Automated IHC Analysis of Co-localized Biomarkers by Light Microscopy

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Technology description

Researchers in Weill Cornell Medical College's Pathology Department have developed an immunohistochemical (IHC) method for double staining tissue samples for analysis under the light microscope that can detect two different biomarkers that are co-localized in the same cellular compartment, e.g. the nucleus. This system employs different special chromogens that are not opaque or overpowering, which gives a distinct blended color when the two chromogens are co-localized. When the biomarkers are not co-localized, each chromogenic product displays its own distinctive color and any cell that does not express either marker is not visible. The method can be assessed by either routine manual microscopy or via an algorithm employing an automated image analyzer to detect the number of cells expressing each biomarker, and the percent of them that also express the other. Typically co-localized biomarker studies employ fluorescence microscopy, which requires expensive optical equipment, a dedicated darkroom, and fresh frozen, unarchived samples -- all unavailable in the typical diagnostic lab. Instead, this IHC technology enables widespread co-localization assessment of paraffin-embedded and archived samples.

This sytem is particularly useful in the many clinical situations where it is important to pinpoint which cells in a tissue sample express a specific biomarker. For example, in bone marrow cancers, such as multiple myeloma, lymphoma, or leukemia, the cancer cells are often a minority of bone marrow cells, scattered amongst many non-cancerous hematopoietic cells. This assay currently is being used effectively to detect the percentage of the cancer cells in the bone marrow that have entered the cell proliferation cycle, which shows how fast the cancer is growing and the need for chemotherapy. Also, it is being used to predict and demonstrate the effectiveness of a given chemotherapy in cancer cells. For example, the tests can predict, whether the cancer cells of a specific patient express a protein that is the target of a specific drug, thus predicting efficacy and guiding the selection of therapeutic agents. As shown in the figure below, the effectiveness of therapy can also be assessed by following proliferation markers of the cancer cells after therapy.

The use of this invention to monitor drug response in a lymphoma patient.A patient with a B cell lymphoma in a clinical trial for a drug that, if effective, will cause a reduction of Rb phosphorylation at the 807/811 serine. PAX5 (red chromogen in all images) is a nuclear antigen, present in B cell lymphomas. In this assay, all lymphoma nuclei will appear red. The cell cycle protein (pS807/811Rb) is detected by a blue chromogen. In the resultant slides, a lymphoma cell (red) that is cycling (blue)

attains a double stained nucleus (purple). Because there is no nuclear counter stain, non-lymphoma / non-cycling cells are not visible. The output is the percentage of cycling lymphoma cells (purple) as a percentage of all lymphoma cells (purple + red). By image analysis, Rb phosphorylation at serine 807/811 (pS807/811Rb) was found in 84.08% of lymphoma cells before treatment but was reduced successfully to only 2.14% by day 21 of therapy.

Institution

Cornell University

Inventors

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