Calcyclin Levels Determined by High-Throughput SRM in Serum Samples of Pre-Eclampsia Patients

Introduction

Pre-eclampsia (PE) is a pregnancy-specific disease that complicates 2-8% of all pregnancies. It is associated with serious maternal and perinatal morbidity and mortality. Endovascular remodeling and invasion of the spiral arteries is impaired which results in reduced and irregular placental perfusion. This in turn leads to placental oxidative stress, resulting in endoplasmic reticulum related impaired protein synthesis.

We previously demonstrated that in placental tissue of patients with PE, calcyclin expression is significantly higher compared to controls. An example of data is shown in Figure 1. We developed a microfluidic LC-MS based assay to evaluate this observation by means of Selected Reaction Monitoring (SRM) in serum fractionated by strong cation exchange (SCX) chromatography. Using this technique, we were able to quantify calcyclin at low attomole levels in serum.

Methods

Sample preparation sera

Off-line fractionation of digest sera was conducted with a 2 µm SCX column using a gradient from 15 to 30% buffer B (450 mM KH2PO4, pH 2.5) to 15 mM KH2PO4 (pH 2.5) at 200 µL/min. The average peak width was reduced by a factor of 2.5 and the retention time by a factor of 2. The SRM chromatograms in Figure 4 compare the retention times and peak widths for the corresponding plasma samples and show an increased background noise for certain peptides; however, this trend was not observed for all peptides of interest and in current studies. Increase in the resolution of the quadrupole mass analyzers, shown in Figure 6, was found to be beneficial for certain peptides, i.e. S9N8 improved by a factor of 3.5 and S10N6 by a factor of 2.5. This observation holds true for quantification of calcyclin in pre-eclampsia samples, for both peptides 152 ± 4.5 and 186 ± 18.5 ng/ml, respectively.

Thrupeutic

The SRM chromatograms in Figure 4 compare the retention times and peak widths for the corresponding plasma samples and show an increased background noise for certain peptides; however, this trend was not observed for all peptides of interest and in current studies. Increase in the resolution of the quadrupole mass analyzers, shown in Figure 6, was found to be beneficial for certain peptides, i.e. S9N8 improved by a factor of 3.5 and S10N6 by a factor of 2.5. This observation holds true for quantification of calcyclin in pre-eclampsia samples, for both peptides 152 ± 4.5 and 186 ± 18.5 ng/ml, respectively.

Results

The specificity of the assay was examined by reducing the SCX fractionation width, data not shown, detection of samples obtained from two pregnant controls. The specificity of the assay was also illustrated by the use of a 150 µm ID microfluidics cartridge and the SRM analyses were performed with a 75 µm ID microfluidic column (top) and 150 µm ID microfluidic column (bottom).

Conclusion

An SRM assay was developed and validated for the measurement of calcyclin in serum samples. The use of a 150 µm ID microfluidic device increased throughput of analyses by a factor of 4 without compromising the specificity of the measurements. Improvements in S/N were observed for increased resolution of pre-fractionation and by increasing the sensitivity of detection in the quadrupole mass analyzers. Reduction of the SCX fractionation width, data not shown, demonstrated a significant increase in peak resolution for calcyclin. This work was in part supported by the Coolsingel Foundation.

Acknowledgement

This work was in part supported by the Coolsingel Foundation.

Reference