CHARTING EXTRACELLULAR TRANSCRIPTOMES IN THE HUMAN BIOFLUID RNA ATLAS

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Introduction

Extracellular RNAs in biofluids have emerged as potential biomarkers for disease. Where most studies focus on plasma or serum, other biofluids may contain more informative RNA molecules, depending on the type of disease. Here, we present an unprecedented atlas of messenger, circular and small RNA transcriptomes of a comprehensive collection of 20 human biofluids[1]. By means of synthetic spike-in controls, we compared RNA content across biofluids, revealing a more than 10 000-fold difference in RNA concentration. The circular RNA fraction is increased in nearly all biofluids compared to tissues. Each biofluid transcriptome is enriched for RNA molecules derived from specific tissues and cell types. In addition, a subset of biofluids, including stool, sweat, saliva and sputum, contains high levels of bacterial RNAs. Our atlas enables a more informed selection of the most relevant biofluid to monitor particular diseases. To verify the biomarker potential in these biofluids, four validation cohorts representing a broad spectrum of diseases were profiled, revealing numerous differential RNAs between case and control subjects. Taken together, our results reveal novel insights in the RNA content of human biofluids and may serve as a valuable resource for future biomarker studies.



Study flowchart

Abbreviations AqF: aqueous fluid; BAL: bronchial lavage fluid; CSF: cerebrospinal fluid; mRNA: miscellaneous RNA; piwi-interacting RNA; piwi-interacting RNA; piwi-interacting RNA; piwi-interacting RNA; piwi-interacting RNA; miscellaneous RNA; miscellaneou References

[1] Hulstaert et al, 2019, BioRxiv; [2] Simon et al, GigaScience, 2018; [3] Rozowsky et al, Cell Syst, 2019; [4] Lorenzi et al, bioRxiv, 2019 [5] Baron et al, Cell Syst, 2016

Results

More than 1000-fold difference in RNA concentrations amongst different biofluids



biofluid	number of mRNAs	number of miRNAs
amniotic fluid	10 531	119
aqueous humor	107	20
ascites	5578	75
BAL	3565	126
bile	2279	45
breastmilk	11 607	213
colostrum	11 914	229
CSF	438	32
gastric fluid	9288	21
pancreatic cyst fluid	13 722	129
PFP	2699	95
PPP	4548	113
PRP	5440	192
saliva	6353	110
seminal plasma	11 868	211
serum	4152	122
sputum	7738	91
stool	134	19
stool Calex	135	18
sweat	410	45
synovial fluid	1614	122
tears	13 366	231
urine	2094	41

Fig 1. (A) Relative RNA content per biofluid. Every bar represents the mean relative RNA concentration per biofluid. (B) Correlation between the relative small RNA and the mRNA concentration. The Spearman correlation coefficient is 0.83 (p-value = 1.76 x 10⁻⁶). (C) The number of mRNAs and miRNAs with at least 4 unique read counts in both replicates is shown per biofluid. Note that the results are impacted by the sequencing depth (average sequencing depth of 11 million read pairs for mRNA capture sequencing; 9 million read pairs for small RNA sequencing).

Stool and saliva contain a high fraction of reads mapping to bacterial genomes





Biomarker potential of mRNA in sputum, urine and CSF

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