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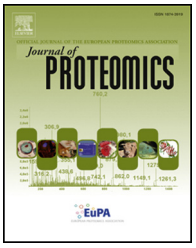

A proteomic approach to monitor the dynamic response of the female oviductal epithelial cell surface to male gametes

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ABSTRACT

Sophisticated strategies to analyze cell surface proteins are indispensable to study fundamental biological processes, such as the response of cells to environmental changes or cell–cell communication. Herein, we describe a refined mass spectrometry-based approach for the specific characterization and quantitation of cell surface proteins expressed in the female reproductive tract. The strategy is based on *in situ* biotinylation of rabbit oviducts, affinity enrichment of surface exposed biotin tagged proteins and dimethyl labeling of the obtained tryptic peptides followed by LC–MS/MS analysis. This approach proved to be sensitive enough to analyze small sample amounts (<1 μg) and allowed further to trace the dynamic composition of the surface proteome of the oviductal epithelium in response to male gametes. The relative protein expression ratios of 175 proteins were quantified. Thirty-one of them were found to be altered over time, namely immediately, 1 h and 2 h after insemination compared to the time-matched control groups. Functional analysis demonstrated that structural reorganization of the oviductal epithelial cell surface was involved in the early response of the female organ to semen. In summary, this study outlines a workflow that is capable to monitor alterations in the female oviduct that are related to key reproductive processes *in vivo*.

Biological significance
The proper interaction between the female reproductive tract, in particular, the oviduct and the male gametes, is fundamental to fertilization and embryonic development under physiological conditions. Thereby the oviductal epithelial cell surface proteins play an important role. Besides their direct interaction with male gametes, these molecules participate in signal transduction and, thus, are involved in the mandatory cellular response of the oviductal epithelium. In this study we present a refined LC–MS/MS based

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workflow that is capable to quantitatively analyze the expression of oviductal epithelial cell surface proteins in response to insemination *in vivo*. A special focus was on the very early interaction between the female organ and the male gametes. At first, this study clearly revealed an immediate response of the surface proteome to semen, which was modulated over time. The described methodology can be applied for studies of further distinct biological events in the oviduct and therefore contribute to a deeper insight into the formation of new life.

1. Introduction

Cell surface exposed molecules are essential in transduction of signals and communication of the cell with its surroundings [1]. These molecules exert also a fundamental role in further key cellular processes, including cell adhesion and ion and solute transport [1]. Because of their importance, their quantitative and qualitative expression analysis in relation to defined physiological and pathological cellular processes is of high interest. Due to the generally low cellular abundance of cell surface proteins, their analysis requires an elaborate strategy [2]. Such a strategy comprises usually an enrichment or purification step for these proteins [3,4], followed by their qualitative and quantitative analysis. Therefore, two-dimensional gel electrophoresis (2D-GE) is a common analytical tool [5–7]. However, some drawbacks of 2D-GE, including noticed underrepresentation of membrane, heavy, and low abundant proteins [8] impelled researchers to use alternative methods as well. Besides, being very helpful for the identification of 2D-GE-separated and quantified cell surface protein spots [9], liquid chromatography–mass spectrometry (LC–MS/MS) can also be used for the global quantitative analysis of such proteins. A study published by the Luesch group described the use of isobaric tags for relative and absolute quantification (iTRAQ) for the LC–MS/MS based analysis of biotinylated and enriched cell surface proteins [10]. The popular iTRAQ technology allows the multiplexed quantification of up to eight samples, making it superior over other techniques. However, the high cost of labeling reagents may severely increase the price of the experiment in large-scale analyses [11]. A stable isotope dimethyl labeling approach presents a more economic option and its efficiency and reliability were shown in diverse studies [12], including our previous ones [13].

Despite advances in proteomic technologies, a main challenge is still the handling of limited sample amounts, e.g. biological material obtained from *in vivo* studies. Therefore, the sample preparation step allowing a bottom-up proteomic approach remains complicated.

In the present study we focused on the optimization and establishment of a sensitive gel-free mass spectrometry based approach for quantification of cell surface proteins of the oviductal epithelium. These molecules play not only a major role in direct spermatozoa-oviduct binding (reviewed in Ref. [14]), but moreover take part in communication between the female organ and the male gametes. Consequently, epithelial surface proteins might be involved in the establishment of the optimal milieu for further reproductive processes [15,16]. Thus, identifying and quantifying the proteins lining the oviductal lumen is highly desirable to get a deeper insight into the spermatozoa-induced signaling events in the female reproductive tract that precede fertilization and early embryonic development. In 2006, Sostaric et al. [17] performed an *in vitro* study to profile the cell surface proteome of cultured oviductal epithelial cells. However, so far neither an optimal strategy has been adapted or has been applied to study the dynamic composition of the cell surface proteome in the oviduct in relation to key reproductive processes including insemination/mating.

The procedure described here comprises *in situ* surface biotinylation of oviducts, isolation of oviductal epithelial cells (OEC), affinity enrichment, solubilization and digestion of cell surface proteins followed by dimethyl labeling of tryptic peptides. This strategy was first evaluated as appropriate method for the LC–MS/MS based quantitative analysis of the obtained sample material. In the next step it was used to monitor the expression changes of cell surface exposed molecules in response to male gametes. Samples were obtained from *in situ* biotinylated rabbit oviducts isolated at three early time points after intrauterine insemination, namely immediately (designated 0 h), 1 h and 2 h, and instantly processed. The obtained data indicate that the applied workflow is capable to observe a specific semen response in the oviduct over time. Primarily structural molecules and proteins related to cell junction signaling are involved in this response. Thus, the applied strategy opens up new possibilities to study the mechanisms that regulate fundamental reproductive processes.

2. Materials and methods

All chemicals, if not specifically stated otherwise, were purchased from Sigma-Aldrich, Steinheim, Germany.

2.1. Primary antibodies

Rabbit polyclonal anti-peroxiredoxin-3 (PRDX3) IgG, mouse monoclonal specific for β4 tubulin and rabbit polyclonal specific for oviduct specific glycoprotein 1 (OVGP1) were obtained from Abcam (Cambridge, UK). Mouse monoclonal specific for β tubulin was purchased from Antibodies online (Aachen, Germany). Rabbit anti-pan cadherin was purchased from Invitrogen (Camarillo, CA, USA) and rabbit anti-epidermal growth factor (EGF) receptor was obtained from Cell Signaling Technology (Danvers, MA, USA).

2.2. Experimental setup

Animal experiments were approved by the "Regierung von Oberbayern" in Munich, Germany (reference number

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