JPH RECODE Maret 2020; 3 (2): 119-126
http://e-journal.unair.ac.id/JPHRECODE

METHYLATION RELATED TO BENEFIT AND HARM IN RNAI APPLICATION: AN EPIGENETIC QUASI SYSTEMATIC REVIEW

Keuntungan dan Kerugian Methylation dalam Penerapan RNAI: Sebuah Review Sistem Quasi Epigenetic

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ARTICLE INFO

Article History:

Received:
August, 26th, 2019

Revisid:
From October,
27th, 2019

Accepted:
Nopember, 13th, 2019

Published online
March, 30th, 2019

ABSTRACT

Background: Double stranded RNA (dsRNA), siRNA, miRNA, RNAi, induce DNA methylation in plants and in mammalian cells, including human. Methylation of CpG island and repeat CGG DNA is high prevalence cases in tropical rain forest, but had been neglected till now. **Purpose:** Aims are knowing the effect of gene silencing to the environment, whereas RNAi cause hypermethylation. **Methods:** The manuscript was Quasi Systematic Review with Bayesian Analysis. **Results:** The result used Science Direct search engine, 935 references are caught, plus 11 references from already recorded in Mendeley library and after screening the abstract or title, 920 are excluded with the not relevant either duplication analyzed with the newest Bayesian network to answer the hypothesis. Screening full text of the 18, plus 10 references from already recorded in Mendeley library, then 16 full text were chosen. The chasing 28 full text were check and recheck with meta-analysis of RNAi-methylation using Science Direct (12 references). **Conclusion:** All of the studies described above indicated that RNAi and CRISPR/Cas9- mediated editing in plant, fishery, mammals, human, based on methylation and demethylation technique set-up by siRNA, miRNA or CRISPR/Cas9.

Keywords: hypermethylation, CpG Island, RNAi, gene silencing, fish breeding

ABSTRAK

Latar Belakang: *RNA untai ganda (dsRNA), siRNA, miRNA, RNAi, menginduksi metilasi DNA pada tumbuhan dan dalam sel mamalia, termasuk manusia. Metilasi pulau CpG dan pengulangan DNA CGG merupakan kasus prevalensi tinggi di hutan hujan tropis, tetapi hingga kini terabaikan.* ***Tujuan:*** *Bertujuan untuk mengetahui efek pembungkaman gen terhadap lingkungan, dimana RNAi menyebabkan hipermetilasi.* ***Metode:*** *Jenis artikel adalah Quasi 'Systematic Review' dengan Analisis Bayesian.* ***Hasil:*** *Hasil menunjukkan bahwa Menggunakan mesin pencari Science Direct, 935 referensi tertangkap ditambah ditambah 11 referensi dari yang sudah direkam di kepustakaan Mendeley, dan setelah menyaring abstrak atau judul, 920 dikeluarkan karena duplikasi atau tidak relevan, dianalisis dengan jejaring Bayesian terbaru untuk menjawab hipotesis. Menyaring teks lengkap dari 18, ditambah 10 referensi dari yang sudah direkam di kepustakaan Mendeley, kemudian 16 teks lengkap dipilih. Sejumlah 28 teks lengkap yang diburu, diperiksa dan periksa kembali dengan meta- analisis RNA-metilasi menggunakan Science Direct (12 referensi).* ***Kesimpulan:*** *Semua penelitian menjelaskan bahwa RNAi dan CRISPR/Cas9-dimediasi di tanaman, perikanan, mamalia, manusia, berdasarkan pada teknik metilasi dan demetilasi yang diatur oleh siRNA, miRNA atau CRISPR/Cas9.*

Kata Kunci: *hipermetilasi, pulau CpG, RNAi, pembungkaman gen, budidaya ikan*

INTRODUCTION

Double stranded RNAs (dsRNAs) may cause sequence specific DNA methylation in crops to regulate the silencing or expression of genes and recently applicable in mammalian cells. Silencing in a gene by methylation could effect to be cancerous, brain and behavior abnormalities like Parkinson Diseases, Alzheimer, Cognitive disorder, disturbing in immune systems etc. After RNAi mediated depletion of DNMT1, hypermethylated CpG island is preserved human colorectal cancer (CRC) cells (Ting, 2004). RNAi (20-25 bp) is a novel method of sequence-specifically to Knock-Out (KO) gene that works by putting short sequences of RNA into cells (Swanton *et a*l., 2004).

The short sequences of RNA match part of a target organism's gene sequence. Working in the cytoplasm of the cell, RNA interference (RNAi) thus does not modify the DNA. Long dsRNA (200-800 bp) can initiate RNAi in plant, worms, insect, even shrimp. This dsDNA may be a new raw material for herbicide, insecticide, fungicide and anti-viral applications in agricultural, fish breading as well as environmental applications. Simply spray, injection, or feeding of dsRNA can be used to protect plants against several viral infections. If insect is feeding on the crop, it takes up the dsRNA and decrease essential protein in the insect, leading to the insect death become the based of protection of the crop plant. RNAi, DNA methylation, and silencing gene is now a cancer therapy.

Hypo methylating agent (HMA) are now and then cause methylation CGG instability especially in DNMT and TSG, so prevention is much better than handling side effect problems of these methylation which is though to be save because using RNAi to KO in fishery, orchid (Kui *et al*., 2017) and fruit breedings could not insert by simply eating or spraying, its needs special condition such e. electrostatic (Choi *et al*., 2014) vibration, pH, etc. RNAi for cancer therapy is a treatment now and then (Reautschnig *et al.,* 2017).

RNAi-methylation KO pathways to brain and behavior disorder (down syndrome, autism, Parkinson, LGBTQA) have the different of CpG island methylation and CGG repeat-methylation (Mutalib, *et al*., 2017). This CpG island methylation of p53 is now the target therapy for cancer with or without RNAi. Hypothesis: Methylation in brain behavior disorders is caused by RNAi. Therefore, purpose of this current study is verifying methylation caused by RNAi which is now used broadly in cancer therapy and fish and plant breeding to silence gene, while breeding could be done in one village.

METHOD

In this study, a Quasi PRISMA Systematic Review with Bayesian Analysis Using Science Direct and other search engine was used. Keyword used was RNAi and methylation. With a Bayesian netwok analysis RNAi also included dsRNA, siRNA and miRNA. Methylation included hypermethylation and excluded hypomethylation. Gene silencing, Knock out are also included. Nine hundred and thirty-five references are caught with Science Direct search engine, and after screening the abstract or title, 920 are excluded since not relevant either duplicated with the aims analyzed using the newest Bayesian network to answer the hypothesis. Screening full text of the 18, plus 10 references from already recorded in Mendeley library, then 16 full text were chosen. The 28 chasing full text were check and recheck with meta-analysis of RNAi-methylation using Science Direct (12 references).

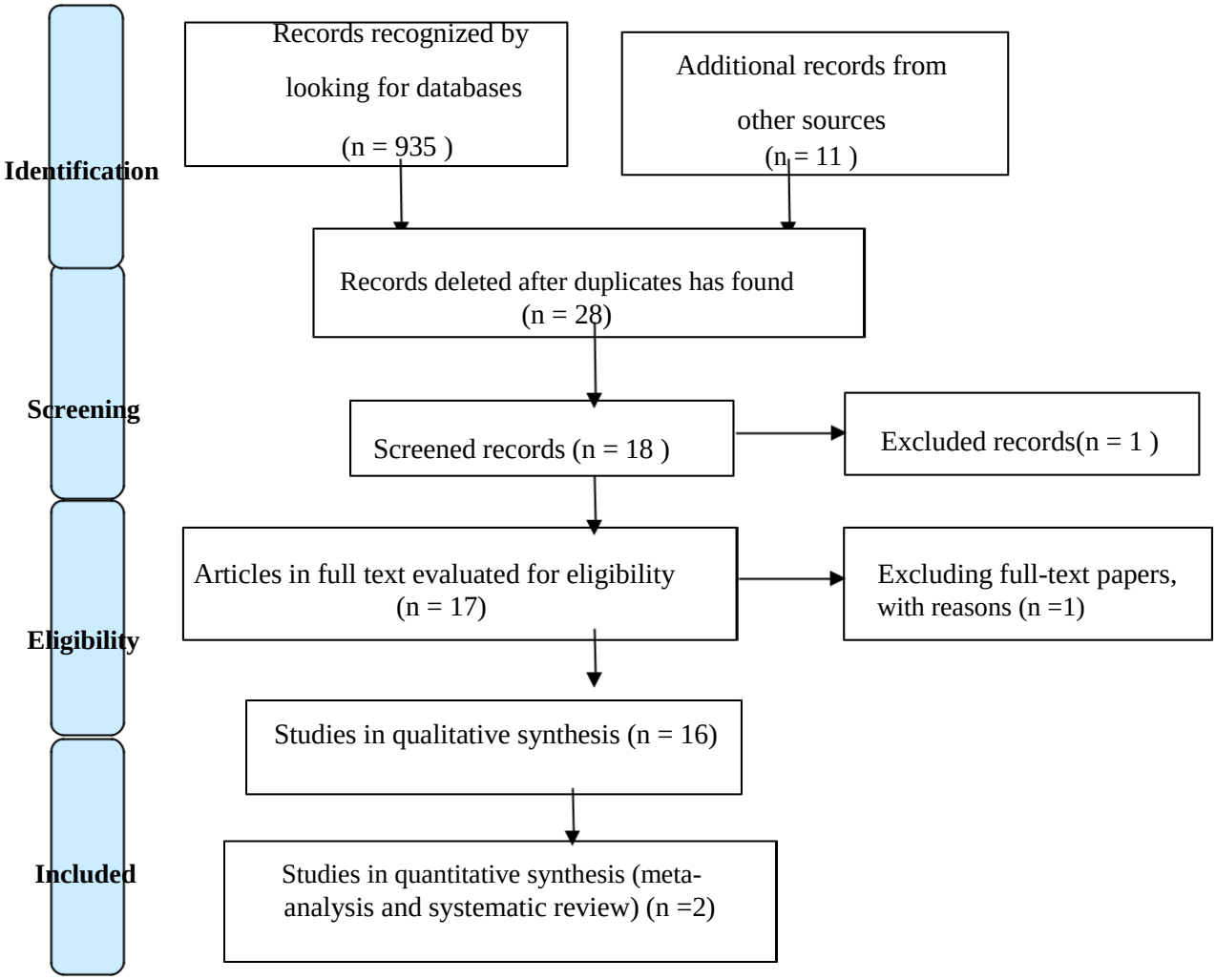

Figure 1. Flowchart RNAi-methylation using Science Direct Search engine

RESULT

DNA methylation is an epigenetic modification that supply phenotype stability and diversity factors. It is influenced by both genetic sequence variation and environment. The using of RNAi to silence gene in plant, worm, cancer, model, tissue like brain and cells are included. References which discuss RNAi techniques and effect of methylation are excluded. Benefits and harms outcome of each references could be seen in Table 1. Effect estimated to methylation of CpG island and CGG is parallel and the using of hypomethylating agent are known widely. DNA methyltransferase gene (DNMT) is revealed by 4 studies in the Table 1 (Ting *et al*., 2004; Nechaev *et al.*, 2013; Mutalib *et al*., 2017; De Carvalho *et al*., 2012)). Tumor Suppressor gene such as p53 should not be methylated (Mutalib *et al* ., 2017) have been proven in the last Reference in Table 1 (De Carvalho *et al*., 2012), where DNA methylation-mediated gene silencing and in further experiments showed that these genes must be silenced by DNA methylation for cancer cell survival, identifies by DNA methylation screening

Table 1. Sixteens References Which Tell The Relation of RNAi Methylation in Plant and Human Cancer

Study, years	Design	Population	RNAi	Methylation
Vinutha *et al*., 2018	Pre-post	Plant	Tomato gemivirus	RNA-dependent DNA (RdDM)
Muthusamy *at al*., 2010	Epigenetic regulation	Cancer	Genome-wide RNAi screens	DNA
Reautschnig *et al*., 2017	Review	Medical application	RNAi and aptamer tech.	Stabilized oligomer
Xie and Yu, 2015	Epigenetic regulation	Plants	si-RNA	DNA
Holoch and Moazed, 2015	Epigenetic regulation	Eukaryotic cells	siRNA-long non coding RNA (lncRNAs)	DNA & histone Expression, stability, defense
Yu *et al*., 2018	Epigenetic	Histone PTM positive feedback	siRNA amplification	Maintain silencing
Braga *et al*., 2014	Epigenetic	Primary and metastatic EOC*	Not CASPASE-8	CpG island methylation
Reinhard and Wagner, 2017	Epigenetic	Targeted cells	Synthetic siRNA Cationic oligomers	Knockdown
Gentile *et al*., 2017	Epigenetic	Subcutaneus Human lung cancer	Synthetic RNAi-based Rx/	Cationic liquid crystalline nanoparticles
Titze *at al*., 2017	Epigenetic Pharmaceutical Market	Different drug targets, organ, administration	siRNA and miRNA	Wide avenue for drug development
Zhu *et al*., 2018	Epigenetic	Caenorhabitis elegans	W-gRNAi screen	Lipid metabolism gene
Wang *et al*., 2018	Epigenetic	Silkworm genome	RNAi KO	DNA 6mA methyltransferase
Ting *et al*., 2004	Epigenetic	Colorectal Human Cancer	RNAi-mediated DNMT1 depletion **	Maintaining hypermethylation of the CpG Island
Nechaev *et al*., 2013	Epigenetic	Toll-like receptor 9 (TLR9)	CpG-siRNA***	CpG hypermethylation
Mutalib *et al*., 2017	Epigenetic SR	Brain and Behaviour	Knock Out DNMT	CGG and CpG methylation
De Carvalho *et al*., 2012	Epigenetic MA	Survival of Cancer cells	Highly impaired DNMT	DNA methylation-mediated gene silencing

*Epithelial ovarian cancer (EOC)
**The primary mammalian DNA methyltransferase, DNMT1
**Dicer substrate siRNA equipped with CpG oligodeoxyribonucleotides

DISCUSSION

Double Knock Out (DKO)

In Table 1, we can seen the report of KO gene mediated by RNAi in relation with methylation. A knock out (KO), DKO, TKO

(Turbo Knock Out) are used for gene silencing in Genetic Modified Organism (GMO) and Human. Broad variety of organism such as crops (post transcription gene silencing) and mammalian cells including human cells, were observed using the RNAi method (Knock-out / KO). The latest repertoire of human RNAi occurences has nowbeen expanded by describing siRNA (21-23-ntdsRNA) induced transcriptional silencing trough promoter methylation i.e. methylation in CpG island of TSG p53 and CGG repeat DNA. One-on- one RNAi target gene is cost-ineffective and time consuming, and siRNAs can impact near perfectly complemen-RNAs, called an off target impact that silence several genes at the same time as a closely constructed one (Double KO (DKO) and Turbo KO (TKO) e.g. better pest resistance, new flower colours, greeter productivity, high inflorescence and longer shelf-life after harvest (Kui *et al*., 2017).

RNAi Methylation for Cancer Therapy

Several methylation related therapy mostly cancer could be seen in Table 1. In big late stage clinical trials, several RNA therapies are being assessed with various modes of action, and many more are being assessed in early clinical development. Messenger RNA (mRNA) is an attractive drug target for therapeutic interventions. Hundred of patients are enrolled in large-scale testing of scale testing of mRNAs for cancer control, small -scale RNAs (siRNA) for renal and hepatic disorders treatment. In people's cells, it is more difficult to insert the RNA, special technique e.g. electrostatic force (Reinhard and Wagner, 2017), vibration and PH, lipophilisation or liposome are needed. This RNAi therapeutic has been performed by small interfering RNA (siRNAs) and microRNA (miRNAs) inhibitors (Gentile *et al*., 2017). Abnormal methylation of DNA in cancer and neurological disorders wa s noted (Mutalib *et al*., 2017; De Carvalho *et al*., 2012). Mutation of Mutation of oncogene K-ras and Tumor Suppressor Gene TP53 have a strong link with CRC and Lung adenocarcinoma. Methylation, mRNA splicing, miRNA is an effective method tor pathogenesis study.

Differentially methylated genetic regions could be analyzed case by cases. DNA methylation or changes in p53-mediated transcriptional is critical in most cancer (Liu *et al.*, 2016), and it could be seen in tropical rainforest area with high prevalence in GMO and AFB1 exposure which induce p53 mutation.

RNAi-Methylation Process

MicroRNAs (miRNAs) are tiny noncodi ng RNAs with a single strand, by using the miRNA induced silencing complex (miRISC) to target mRNAs, miRNAs cause RN A interference (RNAi), by utilizing the miRNA-induced silencing complex (miRISC) to target mRNAs. DKO, TKO silencing. S-Adenosyl-l-methionine synthetase (SAMS) catalyzes for the production of SAM (S-adenosyl-1methionine), a universal donor of methyl in cell biochemical responses (Li *et al*., 2011).

RNAi in The Market

Small to long non-coding RNAs (lncRNAs), have emerged as key regulators of gene expression, genome stability and defence against foreign genetic elements could be seen in Table 1. RNA-mediated epigenetic regulation of gene expression (Holoch and Moazed, 2015)

Ten years after the discovery of dsRNA gene silencing by Fire and Melo's Nobel Prize, notable progress was made in RNAi (Almeida *et a*l., 2017). Changes in the chemical structure of synthetic oligonucleotides (Li *et al*., 2011) make it more stable and specific, and these approaches have developed potential in the RNAi pharmaceutical industry market, particularly in researching new drug targets. Addresses the PF-04523655, TKM-080301, Atu027, SYL040012, SYL1001, siG12D-LODER (phase 2), QPI-1002, QPI-1007, and patisiran (phase 3) nine small-interfering RNAs (siRNAs) and one distinctive microRNA (miRNA) inhibitor that entered stage 2-3 clinical trials. With regard to miRNAs, their content may be down-or up-regulated using mimics of miRNA (AntimiRs) or miRNA. Miravirsen is a hepatitis C virus infection anti miR-122 (Almeida, 2017). RNAi technology is easy to find nowadays and RNAi definitely opens the door to drug development (Almeida, 2017). Taking into consideration: various drug objectives (i.e. p53, mutated p21, p16, caspase, mutated KRAS, microRNAs); therapeutic circumstances, including renal injury,

cancer, viral hepatitis, HCC; and administration routes (ocular, intravenous, subcutaneous, intratumoral.) (Almeida, 2017) Although delivery, dose, toxicity, cost, and biological are still challenges (Almeida, 2017).

Cases with DNA Gene and Promoter Methylation Unstability

Nearly 97% of the genome is non-coding DNA in humans, so changes in these sequences are frequently noted in clinical and specific malfunction; for example, autism, Parkinson, fragile X syndrome (CpG island promoter methylation related CGG repeat) have been found to be associated with introns-derived miRNAs. Clustered frequently interspaced brief palindromic repeat / CRISPR- associated protein 9 (CRISPR / Cas9) also has DKO / TKO but with stable genomic change that can readily be preserved in offspring (Liu, 2016) which is a significant benefit in breeding programmes, whereas RNAi with unstable methylation has only about 50 generation.

CRISPR/Cas9

CRISPR, a bacterial adaptive immune system of type II, has been altered to target the Cas9 nuclease with sequence-specific RNAs for genome editing to the required genomic loci. Fusion of deactivated Cas9 (dCas9) (Xie and Yu, 2015). The enzymatic domain Tet1 or Dnmt3a enables targeted DNA methylation to be established. Investigate the locus-specific functional importance of DNA methylation by providing strong instruments with dCas9-Tet1 and dCas9-Dnmt3a. They intended 4gRNAs for all 14 CpGs in the Snrpn promoter region to activate GFP expression by dCas9-Tet1. De novo methylation of particular CpGs supports gene expression silencing sequences. Therefore, dCas9 fusion construct described either effectively demethylated methylated sequences (dCas9-Tet- 1) or de novo methylate un-methylated sequences (dCas9-Dnmt3a) when targeted by particular RNAs. So methylation or demethylation are exist in CRISPR/Cas9 technique (Liu *et al.*, 2016). After all, RNA-directed methylation of DNA by DNA methyltransferase (DNMT) in plants (Movahedi *et al*., 2015). Das 2016, also reported miRNA, a class of short non-coding RNAi were higher in cancer than other diseases in human, which parallel with what Holoch and Moazed (2015) finds in genome stabilizing methylation (Table 1).

Limitation

Sensitive topic is the largest limitation of this study. Low bias and risk using the network of Bayesian is used in diction when to deeply open and when should do softly open the RNAi-methylation hope and problems. This study using quasi-SR and not full SR have gain the relevant and duplicate hidden several decades of RNAi-methylation in food and energy new global market whereas tropical rainforest is the cultivated land. Fish breeding as agriculture example in one village is now booming (Ardian, 2018) in tropical 2018 rainforest with no more safety superior GMO test since 2001, but well in paten and varietas legacy (Adlhiyati, 2009).

Nowadays, in- silico studies about RNAi (Gabriel *et al*., 2019), CRISPR/Cas9 (Nakayasu *et al*., 2018), CpG methylation (Desai and Chauhan, 2016) and demethylation (Song *et al*., 2019) complete the epigenetic modification in plant and fishery breeding and cancer therapy in fulfiling the the hope of many parties.

CONCLUSION

All of the studies described above indicated that RNAi and CRISPR/Cas9-mediated editing in plant, fishery, mammals, human, based on methylation and demethylation technique set-up by siRNA, miRNA or CRISPR/Cas9.

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