专题研究

精子携带的HBV DNA在小鼠早期胚胎中的复制与表达

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摘要 背景与目的: 研究乙型肝炎病毒(Hepatitis B virus,HBV) DNA在早期胚胎中能否复制与表达,探讨HBV经雄性生殖细胞垂直传递的可能性。 材料与方法: 成熟雄鼠麻醉后双侧睾丸注射经脂质体DOSPER包裹的HBV质粒,手术后雄鼠与超排雌鼠合笼交配,用荧光原位杂变(Fluorescence in situ hybridization,FISH)分别检测单细胞胚和二细胞胚间期核中HBV DNA的存在与复制,用逆转录聚含酶链反应(Reverse transcriptase-polymerase chain reaction,RT-PCR)和免疫荧光方法检测HBV基因在二细胞胚胎中的表达。 结果: 在单细胞胚和二细胞胚间期核内发现HBV DNA阳性杂交信号,RT-PCR可以扩增出HBx基因的特异条带,免疫荧光也可见到HBsAg阳性信号。 结论: HBV DNA进入雄性生殖细胞后,可以整合到宿主基因组内,通过自然受精随精子进入卵母细胞,并在早期胚胎中复制与表达。提示HBV有可能通过雄性生殖细胞进行垂直传递。

关键词 乙型肝炎病毒DNA; 复制与表达; 早期胚胎; 垂直传递

Replication and Expression of Sperm-mediated HBV DNA in Early Mouse Embryo

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Abstract BACKGROUND & AIM: To explore the feasibility of hepatitis B virus (HBV) vertical transmission via male germ line, the replication and expression of sperm-mediated HBV DNA in mouse embryo were studied. MATERIAL AND METHODS: Young adult male mice were injected with HBV DNA-liposome complex. These males were serially mated with superovulated females 2 days after injection. Fluorescence in situ hybridization (FISH) was carried out to confirm the integration of HBV DNA into male pronucleus and its replication with cell division in embryonic development. (Reverse transcriptase polymerase chain reaction, RT-PCR) and immunofluoresence assay were performed to observe the expression of the HBV gene in two-cell stage. RESULTS: FISH demonstrated that the male pronuclei in some one-cell embryos and the each nucleus in some two-cell embryos presented positive signals. RT-PCR showed the specific bands of cDNA of HBx DNA in some two-cell embryos. Immunofluorescence assay presented the positive signals for HBsAg in some two-cell embryos. CONCLUSION: Our results provided the direct evidence for that HBV DNA are able to transmit vertically to next generation via male germ line.

Keywords hepatitis B virus DNA; replication and expression; early embryo vertical transmission

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