精蟲*LRWD1*基因啟動子的高度甲基化研究

Hypermethylation of the Leucine-Rich Repeats and WD Repeat Domain Containing 1 (LRWD1) Promoter in sperm

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Abstract

Study Question: LRWD1 (*Leucine-Rich repeats and WD repeat domain containing 1*) was highly expressed in the testes, and associated with the sperm morphology and motility. The objective of this study was to investigate the association between the DNA methylation and LRWD1 expression in human sperms.

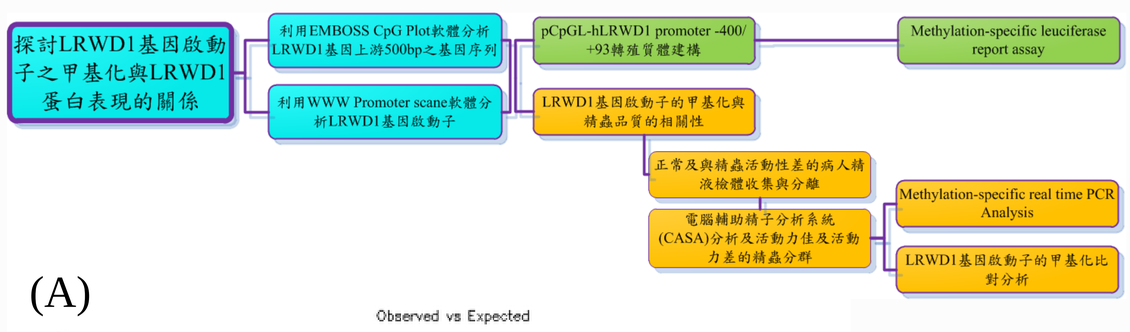
Study Design, Size, Duration: To assess whether the DNA methylation play an important role in transcriptional regulation of LRWD1, we used the EMBOSS CpG Plot to analyze the 500 bp segment immediately upstream to the LRWD1 translational start site (TSS) and revealed the CpG islands are located approximately between positions -253 and +5 from the LRWD1 transcription start site.

Materials, Setting, Methods: A total of 34 CpG sites were located within the predicted CpG islands (total length 258 bp). In the CpG islands contain the LRWD1 core promoter at -198 to +1 from the TSS, that containing the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and NRF2 (nuclear factor (erythroid-derived 2) -like 2) binding sites at −133 to −125 and −9 to −5, respectively. We constructed LRWD1 promoter (-400 to +93) in pCpGL vector (pCpGL-hLRWD1/-400/+93), and LRWD1 promoter activity was analysis by luciferase assay.

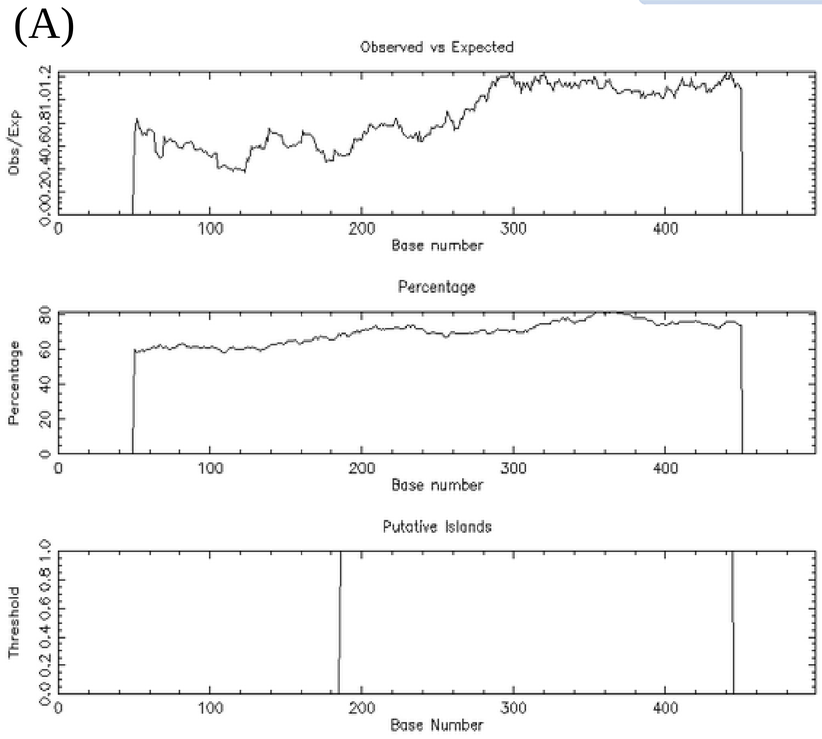
Main Results: The promoter activity of LRWD1 promoter previous treated by *Sss*I methylase previous was showed a significant decrease (~100 fold) than unmethylation control. In the quantitative methylation-specific PCR (qMSP) showed the DNA hypermethylation of NRF2 binding site of LRWD1 promoter was negative ly correlated to sperm motility.

Conclusion: All together, we found that the DNA hypermethylation of LRWD1 promoter would interfere with the expression of LRWD1 in human sperms.

Flow Chart and Methods



(A)

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CPGPLOT islands of unusual CG composition
EMBOSS_001 from 1 to 500

     Observed/Expected ratio > 0.60
     Percent C + Percent G > 50.00
     Length > 200

 Length 259 (187..445)
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EMBOSS CpG Plot to analyze the 500b.p (-439~+61 b.p) fragment immediately upstream to the LRWD1 translational start site.

(B)

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hKRWD1 -439/+261 seq

                                  -430       -420       -410       -400       -390       -380       -370       -360       -350       -340
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
[hLRWD1 -439/+261]   GGTGACCTGTAATCGAACCCACCCACGTTCCAGCTCCTTCCCCACTCCCAAGCCCGCTCCTTAAAGCCCTCATCTCCTCACCCTGCATCACCCCTACCGT
                                                                  Primer:BC009436-BAC 2F-2
                                  -330       -320       -310       -300       -290       -280       -270       -260       -250       -240
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
[hLRWD1 -439/+261]   AAGGGTCCCTCACAGGCTTCGATCCCGGCCCCTATCATGGCCAAGCTGGACCTCAGTTTCCCCAGATGCAGAGCGACGTTTGTGGGCGCGGCCCCTCTCC
                                                      Primer:BSP-NFkB+NRF2-F4                                  CpG islands→
                                  -230       -220       -210       -200       -190       -180       -170       -160       -150       -140
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
[hLRWD1 -439/+261]   CACCCCTTACCGCTGGGGGCAGCTCCCAGGGCGGGTCACTGCCGCGCTGCCGCTCGCAGATCAGACAGGTCCGGATGCCCTTGCAACCGCATTCCCGAAG
                                  -130       -120       -110       -100       -90        -80        -70        -60        -50        -40
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
[hLRWD1 -439/+261]   GACTTCGGGGGTCTCGGCGGCAGCCGCCGCCATCGCGCCGTCCGCGTGGCCAGTGCGCAGGCGCGGCCGTGGGGGCCGCTGGGAGTTGTAGTCCGCGCCG
                                   NFkB
                                  -30        -20        -10        1          11         21         31         41         51         61
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
[hLRWD1 -439/+261]   GGCGGGGGCACGAGGCCTGGGTTCCGCTTCCTTCCGGTCGTTAACGCCCACGGGCTCGCGCGGCGCCGCCTCCTGGGCTCAGTTACCGCGGACGCCAGTG
                                                    NRF2                ←CpG islands
                                   71         81         91         101        111        121        131        141        151        161
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
[hLRWD1 -439/+261]   CCGGGCTCCAGGAGACGCAGGGCGACGCCACACGCCGGGGTGGCCGACTGGGTCAGCGCGGGCTGCGCCTCCTCGCCATGGGCCCCCTCTCGGCGCGGCT
                                  Primer:BC009436-BAC 1R-2
                                   171        181        191        201        211        221        231        241        251        261
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
[hLRWD1 -439/+261]   GCTAATGCAGCGCGGGCGCCCCAAGAGCGACCGGCTGGGGAAGATCCGGAGTCTGGAGTAAGAGCCGGGCAGCGGGTGAGGCTGTGTCCTCGGGGCCGGG
                                                                                  Primer:BSP-NFkB+NRF2-R4
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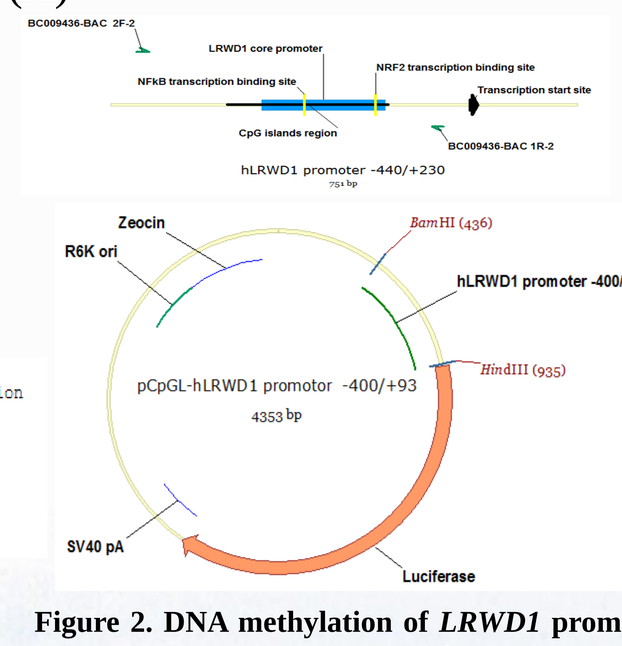
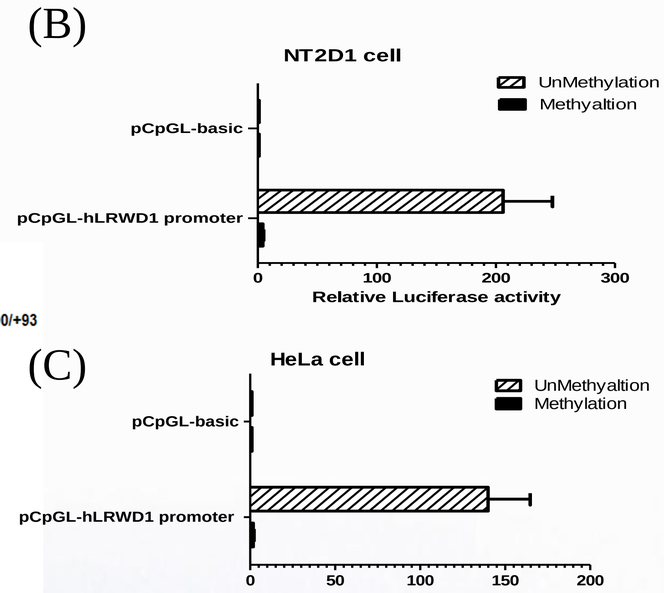
Figure 1. The CpG islands prediction of *LRWD1* promoter.
(A) The CpG islands of *LRWD1* were predicted by online bioinformatics tools (http://www.ebi.ac.uk/emboss/ cpgplot/). The CpG islands are located approximately between positions -253 and +5 upsteam the LRWD1 transcription start site. Criteria used for CpG islands prediction were: Island size > 200, G+C Percent > 50.0, and Obs/Exp > 0.6. (B) The sequence of the CpG islands of LRWD1 promoter that contain the NF-κB and NRF2 binding sites at −133 to −125 and −9 to −5 bps, respectively.

(A)



(B)



(C)

Figure 2. DNA methylation of *LRWD1* promoter -400/+93 was associated with *LRWD1* gene expression. (A) Construction of the plasmid pCpGL-hLRWD1 promoter -400/+93 bps. DNA methylation was important for *LRWD1* promoter activity. NT2/D1 (B) and HeLa cells (C) were transiently transfected with luciferase reporter plasmids driven by either no promoter (pCpGL-basic), unmethylation and methylation 493 bps human *LRWD1* promoter fragment including (pCpGL-hLRWD1 promoter -400/+93). The cells were also cotransfected with the pRL-TK reporter plasmids. Luciferase activities were normalized to pRL-TK reporter activities and shown as fold induction compared with the empty vector control Means±S.D. of triplicates from three independent experiments were shown.* P< 0.005

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                                  -310       -300       -290       -280       -270       -260       -250       -240       -230       -220
                     ...|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|.
[Normal-313/+225]    GGCCCCTATCATGGCCAAGCTGGACCTCAGTTTCCCCAGATGCAGAGCGACGTTTGTGGGCGCGGCCCCTCTCCCACCCCTTACCGCTGGGGGCAGCTCC
                     ::||||:::|::::||:::|:::::||:|:::::||||:::::|:::::::++++:||||:|:|||:||||:::|++|:|:::::|:::|::
[BSP-gDNA -313/+225] GGTTTTTATTATGGTTAAGTTGGATTTTAGTTTTTTTAGATGTAGAGCGACGTTTGTGGGCGCGGTTTTTTTTTTATTTTTTATCGTTGGGGGTAGTTTT
                                  Primer:BSP-NFkB+NRF2-F4                                  CpG islands→
                                  -210       -200       -190       -180       -170       -160       -150       -140       -130       -120
                     ...|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|.
[Normal-313/+225]    CAGGGCGGGTCACTGCCGCGCTGCCGCTCGCAGATCAGACAGGTCCGGATGCCCTTGCAACCGCATTCCCGAAGGACTTCGGGGGTCTCGGCGGCAGCCG
                     |::::++:::|:|::|++++|::|++|:++|::::|:::|::::|++::::|||:::|::|++|:::||++::::::|::++::::::|:++:++:|::|++
[BSP-gDNA -313/+225] TAGGGCGGGTTATTGTCGCGTTGTCGTTCGTAGATTAGATAGGTTCGGATGTTTTTGTAATCGTATTTTCGAAGGATTTCGGGGGGTTTCGGCGGTAGTCG
                                                                                                                    NFkB
                                  -110       -100       -90        -80        -70        -60        -50        -40        -30        -20
                     ...|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|.
[Normal-313/+225]    CCGCCATCGCGCCGTCCGCGTGGCCAGTGCCGCAGGCGCGGCCGTGGGGGCCGCTGGGAGTTGTAGTCCGCGCCGGGGCGGGGGCACGAGGCCTGGGTTCCG
                     |++||::++++|++:|++++:::||::::++|:::++++:|++:::::::|++|::::::::::::|++++|++::++::::|::++::::|:::::::|++
[BSP-gDNA -313/+225] TCGTTATCGCGTCGTTCGCGTGGTTAGTGCGTAGGCGCGGTCGTGGGGGTCGTTGGGAGTTGTAGTTCGCGTCGGGCGGGGGTACGAGGTTTGGGTTTCG
                                  -10        1          11         21         31         41         51         61         71         81
                     ...|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|.
[Normal-313/+225]    CTTCCTTCCGGTCGTTAACGCCCACGGGCTCGCGCGGCGCCGCCTCCTGGGCTCAGTTACCGCGGACGCCAGTGCCGGGCTCCAGGAGACGCAGGGCGAC
                     |::|||::|++::++::::++::::++::::|:++++++:++|++|:::||::::|:|:::::|++++::++||::::|++::|:|::::::++|::::++:+
[BSP-gDNA -313/+225] TTTTTTTTCGGTCGTTAACGTTTACGGGTTCGCGCGGCGTCGTTTTTTGGGTTTAGTTATCGCGGACGTTAGTGTCGGGTTTTAGGAGACGTAGGGCGAC
                                  NRF2               ←CpG islands
                                   91         101        111        121        131        141        151        161        171        181
                     ...|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|.
[Normal-313/+225]    GCCACACGCCGGGGTGGCCGACTGGGTCAGCGCGGGCTGCGCCTCCTCGCCATGGGCCCCCTCTCGGCGCGGCTGCTAATGCAGCGCGGGCGCCCCAAGA
                     +||:|:++|++::::::|++:|:::::|::++++::|::++||:||:++||:::::|||||:|:++:++:::|::|::::::|::++++::++||||::::
[BSP-gDNA -313/+225] GTTATACGTCGGGGTGGTCGATTGGGTTAGCGCGGGTTGCGTTTTTTCGTTATGGGTTTTTTTTCGGCGCGGTTGTTAATGTAGCGCGGGCGTTTTAAGA
                                   191        201        211        221        231
                     ...|....|....|....|....|....|....|....|....|
[Normal-313/+225]    GCGACCGGCTGGGGAAGATCCGGAGTCTGGAGTAAGAG
                     :++:|++:|::::::::::|++::::|::::::::::
[BSP-gDNA -313/+225] GCGATCGGTTGGGGAAGATTCGGAGTTTGGAGTAAGAG
                                  Primer:BSP-NFkB+NRF2-R4
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Figure 3. Prediction of the CpG islands (CGI) in the promoter region of *LRWD1*. The CpG islands of *LRWD1* were predicted by online bioinformatics tools (http://www.ebi.ac.uk/emboss/cpgplot/). Regions of of unusual CG composition are located from -300 to +200 bps in the promoter region of *LRWD1*. Our prediction showed a total of 34 CpG sites in 500 bps (from -300 to +200 in the promoter region of *LRWD1*). +: CpG sites; : : CT conversion; |: identical residuesA total of 34 CpG sites were located within the predicted CpG islands.

Conclusion and Discussion

A luciferase assay that the methylation of the *LRWD1* promoter by *SssI* methylase showed a significant decrease (~100 fold) in the activity of methylation compared with that of unmethylation pCpGL-hLRWD1 promoter -400/+93. All together, we found that DNA hypermethylation would interfere with the expression of LRWD1 in human sperms. We hypothesized that DNA methylation of *LRWD1* promoter may be one of the important roles in LRWD1 expression.

Acknowledgment

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