

Krüppel-like factor 8 activates the transcription of C-X-C cytokine receptor type 4 to promote breast cancer cell invasion, transendothelial migration and metastasis

Supplementary Materials

The PCR primers used to clone the human CXCR4 gene promoter:

CXCR4p-F, 5'- ACG CGG TAC CGT GCA GCT TAC GGT C -3'

CXCR4p-R, 5'- TGT CCG CTA GCA TGG TAA CCG CTG GTT C-3'.

Primers used to generate the truncation mutants of the human CXCR4 gene promoter:

The CXCR4p-R primer described above was used for constructing all the truncations. All the forward primers listed below contained a KpnI restriction site.

-1520CXCR4p-F, 5'-AAC TGG TAC CGG AAC ACT CCA GAC TG -3'

-1372CXCR4p-F, 5' - TCG GTG GTA CCT ATC TCC GCA GCG-3'

-1230CXCR4p-F, 5'-TGC AAG GTA CCG CCA GTC TGA GAT CG -3'

-397CXCR4p-F, 5'- ACG CGG TAC CGA GAG ACG CGT TCC -3'

-217CXCR4p-F, 5'- ACC TGG TAC CTC CTT CCT CGC GTC TGC - 3'

-150CXCR4p-F, 5'- ATG CGG TAC CCT CGG AGC GTG T - 3'

Primers used to construct the GT-box specific mutants of the human CXCR4 gene promoter:

CXCR4p-mGT1-F, 5'- TCC CCT CTC ACG CCT TCT CCC TC -3'

CXCR4p-mGT1-R, 5'- AAG GCG TGA GAG GGG AGA GGG GCA -3'

CXCR4p-mGT4-F, 5'- CGC CCT CTC AAG TCT CCG CGC CGG AG - 3'

CXCR4p-mGT4-R, 5'- AGA CTT GAG AGG GCG GGG GAG CGA GTG -3'

CXCR4p-mGT5-F, 5'- AGC GGC TCT CAC GCC TGC GGA C- 3'

CXCR4p-mGT5-R, 5'- AGG CGT GAG AGC CGC TGC GGA GAT A- 3'

CXCR4p-mGT7-F, 5'- AGC CAC TCT CAT CCA AGA ACC TGG- 3'

CXCR4p-mGT7-R, 5' - TTG GAT GAG AGT GGC TGG AAG AGG- 3'

These mutant primer sets for each of the mutant GT-box (mGT) were used along with the CXCR4p-F/CXCR4p-R primer set described above.

Primers used to clone the cDNA of the human CXCR4 and the dN20 mutant:

hCXCR4-Sma-F, 5'- TCC CCC GGG CGT GGA GGG GAT CAG TAT ATA -3'

hCXCR4-dN20-Sma-F, 5'- TCC CCC GGG CTA TGA CTC CAT GAA GGA AC -3'

hCXCR4-RI-R, 5'- AGG AAT TCT TAG CTG GAG TGA AAA CTT G -3'

Primers used to construct the pLVZP-CXCR4 and pLVZP-CXCR4-dN20:

pKH3-F, 5'- CCC AAG CTT CTG CAG GTC G - 3'

pLVPZ-CXCR4-Not1-R, 5' - ATA AGA ATG CGG CCG CTT AGC GAA AAC -3'

Primers used for qRT-PCR:

hCXCR4-RT-F, 5' - GGC GTC TGG ATC CCT GCC CT -3'

hCXCR4-RT-R, 5'- TGC GCT TCT GGT GGC CCT TG -3'

hKLF8-F, 5'-TCT GCA GGG ACT ACA GCA AG -3'

hKLF8-R 5'-TCA CAT TGG TGA ATC CGT CT -3'

hGAPDH-F, 5'- TCG TAC GTG GAA GGA CTC A -3'

hGAPDH-R, 5'- CCA GTA GAG GCA GGG ATG AT -3'

The ChIP assay primers directed along the nucleotide sequence flanking the GT1 box of the CXCR4 promoter:

CXCR4p-GT1 -ChIP-F, 5' - TTC CCA AGC CGC GCA CCT CTC -3'

CXCR4p-GT1 -ChIP-R, 5'- ATG CGG TGG CTA CTG GAG CAC TC -3'

The BOP assay oligonucleotides spanning the GT1 site in the human CXCR4 promoter:

WT-sense: 5'-CCC CTG CGC CCT CCT TCC TCG CGT CTG CCC CTC TCC CCC ACC CCG CCT TCT CCC TCC CCG CCC CAG CGG CGC ATG CGC CGC GCT CG-3'

WT-antisense: 5'-CGA GCG CGG CGC ATG CGC CGC TGG GGC GGG GAG GGA GAA GGC GGG GTG GGG GAG AGG GGC AGA CGC GAG GAA GGA GGG CGC AGG GG-3'

mGTI-sense: 5'-CCC CTG CGC CCT CCT TCC TCG CGT CTG CCC CTC TCC CCA AAC ACG CCT TCT CCC TCC CCG CCC CAG CGG CGC ATG CGC CGC GCT CG-3'

mGTI-antisense: 5'-CGA GCG CGG CGC ATG CGC CGC TGG GGC GGG GAG GGA GAA GGC GTG TTT GGG GAG AGG GGC AGA CGC GAG GAA GGA GGG CGC AGG GG-3'.