

Role of platelets in tumor immune modulation via horizontal RNA transfer

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Introduction

Platelets are small anucleated cells of the blood, mainly known for their function in hemostasis. Moreover, they play a crucial role in the immune response but also in cancer. Since platelets are known to form complexes and interact with tumor, endothelial and immune cells, it is assumed that platelets are comprehensive effectors in tumor progression and immunity.

Methodology

Platelet RNA profiling of 55 patients with HNSCC and 17 healthy individuals was done by RNA-sequencing. In vitro transfer of SYTO™ RNaselect™ Green labeled RNA from tumor cells to isolated platelets was studied by flow cytometry. Formation of leukocyte-platelet aggregates in whole blood was assessed by flow cytometry. Platelets were also transfected with eGFP-mRNA and after co-incubation with PBMCs the expression of eGFP in these recipient cells was evaluated by flow cytometry.

1. Differential platelet RNA profiles in HNSCC patients

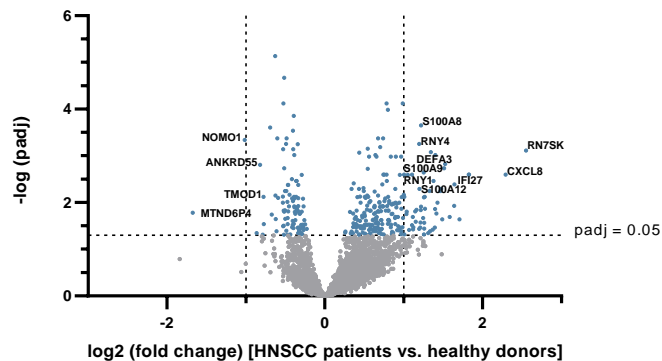


Fig. 1: Volcano plot showing the differential expression analysis ($padj < 0.05$) of platelet RNA from 55 HNSCC patients and 17 healthy individuals. In total we found 56 transcripts that were at least twice as abundant in HNSCC patients as in the healthy group.

2. HNSCC cells transfer RNA to platelets *in vitro*

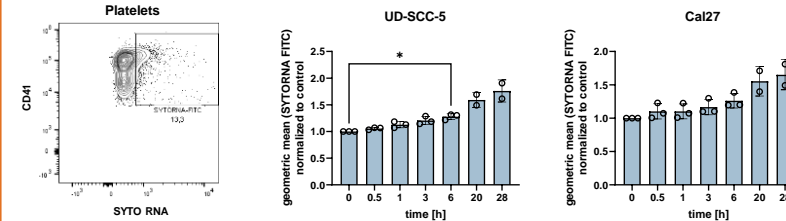


Fig. 2: RNA of HNSCC cell lines was stained with SYTO™ RNaselect™ Green. Following a wash step, platelets were co-incubated with those cells and analyzed for green fluorescence after several time points via flow cytometry. **Transfer of labeled RNA to a fraction of platelets was observable.**

3. Leukocyte-platelet-aggregates in whole blood

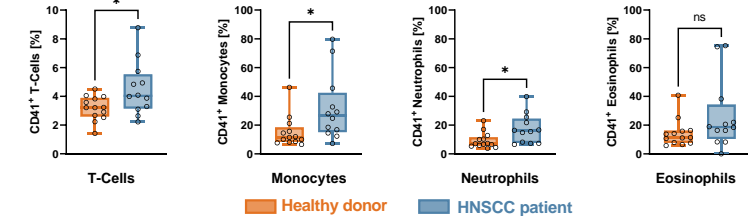


Fig. 3: Whole blood was drawn from healthy donors ($n=13$) and HNSCC patients ($n=12$) and immediately stained to analyze the prevalence of aggregates between different subsets of leukocytes (e.g. CD3, CD14) and platelets (CD41) via flow cytometry. **In HNSCC patients a slight enrichment of leukocyte-platelet-aggregates - especially monocytes - was detected.**

4. Functional RNA transfer from platelets to PBMCs

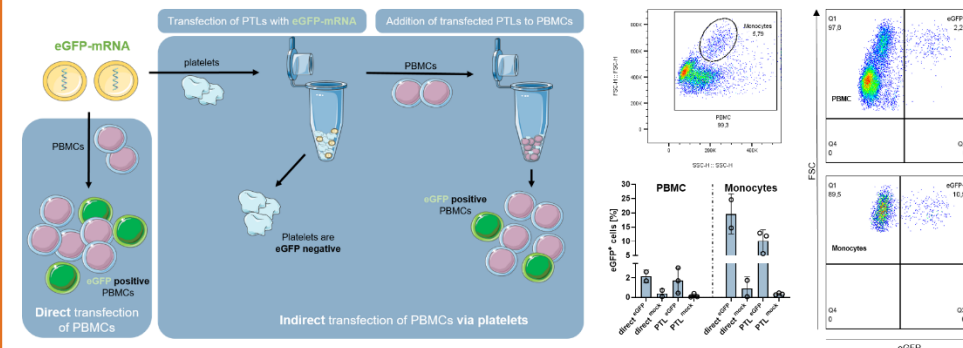


Fig. 4: To study the potentially functional transfer of RNA from platelets to recipient cells, platelets were transfected with mRNA coding for eGFP. After a washing step, those transfected platelets were co-incubated with isolated PBMCs for 24 hours (PTL eGFP). As control, PBMCs were also transfected directly with mRNA for eGFP (direct eGFP). On average, **transfection resulted in a fraction of 2.1% and 1.7% eGFP-positive cells for direct or indirect transfection, respectively.** With respect to the subpopulation of monocytes, the eGFP-positive fraction was even more pronounced with 19.7% and 10.2% for direct and indirect transfection. Importantly, transfected platelets alone did not show eGFP expression (data not shown), **indicating a transfer of functional mRNA and not eGFP protein.**

Conclusion

Platelets of HNSCC patients do show a differential RNA profile compared to healthy individuals (Fig. 1). The presented data points to a direct transfer of RNA from cancer cells to platelets, which could contribute to this finding (Fig. 2). Platelets not only are recipient of mRNA but also are able to pass it on to other cells, where it is actually used for protein synthesis. This could be shown especially for monocytes (Fig. 4). Given the enriched leukocyte-platelet aggregates in HNSCC patients (Fig. 3), this might implicate a role of platelets in tumor immune modulation via horizontal RNA transfer.

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