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Biomarker validation is still the bottleneck in biomarker research

Dear Sir,

In an otherwise excellent review of P4 medicine, Tian *et al.* [1] claim that the selected reaction monitoring (SRM) assay, performed on a triple/quadruple mass spectrometer, enables efficient and specific detection and quantification of potential protein biomarkers in patient tumour tissues and blood samples. They further speculate that biomarker validation time is no longer such a significant issue. These statements are not accurate, and biomarker validation is still the bottleneck for bringing new biomarkers to the clinic. Here are the reasons: it is true that SRM assays can now be designed easily, for just about any human protein, through selection of proteotypic peptides from the SRM Atlas database and that hundreds of proteins can be quantified in multiplexed assays, as we have also demonstrated recently [2, 3]. The difficulty arises when such assays are applied to complex clinical samples such as serum. Because of the presence in serum of very high-abundance proteins (such as albumin and many others), and the expected very low abundance of informative disease biomarkers in serum, direct analysis of such low-abundance proteins by SRM, after sample trypsinization, becomes a major issue. It is possible to quantify in serum, by ELISA, biomarkers such as PSA, down to 0.001 ng/ml [4] but only down to 300 ng mL^{-1} in unfractionated serum by SRM [5]; a mere 300 000-fold difference in sensitivity. Even with PSA enrichment by antibody affinity chromatography, the sensitivity difference is 1000-fold [5]. Some newer affinity purification strategies may be promising, if coupled to SRM, but they are currently more complex, time-consuming and lower throughput than ELISA [6].

In conclusion, the major advantages of SRM assays (multiplexing and access to all proteins, without any need for specific reagents) are well described (something coined as democratization of all proteins, or protein assays for all) in the aforementioned review [1]. However, this is only a dream at present because, for the reasons mentioned earlier, SRM could not quantify proteins in complex mixtures such as serum at the levels necessary for disease diagnostics, or biomarker validation. It is hoped that further advances in sample preparation and mass spectrometry will make this possibility feasible in the future.

Conflict of interest

No conflict of interest was declared.

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