Finding the Sweet Spot for Breast Cancer Detection

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Glycosylation is an important post-translational modification that plays critical roles in cellular physiology, cell–cell interactions, and cancer development and progression. Aberrant glycosylation is a hallmark of the tumor microenvironment, and the activities of specific glycosidases and glycosyltransferases underpin these molecular changes.1 These enzymes are responsible for modifying proteins and lipids with glycans that may modulate their functions and activities. Here, Urano and co-workers exploit the enzymatic activities of the glycosidase α-mannosidase and γ-glutamyltranspeptidase (GGT) to design fluorogenic activity-based probes that enable rapid detection and discrimination of malignant and benign human breast tumors from the surrounding tissues.2 This feat could enable accurate differentiation of breast cancer from healthy tissues during breast tumor resection in the clinic.

Urano and co-workers exploit the enzymatic activities of the glycosidase α-mannosidase and γ-glutamyltranspeptidase to design fluorogenic activity-based probes that enable rapid detection and discrimination of malignant and benign human breast tumors from the surrounding tissues.

To identify a candidate glycosidase biomarker for breast tumors, the authors developed 12 fluorogenic probes bearing different monosaccharide substrates and evaluated them in normal breast tissue and malignant cancer tissues for differential activity. Preliminary screening in the breast tumors led to the identification of two probes based on the enzymatic activities of α-mannosidase and β-hexosaminidase, which showed significant increases in fluorescence signal. The sensitivity and specificity of the α-mannosidase-based probe, termed HMRef-αMan (Figure 1), were 90% and 100%, respectively, so Urano et al. decided to exploit the activity of α-mannosidase to develop chemical tools for identifying malignant breast tumors from the surrounding tissues. Notably, the sensitivity and specificity of their strategy rival those of computed tomography used in the clinic for breast cancer diagnosis.3

The authors developed a 2D fluorescence gel assay combined with peptide mass fingerprinting5 to identify α-mannosidase 2C1 (MAN2C1) as the key enzyme responsible for the increase in fluorescence signal. They also performed immunohistochemical (IHC) staining to corroborate higher levels of MAN2C1 expression in the tumorigenic tissue compared to normal mammary gland and fat tissues. To test the ability of HMRef-αMan to spatially demarcate the tumor tissue, Urano and co-workers incubated a surgically resected breast specimen containing both healthy and cancerous tissue and applied their assay. They found that lesions with a diameter less than 1 mm could be rapidly detected. In addition, IHC staining confirmed that the lesions had elevated MAN2C1 activity.

To differentiate malignant from benign tumors, the authors tested HMRef-αMan in fibroadenoma, phyllodes tumor, and intracystic papilloma. These benign lesions resulted in a stronger fluorescence signal than the malignant tumors, which also correlated with the level of MAN2C1 that they expressed. The sensitivity and specificity of the classification of benign lesions versus malignant tumors were 90% and 80%, respectively, which suggests that HMRef-αMan could be used as a biomarker to differentiate between healthy and diseased tissue.

Urano’s team reasoned that they could build upon their previous expertise with protease-mediated cancer detection tools to discriminate between cancer and benign lesions by developing a GGT-reactive “turn-on” fluorescent probe with...
different emission wavelengths, \textit{gGlu}\textsubscript{2}OMe SiR600 (Figure 1), which is activated by both benign and malignant tumors.\textsuperscript{5–7} In this assay, they used the combination of HMRef-\textit{α}Man and \textit{gGlu}\textsubscript{2}OMe SiR600 probes to visualize the activities of MAN2C1 and GGT in malignant tumors, benign lesions, and normal breast tissue. Excitingly, they found the following: benign lesions were more fluorescent than malignant tumors in the green channel; benign lesions and malignant tumors had similar fluorescence in the red channel; and normal breast tissue did not have fluorescence in both channels (Figure 1). These results suggest that their dual enzyme imaging strategy could be used in a combinatorial fashion to distinguish between benign lesions, malignant tumors, and healthy breast tissue.

Whereas clinical approaches for detecting and delineating healthy from diseased tissues during breast tumor resection have traditionally relied on intraoperative histopathology, this approach offers a noninvasive strategy for assessing surgical margins in the clinic.\textsuperscript{8} This work is a significant advancement in cancer detection because of its novel exploitation of specific glycosidase activity in combination with peptidase activity to achieve increased specificity and sensitivity for discrimination of benign and malignant tissue in breast cancer patient samples.\textsuperscript{9} Furthermore, this work identified MAN2C1 as a potential clinical biomarker for breast cancer. The function of MAN2C1 in breast tumors remains unknown, so understanding the role of this glycosidase in breast cancer may identify potential therapeutic targets for disease treatment.\textsuperscript{10} Interesting future directions also include application of the dual enzymatic activity imaging strategy to breast cancer tissues in a clinical context. While the general applicability of this technique throughout the entire spectrum of breast cancers remains to be seen, the promise of this strategy in the clinical setting is unquestionable.


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Figure 1. Dual detection of \textit{α}-mannosidase and \textit{γ}-glutamyltranspeptidase enzymatic activities in human breast cancer samples using fluorogenic activity-based probes, HMRef-\textit{α}Man and \textit{gGlu}\textsubscript{2}OMe SiR600, respectively, allows detection and discrimination of malignant tumors and benign lesions from the healthy surrounding tissue. Bottom right panel is reproduced with permission from ref 2. Copyright 2020 American Chemical Society.

Furthermore, this work identified MAN2C1 as a potential clinical biomarker for breast cancer.

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Notes

The authors declare no competing financial interest.

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