Abstract

A cocktail of the antibodies against Ki-67 & E-Cadherin is presented. Ki-67 visualizes the nuclei of the proliferating cells, while E-Cadherin visualizes the membrane of all the malignant epithelial cells. This cocktail facilitates the accurate estimation of the proliferative index in infiltrating duct carcinoma of breast, by allowing us to calculate the percentage of the Ki-67 positive malignant cells out of all and only the malignant cells.

Keywords: Ki-67, E-Cadherin, immunostain cocktail, Ki-67 index, infiltrating duct carcinoma of breast

Introduction

Ki-67 (MIB-1) is a proliferative marker which is present in the cell nuclei in all cell cycle phases except the resting phase (G0). The most used antibody for its detection in formalin fixed tissues is a monoclonal antibody against MIB-1. The percentage of Ki-67 positive cells out of the total number of malignant cells is defined as the proliferative index. Generally, elevated proliferative index correlates with a worse prognosis in cancer.

In order to calculate the true proportion of Ki-67 positive cells, all the malignant cells in the field should be counted. This is done usually by counting the hematoxylin stained nuclei. It is important to optimize the degree of counterstaining, given that Ki-67 negative nuclei determine the overall population for calculating the proportion of Ki-67 positive cells. Weak counterstaining can result in overestimation of the Ki-67 index [1].

One should also avoid counting positive Ki-67 stromal or lymphatic cells, because this will also result in overestimation of the Ki-67 index.

Ki-67 index has a prognostic and predictive potential in breast cancer [2], therefore it is very important to recognize the Ki-67 positive and negative malignant cells in order to arrive to the correct index.

E-Cadherin is a calcium regulated adhesion molecule. It decorates the membrane of most normal epithelial cells. Its main use in breast cancer
is to differentiate between lobular carcinoma and ductal carcinoma. A positive stain favors ductal origin of the tumor while the loss of E-Cadherin expression favors the lobular origin of the tumor [3].

Because Ki-67 is a nuclear stain and E-Cadherin is a cell membrane stain we composed a cocktail of the two antibodies; against Ki-67 and against E-Cadherin (Ki&Cad cocktail). It is possible to simultaneously apply the 2 antibodies against 2 compartmentally localized proteins i.e. the membranal E-cadherin and the nuclear Ki-67. Application of this cocktail on the slide allows us to recognize and count all the malignant epithelial cells, and only them. With the aid of this cocktail staining we can give the exact percentage of proliferating malignant cells positive for Ki-67.

**Methods**

We combined two stains into one, Ki&Cad cocktail. 50 microliters of monoclonal mouse anti human Ki-67 antigen Clone MIB1 at a concentration of 1:200 (Dako Cytomation, Denmark) and 50 microliters of Mouse anti-E cadherin (clone :4A2C7 Zymed laboratories San Francisco California) at a 1:50 concentration. The staining of infiltrating duct carcinoma (IDCA) of breast was performed on the Ventana Bench mark according to the manufacturer’s instructions.

**Results**

The cellular localization of the two antibodies was distinct and it was easy to calculate the proliferative index.

Figure 1 shows an area of Infiltrating duct carcinoma (IDCA) of breast. All the malignant cells (95 cells) show a membranal staining of E-Cadherin and 13 of them show nuclear positivity for Ki-67.

In Figure 2 an area of IDCA of breast, immunostained for Ki67 is shown. Some of the positive cells are malignant cells, but most are proliferating lymphocytes.

In Figure 3 an area of lymphocytic aggregate (left) adjacent to the tumor cells is shown. It is obvious that the malignant cells have membranal E-Cadherin staining, while the lymphatic cells, which don’t have membranal staining, should not be taken into account for the proliferative index.
Discussion

Antibody cocktail is a combination of antibodies blended together into one solution, to apply on the tissue section at the same time. An example of this is a Pan Keratin antibody which is a combination of antibodies to the different keratin types and thus will stain the cytoplasm of most epithelial cells.

Another type of cocktail is a cocktail of antibodies against P-63 and P-504S for prostatic biopsies [4,5]. P-63 has a nuclear reactivity while P-504S has cytoplasmatic reactivity (This cocktail is available commercially with double stain visualization).

Our cocktail of an antibody with nuclear reactivity (Ki-67) and an antibody with cell membrane reactivity (E-Cadherin) was not reported previously. In this type of cocktail the difference of the nuclear staining from that of the cell membrane is obvious and easy to visualize, therefore there is no need for double color visualization.

We found that this antibody cocktail is particularly well suited to estimate the proliferative index in infiltrating duct carcinoma of the breast. The advantages of the Ki&Cad cocktail are that it facilitates the accurate estimation of the proliferative index. It is inexpensive as there is no need for the use of a double stain kit. It is easy to perform in any routine immunopathology laboratory and easy to interpret by any surgical pathologist.

References