Article

Regulation of Tissue Factor by CD44 supports coagulant activity in breast tumor cells.

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**Simple summary:** Metastasis and thromboembolic complications are the main cause of cancer-associated death. An overexpression of coagulation factors, and particularly Tissue factor, by tumor cells is a key event implicated in this observed hypercoagulability. Tissue factor is indeed a cellular initiator of the coagulation cascade which has been associated with aggressive tumor phenotypes such as those characteristics of Epithelial-Mesenchymal Transitions (EMTs) and Cancer Stem Cells (CSCs). Understanding molecular mechanisms controlling Tissue Factor overexpression in those tumor phenotypes is thus an important aspect of cancer research. We show here that CD44 (a transmembrane marker of CSC and EMT phenotypes) contributes to regulate TF expression at a transcriptional level, thereby supporting procoagulant properties in tumor cells that facilitate their metastatic spread.

**Abstract** Previous work identified Tissue Factor (TF), a key activator of the coagulation cascade, as a gene induced in cellular contexts of Epithelial-Mesenchymal Transitions (EMTs), providing EMT+ Circulating Tumor Cells (CTCs) with coagulant properties that facilitate their metastatic seeding. Deciphering further molecular aspects of TF regulation in tumor cells, we report here that CD44 and TF coexpress in EMT contexts, and that CD44 acts as a regulator of TF expression supporting procoagulant properties and metastatic seeding. A transcriptional regulatory mechanism bridging CD44 to TF expression was further evidenced. Comparing different TF –promoter luciferase reporter constructs, we indeed found that the shortest -111pb TF promoter fragment harboring 3 Specificity Protein 1 (Sp1) binding sites is still responsive to CD44 silencing. The observation that (i) mutation within Sp1 binding sites decreased the basal activity of the -111pb TF promoter construct, (ii) CD44 silencing decreased Sp1 protein and mRNA levels and (iii) Sp1 silencing diminished TF expression further points to Sp1 as a key mediator linking CD44 to TF regulation. All together, these data thus report a transcriptional regulatory mechanism of TF expression by CD44 supporting procoagulant activity and metastatic competence of CTCs.

**Keywords:** Tissue Factor; CD44; Epithelial-Mesenchymal Transitions; Metastasis; Coagulation