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Review Article

Tumour and microparticle tissue factor expression and cancer thrombosis

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ABSTRACT

Cancer is frequently complicated by venous thromboembolic events (VTE), which pose a significant health burden due to the associated high morbidity and mortality rates, yet the exact details of the pathophysiological mechanisms underlying their development are yet to be fully elucidated. Tissue factor (TF), the primary initiator of coagulation, is often overexpressed in malignancy and as such is a prime candidate in predicting the hypercoagulable state. Further exploration of this potential role has identified increases in the number of TF-expressing microparticles (MP) in the circulation of cancer patients, in particular in those known to have high incidences of thromboembolic complications. The risk of VTE in cancer is found to be further elevated by chemotherapy. Chemotherapy may, in eliciting cancer cell apoptosis, result in an increase in release of circulating procoagulant MP. We discuss a potential role of elevated tumour TF expression and increased circulating TF-positive MP in predicting VTE risk.

Contents

VTE in Cancer

Aberrant systemic activation of the blood coagulation system is found to invoke a characteristic hypercoagulable state in many cancers [1]. As a result of this, the individual may exhibit a marked predisposition towards the development of thrombosis. Such venous thromboembolic events (VTE), mainly deep vein thrombosis of the lower extremities and pulmonary embolism, often pose significant complications to the malignant state and, in this way, comprise the second leading cause of death in cancer patients [2,3]. A high rate of VTE can therefore be considered predictive of poor prognosis. Specifically, VTE has been regarded as a significant predictor of decreased 2-year survival [4], as exemplified in studies of breast cancer and lung cancer [5,6]. This is supported by studies that indicate significant improvement in cancer survival can be attained through the administration of low molecular weight heparins (LMWH) [7,8].

Since the founding observations of a link between cancer and thrombosis by French physician Armand Trousseau in 1865, substantial supporting epidemiological evidence has been accumulated, yet a definitive explanation of the associated fundamental pathophysiology

Abbreviations: fVII, factor VII; fVIIa, activated factor VII; fXa, activated factor X; HNSCC, head and neck squamous cell carcinoma; LMWH, low molecular weight heparin; MM, multiple myeloma; MP, microparticles; PCA, procoagulant activity; PE, phosphatidylethanolamine; PS, phosphatidylserine; TF, tissue factor; VTE, venous thromboembolic events.

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remains to be elucidated. The annual incidence of VTE in cancer is estimated at approximately 1 in 200 (0.5%), around four- to five-fold higher than that of the general population [1,9]. However, this risk is not uniformly distributed across different cancer types. While many types of cancer have been studied and their associated relative risk of VTE assessed, these data are inconsistent, as studies vary with regard to patient population, treatment regimen, duration of follow-up period, period of study, and method of detecting and reporting VTE. Nonetheless the general consensus, when adjusted for disease prevalence in the general population, is that the cancers most consistently associated with the highest VTE incidence are pancreas, brain, stomach, ovary, kidney, uterus and lung, whereas a comparatively lower incidence is found with prostate, breast and melanoma [1,10,11]. In addition, high rates are reported among haematological malignancies, particularly in lymphoma and multiple myeloma (MM) [1,11]. Furthermore, risk-adjusted models have suggested that advanced, metastatic disease in particular is associated with both a high incidence of thromboembolic complications, and increasingly poor prognosis [12–17]. This considered, it is important also to be aware that the magnitude of the problem is likely underestimated, often as a result of limitations in clinical management [18], and therefore may pose an even greater burden than official figures suggest.

Events found to specifically promote the cancer-associated hypercoagulable state include the increased activation of procoagulant factors with concomitant inhibition of anticoagulant mechanisms, impaired fibrinolysis and release of proinflammatory cytokines [19]. One of the most notable of these, is the characteristic upregulation of procoagulant tissue factor (TF) on the surface of tumour cells [2,20].

The Association of VTE with TF Expression Levels on Tumour Cells

TF acts as the principal cellular initiator of the extrinsic pathway of the blood coagulation cascade and, as a transmembrane glycoprotein receptor, exerts its primary function through a natural high affinity interaction with its ligand, factor VII (fVII), and its activated form, fVIIa. Under physiological conditions, TF expression is restricted to extravascular cells and is only expressed by endothelial cells in response to injury. In malignancy however, high levels of TF expression on the cancer cell surface are believed to contribute to the procoagulant tendencies. Aside from its traditional haemostatic role, surface TF is thought to further potentiate cancer progression, through an enhancement of angiogenesis and by facilitating metastasis, and possesses roles in primary tumour growth [21,22]. *In vitro*, high levels of surface TF expression have been further correlated with cell invasion in pancreatic cancer cell lines [23].

Interestingly, recent studies have concluded that TF cell surface expression was explicitly linked to the procoagulant activity (PCA) of pancreatic, breast, colorectal and head and neck squamous cell carcinoma (HNSCC) tumour cell lines [23,24]. Immunohistochemical staining revealed the highest and most consistent intensity of TF expression in tumours typically associated with high VTE incidence such as lung, HNSCC and pancreatic cancers, with lower and less consistent staining in breast, renal and prostate cancers [25]. In pancreatic cancer patients, a symptomatic VTE rate of 26.3% was found in high TF-expressing carcinomas compared with 4.5% in those with low expression [26] and additionally, as with VTE incidence, TF expression has further been associated with poor histologic grade and worsened prognosis [27,28].

Furthermore, increased expression of TF on the surface of tumour cells has been demonstrated to correlate with higher circulating levels *in vivo* [2]. While the blood of healthy individuals is found to contain very low levels of functional blood-borne TF [29], both tumour tissue TF expression and plasma TF concentration are found to be elevated in breast cancer patients [30] and TF levels are also significantly elevated in the serum of ovarian cancer patients [31]. Further to this, the enhanced levels of circulating TF antigen in mice bearing tumours derived from human colorectal cancer cell lines were shown to be predominantly tumour cell-derived using a human TF-specific ELISA [2].

Microparticles and Their Potential Contribution to VTE in Cancer

Microparticles (MP) are phospholipid vesicles, 0.1-1μm in diameter, derived through blebbing of the cell plasma membrane [29,32]. Their formation and roles in cancer progression have been recently reviewed [33]. In normal cells, MP formation is initiated as a consequence of activation or apoptotic signals [34,35]. Under resting conditions, aminophospholipids phosphatidylserine (PS) and phosphatidylethanolamine (PE) are concentrated almost exclusively in the inner (cytoplasmic) leaflet, while the outer (exoplasmic) leaflet is enriched in choline phospholipids sphingomyelin and phosphatidylcholine. MP release is ultimately dependent on the transverse migration and externalisation of the procoagulant phospholipid PS in response to an increase in cytosolic calcium and a concurrent increase in scramblase enzyme activity, with resultant imbalance in membrane phospholipid asymmetry [34,36–38]. PS acts synergistically with TF to amplify its role in the initiation of blood coagulation [38,39] by extending the available phospholipid surface area for binding of coagulation factors and so facilitating the assembly of tenase and prothrombinase complexes responsible for thrombin generation [29,36,38].

Although detectable in the circulation of healthy subjects, MP numbers are generally found to increase among cancer patients [40–42]. While in healthy individuals, endothelial cells and platelets constitute the major sources of MP generation [19,43], current knowledge indicates that these additional TF-positive MP derive from the tumour cells themselves [43]. As the composition of MP typically reflects the antigenic profile of the parent cell from which they originate [29,44–46], it follows that those tumour cells expressing high levels of TF can be assumed to give rise to TF-positive MP. Moreover, in a large proportion of disseminated breast and pancreatic cancer patients, MP-associated TF activity could be further correlated with the presence of MP expressing the tumour cell antigen, MUC1, a transmembrane glycoprotein which is frequently overexpressed in epithelial malignancies [47]. These findings are in agreement with a second study in which approximately half of the TF-positive MP were also positive for MUC-1 [48]. Additionally, phenotypic analysis of vesicles shed by tumour cell lines showed expression of tumour-associated surface determinants [45,49], as did MP measured in samples from gastric and ovarian cancer patients [40,50]. Moreover, circulating human TF antigen could be detected in plasma samples from mice subcutaneously injected with colorectal cancer cells [2] and those orthotopically injected with pancreatic cancer cells [51]. The tumour origin of these MP is further supported by the findings of Zwicker et al. [48], who reported a significant decrease in levels of TF-positive MP in pancreatic cancer following radical pancreatectomy, and has been subsequently verified in a later study in a number of patients [52].

The first demonstration of tumour shedding of MP being linked to the hypercoagulable state of cancer dates back to 1981 [53]. Indeed MP are considered to constitute the predominant source of TF activity released from human cancer cells [54], rather than the alternatively spliced soluble form of TF, whose procoagulant potential is a topic of controversy [55,56]. *In vitro*, these MP exhibit strong PCA in one-stage clotting or thrombin generation assays [51,57] mainly as a result of PS and PE surface exposure but also due to the presence of active TF on their surface. Also, a separate *in vitro* study concluded that high TF expression in pancreatic cancer cell lines correlated with the ability of their conditioned cell-free media to support clotting, and also with the greatest PCA, according to prothrombin time assays [58]. In support of this are reports of elevated TF-positive MP levels [59], and also higher MP-TF activity [60] in cancer patients compared with healthy controls. Furthermore, real-time microscopy indicates