

THERANOSTICS: PAST, PRESENT AND FUTURE

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ABSTRACT

The ever increasing cost in the healthcare services due to new treatments (both newer drugs or modalities), has led the researchers to look critically at cost cutting measures. The significant pressure on the health industry to maintain innovation and product enhancement has led to discovery of important diagnostic techniques which have bearing on the in the early diagnosis and planning the treatment strategy. Theranostics is defined as diagnostic tests that are directly linked to the application of specific therapies. The estimation of status of 'HER-2' in breast cancer patients which can determine the further management and outcome of the disease is one such example that is very much practiced in now a days. The Applications nanoparticles and cancer biomarkers have greatly contributed to the field of medicine as theranostic agents. The selective applications of these have the potential to positively impact the challenges such as reducing risk, costs and enhancing the success of medicines.

KEY WORDS

Theranostics, Herceptin, Magnetic Nanoparticles, EGFR gene mutation.

INTRODUCTION

The diagnostic tests are indispensable in the management of most of the diseases. These diagnostic tests may be as simple as routine blood or urine tests, or may be as complex as tests involving FISH, RT-PCR etc. There is always an attempt to make these tests not only simple but also more specific. In recent times there is a growing demand for the search of such diagnostic tests that are not only have a potential bearing on diagnosis of a disease but also help to decide the course of management in patient.

The term theranostics was probably first used by PharmaNetics president and CEO John Funkhouser in describing his company's business model in developing diagnostic tests directly linked to the application of a specific therapies. As we all know diagnostics mean the ability to define a disease state. Theranostics mean the ability to affect therapy or treatment of a disease state. In other words these diagnostic tests will decide the treatment plan apart from efficiently diagnosing the disease. The diagnostic

tests such as tensilon (Edrophonium) test and International Normalized Ratio (INR), Dexamethasone suppression test are some of them to mention, which are being used in varying capacity in clinical practice. We are going to discuss some newly invented diagnostic tests which are being used in therapeutics and or may be used in future.

ROLE OF HER2 IN BREAST CANCER TREATMENT

Breast cancer is a severe and life threatening disease and one of the common malignant tumor affecting females. It has been shown that approximately 30% of stage IV breast cancers over express Human Epidermal growth factor Receptor-2 (HER-2) which is linked with more aggressive disease and a poorer overall prognosis. During 1980s to 1990s many reference labs used to offer HER-2 testing via a variety of 'home-brew' methods that has led to variation from lab to lab. This could be stumbling block in determining the therapeutic strategy while interpreting the results of this test.

Genentech firm addressed this risk by collaborating with Dako to produce a diagnostic test that could be used in any laboratory thereby standardizing testing and reducing lab to lab variation. Dako's HercepTest® for diagnosis of HER-2 over expression as well as Genentech's Herceptin®, or Trastuzumab, is a humanized monoclonal antibody to HER-2 which is effective by binding to the HER-2 expressed on the cell surface of the tumour cells were approved by FDA on Sept 1998 (1).

With advent of simultaneous approval for both Genentech's Herceptin® for the treatment of Stage IV breast cancer and Dako's HercepTest® for diagnosis of HER-2 over expression has changed the treatment strategy of patients with breast cancer who over express HER-2. In addition, the availability of HER-2 testing has moved into improved DNA-based tests with Abbott receiving FDA approval for a DNA-based HER-2 test in 2002. The detection of HER-2 over expression in breast cancer patient and treatment of HER-2 positive patients with Trastuzumab is one classical example for theranostic that has been widely employed all over the world. The application of the test and further use of Trastuzumab has immensely benefited breast cancer patients and changed the course of treatment dramatically.

Lyme disease:

Current assay used in Lyme disease employs ELISA followed by western blot technique. However it has limitation in picking up the early infection because of significant lack of specificity, sensitivity and high level of cross reactivity for antigens from other bacteria. Detection of five peptide epitopes with high specificity to *Borrelia* has opened up new hopes in the early detection of said infection. The researchers also focused on *Borrelia* surface protein OspC as a source of epitope. This is required for transmission of bacteria from ticks to humans. Arnaboldi and colleagues were successful in identifying the 3 peptides which bound to more than 50% of the Lyme samples. Subsequently they also showed that ELISAs confirming one of these epitopes, OspC1. This method detects Lyme disease in more than 75% of the cases in the early phase and is highly conserved too. It is therefore a good candidate to form part of an assay to reliably detect early-stage Lyme and ensure timely treatment of the disease (2).

Atherosclerosis and LpPLA2:

Atherosclerosis is a widely acknowledged factor in cardiovascular disease which is a leading cause of death in the developed countries. It is needless to say that, atherosclerosis a major cause of cardiac death in third world country as a consequence of rapid urbanization, change in life style and many more factors. In the recent years more focus is given to preventing the of atherosclerosis, early detection rather than treating the consequence of this disease.

Lipoprotein Associated Phospholipase A2 (Lp-PLA2)

There is a long standing evidence to show the role of Lp-PLA2 in atherosclerosis. There is also ample evidence to demonstrate that Lp-PLA2 generates inflammatory substances that inhibition of Lp-PLA2 reduces atherosclerosis in animal models. West of Scotland Coronary Prevention Study Group (WOSCOPS) published data demonstrating the predictive value of Lipoprotein-Associated Phospholipase A2 (Lp-PLA2) as an independent predictor of coronary artery disease. This data has reinforced ongoing research into LpPLA2 as both a predictive marker for atherosclerosis and as a potential target for new classes of atherosclerosis drugs. Currently drugs targeting Lp-PLA2 are in the initial phase of clinical trial.

GSK postulated a role for Lp-PLA2 in atherosclerosis in the early 1990s using gene-sequence databases to clone the gene encoding production of Lp- PLA2 for further study. Laboratory studies have since demonstrated that Lp-PLA2 generates inflammatory substances, and that inhibition of Lp-PLA2 reduces atherosclerosis in animal models. Currently, GSK is progressing drug candidates towards initial safety trials in human subjects.

The development of a diagnostic test for LpPLA2 and drugs targeting the LpPLA2 molecule are progressing in parallel with each potentially supporting the other. It is clear that the existence of the LpPLA2 test, and its potential acceptance in routine risk assessment, has the potential to identify patients for a future drug and increase awareness of the target in the medical community (3).

Molecular Cancer Biomarkers as theranostics:

Molecular biomarker assays such as DNA and RNA testing are widely used in clinical oncology for confirming histological finding that aid in diagnosis

whenever traditional methods fails to do so. One such example is RT-PCR or FISH technique which are used as molecular diagnostic methods in acute promyelocytic leukemia. However when the morphology is atypical, differentiation of essential thrombocythemia from reactive thrombosis is made by detection of clonality marker JAK2 V617F mutation in the essential thrombocythemia (4, 5, 6).

Since these markers either predict the clinical outcome or treatment response, these can be called as Predictive markers (4). These are in turn classified into positive therapeutic predictors or negative therapeutic predictors. As per the definition of theranostic, the predictive marker aptly fits into that. Epidermal growth factor receptor mutation (EGFR) in Non Small Cell Lung Carcinoma (NSCLC) and Human Epidermal growth factor Receptor- 2(HER-2) gene amplification in breast cancer are positive predictor markers. The Kirsten Rat Sarcoma (KRES) viral oncogene homolog gene mutation in metastatic Colorectal Carcinoma is a negative predictor marker. Before these predictors become routine in clinical practice, they must undergo considerable validation process for analytical performance and distinguishing normal from the disease state. The documentation of their clinical utility in terms of survival benefit must also be done before it is routinely employed in diagnosis (7).

Positive therapeutic predictors - EGFR gene mutation in NSCLC: Treatment responsiveness of non small cell lung carcinoma with gefitinib and erlotinib are highly correlated with activating mutations of EGFR gene. These mutations occur at tyrosine kinase domain of EGFR gene which preferentially involve lung cancer in females, non smokers, Adenocarcinoma and of East Asian ethnicity (8). Patients with NSCLC having exon 19 deletion seems to respond better with gefitinib and erlotinib than those of L858R mutation (9). Apart from this detection of different mutations of EGFR gene in various lung cancers enables the distinction between synchronous primary from intra pulmonary metastasis and metachronous primaries from disease recurrence (10).

As we have already discussed, HER-2 assay has a significant bearing in the diagnosis and further management of breast cancer as it is well known now that HER-2 positive status (amplification) responds

better to Trastuzumab therapy. Hence it is a positive predictor marker. Likewise cituximab, an anti EGFR monoclonal antibody was found to benefit colorectal cancer patients with wild type KRAS tumors than those tumors harboring mutations in codons 12 and 13 of KRAS gene. Detection of KRAS gene mutations in patients with colorectal cancer acts as a negative predictor marker (11).

Nanoparticles as diagnostic aids:

Nanoparticle refers to particles roughly measuring around 100 nm . Integration of therapeutic compounds into nanoparticles with the diagnostic agents refers to theranostic nanoparticles. Food and Drug Administration (FDA) has approved iron oxide, gold, protein, liposomes and synthetic polymers as nanoparticles that can be employed in medicine (12, 13). These nanoparticles will not only have a high ratio of surface area to volume but also have tunable optical electronic, magnetic and biologic properties which can be transformed to have different sizes, shapes, chemical compositions and surface chemical characteristics. (12,13). Super paramagnetic iron oxide nanoparticle (SPION) have unique magnetic properties, due to which they show excellent tumor-targeting efficiency, and this paves the way for effective personalized cancer treatment. (14).

Theranostic Nanoparticles and their applications:

Theranostic nanoparticles that simultaneously deliver both imaging and therapeutic agents have gained significant attention for disease management in recent times. Conventional nanoparticles have been previously used to achieve each aspect of disease management separately. The shortcomings with this application being multiple administrations that may be required to fulfill the necessary functions, which bring concerns of patient compliance and safety (15). To overcome these limitations, theranostic nanoparticle systems that can perform all the aspects of disease management in a single setting have been developed over the last decade. In this regard, magnetic-based theranostic nanoparticles (MBTN) are of great interest in disease management due to the numerous advantages of these materials possess when in the presence of a magnetic field. Magnetic nanoparticles (MNPs) are multifunctional agents that can be used for site-specific magnetic targeting (16) and as negative contrast agents in magnetic

resonance imaging (MRI) (17). These MNPs are also used for hyperthermia treatment under alternating magnetic fields (18), and in magnetic field-dependent controlled drug delivery applications (16). These make the MNPs an ideal candidate in the development of advanced theranostic systems.

MNPs are composed of ferromagnetic elements such as iron, cobalt, nickel, or their oxides and alloys (19). MNPs made of iron oxide (magnetite Fe_3O_4 or magnetite Fe_2O_3) and gadolinium (chelated organic gadolinium complexes) (20) have been widely used as contrast agents in MRI for biological applications due to their ability to dissociate into iron and oxygen inside the body, which can safely be eliminated and utilized in metabolic and oxygen transport systems (21,22). When these are fabricated into nanoparticles of approximately 10 nm in diameter, iron oxide nanoparticles begin to exhibit a superparamagnetic behavior (superparamagnetic iron oxide nanoparticles, SPIONs) leading to improved dispersive properties in the absence of a magnetic field, and later guided to accumulate to the site of interest in the presence of a magnetic field, which is of great importance in targeted drug delivery applications (16). The advantage of this MNPs being low cytotoxicity and have been approved by the United States Food and Drug Administration (FDA) for clinical MRI applications (22,23). Numerous studies have explored the potential of MNPs as therapeutic and diagnostic agents for the management of diseases such as cancers and cardiovascular diseases.

Chemotherapy:

With increasing prevalence rates of cancer, the management of cancer has become one of the leading research areas to find more effective imaging and therapeutic modalities. Traditional chemotherapies involve delivery of antineoplastic drugs to the cancer patients. The non-specificity of these drugs usually manifest in various side effects as a result of systemic toxicity (24). The development of MBTN can also play a significant role in cancer management, due to their multi-functional capabilities. With MBTN, it is possible to minimize the systemic toxicity by delivering the drugs only to the cancer cells via active/magnetic targeting thereby sparing healthy tissue and/or cells (24). It is postulated that the dual-targeting mechanism

(combination of receptor-mediated and magnetic targeting) may greatly reduce the toxicity of chemotherapeutic reagents by targeting cancer cells only and localizing of these drugs at the tumor site. Further, the chemotherapeutic drugs can be either loaded into the polymer shell or directly coated on the MNPs surface of MBTN. Sun et al. (25) has demonstrated that the extended particle retention and decreased survival rate in tumor cells when treated with methotrexate- and chlorotoxin-conjugated MNPs. Yu et al. (26) had developed doxorubicin-loaded thermally cross linked MNPs that were administered intravenously into the tumor bearing mice to study the multi-functionality of the particles. The nanoparticles preferentially accumulated in the tumor region within 4.5 hours of the administration and were removed from the body within 24 hours. The nanoparticles showed their therapeutic effect within 12 hours of injection and a significant decrease in tumor size was noticed within 19 days of the treatment. Further, the MR imaging showed a strong negative contrast with the darkening of tumor region, indicating accumulation of the nanoparticles. These preclinical studies have shown a great promise of these doxorubicin loaded thermally cross linked MNPs as theranostic MNPs in diagnosis (MRI) and treatment (drug release) of cancers.

Hyperthermia

Hyperthermia is a treatment in which high temperatures ($> 41^\circ\text{C}$) are applied to kill cancer cells, as they are more sensitive to high temperatures than healthy cells (18). The metallic and magnetic properties of MNPs make them suitable for hyperthermia treatment (27). It is possible to apply alternating magnetic field on administration and targeting of MBTN to cancer site, in which MNPs vibrate and generate thermal energy as a result of absorption of large amounts of magnetic energy by hysteresis loss (18). Heat generated from the MNPs is affected by magnetic properties, particle size, amplitude and frequency of applied magnetic field, and cooling rate of blood (28). By regulating these factors, the heat generation from MNPs can be controlled. The optimal hyperthermia effect can be achieved with 10 kA/m amplitude and 400 kHz frequency (28). Poloxamer, chitosan, alginate, and polyvinyl alcohol hydrogels loaded with MNPs were

formulated by Renard et al. (29). These are implanted in human cancer tumors xenografted in mice for hyperthermia treatment. In another study, Tseng et al proved that the viability of cancer cells significantly reduced when hyperthermia treatment was conducted using MNPs (30).

Hyperthermia has been used with other forms of therapy including chemotherapy to provide more effective treatment. At high temperatures, cancer cells become more vulnerable and respond to chemotherapeutic drugs or radiation effectively in an accelerated fashion (18). The combination of two therapies i.e. hyperthermia and chemotherapy or hyperthermia and radiation therapy would result in better treatment efficacies. Wang et al synthesized MNPs encapsulated As₂O₃ nanoparticles for treating nude mice bearing xenograft human hepatocarcinoma with both thermal and chemo therapy. The application of two therapies in unison showed significant inhibitory effect over tumors in comparison to controls (31).

Conclusion: New diagnostic tests, discoveries have a potential bearing in the management of various diseases. Even though we discussed a few of them, we could not give an account of many tests, technologies in this article. The future has various challenges in the safe application of these, feasibility being the utmost concern. How the scientific community will address these issues will be an exciting thing to watch. The time will decide the applicability of these therapy oriented diagnostic test to manage various diseases.

Conflict of Interest: None

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