The Molecular Stethoscope: RNA of Drug Metabolising Enzymes in Circulating Vesicles Correlates with their Specific Protein Content in the Liver

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SUMMARY

Background. Precision dosing aims to deliver the right dose of a drug for a specific patient. Multi-'omics' can facilitate generating individualized data by establishing a link between an organ, such as liver, and a minimally-invasive biopsy, e.g. plasma.

Results. Key drug-metabolising enzymes were assessed in non-cancerous liver samples from cancer patients (n=9) and controls (n=5). RNA transcripts (~21,000) were analysed in matched plasma (enzymes and liver specific markers). Exosome shedding (measured as novel liver shedding correction factor, LSCF) was higher and more variable in cancer patients than healthy controls; $LSCF_{(cancer)}$=26.08±19.63 reads per million (rpm), n=9; $LSCF_{(healthy)}$=0.83±0.26 rpm, n=5; *t*-test $p<0.01$). Tissue protein and LSCF-corrected plasma RNA levels were assessed for correlation; major drug-metabolising enzymes were significantly correlated between plasma (RNA) and liver (protein); CYP3A4, CYP2C9, CYP1A2 and CYP2A6.

Conclusions. A liquid biopsy test based on plasma to determine liver content of key enzymes was established with potential applications in pharmacology.

INTRODUCTION

Cytochrome P450 enzymes are responsible for the direct metabolism of >75% of therapeutic drugs [1]. Precision dosing aims to deliver the right drug dose to a specific patient based on individual characteristics. Multi-'omics' and 'liquid biopsy' assays are expected to facilitate the use of precision dosing by linking an organ, such as the liver (site of drug metabolism) to a minimally-invasive biopsy, e.g. blood.

METHODS

Fig. 1 shows a schematic of the applied methodology for both RNA and protein analysis.

Samples. Liver tissue (20-250 mg) and plasma (1-3 ml) samples from the same cancer patients (n=9) were assessed; healthy controls (n=5) were used as baseline.

Protein and RNA quantification. In-house QconCAT [2] methodology was used for protein quantification on nanoHPLC-Orbitrap Elite system (Thermo) with an inclusion list focusing on drug-metabolising enzymes (CYP1A2, 2A6, 2C9, and 3A4). Next generation sequencing (NGS) followed Ampliseq workflow [3] performed at Life Technologies (Thermo, Texas) at a depth of 8 million reads/ml plasma; transcripts for liver enzymes (as above) and markers (A1BG, AHSG, ALB, APOA2, CFHR2, F2, F9, HPX, SPP2, TF, C9, MBL2, SERPINC1, FGB) were extracted.

Data analysis. Expression levels of drug-metabolising enzymes were normalized using a novel liver specific shedding correction factor (LSCF), incorporating a combination of 13 liver specific marker genes in plasma. Appraisal of the marker genes using in-house data and data from the Human Protein Atlas (HPA) database is shown in **Table 1**.

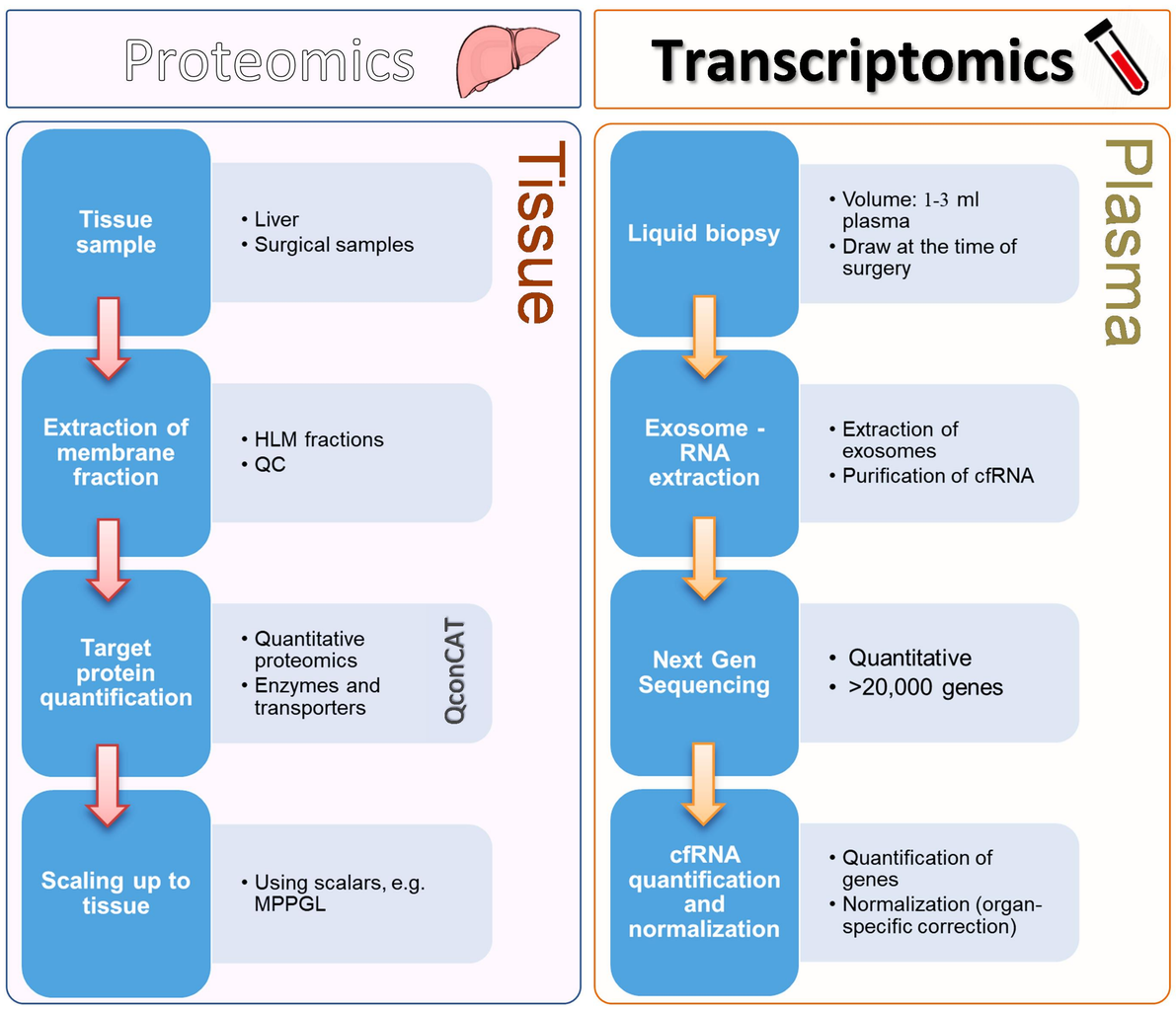

Fig. 1: The proteomic (liver tissue) and transcriptomic (plasma) workflows followed in this study; QconCAT and Ampliseq NGS were used, respectively. cfRNA, cell-free RNA; HLM, human liver microsomes; MPPGL, microsomal protein per gram liver; QC, quality controls.

Table 1: Appraisal of liver specific marker genes used to measure shedding into the blood.

Marker	APOA2	FGB	AHSG	HPX	SERPINC1	F2	CFHR2	F9	SPP2	MBL2	ALB	A1BG	TF	C9
Specificity score (fold) [a]	1068	556	1903	506	670	1472	4661	2258	2145	1414	81	180	12	577
Liver expression [b]	++++	++++	+++	+++	+++	+++	+++	+++	++	++	+++	++	++	++
Detection in plasma (%, n=29)[c]	100	95	97	84	90	87	95	87	74	71	100	84	92	76

[a] *Specificity score is the fold ratio of expression in a particular organ (liver) relative to the organ with the second highest level;* [b] *Tissue expression level: each + sign indicates one order of magnitude;* [c] *Consistency in the detection of marker genes in plasma as % of n = 29 (in house set)*

RESULTS

Exosomal shedding into the blood

The schematic in **Fig. 2 A** explains the concept of RNA shedding from liver. Levels of shedding were drastically higher in cancer patients with higher levels of variability relative to controls (**Fig. 2 B**). This highlights that variability in circulating RNA has two components: variability in expression and variability in shedding, hence the need for correction for variability in shedding before comparing expression levels.

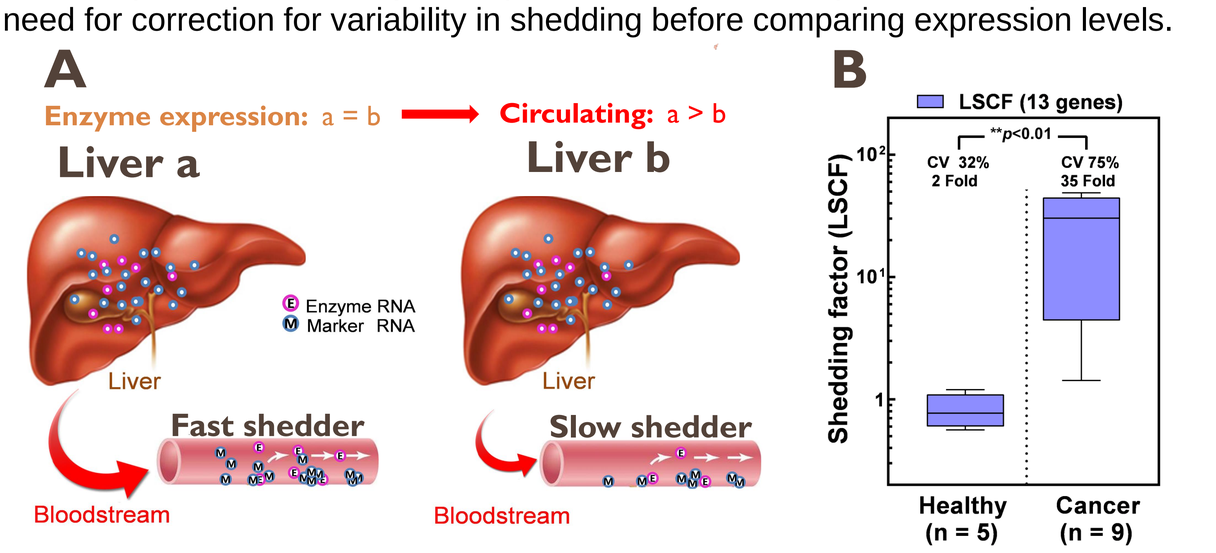

Fig. 2: The concept of exosomal shedding (A) and its measurement using liver specific marker genes. Livers 'a' and 'b' express enzymes 'E' at the same level but shed them in exosomes at different levels. Liver shedding correction factor (LSCF) is computed using 13 liver specific marker 'M' genes.

Correlation between liver enzymes and plasma circulating RNA

Fig. 3 shows liver protein-plasma RNA correlation after normalization. **Fig. 4** demonstrates the effect of normalization on correlation of CYP3A4 as an example.

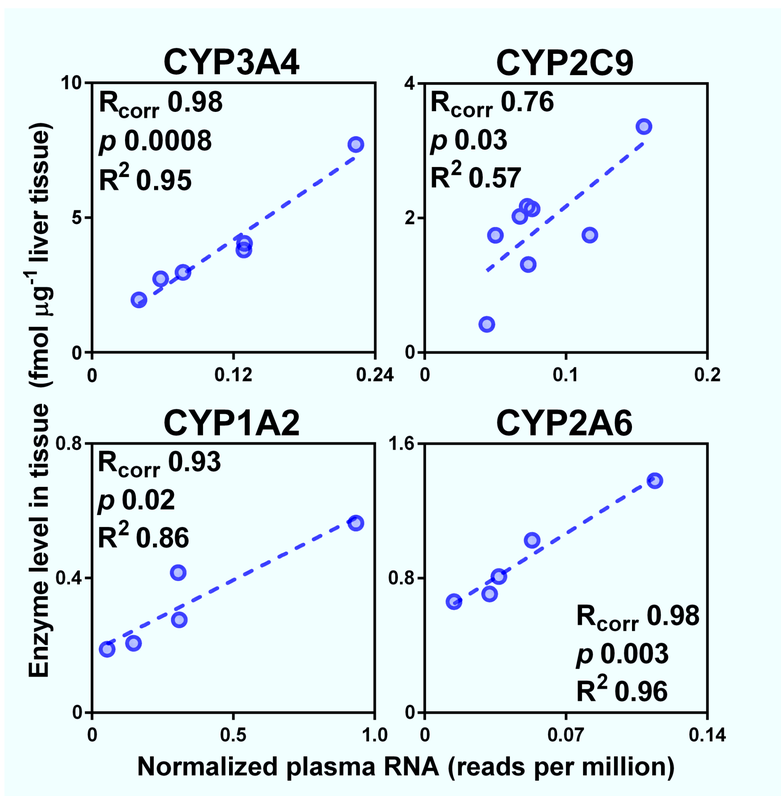

Fig. 3: Correlation between tissue levels of enzymes and RNA levels in plasma after normalization for shedding.

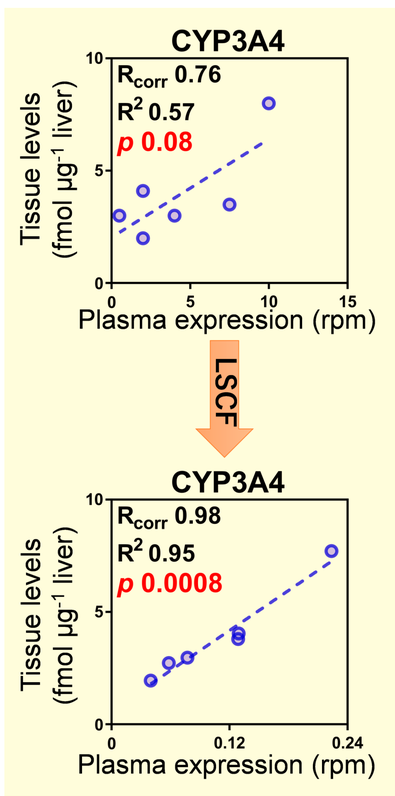

Fig. 4: Correlation before and after correction for shedding (CYP3A4)

CONCLUSIONS

A liquid biopsy test based on plasma samples to determine liver expression of key enzymes was established; this is relevant to: (a) enzymes predominantly expressed in liver and (b) shed into the blood. Correction for shedding variability improved correlations and enabled linking tissue protein content with plasma RNA levels. An application of this technique was demonstrated recently in pharmacology [4]. This technology should facilitate efforts towards precision dosing, an essential element of precision medicine.

References

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