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Reliability of G-Protein-Coupled Receptor Antibodies

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Editorial

Antibodies to 19 subtypes of 1 and -adrenoceptors, muscarinic, dopamine, and galanin receptors, as well as vanilloid (TRPV1) receptors, were shown to be non-selective. These findings show that for antibodies against G-protein-coupled and maybe other receptors, lack of selectivity appears to be the rule rather than the exception. As a result, the formerly widely used validation of such antibodies by the absence of staining in the presence of blocking peptide, i.e. the antigen against which the antibody was generated, is no longer sufficient to confirm specificity. We propose that receptor antibodies be validated using at least one of the following techniques like disappearance of staining in knock-out animals of the target receptor, reduction of staining upon knock-down approaches such as siRNA treatment, selectivity of staining in immunoblots or immunocytochemistry for the target receptor vs. related subtypes when expressed in the same cell line, and/or antibodies raised against multiple distinct epitopes of a receptor yielding venomous antibodies. Other factors to consider while using receptor antibodies in applications like immunohistochemistry and immunoblotting are also mentioned. GPCRs (G-protein-coupled receptors) are key biological regulators. They can be broken down into classes, families, and subtypes. Determining which GPCR subtype is expressed in a specific cell is typically required when assigning specific activities to a GPCR. Because GPCR mRNA and protein expression at the cell surface do not always coincide, protein detection is frequently required. GPCR protein identification can also be used to determine which cell types within a tissue express a certain receptor and/or where subcellular component receptors are found. Furthermore, the density of GPCR expression at the cell surface is dynamically regulated by a wide range of physiological and pathological circumstances, as well as medication therapy. A deeper understanding of such control frequently necessitates precise quantification of protein expression. While antibodies against genetically engineered receptors, such as HA- or His-tagged receptors, can easily be detected, this is not the case for natural receptors. As a result, particular radioligands, otherwise labelled ligands, or antibodies are commonly used to identify native GPCR protein. Because lack of selectivity appears to be the rule rather than the exception when it comes to GPCR antibodies, the scientific community should evaluate what criteria should be used to determine whether an antibody has been validated for its claimed cognate receptor. To start the conversation, we suggest four criteria, at least one of which should be met as reasonable evidence for selection. This will become an editorial policy for all publications that use GPCR antibodies, at least for this Journal. We also believe that antibodies against targets other than GPCR should be subjected to the same rigorous validation procedures. Finally, the multiple studies in this issue of the journal suggest that receptor antibody selectivity is not a problem limited to one or two commercial providers, but rather appears to affect all antibody sources, both commercial and academic. Because the data in this issue of the journal raises more concerns than it answers, the journal invites academic and commercial antibody providers to submit scientific comments on ways for improving antibody selectivity and/or criteria to demonstrate such selectivity.

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