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Membrane type-1 matrix metalloproteinase (MT1-MMP) protects malignant cells from tumoricidal activity of re-engineered anthrax lethal toxin

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Abstract

Protective antigen (PA) and lethal factor (LF) are the two components of anthrax lethal toxin. PA is responsible for interacting with cell receptors and for the subsequent translocation of LF inside the cell compartment. A re-engineered toxin comprised of PA and a fusion chimera LF/*Pseudomonas* exotoxin (FP59) is a promising choice for tumor cell surface targeting. We demonstrated, however, that in vitro in cell-free system and in cultured human colon carcinoma LoVo, fibrosarcoma HT1080 and glioma U251 cells membrane type-1 matrix metalloproteinase (MT1-MMP) cleaves both the PA83 precursor and the PA63 mature protein. Exhaustive MT1-MMP cleavage of PA83 in vitro generates several major degradation fragments with an N-terminus at Glu^{40}, Leu^{48}, and Gln^{512}. In cultured cells, MT1-MMP-dependent cleavage releases the cell-bound PA83 and PA63 species from the cell surface. As a result, MT1-MMP expressing cells have less PA63 to internalize. In agreement, our observations demonstrate that MT1-MMP proteolysis of PA makes the MT1-MMP-expressing aggressive invasive cells resistant to the cytotoxic effect of a bipartite PA/FP59 toxin. We infer from our studies that synthetic inhibitors of MMPs are likely to increase the therapeutic anti-cancer effect of anthrax toxin. In addition, our study supports a unique role of furin in the activation of PA, thereby suggesting that furin inhibitors are the likely specific drugs for short-term therapy of anthrax infection.

Keywords: Tumorigenesis; Furin; Anthrax lethal toxin; Lethal factor; Protective antigen; Cytotoxicity; *Bacillus anthracis*

1. Introduction

Three proteins of anthrax toxin [protective antigen (PA), lethal factor (LF), and edema factor (EF)] are responsible for the pathological symptoms of anthrax (Moayeri, Haines, Young, & Leppla, 2003; Mock & Mignot, 2003; Prince, 2003). During the process of intoxication, the 83 kDa PA monomer (PA83) binds to the cell surface receptor (Bradley, Mogridge, Mourez, Collier, & Young, 2001; Bradley & Young, 2003; Bradley, Mogridge, Rainey, Batty, & Young, 2003;

Abbreviations: DMEM, Dulbecco's MEM; FP59, fusion protein 59 (a chimera in which LF residues 1–254 have been fused with the ADP-ribosylation domain III of *Pseudomonas* exotoxin A); EF, edema factor; LF, lethal factor; lysis buffer, 5 mM Tris–HCl buffer, pH 8.0, containing 1% SDS, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 1 μg/ml pepstatin and 1 mM PMSF; MT1-MMP, membrane type-1 matrix metalloproteinase; MTT, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide; PA, protective antigen

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Scobie, Rainey, Bradley, & Young, 2003) and is then cleaved at the sequence Arg-Lys-Lys-Arg by cellular furin-like proteases (Gordon, Klimpel, Arora, Henderson, & Leppla, 1995). This cleavage releases a 20 kDa N-terminal fragment and a cell-bound, C-terminal 63 kDa protein (PA63). PA63 oligomerizes into a ring-shaped heptamer and exposes the binding sites for EF and LF (Bhatnagar & Batra, 2001; Milne, Furlong, Hanna, Wall, & Collier, 1994; Mock & Fouet, 2001; Singh, Klimpel, Goel, Swain, & Leppla, 1999). The amino terminal ends of both EF and LF bind to PA and are responsible for translocation in the PA63 heptamer pore while the respective C-terminal parts of EF and LF exhibit the adenylate cyclase and proteinase activity, respectively (Beauregard, Collier, & Swanson, 2000; Chauhan & Bhatnagar, 2002; Duesbery et al., 1998; Leppla, 1982; Liu & Leppla, 2003). LF is a zinc-dependent metalloprotease which cleaves a specific bond at the amino terminal region of mitogen-activated protein kinase kinases (MAPKKs), destroying their ability to signal and blocking the essential signal transduction pathways (Bardwell, Abdollahi, & Bardwell, 2004; Chopra, Boone, Liang, & Duesbery, 2003). The complex formed between PA63 and EF or LF is internalized into the cell by receptor-mediated endocytosis (Abrami, Liu, Cosson, Leppla, & van der Goot, 2003). Following endocytosis and release into the cytosol, LF and EF attack their targets and exert their toxic effects.

MMPs are zinc metalloenzymes that are involved in the breakdown of extracellular matrix proteins, cytokines, and cell receptors (Egeblad & Werb, 2002; Overall & Lopez-Otin, 2002). A transmembrane domain and a cytoplasmic tail distinguish membrane type-1 matrix metalloproteinase (MT1-MMP) from soluble MMPs (Nagase & Woessner, 1999; Seiki, 2003; Seiki & Yana, 2003). Invasion-promoting MT1-MMP is ideally positioned to play a critical role in matrix degradation, modification of cell receptors and in the activation pathway of soluble secretory MMPs (Belkin et al., 2001; Deryugina et al., 2001; Deryugina, Ratnikov, Postnova, Rozanov, & Strongin, 2002a; Hotary et al., 2003; Kajita et al., 2001). In agreement, MT1-MMP is directly involved in malignant progression and metastasis and is strongly associated with the most aggressive, invasive malignant cells (Belien, Paganetti, & Schwab, 1999; Deryugina, Soroceanu, & Strongin, 2002b; Habelhah et al., 1999; Hornebeck, Emonard, Monboisse, & Bellon, 2002; Nabeshima et al., 2000; Zucker et al., 2000).

Here, we report that MT1-MMP cleaves protective antigen in cell-free system in vitro and in cultured cells. In cells, MT1-MMP cleavage results in the protective antigen shedding causing the reduction of the levels of protective antigen associated with the cell surface and repression of the general tumoricidal effect of lethal toxin.

2. Materials and methods

2.1. Reagents

All reagents were from Sigma (St. Louis, MO) unless otherwise indicated. A hydroxamate inhibitor GM6001 and rabbit polyclonal antibodies AB815 against the hinge region of MT1-MMP were from Chemicon (Temecula, CA). A furin inhibitor decanoyl-Arg-Val-Lys-Arg-chloromethylketone (dec-RVKR-cmk) was from Bachem (King of Prussia, PA). A murine monoclonal antibody MON-152 against furin was obtained from Alexis (Carlsbad, CA). The recombinant version of the catalytic domain of MT1-MMP (MT1-CAT) was expressed in *Escherichia coli*, purified from inclusion bodies and refolded to restore its native conformation as described previously (Ratnikov et al., 2000). PA83 and PA63 were obtained from List Labs. TIMP-2-free pro-MMP-2 was isolated from conditioned medium of p2AHT2A72 cells derived from HT1080 fibrosarcoma cell line sequentially transfected with E1A and MMP-2 cDNAs (Strongin, Marmer, Grant, & Goldberg, 1993; Strongin et al., 1995). The LF/*Pseudomonas* exotoxin FP59 chimera was purified according to the published protocols (Liu et al., 2001; Varughese et al., 1998).

2.2. General methods

Immunoprecipitation of furin and MT1-MMP from cells surface-biotinylated with membrane-impermeable EZ Link Sulfo-NHS-LC-Biotin (Pierce, Rockford, IL) was accomplished as described earlier (Deryugina et al., 2001; Rozanov et al., 2001). The gelatinolytically active species of MMP-2 were identified by zymography in 0.1% gelatin–10% acrylamide gels