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] Division of Biology and Genetics, Department of Molecular and Translational Medicine, University of Brescia, Viale Europa 11, 25123, Brescia, Italy

[2] Neurosurgical Department, ASST Cremona, Viale Concordia 1, 26100, Cremona, Italy

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, *tumour suppressor miRs* dysregulated in GMB.

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.

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; ii) a trend of downregulation of miR-21-5p and miR-23b-3p in GMB patients at diagnosis compared to hC; iii) a trend of upregulation of each miRNA in GMB patients one month after surgery compared with the levels measured at diagnosis, in particular 3.02, 6.2 and 1.7 fold increase for miR-21-5p, miR-23b-3p and miR-34a-5p, respectively.

CONCLUSIONS: In this pilot study we reported 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.