Protein-Profiling of Genomic Instability in Endometrial Cancer

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Confirmation by LC-MS/MS

Picked protein spots were processed and digested with trypsin using a robotic protein handling system (MassPREP, Waters) as specified above. Peptides were extracted with 30µL 5% formic acid / 2% acetonitrile, followed by extraction with 24µL 2.5% formic acid / 50% acetonitrile. Tryptic fragments were analyzed using a quadrupole time-of-flight mass spectrometer (Q-TOF Premier API, Waters, Milford, MA, USA) with a standard Z-spray source coupled to a Waters nanoAcquity system. The eluate from the CapLC system was electrosprayed using a PicoTip EMITTER (SilicaTip, New Objective Inc). Samples were desalted with a Waters Symmetry C18 column using 100% water / 0.1% formic acid with a flow rate of 15µL/min. Peptides were then separated on the analytical column (BEH C18, Waters) with a solvent system of 100% water / 0.1% formic acid (solvent 1) and 100% acetonitrile / 0.1% formic acid (solvent 2). Finally, the columns were washed 30min with a linear gradient of 3-60% solvent 2 at a flow rate of 300nL/min. The capillary voltage was 2.3kV, the cone and the extraction cone energy voltage were 40 and 2.5V, respectively. The collision gas was argon. Multiply charged ions were selected for collision-induced dissociation using automated switching between MS and MS/MS modes. DDA (Data Dependent Acquisition) was used over a mass range between 300-2000*m/z* with a scan time of 1 minute. The collision energy was automatically alternated between 25 to 45eV depending on the mass and charge states for MS/MS. Data analysis was performed using PLGS 2.3 (ProteinLynx Global SERVER 2.3, Waters) software and MassLynx peptide sequence software (version 4.0, Waters).