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LETTER TO THE EDITOR

Sperm Biology

Isolation and characterization of detergent-resistant membranes from rat spermatozoa

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Dear Editor,

We present herein the isolation and characterization of detergent-resistant membranes (DRMs) from rat spermatozoa. This is the first study on rat sperm DRMs.

Detergent-resistant membranes including lipid raft and caveolae are cholesterol- and sphingolipid-rich microdomains of the plasma membrane that concentrate components of certain signal transduction pathways.[1] They are receiving increasing attention as devices involved in the regulation of membrane fluidity and membrane protein trafficking. Until now, DRMs have been identified in spermatozoa from different species, including human, mouse, porcine and bovine.[2–5] Increasing evidence has shown that these microdomains play important roles during sperm maturation in the epididymis and serve as platforms for the assembly of key recognition molecules that are involved in sperm–zona pellucida interaction on the sperm surface during capacitation.[6] However, until now, no any studies on rat sperm DRMs have been reported. In the present work, we focused on the biochemical isolation and characterization of rat sperm DRMs.

The DRMs were isolated by the use of sucrose density centrifugation. This method relies on the unique lipid composition (with enrichment in sphingolipid and cholesterol) of lipid rafts and caveolae, which makes these membrane domains resistant to solubilization in certain conditions (for example, noninonic detergents), as well as more buoyant than other cellular components. Spermatozoa were released from the cauda epididymidis of rats, washed with phosphate-buffered saline and then resuspended in 2-(N-morpholino) ethanesulfonic acid (Mes)-buffered saline (MBS: 25 mmol l^{-1} Mes, 150 mmol l^{-1} NaCl, pH 6.5) containing 1% (*v/v*) Triton X-100 and protease inhibitor mix (Sigma P-8340, diluted 1:100) and kept on ice for 30 min. This lysate was then centrifuged at 900 g, at 4°C for 15 min to sediment debris. The 2 ml supernatant was mixed with the same volume of 80% MBS-buffered sucrose solution in an ultracentrifuge tube, and a sucrose gradient (containing 4 ml of 30% sucrose in MBS and 4 ml of 5% sucrose in MBS) was layered on top of this detergent extract followed by ultracentrifugation (200 000 *g*) for 20 h at 4°C in a Beckman SW41 rotor. After centrifugation, gradient fractions (1 ml) were collected from the top for further analysis. As shown in **Figure 1a**, the DRM fraction appeared as an opalescent band in the low density fraction of the gradient (fraction No. 6). The total protein in each fraction also can be used as an indicator of appropriate fractionation. **Figure 1b** shows that the majority of proteins in the Triton X-100 sperm lysate was found in the bottom fractions, however, the DRM fraction (No. 6) contained higher protein levels than the surrounding fractions (No. 3–5 and 7–8).

Caveolin-1 and flotillin-2 are two widely studied markers for DRMs, and both of them are significantly enriched in DRMs of somatic cells and spermatozoa.[7] Therefore, we detected their distribution in the sucrose gradient fractions by western blots with caveolin-1 and flotillin-2 antibodies. As shown in **Figure 1c**, flotillin-2 (42 kDa) existed mainly in the DRM fraction No. 6; however, caveolin-1 was only present in non-DRM fractions No. 8–12. The distribution pattern of flotillin-2 was identical to that in previous studies on human and mouse somatic cells and porcine sperm DRMs.[4] However, the pattern of caveolin-1 partitioning throughout the gradient differed dramatically

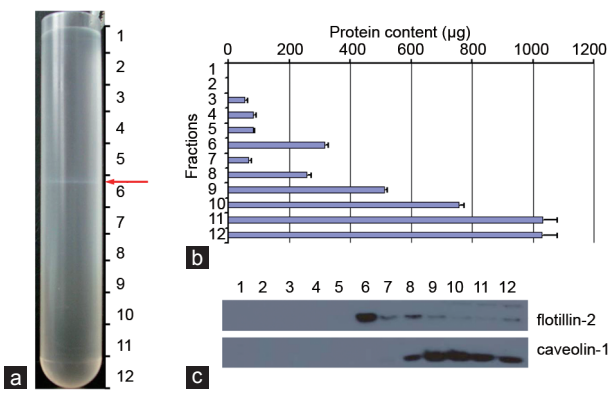

Figure 1: (**a**) Distribution of detergent-resistant membranes (DRMs) in the sucrose density gradient of rat sperm Triton X-100 lysate. Numbers on the right of the tube denote 1 ml fractions from top (No. 1) to bottom (No. 12) of the tube. Note that DRMs were localized at fraction No. 6 (appears as an opalescent band). (**b**) The total protein content of 12 membrane fractions was determined. The data are presented as the mean ± standard deviation of three replicates. (**c**) Caveolin-1 and flotillin-2 differentially target DRMs of rat spermatozoa. Sucrose density gradient fractions of 1% Triton X-100 extracted rat sperm lysates were analyzed for caveolin-1 and flotillin-2 immunoreactivity. Flotillin-2 floats in DRM fraction whereas caveolin-1 is found at the bottom of the gradient in the non-DRM fractions.

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from the previous results in human and mouse somatic cells and mouse spermatozoa.[8] This discrepancy most likely reflects species differences or the presence of caveolin-1 in different sub-types of membrane regions isolated in the bottom, non-DRM fractions.

In summary, our results represent the first characterization of rat sperm DRMs, and may contribute to a better understanding of the sperm fertilizing potential acquisition mechanism.

AUTHOR CONTRIBUTIONS

SGH performed the isolation of DRMs by sucrose density centrifugation. XQL detected the protein amounts in different gradient fractions. SGH and CHT performed western blot analysis. SGH drafted the manuscript, which was revised by YLZ and YS. YLZ and YS supervised the study design and coordination. All authors have read and approved the final manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

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REFERENCES

1 Brown DA, London E. Functions of lipid rafts in biological membranes. *Annu Rev Cell Dev Biol* 1998; 14: 111–36.
2 Cross NL. Reorganization of lipid rafts during capacitation of human sperm. *Biol Reprod* 2004; 71: 1367–73.
3 Nixon B, Bielanowicz A, McLaughlin EA, Tanphaichitr N, Ensslin MA, *et al*. Composition and significance of detergent resistant membranes in mouse spermatozoa. *J Cell Physiol* 2009; 218: 122–34.
4 Bou Khalil M, Chakrabandhu K, Xu H, Weerachatyanukul W, Buhr M, *et al*. Sperm capacitation induces an increase in lipid rafts having zona pellucida binding ability and containing sulfogalactosylglycerolipid. *Dev Biol* 2006; 290: 220–35.
5 Girouard J, Frenette G, Sullivan R. Seminal plasma proteins regulate the association of lipids and proteins within detergent-resistant membrane domains of bovine spermatozoa. *Biol Reprod* 2008; 78: 921–31.
6 Kawano N, Yoshida K, Miyado K, Yoshida M. Lipid rafts: keys to sperm maturation, fertilization, and early embryogenesis. *J Lipids* 2011; 2011: 264706.
7 Nixon B, Aitken RJ. The biological significance of detergent-resistant membranes in spermatozoa. *J Reprod Immunol* 2009; 83: 8–13.
8 Sleight SB, Miranda PV, Plaskett NW, Maier B, Lysiak J, *et al*. Isolation and proteomic analysis of mouse sperm detergent-resistant membrane fractions: evidence for dissociation of lipid rafts during capacitation. *Biol Reprod* 2005; 73: 721–9.

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