J. Cell. Mol. Med. Vol 11, No 6, 2007 pp. 1419

Blocking endogenous peroxidases: a cautionary note for immunohistochemistry

Dear Editor,

In two recent JCMM papers dealing with the staining of various human tissue antigens, divergent approaches for blocking endogenous peroxidase activity were reported. As such, Gray *et al.* [1] inhibited this intrinsic enzyme with a 0.9% hydrogen peroxide (H_2O_2) solution whereas Ranieri *et al.* [2] performed the blockade with a 3% H_2O_2 solution. This arbitrariness has prompted our present letter.

Immunohistochemistry (IHC) applied to formalin-fixed, paraffin-embedded tissue samples is governed by standard operating procedures like any other methodology in biomedicine. One important milestone in IHC standardization has been the revelation of endogenous biotin as a confounding factor along with measures to minimize its contribution to causing staining errors [3–5]. However, IHC pitfalls can also have other origins such as the insufficient quenching of endogenous peroxidases whenever using peroxidase-based detection methods. Despite the existence of expert manuals according to which these enzymes should be inhibited by applying a solution containing 3% H_2O_2 [6] and studies abiding by this recommendation [2, 5, 7–9], some research groups still publish staining approaches in which blockade has erroneously been performed with less concentrated H_2O_2 solutions, e.g. by means of a 0.9% [1] or even 0.3% [10] solution. In this context, it was already shown that, for instance, when endogenous peroxidases were blocked by means of a 0.3% H_2O_2 solution a breast carcinoma was (falsely) positive for a particular protein, yet when blockade was performed by employing a 3% H_2O_2 solution, the same specimen stained negatively for that antigen [9]. It should be emphasized that all other conditions including primary antibody concentrations were identical in the two experimental arms at a given incubation temperature [9].

Therefore, the purpose of this note is to alert investigators to henceforth implement established techniques to avoid this mistake in order to prevent the emergence of staining artifacts associated with falsely positive IHC results and consequently augment the comparability of data generated in different laboratories on the same subject of interest. Given the increasing importance of proteomics and (intracellular) protein biomarkers for the diagnosis of human disease, appropriate countermeasures to avert this immunohistochemical pitfall should have particular relevance.

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doi: 10.1111/j.1582-4934.2007.00185.x