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Epigenetic Biomarkers for Early Esophageal Cancer Detection in North East India

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Abstract: ***Esophageal cancer mortality in North East India is strongly associated with the predominant diagnosis of late stage lesions that hampers effective therapy. Molecular biomarkers for early detection of esophageal cancer are an area of utmost concern in order to control the high incidence and mortality due to esophageal cancer in this region. To date there are no molecular biomarkers that have been used for widespread clinical practice. The emergence of epigenetic biomarkers like methylation markers have ushered in a new hope among the cancer researchers providing new insights in biomarker development for risk assessment, early detection and therapeutic stratification. DNA methylation has rapidly emerged as potential biomarkers in body fluids showing promise to assist the clinical management of esophageal cancer. This review provides an overview of DNA methylation, one of the most common epigenetic events, which profoundly contributes to esophageal cancer initiation and progression. Further it highlights the frequently methylated tumor suppressor and DNA repair genes in esophageal cancer. These frequently methylated genes can be used as biomarkers for early detection of esophageal cancer in North East India after proper validation and verification of these biomarkers in the North Eastern population.***

Keywords: Eoigenetics, Biomarkers, Esophageal Cancer, DNA methylation, North East India

1. Introduction

Esophageal cancer is the eighth most common form of cancer and the sixth most common cause of death from cancer in the world(Arnold, et al., 2014). Globally, there were an estimated 482,300 new esophageal cancer cases and 406,800 deaths in 2012(Ferlay, et al., 2015). The incidence rates vary internationally by nearly 16-fold, with the highest rates found in South East Asia and South East Africa(Arnold, et al., 2014, Parkin, et al., 2014). International comparision of the various population based cancer registries (PBCRs) of India revealed that there is a very high incidence of esophageal cancer in the North East India.Meghalaya, Mizoram and Assam in the North East India has the highest number of esophageal cancer cases(NCRP, 2009-2011).Dietary and environmental factors like tobacco, betel quid and alcohol,smoked and fermented food consumption ,poor nutrition and low intake of fruits and vegetables are the major risk factors associated with esophageal cancer in this region(Phukan, et al., 2001).

Although there is a marked increase in the incidence of esophageal cancer the prognosis for esophageal cancer in the North East India is very poor, with 5-year relative survival rates of 10%(Das, et al., 2015, Talukdar, et al., 2013). The main reason for this poor prognosis is late onset of symptoms when the tumor has metasized and is unresectable(Kato and Nakajima, 2013). Thus, there is an urgent need for rapid and efficient early detection methods for early detection of esophageal cancer at a curable stage.

Esophageal cancer is a multistep process and develops from a hyperproliferative epithelium which progresses into low, intermediate and high grade dysplasia ultimately leading to cancer(Schweigert, et al., 2013). The exact molecular pathogenesis of esophageal cancer is still not fully known but various studies have found that genetic and epigenetic changes play a major role in the initiation of esophageal carcinogenesis. Epigenetic events like aberrant DNA methylation in the important tumor suppressor genes results in disruption of the cell regulatory pathway resulting in cancer(Costa, 2010, Tanzer, et al., 2013). These epigenetic events alongwith genetic mutations promote the normal esophageal epithelium to cancerous epithelium(Tanzer, et al., 2013) . Further, epigenetic events like DNA methylation occur at an early stage of cancer and therefore DNA methylation can be used for development of early detection biomarkers for esophageal cancer(Baba, et al., 2013). The purpose of this review is to summarize our current understanding of previously identified candidate epigenetic biomarkers for esophageal cancer. Most of these aberrantly methylated genes are described as early detection or diagnostic markers, while others might prove useful for estimating prognosis or predicting response to treatment. Timely verification and validation of these biomarkers need to be done in different cohorts of people before using these biomarkers for clinical practice. This will aid in identifying probable biomarkers which can be further tested in the North Eastern population to check the feasibility of using these biomarkers for early detection of esophageal cancer in North East India.

Promoter Methylation of Tumor suppressor genes as epigenetic biomarkers for Esophageal cancer.

Tumor suppressor genes(TSGs) play a very important role in cell growth and proliferation(Agarwal, et al., 2012).Disruption in the function of these genes by methylation results in gene silencing ultimately leading to uncontrolled growth and cancer(House, et al., 2003).DNA methylation is addition of methyl group to 5' carbon of cytosine which brings about conformational changes in the chromatin from open state to closed state chromatin(Lichter, 2008).As a result,the gene code for promoter of the TSG cannot be read by the transcription factors and is not transcribed and translated into its functional protein; hence the gene is silenced(Zhang and Guo, 2010).

DNA methylation signatures have the potential to be used as early detection biomarkers for cancer(Zhang and Guo, 2010). Detecting promoter methylation of TSGs has advantages compared to protein or RNA. Firstly, DNA can be released outside the tumor and is more stable than RNA or protein, which makes DNA markers easier to obtain from body fluids (such as sputum, pancreatic juice, and urine), blood and tissues(Dauksa, et al., 2012). Secondly, PCR based analysis of DNA methylation have relatively high sensitivity(Claus, et al., 2012). Methylation specific PCR (MSP) is able to detect a single methylated allele among 1000 unmethylated alleles, even in the pool of abundant normal DNA (Herman, et al., 1996). Thirdly, the DNA for methylation analysis is chemically stable therefore sample handling requirements are not rigid(Zhang, et al., 2013) . Thus, DNA methylation assays can be exploited as potent noninvasive diagnostic methods for biomarker discovery.

p16 is a G1 cell cycle regulatory gene and an important TSG(Ito, et al., 2007). Inactivation of p16 gene allows the tumor cells to progress through G1 checkpoint of the cell cycle and is found to be associated with cancer development and progression(Shima, et al., 2011). In our previous study on 100 esophageal cancer cases from North East India, 81% of p16 gene was found to be methylated in esophageal cancer patients(Das, et al., 2014). Various studies on p16 gene have reported that p16 hypermethylation is relatively common in esophageal cancer, ranging from 40% to 81%, and is frequently associated with loss of expression and an advanced histological grade of cancer(Viswanathan, et al., 2003, Xu, et al., 2013).Thus,p16 can be exploited as a probable biomarker for esophageal cancer.

The methylation status of REPRIMO, a tumor suppressor gene that regulates p53-mediated cell cycle arrest, was evaluated in 175 endoscopic biopsy specimens and was found to be methylated infrequently in esophageal cancer suggesting this might be a useful biomarker for the early detection of esophageal neoplasia(Hamilton, et al., 2006) .Promoter methylation of various other TSGs viz.tachykinin1 , was detected in precancerous basal cell hyperplasia or dysplastic lesions, indicating their early diagnostic values in esophageal cancer(Jin, et al., 2007). A list of important tumor suppressor genes which are frequently found to be methylated in esophageal cancer and can be used as potent epigenetic biomarkers is included in Table 1.

Promoter Methylation of DNA repair genes as epigenetic biomarkers for Esophageal cancer.

Tobacco and betel nut which is mostly consumed by the North Eastern population contains nitrosamines which form DNA adducts like O6 methylguanine(Zhang, et al., 2003). These adducts intercalate with DNA and cause mispairing resulting in G:C to A:T mutation(Baumann, et al., 2006). MGMT (O6- methyl gyuanine methyl transferase) is an important DNA repair enzyme which specifically repairs the DNA adducts caused by nitrosamines. Impaired activity of the DNA repair enzyme MGMT may result in impaired DNA repairing capacity thereby resulting in accumulation of damaged DNA causing tumor growth(Kuester, et al., 2009).In a study on 100 esophageal cancer patients from North East India,70% of MGMT gene was found to be methylated in case of esophageal cancer(Das, et al., 2014).MGMT gene is frequently found to be methylated in esophageal cancer and can be used as a probable biomarker for esophageal cancer(Shah, et al., 2013, Viswanathan, et al., 2003). Various other DNA repair genes like mismatch repair gene mutL homolog 1 (hMLH1) was reported to be inactivated by genetic or epigenetic alterations in esophageal cancer (Wani, et al., 2012). The fragile histidine triad (FHIT) gene, located at 3p14.2 , plays an essential role in chromosomal abnormality and DNA damage was methylated in 69% of esophageal cancer but not in the matched normal tissues, and this methylation was responsible for decreased FHIT protein level. Loss of FHIT expression was usually observed at initial stages of esophageal cancer and thus might serve as an independent prognostic marker and as a marker for early detection of esophageal cancer (Schildhaus, et al., 2005).Table 1 shows the frequently methylated DNA repair genes which can be used as probable early detection epigenetic biomarker for esophageal cancer.

Table 1: Summary of frequently methylated DNA repair and tumor suppressor genes in esophageal cancer

Classification	Gene	Full Name	Location	Function	Methylation Status
DNA REPAIR GENES	FHIT	Fragile histidine triad	3p14.2	Cell cycle control, DNA damage response,DNA repair	45-69%
	MGMT	O6 methylguanine DNA Methyltransferase	10q26	DNA repair, cell cycle control	33-39%
	MLH1	Human mutL homolog 1	3p21.3	DNA mismatch repair, DNA mismatch repair	23-40%
	MSH2	Human mutS homolog 2	2p21		23-35%
TUMOR SUPPRESSOR GENE	p16 / CDKN2A	Cyclin dependent kinase inhibitor 2A	9p21	Cell cycle control	40-81%
	p14 ARF /CDKN2A	Cyclin dependent kinase inhibitor 2A	9p21	Stabilizing p53,cell cycle control	15-20%
	RPRM	Reprimo	2q23.3		20-30%
	RASSF1A	RAS association domain family 1A	3p21.3	Cell cycle arrest	51%
	APC	Adenomatous polyposis coli	5q22.2	Cell cycle control, apoptosis Cell cycle arrest	50-60%

2. Conclusion

North East India is the hotspot for esophageal cancer with increase in incidence in the recent years(NCRP, 2009-2011). The high incidence may be attributed to the unique dietary habits of these population and mainly the poor prognosis(Phukan, et al., 2001, Talukdar, et al., 2013) .There is an urgent need to control the high incidence and this highlights the need for development of early detection markers for esophageal cancer.

Esophageal cancer pathogenesis is a multistep process controlled by both genetic and epigenetic mechanisms(Zhang and Guo, 2010). Epigenetic silencing of TSGs by promoter methylation is a major event in esophageal carcinogenesis(Chik, et al., 2011). Genome wide and candidate gene methylation assays have identified numerous methylated genes in esophageal cancer in the recent years(Towle, et al., 2013). These epigenetic studies provide new insight about the molecular pathogenesis of esophageal cancer and expand the knowledge of biomarkers for clinical use(Alvarez, et al., 2011). Epigenetic biomarkers like methylation markers are the most promising biomarkers in cancer research and are therefore invaluable tools for early cancer detection, diagnosis, patient prognosis and treatment selection(Lichter, 2008). The biomarkers which have been identified in different study population round the globe need to be validated and verified in the North Eastern population. Further issues regarding the quality control of theses biomarkers need to be properly developed for using these epigenetic biomarkers with greater reliability and reproducibility.Comprehensive identification of each biomarker and identification of methylation gene panel would aid in early diagnosis and prognosis of esophageal cancer in North East India in the near future.

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