Set-up experiments for microRNAs characterization in plasma and circulating tumor cells derived from patients with cutaneous melanoma

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Malignant melanoma is among the most lethal of the cutaneous neoplasms with a 5-year survival of 6% and a median survival of 7.5 months for patients in the later stage of the disease (Luke and Ott, 2014). Acquired resistance may limit the therapeutic potential of currently used drugs (Tronnier et al., 2013, Shtivelman et al., 2014) and novel biomarkers may help clinicians tailor cancer treatments. The present study was aimed at the isolation and characterization of microRNAs (miRNAs) derived from plasma and circulating tumor cells (CTCs) as a non-invasive approach to investigate the changing patterns of drug susceptibility in individual patients. Twenty candidate biomarker miRNAs were selected among those that play a role in cell cycle, survival, proliferation and invasion (Segura et al., 2014). The study received the approval of the local Ethics Committee. Blood samples were collected from healthy volunteers and patients with melanoma at different disease stages (I-IV). Plasma miRNAs were isolated by miRNeasy Serum/Plasma Kit (Qiagen). Before isolating disseminated circulating tumor cells from melanoma patients, set-up experiments were preliminary carried out in samples enriched with cells derived from the A375 human melanoma cell line. We tested an immunomagnetic technique using the antibody mouse anti-MCSP (Melanoma Chonidroitin Sulfate Proteoglycane) conjugated to magnetic beads (CELLection Pan Mouse IgG Kita, Invitrogen) and a system based on the cell separation by density gradient centrifugation (OncoQuick, Greiner Bio-One, Germany). Although immunomagnetic method is commonly used for CTC isolation, we demonstrated by confocal microscopy that seeding dilutions of A375 into normal blood (10 cells per ml) resulted in consistent loss of cells during the immunobead procedure. This was probably due to a scarcity of tumor cells associated to an excessive cell stress during erythrocyte lysis and magnetic separation. At variance with this, cell separation by density gradient centrifugation allowed us to obtain a detectable A375 cells in samples containing only 4 cells per ml of blood (30 cells total). Quantification by Real-Time PCR of the expression levels of selected plasma-derived and CTC-associated miRNAs in patients with metastatic melanoma before and after drug treatment is in progress.

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