Immunohistochemical detection of Ki67 antigen in formalin fixed, paraffin wax embedded sections

We read with interest the recent paper by Torp et al reporting the detection of the Ki67 antigen in formalin fixed, paraffin wax embedded glioblastoma tissue sections using a monoclonal antibody directed against Ki67, purchased from Dako, Glostrup, Denmark. The authors did not recommend the use of this antibody on paraffin wax sections. We have also tested the antibody on sections from routine blocks from human gingiva, tonsils and lymph nodes using different time schedules for microwave pretreatment and application of the primary antibody. Two crucial procedures for detecting Ki67 antigen successfully were identified. Firstly, slides should be pretreated in a microwave oven in citrate buffer (pH 6.0) for over 20 minutes (4 x 5 minutes). Secondly, Ki67 antibody (diluted 1 in 100–200) should be incubated overnight at 4°C to produce strong staining. Torp et al pretreated sections by microwaving for only 10 minutes (2 x 5 minutes) and incubated the Ki67 antibody for only one hour. Under these conditions, we could not achieve satisfactory staining on sections of the three tissues described above. Bankfalvi et al have reported that almost identical results were found with the Ki67 and MIBI antibodies. In their method, microwave heating was carried out for 35 minutes (7 x 5 minutes) and the Ki67 monoclonal antibody was applied overnight.

However, it should be noted that microwave treatment has certain drawbacks compared with autoclave treatment. Human gingival tissue sections were sometimes disrupted and detached from slides during microwave heating, presumably because of the violent boiling procedure, whereas tonsil and lymph node sections were resistant to this procedure even after one hour in our laboratory. Furthermore, another disadvantage of the Ki67 antibody was background and staining on epithelium and various cell types except polymorphons, although these were easily distinguishable from genuinely reactive structures. Thus, Ki67 monoclonal antibody is as useful as MIBI even in formalin fixed, paraffin wax sections, if the tissue sections to be examined can withstand more than 20 minutes in a microwave oven.

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References


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This is the 89th volume in a continuing series on lung biology in health and disease and addresses the exponentially increasing information on adhesion molecules. As with all

texts, we probably have our own views on how they should be presented and developed. Ideally, the book should start with a basic chapter on the whole process of cell recruitment, outlining the rolling of cells, loose adhesion, firm adhesion, and then cell migration. These processes involve different adhesion molecules. With this background, it might have been more appropriate to have started with selecting, proceeded to a chapter on the beta 2 integrins and then the other cell adhesion molecules.

There is some repetition throughout the chapters, as would be expected in such a complicated field, but this certainly does not detract from the strength of the book, which is very comprehensive, well referenced and generally easy to follow. The introduction is good and it is strong from the basic science point of view. It includes a chapter on signal transduction, the lesser known role of adhesion molecules in lung morphogenesis, the adhesion processes between the epithelial cells, and the underlying matrix. There are also chapters on bacterial adhesion, a process which is a prerequisite for tissue infection, and the role of adhesion molecules in malignant metastases. From the clinical point of view, there are sections on experimental lung injury, wound healing, transplantation, and pneumocystis.

One great omission from this book is the genetics of adhesion deficiency. The whole field of adhesion molecules was established following the observation of defective cell migration, shown to be related to deficiency of the adhesion molecules. There have been great advances in the understanding of the basic genetic defect and it would have been appropriate to have had a chapter on this. In contrast, there is little to say about the immune response, which clearly requires very specific cell adhesion and what evidence is included in the text is found in sections such as those on lung transplantation. Again, it might have been more appropriate to have included several chapters on cell specific adhesion processes such as those that involve the lymphocyte or the neutrophil. However, once again, this probably reflects personal preference rather than the criticisms of the book.

In summary, this is an important addition to the lung biology in health and disease series. It contains a lot of information that overlaps other fields of medicine and will appeal to both basic scientists and clinical scientists alike.

R A STOCKLEY


This soft back edition sets out to offer a practical introduction to undergraduates, postgraduate scientists and technicians in the field of electrophoretic protein separation methods. Overall, I found this book to be clearly written, well laid out, amply illustrated, and well referenced. I found the concept of summarising transcription, translation and protein structure in two and a half pages of text quite surprising, though not worrying a little on the basic side. The book concentrates primarily on polyacrylamide gel electrophoresis (PAGE) and gives little space to any other forms of protein electrophoresis.