Small RNA-mediated DNA methyltransferase 1 inhibition causes DNA methylation alteration in mammalian cells

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Background: DNMT1
- Uses hemimethylated DNA as substrate.
- Responsible for CpG methylation maintenance.
- Dysregulation of DNMT1 causes aberrant methylation in cancer.
- Highly abundant transcripts inhibit DNMT1 activity in vivo (asCEBPA).
- LncRNA (Kcnq1ot1) recruits DNMT1 to imprinted genes and somatic DMR.

DNMT1 is loaded with inhibitory RNA

miRNAs are potent DNMT1 inhibitors by competing with hemimethylated DNA substrate
- Methylation assay using a panel of cancer related miRNA oligos
- 200-fold excess of DNA substrate does not fully rescue DNMT1 activity

miR-155 competitively inhibits DNMT1

Fourteen cancer related miRNAs inhibited DNMT1 activity in a methylation assay. Steady state biochemistry experiments indicated miRNA is a competitive inhibitor of DNMT1.

G quadruplex sequence of miR-155 is essential for DNMT1 inhibition
- Truncation of miR-155
- Methylation assay using truncated miR-155

Formation of G-quadruplex by annealing telomeric sequence in KCl buffer
- Truncation analysis identified an essential G quadruplex module in miR-155 required for DNMT1 inhibition. Telomeric oligos with G-quadruplex structure showed a stronger inhibition of DNMT1 activity.

miR-155 causes genome-wide aberrant methylation with emphasis on methylation loss

Exogenous miR-155 induces hypomethylation of the genome. A small shift of hypermethylation of previously hypomethylated region was also observed.

Demethylation occurs in low to moderately methylated CpG dense regions

Average methylation of DMRs
CpG density of DMRs
Exogenous miR-155 induces hypomethylation of low to moderately methylated CpG dense regions.

Hypomethylation is enriched in non-CpG island and intergenic regions

Distribution of hypomethylated CpG sites in CpG island and genic regions

Conclusions
- miRNA inhibits DNMT1 activity by competing with hemimethylated DNA substrates.
- G-quadruplex structure in miRNA is essential for DNMT1 inhibition.
- miR-155 causes genome-wide aberrant methylation.
- Loss of methylation occurs in low to moderately methylated CpG dense regions.