Determination of plasmatic microRNA levels by ddPCR as peripheral biomarkers for IDH-wild type glioblastomas: a pilot study.

Carmine Antonio Donofrio[1-2], Antonio Fioravanti[2], Lucia Riccio[2], Marika Cominetti[2], Maria Rosa Cappelletti[2], Daniele Generali[2], Ilaria Grossi[1], Giuseppina De Petro[1] and Alessandro Salvi[1]

[1] Laboratory of microRNAs, DNA methylation and epigenetics, Division of Biology and Genetics, Department of Molecular and Translational Medicine, University of Brescia, Viale Europa 11, Brescia, Italy (c.donofrio001@unibs.it).
[2] Neurosurgical Department, ASST Cremona, Viale Concordia 1, Cremona, Italy.

1. BACKGROUND

Glioblastoma (GBM) is the most frequent malignant brain tumour in adults with a dismal prognosis and peripheral biomarkers may be useful and effective in managing patients with GBM. The main aim of our study was the use of ddPCR to assess the absolute quantification of the plasmatic levels of three miRNAs as possible GBM-specific biomarkers. We focused on: **miR-21-5p**, an onco-miR overexpressed in blood, tumour tissue and cell cultures derived from patients affected by GBM, **miR-23b-3p** and **miR-34a-5p**, both tumour suppressor miRs dysregulated in GMB.

2. MATERIALS AND METHODS

Eight patients presenting with firstly-diagnosed IDH-wild type GBM and 10 age- and gender-matched healthy control donors (hC) have been enrolled in the study. Peripheral blood samples were collected at diagnosis and one month after surgery. Total RNA was isolated from plasma by means of miRNeasy Serum/Plasma Kit (Qiagen), according to the manufacturer's instructions. **Digital droplet PCR** (ddPCR) was performed to assess the absolute quantification of each miRNA level according to the QX200 ddPCR protocol.
Quantitative variables were expressed as means (± standard deviation) and bivariate comparisons of means were made using the Student's t test.

3. RESULTS

The expression analysis revealed:

i) different levels of each miRNA in hC: 98.74 copies/µL (±123.60), 2.56 copies/µL (±5.57), 0.73 copies/µL (±0.85) for miR-21-5p, miR-23b-3p and miR-34a-5p, respectively (**Figure 1**);

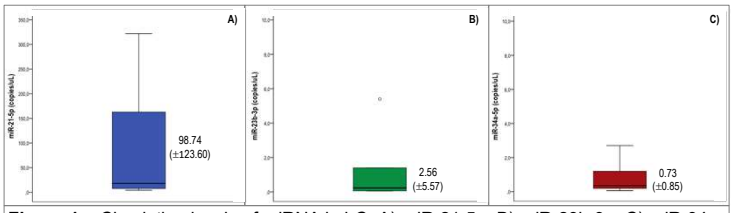

Figure 1 – Circulating levels of miRNA in hC: A) miR-21-5p; B) miR-23b-3p; C) miR-34a-5p.

i) a trend of down-regulation of miR-21-5p and miR-23b-3p in GMB patients at diagnosis compared to hC (**Figure 2**): 24.00 copies/µL (±28.44) compared to 98.74 copies/µL (±123.60) for miR-21-5p (p=0.093) and 0.49 copies/µL (±0.49) compared to 2.56 copies/µL (±5.57) for miR-23b-3p (p=0.272);

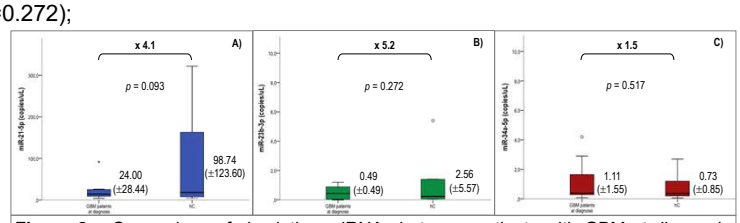

Figure 2 – Comparison of circulating miRNAs between patients with GBM at diagnosis and hC: A) miR-21-5p; B) miR-23b-3p; C) miR-34a-5p.

iii) a trend of up-regulation of each miRNA in GMB patients one month after surgery compared with the levels measured at diagnosis, in particular 3.0, 6.2 and 1.7 fold increase for miR-21-5p (24.00±28.44 vs. 72.40±147.88; p=0.379), miR-23b-3p (0.49±0.49 vs. 3.02±6.88; p=0.317) and miR-34a-5p (1.11±1.55 vs. 1.85±1.87; p=0.406), respectively (**Figure 3**).

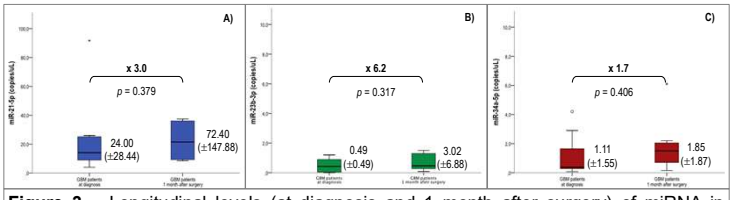

Figure 3 – Longitudinal levels (at diagnosis and 1 month after surgery) of miRNA in patients with GBM: A) miR-21-5p; B) miR-23b-3p; C) miR-34a-5p.

4. CONCLUSIONS

In this pilot study we reported:

i. lower amounts of plasmatic miR-21-5p and miR-23b-3p in patients with GBM compared to hC;
ii. higher amounts of circulating miR-21-5p, miR-23b-3p and miR-34a-5p in plasma of patients affected by IDH-wild type GBM one month after surgery compared to the levels at diagnosis.