

Emostasi e Tumori

GENERATION OF A TARGETING NON-BLOCKING TISSUE FACTOR ANTIBODY FOR SAFE AND EFFICACIOUS CANCER TREATMENTS

A. Metelli[1], P. Nisticò[1], J. Wrangle[2]

[1]IRCCS Regina Elena National Cancer Institute, Roma, Italy; [2]Medical University of South Carolina, Charleston, USA

Background and Aims. Tissue factor (TF) is overexpressed in approximately 80% of human malignancies and is associated with poor prognosis, making it an attractive target for cancer immunotherapy. Tisotumab vedotin (TV), a TF-targeting antibody–drug conjugate, has been approved for the treatment of recurrent or metastatic cervical cancer. However, its clinical benefit remains modest, with a limited improvement in overall survival compared to standard chemotherapy, and its use is complicated by significant adverse events, including severe hemorrhage. Given that TF is the primary initiator of the extrinsic coagulation cascade through complex formation with Factor VIIa (FVIIa), therapeutic strategies that interfere with TF/FVIIa activity may compromise hemostatic balance. Therefore, our aim is to develop TF-targeted therapies that inhibit tumor progression while preserving physiological coagulation.

Methods. Monoclonal anti-TF antibodies were generated using hybridoma technology. Forty clones were screened and compared with tisotumab for binding affinity, avidity, and ability to interfere with TF/FVIIa-mediated coagulation. Binding was assessed by ELISA and flow cytometry, while avidity was evaluated using a urea-based ELISA dissociation assay. Functional blockade of TF activity was determined by prothrombin time (PT) assays and measurement of Factor Xa generation. Epitope competition assays were performed by ELISA to determine whether selected clones shared binding epitopes with tisotumab. For in vivo evaluation, murine Lewis lung carcinoma cells were engineered to express human TF and implanted into mice to assess therapeutic efficacy and safety.

Results. One clone demonstrating strong binding to TF with minimal interference in TF/FVIIa-mediated coagulation was selected for further characterization. Competitive binding assays showed that its interaction with TF was not affected by the presence of tisotumab, indicating recognition of a distinct epitope. In the preclinical model, treatment with the selected anti-TF antibody resulted in significant tumor growth control without evidence of hemorrhagic complications. The antibody was subsequently humanized to enable translational development. Additionally, luciferase-based reporter assays demonstrated that our anti-TF antibody modulates transforming growth factor (TGF)-β signaling, a key pathway driving tumor progression and metastatic dissemination.

Conclusions. We generated and humanized a monoclonal anti-TF antibody that selectively targets TF without disrupting its physiological role in hemostasis. This antibody represents a promising platform for the development of next-generation TF-targeted therapeutics, including bispecific antibodies, T-cell engagers, CAR T-cell therapies, and antibody–-drug conjugates. Our approach aims to exploit TF as a tumor-associated antigen while minimizing the risk of coagulation-related adverse events, thereby advancing the design of safer and more effective TF-based cancer immunotherapies.