Next-generation sequencing miRNA profiling in stool and plasma samples of patients with colorectal cancer or precancerous lesions

Gaetano Gallo1,2, Sonia Tarallo3, Giuseppe Clerico1, Francesca Cordero4, Barbara Pardini5, Giulio Ferrero6, Paolo Vineis1,2, Chiara Albertone1, Alberto Realis1, Alessio Naccaratii, Mario Trompetto1

1) Department of Colorectal Surgery, Clinica S. Rita, Vercelli, Italy
2) Department of General Surgery, University of Catanzaro, Italy
3) Human Genetics Foundation, HuGeF-Torino, Italy
4) Department of Computer Sciences, University of Turin, Italy
5) School of Public Health, Imperial College London, London, UK

INTRODUCTION

MicroRNAs (miRNAs) are small non-coding RNAs (18-25 nt in length) partially complementary to one or more messenger RNA (mRNA) molecules.

• Their main function is to downregulate gene expression.

Dysregulated miRNA expression has been identified in most human malignancies, including colorectal cancer (CRC), where more than 100 miRNAs have been implicated.

• The great potential shown by miRNAs in primary tissues indicate them as a novel class potential diagnostic/prognostic markers.

AIMS OF THE STUDY

In the present project, we investigate miRNA expression profiles in plasma and stool specimens of patients with newly diagnosed sporadic forms of CRC or with CRC precancerous lesions, e.g. colon adenomas and IBD, and healthy subjects recruited at the time of colonoscopy.

• The first hypothesis is that altered miRNA signatures, evaluated in surrogate specimens, could provide a powerful non-invasive and sensitive tool in the early detection of CRC.

• The second hypothesis is that diet and other related factors are involved via miRNA modulation in the cascade of events leading to carcinogenesis and this influence can be assessed also surrogate specimens.

PRELIMINARY STUDY

miRNA expression levels in stool and plasma: a pilot study on different dietary habits

Experimental Design:
We performed a comparison between microRNA expression among groups (n=24) of healthy volunteers with different diets: vegan, vegetarian and omnivorous.

Results:
• miR-92a was differentially expressed between the 3 groups, both in plasma (p<0.0001) and in stool (p= 0.014) samples.
• miR-92a was also inversely related to BMI in the whole group in both matrices (p<0.05).
• miR-92a is a key oncogenic component of the miR-17-92 cluster dysregulated in CRC.

PRELIMINARY RESULTS

miRNA sequencing in stool and plasma samples

While recruitment of samples is still ongoing, we have performed preliminary analyses on a subset of 48 subjects:

• Several differentially expressed miRNAs have emerged after correction for multiple testing comparisons among different categories (CRC, precancerous, inflammation, healthy).
• Interestingly, some miRNAs are repeatedly observed among different categories.
• Some miRNAs were previously observed in literature. Others are new.

METHODS

Next-Generation Sequencing (NGS)
• NGS is a powerful technology increasingly used for miRNA profiling, thanks to decreasing costs and high multiplexing capability.

• We have implemented for the first time this methods in stool samples.

• For plasma samples we investigate miRNAs in exosomes.

CONSIDERATIONS

• Diet and other lifestyle factors seem to influence miRNA expression in stool and plasma samples.
• Both plasma and stool can be used to evaluate miRNA expression levels in studies related to disease.
• Stool miRNAs analysed by NGS from us for the first time seem to provide reliable and comparable results to other specimens.

Study supported by Lega Italiana Per La Lotta Contro I Tumori (LILT - Trento).

REFERENCES