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Neutrophil elastase-dependent cleavage compromises the tumor suppressor role of EMILIN1

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ABSTRACT

Proteolysis of the extracellular matrix (ECM) is a key event in tumor growth and progression. The breakdown of ECM can lead to the generation of bioactive fragments that promote cell growth and spread. EMILIN1, a multidomain glycoprotein expressed in several tissues, exerts a crucial regulatory function through the engagement of α4/α9 integrins. Unlike the majority of ECM molecules that elicit a proliferative program, the signals emitting from EMILIN1 engaged by α4/α9β1 integrins are antiproliferative. In this study, aimed to demonstrate if the suppressor role of EMILIN1 was related to its structural integrity, we tested the possibility that EMILIN1 could be specifically cleaved. Among the proteolytic enzymes released in the tumor microenvironment we showed that neutrophil elastase cleaved EMILIN1 in three/four major fragments. The consequence of this proteolytic process was the impairment of its anti-proliferative role. Accordingly, EMILIN1 was digested in sarcomas and ovarian cancers. Sarcoma specimens were infiltrated by neutrophils (PMNs) and stained positively for elastase. The present findings highlight the peculiar activity of PMN elastase in disabling EMILIN1 suppressor function.

1. Introduction

The microenvironment, in which a tumor originates and that plays a critical role in tumor development and progression (Albini and Sporn, 2007), consists of cells, mainly fibroblasts, immune and vascular cells, soluble molecules and extracellular matrix (ECM) constituents. During tumorigenesis ECM co-evolves generating a complex crosstalk with both positive and negative influences on tumor cells (Mueller and Fusenig, 2004). The quantitative and qualitative changes in the ECM are a key modification of the stromal tumor environment. Many evidences support the concept that several ECM proteins, through their respective integrin receptors, can favor tumor progression and spread (Streuli, 2009). On the contrary, only very few ECM proteins are known to exert primarily a tumor suppressor function. For instance, Trombospondin-1, Trombospondin-2 and Fibulin-2 counteract tumor growth negatively impacting angiogenesis (Bornstein, 2009; Law et al., 2012); LTBP-2 impairs migration and invasion ability of neoplastic cells (Chan et al., 2011). A more direct effect on cell proliferation and survival was described for CCN1, decorin and EMILIN2 (Todorovic et al., 2005; Seidler et al., 2006; Mongiat et al., 2007).

EMILIN1 is an ECM multidomain glycoprotein and it is expressed in lymphatic capillaries, in the walls of large blood vessels, in intestine, lung, lymph nodes, and skin (Bressan et al., 1993; Zanetti et al., 2004; Danussi et al., 2008). EMILIN1 displays strong adhesive and migratory properties for different cell types (Spessotto et al., 2003; Spessotto et al., 2006; Verdone et al., 2008). Beside the functional significance of adhesion and migration as the consequence of the interaction between EMILIN1 and α4/α9 integrin, the striking aspect of this ligand/receptor pair is related to proliferation (Danussi et al., 2011; Danussi et al., 2013). It is generally known that integrin engagement positively regulates cell growth (Streuli, 2009). The finding that EMILIN1 negatively regulates cell proliferation points out a novel function of α4β1 as well as of α9β1 integrin: signals emitting from EMILIN1 engaged by α4/α9β1 integrins are antiproliferative (Danussi et al., 2011). Recent findings obtained in *Emilin1*$^{-/-}$ mice and showing that an EMILIN1-negative or EMILIN1-unfunctional microenvironment promotes tumor cell and metastatic spread suggest that the role played by this ECM glycoprotein is particularly crucial in providing regulation in tumor progression (Danussi et al., 2011; Danussi et al., 2012).

Abbreviations: ECM, extracellular matrix; PMN, polymorphonuclear leukocyte; NE, neutrophil elastase; USTS, undifferentiated soft tissue sarcoma; LMS, leiomyosarcoma; MMP, matrix metalloproteinase.

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Under several pathological conditions including cancer both increased synthesis of certain ECM components and/or increased breakdown with consequent generation of fragments can contribute to tumor growth and progression (Kessenbrock et al., 2010; Lu et al., 2012). The importance of proteolysis in favoring migration and invasion is clear since ECM degradation generates pathways for cell to migrate through physical barriers. Most interestingly, proteolysis of ECM leads to the generation of bioactive fragments that modulate cell growth. The inflammatory cells surrounding and infiltrating the tumor microenvironment display many tumor-promoting effects which accelerate the proliferation and survival of malignant cells, and promote metastatic spread through the release of cytokines, interleukins and enzymes (Mantovani et al., 2008). The presence of infiltrating inflammatory cells such as neutrophils (PMNs) is a peculiar feature of many tumor microenvironments (Dumitru et al., 2013; Smith and Kang, 2013) and neutrophil elastase (NE) profoundly influences cancer growth and development (Houghton et al., 2010). Indeed, a better understanding of molecular direct/indirect mechanisms involved in tumor-associated inflammation could provide new insights for improved treatments in cancer.

In this study we show that EMILIN1 suppressor function was impaired by its cleavage and that NE was the proteolytic enzyme involved in this process. Moreover, we demonstrated that EMILIN1 is digested in sarcomas and ovarian cancers, highlighting that its fragmentation can be a relevant aspect in tumor growth and metastatic process.

2. Results

2.1. EMILIN1 is digested by PMN supernatants

To test if EMILIN1 was digested and to what extent, we incubated EMILIN1 with the different cellular supernatants. Trophoblast (HTR8/Svneo) and decidual stromal cells (DSC) for which a specific involvement was already known in EMILIN1 migratory microenvironment (Spessotto et al., 2006) were used; HT1080, a tumor cellular source of large amounts of several matrix metalloproteinases (MMPs), and inflammatory cells as monocytes, macrophages and PMNs that infiltrate tumors were stimulated with appropriate stimuli to obtain supernatants highly enriched in soluble factors and enzymes. After a 30 min incubation at 37 °C, only supernatants obtained from PMNs fully stimulated with fMLP plus cytochalasin B (CB) were able to digest EMILIN1 (Fig. 1A). Supernatants from fMLP-treated PMNs had a very modest effect and supernatants from resting PMNs were inefficient when compared with fully activated PMNs (Fig. 1B,C), indicating that the degranulation resulting from PMN activation was responsible for EMILIN1 digestion. Accordingly, lysates from resting PMNs were more efficient than lysates from degranulated PMNs (Fig. 1B,D). PMNs, even if highly activated, are able to degranulate only in part their granuli content (Liou and Campbell, 1996), as demonstrated by the enzymatic activity present in the lysate counterparts (Fig. 1D). Neither lysates nor supernatants from TNF-α-stimulated macrophages had any effect (Fig. 1C).

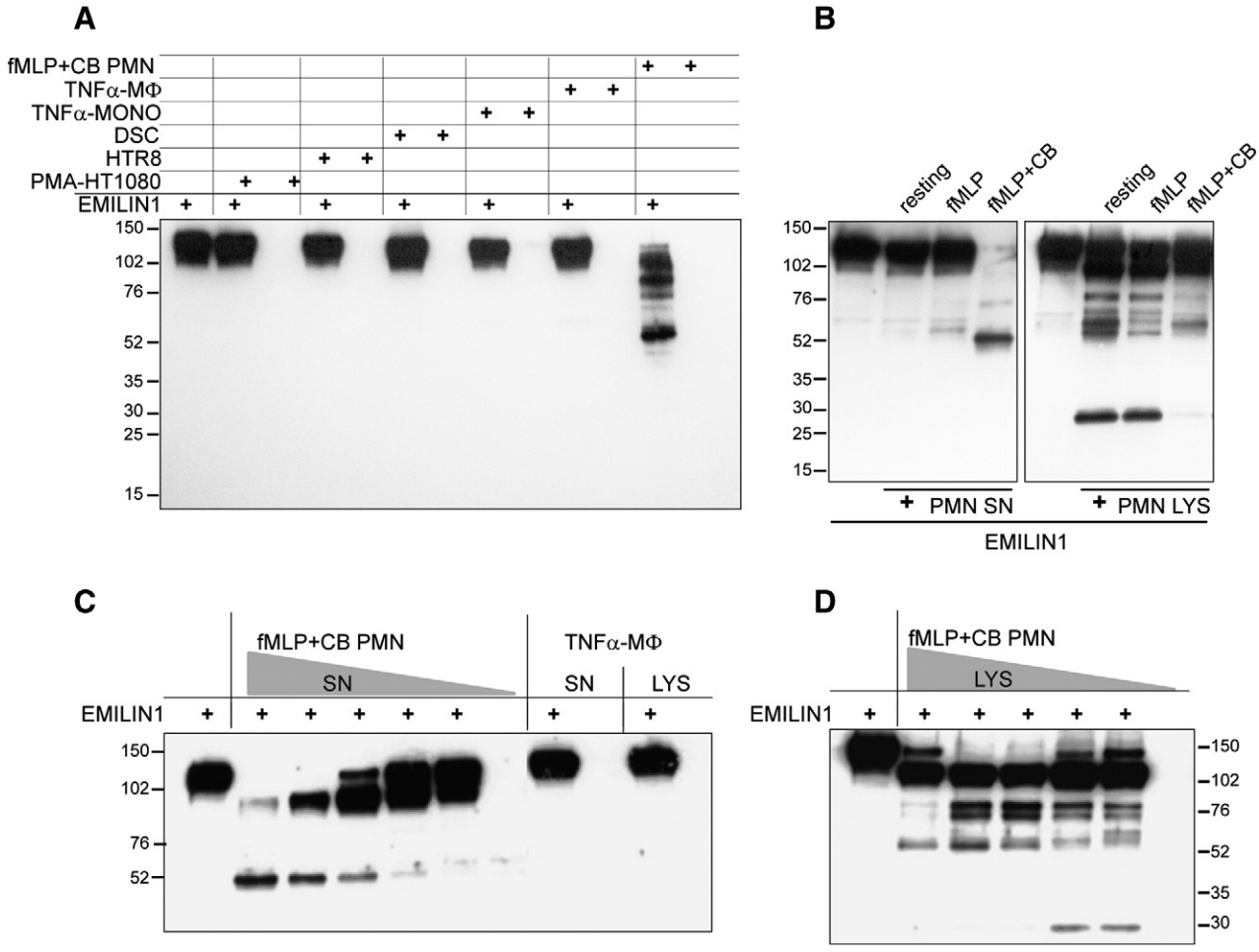

Fig. 1. Supernatants from activated PMNs cleave EMILIN1. A. EMILIN1 (10 μg/ml) was incubated for 30 min at 37 °C with supernatants derived from different cells. B. Supernatants and lysates from resting, fMLP-stimulated or fMLP plus CB-stimulated (fMLP + CB) PMNs were added to EMILIN1 and incubated for 30 min at 37 °C. Increasing concentrations of supernatants (C) or lysates (D) from fLMP + CB-stimulated PMNs were added to EMILIN1. Incubations were done for 30 min at 37 °C and the cleavage products were detected by western blotting with a polyclonal antibody (As556) against EMILIN1. MΦ, macrophages; MONO, monocytes; PMA-HT1080, HT1080 stimulated with PMA (phorbol 12-myristate 13-acetate). Each blot is representative of almost three other experiments with similar results.