Karakterisasi Gen Spag11a pada Epididimis mencit jantan (Mus musculus) Strain DDY : studi mengenai perannya dalam proses Maturasi Sperma = Characterization of Spag11a in the mouse Epididymis : study of its role in Sperm Maturation process / Evelyn Loanda

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Abstrak

ABSTRAK
Latar Belakang : Proses pematangan spermatzoa di epididimis terjadi melalui interaksi antara spermatozoa dengan berbagai protein yang disekresikan oleh epitel epididimis. Gen penyandi protein yang terlibat dalam proses maturasi ini masih banyak yang belum diketahui. Data penelitian sebelumnya menunjukkan bahwa gen-gen yang berperan dalam proses maturasi sperma ekspresinya dipengaruhi oleh androgen. Sperm associated antigen11a (Spag11a) merupakan salah satu gen yang ekspresinya dipengaruhi oleh androgen (Sipila et al, 2006), namun masih belum diketahui apakah Spag11a berperan pada proses maturasi sperma di epididimis.

Tujuan : Mengkarakterisasi gen Spag11a pada epididimis mencit jantan strain DDY

Desain : Penelitian ini menggunakan analisis bioinformatik dan eksperimental

Metode : Struktur gen Spag11a dan deteksi signal peptide dianalisis secara in silico. Quantitative Real time RT-PCR digunakan untuk mengukur ekspresi relatif Spag11a pada analisis spesifisitas jaringan, ketergantungan terhadap faktor endokrin dan faktor testikular. Untuk menganalisis ekspresi gen Spag11a pada tingkat protein dilakukan Western Blot, sedangkan untuk mengetahui lokasi protein SPAG11A pada sel epididimis dilakukan imunohistokimia.

Hasil : SPAG11A termasuk dalam famili protein defensin beta dan analisis signal peptide menunjukan bahwa SPAG11A merupakan protein sekretori. Spag11a dieskpresikan secara spesifik pada organ epididimis, ekspresi di organ lain sangat rendah. Satu hal yang menarik yakni selain menunjukkan spesifisitas organ, Spag11a juga menunjukan spesifisitas regional pada caput epididimis. Ekspresi Spag11a dipengaruhi oleh androgen, penurunan ekspresi Spag11a sangat bermakna (p<0,001) pada hari ke 3 setelah gonadektomi dan mencapai ekspresi paling rendah pada hari ke 5. Ekspresi Spag11a meningkat kembali setelah penambahan testosteron eksogen. Ekspresi Spag11a juga dipengaruhi oleh faktor testikular, dimana pada perlakuan parsial gonadektomi (gonadektomi testis kanan saja) terjadi penurunan ekspresi relatif Spag11a yang lebih cepat dan lebih signifikan pada epididimis kanan dibandingkan dengan epididimis kiri. Pada tingkat protein SPAG11A juga terekspresi secara spesifik pada caput, dan analisis immunohistokimia menunjukan SPAG11A diekspresikan oleh sel prinsipal.

Kesimpulan : Berdasarkan karakter Spag11a yang merupakan gen penyandi protein sekretori, terekspresi

secara spesifik pada caput epididimis dan diregulasi oleh androgen maka dapat disimpulkan Spag11a terlibat dalam proses maturasi sperma. Penelitian lebih lanjut dalam tingkat uji fungsi perlu dilakukan.
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ABSTRACT
Background: Epididymal sperm maturation occurs through interactions between sperm and proteins sereted by epididymal epithelium. Genes encode for proteins involved in the sperm maturation process are still largely unknown. Previous studies showed that genes involved in sperm maturation are regulated by androgen. Sperm associated antigen 11a (Spag11a) is one of the epididymal genes influenced by androgen based on a global DNA microarray analysis (Sipila et al, 2006). However, little is known about the putative role of this gene in the sperm maturation process.

Objective : To characterize expression and regulation of Spag11a genes in the mouse epididymis.

Design : In silico analyses combined with experimental study

Methods : In silico analyses were used to predict Spag11a gene structure and signal peptide. Semi quantitative RT-PCR was used to measure the level of Spag11a expression in the tissue distribution, androgen dependency and testicular factors analyses. Western blot was performed to analyze gene expression at the protein level whereas immunocytochemstry was performed to localize SPAG11A in the epididymal cell.

Results : SPAG11A is member of the defensin beta protein family and constitutes a secretory protein. Spag11a is expressed exclusively in the epididymis and not in other tissues. Moreover, Spag11a shows a region specific expression in the caput, typical for genes that is involved in creating a microenvironment suitable for sperm maturation. Spag11a expression is regulated by androgen. Significant decrease of Spag11a expression was observed after third day of gonadectomy ($p<0.001$). Interestingly, testosterone replacement therapy was able to bring the expression back to the normal level, indicating a high dependency on androgen. Besides androgen, testicular factor also slightly influence Spag11a expression. This was shown by partial gonadectomy experiment in which only the right testis was removed. Spag11a was down-regulated faster on the right epididymal caput compared to the left caput. Spag11a regional expression was also observed at protein level detected by Western immunoblot analyses showing a clear band in caput, not in other regions. Finally, the prediction that SPAG11A is a secretory protein was confirmed by immunocytochemical analyses showing a cell-specific expression in the principal cell. This cell type is known as the main secretor in the epididymal lumen.

Conclusion : Based on the characters of Spag11a, it is most likely that this gene has a specific role in the epididymal sperm maturation. Further investigations using functional assays are needed to confirm the putative role.