0022-1554/92/$3.30
The Journal of Histochemistry and Cytochemistry

Vol. 40, No. 8, pp. 1121–1128, 1992
Printed in U.S.A.

Original Article

Simultaneous Immunostaining Method for Localization of Bromodeoxyuridine and Calcitonin Gene-related Peptide[1]

LUIS M. MONTUENGA, DAVID R. SPRINGALL,[2] JULLIEN GAER, JOHN T. McBRIDE, and JULIA M. POLAK

Departments of Histochemistry (LMM,DRS,JTM,JMP) and Surgery (JG), Royal Postgraduate Medical School, London W12 ONN, United Kingdom.

Received for publication October 7, 1991 and in revised form March 11, 1992; accepted March 31, 1992 (1A2474).

A new simultaneous double immunostaining method has been optimized to localize the DNA synthesis marker bromodeoxyuridine (BrdU) and calcitonin gene-related peptide (CGRP) in endocrine cells of Bouin's-fixed, paraffin-embedded rat lung. Nuclease pre-treatment before immunostaining is compatible with optimal tissue morphology and CGRP antigenicity preservation. Nickel-enhanced development of avidin–biotin–peroxidase staining is used to show CGRP immunoreactivity in black and alkaline phosphatase–anti-alkaline phosphatase is applied to demonstrate incorporated BrdU in red. The present methodology could be useful for studies requiring detection of incorporated BrdU in cells producing regulatory peptides or other labile antigens. (*J Histochem Cytochem 40:1121–1128, 1992*)

KEY WORDS: 5′-bromodeoxyuridine; Calcitonin gene-related peptide; Lung; Endocrine cells; Immunohistochemistry; Double immunostaining method.

Introduction

In 1982, monoclonal antibodies specific for the thymidine analogue 5′-bromodeoxyuridine (BrdU) were produced and applied for the first time to detect DNA replication immunocytochemically (1). Since then, monoclonal antibodies to BrdU have been used increasingly for proliferation studies (2–5). This new method has been compared with the other techniques available for assessment of cell proliferation (6–8). It has been proposed as a faster, easier, and safer alternative to the [^{3}H]-thymidine technique and has the advantage of giving a better morphological definition (9). BrdU is administered shortly before the removal of the tissue (similarly to the [^{3}H]-thymidine technique). Although the monoclonal antibodies recognize BrdU only on monostranded DNA, so that a DNA-denaturing pre-treatment is required, the immunocytochemical demonstration of the incorporation of BrdU is relatively simple.

However, if it is desired to investigate BrdU incorporation in a particular cell type that is not distinguishable morphologically from its neighbors, a second immunostaining may be required, using an antibody specific for that cell type. Several protocols for double immunostaining of BrdU with another antigen have already been described (9–13). A simultaneous double staining method (i.e., one set of staining reactions) would be preferable, but is often prevented by the pre-treatments required for denaturing DNA, which reduce or destroy the antigenicity of the second antigen. Therefore, when double immunolocalization of BrdU and another antigen is intended, a compromise between the optimal conditions for immunocytochemical demonstration of each antigen must be achieved. This applies especially to the fixation and pre-treatment procedures.

Thus far, very few such studies have been carried out with a double immunocytochemical technique on endocrine cells (14,15). The aim of the present study was to set up a simple and reliable double immunocytochemical method for simultaneous demonstration of calcitonin gene-related peptide (CGRP) and BrdU to study the uptake of BrdU by CGRP-immunoreactive cells in the lung of the rat. This involved the optimization of the administration of BrdU, the pre-treatment, and the immunocytochemical methods.

Most of the endocrine cells of the rat lung are known to store CGRP (16). As with other small regulatory peptides, immunocytochemical demonstration of CGRP requires specific conditions (e.g., concerning fixation) for preservation of its antigenicity. Among the several fixation protocols reported so far for BrdU immunocytochemistry (17–19), only a few are suitable for immunostaining CGRP and other regulatory peptides. In addition, in double immunostaining, the DNA-denaturing pre-treatment required for BrdU detection can reduce the antigenicity of the endocrine peptide. Most of the proteolytic pre-treatments reported previously

[1] Supported by the Medical Research Council, The Council for Tobacco Research (USA) and The Spanish Ministry of Education and Science (CICYT project No. PB88-0553) and Beca de formacion del profesorado y personal investigador (LMM).

[2] Correspondence to: Dr. David R. Springall, Dept. of Histochemistry, Royal Postgraduate Medical School, Du Cane Road, London W12 0NN, UK.