Indentification of five circulating microRNAs with high diagnostic values in cutaneous melanoma

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Melanoma is the fourth and sixth most common malignancy in men and women, respectively, and the 5-year survival rate critically depends on disease stage (American Cancer Society, 2016). MicroRNAs (miRNAs) are small, non-coding, single-stranded RNAs endogenously produced by the cells, which regulate the expression of hundreds of target genes. Circulating miRNAs actively secreted by tumor cells or released as the consequence of tumor cell death, have been proposed as potential biomarkers in cancer patients because of their stability in body fluids, resistance to endogenous RNase and constant expression in healthy individuals (Mitchell et al., 2008; Chen et al., 2008).

In the current study, the expression profiles of a selected panel of circulating miRNAs were analysed in plasma samples derived from patients and healthy donors to identify possible candidate biomarkers for diagnosis, prognosis and/or surveillance of human cutaneous melanoma. The study protocol was approved by local Ethics Committee and conducted in accordance to the principles of the Declaration of Helsinki. Blood samples were collected from melanoma patients (n=30) at different disease stages, and from healthy age- and sex-matched volunteers (n=32). Plasma miRNAs were isolated by miRNeasy Serum/Plasma Kit (Qiagen) and real-time PCR were carried out using miRCURY LNA^{TM} Universal RT microRNA PCR system (Exiqon, Denmark). Data analysis was carried out using two different strategies for normalization: Global Mean Normalization (GMN) and NormFinder model.

The GMN approach and NormFinder algorithm provided 13 and 7 significantly dysregulated miRNAs ($p<0.05$), respectively, and those that resulted significantly dysregulated after normalizations and the Bonferroni correction were selected. Circulating miR-15b-5p (p=1.34e-05), miR-149-3p (p=3.40e-12), and miR-150-5p (p=2.85e-12) were up-regulated, while miR-193a-3p (p=1.30e-06) and miR-524-5p (p=2.41e-05) were down-regulated in patients (regardless of disease stage) compared to healthy controls. Linear regression and following receiving operator curves (ROC) analyses were performed to evaluate the diagnostic value of these five selected miRNAs (i.e., the ability to discriminate between cases and controls). The area under ROC curve (AUCs) for individual miRNAs ranged from 0.801 to 0.951. Although the predictive power of all selected miRNAs was clearly demonstrated, miR-150-5p and miR-149-3p gave the best performance (AUCs of 0.9489, 95% CI from 0.8852 to 1.017 and 0.9510, 95% CI from 0.8852 to 1.017, respectively). Noteworthy, predictive performance was further improved when considering the double combination of miR-150-5p and miR-149-3p. The double classifier has indeed an increased area under ROC curve (AUC) of 0.966 (95% CI: 0.938–0.994) with 90% sensitivity, 68% specificity.

In conclusion, our findings identify 5 circulating miRNAs as potential biomarkers in human melanoma worthy of being validated in a prospective clinical study. Interestingly, the miR-149-3p and miR-150-5p signature showed a high capacity to discriminate between cases and controls. The high sensitivity of the combination can be therefore translated in a small percentage of false negatives and a high percentage of true positives results.