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Absence of tissue factor is characteristic of lymphoid malignancies of both T- and B-cell origin

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Summary

Background—Thrombosis is a marker of poor prognosis in individuals with solid tumors. The expression of tissue factor (TF) on the cell surface membrane of malignant cells is a pivotal molecular link between activation of coagulation, angiogenesis, metastasis, aggressive tumor behavior and poor survival. Interestingly, thrombosis is associated with shortened survival in solid, but not in lymphoid neoplasias.

Objectives—We sought to study whether the lack of impact of thrombosis on survival in lymphoid neoplasias could be due to a lack of tumor-derived TF expression.

Methods—We analyzed TF gene (*F3*) expression in lymphoid (N=114), myeloid (N=49) and solid tumor (N=856) cell lines using the publicly available dataset from the Broad-Novartis Cancer Cell Line Encyclopedia (http://www.broadinstitute.org/ccle/home), and in 90 patient-derived lymphoma samples. TF protein expression was studied by immunohistochemistry (IHC).

Results—In sharp contrast to wide *F3* expression in solid tumors (74.2%), *F3* was absent in all low and high grade T- and B-cell lymphomas, and in most myeloid tumors, except for select acute myeloid leukemias with monocytic component. IHC confirmed the absence of TF protein in all indolent and high-grade B-cell (0/90) and T-cell (0/20) lymphomas, and acute leukemias (0/11).

Conclusions—We show that TF in lymphomas does not derive from the malignant cells, since these do not express either *F3* or TF protein. Therefore, it is unlikely that thrombosis in patients with lymphoid neoplasms is secondary to tumor-derived tissue factor.

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Authorship Contributions

GC-M, EB and RF: made substantial contributions to the concept and/or study design, performed research and drafted the manuscript.d

CL-M and ALG-L: performed research and contributed to study design.

Disclosure of Conflicts of Interest

No disclosures.

Keywords

tissue factor; lymphomas; leukemias; cancer; hematological malignancies

Introduction

The occurrence of venous thromboembolism (VTE) in individuals with solid tumors is associated with more than a three-fold higher risk of short-term death, as compared to cancer patients without thrombosis [1,2]. The expression of tissue factor (TF) by cancer cells is one of the key underlying mechanisms linking thrombosis with aggressive tumor behavior [3]. Indeed, TF, a 47 kDa cell-surface trans-membrane glycoprotein that triggers the activation of coagulation by binding to activated factor VII [4], is expressed in variable degrees by most solid tumors, and has been shown to be involved in cell signaling, angiogenesis, inhibition of apoptosis, tumor growth, and metastasis [5,6]. Recent studies show that targeting TF is feasible and may both prevent thrombosis and down-modulate tumor aggressiveness [7,8]. Therefore, accurate information on the types of tumors that express TF, and distinction from those that do not, becomes relevant.

The pathophysiology of thrombosis in individuals with hematological malignancies may differ from that of patients with solid tumors. Interestingly, the occurrence of VTE does not have a negative impact on survival in individuals with multiple myeloma (MM), despite the high rate of thrombosis in the setting of chemotherapy [9,10]. Similarly, VTE in individuals with lymphoma is seen predominantly in association with chemotherapy and, as shown in a large prospective study, does not impact survival [11]. These findings suggest that activation of coagulation in lymphoid-cell-derived tumors may not be driven by TF expressed by neoplastic cells. Indeed, we recently described the absence of expression of the gene that encodes for TF (*F3*) in MM cell lines and in primary patient samples [12]. In order to determine whether other lymphoid neoplasms express TF, we analyzed *F3* and protein expression levels in low- and high-grade lymphoid tumors, as well as in representative myeloid and solid tumors.

Materials and Methods

Gene expression levels were analyzed using the publicly available dataset of the Broad-Novartis Cancer Cell Line Encyclopedia (http://www.broadinstitute.org/ccle/home) comprising 847 solid-tumor cell lines and 114 lymphoid-tumor (84 lymphomas and lymphoid leukemias and 30 myelomas) and 49 acute and chronic myeloid leukemia cell lines. Expression data for each cell line was generated using the Affymetrix HG-U133_Plus_2 arrays. Raw intensity values were generated using Robust Multi-array Average (RMA) algorithm, inter- and intra-array normalized and log2 transformed. RMA log2 values were used to estimate gene expression as follows: cell lines with RMA log2 values below 6.5 were categorized as not expressing F3; cell lines with values between 6.5 and 7.5 were considered as having marginal expression; and cell lines with values above 7.5 were considered as expressing F3 in intermediate to high levels. Immunohistochemistry (IHC) staining for TF protein was performed as previously described [12], using standard procedures on tumor tissue microarrays (TMA) on representative solid-tumor biopsies and myeloid leukemias, including 1 acute promyelocytic leukemia, 1 myelo-monocytic leukemia and 9 leukemias without a monocytic component. In addition 129 lymphoid tumors including 10 precursor-cell acute lymphoblastic leukemias, 99 mature B-cell (9 low-grade and 90 high-grade), and T-cell (20) lymphomas were studied for TF expression. Briefly, slides were deparaffinized for 4 minutes at 72°C using xylene-free dewaxing reagent (EZprep, Ventana Medical Systems) and stained for IHC using a Bench Mark ULTRA

automated slide stainer (Ventana). Following CC1 alkaline antigen retrieval (95°C, 8 min.), TMAs were incubated at 36°C for 4 minutes with primary rabbit polyclonal anti-human TF antibody (dilution 1:75,FL-295, Santa Cruz Biotechnology). Ventana ultraView Universal polymer-based diaminobenzidine (DAB) detection kit was used for visualization of antibody localization. TMAs were counterstained with Harris haematoxylin, and mounted with non-aqueous medium. Patient samples were obtained under informed consent at the Mayo Clinic (primary cell lines were prepared from tumor biopsies for the study of *F3* expression from 90 high grade lymphomas), and at the Instituto Nacional de Ciencias Médicas y Nutrición (tissue microarrays were prepared from diagnostic lymph node or bone marrow biopsies for the study of TF protein expression by IHC). The study was conducted under IRB approval.

Results

In order to determine whether TF is relevant in lymphoid neoplasia biology, we studied TF gene (*F3*) and protein expression in these tumors. We found that *F3* was absent in all precursor and mature lymphoid tumor cell lines including low- and high-grade lymphomas, acute lymphoblastic leukemias (except for a single cell line with low marginal *F3* expression, which interestingly derives from a chronic myeloid leukemia in blast crisis) (Fig. 1). *F3* expression was also absent in primary patient samples from 90 high-grade non-Hodgkin's lymphomas, including diffuse large B-cell (DLBCL) and Burkitt´s lymphomas (data not shown). This contrasted sharply with the common expression levels of *F3* in the solid tumors 74.2% (high in 526/61.5% and marginal in 109/12.7%) (Fig. 1). Notably, Hodgkin´s lymphomas (HL) showed frequent *F3* expression (see supplemental table for GEP values for each tumor).

Gene expression findings were validated using IHC. TF protein was highly expressed in a variety of representative solid tumors, except for renal cell carcinoma (Fig. 2, **panel A**). In contrast, TF was not expressed in any of the lymphoid neoplasias studied of either B-cell or T-cell origin. These included all acute lymphoblastic leukemias, peripheral T-cell lymphomas, high-grade B-cell Burkitt´s and DLBCL (25 germinal center, and 64 non-germinal center phenotype), and low-grade lymphomas (extranodal marginal zone, follicular, mantle-cell lymphoma and small lymphocytic lymphoma) (Fig. 2, **panel B**).

Regarding myeloid-derived neoplasias, *F3* expression was absent in chronic granulocytic leukemia with marginal to inter-median expression in a fourth of acute myeloid leukemias (positive leukemias included 8 with monocytic or myelomonocytic blasts, one acute eosinophilic leukemia, and one promyelocytic leukemia). None of the acute myeloid leukemias studied stained for TF by IHC.

Discussion

Activation of coagulation with or without the development of overt thrombosis is a frequent occurrence in individuals with cancer. Lower-extremity venous thrombosis and pulmonary embolism in individuals with solid tumors constitutes a clinical indicator of an underlying biological advantage to cancer growth and metastasis, as evidenced by worse outcomes in individuals with solid tumors who develop VTE [2]. Patients with hematological malignancies also develop thrombosis particularly in the setting of treatment [13,14]. However, prospective studies have shown that survival is not affected by the occurrence of thrombosis in individuals with lymphoid-cell-derived tumors, including myelomas and lymphomas [9,10]. A recent, large (N=686) prospective study of newly diagnosed Hodgkin´s and non-Hodgkin´s lymphoma reported VTE in 8% and 6.7% of patients respectively, with no VTE-related deaths, and no difference in overall survival in patients with or without VTE. This suggests that the hypercoagulable state in hematological malignancies is

independent of neoplastic behavior, and that the mechanisms leading to thrombosis may differ from those in solid tumors.[11].

There are several molecular links between a prothrombotic state and an oncogenic phenotype [6,15]. Both *in vitro* and *in vivo* experiments point to the expression of TF by malignant cells as one of the key factors in both thrombosis and aggressive tumor behavior [5–8,16–18]. Furthermore, elevated circulating microparticle-associated TF activity has been shown to correlate with poorly differentiated and invasive tumors and poor survival [19].TF has a well characterized role in the inhibition of apoptosis, induction of tumor cell proliferation, angiogenesis, and metastasis, through both clotting-dependent and independent mechanisms [6,7,20]. As reported by others, we too found TF to be expressed at different levels by most solid tumors, with variation within distinct tumor types (Fig. 1). In addition, however, we show that neither *F3* nor TF are found in precursor or mature B-or T-cell lineage lymphomas and leukemias. As background, Mohan Rao showed that TF protein was absent by IHC in a third of epitehlial neoplasias, and in most non-epithelial tumors including 4 lymphomas of unspecified subtype [21]. These findings are in line with the fact that under physiologic conditions, TF is not expressed by lymphocytes, although a subpopulation of lymphocytes of B-cell origin may express functional TF *in vitro,* in response to stimulation with phorbol myristate [22].

Interestingly, we found that Hodgkin´s lymphomas (HL) showed *F3* expression in approximately a third of cases, which may be explained by the fact that malignant cells make up only 1% of the tumor bulk in HL [23]. TF expression likely corresponds to cells from the tumor microenvironment. In line with this hypothesis, the high expression of TF from peripheral blood monocytes of HL patients has been reported to be associated with VTE [24].

Regarding myeloid-cell-derived tumors, *F3* was expressed by a fourth of acute myeloid leukemias (AML), most of which had a monocytic component. The only acute promyelocytic leukemia included, expressed high levels of TF, a finding previously reported by others, and thought to contribute to the procoagulant state in these malignancies [25]. Although TF protein was absent by IHC in all patient-derived bone marrow biopsies with AML, we believe that it is likely that including a larger number of samples would likely detect a subset of positive cases, since tumor-cell-derived TF has been reported in leukemias of monocytic or promyelocytic origin. Furthermore, TF has been shown to be present in the circulation as TF-bearing microparticles derived from myeloid blasts [26,27].

The main weakness of this study is that the data are descriptive, and based on current knowledge of the role of TF in cancer, yet no mechanistic or experimental data are provided. The other important limitation is that we do not evaluate malignant behavior or thrombosis in this study in relation to TF expression. Further work on the mechanisms of thrombosis and the cell of origin of TF in solid versus hematological tumors is warranted.

In conclusion, the absence of TF in hematological tumors contrasts sharply with the frequency of its expression in solid tumors. This fact is relevant, since targeting tumor-associated TF as is currently proposed for solid tumors (through the use of immuno-conjugates, anti-TF antibodies, TF pathway inhibitors, targeted photodynamic therapy, and microRNAs), may not be useful in lymphoid neoplasias [4,28,29]. This does not rule out however, a role for TF from non-neoplastic cell sources (activated endothelium, platelets and or monocytes) as a result of host response to cancer or its treatment, in the development of thrombosis or in the induction of malignant tumor behavior [30]. Indeed blood-borne microparticle-associated TF is increased across individuals with lymphomas and leukemias, and TF mRNA levels have been shown by others to be significantly increased in patients

with lymphoma undergoing chemotherapy [31,32]. Our findings suggest that the source of this circulating TF is most likely not tumor-cell-derived. This opens the question as to whether it is mainly the TF produced by the neoplastic cells that drives malignant behavior in solid tumors. To our knowledge, this is the first report showing that *F3* and TF are not expressed by the neoplastic cells in T- or B-cell lymphoid malignancies, or by most myeloid derived tumors, adding to the understanding of cancer–related thrombosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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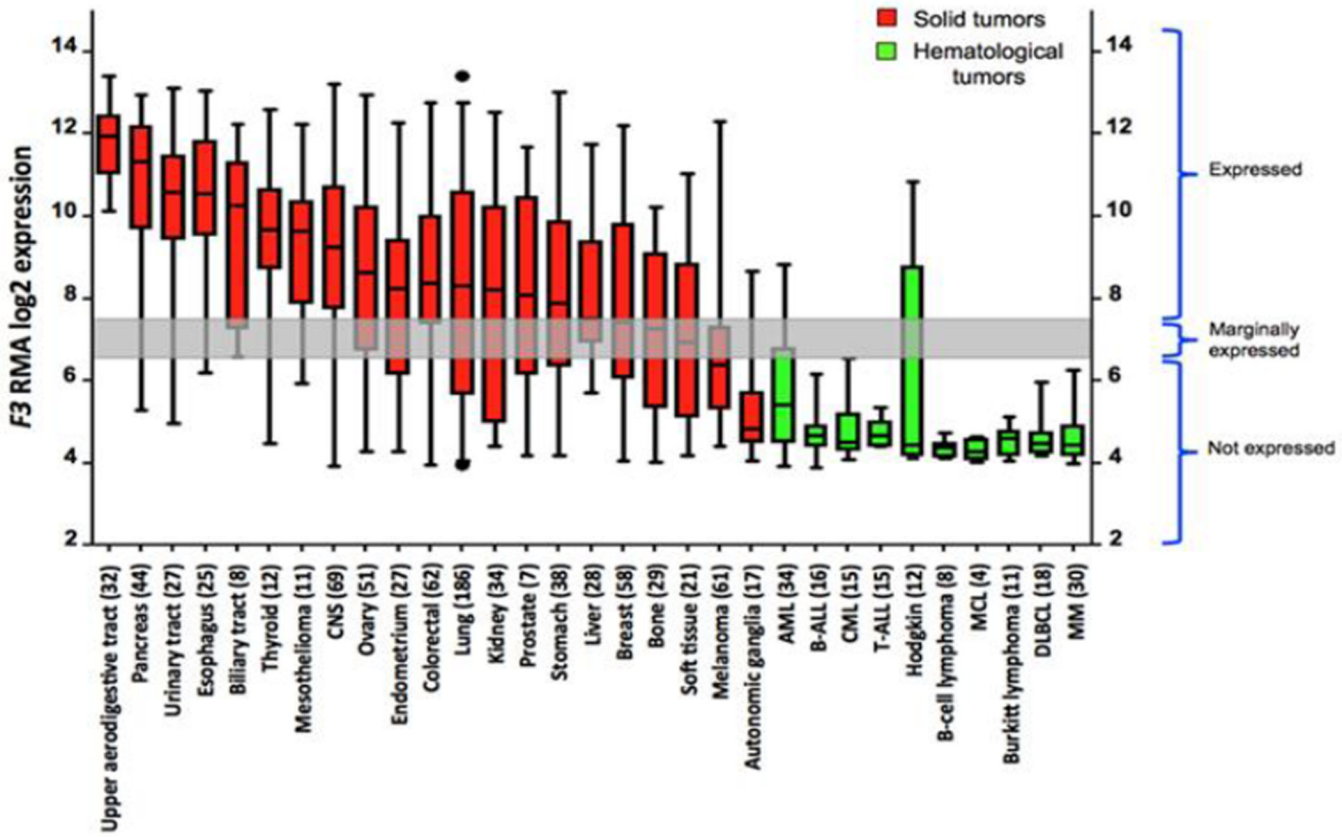

Figure 1. Gene expression analysis of TF gene (*F3*)

The number of cases of each tumor entity are indicated in parenthesis. *F3* expression (RMA log2 expression values) range is depicted in red bars for solid tumors and in green bars for hematological (both lymphoid and myeloid) neoplasias. Estimative of expression levels are as follows: RMA log2 values below 6.5 were associated with lack of expression; values between 6.5 and 7.5 (gray box) as marginally expressed; and values above 7.5 were considered as intermediate to high expression. Results clearly show absence of *F3* expression in cell lines established form all lymphoid neoplasias including progenitor cell-derived acute leukemias, and mature B and T-cell lymphomas. Myeloid derived acute leukemias are negative except for 25%, which show marginal *F3* expression. Finally, a subset of Hodgkin´s lymphomas, which are predominantly (99%) composed of microenvironment, express *F3*. The findings in hematological neoplasms contrast with the significantly higher *F3* expression levels found in most solid-tumor cell lines.
Abbreviations: Acute myeloid leukemia (AML), B-cell acute lymphoblastic leukemia (B-ALL), chronic myeloid leukemia (CML), T-cell acute lymphoblastic leukemia (T-ALL), mantle cell lymphoma (MCL), diffuse large B-cell lymphoma (DLBCL), multiple myeloma (MM).

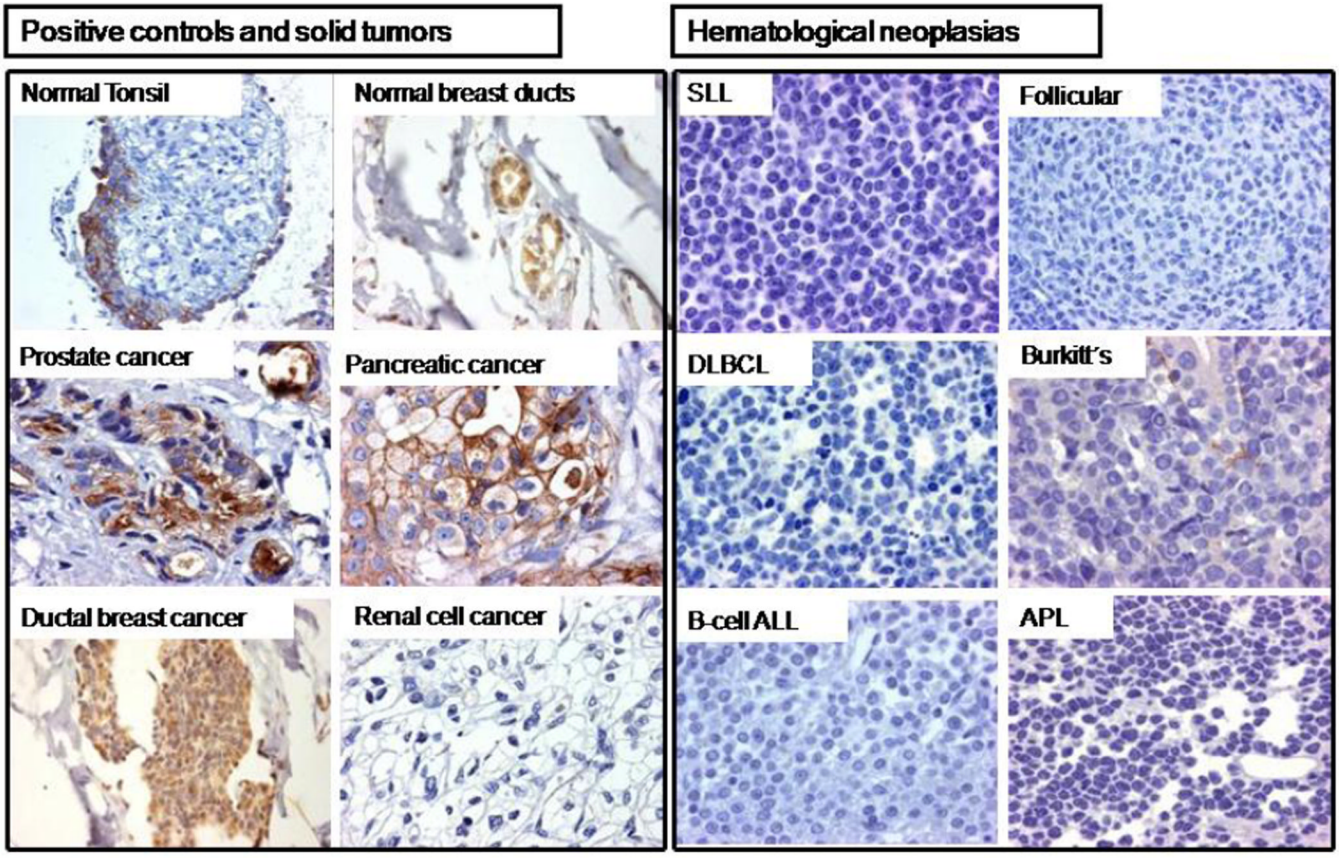

Figure 2. Tissue factor expression in representative tumors by immunohistochemistry (IHC)

(A) Tonsillar epithelium and normal breast ducts were used as positive controls for TF expression, while tonsillar subepithelial connective tissue was used as negative control. TF was expressed by solid tumors (prostate, pancreatic, and ductal breast adenocarcinoma), but not by clear cell renal cell carcinoma. (B) All 129 lymphoid neoplasias were negative for TF expression, including 89 high-grade diffuse large B-cell lymphoma (DLBCL) patient samples (25 with germinal center and 64 with non-germinal center phenotype), as well as 9 low-grade lymphomas including small lymphocytic (SLL) and follicular lymphomas, 10 acute lymphoblastic leukemias (ALL), and 20 peripheral T-cell lymphomas. In Burkitt's lymphoma, the positive TF staining seen in the basement membrane of gastric glands contrasts with the absence of TF in neoplastic cells. TF was absent in this case of acute promyelocytic leukemia (APL).