Letters to the Editor

Mesothelin Is Shed from Tumor Cells

To the Editors: We read with great interest the article by Hellstrom et al. (1) about characterization of soluble mesothelin proteins (SMP) in cancer patients. The authors found that the SMP in ascites from a patient with ovarian carcinoma contained the sequence of extracellular domain of a membrane-bound mesothelin (2, 3) rather than a soluble form speculated previously by Scholler et al. (4). Their results indicate that membrane-bound mesothelin could be shed from the tumor cells to generate SMP detected in patient sera by ELISA. We agree with their conclusion that membrane-bound mesothelin is the predominant protein present in the serum and here provides direct evidence showing that such shedding does in fact take place.

Our experiments are summarized in Fig. 1. Using an SS1P affinity column, we purified SMP from culture supernatants of a well-documented A431/K5 cell line (3) expressing only membrane-bound mesothelin. SS1P is an immunotoxin with an Fv specific for mesothelin. About 100 ng/mL SMP was found in the culture supernatant. The molecular weight of purified SMP was ~40 kDa (Fig. 1A), consistent with the SMP described in Hellstrom et al. (1). The full-length mesothelin precursor proteins were not detected in culture supernatant. After deglycosylation with PNGase, the molecular weight of SMP was ~34 kDa. The SMP bands were cut out from a Coomassie-stained gel, protein in the gel was digested with trypsin, and the tryptic peptides were analyzed using liquid chromatography ion trap mass spectrometry (Fig. 1B). The sequence of NH_2 terminal (EVEK) was determined by automated Edman degradation, and peptides corresponding to multiple internal regions of mesothelin were determined using the mass spectrometry/mass spectrometry mode on the ion trap mass spectrometer. Our results confirmed that the SMP was the extracellular domain of membrane-bound mesothelin shed from cells.

Mesothelin is a glycosyl-phosphatidylinositol–anchored protein. Release of such proteins from the cell surface into the serum and other body fluids can be mediated by phospholipase or proteases (5). To determine whether the shedding of mesothelin is dependent on phosphatidylinositol-specific phospholipase C, we did anti-cross-reacting determinant assay (Prozyme, San Leandro, CA) by Western blot. The SMP was not recognized by anti-cross-reacting determinant (Anti-CRD) antibodies (Fig. 1C).

In conclusion, our data show that the extracellular domain of membrane-bound mesothelin can be shed from tumor cells. The shedding may not require phosphatidylinositol-specific phospholipase C phospholipolysis. The precise mechanisms need further investigation.

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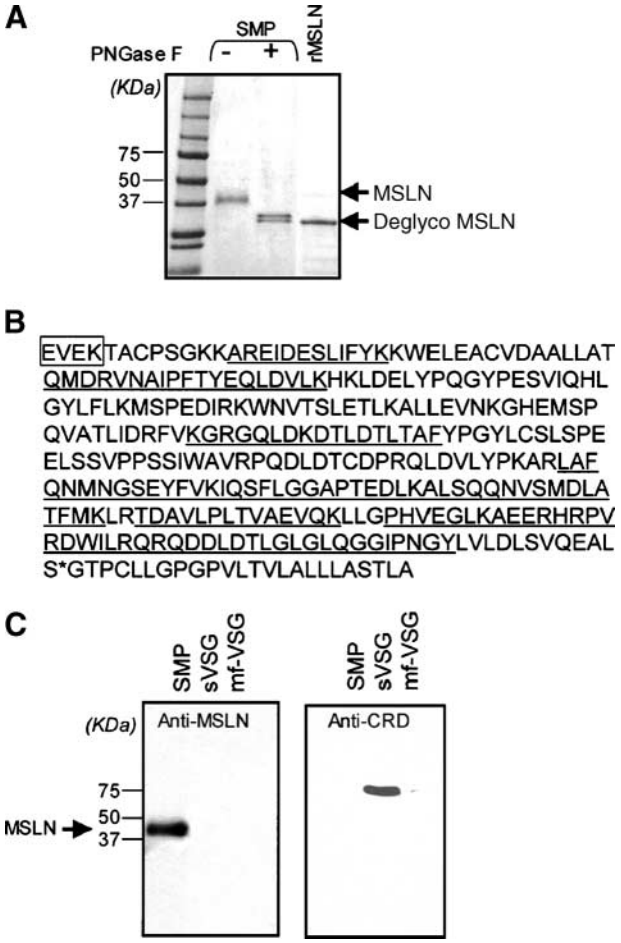

Figure 1. A. SMPs in culture supernatant of A431/K5 cells were purified and analyzed with or without PNGase F digestion by SDS-PAGE. Recombinant bacterial mesothelin proteins (*rMSLN*) were used as a control. *Deglyco,* N-linked deglycosylation. **B.** SMPs were analyzed by mass spectrometry. *Underline,* the identified peptide sequences were aligned with membrane-bound mesothelin (MSLN). *Box,* NH_2-terminal sequence. *, predicted glycosyl-phosphatidylinositol cleavage site. **C.** The SMP was recognized by an anti-MSLN antibody but not by an anti-cross-reacting determinant (Anti-CRD) antibody by Western blot. *sVSG,* soluble form of variant surface glycoprotein; *mf-VSG,* the membrane form of VSG.

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