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Immunodetection of N-Glycolyl GM3 Ganglioside in Formalin-Fixed and Paraffin-Embedded Tissues: A Fact that Needs Further Investigations

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Received Date: 14th April 2018

Accepted Date: 16th April 2018

Published Date: 20th April 2018

Citation: Blanco R (2018) Immunodetection of N-Glycolyl GM3 Ganglioside in Formalin-Fixed and Paraffin-Embedded Tissues: A Fact that Needs Further Investigations. Enliven:Immunol Immunotechnol 5(1):00e1

Editorial

Gangliosides are glycosphingolipids containing at least one residue of sialic acid [1]. These molecules are mostly localized on cell membrane with the sialylated sugar chains protruding out of cells and the ceramide moiety anchor in the outer surface of plasmatic membranes [2]. Traditionally, immunohistochemistry (IHC) has permitted the detection of a variety of gangliosides in normal and malignant human tissues [3]. Among these molecules, N-glycolyl GM3 ganglioside (NeuGcGM3) has been immunohistochemically localized in human tumors, becoming both a prognostic factor and an attractive target for cancer immunotherapy [4,5].

Frozen tissues are considered the most adequate kind of samples for the study of gangliosides. In line with this, an increased expression of NeuGcGM3 was demonstrated in some malignant tumors [5-7] using frozen tissues after 4% paraformaldehyde (methanol-free formaldehyde) fixation. Regrettably, the number of cases included in these studies was limited, due to frozen tissues are not available in sufficiently large quantities in conventional pathology departments. This fact allowed using formalin-fixed and paraffin-embedded (FFPE) samples which are the most common specimens available for NeuGcGM3 analysis after the histopathological diagnosis.

Based on the localization and the chemical composition of NeuGcGM3, at least three questions related with the immunodetection of this molecule in FFPE samples exist: (1) the organic solvents used in the routine tissue processing (e.g. methanol, ethanol) removes an important fraction of NeuGcGM3 from the cell surface, (2) the specificity of monoclonal antibodies (mAb) that usually recognize oligosaccharides chains associated to both glycosphingolipids and glycoproteins [8,9] and (3) the increased content of NeuGcGM3 detected in human tumors since gangliosides, with exception of brain, commonly constitute less than 5% of lipids present in cell membranes [10,11].

It is known that after formaldehyde fixation carbohydrates, lipids and nucleic acids are trapped in a matrix of in solubilized and cross-linked proteins. But, the chemical structure of these molecules is not altered by formaldehyde unless fixation is prolonged for several weeks [12]. Although, commercial formaldehyde solution contains about 10-15% of methanol to prevent the polymerization, it is insufficient to completely remove NeuGcGM3 from human tissues after fixation and subsequent tissue processing [4,13]. However, in cases of lower levels of expression, NeuGcGM3 could be totally extracted from tissue sections, affecting the selection of candidates for immunotherapy.

Alonso et al. reported a comparable reaction of GM3-immunized sera against both paraffin and resins embedding GM3-expressing melanoma tumors, suggesting that the conventional histological processing is incapable to extract and/or damage the antigenic carbohydrate determinants of gangliosides [14]. Tissue processing for electron microscopy using resins such as Epon minimizes the extraction of lipids. In a similar way, Carr et al. reported a strong reactivity against FFPE breast carcinoma sections using sera from selected patients immunized with the NGcGM3/VSSP vaccine. This is a cancer vaccine able to induce a specific immune response against NeuGcGM3 [15].

In addition, P3 mAb was able to recognize Wilm's tumors and non-small cell lung cancer (NSCLC) [6,16] in FFPE samples, while the reactivity of GMR8 Mab was evidenced in the later [13]. These mAb are IgMs that react to NeuGcGM3 and other NeuGc-containing gangliosides [17,18]. However, NeuGcGM3 was the most NeuGc-containing ganglioside detected in NSCLC [13]. Interestingly, Hayashi et al. demonstrated that chloroform-methanol was capable to completely remove the reactivity of GMR8 mAb from NSCLC sections. Furthermore, the conservation of

NeuGcGM3 and NeuGcGD1a in the lipidic fraction of FFPE samples was demonstrated by mean of TLC-immunostaining [13].

The staining of14F7 mAb, a highly specific IgG1 against NeuGcGM3 [5], has been evidenced in a variety of FFPE human tumors [6,16,19]. This mAb is able to discriminate NeuGcGM3 from N-acetyl GM3 (a closely related molecule) [5,20], which is a normal constituent of human cells. Moreover, the specificity of 14F7 mAb reaction was confirmed by enzymatic treatment of FFPE sections [4], although a faintly decreased in the staining after protease exposure was observed. In this regard, it was suggested that 14F7 mAb reacts with the oligosaccharide core of NeuGcGM3 present in glycolipids and glycoproteins [5], potentially enriching the staining of this mAb.

A correlation between the in vivo radioimmunolocalization of NeuGcGM3-expressing breast tumors using 99mTc labeled 14F7 and the reactivity of this mAb in the FFPE counterparts by IHC was also reported [21]. Similarity, the immunostaining of 14F7 mAb in frozen tissues correlated with the FFPE counterparts [7]. Furthermore, it was demonstrated that treatment with ethanol and methanol was unable to extracted NeuGcGM3 from NSCLC sections after formaldehyde fixation [7]. This molecule was also found in lipidic extracts obtained from FFPE samples by mean of TLC-immunostaining with 14F7 mAb and mass spectrometry [4].

Finally, an over expression of GM3 synthase gene usually occurs in malignant cells, inducing an augment in the NeuAcGM3 content [22-24]. In addition, the hypoxic condition of tumors provokes the over expression of sialin, a sialic acid transporter, which favors the absorption of NeuGc from the external medium and its posterior incorporation to newly synthesized glycoconjugates. Consequently, an increase in the amount of NeuGc-containing gangliosides, including NeuGcGM3, takes place in malignancies [24,25]. Moreover, a change from NeuAcGM3 to NeuGcGM3 could also occur in tumors, as it was previously described in animal models [26].

All these facts support the increased proportion of NeuGcGM3 found in FFPE tumor sections. They also support the use of FFPE tissues as a selection tool of cancer patient candidates for specific therapies using NeuGcGM3 as target. However, the molecular basis to understand the mechanism by which NeuGcGM3 is preserved in this kind of samples needs further investigations.

Acknowledgments

The author wants to express grateful to Ph.D. Adriana Carr (Center of Molecular Immunology) for the valuable discussion about the paper as well as to Mr. Rolando Dominguez for his editorial assistance.

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