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3D imaging detection method of HER2: Application of dual conjugated affibody-quantum dots probes and ratiometric analysis

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HER2 overexpression is associated with Breast Cancer (BC) poor prognosis, due to increased metastases and angiogenesis, and decreased apoptosis. HER2 is commonly assessed by immunohistochemistry. Technique that requires extensive sample processing to get thin fixed samples (3-5 m) that are analyzed using standard HER2 detection probes, and subjective algorithms for HER2 interpretation. Consequently, lacks accuracy and reproducibility, and could lead to misdiagnosis. Therefore, we developed a 3D imaging detection method of HER2 using affibody molecules conjugated with quantum dots (Aff-QDs) and ratiometric analysis (RMA). Affibody anti-HER2 and affibody negative control were conjugated by the maleimide reaction with QD605 and QD545, respectively. Fixed HER2+ and HER2-BC spheroids were incubated with a mixture (1:1) of both Aff-QDs, and confocal image stacks were recorded in the z-axis. Images were processed by RMA (AffantiHER2-QD605/Affneg-QD545 fluorescence), to assess the specific HER2 signal. We found that the non-specific accumulation for both Aff-QDs was the same within HER2-spheroids. However, the AffantiHER2-QD605 signal in HER2+ spheroids, was significantly higher (5.91 0.81 F/F0) than that of Affneg-QD545 (2.67 0.56 F/F0, $p<0.05$) and was optimally resolved up to 50 m depth. After RMA, non-specific signals were removed in HER2+ and HER2- spheroids, and no false HER2 signal was found. Therefore, Aff-QDs can efficiently penetrate in spheroids, used as 3D BC models, with minimal sample manipulation; after RMA, specific and objective 3D HER2 result can be obtained. The method proposed here, could reduce the typical problems associated with traditional immunohistochemistry and improves HER2 detection by 3D analysis.

Biography

Perla Pérez-Treviño is a PhD student in Biotechnology from the Tecnologico de Monterrey (ITESM), Mexico. Since 2012 to the present, she has been working as Research Assistant at Institute of Cardiology and Vascular Medicine, Zambrano-Hellion Hospital, School of Medicine, ITESM. She has published two papers as first author in important peer reviewed journals and two more that are in revision. Her work is focused in the study of molecular and microstructural cell alterations during various chronic pathologies, and currently, she is working in assessing the expression of biomarkers in 3D models of cancer cells growth and tumors.

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