

Sulfated CXCR3 Peptide Trap Use as a Promising Therapeutic Approach for Age-Related Macular Degeneration

GukHeui Jo ^{1,†}, Jae-Byoung Chae ^{2,†}, Sun-Ah Jung ¹, Jungmook Lyu ³, Hyewon Chung ^{2,*}
and Joon H. Lee ^{1,*}

¹ Myung-Gok Eye Research Institute, Kim's Eye Hospital, Konyang University College of Medicine, Seoul 07301, Republic of Korea

² Department of Ophthalmology, Konkuk University College of Medicine, Seoul 05029, Republic of Korea

³ Department of Medical Science, Konyang University, Seo-gu, Daejeon 35365, Republic of Korea

* Correspondence: joonhlee@konyang.ac.kr (H.C.); hchung@kuh.ac.kr (J.H.L.); Tel.: +82-2-2637-7871 (H.C.); 82-2-2030-7657 (J.H.L.)

† These authors contributed equally to this study.

Supplementary Material

Supplementary Figures

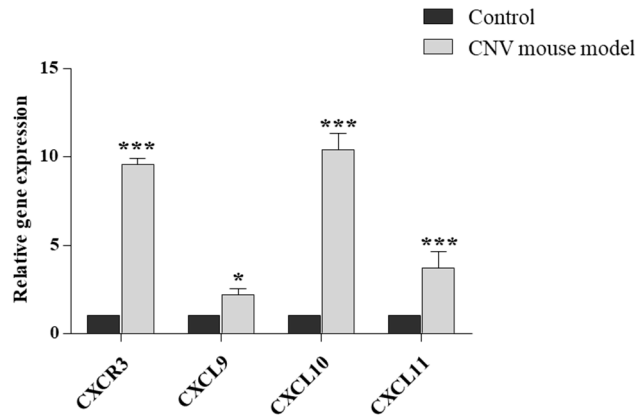


Figure S1. Quantification of mRNA expression of CXCR3 and its ligands (CXCL9, CXCL10, and CXCL11) in the RPE/choroid complex from a control group and a laser-induced CNV mouse model (n=3). *** $p < 0.001$, * $p < 0.05$.

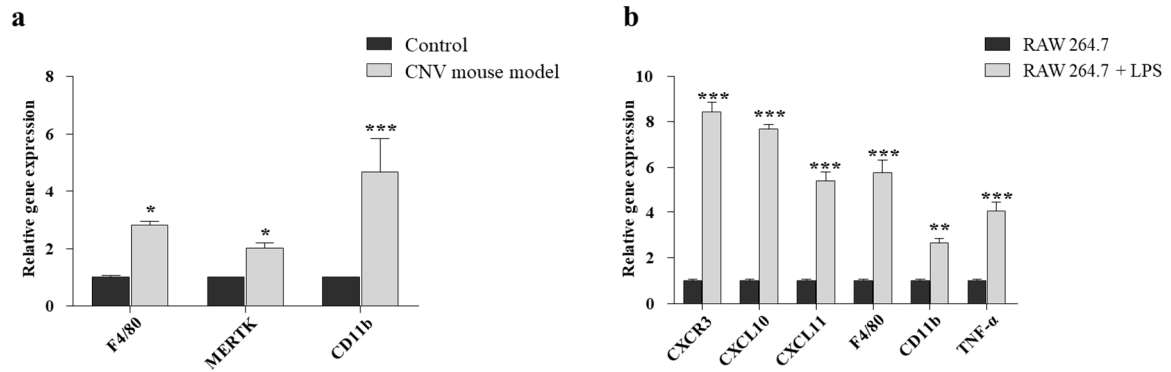


Figure S2. Quantification of mRNA expression of markers related to macrophages in mouse RPE/choroid and RAW 264.7 cells. (a) mRNA expression of macrophage related genes, including F4/80, MERTK and CD11b were increased in RPE/choroid complex from a laser-induced CNV model compared to a control group (n=3). (b) Quantification of mRNA expression in RAW 264.7 cells. In LPS-treated group, the expression of CXCR3, its ligands, and macrophage related genes has increased compared to control group (n=3). *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Supplementary Table

Table S1. CXCR3 primers used in oligo shuffling. In total, 10 oligos (39 nt in length) were synthesized.

Primer	Sequence (5' to 3')
	1F: aaa gat atc atg gtc ctt gag gtg agt gac cac caa gtg
	2F: ctt aat gac gcc gag gtt gcc gcc ctc ctg gag aac ttc
	3F: agc tct tcc tat gac tat ggt gaa aac gag agt gac tcg
	4F: tgc tgt acc tcc ccg ccg tgc cca cag gac ttc agc ctg
	5F: aac ttc gac cgc ctc gag aaa aaa aaa aaa aaa aaa aaa
<i>hCXCR3</i>	3F sulfation: agc tct tcc tag gac tag ggt gaa aac gag agt gac tcg
	1R: ttt ctc gag gcg gtc gaa gtt cag gct gaa gtc ctg tgg
	2R: gca cgg cgg gga ggt aca gca cga gtc act ctc gtt ttc
	3R: acc ata gtc ata gga aga gct gaa gtt ctc cag gag ggc
	4R: ggc aac ctc ggc gtc att aag cac ttg gtg gtc act cac
	5R: ctc aag gac cat gat atc ttt ttt ttt ttt ttt ttt
	3R sulfation: acc cta gtc cta gga aga gct gaa gtt ctc cag gag ggc
	1F:aaa gga tcc atg tac ctt gag gtt agt gaa cgt caa
	2F:gtg ctt gat gcc tcg gac ttt gcc ttt ctt ctg gaa aac
	3F: agc acc tct ccg tac gat tat ggc gaa aac gag agc gac
	4F:ttc tct gac tcc ccg ccg tgc cca cag gat ttc agc
	5F:ctg aac ttt gac cgt ctc gag ttt ttt ttt ttt ttt
<i>mCXCR3</i>	3F sulfation: agc acc tct ccg tag gat tag ggc gaa aac gag agc gac
	1R: aaa ctc gag acg gtc aaa gtt cag gct gaa atc ctg tgg
	2R: gca cgg cgg gga gtc aga gaa gtc gct ctc gtt ttc gcc
	3R: ata atc gta cgg aga ggt gct gtt ttc cag aag aaa ggc
	4R: aaa gtc cga ggc atc aag cac ttg acg ttc act aac
	5R: ctc aag gta cat gga tcc ttt ttt ttt ttt ttt ttt
	3R sulfation: cta atc cta cgg aga ggt gct gtt ttc cag aag aaa ggc

Table S2. Sequence of CXCR3 amino acid and prtein estimated size.

Protein	Type	Amino acid sequence	size
<i>hCXCR3</i>	wt	MYLEVSRQVLDAADFALLENSTSPYDYGENESDFSDSPPCPQDF SLNFDR (52)	5.72 kDa
	sulfation	MYLEVSRQVLDAADFALLENSTSPYDYGENESDFSDSPPCPQDF SLNFDR (52)	5.72 kDa
<i>mCXCR3</i>	wt	MVLEVSDHQVLNDAEVAALLENFSSSYDYGENESDSCCTSPPCPQ DFSLNFDR (53)	5.83 Kda
	sulfation	MVLEVSDHQVLNDAEVAALLENFSSSYDYGENESDSCCTSPPCPQ DFSLNFDR (53)	5.83 Kda

Red: Sulfation site.

Table S3. List of primers used in PCR reactions.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
CXCR3	TACGATCAGCGCCTCAATGCCA	AGCAGGAAACCAGCCACTAGCT
CXCL9	CCTAGTGATAAGGAATGCACGATG	CTAGGCAGGTTTGATCTCCGTTT
CXCL10	ATCATCCCTGCGAGCCTATCCT	GACCTTTTTTGGCTAAACGCTTTC
CXCL11	CCGAGTAACGGCTGCGACAAAG	CCTGCATTATGAGGCGAGCTTG
F4/80	GCTCTCCATCTGCCTTTTCCTG	GGAAGCCAAGTAGAGGTAGTGC
MERTK	ATCATCCTCGGCTGCTTCTGTG	ACGACCAGTTGGGAATCCTCCT
CD11b	TACTTCGGGCAGTCTCTGAGTG	ATGGTTGCCTCCAGTCTCAGCA
TNF-α	GGTGCCTATGTCTCAGCCTCTT	GCCATAGAAGTATGAGAGGGAG