Synthetic Biomarkers for Cancer Detection and Diagnosis
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Critical to the clinical management of cancer is the ability to detect disease and provide molecular information that can be acted on by therapeutics. Current clinical methods in cancer diagnostics include invasive biopsies of tumor tissue, imaging, or measurement of biomarkers in the blood. These existing tools would be enhanced by technology that is minimally invasive and could output functional readouts on tumor activity. Exciting progress is being made on this goal on several fronts, and includes activity-based probes to create "synthetic" biomarkers, methods to image metabolic activity of tumors, and devices for drug micro-dosing. Future efforts are to coordinate the readouts from these diagnostic methods with molecular targeted therapeutics for a unified precision medicine approach for cancer treatment.

Heterogeneity drives challenges in cancer treatment

Cancer is a leading cause of death worldwide and cancer care is estimated to be $147 billion dollars in 2017 in the United States (National Cancer Institute). Despite promising recent advances in cancer treatment, the response of patients to therapies is heterogeneous. The evidence for the heterogeneity of human cancer between individuals, tumors, and even within the same tumor is growing as an increasing number of samples are sequenced and novel methodologies are developed. Adding to the complexity is that heterogeneity is not only encoded by genetics, but can also be influenced by the microenvironment of the cancer cell. At the same time, there are an increasing number of therapeutics that target cancer cells on a molecular basis. This presents the challenge of how to guide clinical decisions to segment patient populations on a quantitative basis to match therapeutics to an individual's cancer in order to maximize the efficacy and minimize the side effects of treatment. Furthermore, technology that is time-resolved would allow longitudinal monitoring of patients to track patient response and capture the development of therapeutic resistance. These challenges are embodied in the NIH's Precision Medicine Initiative, an effort to understand "how a person's genetics, environment, and lifestyle can help determine the best approach to prevent or treat disease."

Cancer biomarkers can guide clinical care

The timeline of cancer development is a complicated landscape and includes risk assessment, screening, diagnosis of disease onset, response to treatment, and monitoring for relapse after treatment. Biomarkers are tools that can provide information about disease status and can answer important questions such as: Who has cancer and how advanced is it? What is the best available treatment for an individual and their cancer? Is the patient responding to treatment? Is there relapse of disease? The answer to these questions can provide valuable insight to inform clinical actions and therefore can improve the clinical management of cancer for reduced side effects and better treatment outcomes. The physical representation of biomarkers come in many forms and can include genetic sequencing information, such as the increased risk for breast and ovarian cancer in individuals with BRCA1 mutations (Shattuck-Eidens et al., 1995), analysis of nanometer scale extracellular vesicles from the urine, such as prostate cancer prediction through sequencing of exosomes (McKiernan et al., 2016), and aberrant overexpression of proteins, such measuring expression levels of HER2 in biopsies to guide treatment of antibody-based therapies (Slamon et al., 2001). Due to the large diversity of individual patients, the future of cancer diagnostics will likely integrate many inherently noisy biomarker signals using methodology developed for 'big data'.

Activity-based nanosensors as theranostics

Although there are promising biomarkers for the diagnosis and monitoring of disease, not all biomarkers are created equal. For example, the serum biomarker, prostate-specific antigen (PSA), was widely adopted to screen men over the age of 50 for prostate cancer starting in the 90s. Although PSA enabled the increased diagnostic sensitivity for prostate cancer, many of the cancers were benign while clinical actions based on the detection of elevated PSA levels increases the risk for complications associated with follow-up procedures such as biopsies and treatment, leading to what has been recognized as “over treatment” (Prensner et al., 2012). Furthermore, the mechanism for the rise of PSA protein levels in the bloodstream remains unclear, and therefore its link to disease progression is not well understood. This scenario represents an opportunity to re-envision biomarkers as tools that encode information that have relevant information for clinical decisions for treatment, such as how an individual tumor might respond to drug treatment. The pairing of diagnostic information with treatment mode is embodied by the term ‘theranostic’, a portmanteau of therapy and diagnostic.

Enzymes are often the target of cancer therapeutics. A particularly important class of enzymes are proteases, which are known to play critical roles during the progression of cancer (Koblinski et al., 2000). Fluorescence-based small molecule probes to functionally image protease activity have been developed as important tools to study biology and can also be used for surgical navigation for total tumor resection (Yim et al., 2018). Other groups have engineered imaging probes that are activated in response to protease activity that fluoresce in the visible and near-infrared wavelengths (Jiang et al., 2004; Weissleder et al., 1999). However, these technologies rely on optical imaging that is limited by imaging depth. As a complementary technology, we have engineered an exogenously administered nanoscale sensor that is actively targeted to tumor cells and sheds analytical fragments in response to tumor-specific proteases that can be detected in the urine (Kwon et al., 2017). The advantage of an exogenously administered synthetic system over a naturally occurring blood biomarker is that analyte generation can be engineered for optimal kinetics, specificity, and amplitude using material design. Furthermore, we found that analyte generation was also dependent ligand-receptor matching between nanosensor and tumors. This technology can be further developed to include a wider array of protease-sensitive analytes and tumor-specific receptors to precisely stratify patients into treatment groups based on receptor expression that can be matched to therapies such as integrin-targeted therapeutics. Urine-based readouts can be paired with spatial information if nanosensors are built with superparamagnetic nanoparticle cores for magnetic resonance imaging (Kwong et al., 2013).

Conclusions

There are an increasing number of molecularly targeted therapeutics available for the treatment of cancer. Diagnostics that have activity-based readouts are promising tools to stratify patients for these increasingly precise treatment regimes. The future of clinical management of cancer will include the integration of diagnostic information with molecularly targeted therapeutics.

References

Jiang, T., Olson, E.S., Nguyen, Q.T., Roy, M., Jennings, P.A., and Tsien, R.Y. (2004). Tumor imaging by means of proteolytic activation of cell-penetrating peptides. PNAS *101*, 17867–17872.

Kwon, E.J., Dudani, J.S., and Bhatia, S.N. (2017). Ultrasensitive tumour-penetrating nanosensors of protease activity. Nat Biomed Eng *1*.

Kwong, G.A., Maltzahn, G. von, Murugappan, G., Abudayyeh, O., Mo, S., Papayannopoulos, I.A., Sverdlov, D.Y., Liu, S.B., Warren, A.D., Popov, Y., et al. (2013). Mass-encoded synthetic biomarkers for multiplexed urinary monitoring of disease. Nature Biotechnology *31*, 63–70.

McKiernan, J., Donovan, M.J., O'Neill, V., Bentink, S., Noerholm, M., Belzer, S., Skog, J., Kattan, M.W., Partin, A., Andriole, G., et al. (2016). A Novel Urine Exosome Gene Expression Assay to Predict High-grade Prostate Cancer at Initial Biopsy. JAMA Oncol *2*, 882–889.

Prensner, J.R., Rubin, M.A., Wei, J.T., and Chinnaiyan, A.M. (2012). Beyond PSA: The Next Generation of Prostate Cancer Biomarkers. Science Translational Medicine *4*, 127rv3-127rv3.

Shattuck-Eidens, D., McClure, M., Simard, J., Labrie, F., Narod, S., Couch, F., Hoskins, K., Weber, B., Castilla, L., Erdos, M., et al. (1995). A Collaborative Survey of 80 Mutations in the BRCA1 Breast and Ovarian Cancer Susceptibility Gene: Implications for Presymptomatic Testing and Screening. JAMA *273*, 535–541.

Slamon, D.J., Leyland-Jones, B., Shak, S., Fuchs, H., Paton, V., Bajamonde, A., Fleming, T., Eiermann, W., Wolter, J., Pegram, M., et al. (2001). Use of Chemotherapy plus a Monoclonal Antibody against HER2 for Metastatic Breast Cancer That Overexpresses HER2. New England Journal of Medicine *344*, 783–792.

Weissleder, R., Tung, C.-H., Mahmood, U., and Jr, A.B. (1999). In vivo imaging of tumors with protease-activated near-infrared fluorescent probes. Nature Biotechnology *17*, 375–378.

Yim, J.J., Tholen, M., Klaassen, A., Sorger, J., and Bogyo, M. (2018). Optimization of a Protease Activated Probe for Optical Surgical Navigation. Mol. Pharmaceutics *15*, 750–758.

Koblinski, J., Ahram, M., and Sloane, B.F. (2000). Unraveling the role of proteases in cancer. Clinica Chimica Acta *291*, 113–135.