Analisis regulasi ekspresi spink2 pada epididimis mencit: studi in silico, peran androgen dan faktor testikuler = Analysis of spink2 expression regulation in mouse epididymis in silico study the role of androgen and testicular factors / Meidika Dara Rizki

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Abstrak

ABSTRAK
Latar Belakang: Pematangan spermatozoa di epididimis terjadi melalui interaksi antara spermatozoa dengan protein yang disekresikan oleh sel epitel yang melapisi duktus epididimis. Sekresi protein tersebut menciptakan microenvironment yang diregulasi oleh gen-gen tertentu. Studi sebelumnya menunjukkan bahwa gen yang terlibat dalam pematangan spermatozoa pada umumnya terekspresi secara spesifik di epididimis dan dipengaruhi oleh androgen. Spink2 merupakan salah satu gen yang terekspresi di epididimis, namun regulasi ekspresinya masih belum diketahui. Tujuan penelitian ini adalah untuk mengkarakterisasi ekspresi dan regulasi gen Spink2 pada epididimis mencit jantan.Metode: Analisis secara in silico digunakan untuk mengetahui struktur gen dan prediksi sinyal peptida, serta domain fungsional gen Spink2. Quantitative real-time RT-PCR digunakan dalam mengukur ekspresi relatif gen Spink2 pada analisis spesifisitas jaringan, ketergantungan terhadap androgen dan faktor testikuler, serta post-natal development.Hasil: Spink2 termasuk dalam famili serine protease inhibitor yang ditandai dengan adanya domain Kazal type 2. Analisis signal peptide menunjukkan bahwa Spink2 merupakan protein sekretori. Spink2 terekspresi di testis dan epididimis, dengan ekspresi tertinggi berada di kaput epididimis. Ekspresi Spink2 pada mencit yang digonadektomi mengalami peningkatan setelah 6 jam, kemudian menurun mulai dari hari ke-1 hingga hari ke-5. Pemberian testosteron mampu mempertahankan ekspresi Spink2 pada 3 dan 5 hari setelah gonadektomi. Selain itu, pada analisis pengaruh faktor testikuler, ekspresi Spink2 menunjukkan adanya regulasi dari faktor testikuler pada semua kelompok setelah dilakukan efferent duct ligation EDL . Spink2 menujukkan regulasi post-natal yakni mulai terekspresi pada umur mendekati 22 hari.Kesimpulan: SPINK2 merupakan protein sekretori yang terekspresi pada kaput epididimis, serta diregulasi oleh androgen dan faktor testikuler. Spink2 tidak terekspresi secara konstitutif. Berdasarkan data tersebut Spink2 sangat berpotensi terlibat dalam proses pematangan spermatozoa di epididimis. Penelitian lebih lanjut diperlukan untuk mengkonfirmasi potensi tersebut.
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ABSTRACT
Background Sperm maturation in the epididymis occurs through interactions between sperm and proteins secreted by epithelium cells lining the epididymal duct. The secretion of these proteins creates a microenvironment that is regulated by certain genes. Previous studies showed that genes which are involved in sperm maturation process are expressed specifically in the epididymis and regulated by androgen. Spink2 is one of the epididymal genes, but the regulation of its expression is still unknown. Therefore, this study was aimed to characterize Spink2 expression and its regulation in the mouse epididymis.Method s In silico analysis was performed to determine the gene structure and identify the signal peptide, as well as the functional domain of Spink2. Quantitative real time RT PCR was performed to measure relative expression

of Spink2 in the analyses of the tissue specificity, androgen dependency, testicular factor and post natal development.Result s Spink2 belongs to the serine protease inhibitor family which is characterized by the presence of Kazal type 2 domain. Signal peptide analysis showed that Spink2 amino acid sequence contains a signal peptide, indicating Spink2 is a secretory protein. Spink2 was expressed specifically in the testis and epididymis, with the highest level of its expression was in the epididymal caput. Spink2 expression increased after six hours and started to decrease on day 1 throughout day 5. Interestingly, administration of exogenous testosterone was able to maintain expression at the physiological level. In addition, Spink2 was slightly affected by testicular factors. During post natal development, Spink2 start to be expressed at day 22 before increased dramatically throughout day 60.Conclusion s Spink2 is a secretory protein that is expressed in caput region of the mouse epididymis and regulated by androgen. Spink2 is not constitutively expressed throughout development. Based on our data, may be involved in epididiymal sperm maturation process. Further studies are required to confirm its role.