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RESEARCH ARTICLE

METHYLATION PROFILING OF TUMOR SUPPRESSOR GENES INVOLVED IN LUNG CANCER

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ABSTRACT

The causes of cancer are due to mutations of key proteins involved in cell cycle regulation, DNA repair enzymes and in some cases inactivation of tumor suppressor genes leads to the growth of tumors. The expression rates of TSGs vary in different stages of cancer as well in various cases of cancers. The inhibitions of TSGs are due to methylation of the DNA. In our present study, we found the genes which are methylated in different types of lung cancers and identified the methylation frequencies. Using the pubmeth database, we have identified the total number of genes undergoing methylation in the various lung cancers. From the total number of genes, we have identified the tumor suppressor genes which are in the methylated state leading to the inactivation of expression and promoting the tumor growth. The further focus on tumor suppressor genes which are methylated is necessary to find a novel way to activate them in the cancerous stage.

Keywords: DNA Methylation, Tumor Suppressor Genes, Epigenetics, Lung Cancer, Gene Expression

INTRODUCTION

Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues. Cancer cells can spread to other parts of the body through the blood and lymph systems[1]. The two main types of genes that are recognized as playing a role in cancer are oncogenes and tumor suppressor genes. Aberrant methylation is a widespread phenomenon in cancer and may be among the earliest changes to occur during oncogenesis. One of the major roles of DNA methylation in mammals is thought to be in control of gene regulation. This is because methylation within gene regulatory regions such as promoters and enhancers generally suppresses their function[2]. Several lines of evidence suggest that the methyl group in some cases directly interferes with the binding of transcription factors that activate transcription. In an alternative process, CpG-binding domain (MBD) proteins bind to the methyl group and subsequently to chromatin remodeling proteins (such as histone deacetylases) to silence the gene by making it transcriptionally unavailable. Genes that are methylated tend to be packaged more tightly, which causes them to be silenced. Genes that are not methylated tend to exhibit looser packaging, which allows their expression[3]. Mutations in certain tumour suppressor genes are most often thought of in association with their inactivation during cancer initiation or progression; epigenetic alterations such as DNA methylation appear to be tightly linked to the sequential non-reversible events of normal tissue differentiation and organogenesis[4].

Hence our objectives of the present study are to understand the lung cancer methylome which leads to identification of genes involved in causing lung cancers. Among the genes identified, tumor suppressor genes involved in lung cancer and their methylation frequencies are keyed out in different types of lung cancers.

MATERIALS AND METHODS

2.1. Retrieval of Lung Cancer Genes Methylation Frequencies Information using PubMeth

Pubmeth is an annotated and reviewed database of methylation in cancer. PubMeth can be queried using the web-interface at http://matrix.ugent.be/pubmeth/search.html in two ways, depending on the researcher's focus: Gene-related: in which cancer types (and subtypes) the genes of interest are reported to be methylated and Cancer-related: which genes are reported to be methylated in the cancer types/subtypes. The Cancer-centric query approach is opted for the retrieval of methylated genes involving in Lung cancer. This type of search is meant to get a quick overview of the genes that are reported in the methylation context in the cancer (sub) types of interest and in which frequency, to explore methylation in the cancer types of interest[5].

2.2. KEGG / Kyoto Encyclopedia of Genes & Genomes Pathway Maps

The KEGG Pathway database is a collection of graphical diagrams (KEGG pathway maps) and associated text information (KEGG pathway entries) for metabolism, various other cellular processes, and human diseases[6]. The KEGG pathway map is a moleculalr interaction/reaction network diagram represented in terms of the KEGG Orthology (KO) groups, so that experimental evidence in specific organisms can be generalized to other organisms through genomic information. Each map is manually drawn with in-house software called KegSketch, which generates the KGML+ file[7].

RESULTS

Table 1: Tumor Suppressor Genes identified in Lung Cancer

S.no	Gene	Description
1	***CDKN2A***	Cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)
2	***RASSF1***	Ras association (RalGDS/AF-6) domain family member 1
3	***DAPK1***	Death-associated protein kinase 1
4	***RUNX3***	Runt-related transcription factor 3
5	***APC***	Adenomatous polyposis coli
6	***CADM1***	Cell adhesion molecule 1
7	***CDKN2B***	Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)
8	***DLC1***	Deleted in liver cancer 1
9	***FHIT***	Fragile histidine triad gene
10	***PRKCDBP***	Protein kinase C, delta binding protein

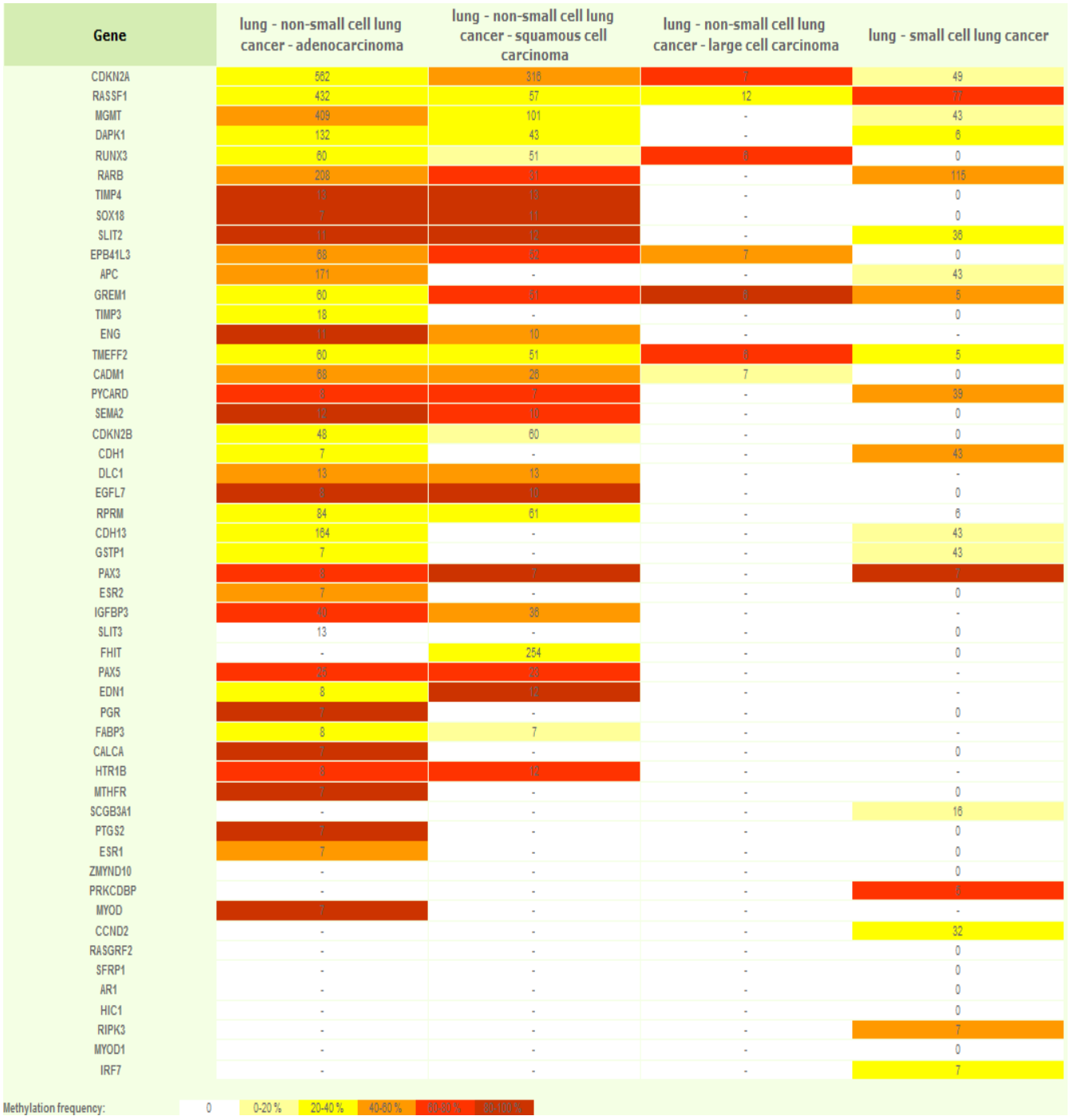

Figure 1: Methylation Frequencies of Genes involved in Lung Cancer

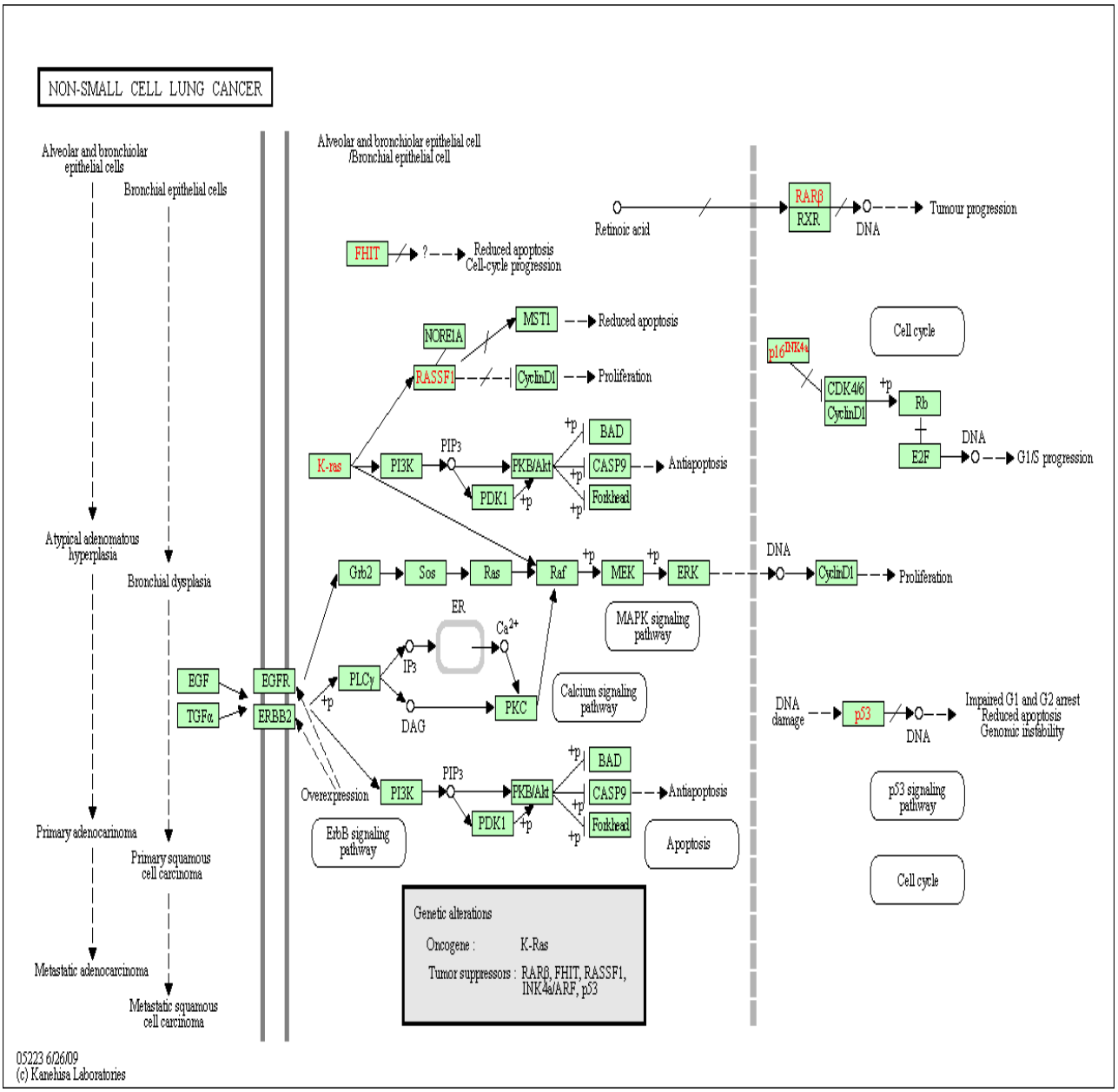

Figure 2: Non-Small cell lung cancer

Table 2: Methylation Frequencies of Tumor Suppressor Genes involved in Lung Cancer

Methylation Frequencies	NSCLC - Adenocarcinoma	NSCLC - Squamous	NSCLC – Large cell carcinoma	SCLC
0 – 20 %	-	CDKN2B RUNX3	CADM1	CDKN2A APC
20 – 40 %	CDKN2A; 2B RASSF1 DAPK1 RUNX3	RASSF1 DAPK1 FHIT	RASSF1	DAPK1
40 – 60 %	APC CADM1 DLC1	CDKN2A CADM1 DLC1	-	-
60 – 80 %	-	-	CDKN2A RASSF1 RUNX3	RASSF1 PRKCDBP
80 – 100 %	-	-	-	-

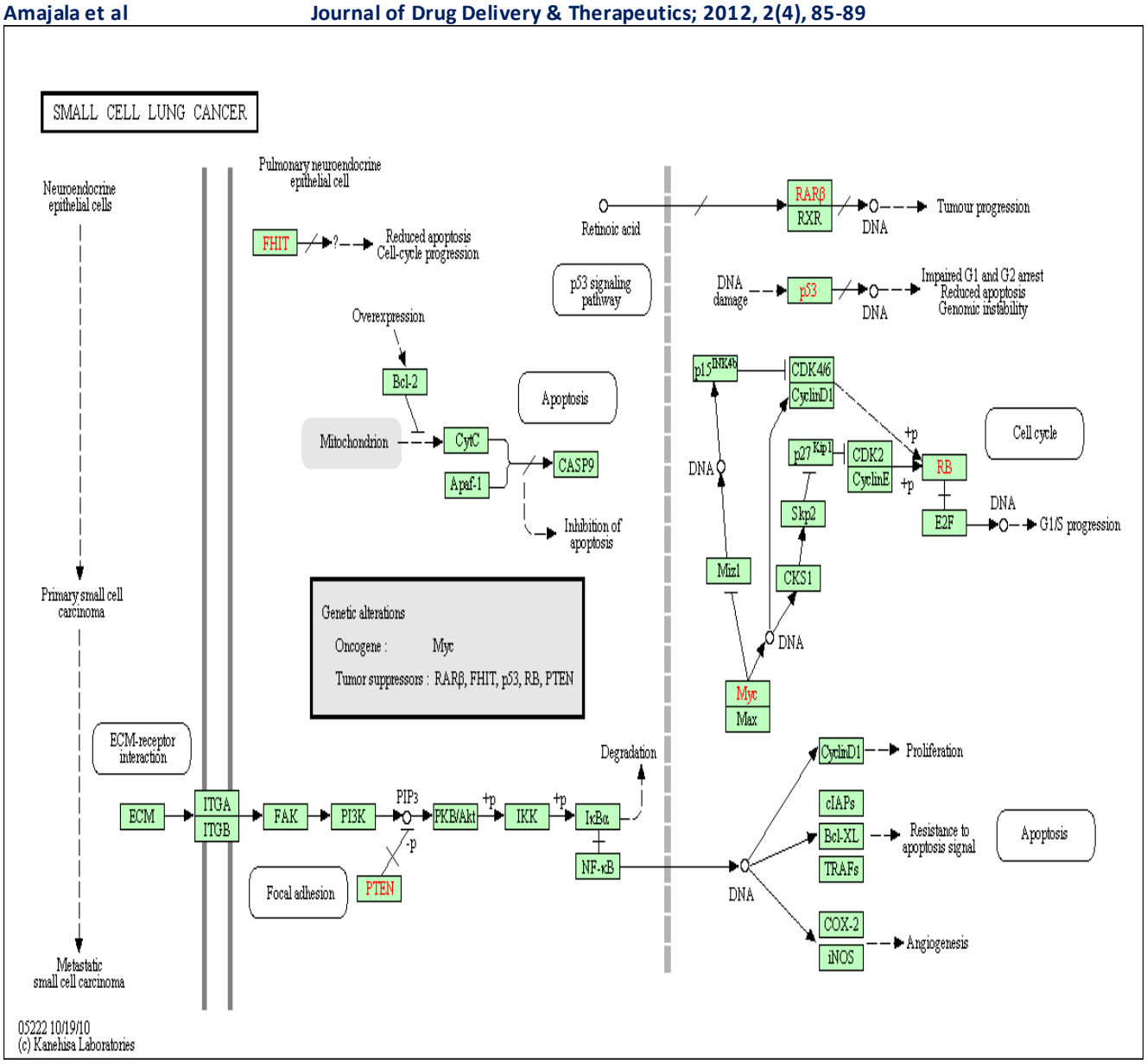

Figure 3: Small cell lung cancer

DISCUSSION

4.1. Identifying the Tumor Suppressor Genes and their Methylation Frequencies

By using PubMeth database, the genes which are found to be methylated in various lung cancer cell lines are depicted in the **figure 1**. There are two different types of lung cancers i.e.., are NSCLC – Non-Small Cell Lung Cancer & SCLC – Small Cell Lung Cancer. The subtypes of NSCLC are: NSCLC-Adenocarcinoma, NSCLC-Squamous, and NSCLC-Large Cell Carcinoma. Among those identified genes, the TSGs are recognized to know how many TSGs are involved in various types of lung cancers. Specific TSGs show different methylation status in respective types of lung cancers. Such kinds of methylation frequencies are also observed in other cancer types where the same TSGs may possess other methylation status.

4.2. Methylation Frequencies of TSGs in Lung Cancer

In different types of lung cancers, it has been observed that no TSGs are likely having the same methylation frequencies / status. In each type of lung cancer different TSGs have different methylation status and these methylation statuses differ for various kinds of cancers also. The identified TSGs are tabulated in the **table 1** and their respective methylation frequencies are tabulated in **table 2**. The dark red coloration indicates the highest methylation frequency of the genes. Among the identified TSGs, the RASSF1 & CDKN2A TSGs are highly methylated and involved in both Non-Small Cell & Small Cell Lung Cancers.

4.3. KEGG - NSCLC & SCLC Pathway Maps

The identification of TSGs in the cancer pathway maps gives an insight to the connectivity of other genes as well associative cellular mechanisms can be understood well. By the pathways, the ability to find the connectivity to other cellular pathways such as apoptosis, cell-cycle progression, etc can be graphically depicted. The pathway

maps provide focused information about the various genes associated, colligated and responsible for various cellular states. From the NSCLC pathway map (**figure 2: NSCLC-Non-Small Cell Lung Cancer Pathway**), it can be depicted that the genes FHIT & RASSF1 are indirectly involved in controlling the various functions like reduced apoptosis, cell-cycle progression & proliferation through other key proteins like CyclinD1 & MST1. From the methylation frequencies analyses, the both TSGs are highly methylated which results them in uncontrolling the key functions of cell-cycle progression & proliferation. In the same manner, the SCLC pathway map (**figure 3: SCLC-Small Cell Lung Cancer Pathway**), also provides information regarding the other TSGs like Myc, RB, p53, PTEN along with the FHIT & CDKN2A genes which are methylated in both the SCLC & NSCLC. These TSGs play a critical functional role in inhibition of apoptosis, degradation, tumor progression, proliferation and angiogenesis.

CONCLUSION

By the PubMeth database studies, we can conclude that the minimum numbers of genes in lung cancer cell lines involved in methylation are 44 genes. Among these 44 genes, 10 are identified as Tumor Suppressor Genes, and their detailed annotated information has been obtained through Genecards database. The 10 specific TSGs having different methylation frequencies have been observed. This indicates that methylation states are varied from one cancer to another type of cancer. The pathway maps focus the colligation of other genes and exploring differential gene connectivity and pathway conservation in identifying the novel ways to activate the tumor suppressor genes.

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CONFLICT OF INTEREST - None

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