MICRORNA COPY NUMBER ABNORMALITIES IN FAMILIAL COLORECTAL CANCER

THESIS
A thesis submitted for the degree of Master of Biomedical Science majoring on Genetic Counseling

by
AVANITA S PRABOWO
G4A008035

POST GRADUATE PROGRAM
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Has been defended in front of the defense committee
on June 6th , 2011
and has been approved by:

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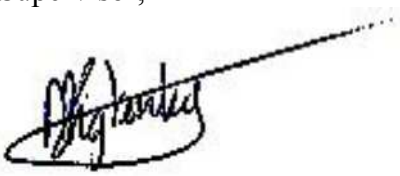	
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DECLARATION

I hereby declare that his submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgement is made in the text.

Semarang, June 2011

Avanita Prabowo

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LIST OF ABBREVIATIONS

aCGH	: array comparative genomic hybridization
APC	: adenomatous polyposis coli
Bp	: base pair
CGH	: comparative genomic hybridization
CLL	: chronic lymphocytic leukemia
CNP(s)	: copy number polymorphism(s)
CNV(s)	: copy number variation(s)
CRC	: colorectal cancer
C13orf25	: Chromosome 13 open reading frame 25
DNA	: deoxyribonucleotide acid
dNTP	: deoxynucleotide triphosphate
FAP	: familial adenomatous polyposis
HNPCC	: hereditary nonpolyposis colorectal cancer
Kb	: kilobase
Mb	: megabase
miRNA(s) / miR(s)	: microRNA(s)
miRISC	: multiprotein RNA-induced silencing complex
PCR	: polymerase chain reaction
qPCR	: quantitave polymerase chain reaction
RISC	: RNA-induced silencing complex

RNA	: ribonucleotide acid
siRNA	: small interference RNA
SNP(s)	: single nucleotide polymorphism(s)
TSG	: tumor suppressor gene
UTR	: untranslated regions
oaCGH	: oligonucleotide array CGH
OG	: oncogene
ORF	: open reading frame

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Supplemental 7. List of recurrent duplication and deletion CNV (affecting miR)

ABSTRACT

MicroRNA Copy Number Abnormalities in Familial Colorectal Cancer

Background
The majority of the familial colorectal cancer (CRC) cases cannot be explained by known gene defects, suggesting the existence of other genetic risk factors. In an approach to identify such risk factors, we recently performed a screen for copy number variations (CNVs) in high-risk CRC patients and found, among other lesions, several small deletions affecting microRNA genes. MicroRNAs are negative regulators of approximately 30% of the genes in the human genome, including numerous tumor suppressor genes and oncogenes.

Methods
In order to comprehensively investigate copy number variation in microRNA genes, and to reveal whether such variations may affect CRC predisposition, we screened for CNVs affecting microRNA genes in 17 unexplained familiar early-onset CRC patients using a custom made ultimate (tiling) resolution oligo array containing 695 miR genes. We performed MLPA, q-PCR and PCR to validate all CNVs candidate.

Results
We found various small (0.2 - 23 kb) constitutional deletions and duplications affecting single microRNA genes. Several of these CNVs, validated using PCR-based technique, were only detected in patients and not in controls. For example miR-646 was found to be duplicated in patient but in 250 controls no duplication was found. MiR-770 was deleted in another patient, whereas no CNVs at this position were found in 94 controls.

Conclusion
We conclude that CNVs of smaller size (<1 Mb) affecting miRNA genes do occur and can be identified using custom oligo array. Nevertheless, further optimization needs to be done to reduce the noise and false positive results generated which complicate the validation process. On the other hand using an in-house database, some of the miRs are indeed affected by polymorphic CNVs and many others are still unknown. Overall we showed CNVs in microRNA genes are more common than previously thought, and some of them may be associated with CRC predisposition.

Keywords: Familial colorectal cancer, copy number variations, microRNA, microarray

ABSTRAK

Kelainan Nomor Salin MicroRNA di Kanker Kolorektal Keluarga

Latar belakang
Sebagian besar kasus kanker kolorektal familial (CRC) tidak dapat dijelaskan dengan kecacatan gen yang diketahui, menunjukkan adanya faktor-faktor risiko genetik. Dalam pendekatan untuk mengidentifikasi faktor-faktor risiko tersebut, kami baru-baru ini melakukan sebuah screening untuk variasi nomor salinan (CNV) pada pasien CRC berisiko tinggi dan menemukan, antara lesi lain, penghapusan beberapa kecil yang mempengaruhi gen microRNA. MicroRNAs adalah regulator negatif sekitar 30% dari gen dalam genom manusia, termasuk gen supresor tumor banyak dan onkogen.

Metode
Dalam rangka menyelidiki secara komprehensif penyalinan nomor variasi dalam gen microRNA, dan untuk mengungkapkan apakah variasi tersebut dapat mempengaruhi predisposisi CRC, kami memeriksa untuk CNV mempengaruhi gen microRNA di 17 pasien dengan CRC pada umur yang muda, yang tidak diketahui penyebabnya dengan awal-awal menggunakan custom made utama (ubin) array resolusi oligo mengandung 695 gen Mir. Kami melakukan MLPA, q-PCR dan PCR untuk memvalidasi semua calon CNV.

Hasil
Kami menemukan berbagai kecil (0,2-23 kb) penghapusan konstitusional dan duplikasi gen mempengaruhi microRNA tunggal. Beberapa CNV ini, divalidasi menggunakan teknik berbasis PCR, yang hanya terdeteksi pada pasien dan tidak di kontrol. Misalnya mir-646 ditemukan digandakan pada pasien tetapi pada 250 kontrol tidak ditemukan duplikasi. Mir-770 telah terhapus pada pasien lain, sedangkan tidak ada CNV pada posisi ini ditemukan di 94 kontrol.

Kesimpulan
Kami menyimpulkan bahwa CNV ukuran lebih kecil (<1 Mb) gen mempengaruhi Mirna memang terjadi dan dapat diidentifikasi dengan menggunakan array oligo kustom. Namun demikian, optimasi lebih lanjut perlu dilakukan untuk mengurangi kebisingan dan hasil positif palsu yang dihasilkan yang mempersulit proses validasi. Di sisi lain menggunakan database di-rumah, beberapa miRs memang dipengaruhi oleh CNV polimorfik dan banyak lainnya masih belum diketahui. Secara keseluruhan kami menunjukkan CNV dalam gen microRNA lebih umum daripada yang diperkirakan sebelumnya, dan beberapa dari mereka mungkin akan dikaitkan dengan kecenderungan CRC.

Kata kunci: kanker kolorektal familial, penyalinan nomor variasi, microRNA, microarray