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行政院國家科學委員會專題研究計畫成果報告

##

豬精子活動力抑制蛋白對豬精子作用之免疫細胞化學研究

The immunocytochemical study of the biological effect of the porcine sperm motility inhibitor on the porcine sperm

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計畫主持人: 鄭海倫 Hellen Jeng

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摘 要

從豬精漿中純化出豬精子活動力抑制蛋白 (Sperm Motility Inhibitor, SMI), 用FITC標示SMI, 再放入豬精子懸浮液中,然後以螢光顯微鏡觀察FITC的標示位置,判斷SMI與精子的binding site, 結果顯示SMI結合在精子鞭毛的中節(midpiece)上。以脾臟內免疫法製出的polyclonal antiserum, 用於精子超薄切片的免疫組織染色, 再用電子顯微鏡觀察,因細胞膜內可見免疫-金顆粒,故判斷SMI可以進入精子細胞的細胞膜內, SMI不是membrane-bound protein 也不是surface protein。

關鍵字: 精漿, 精子活動力抑制蛋白, 免疫化學

ABSTRACT

The sperm motility inhibitor (SMI) was purified from porcine seminal plasma. The fluorescent FITC-labeled SMI was incubated with the washed porcine sperm, which was then observed under the fluorescence microscope. The result revealed that SMI bind on the midpiece of the sperm flagellum. The polyclonal antiserum was induced by intrasplenic immunization method. Using the techniques of western blotting and immunohistochemical electron microscopy, we illustrated that SMI could enter the sperm and therefore, it is not a membrane-bound protein, nor a surface protein.

Keywords: seminal plasma, sperm motility inhibitor (SMI), immunohistochemistry

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簡介背景

由鄭等人(Jeng et al, 1993) 從豬精漿中純化出來的精子活動力抑制蛋白(sperm motility inhibitor, SMI)，經測試得知可以抑制豬精子的活動力，然加入母豬濾泡液後，抑制可被抵消，故此抑制爲一種可逆性的抑制。而後趙等人(Chao et al, 1996) 報導 SMI 爲 Na^+,K^+-ATPase 的競爭性抑制者，且與人類的ß-microseminoprotein 相似。人類的ß-microseminoprotein 有 94 amino acid(Akiyama et al, 1985)，N 端第一個 amino acid 爲 pyroglutamic acid，且有十個 cysteine 爲保留性 amino acid ，豬的 SMI 則只有 91 amino acid (Fernlund et al., 1994)。人類的β-microseminoprotein 與 prostatic acid phosphatase (PAP)，prostate-specific antigen (PSA) 共同爲前列腺的三個主要分泌蛋白，但其功用未明(Lilja et al., 1988)。以 human β-microseminoprotein 爲抗原產生之抗體進行測試，得知氣管，支氣管，胃，十二指腸，結腸，子宮頸等其他器官的分泌物皆有 β-microseminoprotein 的免疫反應 (Weiber et al, 1990)，但鄭等人在先

期實驗中已證實雄性生殖道中，只有前列腺分泌 SMI(submitted data)，鄭與趙等人可算是最早提出此蛋白可能生理功能者。

動物精液由精子與精漿兩大部分所組成，精子經由授精(insemination)過程接近卵子，而達受精事實(fertilization)，其間經過許多步驟與過程，但精漿內的物資大部分被認爲只是提供精子所需營養與游動的能量，並未隨精子進入雌性內部的生殖道，所以欲知 SMI 對精子的真正作用，首先應證明 SMI 有無進入精子內或與精子細胞膜結合。

鄭等人(Jeng and Chang, 1997)已報導 SMI 可降低 spontaneous acromosome reaction 的頻率，與精子之生理功用有關，但不知是否在細胞膜或進入精子內與其他胞器結合。因此作用位置關鍵著 SMI 的生理功能，故本研究乃提出計畫以西方點墨法與穿透式電子顯微鏡層次的免疫細胞化學方法，探討 SMI 對精子的作用。

Materials and Methods

1. 精液收集與豬精子活動力抑制蛋白(SMI)之純化

   參看Jeng et al., 1993.

2. 製備豬精子活動力抑制蛋白(sperm motility inhibitor, SMI)的抗體

   使用紐西蘭大白兔,以 intrasplenic immunity 方法，製備 SMI 的抗體參考 Hong et al., 1989, 再抽取血漿內 polyclonal 抗體，接續下面實驗。

3. Western blotting (Aumuller et al., 1988)

   取豬的精子磨碎作 SDS-PAGE, 再用 SMI 的抗體，作 Western blotting 偵測 SMI 有無進入 sperm 內

4. Immunocytochemical electron microscopic study

   以 paraformaldehyde 固定豬的精子,製成 EM 觀察的超薄切片，看 immuno-gold particle 分佈的狀況,以判定 SMI 可能負責的功能之依據。

Results and Discussions

FITC-labelled SMI indicated that sperm motility inhibitor (SMI) binded on the midpiece of the flagellum of sperm. Immunohistochemical studies indicated that SMI did appear in the intramembranous space of sperm (Figs. 1 and 2).

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A

B

Fig.1 Sperm incubated with FITC-labelled SMI.

A.位相差顯微鏡

B.螢光顯微鏡

Fig.2 Immunohistochemical localization of SMI in sperm under electron microscope.
紅色箭頭指免疫－金顆粒