DNA Integrity Index as a Potential Molecular Biomarker in Colorectal Carcinoma

Thesis

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Rasha Ahmed Abd El Razek

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List of Abbreviations

Abb.	Full term
APC	Adenometous polyposis coli
ACS	American cancer society
AJCC	American Joint Committee on Cancer
antiEGFR	Anti-epidermal growth factor receptor
ALU	Arthrobacter luteus
Bp	Base pair
BCL2	B-cell lymphoma 2
BAX	BCL-2-like protein Associated X
BMP3	Bone morphogenetic protein 3
BRAF	B-Raf proto-oncogene,
CA19-9	Carbohydrate antigen 19-9
CEA	Carcinoembryonic antigen
CtDNA	Cell- tumor DNA
CfDNA	Cell-free DNA
CffDNA	Cell-free fetal DNA
CIN	Chromosomal instability
CRC	Colorectal cancer
C.T	Computer assisted tomography
CIMP	CpG-island methylator phenotype
P16	Cyclin-dependent kinase inhibitor
DII	DNA integrity index
dsDNA	Double-stranded DNA
ECLIA	Electrochemiluminescence immunoassay
FAP	Familial adenomatous polyposis
FIT	Fecal immunochemical testing
GIT	Gastrointestinal tract
GI	Gastrointetinal

List of Abbreviations (Cont...)

Abb.	Full term
gDNA	Genomic DNA
gFOBT	Guaiac fecal occult blood testing
HCC	Hepatocellular carcinoma
HNPCC	Hereditary nonpolyposis colorectal cancer
IBD	Inflammatory bowel disease
IQR	Interquartile range
Kbp	Kilo base pair
KRAS	Kirsten rat sarcoma
LOH	Loss of heterozygosity
LS	Lynch syndrome
M2-PK	M2-pyruvate kinase
MRI	Magnetic resonance imaging
Tm	Melting temperature
mCRC	Metastatic CRC
MI	Myocardial infarction
MSI	Microsatellite instability
MMR	Mismatch repair
MAPK	Mitogen-activated protein kinase
MT-sDNA	Multitarget stool DNA testing
MLH1	MutL homolog 1
MSH2	MutS homolog 2
MSH6	MutS homolog 6
NDRG4	N-myc downstream-regulated gene 4 protein
NIPT	Non-invasive prenatal testing
NSCLC	Non-small cell lungcCancer
Nt	Nucleotide

List of Abbreviations (Cont...)

Abb.	Full term
PCR	Polymerase chain reaction
PK	Proteinase K
qPCR	Quantitive polymerase chain reaction
xg	Relative centrifugal force
rpm	Revolutions per minute
RHD	Rhesus D
SEPT9	Septin 9
SINE	Short interspersed nuclear elements
SD	Standard deviation
TNM	T=tumor Size, N = node Involvement and M = metastasis status
Ct	Threshold cycle.
TGFβRII	Transforming growth factor βreceptor II
TP53	Tumor protein 53
UV	Ultra violet
WHO	World health organization

INTRODUCTION

Colorectal cancer (CRC) is the third most frequently diagnosed malignancy, accounting for approximately 10% of global cancer burden ***(Ng et al., 2017)***.

Survival of CRC patients is significantly associated with the staging of the disease at diagnosis. The five-year survival rate for CRC patients diagnosed in early stage is >90%, while for those diagnosed in late stage is approximately 7%. Therefore, early diagnosis and management of CRC are pivotal in improving treatment outcomes for CRC patients and reducing the disease-related mortality ***(Bresalier et al., 2015).***

Currently, there are several approaches to screen CRC, such as fecal occult blood test (FOBT) and colonoscopy. FOBT is a non-invasive and low-cost method, but its sensitivity for CRC is limited ***(Lieberman, 2009).*** While Colonoscopy is the goldstandard for CRC screen with a specificity of >95%, but it requires bowel preparation and occasionally accompanied with severe complications ***(Toth et al., 2012)***.

Additionally, Established serum tumour markers for CRC, carcinoembryonic antigen (CEA) and Carbohydrate antigen 19-9 (CA19-9), are mainly used in cancer surveillance but have only limited value in early cancer detection as pointed-out by the guidelines of the European Group on Tumor Markers ***(Duffy et al., 2007).***

Since the conventional methods for CRC screening are either ineffective or invasive, more patient-friendly and less-invasive approaches with high sensitivity and specificity are imperative.

In recent years, the investigation of circulating molecular markers in peripheral blood (liquid biopsies) is of great importance owing to their advantages as being easily accessible, reliable, reproducible and early detectable in cancer ***(Schwarzenbach et al., 2011).*** Many blood-based biomarkers like circulating free DNA, microRNAs, circulating tumor cells have been investigated for the diagnosis of cancer ***(Madhavan et al., 2014).***

Cell-free DNA (cfDNA) circulating in the human blood has been suggested to be a promising tumor marker ***(Schwarzenbach et al., 2011)***. However, its level is also elevated in various non malignant disorders such as infectious and autoimmune diseases, stroke, infarction and trauma ***(Holdenrieder et al., 2009).***

Therefore, more specific approaches such as measuring the integrity of cfDNA, which describes the relation between longer and shorter DNA fragments, have been proposed. This approach is based on the findings that cfDNA varies in length according to its mechanism of release from the cell: apoptosis, which usually takes place in normal tissues, results mainly in DNA fragments of 180 bp or less, whereas necrosis,which is the usual form of cell death in cancer tissues, produces longer fragments ***(Jahr et al., 2001).***

In consequence, the DNA integrity as the ratio of longer to shorter fragments is reported to be increased in patients with cancer.

The Arthrobacter luteus (ALU) is the most abundant repeated sequence in the human genome, with a copy number of 1.4 x 10^6 per genome ***(GU et al., 2000).*** The ALU sequences are about 300 base pairs long and are therefore classified as short interspersed nuclear elements (SINEs) that account for more than 10% of the human genome among the class of repetitive DNA elements ***(Hwu et al., 1986).***

Several studies used ALU amplicons for calculation of DNA integrity index (DII) that represents the ratio of repeated sequences of ALU (247 and 115 bp) ***(Umetani et al., 2006a).*** The ALU 115 primers amplify small fragments (truncated by apoptosis) and the ALU 247 primer amplifies longer DNA fragments ***(Feng et al., 2013).***

Aim of the Work

The aim of this study was to assess the clinical utility of the DII as potential biomarker for CRC and to evaluate its correlation with the traditional tumor marker, CEA.