selected miRNAs of *G. max*, *Z. mais* and *M. truncatula, which we found, by in silico analysis, to* have a putative targeting activity on human oncogenes and tumor suppressor genes.

Bioinformatics analysis

1

- Bioinformatics analysis aiming to investigate at large the presence of target sites of plant miRNAs in human coding genes.

Non-redundant mature miRNA plant sequences (3,328), extracted from the miRBase database (Release 21), were used as query sequences for a blast analysis (NCBI blast C++ toolkit) against Ensembl human transcript sequences (Extracted by Biomart functionalities). "–task short" and similarity greater than 90% were used In blast analysis

4,619 putative target identified

Criteria:
- Match on the transcript: 16 bp (max number mismatches: 2-3) at miRNA 5'-end
- Perfect match of the seed
- Region minimum free energy hybridization (RNA hybrid analysis)

214 selected for experimental validation

2

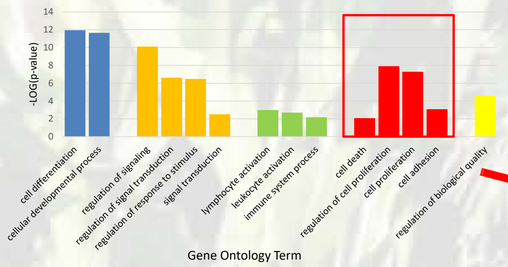
- Comparative analysis of human and plant miRNAs mature sequences to find the plant miRNAs that might mime endogenous miRNAs in human cells

Criteria used: :
-perfect match of seed sequences
- sequence identity greater than 15-16 nt starting from the miRNA 5'-end region
- mismatch permitted: max 1
- common target sites identified by MIRANDA

Plant miRNAs 18 ⇄ hsa miRNAs 16 (SEED MATCH)

1,054 target genes

GO gene enrichment analysis



3

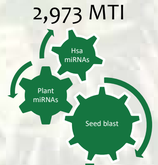
- Bioinformatics analysis to identify plant miRNAs for experimental validation

We used mirTarBase [3] as reference database for data extraction related to human miRNAs and miRNA-target interactions (MTIs) experimentally validated by reporter assay, western blot and RT-qPCR. The plant miRNAs with identical sequence to the seed of selected human miRNAs were used for the experimental approach in order to investigate their functional role on human target genes expression.

miRTarBase

Search for hsa MTI validated by:
- reporter assays
- Western Blot
- qRT-PCR

2,973 MTI



Edible plants

221 plant miRs ⇄ 106 hsa miRs

619 target genes

Gene Ontology Analysis

23 Key TGs in cell proliferation

7 plant miRNAs
(oncogenes and oncosuppressors)

Experimental validation

Experimental validation

Cell proliferation assays by MTT reduction

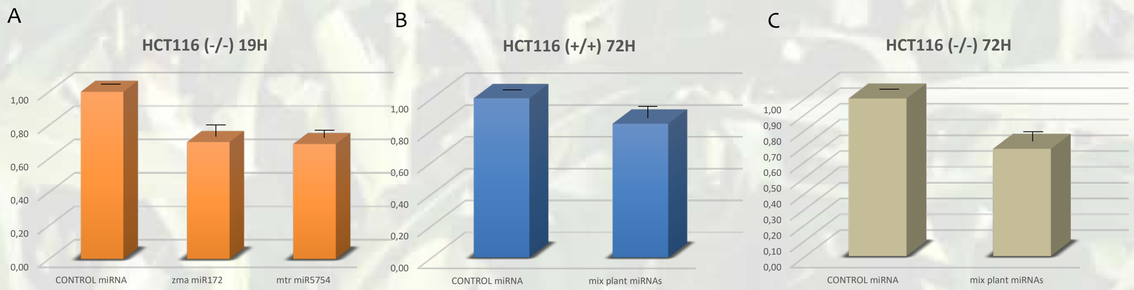
- Human colon cancer cell p53 wild type (HCT116 +/+) and p53 knock out (HCT116 -/-) were transfected with 150pmol of gma-miR-160, gma-miR-4995, gma-miR-4368, gma-miR-5677, gma-miR-4351, zma-miR-172, mtrmiR-5754, a mix of all miRNAs or control miR-mimic (Ambion) using SiPORT NeoFX Transfection Agent (Ambion). 18h and 72h later cells were collected for MTT assay or for RNA and protein extractions.

RNA extraction from cell lines

- Human RNA (mRNA component + miRNA component) was isolated using the RNeasy Plus Mini Kit (Qiagen). The RNA quality was assessed by a 2100 Bioanalyzer (Agilent). Reverse transcription of 10 ng of total RNA was performed using TaqMan MicroRNA RT Kit (Life Technologies). The exogenous miR-mimics expression levels were measured in triplicate by TaqMan MicroRNA Assay (Life Technologies) using the ABI PRISM 7900HT platform (Applied Biosystems®, Life TechnologiesTM) and normalized to snU6 expression.

Sequencing

- Total RNA and total miRNAs were sequenced using TruSeq Stranded RNA kit and TruSeq smallRNA kit according to the manufacturer's instructions (Illumina).



(A) The MTT proliferation assay demonstrated a significant cell proliferation reduction up to 30% of HCT116 p53-/- 19 h after the exogenous zma-miR-172 or mtr-miR-5754 transfection compared to the cells transfected with control miRNA. Moreover, respectively in HCT116 p53+/+ and HCT116 p53-/- cell lines was observed a 20% and 30% of cell proliferation reduction, 72 hours after the transfection of the mix of selected plant miRNAs, compared to the control cells (B-C).
On the basis of the positive results obtained by this pilot experimental validation, we are currently studying in the same cell lines, the effects produced on the entire transcriptome profile (coding and non-coding RNAs) to observe at large and clarify, direct and indirect effects of these plant miRNAs on human cell pathways related to cell proliferation in cancer cells.

1 Choi SW, Friso S (2010) Epigenetics: A New Bridge between Nutrition and Health. Adv Nutr 1(1):8-16. doi: 10.3945/an.110.1004. 2. Zhang L. et al. (2012) Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. Cell Research (2012) 22:107–126. doi:10.1038/cr.2011.158 3. Hsu SD et al. (2014) miRTarBase update 2014: an information resource for experimentally validated miRNA-target interactions. Nucleic Acids Res. 2014 Jan;42(Database issue):D78-85. doi: 10.1093/ nar /gkt1266.