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Autoantibodies as diagnostic and prognostic cancer biomarker: Detection techniques and approaches

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ABSTRACT

Autoantibodies produced by the patients' own immune systems in response to foreign substances are emerging as an attractive biomarker for early detection of cancer. These serum immunobiomarkers are produced in large quantities despite the presence of very less amount of the corresponding antigens, and thus presenting themselves as a novel class of stable and minimally invasive disease biomarkers especially for cancer diagnosis. Although a plethora of research, including conventional molecular biology-based as well as cutting-edge optical and electrochemical strategies (biosensor), have been conducted to detect autoantibodies, most of these strategies are yet to be readily applicable in the off-laboratory settings at clinics. Herein, we detail the biogenesis, diagnostic, prognostic and therapeutic potential of autoantibodies as cancer biomarkers. With the particular emphasis on cutting-edge advances in electrochemistry, optical (surface plasmon resonance) and microfluidics techniques, this review entrusts the unmet needs and challenges of autoantibody detection approaches and provides a future perspective of the presented strategies. We believe this review can potentially guide the researchers towards the development of robust, reliable and sensitive detection strategies for tumor-associated autoantibodies and translation of these biomarkers to real clinical settings for diagnosis and prognosis of cancer.

1. Introduction

Autoantibodies are primarily produced by a small subset of the B cells known as B-1 cells or $CD5^+$ B cells after the immune reactions are directed against one or more of the body's own antigens (self-antigen) (Aggarwal, 2014; Shoenfeld et al., 2013; Elkon and Casali, 2008; Wardemann and Nussenzweig, 2007). They may comprise proteins, nucleic acids, carbohydrates, lipids or various combinations of these biological materials. For example, in systemic lupus and related systemic autoimmune disorders, the dominant antigens are ribonucleoproteins or deoxyribonucleoproteins (Casali and Schettino, 1996; Elkon and Casali, 2008). Autoantibodies may be pathogenic, disease-specific and diagnostic, or of no apparent significance. They bind to non-foreign structures within the body and can be found in most well-defined autoimmune disorders. Low-level of autoantibodies occur naturally in healthy individuals and is more common among older adults (Elkon and Casali, 2008; Llorente et al., 1997). These natural autoantibodies occur in low concentrations and have weak binding affinities. Until recently, it had been thought that high-affinity autoantibodies were only associated with autoimmune conditions. However, there is increasing evidence that these autoantibodies are also involved in chronic malignancies. Several mechanisms have been proposed for the production of autoantibodies in cancer including host-immune reactions to tumor-associated antigens (TAAs), antigenic stimulation because of the destruction of malignant cells, or immune dysregulation induced by the neoplastic process (Caron et al., 2007).

Over the last few decades, the evidence of circulating autoantibodies in the sera of cancer patients has created enormous opportunities by utilizing the immune system as a source of clinically useful cancer biomarker (Finke et al., 2017; Hanash, 2003). Autoantibodies have become of particular interest as a cancer biomarker because they can be easily extracted from the serum *via* minimally invasive blood collection (Anderson et al., 2014; Dudas et al., 2010; Zayakin et al., 2013). Various studies have shown that antibodies to the TAAs are present in the samples of patients with different types of malignancies. Additionally, they show an increased level in very early stages of cancer

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and are observed in the patients with several carcinomas including breast (Anderson et al., 2010a; Bassaro et al., 2017), lung (F.M. Brichory et al., 2001; Chapman et al., 2011), gastrointestinal (Zayakin et al., 2013), ovarian (Anderson et al., 2014), colorectal (Álvarez-Fernández et al., 2016), oesophagal (Chen et al., 2017), hepatocellular (Ying et al., 2017) and prostate (Bradford et al., 2006) cancers. Most extensively studied tumor-associated autoantibodies are the auto-antibodies against p53 (Yadav et al., 2017), L-myc (Yamamoto et al., 1996), glycosylated annexin I, annexin II (F.M. Brichory et al., 2001) or HER2-neu (Caron et al., 2007). Detection of autoantibodies also reported during the cellular alteration to malignancy. Therefore, these circulating biomarkers present themselves as an early reporter of the aberrant cellular mechanism involved in tumorigenesis. For instance, anti-tumor protein p53 antibodies reported to detectable as early as 17–47 months earlier to clinical tumor manifestation in uranium workers with the high risk of lung cancer development (Tan and Zhang, 2008; Zaenker et al., 2016). Furthermore, autoantibodies may be valuable biomarkers as they are stable serological proteins with high levels in serum even during low levels of the corresponding antigens (Anderson and LaBaer, 2005; Lu et al., 2008). Even if different cancer biomarker has discovered, they are yet limited in clinics due to their poor predictive values.

To date, various conventional methods such as enzyme-linked immunosorbent assay (ELISA) or sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS/PAGE) have employed to detect the auto-antibodies in serum (Tan et al., 2009). ELISA is one of the most widely used detection techniques, relying on a sandwich immunoassay (Anderson et al., 2010b; Bassaro et al., 2017; Chapman et al., 2008; Takeda et al., 2001). Improvements in the technologies such as proteomic-based platform have enabled the panel of TAAs that shows better diagnostic value than a single TAA marker. In recent years, much attention has been given to developing new strategies based on electrochemistry (Garranzo-Asensio et al., 2016; Masud et al., 2017; Yadav et al., 2017), microfluidics (Hu et al., 2009)(López-Muñoz et al., 2017) and surface plasmon resonance sensor (Ladd et al., 2009; Soler et al., 2016). Among these techniques, electrochemical biosensors have shown great promise because they are fast responsive, user-friendly and cost-effective. Furthermore, microfluidic-based platforms are well suited for overall analytical performance (Hu et al., 2009).

As the field has progressed rapidly in recent years, several review articles had been published (Bassaro et al., 2017; Fortner et al., 2017; Macdonald et al., 2017; Tan et al., 2009; Wu et al., 2017; Zaenker et al., 2016). Most of these articles are based on the biogenesis, discovery and diagnostic development of tumor-associated autoantibodies. However, there is a lack of systemic study, which discusses the potential challenges associated with the detection method or diagnostic development for tumor-associated autoantibodies. In this review, we not only discuss the role of tumor-associated autoantibodies in cancer diagnosis but also identify the challenges encountered while detecting the autoantibodies. We also propose solutions to translate the autoantibodies from research evidence into clinical practice for diagnosis and prognosis of cancer.

2. Production of autoantibodies

The immune response against solid tumors was established by Baldwin in the 1960s (Baldwin, 1955), showing it as a combination of simultaneous cellular and humoral responses. However, the exact mechanisms of autoantibodies generation and their immunological roles are still unknown. The immune surveillance is considered as one of the most commonly proposed mechanisms, where the immune system recognizes and destroys autologous cells that have become malignant (Anderson and LaBaer, 2005; Thomas, 1982; Zaenker et al., 2016). The autologous proteins of tumor cells are known as TAAs that could be overexpressed, mutated, misfolded, or aberrantly degraded to the extent that the reactive immune response is induced in cancer patients. This reactive immune response produces autoantibodies directed against these miss-presented or misfolded proteins (Järås and Anderson, 2011; Kazarian and Laird-Offringa, 2011). Nevertheless, TAAs or proteins that have undergone alternate post-translational modifications (PTMs) can also be recognized as a non-autologous target for immune response. Their phosphorylation, glycosylation, oxidation, or proteolytic cleavage during the alternate PTMs could generate a neo-epitope or enhance self-epitope presentation, which in turn triggers affinity to the major histocompatibility complex (MHC) or T-cell receptor (Hanash, 2003; Zaenker and Ziman, 2013). The immune response produced against these immunogenic epitopes of TAAs enhances the production of autoantibodies, which are utilized as serological biomarkers for cancer detection (Hanash, 2003; Tan et al., 2009).

Despite the apparent mechanism of the production of auto-antibodies in cancer, it is not entirely known how the modifications of antigens trigger the humoral immune response. Several hypotheses had been proposed such as (i) tumor cell death mechanism is the result of defective apoptosis or ineffective clearance of apoptotic cells (Anderson et al., 2010b; Vogelstein and Kinzler, 2004; Utz and Anderson, 1998), (ii) structural similarities between TAAs and foreign antigens producing a humoral response against these foreign antigens (Coronella-Wood and Hersh, 2003), and (iii) the autoantibodies are produced against intracellular self-antigens that are abnormally presented to the immune system or expressed in cancer cells. A new epitope or neoepitope for antibodies binding is created by the mutations that change the protein structure located within an unexposed region of the protein (Liu et al., 2011; Anderson and LaBaer, 2005; Hanash, 2003). All the mechanisms mentioned above triggers the autoantibody production. However, they follow one of the two pathways for biogenesis. Firstly, the TAAs are presented on the antigen presenting cell and bound by B cell receptor. The B cells with high affinity for these TAAs encounter the antigen, engulf, lyse and display it on their cell surface for recognition and binding by activated T helper cells (Goodnow et al., 2005). Antigenic peptides ingested by antigen presenting cells are bound by class II MHC complex. The binding of the activated T cells to the B cells displaying TAAs signals further release of cytokines and chemokine, leading to B cell proliferation. A large number of lymphocytes against the same antigens are released. Some of these lymphocytes serve as memory cells, and others act as effector cells that produce plasma cells responsible for the systemic release of autoantibodies (Fig. 1). This antibody-TAA binding represents the end stage of humoral immunity facilitating the destruction of modified cells by opsonization and subsequent phagocytosis (Carter, 2001). Also, lack of clearance of apoptotic cells containing self-antigens activated the B cells and produced the autoantibodies.

3. The role of autoantibodies in cancer

Autoantibodies against TAAs present themselves as an efficient cancer biomarker (Pedersen and Wandall, 2011; Zhang et al., 2003). More recently, the use of autoantibodies toward TAAs is profoundly explored in different types of cancers. Accordingly, autoantibodies can be used as potential biomarkers for diagnosis, prognosis and therapeutic drug monitoring of cancers. Although a single autoantibody test lacks the required specificity and sensitivity for cancer screening and diagnosis, tumor-associated autoantibodies offer several distinct advantages including early detection, high stability, and minimally invasive monitoring of cancer.

3.1. Early detection

The immune response to TAAs occurs at an early stage during tumorigenesis. Hence, the autoantibodies produced against TAAs can be detected before any other symptom appears. Many studies have shown the potential of the early detection of autoantibodies even several years before the onset of the clinical symptoms of a malignancy (Chapman et al., 2011; Turnbull et al., 1978). This property of autoantibodies is of

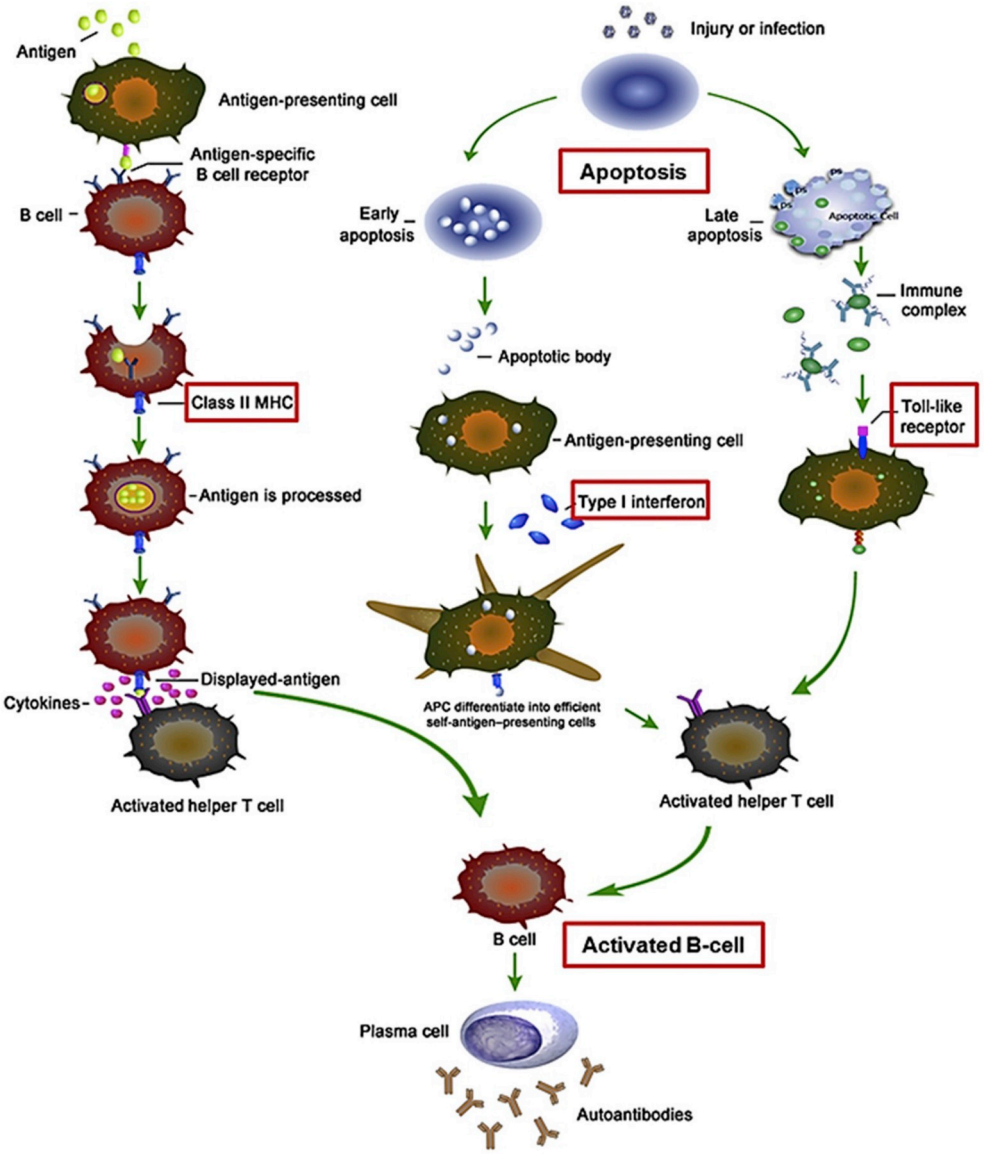

Fig. 1. Schematic illustration of cellular interactions inducing a humoral response. Antigens are presented on the antigen presenting cell and bound by B cell receptor. Antigenic peptides ingested by antigen presenting cells are bound by MHC II and presented on the cell surface. Activated helper T cell detects the antigen peptide complex on B cell, activating them by releasing cytokines. B cell differentiation yields plasma cells which secrete a large number of antibodies and memory B cells for a quicker future response. Also, the lack of clearance of apoptotic cells containing self-antigens plays a crucial role in autoantibody production. In addition to MHC class II complex, toll-like receptor and type I interferon are also regarded as the elements involved in autoantibody production. Reprinted with permission from (Wu et al., 2017).

most significant advantage, as cancer patient's mortality rate significantly decreases if the cancer is detected early. For example, the tumor suppressor gene p53 is one of the most frequently mutated genes in human malignant tumors, and the mutation of the p53 gene can occur before malignant cell transformation in some cancers. Li et al. demonstrated that p53 autoantibodies are significantly correlated with the development of malignancy by using 115 serum samples from a cohort of high risk for cancer (Li et al., 2005). The predictive value of p53 autoantibodies reached 0.76 with an average lead-time to the diagnosis of 3.5 years. Some studies also discovered that p53 autoantibodies had been isolated in smokers before the development of lung cancer (Schlichtholz et al., 1994). Recently, p53 autoantibodies have been demonstrated to be useful for the early diagnosis of many cancers, including colorectal, lung, breast, ovarian and cervical cancer (Anderson et al., 2010b; Pedersen et al., 2013; Soussi, 2000).

3.2. High stability

The autoantibodies are inherently stable, and tumor-associated autoantibodies can persist as long as the corresponding TAA triggers a specific humoral response as antibodies are not affected by proteolysis like other polypeptides. They remain in circulation with half-lives up to 30 days and are more stable outside the body compared to other biomarkers. This persistence and stability of the autoantibodies in the blood sample has an advantage over other biomarkers, including TAAs themselves, which may be rapidly degraded or cleared by the body (Tan et al., 2009). For example, the extracellular protein kinase A (ECPKA) autoantibody, a universal cancer biomarker was reported to be increased in various stages of different types of cancer such as bladder, breast, colon, lung, ovarian, prostate and pancreatic cancers when compared to healthy controls (Kita et al., 2004; Zaenker et al., 2016).

3.3. Non-invasive marker

Current techniques for cancer detection generally require highly invasive tissue biopsy or imaging techniques such as mammography for breast, computed tomography (CT) for lung. These techniques are time-consuming, expensive and associated with pain. Depending on the sensitivity of the employed screening method, these techniques are ill-suited for the early diagnosis of malignant cells (Nagayoshi et al., 2014). The autoantibodies against TAAs are found in the serum, plasma, saliva, cerebrospinal fluids of cancer patients where they are easily accessible for screening (Aggarwal, 2014; Arif et al., 2015). The wide distribution of autoantibodies in biological fluids makes them a

Table 1
Summary of key studies about the prognostic potential of autoantibodies in cancer. (Abbreviation: T.A.: Tumor Antigen).

Patient population	T.A.	Tumor type	Outcome	Reference
Patient with colorectal cancer (n = 40)	P53	Colorectal cancer	Twenty-seven (96%) of 28 patients, who had p53 autoantibodies and whose cancer was completely removed, had no such antibodies after resection and no recurrence after 7–26 months.	Takeda et al. (2001)
Breast cancer patients (n = 165)	P53	Breast cancer	Decreased survival. Survival was correlated with anti-p53 antibody level; the higher the antibody level, the shorter was the survival.	Lenner et al. (1999)
Invasive serous ovarian cancer (n = 60)	P53	Ovarian cancer	Increased survival. a p53 autoantibody is associated with a modestly favorable prognosis for serous ovarian cancers.	Anderson et al. (2010b)
Patients with ovarian cancer (n = 104)	P53	Ovarian cancer	Overall survival was increased for patients with antibodies to p53 when compared with patients without p53 antibodies.	Goodell et al. (2006)
Patients with colorectal tumors (n = 1209) 998 were diagnosed with colorectal adenocarcinoma and 211 with colorectal polyps	P53	Colorectal cancer	Decreased survival time for the patients who had high p53-Ab levels	Tang et al. (2001)
Patients with lung cancer (n = 125) 14 from small cell lung cancers (SCLC) and 111 non-SCLCs (NSCLC)	P53	Lung cancer	The p53 autoantibody-positive patients had a worse prognosis than the p53 autoantibody negative patients	Lai et al. (1998)
Group 1 (n = 50) patients who died of metastases within 7 years after complete surgical treatment; group 2(n = 50) patients who were matched with group 1 for six standard prognostic features	TA90	Melanoma	Decreased survival	Litvak et al. (2004)
Patients with glioblastoma (n = 62)	GLEA2	Glioblastoma	Prolonged survival of patients positive for GLEA2 autoantibodies with a median survival time of 17.4 months.	Pallasch et al. (2005)
Patients histopathologically diagnosed as invasive ductal cell carcinoma (IDC) of the pancreas (n = 36)	MUC1	Pancreatic cancer	The survival time of the patients was significantly longer	Hamanaka et al. (2003)
Patients with breast cancer (n = 133)	TF	Breast cancer	The high levels of anti-TF antibody before surgery are associated with better survival of stage II breast cancer patients.	Kurtenkov et al. (2005)
Patients with head and neck cancers (HNC) (n = 156)	HPV, E6, and E7	Head and neck cancer	Decreased recurrence occurred among those with antibodies to both E6 and E7	Kurtenkov et al. (2005)
Patients with esophageal squamous cell carcinoma (n = 134)	CDC25B phosphatase	Esophageal cancer	Decreased survival	Dong et al. (2010)
Patients with colon cancer (n = 58)	CEA	Colon cancer	Patients with detectable amounts of circulating IgM anti-CEA antibodies had a significantly better 2-yr survival compared to the rest of the patients	Albanopoulos et al. (2000)
Patients with breast cancer (n = 52)	CEA	Breast cancer	The presence of anti-CEA antibodies is associated with improved recurrence-free survival	Haidopoulos et al. (2000)

promising biomarker for less invasive diagnosis and more personalized monitoring of diseases. Also, the sample collection is more simplified as a result of the long half-life, i.e., seven days of the autoantibodies that minimize the hourly fluctuations.

4. Clinical applications of autoantibodies

4.1. Diagnostic potential of autoantibody biomarker

More recently, the use of autoantibodies toward TAAs is gaining much scientific interest as these TAAs have been detected in serum prior to the appearance of cancer clinical symptoms and may, therefore, serve as a diagnostic biomarker (Lu et al., 2008; Madrid, 2005). For instance, CA 15-3, CA 27-29 and CEA have used for the diagnosis of breast cancer; however, due to the low sensitivity and specificity, they have been used for advanced stage diagnosis and prognosis of cancer (Duffy, 2006). Some of the antibodies, for example, HER 2/neu have been detected in the early stage of breast cancer. Conversely, these antibodies can also be present in other types of cancer, thus limiting their application as a reliable diagnostic biomarker for breast cancer detection (Madrid, 2005). The addition of three TAAs, protein 16 (p16), p53, and avian myelocytomatosis viral oncogene homolog (c-myc) increases the sensitivity of the autoantibodies in breast cancer patients by 44% and specificity by 97.6%. For instance, Chapman et al. tested 94 healthy controls, 97 primary breast cancer sera, and 40 ductal carcinomas in situ (DCIS) sera against seven antigens including HER 2, c-myc, p53, BRCA1, BRCA2, NY-ESO-1, and MUC1 (Chapman et al., 2007). For the individual marker, the specificity of the assay was in between 91% to 98%. The individual autoantigen sensitivity was 3%–23% in the DCIS sera, 8%–24% in primary cancer sera. The sensitivity increased up to 64% for primary breast cancer and 45% for DCIS when the panel of autoantigens combined.

Following that, numerous studies have conducted to prove the potentiality of autoantibodies in early diagnosis of lung cancer. Brichory et al. reported the discovery of autoantibodies against protein gene product 9.5 (PGP 9.5) and annexins I and II in the patient with lung carcinoma (F. Brichory et al., 2001, F.M. Brichory et al., 2001). In this study, 60% of these patients reacted against glycosylated annexin I and II, but healthy controls showed no reaction. A study by Pereira-faca et al. demonstrated the14-3-3 theta autoantibody as a potential biomarker for the early diagnosis of lung cancer (Pereira-Faca et al., 2007). They tested the presence of this autoantibody in 45 patients with newly diagnosed lung cancer, 18 with pre-diagnosed lung cancer and 62 with healthy controls. In another study He et al., found the autoantibodies against alpha-enolase in 28% of 94 lung cancer patients (P. He et al., 2007). To date, CEA is the only serological biomarker for diagnosis of colon cancer. However, it is limited by low sensitivity and specificity (Liu et al., 2009). Many autoantibodies associated with colon cancer had been reported so far such as GA-733-2 (Mosolits et al., 1999), p53, Fas/CD95 (Reipert et al., 2005) and p16 (Cioffi et al., 2004). Reipert et al. investigated reactivity against first apoptosis signal (Fas) receptor in sera from 38 healthy controls, 38 patients with colorectal adenocarcinoma (Reipert et al., 2005). In the study, they did not find any reactivity against healthy controls. Reactivity to p53 had detected in precancerous lesions and people with high risk of colon cancer, for example, people with ulcerative colitis. However, autoantibodies to p53 have suggested as only a supplement to colonoscopy (Cioffi et al., 2004). Similarly, autoantibodies to TAAs have identified in the samples of the patients with hepatocellular carcinoma (HCC) at the early stage of liver diseases (Zhang et al., 2001). These TAAs are believed to be the potential biomarkers for the early detection of autoantibodies against the development of HCC malignancy. The more established HCC associated TAAs are p53 (Soussi, 2000) and p62 (Zhang and Chan, 2002). With more recent and extensive studies on the production of autoantibodies in cancer, they are attracting significant attention as potential biomarkers for cancer diagnostics (Tan and Shi, 2003).

4.2. The prognostic potential of autoantibodies

In addition to diagnostic significance, autoantibodies can also use for prognosis or monitoring response to therapy. Many autoantibodies have correlated with clinical prognosis (Anderson et al., 2010b; Hamanaka et al., 2003; Pallasch et al., 2005) (Table 1). Prognostic biomarkers are essential for targeting cancer therapies to high-risk populations. Recently, molecular prognostic markers are used for the differentiation of tumor cells/tissues with normal cells/tissues with similar cytology/histology. A tumor antigen-specific autoantibodies have also suggested as reporters of cancer progression. Nevertheless, very few autoantibodies have been assessed so far as prognostic biomarkers of cancer (Heo et al., 2012). The most commonly studied autoantibody to predict the prognosis of cancer is the p53 autoantibody. Cai et al. reported that monitoring the serum p53 antibodies level can be a useful tool for evaluating the response towards before and after the treatment of esophageal cancer (EC) patients with radiotherapy (Cai et al., 2008). They showed that the availability of p53-antibodies in patients' serum was associated with histological grade, stage of the disease and lymph node metastasis in EC.

Although the correlations between some autoantibodies and the prognosis of cancer patients have been established, it is still early to merely use autoantibodies to predict the therapy outcome in routine clinical practice. There are various challenges associated with the evaluation of prognostic autoantibody biomarkers. First, the clinical sample should be derived from the patients receiving uniform therapy before the onset of the treatment (Türeci et al., 2006). Large-scale and well-defined populations are needed to evaluate the prognostic value of autoantibody biomarkers systematically. Next, prognostic significance must be independent of recognized clinical variables, such as stage, grade, and molecular subtype.

4.3. The therapeutic target of autoantibodies

Depending on the cancer type and tumor status, different changes in the specific antibody titer had been demonstrated. Many studies suggest that TAAs can be the potential targets for immunotherapy (Knutson and Disis, 2005; Rosenberg, 1997). The mechanism of production of autoantibodies by the humoral immune response in cancer patients would help the development of novel therapeutics for poorly curable cancers. Based on the recognition by humoral or cellular immune responses in the autologous human host, tumor antigens have provided the cancer researcher with powerful tools to identify attractive new targets for vaccine-based therapies (Sangha and Butts, 2007). The immune system can act as a very sensitive reporter for identification of new altered proteins that are different from self-proteins. Therefore, this strategy may help to detect proteins that undergo alterations during the tumorigenesis. These proteins could become therapeutic targets. For instance, there is the occurrence of autoantibodies against a number of kinases in colorectal cancer as described by Babel et al. (2009). In most of the candidates, TAAs were kinase proteins, and these kinases are frequent targets for therapeutic drugs. The diversity of patient populations might result in different responses to immunotherapy. Hence, personalized profiles of TAAs and autoantibodies should be used to identify therapeutic targets to develop vaccines for targeted immunotherapy against cancer (Casal and Barderas, 2010). In addition, monoclonal antibodies such as an antibody against HER2/*neu* is used as a therapeutic agent against breast cancer (Slamon et al., 2001) and cancer-testis antigens are currently using for vaccination in cancer patients (Jäger et al., 1999).

5. Detection of autoantibodies: grand challenges

Having the significance potentiality of autoantibodies as biomarkers, several significant challenges still need to be considered and addressed. These challenges include biological, technical and clinical,

which are discussed more details in the following sections.

5.1. Biological challenges

A disease marker can be considered as an ideal biomarker, if it is readily available in an accessible body fluid, such as serum or plasma, can be measured accurately and can predict disease, can track response to therapeutic interventions, can predict side effect profiles and monitor disease progression and recurrence. While autoantibodies fulfill all these requirements, the application of autoantibodies is still limited in clinics due to several biological challenges, as described below.

5.1.1. Inhibition of generation of autoantibodies

The natural inhibition of production of autoantibodies has also considered as one of the major challenges of using autoantibodies as potential detection marker (Dudas et al., 2010). This inhibition of autoimmune response suppresses the formation of antibodies against the TAAs. Several mechanisms inside the human body inhibit the formation of antibodies against overexpressed proteins or cellular variations. One mechanism of inhibition is the production of TAAs in the epithelial cells of thymus compared to their natural production site like in the case of melanoma antigen. Melanoma antigens are usually expressed in the male testicular cells. Additional mechanisms associated with autoantibodies inhibitions are *i)* downregulation of antigen presenting cell, MHC class I molecules (Hofbauer et al., 2005) *ii)* lack of expression of co-stimulatory molecules on the tumor cells (Chen et al., 1993) *iii)* tumors producing immunosuppressive factors like TGF-, IL-10 and VEGF (Kusmartsev and Gabrilovich, 2006).

5.1.2. Insufficient immune response

The immunogenicity of a tumor depends on several factors that may be variable even among tumors of a similar type. Due to the difference in tumor size, level of expression and difference in PTMs and variability in the immunogenicity, it is challenging to consider autoantibody as a general marker for diagnostics (Kolch and Mischak, 2005). The level of expression, PTM, and variations in protein processing are of great importance in TAAs as they impart different immunogenicity to the same protein. Furthermore, the specific immune response to an antigen is also dependent on the structure of the highly polymorphous MHC molecules.

5.2. Technical challenges

5.2.1. Sample processing and choice of sample source

The concentration of autoantibodies can be affected by various pre-analytical factors, which need to be standardized such as blood collection, serum or plasma processing, the time interval between collection and centrifugation, and the storage conditions. The variability in the kinds of patients studied as well as inconsistency in withdrawing the blood from cancerous and control samples may also cause differences in levels of the particular analytes measured (McIntosh et al., 2008). For instance, longer storage duration of specimens could make bias for discriminating the prostate cancer from the noncancer group.

5.2.2. Limited comparability

Due to platform-dependent variation such as, some studies used healthy participants as controls, while the others used a mix of non-cancer patients which makes it difficult to draw comparisons between the studies (Dumstrei et al., 2016).

5.2.3. Nonspecific response from biomolecules

The clinical sample may have complex biological environments containing various unknown cells and other biomolecules. These molecules can non-specifically attached to the sensor surface resulting in false-positive responses. Therefore, a suitable blocking agent such as mercaptoethanol, mercaptohexanol, poly (ethylene glycol), or bovine serum albumin can be used to prevent nonspecific bindings (M.N. Islam et al., 2017). Some TAAs such as p53 and ECPKA are present in various types of cancer and thus lack discrimination power in diagnosing a specific type of cancer (Tan et al., 2009; Zaenker and Ziman, 2013).

5.2.4. Low sensitivity and specificity

Detecting a single autoantibody lacks the specificity and sensitivity required for cancer screening and diagnosis. Autoantibodies against particular TAAs are found in only 10–30% of patients (Solassol et al., 2011). This is due to the heterogenic nature of cancer. The same type of cancers are composed of different biological subtypes, and the patients develop an immune response to different sets of an antigen (Desmetz et al., 2011). Hence, no single protein is likely to detect a particular cancer type. Instead, a specific combination of autoantibodies could detect cancer such as prostate cancer better than the reference test (prostate-specific antigen (PSA) test) (Caron et al., 2007). The combination of TAAs showed an increase in specificity. However, it was not sufficient to build a reliable screening test. The sensitivity and specificity of autoantibodies also differ from a sample source and platform variation such as study design, the ethnicity of patients, sample types, detection methods, and so on.

5.2.5. Limited efficiency

The methods available are mostly focused on discovering the autoantigen and then probe for subsequent autoantibodies using the patient sera. It is also interesting to note that most of these techniques lack the capture of cancer-related PTMs (Baldwin, 1966; Mintz et al., 2003). It is crucial to detect these PTMs as they are one of the major contributors to the formation of autoantibodies. Technically, all the available assays are limited by ambiguity in the clinical application even if reactive autoantigens (or the corresponding autoantibodies) are detected. Protein microarrays can only provide a relative comparison between samples for each spot and do not provide any quantitative estimation of the level of autoantibody. In addition, large-scale clinical trials covering a wide range of population are necessary to validate the results of these methods when employed for biomarker discovery. This has opened avenues for modern immuno-assays to play an essential role in providing a stable and reliable method for autoantibodies detection.

5.3. Clinical challenges

To establish an autoantibody-based cancer detection, it requires large-scale clinical studies to evaluate the efficiency and reliability of the marker. For each targeted autoantibody, multiple numbers of cancer patients should be analyzed. Along with the cancer patients, the test should be evaluated from large numbers of healthy individual as controls. In follow up study, it is also essential to differentiate between the false positive signals from the true positives ones.

6. Cutting-edge advances in the detection of autoantibodies

6.1. Electrochemical approaches

The electrochemical technique is a highly suitable method for biomolecular analysis due to its inherent advantages, including high sensitivity and specificity, fast-responsive and compatibility with miniaturization, and relatively low-cost detection (Haque et al., 2017; Hossain et al., 2017). In electrochemical detection, a recognition element, *i.e.*, an antigen interacts with the target antibodies selectively that are present in the serum matrices. The electroactive molecules, tagged with a detection antibody are then employed to obtain a measurable electrochemical signal to quantify the number of antibodies. The readout is generally voltammetry (i.e., cyclic voltammetry, linear sweep voltammetry, differential pulse voltammetry, square wave voltammetry, and stripping voltammetry), amperometry, and impedimetric techniques.

Over the past few years, several electrochemical assays have been developed for the detection of autoantibodies in cancer. Recently, Asensio et al. developed a biosensor based on magnetic microcarriers modified with covalently immobilized HaloTag fusion p53 protein on an electrochemical detection system (Garranzo-Asensio et al., 2016). The biosensor utilizes two main steps: magnetic capture and electrochemical detection. In the first step, the magnetic microcarriers were modified with covalently immobilized HaloTag fusion p53 protein for the selective capture of specific autoantibodies against the immobilized protein present in the sera of patients. The attached autoantibodies were then detected with a HRP conjugated secondary anti-human IgG. After the magnetic capture of magnetic bead's bearing the immunocomplexes onto screen-printed carbon working electrodes, the bio-recognition event is monitored by amperometric readout (cathodic current) generated by the enzymatic reduction of H_2O_2 mediated by HQ, presenting the level of p53-autoantibodies in the sample. The proposed biosensor had been applied for the analysis of sera from 24 patients with high-risk of developing colorectal cancer and 6 from patients already diagnosed with colorectal and ovarian cancer. They demonstrated the potential of this method to detect the very low concentration of p53 autoantibodies with a limit of detection (LOD) of 0.34 U/mL. Recently, we have developed an electrochemical-based technology platform, where uniquely engineered gold loaded mesoporous iron oxide nanocube ((Au@$NPFe_2O_3NC$) were utilized to the capture the autoantibodies (p53) from clinical samples (Yadav et al., 2017). In this method, the advantageous of Au@$NPFe_2O_3NC$-based capture and purification were combined with inexpensive, and portable nature of the electrochemical and naked eye colorimetric readouts, as shown in Fig. 2. The nanocubes initially modified with the p53 protein and dispersed into the serum sample where they bound to p53-specific autoantibodies. After the magnetic isolation and purification of nanocube-attached target p53 autoantibody in an eppendorf tube, an horseradish peroxidase (HRP)-modified secondary antibody was used to recognize the autoantibody and catalyze the enzymatic oxidation of the 3,3′,5,5′-tetramethylbenzidine (TMB)/H_2O_2 system to generate detectable signals (colorless to blue). For electrochemical detection, the colored (yellow) solution was pipetted onto the SPGE electrode, and the amperometric response was measured This method has excellent sensitivity with the detection limit of 0.02 U/mL. However, this method relies on expensive HRP enzyme and suffers from low sensitivity.

In order to enhance the sensitivity and making it more cost-effective, the intrinsic peroxidase mimetic activity of Au@$NPFe_2O_3NC$ was utilized to replace HRP for signaling event (Masud et al., 2017). In this study, a neutravidin-modified screen-printed carbon electrode was functionalized with biotinylated p53. Next, serum/plasma samples containing p53-specific autoantibody were incubated onto the electrode surface followed by the incubation with IgG/Au−$NPFe_2O_3N_C$ nanocatalysts. The surface-attached nanocatalysts catalyzed the oxidation of TMB in the presence of H_2O_2 and produced a blue-colored complex product (naked eye), which turned into yellow after the addition of an acid to the reaction media, facilitates both colorimetric and electrochemical detection (LOD 0.08 U/mL) (Fig. 3). The naked-eye detection has additional advantages as a first-pass screening test and can easily integrate into a low-cost point of care (POC) diagnostic device and primarily be applied to screen a large population for potential cancer detection in developing countries. Having cost-effectiveness, portability, and potentiality of the rapid screening test or quantitative evaluation, the reported assay opens a new pathway for diagnosis in clinics.

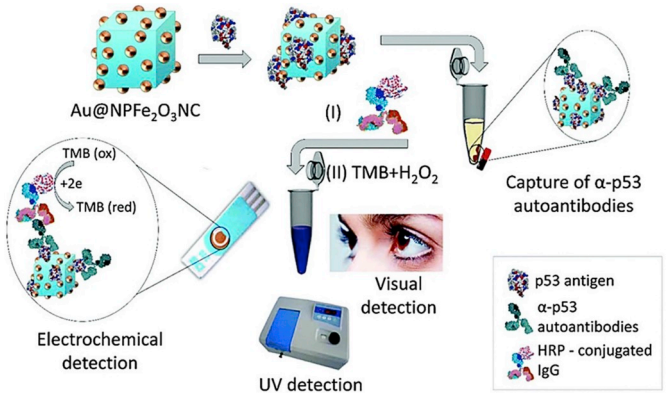

Fig. 2. Schematic representation of the electrochemistry-based detection of p53 autoantibodies. p53-Functionalized Au@$NPFe_2O_3NC$ were used as "dispersible nanocapture agents' for capturing the target autoantibodies in serum. After magnetic purification and separation, these bionanoconjugates were treated with HRP-IgG antibodies and TMB-substrate solution. The level of autoantibody concentration against the p53 antigen was detected by the naked eye, UV–vis and electrochemical detection techniques. Reprinted with permission from (Yadav et al., 2017).

6.2. Optical approaches

Surface plasmons resonance (SPR) is one of the most common label-free optical techniques used for clinical and medical diagnostics. It allows real-time and quantitative detections of interaction among biomolecules. Although it is a label-free technique, direct detection of analytes is possible using a surface plasmon resonance (SPR) sensor. Also, sandwich assays similar to ELISA involving secondary antibodies for either verification or amplification of the sensor signal can also be performed. Furthermore, it offers the analysis of receptor–target interactions with a wide range of molecular weights and affinities (Nguyen et al., 2015). Recently, SPR sensors have been applied to many clinical and medical diagnostics (Englebienne et al., 2003; Thaler et al., 2005). In SPR, surface immobilized molecular interaction between target autoantibodies and specific bioreceptor causes a change in the refractive index, which is measured by the transducer in real-time (Li and Li, 2019) (Fig. 4). The biosensor is based on wavelength modulation and the Kretschmann geometry of the attenuated total reflection method (Zhang et al., 2013). Soler et al., designed a nanoplasmonic based biosensor for the detection and quantification of a specific colorectal cancer-related autoantibody (Soler et al., 2016). The method employs a refractometric nanoplasmonic biosensor whose configuration is based on the localized surface plasmon resonance (LSPR) of gold nanodisks. The gold nanodisks were fabricated by hole-mask colloidal lithography that gives reproducible results, with a controlled density of disks on the surface. The method is sensitive to refractive index changes, which can detect as variations of the LSPR that permits the real-time monitoring of the biorecognition events under label-free conditions. Since the method does not need any labels or sample treatment, it offers rapid and sensitive detection of autoantibodies in serum or plasma. The performance of the biosensor assay has demonstrated for the determination of autoantibodies against two crucial CRC antigens: GTF2b and EDIL3. The study shows limits of detection around 1 nM (150–160 ng mL^{-1}). Ladd et al. also used surface plasmon resonance sensors to detect autoantibodies against carcinoembryonic antigen (CEA), an established biomarker for germline cancer. The sensor was able to differentiate between the cancerous samples and the controls with a confidence interval of 95% when benchmarked against ELISA (Zhang et al., 2013).

On the other hand, surface enhanced Raman spectroscopy (SERS) based technique is proved a powerful tool that allows sensitive detection of low concentration analytes through the amplification of electromagnetic fields generated by the excitation in metallic nanostructures of SERS substrates. This technique has been used for analyzing different biomarkers including autoantibodies for rheumatoid arthritis (Chon et al., 2014). Recently, a SERS-based immunoassay using functional nano-tags has become a promising alternative for sensitive detection in ELISA (Han et al., 2008). This technique has attracted significant attention because of its rapid and sensitive detection capability.

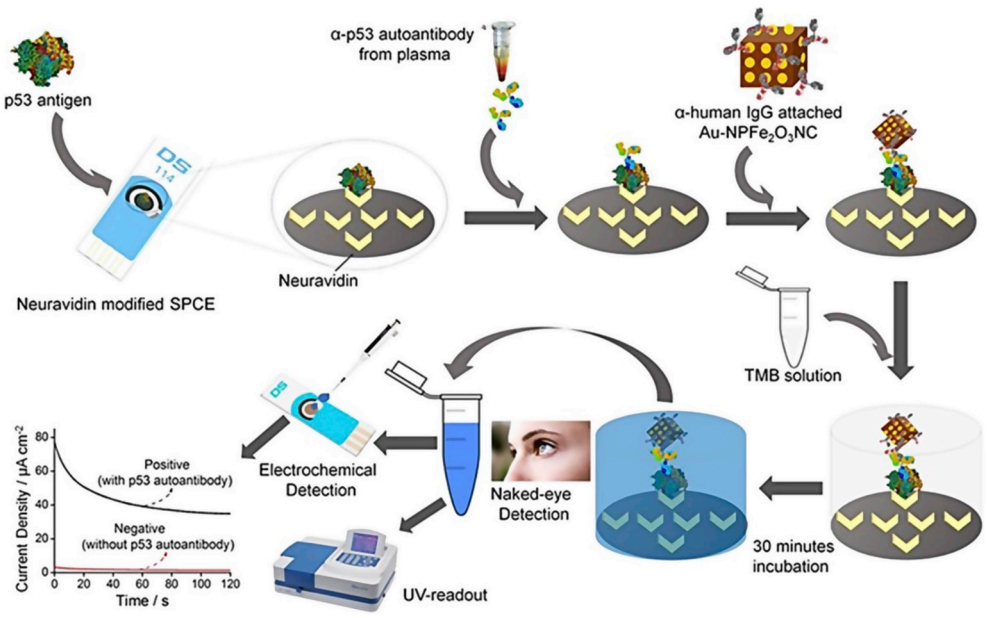

Fig. 3. Schematic representation of the electrochemistry-based detection of tumor-associated p53 autoantibody. A neuravidin-modified screen-printed carbon electrode was functionalized with biotinylated p53. Serum/plasma samples containing p53-specific autoantibody were then incubated onto the electrode surface followed by the incubation with IgG/Au−$NPFe_2O_3$NC nanocatalysts. The surface-attached Au−$NPFe_2O_3$NC nanocatalysts catalyzed the oxidation of TMB in the presence of H_2O_2 and produced a blue-colored complex product (naked eye), which turned yellow after the addition of an acid to the reaction media. The level of p53 autoantibody was detected via measuring the intensity (UV–vis) and amperometric current generated by the yellow product. Reprinted with permission from (Masud et al., 2017). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Chon et al. developed a method for anti-cyclic citrullinated peptide (CCP) autoantibodies using a SERS-based magnetic immunosensor (Chon et al., 2014). In the method, sandwich immunocomplexes, including magnetic beads were immobilized on the wall of a microtube using a magnetic bar, and then their SERS signals were measured. This technique overcomes the slow immunoreaction problems caused by the diffusion-limited kinetics on a solid plate since the reaction occurs in solution. The assay time was less than 1 h with the lower detection limit of 13 pg mL^{-1}. Although this assay was reported to demonstrate the autoantibodies against rheumatoid arthritis, its ability to measure target antibodies up to picogram concentrations suggests that the device may further be applied for detection of autoantibodies against TAAs for early diagnosis of cancer.

6.3. Microfluidic approaches

In recent years, microfluidic-based technologies have shown great promise in producing novel manipulation techniques for biological applications (Kashaninejad et al., 2016, 2018). Compared to conventional methods, microfluidics-based immunoassays entails several advantages including less amount of reagents and samples (down to femtoliters) consumption, automation (fluid handling steps is integrated), high potential for multiplex detection with minimal sample handling and processing. This technique can speed up antibody-antigen interaction due to high surface-area-to-volume ratios and smaller length scales, and improve analytic efficiency by reducing analysis time and sample volume, enhance sensitivity and selectivity and allow for

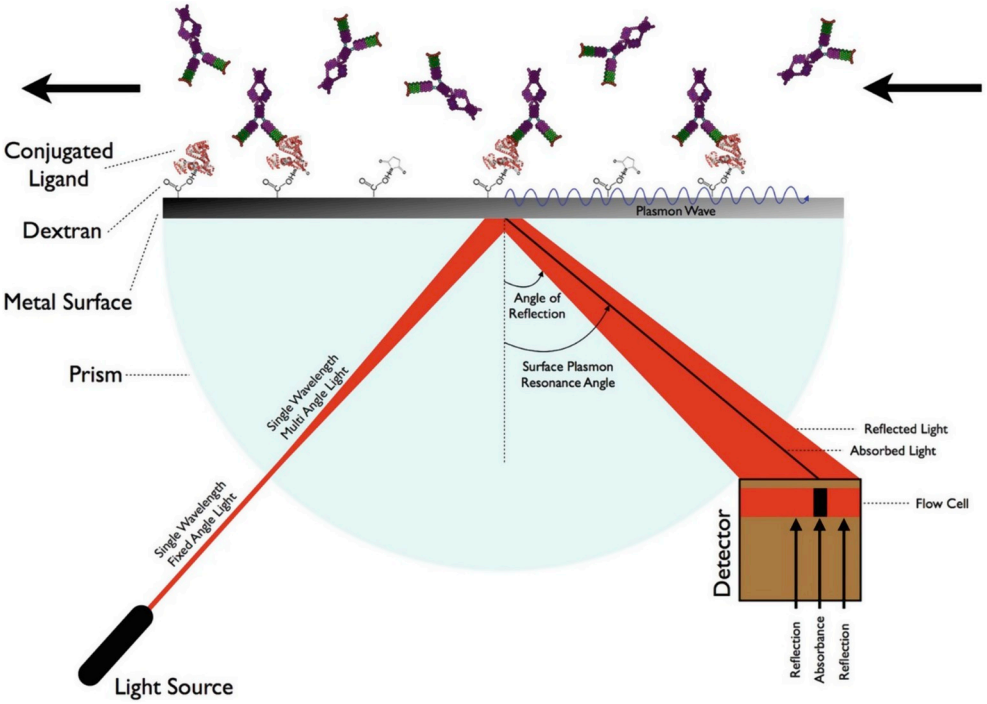

Fig. 4. The basic structure of the SPR chip, where dextran modified a gold or silver metal chip was employed to immobilize the target. The monochromatic laser beam enters the prism and generates different metal angles. The metals absorb and change transform their energy into plasma waves. Interactions of the immobilized target change the equivalent amount of resonance angle and hence gives the target specific SPR signals. Reprinted with permission from (Li and Li 2019). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

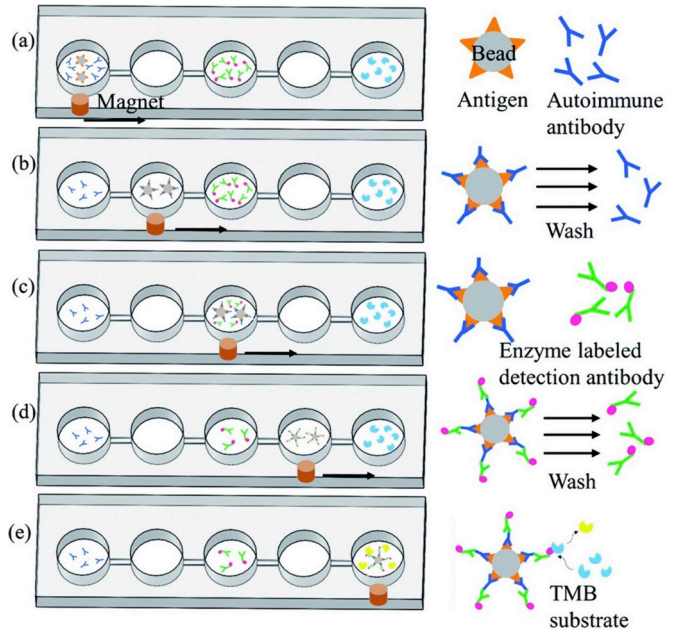

Fig. 5. Schematic representation of microfluidics immunoassay chip for autoantibody detection. p53 antigen modified magnetic bead capture target p53 from saliva sample (a) followed by magnet beads washing and purification in next well (b). HRP conjugated anti-human IgG antibody was then employed to form the immunocomplex (c); which were subsequently pass to next well and washed to remove loosely attached or unbounded HRP/IgG (d). At the fifth well, TMB substrate solution was added to get target specific signal (blue color generation) using a microplate reader (e). Reprinted with permission from (Lin et al. 2018). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

miniaturization of analytic devices (Kricka, 1998). These advantages make microchips to be widely used in clinical diagnosis (Länge et al., 2006), biomolecular separation (X. Chen et al., 2007) and cell handing systems (Yi et al., 2006). Recently, Hu et al. developed a microfluidic protein chip for an ultrasensitive and multiplexed assay of Cancer Biomark. (Hu et al., 2009). In the study, aqueous-phase-synthesized CdTe/CdS quantum dots (aqQDs) employed as fluorescent signal amplifiers to improve the detection sensitivity. They designed a versatile fluorescent probe by conjugating secondary antibodies, aqQDs, and found that the aqQD-based protein chip could rapidly detect carcinoma embryonic antigen (CEA) and alpha-fetoprotein (AFP) with high sensitivity and selectivity, even in human serum and in the format of both sandwich immunoassay and reverse phase immunoassay. Lopez-Munoz et al. have introduced simple label-free integrated plasmonic biosensor based on commercial Blu-ray discs (López-Muñoz et al., 2017). Blue-ray discs are top-down fabricated optical discs generate the plasmonic effects in the visible range. With the integration of this nanostructured plasmonic biosensor based on Blu-ray discs with microfluidics, they achieved pM detection limit for the detection of colorectal cancer-specific antibodies against the GTF2b proteins. Recently, a microfluidic chip combining magnetic immunoassay was reported to detect p53 autoantibodies present in saliva (Fig. 5). Each reaction well of the chip was separated by microvalves to avoid cross-contamination and enable magnetic bead-based target guiding. After forming an immunocomplex with target p-53 and HRP-labelled antibody, TMB substrate solution was employed to read out the signals. However, besides superior advantages of microfluidic platforms, some difficulties need to considered and addressed such as complete automation, user-friendly operation, inexpensive and easy platform, and portability before applying them in clinics.

7. Conclusion and future perspectives

This review highlighted the recent advances in the detection of autoantibodies. We also addressed the significant technical and biological challenges of these strategies. The primary challenge with most of the proteomics-based techniques is that they fail to detect antigens with PTMs. Some advanced, simple, non-invasive screening methods are expected in the clinical practice to detect more informative targets including proteins and relevant PTMs. We also focused on the cutting-edge advances in the detection of autoantibodies using electrochemical, optimal and microfluidic approaches. Considering the potential of microfluidic platforms, in particular, droplet-based microfluidic, along with the recent advances in single cell screening and analysis, we envision that more advanced microfluidic-based systems will be developed for autoantibody detection and therapeutic monitoring.

Current research in this field is still an emerging stage. Given the limited diagnostic potential for single markers, the combination of multiple markers is needed to enhance the overall sensitivity. Detection of autoantibodies against cancer is a promising biomarker for early diagnosis of cancer if a sensitive and specific tool is developed. Novel nanoparticle-based technologies have proved to be a powerful tool for autoantibodies detection in cancer patients. The nanostructure-based platform possesses the potential to be further miniaturized and developed into POC diagnostic devices for easy quantification of autoantibodies. Considering the enormous potentials of autoantibodies against TAAs in cancer diagnosis, more attention needs to be paid to develop an efficient and reliable biosensor. We believe that the final requirement for translating the existing approaches to the POC platform is the development of fully automated and integrated biosensors capable of high-throughput screening of autoantibodies in clinical samples.

CRediT authorship contribution statement

Sharda Yadav: Data curation, Investigation, Writing - original draft, Writing - review & editing. **Navid Kashaninejad:** Writing - original draft. **Mostafa Kamal Masud:** Writing - original draft. **Yusuke Yamauchi:** Writing - original draft. **Nam-Trung Nguyen:** Writing - original draft. **Muhammad J.A. Shiddiky:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing - original draft, Writing - review & editing.

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