Protein-Profiling of Genomic Instability in Endometrial Cancer

Timo Gemoll[1,2,3], Jens K. Habermann[1,2], Johanna Lahmann[2], Silke Szymczak[4], Caroline Lundgren[5], Nana K. Bündgen[2], Thomas Jungbluth[2], Britta Nordström[5], Susanne Becker[3], Marta I. Lomnytska[3,5], Hans-Peter Bruch[2], Andreas Ziegler[4], Ulf Hellman[6], Gert Auer[3], Uwe J. Roblick[1,2], and Hans Jörnvall[1]

(1) Department of Medical Biochemistry and Biophysics, Karolinska Institutet, SE-17177 Stockholm, Sweden
(2) Department of Surgery, University of Lübeck, D-23538 Lübeck, Germany
(3) Karolinska Biomic Center, Karolinska Institutet, SE-17176 Stockholm, Sweden
(4) Institute of Medical Biometry and Statistics, University of Lübeck, D-23562 Lübeck, Germany
(5) Department of Oncology, Radiumhemmet, Karolinska University Hospital, SE-17176 Stockholm, Sweden
(6) Ludwig Institute for Cancer Research, Ltd., SE-75124 Uppsala, Sweden

Address for correspondence:

Professor Hans Jörnvall, MD
Department of Medical Biochemistry and Biophysics
Scheeles väg 2
Karolinska Institutet
SE-17177 Stockholm, Sweden
Phone: +46-8-524 877 02
Fax: +46-8-33 74 62
Mail: Hans.Jornvall@ki.se

&

Jens K. Habermann, M.D., Ph.D.
Laboratory for Surgical Research, Dept. of Surgery
University Clinic Schleswig-Holstein, Campus Lübeck
Ratzeburger Allee 160
D-23538 Lübeck, Germany
Phone: +49-451-5002676
Fax: +49-451-5002069
Mailto: Jens.Habermann@gmail.com

In-gel digestion and mass spectrometry (MALDI-ToF-MS)

Spots of interest were manually cut from the 2-D gels in a LAF bench with a scalpel and transferred to sterile Eppendorf tubes. The pieces of gel were washed with water and destained in freshly prepared destaining solution (30mM potassium hexacyanoferrate: 100mM sodium thiosulfate, 1:1 v/v) until the brown color disappeared. Trypsin (80ng in 50mM ammonium bicarbonate/10% acetonitrile) was added and incubation was carried out at 30°C overnight. Digestion was stopped by acidification with trifluoroacetic acid (TFA) and the resulting tryptic peptides were diluted with 20µl of 0.1% TFA. For matrix-assisted laser desorption/ionization (MALDI), the peptide extracts were concentrated and desalted with µC18 ZipTips (Millipore, MA, USA) by siphoning about 10 times and washing twice with 15µl 0.1 % TFA. The tryptic fragments were eluted directly onto the target plate with 75% acetonitrile / 0.1% TFA containing half saturated alpha-Cyano-4-hydroxy-cinnamic acid as matrix. They were analyzed by matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-ToF-MS) in an Ultraflex III TOF/TOF instrument (Bruker Daltonics, Bremen, Germany). The peptide mass fingerprints (up to 100 observed peptides per spot with a S/N greater than six) were analyzed using the FlexAnalysis v3.3 software (Bruker Daltonics, Bremen, Germany). Database searches were carried out with the software package ProFound (release February 2010, http://prowl.rockefeller.edu) in the non-redundant protein database of the National Center for Biotechnology Information (NCBInr, v2010/02/20 with 10,448,260 sequences). “Mammalia” was chosen for the taxonomic category. All peptide masses were used monoisotopic and [M+H]+ (protonated molecular ions). Search parameters

included carbamidomethylation of cysteine by iodacetamide as fixed modification, and oxidation of methionine as variable modification. One missed trypsin cleavage was allowed. Only protein hits with three or more matching peptide masses and a sequence coverage of at least 10% were considered. Internal calibration was achieved by analysis of autolytic trypsin cleavage products resulting in an accuracy of ±0.04 Da. Judgement of significance was based on the expectation-value, number of matching peptide masses, and agreement between experimental and theoretical physical properties of the proteins.