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Alterations in manganese, copper, and zinc contents, and intracellular status of the metal-containing superoxide dismutase in human mesothelioma cells

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Abstract

Background and aim: Molecular diagnostics and therapeutics of human mesothelioma using disease-related markers present major challenges in clinical practice. To identify biochemical alternations that would be markers of human mesothelioma, we measured the intracellular steady-state levels of biologically important trace metals such as manganese (Mn), copper (Cu), and zinc (Zn) in a human mesothelial cell line, MeT-5A, and in five human mesothelioma cell lines (MSTO-211H, NCI-H226, NCI-H2052, NCI-H2452, ACC-MESO-1) by inductively coupled plasma-mass spectrometry (ICP-MS). We also aimed to investigate whether the alterations were related to the intracellular status of metal-containing superoxide dismutase (SOD).

Results: There were no significant differences in the contents of the trace metals among MeT-5A, MSTO-211H, and ACC-MESO-1 cells. However, each of the other three mesothelioma cell lines had a unique characteristic in terms of the intracellular amounts of the metals; NCI-H226 contained an extremely high level of Mn, an amount 7.3-fold higher than that in MeT-5A. NCI-H2052 had significantly higher amounts of Cu (3.4-fold) and Zn (1.3-fold) compared with MeT-5A. NCI-H2452 contained about 5.8-fold the amount of Cu and 2.5-fold that of Mn compared with MeT-5A. As for the intracellular levels of copper/zinc-SOD (Cu/Zn-SOD) and manganese-SOD (Mn-SOD), those of Cu/Zn-SOD were relatively unchanged among the cells tested, and no notable correlation with Cu or Zn contents was observed. On the other hand, all mesothelioma cells highly expressed Mn-SOD compared with MeT-5A, and a very high expression of the enzyme with a robust activity was observed in the two mesothelioma cells (NCI-H226, NCI-H2452) containing a large amount of Mn.

Conclusions: In comparison with MeT-5A human mesothelial cells, some human mesothelioma cells had significantly higher amounts of Mn or Cu and one mesothelioma cell had a significantly higher amount of Zn. Interestingly, all mesothelioma cells overexpressed Mn-SOD compared with MeT-5A, and the cells whose Mn-SOD activity was increased contained higher amounts of Mn. It seemed that intracellular Mn content was positively

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correlated with Mn-SOD, suggesting that the intracellular Mn level is associated with Mn-SOD activity. These biochemical signatures could be potential disease-related markers of mesothelioma.

Keywords: Mesothelioma; Trace element; Superoxide dismutase

Introduction

Mesothelioma is a rare, refractory malignancy that is derived from mesothelial cells lining the pleural and peritoneal cavities. The causal relationship between asbestos exposure and the onset of the disease has been well-established epidemiologically. Since the use of asbestos rapidly increased during the second half of the twentieth century, it is expected that the incidence of the disease will increase in industrialized countries, including Japan [1]. From the clinical point of view, the accurate and specific diagnosis of the disease in its early stage is a matter of considerable urgency, highlighting the importance of identifying disease-related biomarkers that could be applied for the early detection of the disease.

Recently, we have shown that asbestos exposure induces ferritin heavy chain protein that suppresses reactive oxygen species (ROS) toxicity [2]. We demonstrated that iron in asbestos, which generates ROS in cells, induced the iron-binding protein, and this induction might potentiate mesothelioma development. That study prompted us to investigate the role of trace elements and its binding proteins in mesothelioma pathogenesis. In fact, it has been suggested that some trace metals and their interactions with various biomolecules are related to the carcinogenic process [3]. In some tumors, including those in lung, kidney and liver, altered intracellular amounts of metals have been reported [4–6]. However, to our knowledge, the intracellular status of trace metals in mesothelioma cells has not yet been assessed.

Interestingly, it has been shown that mesothelioma cells show an increased activity of manganese-superoxide dismutase (Mn-SOD) and, to a lesser extent, copper/zinc-superoxide dismutase (Cu/Zn-SOD), compared with normal tissues or other cancers [7]. Since these enzymes require Mn, Cu, and Zn for their enzymatic activity, we hypothesized that the intracellular contents of these metals in mesothelioma cells are possibly altered and that alterations might be potential biochemical markers for the diagnosis and therapeutics of mesothelioma. To explore this hypothesis, we measured the intracellular amounts of the trace metals in various human mesothelioma cell lines using inductively coupled plasma-mass spectrometry (ICP-MS) with a highly sensitive, quantitative method [8]. In addition, we determined the cellular status of Mn-SOD and Cu/Zn-SOD with the aim of determining whether the levels were associated with the trace element contents. Herein, we show the alterations in the trace metals and the metal-containing SOD in mesothelioma cells and discuss their potential use as disease-related biomarkers.

Material and methods

Cells

The immortalized human pleural mesothelial cell line MeT-5A and the human mesothelioma cell lines MSTO-211H, NCI-H226, NCI-H2052, and NCI-H2452 were purchased from ATCC (American Type Culture Collection, Manassas, VA, USA). ACC-MESO-1 [9] was purchased from RIKEN BRC cell bank. MSTO-211H was established from a patient with biphasic mesothelioma. NCI-H2452 has epithelial morphology, and the subtype of ACC-MESO-1 is epithelioid [10]. They were grown in RPMI 1640 medium (Sigma-Aldrich, St Louis, MO, USA) supplemented with 10% fetal bovine serum (JRH Biosciences Inc., Lenexa, KS, USA), 50 U/mL penicillin, and 50 μg/mL streptomycin (Invitrogen Corporation, Carlsbad, CA, USA). They were passaged twice a week when reaching 80–90% confluence in 100-mm dishes.

Inductively coupled plasma-mass spectrometry (ICP-MS) analysis

All cell lines were maintained in 100-mm dishes under 5% CO_2 at 37 °C for 3 days before sample preparation for ICP-MS without medium change. On the day of sampling, the cells were washed three times with 1 mol/L Tris-HCl (pH 7.4, Fluka, St. Louis, MO, USA) containing 8.9 g/L NaCl (Sigma-Aldrich), and the supernatant was removed completely. The cells and the culture medium were lysed in 5 mL concentrated HNO_3 (TAMAPURE AA-100, Tama chemicals, Kawasaki, Japan). The lysates were transferred to a Teflon^{TM} beaker, placed on a hot plate, and evaporated at 130 °C for 4–5 h. After drying, the residue was dissolved in 1 mL concentrated HNO_3, and 0.5 mL hydrogen peroxide was added in order for the remaining organic materials to decompose. Samples