Review article

Paraneoplastic antigens as biomarkers for early diagnosis of ovarian cancer

Madhumita Chatterjeea, Laura C. Hurleya,b, Michael A. Tainskya,b,c,d,*

a Department of Oncology, Wayne State University School of Medicine, Detroit, MI 48201, United States
b Cancer Biology Graduate Program, Wayne State University School of Medicine, Detroit, MI 48201, United States
c Molecular Therapeutics Program, Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI 48201, United States
d Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI 48201, United States

A R T I C L E   I N F O

Keywords:
Ovarian cancer
Paraneoplastic neurological syndrome (PNS)
Onconeural autoantibodies
Onconeural antigen
Tumor associated antigen (TAA)
Diagnostic biomarker

A B S T R A C T

Paraneoplastic syndromes are a group of rare disorders that can be triggered by an abnormal immune response to proteins from tumors of the lung, ovary, lymphatics, or breast. Paraneoplastic clinical syndromes affect < 1% of patients with cancer; however, the frequency of subclinical levels of paraneoplastic autoantibodies in asymptomatic patients with cancer is unknown. Numerous studies have reported that ovarian cancer patients show signs of paraneoplastic neurological syndromes (PNSs) before or after their cancers are diagnosed. PNSs arise from a tumor-elicited immune response against onconeural antigens that are shared by tissues of nervous system, muscle, and tumor cells. Studies on the serum IgGs obtained from ovarian cancer patients have indicated the presence of onconeural antibodies in the absence of any PNS symptoms. The occurrence of PNSs is low in ovarian cancer patients and it can be accompanied by onconeural antibodies. The diagnosis of PNSs is accompanied by a suspicion of a malignant tumor such that neurologists typically refer such patients for a tumor diagnostic workup. There will be tremendous utility if subclinical levels (without paraneoplastic neurological symptoms or myositis) of these autoantibodies to paraneoplastic antigens can be exploited to screen asymptomatic high-risk patients for ovarian cancer, and used as markers in immunoassays for the early detection or recurrence of ovarian cancer. Ovarian cancer overall survival is likely to be improved with early detection. Therefore, a panel of onconeural antigens that can detect paraneoplastic autoantibodies in patient sera should provide diagnostic utility for an earlier therapeutic intervention. Here we review the usefulness of PNS and other paraneoplastic syndromes and their association with paraneoplastic antigens to exploit these autoantibody biomarkers to form diagnostic multi-analyte panels for early detection of ovarian cancer.

1. Introduction

1.1. Historical background of the discovery of paraneoplastic syndromes

Paraneoplastic syndromes are rare heterogeneous disorders that are characterized by the presence of endocrinological, neurological or dermatological syndromes. These disorders arise from the secretion of hormones from the tumor, or can be an autoimmune response elicited by tumor cells against onconeural antigens common to both the nervous system and to an underlying tumor (Pelosof and Gerber, 2010). The occurrence of paraneoplastic symptoms leads physicians to explore for the presence of cancer as the symptoms can appear prior to clinical manifestation of cancer. In 1825, Armand Trousseau first described the existence of a paraneoplastic syndrome called “Trousseau’s Syndrome” in a gastric cancer patient who was also diagnosed with venous thrombosis. It has been reported that pancreatic, lung, and gastric cancer are associated with this syndrome, which typically appears months to years before the clinical diagnosis of a tumor (Callander and Rapaport, 1993). Hermann Oppenheim in 1888 was the first to suggest that neurological symptoms in patients with cancer could be directly connected to the underlying tumor (Schulz and Pruss, 2015). In 1912, Harvey Williams Cushing reported an endocrinological syndrome caused by a malfunction of the pituitary gland which he termed “Cushing’s syndrome” (Cushing, 1994). Li et al. reported the incidence of Cushing’s syndrome due to the presence of a multiple endocrine neoplasia type-1 (MEN-1) associated thymic neuroendocrine tumor (Th-NET). In 1948, Derek Ernest Denny-Brown documented a case study of two patients who had primary simple degeneration of the dorsal root ganglion cells associated with a primary degeneration of the muscles called “polymyositis”. Both of the patients who presented symptoms of severe neuropathy and ataxia had previously been diagnosed with bronchogenic pulmonary carcinoma (Denny-Brown, 1948). In 1929, Casper and in 1951, Brain et al. reported case studies that demonstrated the association of subacute cortical cerebellar
degenerated with cancer (Brain et al., 1951). In 1968, Corsellis et al. defined “paraneoplastic limbic encephalitis” (PLE) in a study of three patients in which one patient developed memory loss that increased over a period of months and the two other patients had bronchial carcinoma associated with dementia (Corsellis et al., 1968). In 1985, Graus et al. reported the presence of neuronal antinuclear autoantibodies in four patients with subacute sensory neuropathy and small cell carcinoma of the lung (Graus et al., 1985).

The discoveries of various endocrinological, neurological and dermatological syndromes that are caused by the underlying cancer, have led neurologists to coin the term “paraneoplastic syndromes”. Paraneoplastic neurological disorders occur in the central or peripheral nervous system and can result in muscle weakness and brain degeneration, leading to immobility and death. Clinical symptoms of paraneoplastic syndromes may include loss of muscle tone, slurred speech, memory loss, vision problems, dementia, ataxia, seizures, and sensory loss in the limbs. An international panel of PNS experts has recommended consensus criteria for diagnosis of PNS (Graus et al., 2004). Paraneoplastic syndromes, if diagnosed correctly by the physician, may help to diagnose an underlying cancer before its clinical symptoms. The epidemiology of the paraneoplastic neurological disorders varies from cancer to cancer. Studies have shown that approximately 0.5–1% of all cancer patients have a clinically diagnosed PNS (Rees, 2004). This review will focus on the incidence and association of various PNSs with ovarian cancer, the pathogenesis of PNS in ovarian cancer, and the potential for onconeural antibodies to be useful tools to detect ovarian cancer at an early and potentially curable stage.

2. Incidence of paraneoplastic syndrome in ovarian cancer

Symptomatic paraneoplastic disorders are rare in patients who have gynecological cancers, and the incidence of occurrence is approximately 1 per 1000 new cases (Rees, 2004). PNSs that are most commonly associated with ovarian cancer include paraneoplastic cerebellar degeneration, dermatomyositis and polymyositis (Zahr and Baer, 2011). Women presenting with these syndromes are referred for evaluation of ovarian cancer. It has been reported that 0.1% of patients with subacute cerebellar degeneration develop ovarian carcinoma (Abrey and Dalmau, 1999). Symptoms of paraneoplastic cerebellar degeneration include ataxia, lack of balance, speech dysfunction, and nystagmus. Polymyositis is an inflammatory myopathy resulting in muscle weakness, and is associated with dermatomyositis, in which inflammation manifests in skin rashes and can co-occur with muscle weakness. Patients with either syndrome are at higher risk for malignancy.

In 1965, a survey of incidence of carcinomatous neuromyopathy in cancer patients was reported by Croft and Wilkinson. In the survey, out of 55 patients with ovarian cancer, only 9 patients were reported to have carcinomatous neuromyopathy (Croft and Wilkinson, 1965). Dalmau et al. assessed 121 neurologic consultation reports that were obtained from 83 ovarian carcinoma patients who were seen between 1993 and 1996. In that study, 38 patients were reported to develop peripheral neuropathy after the completion of chemotherapy. PNSs were observed in 4 patients; 1 patient was diagnosed with dermatomyositis and 3 patients were diagnosed with subacute cerebellar degeneration (Abrey and Dalmau, 1999). In 2010, a population based European study was reported by Giometto et al. that represented PNS association of 979 patients (968 patients had definite PNS and 11 patients had possible PNS; 899 patients had data available) recruited between 2000 and 2008 by applying the diagnostic criteria provided by Graus et al. (Graus et al., 2004). Analyses of the data that was collected from PNS Euro network database showed that 94 out of these 899 (10.5%) had ovarian cancer patients associated with a PNS (Giometto et al., 2010). In 2001, Hill et al. reported a pooled analyses of the incidence of dermatomyositis and polymyositis in cancer patients using the national data obtained from Swedish National Board of Health that spanned between 1964 and 1983, Finnish National Board of Health from 1969 to 1985, and Danish Hospital Discharge Registry from 1977 to 1989. Their study population was comprised of 618 patients with dermatomyositis, out of which 198 had cancer and 115 out of 198 patients were diagnosed with dermatomyositis prior to the cancer development. Their study showed that the standardized incidence ratio (SIR) was 10.5, at 95% confidence level (CI (6.1–18.1)) for women who developed dermatomyositis prior to diagnosis of ovarian cancer, indicating a strong association of dermatomyositis prior to symptoms of ovarian cancer (Hill et al., 2001).

3. Presentation of paraneoplastic syndromes with ovarian cancer

Numerous studies detailed the findings of PNSs with ovarian cancer. In 2000, Forgy et al. documented a case report of a patient who was diagnosed with two PNSs, nephrotic syndrome and paraneoplastic cerebellar degeneration prior to the diagnosis of ovarian cancer. CT scan and ultrasound were performed later for the evaluation of malignancy that revealed the presence of 5 cm ovarian mass and multiple paraaortic lymph nodes. Tirmaz et al. reported a case history of a 75-year-old woman with stage III serous ovarian carcinoma who developed peripheral mixed sensory and motor neuropathy after surgery and chemotherapy. Eleven months after her last chemotherapy, the PNSs persisted. She developed ataxia and pseudoathetosis beside other prevailing PNSs (Tirmaz et al., 2014). Li et al. presented a case study of a 37-year-old woman who developed paraneoplastic cerebellar degeneration and limbic encephalitis following Hepatitis-B vaccination. The symptoms included ataxia, slurred speech, involuntary movements of arms, depression, aggressive behavior, dysphagia and hypomnesia. The occurrence of paraneoplastic Yo antibodies were confirmed in both CSF and serum with a concurrent serum CA125 highly elevated at 2752 U/ml. The patient was diagnosed with high-grade, stage IIc ovarian serous papillary cystadenocarcinoma. After the completion of chemotherapy and cytoreductive surgery no ovarian cancer recurrence was observed after 38 months from diagnosis (Li et al., 2015). Hong et al. reported a case study of a 48-year-old patient who had multiple erythematous skin rashes on her face, forehead, knuckles and anterior chest area. Bilateral knuckles were found to have Gottron’s papules with impairment of her speech and hearing as well as weakness in muscular strength around her shoulders and leg with concurrent increased levels of aspartate aminotransferase (148 IU/l), alanine aminotransferase (130 IU/l), total creatine kinase (1190 U/l) and CA125 (543 IU/ml). The patient was diagnosed with dermatomyositis. Based on transvaginal ultrasound and MRI, the patient underwent optimal debulking, total hysterectomy and bilateral salpingo-oophorectomy due to the presence of stage IIIC high grade ovarian serous carcinoma. After the surgery, all PNSs disappeared (Hong et al., 2015). Scholz et al. reported a case study of a 45-year-old woman who had problem walking because of the development of generalized shivers. She was diagnosed with opsoclonus, associated with conjugated eyes that showed movement arrhythmically in all directions. Myoclonic speech associated with dysarthria was noted. PNSs, such as truncal ataxia and limb ataxia were also observed with elevated levels of carcinoembryonic antigen (5 ng/ml) and CA125 (2161 units/ml). The presence of tumors on both her right and left ovaries was confirmed after an abdominal CT and laparotomy with a diagnosis of stage IIIc ovarian carcinoma (Scholz et al., n.d.). Appearance of Cushing syndrome in a 61-year-old woman prior to the diagnosis of ovarian cancer has been reported. Two-site immunoradiometric assay (IRMA) was performed to measure the levels of Adrenocorticotropic hormone (ACTH) and its precursors with blood serum and urine analysis revealing the elevated CA125 level (214.6 U/ml) and cortisol level (496 nmol/24 h). Pelvic ultrasound followed by laparotomy confirmed the presence of multicystic right ovarian mass confirmed at surgical resection as ovarian carcinoma. Hydrocortisone treatment followed by chemotherapy resulted in a long disease free interval as indicated by her post 4 years of follow-up (Al Ojaimi, 2014).
In another case study, a 60-year old woman was first diagnosed with non-seminomatous ovarian cancer and later developed paraneoplastic syndrome, Acrokeratosis paraneoplastica. Ovarian cancer was suggested by an abdominal CT scan and by the rise in CA125 value (305 U/ml). Before undergoing surgery, she also developed laminar hyperkeratosis on both of her feet as confirmed by physical examination and punch biopsy of her skin (Hempen et al., 2015). Masui et al. reported the diagnosis of paraneoplastic Trousseau’s syndrome in a 46-year old woman who had ovarian cancer. The patient presented multiple infarctions in her right brain, pulmonary embolism and deep vein thromboses in the area of right renal infarctions (Saho et al., 2014). Zivaljevic et al. reported a case study of a 50-year old woman who was suffering from muscle weakness and pain in her arms and shoulders. She was diagnosed with Eaton-Lambert syndrome. Treatments for PNS greatly improved her condition. She was later diagnosed with ovarian cancer (Zivaljevic et al., 2005). In 2015, Batycka-Baran et al. reported a case study of a 70-year old woman who was suffering from a very rare paraneoplastic syndrome called, erythema annulare centrifugum, for 3 years as revealed by erythematous lesions on the upper regions of thighs. She also had a history of colon cancer but did not have recurrence after surgery followed by chemotherapeutic treatments. Further blood tests revealed elevation of CA125 level and the reports from transvaginal ultrasound indicated the presence of cystic tumor on the left ovary that was later confirmed by serous papillary adenocarcinoma G2 by histological examination. After the surgery was performed, her skin lesions resolved (Batycka-Baran et al., 2015).

As paraneoplastic symptoms occur long before the diagnosis of cancer, accurate diagnosis of specific type of paraneoplastic syndrome by a neurologist can provide an indication of ovarian cancer. Further confirmation of the ovarian cancer diagnosis come from transvaginal ultrasound, abdominal PET-CT scan followed by blood test for detecting levels of CA125, as well as both blood and CSF for testing for the presence of specific onconeural antibodies. A precise diagnosis of paraneoplastic syndrome can be extremely beneficial for early detection of ovarian cancer that might be helpful in initiating antineoplastic treatments, overall survival and paraneoplastic syndrome management.

4. PNS pathogenesis in ovarian cancer

The development of PNS is commonly a result of autoimmunity. The tumor associated antigens (TAAs) can elicit an immune response to onconeural antigens expressed in certain normal tissues, such as neuronal or muscle tissues breaking immune tolerance and resulting in autoimmunity. Only a fraction of tumor-bearing patients with elevated titers of paraneoplastic autoantibodies will develop PNS. Paraneoplastic onconeural antigens are shared by nervous tissues and ovarian tumors. The pathogenesis of myositis is reported to be mediated by cytotoxic T-cells, as evidenced by an infiltration of CD8 + T-cells in the muscle of myositis patients, which are recruited by local inflammation (Carstens and Schmidt, 2014). Dermatomyositis symptoms are caused by immune-complexes binding to endothelial cells, activating the complement system and resulting in cell lysis and capillary destruction through the membrane attack complex (Carstens and Schmidt, 2014). Darnell et al. reported that the tumor-specific expression of CDR2 in neurologically normal patients with ovarian cancer. In this study, tumor specimens lyses were prepared from 20 ovarian cancer patients were probed with sera obtained from paraneoplastic cerebellar degeneration patients on western blot and 13/20 tumor lyses showed the expression of CDR2 (Yo protein) both in cerebellar neuronal tissue and ovarian tumors (Darnell et al., 2000). Expression of CDR2 was also observed in ovarian cancer patients who had no clinical manifestation of paraneoplastic cerebellar degeneration or circulating anti-Yo antibodies (Darnell et al., 2000). Therefore, the expression of onconeural antigens and their association with their respective antibodies does not always associate with the appearance of PNS (Darnell et al., 2000). Cross-reactivity of tumor and nervous tissue alone is insufficient to cause a PNS and other factors are necessary in PNS development including enhanced cytokine production, increased MHC-I expression, and infiltration of CD8+ T-cells to the tissue site (Dalkas, 2004). The occurrence of antibodies in serum and cerebrospinal fluid (CSF) that recognize the antigens shared by neurons and tumor cells has been reported. Symptoms directly resulting from antibody effects can be caused by additional antigen targets located extracellularly on the cell membrane. For example, CDR2L (a member of CDR family) has 50% sequence homology to CDR2 and its expression has been observed in both ovarian tissue and cerebellar Purkinje cells. Eichler et al. has reported that in a study population comprising patients with ovarian cancer, breast cancer and PNS patients with Yo-positive antibodies, those patients who had paraneoplastic cerebellar degeneration, harbored antibodies directed against both CDR2 and CDR2L (Eichler et al., 2013). For cytoplasmic antigens, PNS development has been reported to be T-cell mediated as directly demonstrated by detection of paraneoplastic Yo antigen specific cytotoxic T-cells in the blood sample of patient with paraneoplastic cerebellar degeneration (Albert et al., 1998).

5. Clinical use of paraneoplastic syndrome associated antibodies for diagnosis of ovarian cancer

Onconeural antibodies are more important in the diagnosis and management of PNS. These antibodies are often specific for the PNS-associated malignancy rather than for a particular neurological syndrome. Multiple antibodies may coexist in a given cancer patient. While investigating a patient for suspected PNS, the entire range of onconeural antibodies should be analyzed for proper diagnosis. As the immune system is implicated in PNSs, autoantibodies detected in patient sera may represent reliable diagnostic analytes. While a significant portion of patients with PNSs will have an underlying tumor, a small fraction of tumor-bearing patients has PNS. However, the absence of a PNS does not signify an absence of paraneoplastic autoantibodies. Cancer patients without PNS can harbor detectable onconeural autoantibodies, although at a lower titer than those patients presenting with a PNS. Therefore, paraneoplastic antibodies represent a stable analyte for the early detection of ovarian cancer. Numerous reports have documented the presence of different onconeural antibodies in ovarian cancer patients which are listed in Table 1.

5.1. Paraneoplastic cerebellar degeneration, paraneoplastic encephalomyeloneuropathy and encephalomyelitis-associated onconeural antibodies in ovarian cancer

Yo antibody is also known as Purkinje cell cytoplasmic antibody type 1 (PCA-1), is targeted against CDR2 antigen that has been shown to be expressed in both tumor cells and Purkinje cells, and is likewise associated with ovarian cancer and paraneoplastic cerebellar degeneration. Monstad et al. determined the prevalence of Yo antibodies in a study population comprising 557 ovarian cancer patients and 253 breast cancer patients, few of which were associated with PNS. The frequency of Yo antibody association with ovarian cancer was found to be 13/557 (2.3%), as opposed to 4/253 (1.6%) patients with breast cancer. Only 2/13 ovarian cancer patients had paraneoplastic cerebellar degeneration prior to diagnosis of ovarian cancer (Monstad et al., 2006). For paraneoplastic autoantibodies to be clinically useful for ovarian cancer diagnostics, panels of multiple antibodies will need to be employed. Karasnoudis et al. reported a case study of a 60-year patient who initially had paraneoplastic cerebellar degeneration. After performing paraneoplastic antibody screening, only Zic4 antibody titer in serum was found to be elevated. CSF also showed presence of Zic-4 antibodies. Thoracic and abdominal CT scans revealed the presence of a tumor in the right ovary and diagnosis of ovarian adenocarcinoma was confirmed (Karasnoudis et al., 2011). Hofterberger et al. reported the appearance of carbonic anhydrase-related protein VIII (CARP VIII)
### Table 1

| Paraneoplastic syndrome in association with ovarian cancer | Onconeural antibodies targeting paraneoplastic antigens in PNS-associated ovarian cancer | Function of paraneoplastic antigens that elicit immune responses to generate onconeural antibodies | NCBI reference sequence accession number | References |
|----------------------------------------------------------|------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|----------------------------------------|-----------|
| Paraneoplastic cerebellar degeneration                    | Yo antibody or Purkinje cell cytoplasmic antibody type 1 (PCA-1) targets CDR2 antigen | CDR2 interacts with c-myc and downregulates c-myc dependent transcription in tumor cells. CDR2 is involved in mitotic cell division. | NM_001802                              | O'Donovan et al. (2010); Takanaga et al. (1998) |
| Paraneoplastic cerebellar degeneration                    | Zk-4 antibodies                                                                   | Zk proteins are transcription factors that have zinc finger domains that bind DNA of the target genes to regulate their transcription activity. The expression of these genes occurs during the development of cerebellum. | BC136339                                | Houtmeyers et al. (2013); Keraounoudis et al. (2011) |
| Paraneoplastic cerebellar degeneration                    | Carbonic anhydrase-related protein VIII (CARP VIII) antibodies                   | CARP VIII protein is related to carbonic hydrase family but does not have catalytic activity of hydration of CO2. It is expressed in brain Purkinje cells and also in different cancers like lung and has been reported to cause proliferation and invasion of tumor cells thus resulting in tumor growth and progression. | NM_004056; Tetsuka et al. (2013)         | Akisawa et al. (2003); Aprutwar et al. (2010) |
| Paraneoplastic cerebellar degeneration                    | Creatine kinase B (CKB) antibodies                                                | Creatine kinase isoenzyme is highly expressed in brain. CKB is involved in reversible catalytic transfer of phosphate between creatine kinase and ATP, thus maintaining energy homeostasis in the cells. | NM_001823; Yoshida et al. (2004)         | Tetsuka et al. (2013) |
| Paraneoplastic encephalomyeloneuropathy                   | P/Q and N type calcium-channel antibodies                                          | Neurotransmission is triggered by Calcium influx through P/Q and N type calcium-channel at the central and peripheral synapses. | X99897; NM_01635; Yoshida et al. (2004)  | Currie and Fox (1997); Lenon et al. (1995) |
| Eencephalomyelitis                                        | Amphiphysin antibodies                                                             | Amphiphysin is mostly present in the brain tissue and exist in two isoforms, and its function is as a tumor suppressor depends on Trim33 dependent degradation of β-catenin. | N/A                                     | Fischers et al. (2013) |
| Myositis                                                  | Jo-1 autoantibodies that target the Histidyl-RNA synthetase antigen (HARS) or Jo-1 antigen | HARS catalyzes the transfer of Histidine amino acid to its cognate tRNA during protein synthesis. HARS is responsible for causing autoimmunity in antinucleosynthetase syndrome by producing Jo-1 antibodies that undergo class switching and affinity maturation that are driven by T-cell activation which ultimately causes inflammation and tissue damage. | AAAX9363.1; Raben et al. (1994); Zahr and Baser (2011) | |
| Myositis, Myasthenia gravis                              | SRP-54 autoantibodies                                                             | Signal recognition particle 54 kDa protein, one of six subunits in the signal recognition particle ribonucleoprotein complex. | U51920.1; Hainzl et al. (2002); Suzuki et al. (2015) | |
| Myositis, Myasthenia gravis                              | Contactin antibodies                                                              | Contactin aids in actin assembly by binding actin. It is a signaling protein involved in tumor invasion as well as cell adhesion, migration and endocytosis. | BC008799.2; Berrih-Aknin (2014)         | |
| Dermatomyositis                                           | Tif-1 gamma (Trim33) autoantibodies or anti-p155/140                               | Tif-1 gamma (Trim33) is a E3 ubiquitin ligase. Its function as a tumor suppressor depends on Trim33 dependent degradation of β-catenin. | NG_02287.1; Maxia et al. (2016); Xue et al. (2015) | |
| Dermatomyositis                                           | NXP-2 autoantibodies, or anti-MJ antibodies                                       | NXP-2 protein is a nuclear matrix-associated protein. It plays an important role in RNA processing due to its RNA binding activity. It is involved in RNA metabolism. | NM_013558.2; Ishikawa et al. (2012); Kimura et al. (2002) | |
| Dermatomyositis                                           | CDR2L antibodies                                                                  | The function of CDR2L is currently unknown. It is a paralog of CDR2 with 50% sequence homology. | NM_014603; Eichler et al. (2013)         | |
| Idiopathic inflammatory myopathy, Sjo¨gren’s syndrome and Systemic lupus erythematous (SLE) | Ro52 autoantibodies                                                              | Ro52 protein contains 3 motifs, namely, RING-finger motif, a B-box, and a coiled-coil domain and so it belongs to tripartite motif (TRIM) family. Ro52 protein has E3-ligase activity and its overexpression in B cells reduces cell proliferation and causes an increase in apoptotic cell death. | NM_003141.3; Estupina et al. (2006); Rutjes et al. (1997) | Idiopathic inflammatory myopathy, Sjo¨gren’s syndrome and Systemic lupus erythematous (SLE) |
| Antiphospholipid antibody syndrome                        | Phospholipid antibodies                                                            | Phospholipids can form lipid bilayers that are major components of cells. Different enzymes act on phospholipids to form secondary products that act as a second messenger in signal transduction pathways. In prostaglandin signaling pathways, phospholipids act as a prostaglandin precursor substrate for lipase enzyme. | N/A                                      | Lands and Samuelsson (1968); Ruffatti et al. (1994) |
| No association of ovarian cancer with paraneoplastic syndrome | Ri antibodies bind to Nova-1 (neuro- oncological ventral antigen-1)               | Nova-1, also called paraneoplastic opsoclonus-myoclonus ataxia (POMA) antigen, is a sequence specific RNA binding protein that is involved in the splicing of neuronal pre-mRNA. | NM_002515; Drilce et al. (1997); Jensen et al. (2000) | |

Table 1 provides a brief description of various paraneoplastic antigens that are capable of eliciting immune responses in ovarian cancer that may be associated with different paraneoplastic syndromes. Different functions of these paraneoplastic antigens along with their NCBI reference sequence accession numbers are also listed in Table 1.
antibodies in association with paraneoplastic cerebellar degeneration at the time of ovarian adenocarcinoma tumor recurrence in a 69-year woman (Hoftberger et al., 2014). CARP VIII protein is expressed in the brain Purkinje cells, however strong expression of CARP VIII protein has been observed in lung cancer and has been linked to its higher proliferative and invasive properties that are essential for tumor growth and progression (Akisawa et al., 2003). Lennon et al. investigated the frequency of anti-P/Q and N type calcium-channel antibodies in a study population of cancer patients. Of 70 small-cell lung, ovarian or breast carcinoma patients who were associated with a paraneoplastic encephalomyeloneuropathy, 2/19 (5%) ovarian cancer patients were reported to harbor antibodies against P/Q-type and N-type calcium channels. The calcium channel antibodies were detected in human cerebellar and cerebral cortical tissues (Lennon et al., 1995). Antoine et al. reported the occurrence of amphiphysin antibodies in ovarian cancer patients in a study comprised of 2800 patients but only 5 were selected after pre-screening the sera for the presence of amphiphysin antibodies. Among 5 patients, who were diagnosed with encephalomyelitis prior to ovarian cancer diagnosis, one was found to have circulating amphiphysin antibodies (Antoine et al., 1999).

5.2. Myositis-associated onconeural antibodies in ovarian cancer

Dermatomyositis and polymyositis are paraneoplastic syndromes that can occur together or alone and symptoms can be followed by a diagnosis of ovarian cancer. The Jo-1 autoantibody that recognizes the Histidyl-tRNA synthetase is an antibody specific to myositis (Zahr and Baer, 2011). Chatterjee et al. have independently identified an epitope of the anti-Jo-1 target, Histidyl-tRNA synthetase, through a phage-display screening of serum IgGs obtained from ovarian cancer patients. This epitope, when combined in a panel of 3 antigens, had the ability to predict ovarian cancer recurrence 9 months prior to the standard clinical recurrence criteria including CA-125 (Chatterjee et al., 2012). Other autoantibodies found in the serum of myositis patients include anti-Ro52, anti-PL-7, anti-PL-12, anti-Mi-2, anti-PM-Scl75, anti-PM-Scl100, and anti-Ku (Cruellas et al., 2013). Patients with inflammatory myopathies that are positive for anti-Jo-1 are often positive for anti-Ro52. In one study examining the sera of 112 patients with inflammatory myopathies, 21% of patients were anti-Jo-1 positive, 20% of patients were anti-Ro52 positive, and 58% of those anti-Jo-1 positive patients were also positive for anti-Ro52 (Rutjes et al., 1997). In a study of 89 anti-Jo-1 positive patients with antisynthetase syndromes including polymyositis and dermatomyositis, 36 were also Ro52 positive. It was also found that when Jo-1 and Ro52 antibodies co-occurred, the risk of malignancy was associated with malignancies reported of colon, breast, ovarian, and esophagus (Marie et al., 2012). Therefore, these two antigens together on a panel could increase cancer diagnostic specificity of an autoantibody classifier. Screening of high grade serous ovarian carcinoma (HGSOC) sera was performed on diagnostic myositis antigen line blots (Mi-2, Ku, PM-Scl100, PM-Scl75, Jo-1, SRP, PL-7, PL-12, Ej, Oj, Ro-52; Ravo PNS Blot, Ravo Diagnostika, Freiburg, Germany) and on general paraneoplastic diagnostic line blots (GAD62, SOXI, Ma2, Ma1, Amphiphysin, CRMP5, Ri, Yo, HuD; Euroimmun myositis profile, Euroimmun US, Mountain Lakes, NJ). Reactivity was found with 15/20 antigens in HGSOC patient sera (Hurley et al. unpublished data). Furthermore, this onconeural autoantibody reactivity was observed in patients’ serum samples drawn at times when they had CA125 values below the clinical cutoff value, 35 U/ml (Chatterjee et al. unpublished data). Additionally, reactivity of multiple paraneoplastic antigens including anti-Jo-1, anti-Yo and anti-Ro52, was observed with serum IgGs obtained from single patients. Jo-1 positive ovarian cancer patient sera with co-occurrence of Ro52 autoantibodies were observed by screening of paraneoplastic syndrome patient sera and ovarian cancer patient sera against paraneoplastic diagnostic line blots and western blot screening with recombinant full-length proteins (Chatterjee et al. unpublished data). Ishikawa et al. reported a study of screening of patients with connective tissue disease including myositis and dermatomyositis for the detection of autoantibodies that target nuclear matrix protein 2 (NXP-2). Out of 206 patients screened, 6 were positive for NXP-2. The study showed that 1 out of these 6 patients had dermatomyositis diagnosed at the same time as diagnosis of ovarian cancer. The patient was negative for antibodies to transcription intermediary factor-1gamma (Tif1-gamma) but positive for antibodies to NXP-2 (Ishikawa et al., 2012). In another study of patients with inflammatory myopathies screened against an inflammatory myopathy immunoproteome test, 11/80 patients tested positive for an inflammatory myopathy associated antibody, and 5/11 of those patients had a cancer. Out of these 5 cancer cases with positive inflammatory myopathy immunoproteomes, 1 of the cases was a woman with ovarian cancer who tested positive for Tif1-gamma (Masiak et al., 2016). Fiorentino et al. reported that in a cohort of 111 patients at the Stanford University Dermatology Clinic and a cohort of 102 patients at the Johns Hopkins Myositis Center, positivity to either NXP-2 or Tif1-gamma was present in 83% of patients with Cancer-Associated Dermatomyositis (Fiorentino et al., 2013). Suzuki et al. reported the presence of anti-SRP54 antibodies in 100 patients who had an inflammatory myopathy, 5 of whom had a malignancy, including 1 ovarian cancer (Suzuki et al., 2015).

5.3. Occurrence of autoantibodies in ovarian cancer with or without the association of other paraneoplastic syndromes

Appearance of onconeural autoantibodies has been reported in ovarian cancer in association with other paraneoplastic neurological disorders. One case study was reported of a 76-year woman who had symptoms such as, agitation, symptoms of depersonalization, and visual hallucinations that was later diagnosed as paraneoplastic Trigeminal Neuralgia using magnetic resonance imaging (MRI) and electroencephalography (EEG). After performing paraneoplastic screening test for the presence of onconeural antibodies, only Hu antibodies were found to be present in her serum. Chest and abdominal CT scan revealed a large mass on her left ovary classified as ovarian intestinal-type mucinous tumor after the surgery (Kalanie et al., 2014). An initial diagnosis of antiphospholipid antibody syndrome (APS) in a 41-year-old woman resulted in a clinical manifestation of thromboembolism (paraneoplastic in nature) with the appearance of anti-phospholipid antibodies resulting from an ovarian endometrial adenocarcinoma. The symptoms of APS disappeared after the surgical removal of the tumor (Rufatti et al., 1994). In some cases, onconeural antibodies appeared without the development of paraneoplastic syndromes in ovarian cancer patients. Drlicek et al. investigated the prevalence of Ri autoantibodies in ovarian cancer patients that bind to Nova-1 (neuro-ocular ventral antigen-1), a neuronal antigen that takes part in splicing of pre-messenger RNA. Immunocooming of 300 serum samples obtained from 181 ovarian cancer patients using immunofluorescence technique followed by immunoblotting indicated that 11/181 ovarian cancer patients had anti-neuronal antibodies, of which, 4/11 patients had anti-Yo antibodies and 7/11 patients had anti-Ri antibodies. The range of the titers was 1:400–1:204,800. Follow-up of all these patients indicated no development of PNS within 2 years of their initial diagnosis. Thus, the occurrence of onconeural antibodies in ovarian cancer patients is not always accompanied by the development of PNSs (Drlicek et al., 1997).

5.4. Occurrence of onconeural autoantibodies in recurrent ovarian cancer

In addition to early detection, paraneoplastic autoantibodies can also indicate tumor recurrence and can be considered as biomarkers for disease monitoring during the patient follow up. It has been reported that return of symptoms of a PNS symptoms after successful treatment of the tumor can indicate tumor recurrence (Zahr and Baer, 2011). The titer of a paraneoplastic antibody against Ma-2 to act as a sensitive and specific predictor of recurrence has been demonstrated in small
6. Conclusion

The diagnosis of ovarian cancer has been challenging due to a dearth of biomarkers for sensitive and specific screening tests. Paraneoplastic neurological disorders that appear in ovarian cancer patients are considered to be remote effects of cancer and may occur in association with onconeural antibodies. Panels of paraneoplastic antigen targets of these onconeural antibodies may be useful in developing diagnostic immunoassays for early detection of ovarian cancer and its recurrence in a clinical setting.

Conflict of interest

Dr. Tainsky is a paid consultant to Avant Diagnostics on ovarian cancer biomarkers. Dr. Tainsky and Dr. Chatterjee are coinventors on patents issued and pending for autoantibody biomarkers.

Acknowledgements

The authors thank Dr. Robert Morris, Wayne State University School of Medicine, Department of Oncology, for helpful suggestions on the content of this manuscript. We also acknowledge the Ruth L. Kirschstein National Research Service Award T32-CA009531 for generously supporting the trainee Laura C. Hurley.

References

Abrey, L.E., Dalman, J.O., 1999. Neurologic complications of ovarian carcinoma. Cancer 85 (1), 127-133.
Cui, T., Hurtig, M., Elgue, G., Li, S.C., Veronesi, G., Essaghir, A., Demoulin, J.B., Pelosi, G., Croft, P.B., Wilkinson, M., 1965. The incidence of carcinomatous neuromyopathy in patients with carcinoma. Hormones Athens 13 (1), 140-145.

Albert, M., Darnell, J.C., Bender, A., Francisco, L.M., Bhardwaj, N., Darnell, R.B., 1998. The JAKs: cytokine receptors as ubiquitin ligases. Nature 395 (6696), 749-752.

Baker, R., Hynes, J., 2009. The role of cancer-associated dermatomyositis and inclusion body myositis. Curr. Opin. Neurol. 17 (5), 561.

Chatterjee, M., Dyson, G., Levin, N.K., Shah, J.P., Morris, R., Munkarah, A., Tainsky, M.A., 2017. A novel peripheral blood signature of ovarian cancer patients. J. Immunol. 198 (7), 2795-2804.

Eichler, T.W., Totland, C., Haugen, M., Qvale, T.H., Mazengia, K., Storstein, A., Haukanes, B.I., Vedeler, C.A., 2013. CDR2L antibodies: a new player in paraneoplastic cerebellar degeneration. J. Neuroimmunol. 257 (1–2), 3-12.

Berrih-Aknin, S., 2014. Cortactin: a new target in autoimmune myositis and Myasthenia Gravis. Autoimmun. Rev. 13 (10), 1001-1003.

Asahina, Y., Nishimori, I., Taniuchi, K., Okamoto, N., Takeuchi, T., Sonobe, H., Ohtsuki, R., 2014. The newly identified human nuclear protein NXP-2 possesses three coiled-coil domains and its association with carcinoma. Brain 91 (3), 481–492.

Sato, F., Tobinai, K., 2015. T-cell receptor use in ovarian cancer patients with anti-Ro/SS-A autoantibodies. J. Immunol. 195 (9), 4201–4205.

Keranen, A., Ryynänen, R., Varila, J., Roponen, K., Pohjalainen, T., 2003. The relationship between TNF-α levels and response to chemotherapy in ovarian cancer patients. Acta Oncol. 42 (1), 77–83.

Hempen, U., Amartsaz, E.P., Kamaraschev, J., Fink, D., Dedes, K.J., 2014. Acroleinotaxis of paraneoplastic sera in ovarian carcinoma: case report. BMC Cancer 15, 507.

Hill, C.L., Zhang, Y., Sigirivelu, B., Fukuda, E., Mellemkjaer, L., Airo, E., Arias, S.P., Selden, D., 2001. Frequency of autoimmune myositis and polymyositis: a population-based study. Lancet 357 (9250), 96–100.

Hoffberger, R., Sabater, L., Velasco, F., Ciordia, R., Dalmau, J., Graus, F., 2014. Carion antibodies: a marker for ovarian cancer. J. Neuroimmunol. 257 (1–2), 85–93.

Houmeyries, R., Souppouy, J., Teijpar, S., Arkell, R., 2013. ZIC gene family encodes multi-functional proteins essential for patterning and morphogenesis. Cell. Mol. Life Sci. 70 (10), 3791–3811.

Ishikawa, A., Muro, Y., Sugura, K., Akiyama, M., 2012. Development of an ELISA for detection of autoantibodies to nuclear matrix protein 2. Rheumatology (Oxford) 51 (7), 1181–1187.

Jen, K.B., Bredge, B.K., Stefani, G., Zhong, R., Buckanovich, R.J., Okano, H.J., Yang, Y.Y., Darnell, R.B., 2000. Nova-1 regulates neuron-specific alternative splicing and is essential for neuronal viability. Neuron 25 (2), 359–371.

Jett, J.R., Peel, J.J., Fredericks, L., Jewell, W., Pingleton, W., Roberts, J.F., 2014. Audit of the autoantibody test, EarlyCD8(tl)-lung, in 1600 patients: an evaluation of its performance in routine clinical practice. Lung Cancer 83 (1), 51–55.

Kalinke, H., Harandi, A.A., Mardani, M., Shahverdi, Z., Morakabati, A., Alidasi, S., Heydari, D., Soroush, Z., Pakhaman, H., Hoseinzeidah, Z., 2014. Trigeminal neuralgia: role of anti-periaxin antibody induced by a borderline ovarian mucinous tumor. Case Rep. Neurol. 6 (1), 7–13.

Keranosoudis, A., Rockhoff, M., Fedelein, J., Gold, R., Krogias, C., 2011. Isolated ZIC4 antibodies in paraneoplastic antibodies syndrome with an underlying ovarian tumor. Arch. Gynecol. Obstet. 284 (4), 651–655.

Kimura, Y., Sakai, F., Nakano, O., Kishaki, S., Segigami, H., Sawamura, T., Sadano, H., Osumi, T., 2002. The newly identified human nuclear protein NXP-2 possesses three distinct domains, the nuclear matrix-binding, RNA-binding, and coiled-coil domains. J. Biol. Chem. 277 (23), 20661–20671.

Knudsen, L.W., Samuelsson, B., 1968. Phospholipid precursors of prostaglandins. Biochim. Biophys. Acta 164 (2), 426–429.

Lennon, V.A., Kryzer, T.J., Griesmann, G.E., O’Sullivanbrand, P.E., Windebank, A.J., Wopman, A., Miljanich, G.P., Lambert, E.H., 1995. Calcium-channel antibodies in the Lambert-Eaton syndrome and other paraneoplastic syndromes. N. Engl. J. Med. 332 (22), 1467–1474.

Liu, Z., Zhang, L., Jiang, X., Wei, X., Ren, Y., 2016. Expression and clinical significance of CK20 protein in ovarian cancer. Tumour Biol. 39 (2), 123215.

Liu, Z., Cao, D.Y., Shen, K., Yang, J.X., 2015. A rare case of ovarian cancer presenting with paraneoplastic cerebellar degeneration and limbic encephalitis. Chin Med. J. (Engl.) 128 (19), 2685–2687.

Liu, Z.H., Chen, J.X., Ou, W.B., Zhang, Q., Xu, Z.R., Zhan, Y., Ma, L., Huang, T., Yan, Y.B., Zhou, H.M., 2013. Knockdown of creatine kinase B inhibits ovarian cancer progression by decreasing glycolysis. Int. J. Biochem. Cell. Biol. 45 (5), 979–986.

Maa, P., Brunner, E., Hulsenboom, E., VanDuijn, M., Schreurs, M.W., Hooijkaas, H., Smitt, P.A., 2013. Multiplex serology of paraneoplastic antineuronal antibodies. J. Immunol. Methods 391 (1–2), 10–20.

Masi, A., Kuczynska, Z., Czuczyszyn, Z., Zdrojewski, Z., 2009. Antibodies to anti-TIF1-gamma antibodies. Reumatologia 54 (1), 14–20.

Matsui, S.E., Kröninger, A., Yamaguchi, T., Saitou, N., Kuroda, T., Okita, Y., Hojo, H., Maeda, H., 2004. Anti-Ro52 antibodies are associated with anti-Ro52 antibody-positive patients with anti-Ro52 antibody-positive patients. Autoimmunity 37 (5), 387–400.

Matsui, S.E., Kuroda, T., Okita, Y., Hojo, H., Maeda, H., 2004. Anti-Ro52 antibodies in sera from patients with idiopathic inflammatory myopathy. Clin. Exp. Immunol. 133 (3), 434–438.

Matsui, S.E., Kuroda, T., Okita, Y., Hojo, H., Maeda, H., 2004. Anti-Ro52 antibodies in sera from patients with idiopathic inflammatory myopathy. Clin. Exp. Immunol. 133 (3), 434–438.

Matsui, S.E., Kuroda, T., Okita, Y., Hojo, H., Maeda, H., 2004. Anti-Ro52 antibodies in sera from patients with idiopathic inflammatory myopathy. Clin. Exp. Immunol. 133 (3), 434–438.
Schulz, P., Pruss, H., 2015. “Hirnsymptome bei Carcinomatose” – Hermann Oppenheim and an early description of a paraneoplastic neurological syndrome. J. Hist. Neurosci. 24 (4), 371–377.

Smith, C.G., 2017. A resident’s perspective of ovarian cancer. Diagnostics Basel 7 (2).

Suzuki, S., Nishikawa, A., Kuswana, M., Nishimura, H., Watanabe, Y., Nakahara, J., Hayashi, Y.K., Suzuki, N., Nishino, I., 2015. Inflammatory myopathy with anti-signal recognition particle antibodies: case series of 100 patients. Orphanet. J. Rare. Dis. 10, 63.

Takanaga, H., Mukai, H., Shibata, H., Toshimeri, M., Ono, Y., 1998. PKN interacts with a paraneoplastic cerebellar degeneration-associated antigen, which is a potential transcription factor. Exp. Cell Res. 241 (2), 363–372.

Tetsuka, S., Tominaga, K., Ohba, E., Kurowi, K., Sakashita, E., Kasahima, K., Hamamoto, T., Namekawa, M., Morita, M., Natsui, S., Morita, T., Tanaka, K., Takiyama, Y., Nakano, I., Endo, H., 2013. Paraneoplastic cerebellar degeneration associated with an onconeural antibody against creatine kinase, brain-type. J. Neurol. Sci. 335 (1-2), 48-57.

Tirmazy, S.H., Tirmazy, S.H., Bhana, R., 2014. A rare case of anti-yo antibody associated paraneoplastic sensory polyganglionopathy in a patient with ovarian cancer. Middle East J. Cancer 5 (3), 179–182.

Xue, J., Chen, Y., Wu, Y., Wang, Z., Zhou, A., Zhang, S., Lin, K., Aldape, K., Majumder, S., Lu, Z., Huang, S., 2015. Tumour suppressor TRIM33 targets nuclear beta-catenin degradation. Nat. Commun. 6, 6156.

Yoshida, Y., Kinuta, M., Abe, T., Liang, S., Araki, K., Cremosa, O., Di, P.G., Moriyama, Y., Yasuda, T., De, C.P., Takei, K., 2004. The stimulatory action of amphiphysin on dynamin function is dependent on lipid bilayer curvature. EMBO J. 23 (17), 3483-3491.

Zahr, Z.A., Raser, A.N., 2011. Malignancy in myositis. Curr. Rheumatol. Rep. 13 (3), 208-215.

Zhu, Q., Han, S.X., Zhou, C.Y., Cai, M.J., Dai, L.P., Zhang, J.Y., 2015. Autoimmune response to PARP and BRCA1/BRCA2 in cancer. Oncotarget 6 (13), 11575-11584.

Zivaljevic, M., Popovic, S., Vujkov, T., 2005. Lambert-Eaton myasthenic syndrome—a rare manifestation of paraneoplastic syndrome in ovarian cancer—case report. Med. Pregl. 58 (9-10), 495–497.