Does the increase of exosomal microRNAs reflect an activated immune system in melanoma?

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Introduction

Cutaneous malignant melanoma is a form of skin cancer that accounts for 65% of skin cancer-related deaths[1]. The incidence increases continuously, and while early detection leads to nearly 100% survival rates, the mortality raises to greater than 80% for patients with advanced disease[2]. One of the new classes of potential cancer biomarkers are microRNAs.
MicroRNAs are non-coding RNAs that suppress the translation of their target mRNAs by binding to the 3' untranslated region[3]. On the one hand, melanoma-derived exosomes are discussed as vesicles for degradation of anti-tumor microRNAs. On the other hand, exosomal microRNAs might be active in recipient cells[4], e.g., by repressing anti-tumorigenic immune responses. To investigate these possibilities, we profiled the microRNA content of exosomes from melanoma cell lines and plasma of melanoma patients. Informed consent was collected according to guidelines for medical and research ethics.

1 Identification of microRNAs selectively exported via exosomes

We investigated the microRNA expression (Agilent Microarray, 8×60K human V16 miRNA) of four primary melanoma cell lines and their respective exosomes isolated from cell culture supernatant by ultracentrifugation. To detect microRNAs selectively exported via exosomes, we analyzed only microRNAs with a reliable mean signal intensity of at least 50 in exosomes. While most of the microRNAs were detected at similar levels in cells and their exosomes and ten with higher signal intensities in cells, we identified a set of five microRNAs (miR-142-5p, miR-144-3p, miR-150-5p, miR-223-3p, and miR-451a) with significantly higher signal intensities in exosomes compared to the originating cells (fig. 1A,B; $p < 0.01$). This indicates that these microRNAs were selectively exported via exosomes.
Transfection of the exosomal microRNA miR-451a into a melanoma cell line did not induce tumor-suppressive effects in these cells (data not shown).

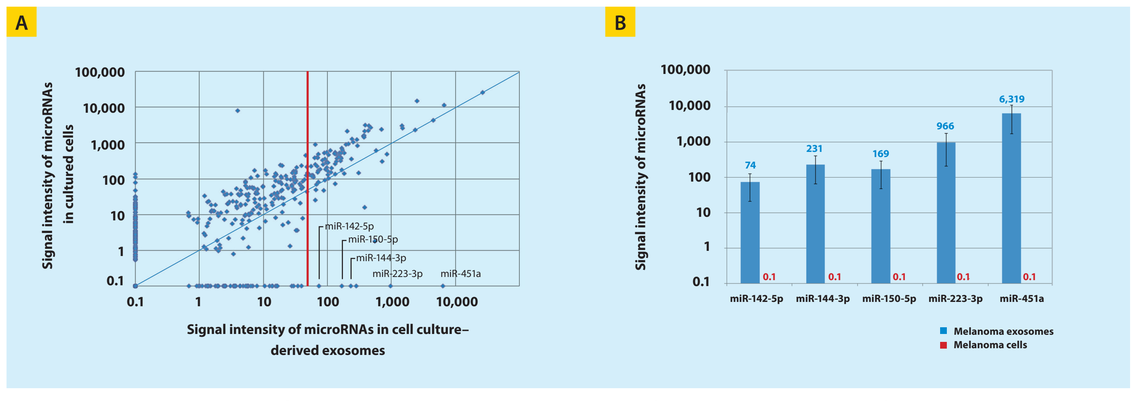

Figure 1: (A) Mean signal intensities of microRNAs in melanoma cell lines vs. microRNAs from the respective exosomes (four independent microarray experiments, non-normalized data). The red line indicates the threshold of a mean signal intensity of 50 in exosomes. (B) Signal intensities of five microRNAs with significantly higher signal intensities in exosomes compared to the originating cells ($p < 0.01$).

2 Exosomal microRNAs from cell culture are also detectable in melanoma plasma

Next, we compared melanoma cell culture–derived exosomes with plasma exosomes from twelve different melanoma patients. The selectively exported microRNAs (fig. 1) were also detected in the plasma exosomes from melanoma patients (indicated by red dots in fig. 2A). Unexpectedly, the microRNA profiles of plasma exosomes differed strongly from the cell culture exosome profiles (coefficient of variation >1). We also detected an additional 97 microRNAs in plasma exosomes compared to cell culture–derived exosomes (fig. 2B). This might be explained by a complex mixture of exosomes from distinct origins in plasma.

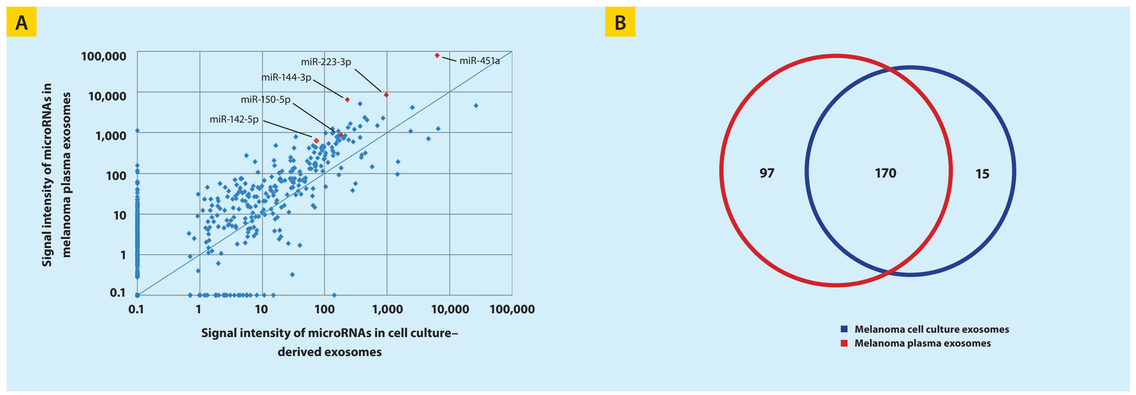

Figure 2: (A) Mean signal intensities of microRNAs in exosomes from melanoma plasma samples (n=12) vs. melanoma cell culture–derived exosomes (n = 4, non-normalized data) (B) Venn diagram representing the number of detected exosomal microRNAs from plasma of melanoma patients and melanoma cell culture, respectively.

3 The amount of exosomal microRNAs is increased in melanoma plasma

We next compared the exosomal microRNA profiles in plasma samples from 12 melanoma patients and 13 healthy donors. Strikingly, all detected microRNAs showed an increase in signal intensity in the samples from melanoma patients (fig. 3). The mean ratio of signal intensities from patient samples vs. samples from healthy donors amounted to 9.6. Although the mean ratio varied among the 12 patients, the signal increase across all microRNAs was highly significant ($p < 0.01$) in patients compared to healthy donors (fig. 3B). The increased amounts of microRNA was confirmed for selected microRNAs by qRT-PCR (data not shown). It has not been clarified yet whether the elevated microRNA levels might be a consequence of a higher exosome concentration or a higher exosome load in the plasma of those patients[5,6].

Surprisingly, the microRNAs that were shown to be selectively exported from melanoma cells via exosomes in culture (fig. 1) did not deviate from the mean signal increase in plasma samples from melanoma patients (fig. 3A). On average, plasma exosome microRNA profiles in samples from melanoma patients and healthy donors showed a minor variation (coefficient of variation <1). The signal intensity of single microRNAs varied strongly among the patients (104 out of 175 microRNAs with a coefficient of variation >1). Therefore, our microRNA analysis did not cluster melanoma patients and healthy donors (data not shown). A Significance Analysis for Microarrays (SAM) did not identify microRNAs that deviate from the mean signal increase across all microRNAs in melanoma patients vs. healthy donors.

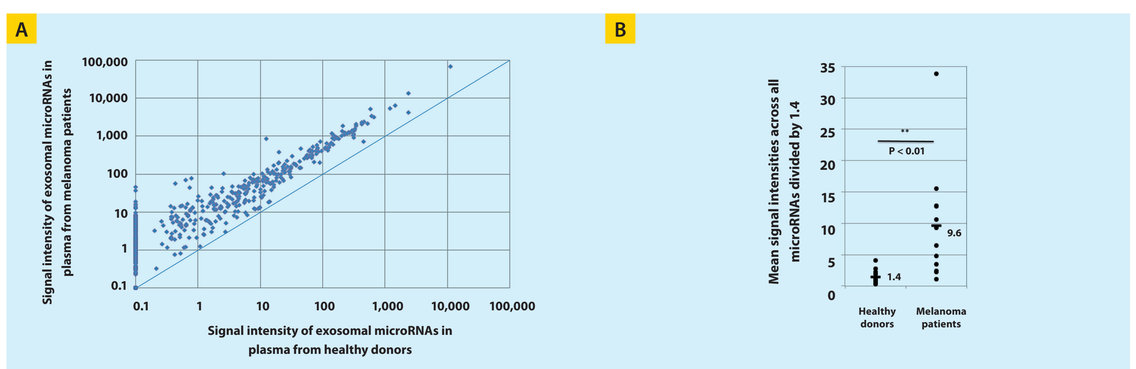

Figure 3: (A) Mean signal intensities of microRNAs in plasma exosomes from melanoma patients (n = 12) vs. healthy donors (n = 13; non-normalized data). (B) Data indicate the ratio defined by the mean signal intensities across all microRNAs in a single healthy donor (n = 13) or melanoma patient (n = 12) divided by the averaged mean signal intensities from all healthy donors (this average amounts to 1.4).

4 The exosomal microRNA level decreases after removal of dysplastic nevi

Two of the samples from supposedly healthy donors showed elevated exosomal microRNA levels as compared to the mean of the control samples. In the follow-up care, dysplastic nevi were diagnosed in these two donors. After surgery, the levels of exosomal microRNAs returned to normal in samples from both donors (fig. 4A,C vs. B,D).
In contrast, one patient showed an unremarkable exosomal microRNA level accompanied by widespread metastatic growth (data not shown). Since the microRNA profiles of patients differ from those of melanoma cell culture exosomes (fig. 2), we think that most plasma exosomes do not originate from the tumor. Instead, we propose an indirect effect: The increased exosomal microRNA level in plasma could be due to an immune response against the tumor, where tumor cells might induce an enhanced exosome production by immune cells (fig. 5).

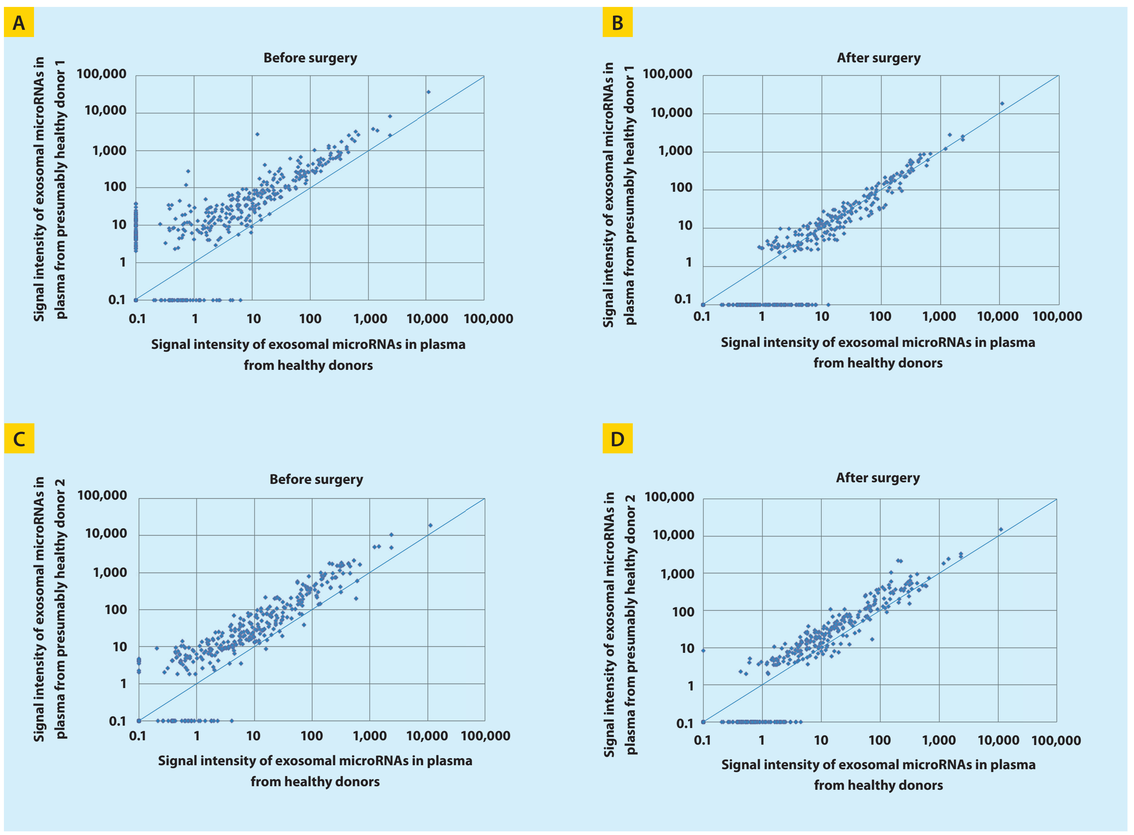

Figure 4: Mean signal intensities of microRNAs in plasma exosomes from 13 control samples from healthy donors vs. samples from two particular donors. (A,C) before and (B,D) two weeks after surgery (non-normalized data).

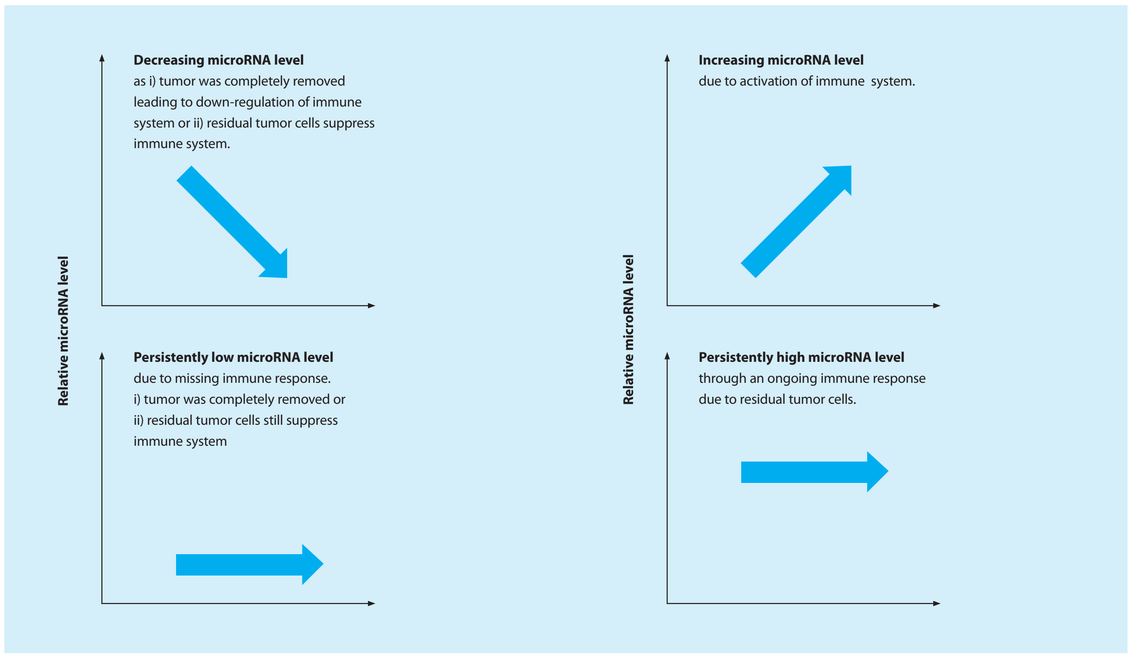

Figure 5: Hypothesis for trends of exosomal microRNA levels after surgery based on an interplay between immune system and tumor.

Conclusion

- Some microRNAs are selectively exported from primary melanoma cell lines via exosomes.
- We detected an additional 97 exosomal microRNAs in melanoma plasma compared to melanoma cell culture, reflecting a mixture of exosomes from distinct origins.
- The amount of exosomal microRNAs is significantly increased by 9.6-fold on average ($p < 0.01$) across all microRNAs in melanoma plasma indicating an increased amount of exosomes or a higher exosome load.[5,6]
- Elevated exosomal microRNA levels returned to normal in two patients after surgery of dysplastic nevi.

Outlook

An increase of the exosomal microRNA level in plasma could indicate an immune response against the tumor. In our future research, we aim to find out whether an increased microRNA level in plasma exosomes represents an anti-tumor response linked to a beneficial effect.

References
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