

Modulation of DNA methylation influences cartilage formation in murine chondrogenic models

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This PDF file includes:

- Sequences of primer pairs used for the PCR assay analyses.
- Sequences of primer pairs used for the RT-qPCR reactions.
- Sequence data of the 3'UTR regions of *Dnmt3a*, *Ogt* and *Tet1* genes with insert flanking T7 promoters for antisense probe preparation. These regions were cloned into pDrive vector and later amplified for digoxigenin-labelled RNA probe preparation.

Epig.Modif.	Gene	Forward	Reverse
DNA methylation	Dnmt1	CAGAGGAGAGAGACCAGGATAA	GCTGTTACCTCTTCCAGTTTCT
	Dnmt3a	CAAGGGACTTTATGAGGGTACTG	TTCTCAAAGAGCCAGAAGAAGG
	Dnmt3b	GAACCCTGGAGCTGCTATATG	CAACTTGGGTGGCTCAAATTC
	Tet1	CCAAGTGGGTGATCAGAAGAA	CACAGCAGGATAAGGACAATA
	Tet2	AGGATGCAATCCAGACAAAGA	GCTTTACCTTCTCTCCATACC
	Tet3	GAACTCATGGAGGATCGGTATG	CAGTGTGTGTCTTCGGATCA
	Ogt	GACTGGCGACTACACAGATTA	GGGAACGGGTGGTTACAATA
Histone acetylation	Atf2	ATGGCAGTGGATTGGTTAGG	GAGAAGCCGGAGTTTCTGTAG
	Crebbp	CAACAAACCATCCTGGGATCT	GGGTCTATGGGATTTGGGTTAC
	Ep300	GGCAATGCTGGCAGTTTATT	TTCCCATCTTAAGTGTTGGG
	Hat1	GCCTGAAGATCTTGCTGTACTA	TCATCTGCCTCCACACAATC
	Kat2a	GCTCTTGGGAATGGTAGTAGATG	GCAAGAGCTTGAAGAGGTAGAA
	Kat2b	GAGGAGACCTCCAGCAGATAAT	TGAGAAACGTGAGCAGCAAG
	Kat5	TTTCCCTCAGAATGGGTCAG	CATCAGTGCCCAAGCAATTAG
	Kat6a	CCCATCTGTAGCTTCTGTCTTG	CACGATGGATGACCGCTATT
	Kat6b	GTGCCGATCCCATCCAATA	ACAGGATGGGTGTCCAATA
	Kat8	TCTATGTCTACTATGTGGGCTTTA	GCCAGCTCACTCAGGTATTT
Histone deacetylation	Hdac1	GATGCAGAGATTCAATGTTGGTG	GATGTCCGTCTGCTGCTTATTA
	Hdac2	CCTCATAAAGCCACTGCTGAA	CCGACGTAAATCTCTGCATCT
	Hdac3	TGATGACTGCCAGTGTTTC	GGCCCAGTTGATGGCAATA
	Hdac4	CTTGAGGGCCGCTTTATCA	GGAAAGAAACACAACCAAGTCTATC
	Hdac5	AGGAGGAGCTGGAGAAACA	GATGGCACTCTCTTTGCTCTT
	Hdac6	CCCAATCTAGCGGAGGTAAAG	GTTCCAGATCCAGCCCTTGAA
	Hdac7	CAGAAGCTGGCTGAAGTGAT	GCTCAAGAGTTCTGTAGGGAATAC
	Hdac8	CCTTCCACACTGATGCCATATC	GGCAGTCATAACCTAGTCCATATT
	Hdac9	GGAGCACATCAAGGAATTCTA	GCCTCTCTACTTCTGTCTTG
	Hdac10	AGACCCAGACCCTGGATAAA	AAAGGTGTCCGGGTGAAAG
	Hdac11	GAGCTGAAGTGGTCCTTTGT	TCCTCTGCACAAGGAAGTTG
	Sirt1	GGATCCTTCAGTGTCATGGTT	CACCGAGGAACCTGATTAAA
	Sirt2	CCAACCATCTGCCACTACTT	CGTGTCTATGTTCTGCGTGTA
	Sirt3	GCCCAATGTCACTCACTACT	GATCCCAGATGCTCTCTCAAG
	Sirt4	CGCTGCTCAAGATCCCTAAG	GGGCAGCTCTCATTTCTGTAA
	Sirt5	GTGTCTAGTGGTGGGAACATC	GGGTGGTCTCCATGTTAACT
	Sirt6	CCCAAGTGAAGACGCAGTA	CAGTCCAGAATGGTGTCTCTC
	Sirt7	GTGGTGTCTCAGAACTGTGATG	CAGGAGGTGCAGACTTCAATATAC

Table S1. Sequences of primer pairs used for the PCR assay analyses. The table continues on the next page.

Histone methylation	Ash1l	GAGAGAGGAACTTCGTGCTAAA	TGTACAGCGATGCTGAGTG
	Dot1l	CATCACTACGGAGTGGAGAAAAG	ACCTCGTTCCAGTGTGTATTC
	Ehmt1	GTCTGACCTGAGTTCTGAATCC	CTGCTCGGCTTCTTTCTACTT
	Ehmt2	ACAACGCACGCCACTAAT	CATCCTCTTCCTTGCTGTAGAC
	Ezh1	GGAGCAAAGGCTCTGTATGT	GGCTGGACACGAAGTTTCT
	Ezh2	CATCCCGTTAAAGACCCTGAA	ACAGTTTCGTCTTCCACCATAA
	Fbxo11	GATGGATTTGCTGCAGGTATTG	GCCTCCAAATCGGTTGTTAAAG
	Kmt2a	CCAGACCCTCCTGTTCTTACT	CGCTGAACTCCAACACAGATAC
	Kmt2b	GCTGGACTGGAAAGCAGAA	CTAGGTCGCAATCCTCCTTAAA
	Kmt2c	TGTAAAGGCCTCACACCTTG	GGAGTGGACAAACTGCTTACT
	Kmt2d	GAGACATGTGACAAAGGGTATCA	CATAGCCGGCATGTCTTACA
	Kmt2e	AGCCCAGGAGTTTGATAAAG	AGGAACTTCATCACCACCTTCA
	Prdm4	GACACACCAATAGAGAGCAGAG	ATGGAAGGACACCAACAACA
	Prmt3	AGACTTAAGAATCCTACGGTTGAA	GGAGTAGACACAGGCTCATAAAG
	Prmt4 (Carm1)	GGCGATTTGCACAGGATAGA	GGAGCCAATGAAAGCAACATC
	Prmt6	GGTGAACAAGATACGGACAT	CTCATGGTCTCCCACTTTGTAG
	Setdb1	ATCAGCTCAAGATGTCCAGAAG	CCTGACCCAAGGTTCTTTTAT
	Setdb2	TCTGCCACAAATGGAGACTATG	CACTCTGAGTCACAGGTGTATG
	Smyd1	GGAAGTGGTGAAGGAGATGATAC	GCTTCACAACCTCGTGATACA
	Smyd2	CGGCGATATTCCCTGATGTT	TCCTGTACAGCTCTGACTTCT
	Suv39h1	CTCTGCATCTTCCGCACTAAT	CTGAGGTAATAATCTCTCCACATAC
	Suv39h2	GTGAATCATAGTTGTGACCCAAATC	CAGCTCTTCTCCAGCGTTTAT
	Suv420h2	CTGCTCTCAAGACCCACATTT	AAGCACGGGTAGACACAATC
Histone demethylation	Jmjd1c	GCCATGATGCCAACAAGATATG	GTACAAAGAACCCTGGCAAATG
	Kdm6b	GAGGAACCAGACAGCACTAC	CTTCCACCTCTTGGCATCA
	Jmjd4	AAGTATGGAGACGCGGTTG	GTAAGTATATAGTCGCGGAAGG
	Jmjd6	CCATTGCCATCACCAGAA	GCTCCTGTTTCAAGATCCTATACC
	Jmjd7	CTTGACAAGGACCACTATGA	TGCTGGTGTGTAGAGATTGTAAG
	Jmjd8	GAATTGCAGGAGCTGGATCT	TGCTTCTCAGGAGGGTAGAG
	Kdm1a	TGGGATCAGGATGATGACTTTG	GCACTGCTGTGTTCAAGTTAAT
	Kdm1b	GACTCAAATCTCCAGTGCAAAG	GATGGCACCTCTCTGTAGTATG
	Kdm3a	TCATCCACAGCAAAGACAGAA	GGCCTCTTGATGAATCAGAA
	Kdm3b	GTGACCAAGCAGAGGATTCA	TTGCTCTTCTCCAACCTTCC
	Kdm4b	CAGCCGGATTACACAGGAAG	TGGACTCAGCGCAGTTAAAG
	Kdm4c	AGTTGTTATGGTGTCCCTTCTC	GAGCCCACTGATTGTTCTTAGT
	Kdm5a	CCTGAATGAACTTGAGGCAATG	CACCACAGGGATCTTCAATGTA
Chondrogenesis	Acan	TGGAGGTCATAGTGAAGGTATTG	ATGATGGCGCTGTTCTGTAG
	Col2a1	TGGCTTCCACTTCAGCTATG	GGTAGGCGATGCTGTTCTT
	Prg4	TGGAGGACTAACAGGGAAGATA	CTGAATGTTGCCACCTCTCT
	Alpl	GCCATGACATCCCAGAAAGA	GCCAGACCAAAGATGGAGTT
	Col1a1	ACAAGGTGACAGAGGCATAAA	ACCAGGAGAACCAGGAGAA
	Col10a1	AACGCCACAGGCATAAA	CTCCTCTTACTGGAATCCCTTTAC
	Spp1	CTTTCACTCCAATCGTCCCTAC	TGGCATCAGGATACTGTTTCATC
	Znf219	CGGCCACCCAAGAAGAAA	CGCCGAGCGGAAAGATT

Table S1. Sequences of primer pairs used for the PCR assay analyses.

Gene	Accession number	Primer sequence	Amplicon size
1. Epigenetic marker genes			
<i>Dnmt3a</i>	XM_006514953.3	CTGTCCCTACGACCAGTGC CTGATGTCAAGCCCTCGGAA	110
<i>Tet1</i>	NM_001253857.2	CTGGGGCCATCCAAGTCAAT TGTGTGAACCTGATTTATTGTGGT	120
<i>Ogt</i>	NM_139144.4	TTCAGTATTCTGTGCCGCC TCGTTGGTTCTGTACTGTCCG	188
2. Chondrogenic marker genes			
<i>Sox9</i>	NM_011448.4	GGAAGTCGGTGAAGAACGGA AGATTGCCAGAGTGCTCG	158
<i>Col2a1</i>	NM_031163.3	TCATCTTGCCGCATCTGTGT TGCCCTTTGGCCCTAATTT	164
<i>Acan</i>	XM_006540566.1	ATTCCCGCCACCTACCT GCTGACTAGGTTTCGGAGCA	190
3. Reference genes			
<i>Actb</i>	NM_007393.5	AGATCAAGATCATTGCTCCTCCT ACGCAGCTCAGTAACAGTCC	174
<i>Sdha</i>	NM_023281.1	TCGACAGGGGAATGGTTTGG GGA CTCTCCGAGCTTCTG	110

Table S2. Sequences of primer pairs used for the quantitative real-time PCR analyses. Nucleotide sequences, gene accession numbers and amplicon sizes are displayed for each primer pair.

>T7promoter-Dnmt3a (anti-sense) Clone#:D7

chr12:3,907,819-3,908,735

GCCAAGCTCTAATACGACTCACTATAGGGGAAAGCTCGGTACCACGCATGCAGACGCGTTACGTATCGGATCCAGAATTCAGATTGCACG
CGAGTCTGGATAAATCCACACTCAGCTTCCCCAGGGTCCGACCCTCAGCTGAGCCCCCTTTTGGCAGGTGAAGCTAGGCATGGGGC
AGGGGTGGGGGGTGATATGGGTCATCCTTCAGTTTGTGTTAAACCCCCCTCCAGCTGCTCCCCCTCTCCCATCCTGGGCTGAAACCCCTTTG
CACAGAGGGCCAGGCGGAAGCTGATGTCTTTGCTCATCTCGCTGTTTTGAAAGCACCATTATGAAGTCGGGATGTACAGTAGTTAACAG
TACCTTTTATATATATATATCTCATATCTATCATATATATATAAACTGTAAACAAGAGGTAACAGCGGCTTCTAGAACTGGTTTTCTT
CAAGGTTTCCGTGTGTGGTAGGCACCTGAAATACTGTAGAAAAGTGTCTGGTGTGGTGTCTCTCAGCGCCCTGCTGCTAGTTGGGTCT
GTTTTGCATGACCAGGAATGGTGTCTGTTGAGACTGCTCGGGGTGGGAGAGGAGACTCTGCCTCCGAGGGTCTCTATTCTCTTCTGT
TGTGCTCTCTATCTGATCAGGCTAGAGACAACCAAAGAGATATATAGAAAAGGAAAAAAAAAAAAACCAATATTTTAAAGTTTTATAGAAT
TTTCCCTCTCTCCCTTTCCCTCTCCAAGGCATTTCTACTTTTTGTGTTACTGATTTTGCTGCAATAACTCTCTTGTTCAGTCACAGCA
AGAAACAAAAACCCAAACAAACAGAAAACCCCTCTGAAAAAGAGTAGAAAACAAAAAGTTGCTCTCTAGGACCGAGGGGAAGAAGGG
GAGGGGCCTATTTTGTCTTTTAGATAGGACTGAAGGTTAACATTGAAAACCTCAGGAGATGATGTCCAACCCCTTTTGAAGGCCAAGCCC
TCCGGCGTTTTCTCTCTGCGGTTTGTCTTAAATTCCTTTTTCTTCTGCGGTGCTGAACCTTTCTCCGTCCTCTCGTTCTTGGGGGT
TTAATGGTTTTGTTTTGTTTTGGTTTTGTTGTTGTTAAATTTTTTATCATCACTACTTCAGTTTGCCCCCATGTCCCTTAACCACA
AATCACGAATTCGTCGAAAAGCTTCTCAAGCCTAGGCTAGCTCTAAACCAACGTGTGGGGGGCCCAACTCCCGGCCCTTGAATTTCTA
TAAGGTCACCTAAAGGGCCGACAATTCATGGGCCCGCGTTTTACAACGCCGGGACTGGA AAAACCCCTGGGGTTACCCAATTT

>T7promoter-Ogt (anti-sense) Clone#:O2

chrX:101,682,485-101,683,489

CCAAGCTCTAATACGACTCACTATAGGGGAAAGCTCGGTACCACGCATGCAGACGCGTTACGTATCGGATCCAGAATTCGTGATTCTACC
AAGGCCGAATCCATAGAGTTTGAAGCTAAGTCCAGACATTCTAACACAGTCTAAAAATAAAATAACTGCCTGTATAGAGATGCTTCTTC
AGAGCGGTGAACACCATTTAAGTACTGGAGAGAAGTGAATTTACTAACCAACATTTGAATACTTATATACTTACCTGGGATTTTCAT
TTCTGCTGAAAGAAATGGGAAGAACAGGACTCACTTTAAAAAAAAAAAAACAACTTTAGAAAGGAAGGTAAAAATCTTATCACACTATTA
CTTTTGAAGCAGCATAGGAGGGGCACTCAAGGGCTGAAACACTGGTGAACAGGTAAACCTTAGGCAGAAAAAACCCCTATATTATTA
TCAATAACAGTACCACAACCTGTGACCTTCATGATTATTAATCTCACTCCTAAGAATTTTCATTTGGCACCAGATGGCATGTTTCGAGAGAAA
CACCTTGGGATGTCTGAAAGCAGACATGGGTTTGTGTAGCAGTCACAGGAGAATTTAACGGGAGCTGGGGTGTGTAAAAACAGGCTGAAAA
GTCTTAAGTCATCATCTAAACAAAGCACTGACAGTGCCAAGCATTAGGTCAGACACTAAAATGACTGATATAGGCTCATGTGGTTTTATA
AAACCTATAAAAAGACTACACCAGCAAGGTCCCTGTCAACCTGTGAGAGTTCAGACACTAAAACAGGAAGCAACATTTAGTTTTAAAC
CTCAGAAGAATCAAGATCACTTCACATGAGTAAAAATACCTGAAAGCAAAACATTTTAAAAGCTCCAAAACCCAAAATATAAAGAAAAA
AAAAAGGATCAGAAGACTTGATCAATCCACGGCATCACAAAACGGCTGGTTAATCTCTATCTCCCTCCACATTAAGCATCCCATGGAA
GACCAAGGGAGACCAGACCTTCAGACCTGTGCACCACCTGTTTGCTGCAAACTCTAAGAGATTCTTGTGGAATAGCATTTCTTCTT
TCTTGTCTCTCCCTATCATGCAGAATCTGAATTCGTGCAACAAGCTTCTCGAGCCTAGGCTAGCTCTAGACCACACGTGTGGGGGGCCCC
AACTCCCCGGCCGCTGTATTCCATAGGGGCCACCTAAATGGGCCGCACAATTCACTGG

>T7 prom-Tet1 (anti-sense), Clone#T1

chr10:62,811,427-62,812,547

CCAAGCTCTAATACGACTCACTATAGGGGAAAGCTCGGTACCACGCATGCAGACGCGTTACGTATCGGATCCAGAATTCGTGATTGGGTA
AGGGTGACAAATGCTAACGCAAGCATGTCCCTCAATTATCTCTATAGAATATTTCTTCTCATTTGGGAAGGACATGTCTCTTAATTAT
GTATTTTCTAATCTTGAACACTGGGAACCTTTGACTGATCCCAATTTGCCTCCTTTTCTTTGAAAACACCTGTAATTGGGTATATA
GTTAATTGACAGGTGACTTCAGAAGACAATATTTTCTAGTTAAAGAGGGGAAATTGATATTGGTAGCATCTTGATTTAAATTTTT
TTTTTTAGAGACCTCTATTCTATAAGTAAGAACAAGAGCCGTGTGTTATTTGAGTACAGGCTGAGGTTAACGGGATGGGACTCTACA
GAGCAAGAACAATGACTTCTCTGGTATTCAATATGCACACATAATAGACAATAGGATTCTGCTACTATACTATTTCAGTTACTCTT
AAAAGTAGAAAGTGCTTTTTTAAATATATACATTTATAGTTAGCGAACAGCTTCCAACCATCATGAGAATAGAGAACTACTTTATATAG
GAGAAATGTTTTAAGAGAAAAAGCATTTTCATTGGTTTACTTCAAGAAATGTGCAGACACGATGGCAATGATTGGAAGAGGCTTGAGCA
ATGGCACCAGAAAAAATCACTCAGCTCATCACTCCGTGTGTTGATGTGGTTAAAGCACCAGGAAGTGTGTAGAGTTAACACTACATCT
TGGTCTTTACTTCTTATTGCAGGAACCTGTTATAAACCTCTGAATAGTAAACAAACCCGGGGGAAAGCACCAGACTCTATGGTTCTAG
CATGGCCAACACCTAACCAAGATGTTCCCTTAAAGTATTTAAACTATAAAAAAGTTTATTTTTGCTGAAAATGTTACCTTCTACCAATT
CTCAAAATGTAATCACAGTTAAGTATTACCCCTTTTTTTTTTTTTTTTAAATATTACGTCGGAATTGAAATGGGCGAAACAAAGCTTCC
GTAAACAACAAAATGACTTTCTTTTAAACAGCACCGGAAAAAACAAAAAACCAAGAAAACCAACCAACCAACAAAAATTTCCCTCT
ACCCCCCTTTAGCCAAGGGCTTTACTTGGAGAAAAGCCTTTAGACCCACCGATTGGAAGGGAATCTGAATTCGTCCAAAACTTTCCCA
AGCCTAGGCTAACTCTAAACCAACCGTGGGGGGGGCCCCAACTTCCGGGCCCTTGAAATCCTTAAGGGGCACCTAAAGGGCCGCCCC
AAATTTCCCGGGGCCCGCCTTTTTACAAGGCCGGGACTGGGAAAAACCTGGGGGGTTACCAATTAACCCCTTGGCGAAAAATCCC
CCTTTTCCCCCGTGGGGCGTATAAGACAAGAGG

Table S3. Sequence data of the 3'UTR regions of *Dnmt3a*, *Ogt* and *Tet1* genes with insert flanking T7 promoters for antisense probe preparation. These regions were cloned into pDrive vector and later amplified for digoxigenin-labelled RNA probe preparation.