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Commentary

Circulating microRNAs as Candidate Biomarkers for the Surveillance of Melanoma Patients

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miRNAs are small non-coding RNAs affecting the expression of many genes and are regarded as crucial regulators involved in the fine-tuning of all cellular processes. Organ- and disease-specific miRNAs have been described. They are not only found within cells but also in body fluids, where they are stable and can easily be detected, e.g. by RNA-Seq or qPCR. Thus, miRNAs seem ideal biomarkers for diagnosis and prognosis of a variety of diseases, including cancer. This applies in particular to cell-free miRNAs from body fluids because they are readily accessible in a cost-effective way (see e.g., Schwarzenbach et al., 2014).

The study by Stark et al. (2015b), published in this issue of *EBioMedicine*, describes a panel of miRNAs as potential biomarkers in melanoma, for diagnosing early recurrence and for estimating survival. Diagnostic miRNA biomarkers might be particularly useful as a screening tool for developing metastatic lesions and for the follow up of stage III patients. Moreover, miRNA biomarkers with predictive value could be supportive in the clinical management of melanoma: e. g. patients with a low-risk score could be spared from aggressive adjuvant treatment and expensive imaging surveillance.

The authors started with the analysis of 17 miRNAs, the selection criterion being "high enrichment in melanoma": most of the included miRNAs were expressed at least 15-fold higher in a set of 55 melanoma cell lines compared to 34 other solid cancer cell lines (Stark et al., 2015a). When analyzing stage III and stage IV melanoma tissues, Stark and colleagues could identify miRNAs which were predictors of tumor stage, recurrence and overall survival. A subset of 7 miRNAs (MELmiR-7 panel) was later derived, which was particularly informative when analyzing the sera of melanoma patients. Within the MELmiR-7 panel, combinations of 4 or 5 (serum) miRNAs were identified which allowed for discrimination of different disease stages with high sensitivity and specificity and, importantly, with a better diagnostic score than currently applied serological tests based on LDH and S100B.

Intuitively, one might have expected that the expression of the "melanoma-enriched" miRNAs included in the analyzed panels would increase in tissue and serum as a function of melanoma progression. Intriguingly, however, the expression level of the majority of the miRNAs was lower, not higher, in the serum of melanoma patients compared to healthy controls and was also lower in stage IV versus stage III tissue samples. Possibly, the differentially expressed serum miRNAs do not derive from the tumor cells, but from other sources like blood cells or endothelial cells. As discussed by the authors, the observed decreased expression of miRNAs in the serum might also reflect a systemic response to the tumor, e. g. based on a different cytokine pattern elicited by the cancer cells and their microenvironment.

Another interesting finding was that the serum expression level of miR-211 in stage IV patients is indicative for their survival time, a high expression correlating with shorter survival. MiR-211 is a known lineage-specific miRNA under MITF control, and a high serum level in late-stage melanoma patients has been reported before (Margue et al., 2013; Margue et al., 2015; Saldanha et al., 2013; Stark et al., 2015a). Many of the 17 miRNAs analyzed in the present study had not been in the focus of previous studies describing melanoma-specific miRNA signatures with potential diagnostic and predictive value (e. g. Friedman et al., 2012; Greenberg et al., 2013; Saldanha et al., 2013). We recently analyzed a larger panel (88 serum miRNAs), selected on the basis of whole miRNome arrays of serum samples of melanoma patients and healthy controls (Margue et al., 2015). Also in our study, miR-211 was among the miRNAs which could be used to discriminate stage IV tumors from healthy serum while miR-16 was rather down-regulated in contrast to the up-regulation reported by Stark. However, miR-16 levels need to be interpreted with caution as it has been shown to be highly expressed in erythrocytes, therefore it may be considered a marker of hemolytic serum (Pritchard et al., 2012).

It will be important to see how well the results of the present study involving a sensitive method of detection and relatively large cohorts of patients and controls can be reproduced in other centers. As reviewed recently by Jarry et al. (2014), non-congruence between results of different studies on circulating miRNAs in oncology has been a frequent observation, due to the inherently low concentration of secreted miRNAs, the use of different platforms and many possible technological pitfalls. Similar to previous initiatives regarding qPCR (MIQE) and microarray analysis (MIAME), common guidelines regarding quality controls of the various pre- and post-analytical steps (for extraction methods, pre-amplification steps, normalization factors, possible blood cell contaminations, bioinformatic analysis, etc.) and the documentation thereof would greatly facilitate the definition and refinement of robust miRNA biomarkers for the benefit of patients with melanoma and other cancers.

The present publication rightfully underlines that a reasoning such as 'miRNAs released from the tumor reflect miRNA expression of

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the malignant cells' and therefore 'serum miRNAs reflect tumor burden' is certainly too simplistic. An area of ongoing investigation is the selective sorting of intracellular miRNAs into exosomes which are then released from the cells. It seems clear that this is not a passive process; consensus sequences within miRNAs might facilitate their secretion (Villarroya-Beltri et al., 2013). It can be envisaged that the process of miRNA secretion is regulated and that cancer cells might exploit this process to secrete or retain different miRNAs to gain selective growth advantages. Another fascinating field of research is the investigation of possible "messenger functions" of secreted miRNAs regarding metastatic spread or drug resistance.

The study by Stark and colleagues adds novel information to the miRNA biomarker field in melanoma. It will now be interesting to see in future cohorts how the MELmiR-7 panel will perform in the surveillance of stage III patients to allow for an earlier detection of tumor recurrence, so that adjuvant, systemic or targeted therapies can be offered at an earlier time point, for the benefit of the patients affected by this fatal disease.

Disclosure

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