Development of ELISA Measurement for Urinary 3-Hydroxyproline containing Peptides and its Preliminary Application to Community Healthy Persons and Cancer Patients

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Introduction: As basement membrane is degraded and lymph vessels, the increased excretion of endogenous 3-hydroxyproline (3-Hyp) is expected in cancer patients because 3-Hyp is the unique component of type IV collagen in basement membrane. We developed ELISA method to measure 3-Hyp containing peptides in urine and report preliminary application for cancer screening.

Methods: Polyclonal antibodies were made against a synthetic peptide of 10 amino acids including putative prolyl 3 hydroxylation product in collagen sequence. Competitive ELISA method using the antigen peptide was developed to measure urinary 3-Hyp containing peptides and applied to healthy controls and cancer patients.

Results: The ELISA assay detected the antigen peptide mixed in urine in the range of 0.1 μg/ml to 80 μg/ml. One hundred and eighty healthy controls and 22 cancer patients samples were assayed by this method. The values in controls were 2.44 ± 1.90 (SD) μg peptide/gm creatinine for 52 men (with a range from 0.65 to 10.51) and 2.87 ± 2.01 (0.94 to 17.31) for 128 women. The values in 22 cancer patients unexpectedly showed the very low value, 0.110 ± 0.137 (p<0.001). As reported previously, endogenous urinary excretion of 3-Hyp measured by an amino acid analysis showed very low levels in healthy controls and high levels in cancer patients, but this ELISA study showed the opposite results. This suggested that cancer tissues have high levels of MMPs and/or peptidase activities that could degrade 3-Hyp-containing polypeptides.

Conclusions: The competitive ELISA assay to measure urinary 3-Hyp containing peptides showed the difference between healthy control and cancer patient samples.

Insulin–like growth factor binding protein-related protein 1 (IGFBP-rP1) modulates tumor cell adhesion with laminin-332 (laminin-5)

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Objective: Insulin–like growth factor binding protein-related protein 1 (IGFBP-rP1), a member of IGFBP superfamily, was originally identified as a tumor-derived, cell adhesion factor (TAF) that interacts with heparan sulfate proteoglycans. This protein is highly accumulated in blood vessels of tumor tissues and has recently been reported to have tumor-suppressing activity. However, exact function of IGFBP-rP1 remains unknown. On the other hand, laminin-332 (Lm5) is an important basement membrane protein which has potent cell adhesion and migration activities. Lm5 is also involved in tumor growth and invasion. In this study, we attempted to characterize the cell adhesion activity of IGFBP-rP1 and its functional interaction with Lm5.

Methods: The cell adhesion activities of purified IGFBP-rP1 and Lm5 were analyzed using human colon adenocarcinoma cell line DLD-1. DLD-1 cells introduced with a control vector or an IGFBP-rP1-expression vector were also used.

Results: When IGFBP-rP1 alone was coated on plastic plates, it scarcely supported adhesion of DLD-1 cells to the substrate. However, when IGFBP-rP1 was co-coated with Lm5 at 0.2 μg/ml, Lm5 alone did not support cell adhesion, the cell adhesion and spreading were strongly promoted depending on the amount of IGFBP-rP1. This synergistic cell adhesion activity of IGFBP-rP1 was efficiently blocked by heparin, anti-integrin-α3 or β1 antibody, whereas heparin did not inhibit the cell adhesion to Lm5 (0.5 μg/ml) alone. This suggests that IGFBP-rP1 efficiently promotes the integrin-mediated cell adhesion to Lm5 by binding heparan sulfates on cell surface. We also found that IGFBP-rP1-expressing DLD-1 cells attached on plates more efficiently than the control cells.

Conclusions: These data indicate that IGFBP-rP1 modulates tumor cell adhesion to Lm5 and possibly other integrin-dependent substrates. It seems likely that IGFBP-rP1 produced by tumor cells or stromal cells affects tumor growth by the synergistic action with Lm5 at invasion fronts.