Identification of a protein involved in the specific interaction between spermatogenic and Sertoli cells of the rat

メタデータ	言語: eng 出版者: 公開日: 2017-10-05 キーワード (Ja): キーワード (En): 作成者: メールアドレス: 所属:
URL	http://hdl.handle.net/2297/16169

氏　　名	前　田　智　司
生　年　月　日	
本　　籍	東京都
学 位 の 種 類	博士（薬学）
学 位 記 番 号	博甲第260号
学位授与の日付	平成10年3月31日
学位授与の要件	課程博士（学位規則第4条第1項）
学位授与の題目	Identification of a protein involved in the specific interaction between spermatogenic and Sertoli cells of the rat （精子形成細胞とセルトリ細胞の特異的な接着を規定するタンパク質の同定）
論文審査委員	（主　査）中西　義信 （副　査）大場　義樹，正宗　行人，松永　　司，山口　正晃

学 位 論 文 要 旨

The mammalian spermatogenic pathway is a complex process that involves the proliferation of the testicular stem cells called spermatogonia, the meiotic division of spermatogonia giving rise to haploid spermatids, and the morphogenic differentiation of spermatids into spermatozoa. This whole process continues for more than four weeks in the seminiferous tubules of the mammalian testis. Throughout the spermatogenic pathway, spermatogenic cells keep a close contact with Sertoli cells, testicular somatic cells. Sertoli cells have thus been presumed to play an important role in the spermatogenic differentiation, transferring many materials and signals to spermatogenic cells. Although a number of proteins with cell adhesion activity exist in both spermatogenic and Sertoli cells, it is not certain whether any of them are truly involved in the specific association between the two cell types. In this study, I attempted to identify a protein responsible for this association using an expression cloning method.

1) Establishment of cell adhesion assay

When rat testicular cells were primary co-cultured, spermatogenic cells were maintained in association with Sertoli cells that grew as a monolayer. In order to identify the molecule(s) responsible for the specific interaction between the two cell types, I established a quantitative cell adhesion assay using spermatogenic and Sertoli cells isolated from a primary culture.

In each cell adhesion assay, the number of spermatogenic cells bound to Sertoli cells was determined and shown relative to the input cells, taken as 100; i.e., the cell adhesion index. Under the established conditions, spermatogenic cells prepared from a 3-day co-culture routinely gave indexes of 13~30.

2) Molecular cloning of spermatogenic cell protein responsible for spermatogenic-Sertoli interaction

I decided to identify a cell adhesion molecule(s) in spermatogenic cells employing an expression cloning method. I first sought a culture cell line suitable for the host for cloning. The requirements for host cells were as follows; the cells grow in suspension, do not bind to Sertoli cells, and express the SV40 large T-antigen that allows a plasmid containing the SV40 replication origin to replicate extrachromosomally. A human T-cell-derived cell line Jurkat Tag met all the requirements, showing an adhesion index of about 1/5 of that with control spermatogenic cells.

A cDNA library with a complexity of 2×10^6 was prepared from the mRNA of spermatogenic cells using a mammalian expression vector pcDNA I/Amp that contains the SV40 replication origin. The library was introduced into Jurkat Tag cells by electroporation, and the cells that acquired the ability to bind to Sertoli cells were selected in the cell adhesion assay. After multi-round of such functional screening, four cDNA clones were eventually isolated.

Two of the four cDNA clones (#78 and #97) were brought to the final examination for the binding to Sertoli cells. To do so, their inserts were isolated and recloned with the pHook-2 vector, which expressed a single-chain antibody against the phOx hapten and allowed selection of the transfectants that express an introduced DNA using latex beads containing the hapten. The inserts of #78 and #97 clones were ligated with the vector and the resulting DNA were introduced into Jurkat Tag cells, and cells expressing the cDNA were purified. These cells were analyzed for the binding to Sertoli cells, together with Jurkat Tag cells expressing the empty vector (negative control) and spermatogenic cells (positive control). The clone #97 showed a significant binding activity comparable to that of control spermatogenic cells while the cells expressing clone #78 did not bind to Sertoli cells. These results indicated that clone #97 was the one which coded for the spermatogenic protein responsible for the binding of spermatogenic cells to Sertoli cells. The reason for a failure of detecting the activity with the clone #78 is not clear at present, but it may be possible that the protein encoded by the clone was not properly expressed under this experimental condition. I went further to determine the property of the #97 clone-encoded protein.

3) Characterization of cell adhesion protein

The #97 cDNA clone covered a DNA region of about 1.3 kbp in length including an open reading frame for 243 amino acid residues. A predicted molecular mass of the encoded protein was 27 kDa.

There was no canonical N-linked glycosylation sites in the deduced amino acid sequence. This protein was likely to be secretory since there was a presumed signal peptide; 23 amino acid residues at the N-terminus were all hydrophobic. Another characteristic amino acid sequence was a Cys-rich region in the C-terminal half. In particular, five Cys residues appeared every nine amino acids near the C-terminus. A database search told me that the protein of interest was a previously-identified testicular protein. The entire amino acid sequence of this protein showed a significant similarity to that of a testicular protein called Tpx-1 or AA1; 85, 65, and 68% identity with mouse, guinea pig, and human proteins, respectively. I thus concluded that this protein is the rat homologue of Tpx-1 whose function has remained unclear. Tpx-1 is a member of the CRISP (standing for Cys-rich secretory proteins) family of proteins, which contain the N-terminal signal peptide and are rich in Cys residues at the C-terminal half.

I then carried out the structure-function analysis of rat Tpx-1. Mutated Tpx-1 proteins which had various external and internal deletions were expressed in Jurkat Tag cells, and those expressing the proteins were selected and analyzed in the cell adhesion assay. The N-terminal region, including the putative signal peptide and next 79 amino acids, was shown to be sufficient for the entire binding activity. This region contained neither known domain structures required for cell adhesion activity nor other characteristic amino acid sequences.

Since Tpx-1 possessed an N-terminal signal peptide-like sequence, it was presumed to be secreted. I thus determined its subcellular localization. Rat Tpx-1 cDNA was manipulated so that GFP was attached to the C-terminus of Tpx-1, and the resulting DNA was introduced into Jurkat Tag cells by electroporation. When the cells expressing the Tpx-1-GFP fusion protein were analyzed under a confocal laser microscope, the fusion protein appeared to be present in the cytoplasm associated with membranous structures (most likely the endoplasmic reticulum) while GFP alone was evenly distributed in the cytoplasm and the nucleus. It is thus likely that rat Tpx-1 undergoes the endoplasmic reticulum-mediated secretion.

The above results showed, for the first time, that Tpx-1 is a testicular cell adhesion molecule responsible for the specific interaction between spermatogenic and Sertoli cells.

学位論文審査結果の要旨

前田智司から提出された学位論文について，各審査委員による査読の後に平成10年1月28日に口頭発表会が公開で行われた。同日に上記5名の審査委員によって最終の審査委員会が開かれた結果，以下の理由により当該論文は博士(薬学)の学位を授与するに値すると判定された。

この論文は，哺乳動物の雄性生殖細胞に存在する細胞接着因子の分子クローニングを記述したものである。前田は，生殖細胞とセルトリ細胞との接着アッセイ系を確立し，それを利用した発現クローニング法により生殖細胞に存在する接着因子のcDNA単離を試みた。ラット生殖細胞のmRNAにより作製したcDNAライブラリーをセルトリ細胞には接着しない培養細胞に導入し，接着性を獲得した細胞を選別した。最終的に得られたcDNAクローンは，副精巣に存在するTpx－1と呼ばれるタンパク質のラットホモログをコードしていた。この分子は，既知の細胞接着因子とは異なり，シグナルペプチドを持つ分泌性のタンパク質であった。また，セルトリ細胞との接着活性には，シグナルペプチドを含むアミノ末端側102個のアミノ酸残基で十分であった。

この段階では，Tpx－1が生殖細胞とセルトリ細胞との特異的な接着を規定するとは結論できない。しかし，これまで役割の不明であったTpx－1が細胞接着因子としての機能を有することを見いだした点は重要である。この研究では従来とは異なるアプローチがとられ，精巣内の細胞接着因子について新しい知見を提供した。得られた成果は，哺乳動物精子形成機構の解明に寄与するものと評価される。