Identification of the 14-3-3 zeta and epsilon isoforms in mouse estrous uterine fluid

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摘要

在小鼠的動情週期時，子宮內膜細胞在動情前期時增生相當明顯，而動情期時會顯著性的死亡。從死去的子宮內膜細胞所釋放的某些蛋白不僅有助於充當監測其死亡的生物指標，或許可以經由這些蛋白的足跡，拼湊出子宮內膜死亡的輪廓。而這些釋出的蛋白最有可能會存於子宮腔液中。因此我們透過研究動情期與動情前期腔液中明顯差異的蛋白，作為尋找這些指標蛋白的方法。利用 SDS-PAGE 比較兩個不同時期子宮腔液的蛋白形態，進而找到一個約 30 kDa 的蛋白，具有動情期腔液內之含量顯著大於動情前期的特性。將此蛋白帶切開，並以胰蛋白酶作用，再利用 LC/MS/MS 分析。經由 Peptides 片段的分析，證實內含 14-3-3 epsilon 與 14-3-3 zeta。免疫分析的結果證實 14-3-3 zeta 在動情期腔液內之含量遠大於動情前期。將動情期的子宮腔液以分子篩 Sepharcyl S-200 進行分離後，經由決定收集管中 14-3-3 zeta 與 14-3-3 epsilon 的分佈形態並比對，得知它們呈現 dimer form 形式但卻無同收集管(Co-fractionation)之特性。而且 14-3-3 epsilon 並無法與 14-3-3 zeta 抗體產生共同沈殿作用。總之、這個研究認定，透過動情期腔液中 14-3-3 zeta 量的分析，可以充當小鼠子宮內膜破壞的生物指標(biomarker)。

關鍵字：endometrial damage、14-3-3 epsilon、14-3-3 zeta、LC/MC/MS、生物指標

Abstract

During the mouse estrous cycle, the endometrium prominently proliferates in proestrus and dramatically degenerates in estrus. Proteins released from the degenerating endometrial cells may not only serve as surrogate markers for endometrial damage potentially measurable in accessible luminal fluids, but the profiles of protein efflux may reflect the underlying mechanisms for exclusion of endometrial cells. To determine the potential biomarker of degenerating endometrial cells, we studied the differential proteins of uterine luminal fluid (ULF) between estrus and proestrus. In comparison of two patterns of ULF constituents resolved by SDS-PAGE, an approximately 30 kDa proteins was predominately shown in estrous ULF distinct from proestrous ULF. The protein band from SDS-PAGE was subjected to tryptic digestion, followed by LC/MS/MS sequences analysis and identified as 14-3-3 epsilon and 14-3-3 zeta from peptide fragments analysis. Immunoblot analysis revealed that zeta forms were specifically increased in ULF at estrus distinct from proestrus. After separation of mouse estrous ULF from Sephacryl S-200 chromatography, two 14-3-3 isoforms were predicted to be a dimmer form, but not co-fractionated in the gel filtration fractions. In addition, the 14-3-3 epsilon was not co-immunoprecipitated with anti-14-3-3 zeta antibody from the same fraction. Taken together, this study has determined that the analysis of 14-3-3 zeta levels from the estrous ULF could be useful as a biomarker for mouse endometrial damage.

Keywords：*endometrial damage、14-3-3 epsilon、14-3-3 zeta、LC/MC/MS、biomarker*