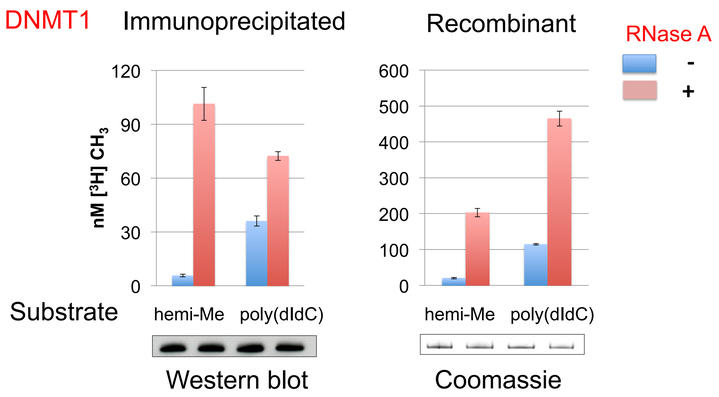
Small RNA-mediated DNA methyltransferase 1 inhibition causes DNA methylation alteration in mammalian cells

Guoqiang Zhang, Sriharsa Pradhan
New England Biolabs Inc, 240 County Road, Ipswich, MA 01938

Background: DNMT1

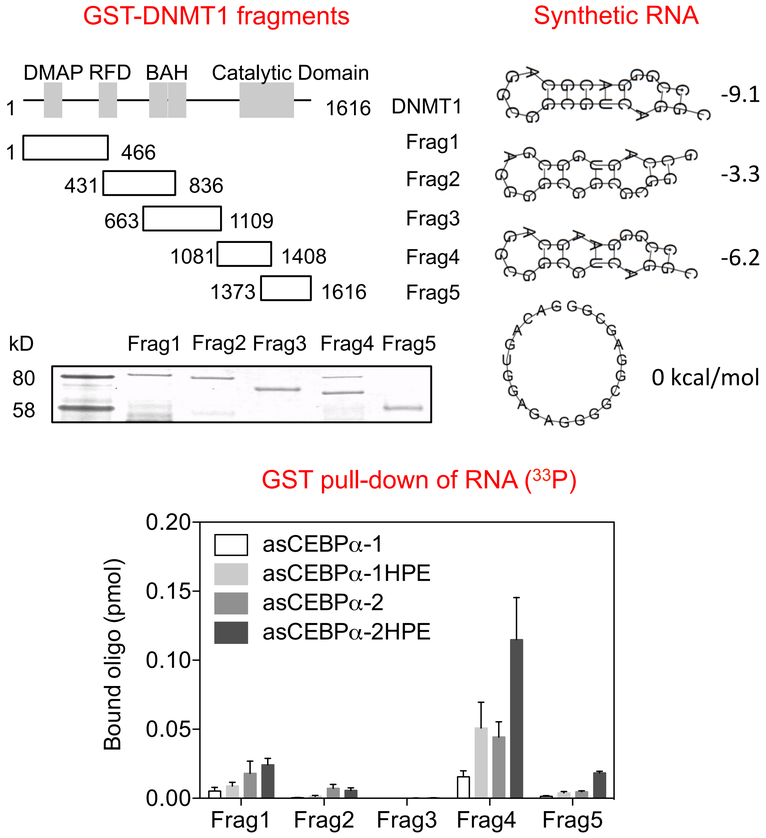
- Uses hemimethylated DNA as substrate.
- Responsible for CpG methylation maintenance.
- Dysregulation of DNMT1 causes aberrant methylation in cancer.
- Highly abundant transcripts inhibit DNMT1 activity *in vivo* (asCEBPA) .
- LncRNA (Kcnq1ot1) recruits DNMT1 to imprinted genes and somatic DMR.

DNMT1 is loaded with inhibitory RNA



Methylase assay using DNMT1 purified from baculovirus or immunoprecipitated from HEK293T. Adding RNase A to reaction increased immunoprecipitated DNMT1 activity by ~18 fold.

RNA binds to DNMT1 catalytic site

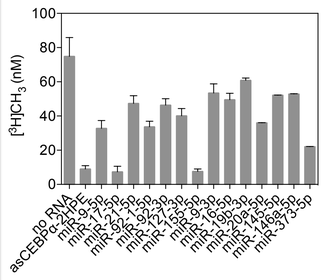


GST pull-down of RNA (^{33}P)

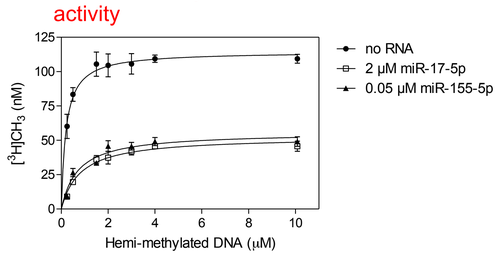
GST pull-down experiments using radioactively labeled small RNA molecules indicated that the DNMT1 fragment containing the catalytic region binds RNA. This binding is correlated with the calculated free energy.

miRNAs are potent DNMT1 inhibitors by competing with hemimethylated DNA substrate

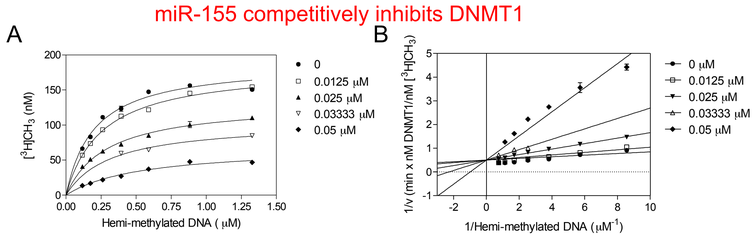
Methylase assay using a panel of cancer related miRNA oligos



200-fold excess of DNA substrate does not fully rescue DNMT1 activity



miR-155 competitively inhibits DNMT1



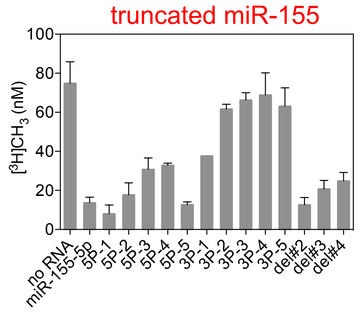
Fourteen cancer related miRNAs inhibited DNMT1 activity in a methylase assay. Steady state biochemistry experiments indicated miRNA is a competitive inhibitor of DNMT1.

G quadruplex sequence of miR-155 is essential for DNMT1 inhibition

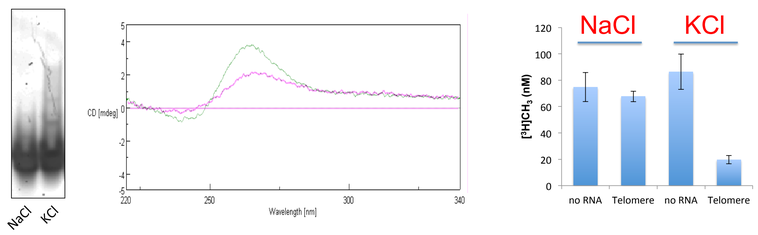
Truncation of miR-155

ID	Sequence
miR-155-5p	UUAAUGCUAAUCGUGAUAGGGGU
5p-1	~~UU~~AAUGCUAAUCGUGAUAGGGGU
5p-2	~~UUAA~~UGCUAAUCGUGAUAGGGGU
5p-3	~~UUAAUG~~CUAAUCGUGAUAGGGGU
5p-4	~~UUAAUGCU~~AAUCGUGAUAGGGGU
5p-5	~~UUAAUGCUAA~~UCGUGAUAGGGGU
3p-1	UUAAUGCUAAUCGUGAUAGGG~~GU~~
3p-2	UUAAUGCUAAUCGUGAUAG~~GGGU~~
3p-3	UUAAUGCUAAUCGUGAU~~AGGGGU~~
3p-4	UUAAUGCUAAUCGUG~~AUAGGGGU~~
3p-5	UUAAUGCUAAUCG~~UGAUAGGGGU~~
del#2	UU~~AAU~~GCUAAUCGUGAUAGGGGU
del#3	UUAAU~~GGU~~AAUCGUGAUAGGGGU
del#4	UUAAUGCU~~AAU~~CGUGAUAGGGGU

Methylase assay using truncated miR-155

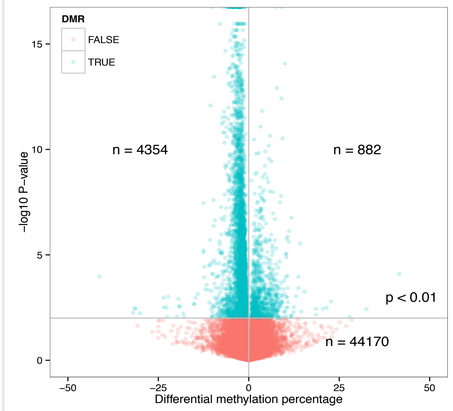


Formation of G-quadruplex by annealing telomeric sequence in KCl buffer



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.

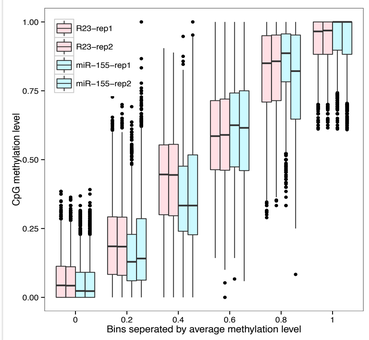
miR-155 causes genome-wide aberrant methylation with emphasis on methylation loss



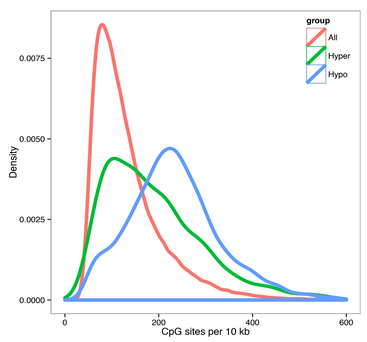
HCT116 → miR-155 transfection → Genomic DNA prep → RRBS

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



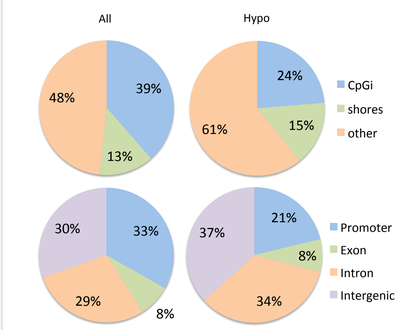
Average methylation of DMRs



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.

NEW ENGLAND BioLabs Inc. enabling technologies in the life sciences