Opinion

Pharmaceutical BIOPROCESSING

Characterization and validation of antibodies

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Antibody characterization and validation are often considered parallel requirements which go hand in hand in determining the properties governing the utilization of antibodies in several applications.

Characterization encompasses the essential attributes which are the core information for any antibody, namely the character of the antibody molecule and therefore the specific preparation during which it's being supplied (serum, purified IgG, affinity-purified, etc.), knowledge of its binding specificity (identity of the target recognized at both the entire molecule and epitope level), cross-reactivity (identity of non-target reactants and therefore the extent of off-target binding), affinity binding constant (both equilibrium and kinetic parameters), the antibody sequence and ultimately its combining site structure when complexed with the target. Typical characterization methods include enzyme-linked immunosorbent assays (ELISA, target reactivity), surface Plasmon resonance (SPR, affinity determination), peptide arrays (epitope mapping), protein arrays (specificity screens), variable (V)-gene cloning (sequencing) and X-ray crystallography (native and complexed structure determinations).

Validation extends these properties to the criterion of 'suitability for particular applications', which within the case of antibodies are legion and include immunoprecipitation (IP), western blotting (WB), sandwich assays, immunohistochemistry (IHC), immunocytochemistry (ICC), flow cytometry, proximity ligation, intracellular and in vivo action, and lots of others. On the face of it, the high failure rate among antibody reagents in these techniques could seem surprising: the main distinction between native, fixed and denatured protein structures is documented, requiring appropriate reagents for those categories, but within them a reliable outcome with a target-specific binder would, perhaps naively, be anticipated. If that were the case, only three sorts of test would be needed, IP, IHC/ICC and WB. However, applications are increasingly specialized in order that other considerations (e.g. how precisely the sample is fixed or denatured, the composition and complexity of the sample, incubation conditions, etc.) can moderate antibody functionality. Moreover, target proteins of an equivalent primary sequence may exhibit subtle cell- or tissue-specific differences which may alter their conformations and their epitopes, like post-translational modifications (PTMs, e.g. glycosylation, phosphorylation), interacting proteins, etc. As antibodies are themselves proteins, their conformational diversity and PTMs also can impact their binding characteristics, whether produced recombinant or from natural sources. Thus, specificity, the foremost familiar property of antibodies, and its thorough and exact delineation for each case, seems to be a serious a part of the matter. Validation of specificity for the 'real world' applications during which it'll be used, namely against the target expressed at endogenous levels in cells and tissues, is that the ultimate consideration that, from a practical standpoint, exceeds simple characterization performed against one or set of purified or exogenously overexpressed targets.

We provide one example of a dataset showing application-specific differences in validation of a sizeable collection of target-specific monoclonal antibodies (mAbs). During this case, 96 mAbs were selected on the idea of overall immunoreactivity in ELISA, either against cells over-expressing the target protein or purified target protein. This complete set of

ELISA-positive mAbs was then assayed for efficacy and specificity in distinct applications in native brain tissue samples, namely WB, IHC and specialized IHC within the sort of plastic embedded sections prepared for Array Tomography. These results underscore that distinct mAbs (or for that matter the other binder type) could also be suitable for a specific assay, but unsuitable for an additional even highly related assay, which validation must be performed for every intended purpose. They also speak to the necessity for transparent reporting of the precise nature of any prior antibody validation, enabling it to be thoughtfully evaluated in reference to the user's needs.

These issues aren't restricted to antibodies but are equally applicable to non-antibody protein binding molecules, like DARPins, aptamers, monobodies, affimers and other molecular entities, all of which exert an impact through their ability to bind to a protein target. Moreover, they hold true the maximum amount for binders made through recombinant technologies as for the classical pAbs and mAbs. Purified pAbs often have excellent monospecificity, especially when affinity purified, and within the Human Protein Atlas validation results for an outsized number are presented transparently. While in theory mAbs have the capacity to be more specific, they often exhibit entirely unexpected strong cross-reactions, where an epitope aside from that intended fortuitously interacts well with the combining site. Although this has the potential to be amplified in polyclonal preparations, during which the constituent antibodies could each exhibit distinct properties, cross-reactivity during a pAb could also be diluted call at many cases by being a mixed population, while reactivity with the target is common to all or any its components. In some cases, mAb cross-reactivity has been analyzed by X-ray crystallography; indeed, it's possible to pick for useful bispecific interactions within the same combining site. It's perhaps too early to know fully the specificity characteristics of the non-antibody binders where a smaller range has been produced, except for which many of an equivalent principles will likely hold.