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Lung adenocarcinoma and antiphospholipid antibodies ☆

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ABSTRACT

Thrombosis is a frequent finding in cancer patients, being referred to as a poor prognostic factor. The mechanisms underlying the thrombophilic state in malignancy are not well elucidated but involve a complex interaction between tumor and host cells as well as the hemostatic system. A number of studies have demonstrated the presence of antiphospholipid antibodies (aPL) in cancer patients, suggesting a potential role in tumor-associated thrombosis. A prospective analysis has been performed in a group of lung adenocarcinoma patients in respect to the presence of aPL and thrombotic manifestations. Lupus anticoagulant (LAC) was identified in 61 out of 105 patients and it correlated highly with thrombosis (22/61, LAC positive vs 2/44, LAC negative RR = 7.93; $p<0.001$). On the other hand, patients that displayed IgM anti-β2-glycoprotein I (aβ2GPI) (22/80) showed an unexpected decrease in thrombosis risk (2/22, with IgM aβ2GPI vs 18/58, without IgM aβ2GPI RR = 0.29; $p = 0.04$). Considerations on the mechanisms that link cancer, thrombosis and aPL are discussed in this article.

Contents

1. Cancer and thrombosis

Thrombosis is a common finding in cancer patients [1,2] and has been referred to as a marker of poor prognosis [3,4]. In fact, it is reported that coagulation activation markers are found in a high number of cancer patients despite the absence of thrombotic complications [5,6]. A number of mechanisms have been proposed to explain such observations [7], including: i.: Increased expression of procoagulant proteins,

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such as the clotting initiator tissue factor, by tumor cells; ii.: Production of inflammatory cytokines with subsequent activation of vascular cells, including platelets, endothelial cells and monocytes; iii.: Direct tumor-cell interactions also resulting in activation of vascular cells; and iv.: Release of tumor-derived procoagulant microvesicles [8].

Procoagulant properties have been associated with more aggressive tumor behaviour and several lines of evidence demonstrate that blood clotting activation is intimately related to tumor progression and metastasis [9]. In addition to coagulation proteins, a family of G protein-coupled-receptors known as protease-activated receptors (PARs) has been implicated in tumor biology [10]. These receptors might be activated through proteolytic cleavage by blood coagulation enzymes thus eliciting the production of several pro-tumoral factors including cytokines, angiogenic factors and metalloproteases among others [10]. Therefore, it is proposed that blood coagulation enzymes could interfere with tumor biology through both hemostatic and nonhemostatic mechanisms [11].

2. aPL/APS in cancer

Antiphospholipid syndrome (APS) is an acquired autoimmune prothrombotic condition, being the most common acquired thrombophilia [12]. It is characterized by arterial and/or venous thrombosis and recurrent fetal loss associated with the persistent presence of antiphospholipid antibodies (aPL) [13]. In fact, aPL are highly heterogenous and include antibodies that recognize proteins that bind anionic phospholipids, such as β2-glycoprotein I (β2GPI) and prothrombin, as well as those that recognize negatively charged phospholipids as antigens, such as cardiolipin and phosphatidylserine (PS) [14].

Several studies have demonstrated a high prevalence of aPL in cancer patients [15,16]. Given the involvement of aPL in autoimmune disease-related thrombosis, it is argued that they might play a role in the cancer-associated prothrombotic state [17]. A particularly serious and often fatal development of APS is termed catastrophic antiphopholipid syndrome (Asherson's Syndrome), which is characterized by the rapid progression in the development of fulminant thrombotic complications that predominantly affect small vessels [18]. In this context, Asherson's Syndrome has been reported as occurring in cancer patients [15,19].

In a previous retrospective study, we demonstrated a high incidence of aPL, as demonstrated by the presence of lupus anticoagulant (LAC), in a group encompassing several distinct cancer types [20]. This study showed an important correlation between the presence of LAC and thrombosis. This finding contrasted with that reported by Genvresse et al. which showed high prevalence of anticardiolipin (aCL) and anti−β2GPI (aβ2GPI) antibodies in patients with non-Hodgkin's lymphoma, both presenting poor correlation with thrombosis outcome [21].

3. Lung cancer, thrombosis and aPL

Lung cancer is the leading cause of cancer-related death and thus a major health problem [22]. Despite advances in cancer detection and new chemotherapeutic agents, the prognostic outcome of lung cancer patients has improved only minimally. Adenocarcinoma is the most frequent histological type of lung cancer, accounting for 50% of all lung cancers. Remarkably, a retrospective study demonstrated that the thrombotic risk in lung cancer patients is 20-fold higher than in the general population [23]. In addition, this study showed a three-fold higher thrombosis incidence in lung adenocarcinoma than in lung squamous cell carcinoma patients [23].

Table 1
Association between thrombosis and aPL in lung adenocarcinoma patients.

aPL status	Thrombosis group *n* (%)	Non-thrombotic group *n* (%)	Thrombosis risk
LAC (+)	22 (36)	39 (64)	RR = 7.93
LAC (−)	2 (4)	42 (96)	$p<0.001$
IgM aβ2GPI (+)	2 (9)	20 (91)	RR = 0.29
IgM aβ2GPI (−)	18 (31)	40 (69)	$p=0.04$

LAC activity was evaluated using plasma activated partial thromboplastin time with the diluted Russell's viper venom on an automated coagulometer CA-1500 (Dade Bhering, Marburg, Germany) in citrated peripheral blood. If the clotting time was prolonged the mixture test was performed, and if it was still prolonged, the correction test was performed. Antibodies against β2GPI were measured using an enzyme-linked immunosorbent assay kit (Lousville aPL Diagnostics, Seabrook, TX, USA) according to the instruction of the manufacturer. Thrombosis was diagnosed by imaging techniques (Color-Doppler ultra-sound, CT scanning, magnetic resonance imaging, and pulmonary scintigraphy).

A prospective study involving 105 consecutive and unselected patients with lung adenocarcinoma, most of them (82%) in advanced disease stages (III and IV), was performed in order to correlate thrombosis and aPL levels [24]. A relationship between the incidence of thrombosis with LAC activity (58%) but not aCL presence (10% IgG; 9% IgM) was observed. Moreover, IgM aβ2GPI was found in 26% of the patients, whereas IgG aβ2GPI was present in 5%. Importantly, the aPL status was maintained in surviving patients six months after the first evaluation. Patients were periodically screened for detection of thrombosis and several hemostatic factors known to correlate with prothrombotic conditions (protein C, antithrombin, fibrinogen, factor VIII, factor IX and D-dimer) have been measured. A high thrombosis incidence (24%) was found and a great number of patients presented elevated levels of factor VIII, factor IX, D-dimer and fibrinogen. Interestingly, it was observed a high correlation between LAC activity and thrombosis occurrence (Table 1). This is in accordance with Galli et al. that related LAC to a strong risk factor for thrombosis development in APS [25]. Interestingly, LAC activity was significantly correlated with lower survival rates in lung adenocarcinoma patients. Therefore, it is possible that aPL may serve as a prognostic factor in lung adenocarcinoma, as previously suggested for aggressive non-Hodgkin's lymphoma [26].

It has been demonstrated that IgG aβ2GPI but not IgM aβ2GPI antibodies are significantly correlated with thrombosis risk in non-cancer patients [27]. Moreover, high titers of IgM aPL are unrelated to pathogenicity in patients with non-Hodgkin's lymphoma [28]. Remarkably, our study demonstrated a strong negative correlation between IgM aβ2GPI and thrombosis occurrence and survival in lung cancer patients [24]. However, it remains to be determined which mechanism(s) could be associated with this observation.

4. Generation of aPL in cancer

Several mechanisms have been proposed to explain aPL generation in autoimmune diseases [14]. Therefore, excessive generation of anionic phospholipid membranes has been regarded as a potential source of immunogens for aPL production [29]. Anionic phospholipids, such as PS, are normally absent from the extracellular surface of cell membranes, but they redistribute from the inner to the outer layer during apoptosis. In this context, it has been demonstrated that β2-GPI bound to apoptotic but not viable cells thus inducing aPL production *in vivo* [29]. More recently, Yamaguchi et al. demonstrated that binding of β2GPI to anionic membranes facilitates processing and presentation of the cryptic β2GPI epitope that activates pathogenic autoreactive human T cells [30]. In this context, it has been reported that viable tumor cells [31,32] as well as tumor blood vessels [33] shows increased exposure of anionic phospholipids on the outer layer of their membranes. Therefore, tumor microenvironment may be a source of anionic lipid surfaces that facilitate aPL production.

Microvesicles are membrane-derived fragments that are shed from a variety of cells upon energy-dependent mechanisms [34]. It is well established that cancer patients exhibit an increase in circulating microvesicles with externalized PS, being these structures possibly involved in the prothrombotic state [8]. We observed an important elevation in circulating microvesicles in lung adenocarcinoma patients, as compared to healthy individuals. However, future studies are necessary to determine whether this condition favors the production of aPL in cancer as well as in other diseases.

5. Summary

A prospective study involving 105 patients diagnosed with lung adenocarcinoma demonstrated a high prevalence of aPL. Remarkably, the incidence of thrombosis and LAC presence were independently correlated with decreased survival rates. A very intriguing finding was that IgM aβ2GPI was frequently found in lung adenocarcinoma patients and inversely correlated with thrombosis outcome. These findings demonstrate that in lung cancer, and possibly in other cancers, there is a strong integration between the immune system, blood coagulation and tumor biology.

Take-home messages

- Thrombosis is a frequent finding in cancer patients.
- Lung adenocarcinoma patients have a high frequency of antiphospholipid antibodies.
- Lupus anticoagulant is associated with increased thrombosis risk in lung adenocarcinoma patients.
- IgM Anti-β2-glycoprotein I antibodies are related with decreased thrombosis risk and increased survival in lung adenocarcinoma patients.

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The proteasome inhibitor bortezomib depletes plasma cells and protects mice with lupus-like disease from nephritis

Autoantibody-mediated diseases like myasthenia gravis, autoimmune hemolytic anemia and systemic lupus erythematosus represent a therapeutic challenge. In particular, long lived plasma cells producing autoantibodies resist current therapeutic and experimental approaches. It was previously shown that the sensitivity of myeloma cells toward proteasome inhibitors directly correlates with their immunoglobulin synthesis rates. Therefore, it was hypothesized that normal plasma cells are also hypersensitive to proteasome inhibition owing to their extremely high amount of protein biosynthesis. In this study, **Neubert K. et al. (Nat Med 2008; 14: 748–55)** show that the proteasome inhibitor bortezomib, which is approved for the treatment of multiple myeloma, eliminates both short-and long-lived plasma cells by activation of the terminal unfolded protein response. Treatment with bortezomib depleted plasma cells producing antibodies to double-stranded DNA, eliminated autoantibody production, ameliorated glomerulonephritis and prolonged survival of two mouse strains with lupus-like disease, NZB/WF1 and MRL/lpr mice. Hence, the elimination of autoreactive plasma cells by proteasome inhibitors might represent a new treatment strategy for antibody-mediated diseases.

Antigen receptor editing in anti-DNA transitional B cells deficient for surface IgM

In response to encounter with self-Ag, autoreactive B cells may undergo secondary L chain gene rearrangement (receptor editing) and change the specificity of their Ag receptor. Knowing at what differentiative stage(s) developing B cells undergo receptor editing is important for understanding how self-reactive B cells are regulated. In this study, **Kiefer K. et al. (J Immunol 2008; 180:6094–106)** report that dsDNA breaks in mice with Ig transgenes coding for anti-self (DNA) Ab, were found indicative of ongoing secondary L chain rearrangement not only in bone marrow cells with a pre-B/B cell phenotype but also in immature/transitional splenic B cells with little or no surface IgM (sIgM(-/low)). L chain-edited transgenic B cells were detected in spleen but not bone marrow and were still found to produce Ab specific for DNA (and apoptotic cells), albeit with lower affinity for DNA than the unedited transgenic Ab. The authors conclude that L chain editing in anti-DNA-transgenic B cells is not only ongoing in bone marrow but also in spleen. Indeed, transfer of sIgM (-/low) anti-DNA splenic B cells into SCID mice resulted in the appearance of a L chain editor (Vlambdax) in the serum of engrafted recipients. Finally, the authors also provide evidence for ongoing L chain editing in sIgM (low) transitional splenic B cells of wild-type mice.

Antibodies to mitotic spindle apparatus: clinical significance of NuMA and HsEg5 autoantibodies

The clinical associations of NuMA and HsEg5 antibodies, the main anti-mitotic spindle apparatus autoantibodies, remain unclear due to their extremely low prevalence. Here, the clinical data of 40 anti-NuMA- (0.87 per thousand) and 7 anti-HsEg5- (0.15 per thousand) positive patients detected during routine immunofluorescence examination of 45,804 sera is analyzed. **Mozo L. (J Clin Immunol 2008; 28 285–90).** Antibodies to HsEg5 did not associate with any specific pathology. NuMA positivity associated with a diagnosis of connective tissue disease (CTD) in 18 patients (45%), primary Sjogren or sicca syndrome and undifferentiated connective tissue disease being the most represented. Seven patients (17.5%) were diagnosed with different organ-specific autoimmune diseases, whereas in the other 15 patients (37.5%), no autoimmune pathology could be documented. Therefore, although both anti-mitotic spindle apparatus antibodies are not associated to a defined autoimmune pathology, the presence of NuMA antibodies, mainly at high titers, may be an indication for a more extensive screening of CTD.