Public Abstract

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DIAGNOSTICS AND MOLECULAR IMAGING

The rise of targeted therapy in cancer treatment has created a strong need for characterization of a patient's tumor before receiving treatment. Many effective cancer drugs are now being targeted at specific proteins in the tumor, thus only patients who have tumors which express these proteins in appreciable amounts will respond to these kinds of therapy. The most popular method of diagnosing patients is through the practice of immunohistochemistry (IHC), where biopsied patient tissue is subjected to testing for protein expression. IHC works by incubating a primary antibody towards the target protein, then targeting this primary antibody with a secondary antibody containing a reactive enzyme – most commonly, horseradish peroxidase (HRP). This method is expensive, contains many steps, involves varying amounts of amplification due to enzyme reactivity, and is only as specific as the primary antibody. Patients receiving treatment using popular drugs targeted at common proteins such as EGFR, cMET, and PD-L1 have shown varying degrees of responses based on initial IHC diagnosis, even when using FDA-approved diagnostic kits.

Due to the discrepancies seen between diagnosis and drug efficacy, we have developed new methods utilizing small nano-scale compounds that utilize peptides to target protein biomarkers in human tissues. Peptides which are targeted towards receptors contain only the amino acid sequences which are sufficient for protein binding. Due to their tailored specificity, low cost, scalable production, and ease of modification, peptides can be an attractive method of investigating protein content in human tissues. We investigated the use of peptides combined with imaging agents as diagnostic methods to compare with immunohistochemistry. Gold nanorods (GNR) scatter light efficiently in the dark field, and their high surface-area-to-volume ratio allows each nanorod to be coated with many targeting peptides, enhancing specificity of each nanoparticle for the receptor of interest. We first investigated attachment of peptides to (GNR) that can be used to diagnose common biomarkers EGFR and cMET in tumor tissues. EGFR is one of the most commonly overexpressed proteins in human cancers, and many EGFR-targeted drugs have shown improvement of progression-free survival in patients. During the course of EGFR-targeted treatment it is common that a patient will eventually develop resistance to the EGFR-targeted drugs. One such mechanism is the circumventing of EGFR pathway through upregulation of the cMET protein on the tumor surface. Once EGFR is internalized and cMET is the dominant pathway, patients will stop responding to EGFR-targeted drug and the tumor will continue proliferation. There are numerous cMET drugs on the market for second or third line therapy when resistance occurs with this mechanism, however diagnosis of the cMET biomarker has become controversial due to poor diagnostic results using the current standard IHC methods. We thus followed up our EGFR diagnostic study by investigating the cMET protein using the same GNR platform with a cMET-targeted peptide. The GNR-based histochemistry platform shows specificity for the targeted receptors in tumor cell lines and patient tissues, and is able to detect a range of protein expression, rather than relying on binary pathology grades of 1+, 2+, or 3+ expression. EGFR and cMET are two popular biomarkers targeted by pharmaceuticals, and have seen recent success when combined with immune checkpoint inhibitors. The current surge in immuno-oncology has shown excellent response of patients to drugs that inhibit common immune checkpoints such as the interaction between immune receptor Programmed Death 1 (PD-1), expressed on immune cells, and its ligand, PD-L1, expressed on tumor cells. The binding of PD-1 on T cells to PD-L1 on tumor cells will stop the T cells from destroying the tumor. Inhibition of immune checkpoints restore lost host immune function by allowing T-cells to recognize tumor cells as foreign. As with EGFR and cMET, there has been much debate over whether

current methods of diagnosing PD-L1 levels in patients are sufficient due to patient responses varying with respect to the diagnostic recommendation. We extended our peptide-based diagnostic method to investigate PD-L1 by analyzing the crystal structures of PD-L1 and PD-1 and synthesizing a peptide that is specific for the binding region of PD-L1. Using this sequence, we combined our PD-L1 peptide with a biotin molecule, to allow for conventional IHC, and a Cy5 fluorophore to do fluorescent investigations of PD-L1 levels in patient tumor tissues. When compared head-to-head with the current FDA-approved PD-L1 diagnostic standard, the peptide-based method shows high specificity for tumors in tissues that the FDA-approved diagnostic fails to recognize. Due to these results, we believe that peptide-based histochemistry can be used as a specific, cheap alternative to conventional antibody-based IHC.