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Editorial: Is immunochemistry sufficient for protein detection, characterisation and identification?

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In the vast majority of studies, results exclusively rely on immunochemical data including immunoblotting, ELISAs, immunohistochemistry, to name a few. For this purpose not only monoclonal but also polyclonal antibodies were used. Therefore the outcome is based on fidelity of specificity and represents ''immunoreactivity''. In many cases antibodies are commercially available and were generated based upon a small peptide used as an immunogen and the control carried out is to run e.g. western blots with preincubation of the antibody with this peptide thus going in circles. Very often the antibody is generated by using recombinant protein expressed in prokaryotes for the immunisation, taking into account that E. coli has a different codon usage and no copy editing. Another major problem is ignored very often, i.e. the relevance of posttranslational modifications and splicing variants.

In our experience a mixture of many splicing variants are represented by a single band and therefore are detected and eventually being quantified together and shifts of individual splicing form variants are not considered. It is well-known and documented that antibodies directed against a given protein do not detect its posttranslational modification as e.g. phosphorylation, glycosylation, etc. and this is true also the other way around. One may add here a large series of confounding factors and in my opinion, work on proteins should be confirmed by protein chemical methods, as MALDI-TOF, MALDI-TOF-TOF, Q-TOF, LC-MS, LC-MS/MS.

Of course this kind of verification is limiting protein research work and makes it much more expensive and time consuming, but this price must be accepted for the sake of specificity and solid results.

With the rapidly growing proteomics techniques and laboratories getting more and more equipped with mass spectrometry instrumentation, this aim will be followed almost automatically. Of course, there are also limitations and pitfalls in proteomic technology but these can be handled by the experts (Fountoulakis, 2001; Lubec et al., 2003).

In ''Amino Acids'' a series is being started to regularly publish short communications about unambiguous identification of proteins and this is an invitation to contribute. Protein identification will also be a main subject at the forthcoming International Congress on Amino Acids and Proteins, August 8^{th}–12^{th}, 2005 in Vienna.

References

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