JADAVPUR UNIVERSITY

MASTER'S DEGREE THESIS

Identifying Rare Co-methylation for Head and Neck Squamous Carcinoma by applying Random Clustering Techniques

A thesis submitted in fulfilment of the requirements for the degree of Master of Technology in Computer Technology

BY
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MASTER OF COMPUTER TECHNOLOGY

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May 31, 2019

Declaration of Authorship:

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Abstract

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Head and Neck Squamous Carcinoma is one of the most common aggressive cancers. Considering the previous advancement, this cancer posses poor survival rate. Although it has been observed that the early detection of this cancer can enhance the survival rate up to 80 %. However, conventional studies are mostly focusing on the well-known and densely connected prognostic biomarkers. Follow that, the rarely associated significant prognostic markers are being neglected. To address such consequences, a frame has been proposed applying two exclusive layers of clustering techniques Viz., DBSCAN and K-means respectively. Initially, the complete list of differentially co-methylated samples is selected. Applying DBSCAN based random clustering rarely differentially co-methylated samples are being identified. Subsequently, the rate of co-methylation has been calculated among the selected samples. Again, based on the rate of co-methylation, the selected samples have been clustered applying K-means. Top sample from each cluster viz., MGRN1, WWTR1, BAG2, CXCR4, MCL1 has been shown as an influential rarely connected prognostic marker. To understand the biological implication of the samples, pathway and gene ontology analysis have been performed. Finally, the study has been re-verified through literature study.

Keyword: DBSCAN, K-means, Rare Methylation, Head and Neck Squamous Carcinoma, Co-methylation

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Chapter 1:

Introduction:

In modern world cancer is one of the threated disease. Day by day the number of cancer patient increase around the whole world. Due to increase of tobacco and alcohol consumption, heavy air pollutions people around the world suffering by this terrible disease. According to survey of National Cancer Institute of United States approximately 65,000 people diagnosed HNSC this year [7]. There are lots of researches going on HNSC around the world.

Based on the different research on HNSC genes there are lots of ways to identify this particular type of cancer. Existing methods to observe the HPV infected CpG methylated regions of different genes based on different tests. There are lots of methods to analyse the methylation data of HNSC cancer genes. Existing researches proved that it can be analyse from DNA methylation biomarkers, other ways like epigenetic modification of genes. In our project work we are working on the rare methylation gene of HNSC cancer genes.

1.1 HNSC cancer:

HNSC stands for Head and Neck Squamous Cell Carcinoma, among the all cancers in the world head and neck cancer is the sixth common cancer. In this cancer the development of malignance tumor in our mouth, neck, larynx etc. different positions. Due to consumption of alcohol and smoking increase the rate of the HNSC cancer around the world. In this type of cancer flat squamous cell developed around the head and neck. HNSC can begin with salivary gland, oral cavity, pharynx etc. in different parts of mouth and neck [27].

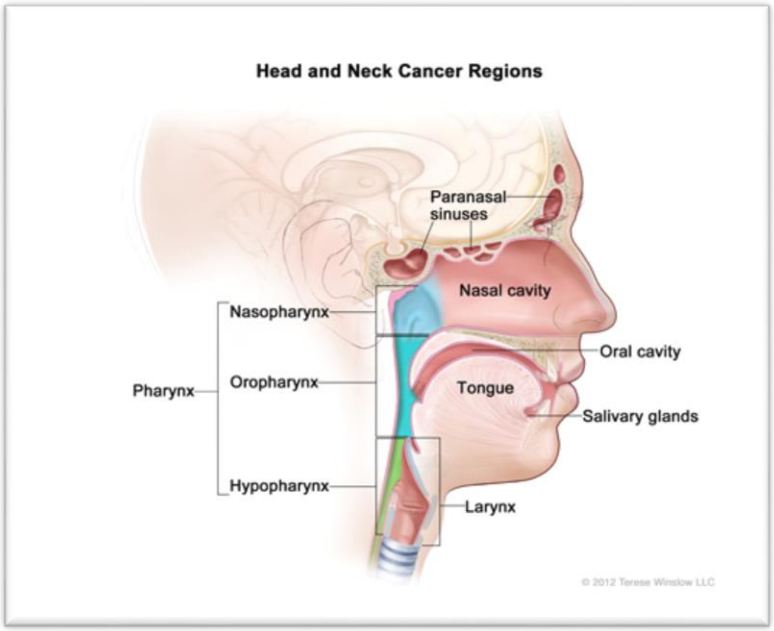

Fig 1.1: Head and Neck cancer region

Source: https://www.cancer.gov/types/head-and-neck/head-neck-fact-sheet

Fig 1.1 shows the regions which places are effected by HNSC. The primary symptoms of this cancer are not look like dangerous in the initial states like lymph node observed outside of the neck, bleeding from mouth, ulcer in mouth and lips and which never cure or heal, difficulties in swallowing food and weight loss, lump in leaps and neck. These are the primary symptoms of HNSC cancer, if it will not diagnose properly infection scattered and turn into cancer.

Mouth: Highly consumption of alcohol and tobacco increase the rate of this type of cancer. First stage there are bleeding in mouth, painless ulcer in the mouth and not cure from long time, lump in the mouth cavity, bad odour etc. are observed. It is not treated the symptoms turned into HNSC. Most of the cases squamous Cell developed in mouth and turned into HNSC cancer [12].

Nasopharynx: In this position nasal cavity and Eustachian tubes connect to each other by this part. Primarily lump can be feel, sometimes problem in swallowing, pain in the particular part. In future this may cause HNSC cancer.

Larynx: This position contains the vocal cord of human. Heavy consumption of tobacco increase the risk of HNSC cancer in vocal box. It starts with vocal sound problem from a long time. It turns to cancer.

Trachea: This position contains our salivary gland. Tumor developed in our salivary gland and in future this turns into cancer. This is the rare type of cancer among the all HNSC cancers [12].

There are different types of head and neck cancer which are described below.

a. Laryngeal and hypopharyngeal cancer: Larynx contains our vocal cord which help us to speak. It contains a tube shaped organ situated in the neck. It help us swallowing, talking and breathing. Larynx contains three parts glottis, supraglottis and subglottis. The surrounded part of the larynx is called the Hypopharynx. It is also known as gullet. The starting part of hypopharynx which situated behind the nose called nasopharynx and ended up in the larynx. This is also called throat. This type of cancer starting by developing by tumor formation. In this region the abnormal growth of cells occurred, due to that growth the tumor is formed and spread across the region due to malignancy. This type of tumor is cancerous in nature and also responsible for spreading cancer across the region. 95% case of this cancer it is caused for squamous cells formation.

b. Nasal cavity and paranasal sinus cancer: The nasal cavity region started behind the nose and extended to throat positions. Around the nasal cavity paranasal sinus situated which is filled with air. Due to abnormal growth of cell growth and other causes.

Squamous cell carcinoma is one of the common reason for this types of cancer development. Thin flat layer of squamous cell developed in this region, which is the most common type for this type of cancer. Beside of that there are also have different types like Adenocarcinoma, Melanoma, Inverting papilloma etc.

c. Oral and oropharyngeal cancer: Mouth and tongue are the main parts of oral cavity. Rest of those part tonsil is also include in the oral cavity. For this types of cancer 90% cases based on squamous cell carcinoma. As the effect of carcinoma cancer flat thin layer developed in mouth and throat. The development of this can be seen in Tongue, Gum, Floor of mouth, Tonsils, oropharynx. Sometimes painless ulcer observed in the mouth floor. It may also developed cancer in future [12].

d. Salivary gland cancer: Salivary gland is one of the most important gland. It produces saliva which helps to moist the mouth cavity. Rest of that saliva contains some important enzymes which helps to digest food initially and helps to swallow food. Another most important role play saliva to prevent infection by destroying germs in the mouth cavity. There are three major parts in salivary gland, they are Parotid Gland, Submandibular Gland and Submaxillary gland. For salivary gland cancer tumor is the most responsible. More than 80% tumors grow up in the parotid gland, but other two glands has less percentage compare to parotid gland.

1.2 Epigenetic Alteration:

DNA methylation is one of the important phenomena in the DNA sequence. DNA consists of four different unique nucleotide molecules, they are cytosine, guanine, adenine and thymine. With the 5th position of the cytosine molecule methyl group added and produce 5-methyl cytosine. DNMT or DNA Methyl Transferases a group of enzymes help for methylation and maintain it (DNMT1, DNMT3a, and DNMT3b). There are almost 70% genes are methylated. In case of promoter region which contains regulatory elements and controls the transcription, after methylation occurred in that region produce the inactivation of silencing of genes. Besides of DNA methylation there are also demethylation occurs which balance the methylation process.

In another case besides of DNA methylation histone methylation is also an important phenomena. In a DNA strand wrapped around the histone. There are four types of histones, H2a, H2b, H3 and H4. Among the H3 and H4 are the activated histones among the four histones. Histone modification occurred due to addition of two or more than methylation group added with it. Histone methylation also responsible for activating and deactivating for different positions of body to maintain the balance. Histone methylation is also responsible for deactivation of chromatin. But sometimes the balance hamper then abnormal methylation occurred. Due to hamper and disruption of this pattern it may cause cancer.

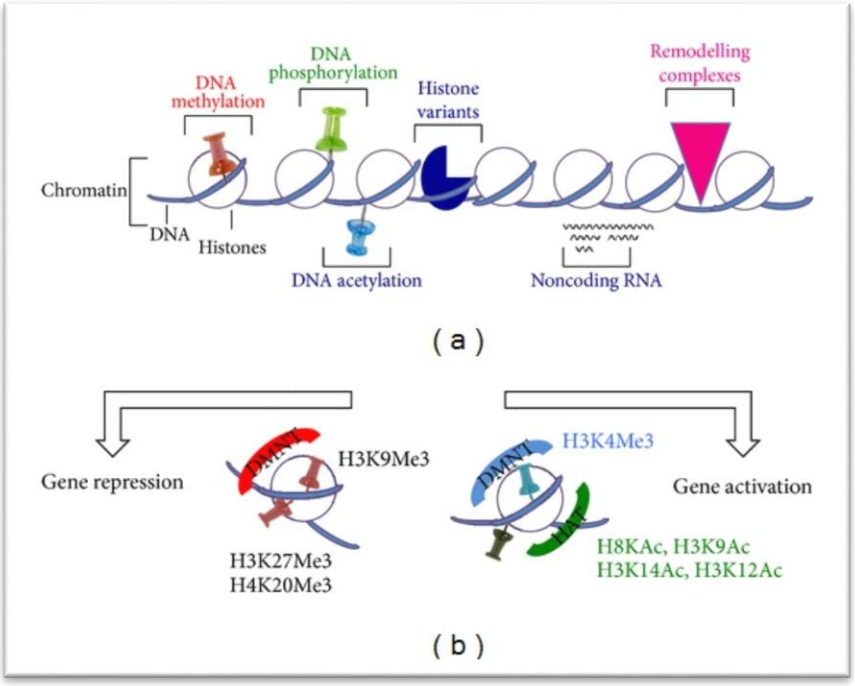

Fig 1.2: Histone modification

Source:https://www.researchgate.net/profile/Lorena_Perrone/publication/264166836/figure/fig1/AS:202883799752704@1425382675728/Scheme-of-histone-and-epigenetic-modifications-a-Chromatin-structure-can-be-modified.png

Fig 1.1 shows the histone modification process. Where adding different groups caused activation and deactivation of chromatin. Which responsible for activation of tumor silencing genes. Also maintain the balance of healthy life.

In DNA methylation DNMT responsible for methylation, other hand TET is responsible for Demethylation. But if the balance of methylation and demethylation hampered then abnormal methylation occurred. Due to the abnormal methylation the promoter regions get hyper methylated, as a result mutated gene cannot be repaired, abnormal protein formation occurred. Due to silencing of the tumor suppression genes firstly tumor growth occurred, after that malignance tumor produced which is responsible for the cancer formation. This is called the epigenetic alteration.

For the HNSC cancer squamous cell carcinoma observed in oral cavity, gum and other sections in mouth. Tumor development can be observed in trachea and other regions.

1.3 Motivation:

Our work is to find the rare co-methylation genes of HNSC cancer. From TCGA data portal we have taken the beta value of methylation of different cancer genes of different cancer patients. Based on the beta value of methylation we construct a correlation matrix which indicates the linear relations between each genes. The next step we perform DB-Scan clustering on the correlation matrix. The genes which are outside of cluster they are selected and identify as rare methylation genes. From that genes we perform correlation again and apply K-Means clustering method on them. Those genes of each clusters which has the minimum distance from centroid of each cluster are denoted as highly influential rare cancer genes of HNSC cancer.

We have observed many works based on HNSC cancer, but we observed that the every researches are working on the common cancer genes of HNSC cancer. We unable to find any works on rare cancer genes of HNSC cancer. So apart from the conventional path we try to do our works on rare cancer genes. Based on the rare genes we have performed the required steps of calculations and find the most influential rare cancer genes of HNSC cancer.

Chapter 2:

Literature Survey:

We have studied the existing works and papers on methylation of HNSC cancer genes. From the existing works which guided us to progress on our works to proceed. Last few years there are lots of works on methylation of HNSC cancer genes. Based on the observation of tobacco and alcohol consumer patients, the main objective is to find the DNA methylation biomarkers of the HNSC genes and find CpG loci which are most responsible for HNSC cancer [Scott M. Langevin, Devin C Koestler, Brock C Christensen, Rondi A Butler, John K Wiencke, Heather H Nelson, E Andres Houseman, Carmen J Marsit, and Karl T Kelsey; 2012 Mar 1]. On other hand observed on promoter hyper methylation of the gene inactivate the silencing of the tumor suppressor genes, testing on highly methylated genes(98%) of the salivary rinse and tumor samples of squamous cell carcinoma of different patients and observe the malignancy of tumor and analysis of the hyper methylation of the promoter region of the testing genes (KIF1A and EDNRB)[Semra Demokan and Nejat Dalay; 2011 Jul 9].

2.1 Epigenetic Modifications and Head and Neck Cancer: Implications for Tumor Progression and Resistance to Therapy

Based on the different observations of methylation of DNA, histone modification and miRNA alteration are studied to differentiate between normal genes and cancer genes. Also observe the epigenetic modification and also find the hyper methylation regions to detect the tumors. Based on the required observations try to enhance the resistance therapy to prevent HNSC cancer [Rogerio M. Castilho, Cristiane H. Squarize and Luciana O. Almeida; 2017 Jul 12]. In this paper the work is on to study the epigenetic modification on various factors on DNA methylation, histone modification, miRNA alteration.

Studying on the different epigenetic alteration and DNA methylation of the required genes. From the taken genes there are normal and cancer genes. Among those genes study the epigenetic alterations and DNA methylation of each genes. DNA methylation is basically adding the methyl group to the 5-carbon and form methyl cytosine. 5’ region is called promoter regions of genes referred as CPG Island. Those genes which are unmethylated are transcriptionally active genes. There are three different genes which helps for methylation process they are DNMT1, DNMT3a, and DNMT3b. This is also responsible for chromatin modification. To study this histone modification also which helps activation of tumor suppression gene. Loss of DNA methylation also responsible for epigenetic alteration. For this epigenetic alteration benign and malignant cancer cell developed here. Research also prove that the DNA hyper methylation is responsible for cancer development.

DNA methylation profiling used to distinguish between cancer genes and normal genes. This is also helps to identify the sub type of cancer. There are different studies on DNA methylation to identify the tumor formation and development of HNSC cancer.

DNA hypermethylation is also responsible for epigenetic alteration and histone modification.

Tobacco associated in the cancer gene also development of squamous cell in oral cavity and further development of HNS. Also study the DNA hypermethylation which responsible for HNSC cancer of different genes

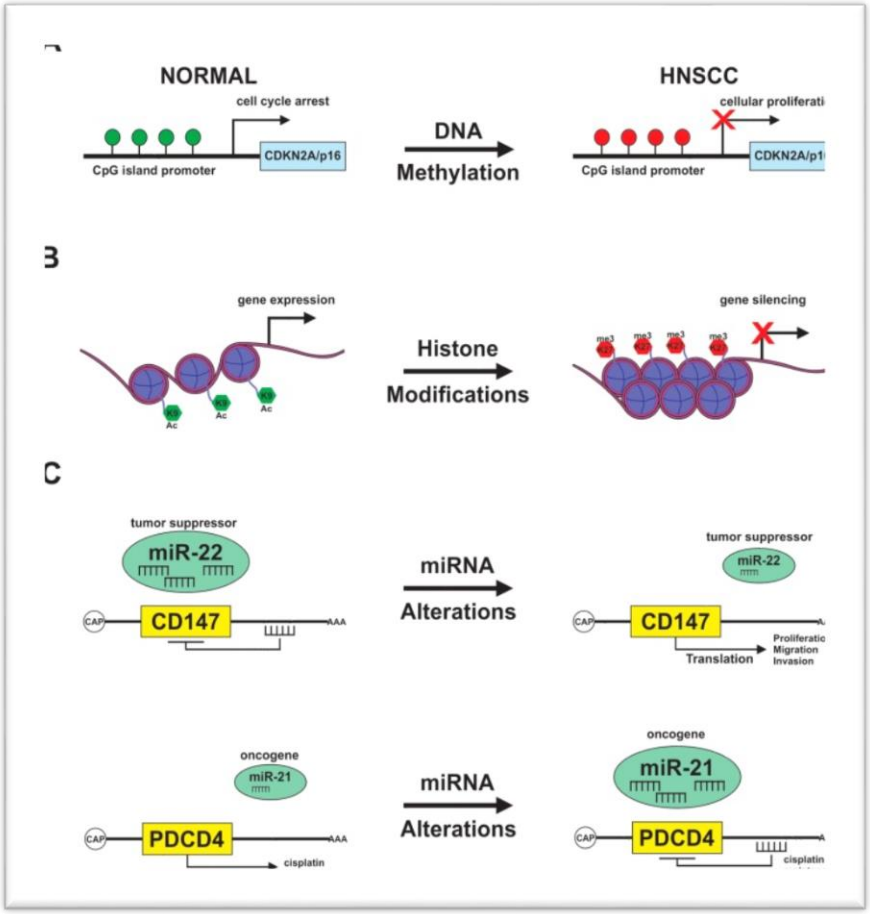

Fig 2.1: Epigenetic alteration overview.

Source:https://www.ncbi.nlm.nih.gov/core/lw/2.0/html/tileshop_pmc/tileshop_pmc_inline.html?title=Click%20on%20image%20to%20zoom&p=PMC3&id=5535996_ijms-18-01506-g002.jpg

There are also study on histone modification and chromatin activation of different genes which are responsible for HNSC cancer development. Observation of different genes and their histone modification to find the cancer progression of genes.

Main aim is to observe the epigenetic modification for tumor detection and emerging Epi-drugs capability for HNSCC to therapy. From the observation of epigenetic modification sorted out the hyper methylated genes of HNSC in tabular format.

From hyper methylation try to find the inactivation of tumor suppression gene and besides that to study on the miRNA. The aim of the project is to development of epigenetic marker for early detection of tumor HNSC and maintain the continuous observation.

This paper concluded that based on the observations of the epigenetic modification and other criteria to detect the salivary tumor stem cells and observe the novel epigenetic modification and tumor sensitivity. From the study to design novel drug for the therapy of the cancer.

2.2 Role of DNA methylation in head and neck cancer:

Head and neck cancer is the sixth most observed cancer among all cancers around the world (Crowe et al. 2002; Ohshima et al. 2005). Due to consumption of tobacco and alcohols increase the risk of HNSC in oral cavity, larynx and pharynx cancer (Ohshima et al. 2005). Viral infection, allergic reaction and other factor cause acute laryngitis and slowly it turns to cancer (Kumar et al. 2000). Epigenetic alteration is also responsible for development of cancer. Besides of that DNA methylation also affects. There are two types methylation occurred, they are hypomethylation and hypermethylation.

One side DNA hypomethylation responsible for activation of oncogenes and instability occurs in chromosome. Oncogenes which is responsible for the cancer and malignant tumor formation. Other hand DNA hypermethylation inactivation of the tumor suppression genes.

In tumor formation alteration and change in methylation can observed in many case of tumor formation (Jones and Baylin 2002). For hypermethylation identification of crucial gene which is responsible for HNSC cancer. Hypermethylation inactivate the tumor suppression gene and helps to discovery of new biomarker. From the study it proves that DNA methyltransfrase, histone modification can responsible for epigenetic modification, which can change in oncology and responsible cancer development (Ren et al. 2011; Wagner et al. 2010).

Here study on promoter methylation occurred from the required sets of genes. From the study on HNSC cancer patients of U.S.A. it is find that highly methylation in *KIF1A* and *EDNRB* genes were observed. The level of methylation is 97% and 98% respectively [1].

From the study it shows from the promoter hyper methylation of the HNSC cancer genes of 24 TSG via candidate gene approach (Yalniz et al. 2011) CHFR, RARβ, DAPK1, and RASFF1A genes are the most common methylated genes of HNSC. Around the world research proves that the role of tobacco responsible for HNSC cancer in oral cavity and HPV infection.

From the set of testing it is examined that (*CYP1A1*, *CYP2A13*, and *GSTM1*) these genes found in the high methylated level (27.4–58.1%) in the tumors of the observing sample [1]. Also observe that hMLH1 and hMSH2these genes take responsibilities for oral carcinogenesis and formation of malignancy occurred (Czerninski et al. 2009).Observing hypermethylation on the patients having genes p16, p15, hMLH1, MGMT, and CDH1 are frequently suffering from oral cancer. Having higher methylation of gene p14ARF higher chance of oral cancer compare to rest of the genes.

DNA hyper methylation responsible for the inactivation of tumor silencing genes. In this case DNA methylation plays many important roles for development and growth of tumor (Jones and Baylin 2002; Momparler and Bovenzi 2000).

From the study there are different sets of genes are selected for test. From the different steps of testing on different genes and observe their methylation. From those genes prediction of hyper methylation of the gene which are responsible for the tumor. Different factors are also included to detection of cancer and other factors by performing different steps.

2.3 Analysis of Site-Specific Methylation of Tumor-Related Genes in Head and Neck Cancer: Potential Utility as Biomarkers for Prognosis:

In the case of HNSC cancer oral cavity, pharynx and larynx are affected by the solid tumor growth. Besides that smoking and drinking alcohol increase the risk of the HNSC cancer. The main aim of this study is to find the effect of hyper methylation in the tumor related genes (Kiyoshi Misawa, Daiki Mochizuki, Atsushi Imai, Masato Mima, Yuki Misawa and Hiroyuki Mineta; 22 January 2018).

Based on the study of 178 patients of HNSC cancer, among the total number of samples there are 153 patients are male and rest 25 are female. 75% of the total patients have advanced stage of this disease. Observing on the tumor position in different positions larynx, oral cavity and hypopharynx. From each cases observing the methylation level. Promoter hyper methylation can be found from their frequency levels. There are hyper methylation of 30 genes shown, but among them 5 genes (SST, SSTR1, HCRTR2, NPFFR1, and NPFFR2) have high level of methylation, more than 70% [15].

Observing on the different genes it can be observe that for laryngeal cancer development CDH13, p16, RASSF1A, GAL, NPY, and NPY1R have high chance. For hypopharyngeal cancer CDH13, p16, MGMT, GAL, NPY, GALR2, and VEGFR3 are responsible. Another thing also observed that CDH13 and GAL are responsible for oral cancer formation. A correlation between normal genes and tumor genes is shown here. The frequency of oral cavities with respect to the larynx and hypopharynx is higher ((p<0.001andp=0.006) [15].

Analysis of different genes of different ages (>70 and <70). To check study the survival rate. Laryngeal cancer patient having p16 and COL1A2 where promoter methylation observed have poor survival rate. Hypermethylation of DAPK, TAC1, GALR1, NPY1R, SSTR1, and VEGFR3 have less survival rate of oral cancer.Observing on the table it is found that in hypopharyngeal cancer GAL gene has lower frequency, but for laryngeal cancer it has quiet high frequency compare to hypopharyngeal cancer.

Methylation Index is also calculated by calculating the ratio of the number of methylated genes with respect to the total number of genes. Methylation Index of the 30 tumor related genes from the total number of 178 patients are sufficiently higher. Overall from this paper we can say that promoter methylation of specific tumor related genes increase the risk of HNSC cancer.

2.4 Promoter methylation in head and neck tumor genesis:

Over United States 50,000 patients diagnosed HNSC cancer in every year. This cancer has the the high mortality rate, due to having its different natures this type of cancer is very difficult to diagnose. Now a days there are many types of way of identification to detection of this types of cancer. There are so many epigenetic studies but among them DNA methylation is the best and easiest way by to detect HNSC cancer.

In DNA methylation CH_3 added with the cytosine group. DNA hyper methylation show that the modification of DNA [Josena K. Stephen, Kang Mei Chen, Shaleta Havard, Glynis Harris, and Maria J. Worsham1; 2013 Jul 25].

In this paper calculate the methylation status of each gene with the help of MS-MLPA and detect the promoter methylation by conventional specific methylation method.

Initially there are some selected genes are taken for test. After testing it is found that 22 tumor suppressor genes which are responsible for HNSC cancer.

RARβ and APC in this two gene promoter methylation observed tumor in the early and late stage [9]. Beside that there are observing of different genes, there are different values of percentage of different HNSC cancer rate. It shows that having hyper methylation in promoter region in APC gene there are 25% chance of oral cancer.

There are different processes of and different ways to detect the promoter hyper methylation. It is also proved that among the all methods epigenetic events of promoter hyper methylation is the most efficient way to detect cancer.

2.5 Frequent Promoter Hyper methylation of tumor related genes in head and neck squamous cells carcinoma:

Due to having epigenetic alteration there are different there are inactivation of tumor suppressor gene occurred, besides that there is development of squamous cells in head and neck. In this study observation of promoter methylation of HNSC cancer genes occurred [Katrin Steinmann, Annett Sandner, Undraga Schagdarsurengin, Reinhard H. Dammann; 2009 Dec 1].

There are 15 genes are analysed. They are RASSF1A, p16, MGMT, DAPK, RARβ, MLH1, CDH1, GSTP1, RASSF2, RASSF4, RASSF5, MST1, MST2, LATS1, LATS2. After calculated the frequencies it shows that the tumor related genes have high frequencies (42%) more than the normal genes. MST1 has the highest frequency 96%, which is frequently observed in HNSC cancer genes. Other hand MST2 has 4% frequency which is lowest. So having lowest frequency MST2 is hard to observe.

Now different stages of HNSC cancer of different tumor related genes methylation observed in different patients. Initial stage having almost same methylation level, which is realy hard to distinguish ($p=0.037$). In advanced tumor stage it is noticed that RASSF5 gene has higher methylation ($p<0.05$). It is noted that in initial stages of given tumor related genes have similar or nearly similar frequency. The hyper methylation of tumor related genes in the promoter regions inactivate the tumor suppression genes, which helps to develop the tumor and responsible for HNSC cancer.

Chapter 3:

Materials and Method:

For our work on HNSC cancer gene taking the beta value of methylation from TCGA data portal. TCGA (The Cancer Genome Atlas program) started on 2005 to find the genetic mutation which are responsible for cancer. TCGA contains different types of cancer genes of different patients. There are 4215 genes of HNSC of 580 patients, among the 580 samples of patients there are 550 samples are tumor samples and rest 30 samples are normal gene. Beta value of methylation indicates the methylation level of required gene. The value lies in between 0 to 1, where the value 0 indicates the gene is not methylated and for 1 it is fully methylated.

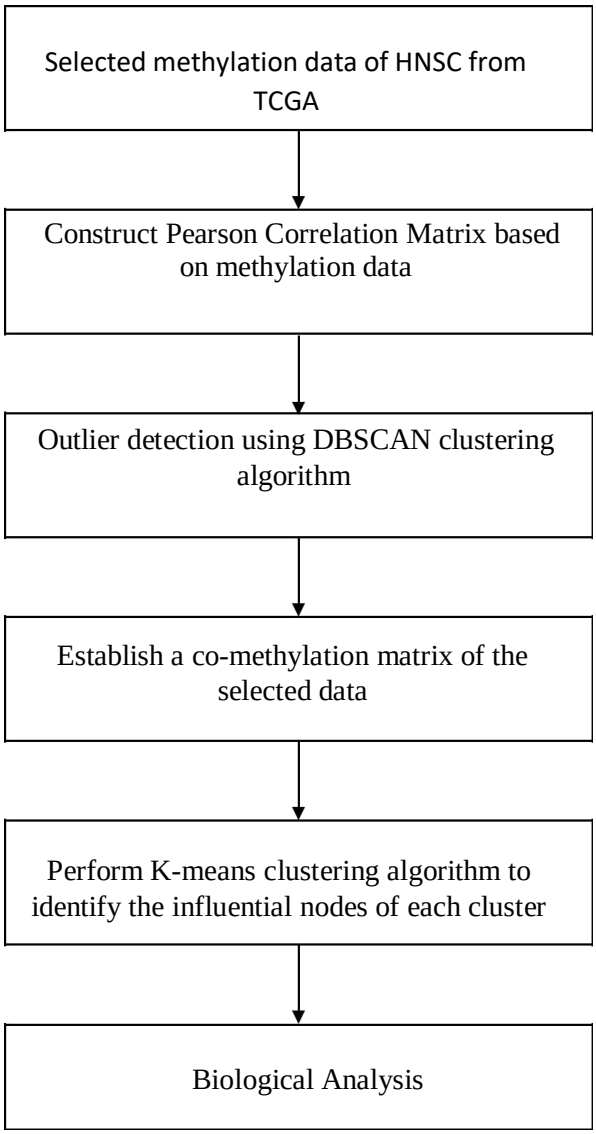

Fig 3.1: The Flowchart of the proposed framework

Fig 3.1 shows the flow of steps which proceed through the entire works. From the raw data of the beta value of methylation data of HNSC cancer gene we have calculated Pearson Correlation matrix. The required mathematical formula of Pearson Correlation is given below.

$$\boldsymbol{r} = \frac{\sum((\boldsymbol{g1}-\boldsymbol{g1'})(\boldsymbol{g2}-\boldsymbol{g2'}))}{\sqrt{\sum(\boldsymbol{g1}-\boldsymbol{g1'})^2 \sum(\boldsymbol{g2}-\boldsymbol{g2'})^2}} \quad (1)$$

The equation (1) describes the mathematical formula of Pearson Correlation where r is the Pearson Correlation coefficient. Where g1 and g2 is the beta value of methylation of the gene and g1' and g2' is the average beta value of methylation value of the genes. Using this formula calculating correlation of the gene with respect to all other genes. Correlation denotes the linear relation between one genes with respect to all other genes. Where the values lies between 1 to -1.

From the Correlation matrix DB-Scan clustering framework is applied to detect the outliers. Where the main target is to find the rare methylation genes which are in the outliers. The mathematical expression is given below.

$$\boldsymbol{N_{\varepsilon}(g1)}:\{\boldsymbol{g2}|\boldsymbol{d(g1,g2)} \leq \boldsymbol{\varepsilon}\} \quad (2)$$

From equation (2) ε is the radius of the circle of neighbourhood where g1 has the more number of points which is equal to the minimum number of points and g2 has less point. But here in our works from the outside of the cluster circle outliers are taken for further analysis which contains the rare methylation genes. The genes which are in between the neighbourhood circle or adjacent neighbourhood are in the clusters.

From the outliers collect the beta value of methylation from the TCGA data table and perform Correlation again of the outlier genes. Now we use K-Means clustering framework in the Correlation matrix.

$$\boldsymbol{K(V)} = \sum_{\boldsymbol{j=1}}^{\boldsymbol{c}} \sum_{\boldsymbol{k=1}}^{\boldsymbol{c_i}} (\left|\left|\boldsymbol{g_j}-\boldsymbol{g_k}\right|\right|)^2 \quad (3)$$

Equation (3) is the mathematical expression of K-Means clustering method c is the number of cluster centres and c_i denotes the number of points in the i^{th} cluster. $\left|\left|g_j - g_k\right|\right|$ is the Euclidean Distance. Where g_j are the methylation are values of the set of genes and g_k is the set of centres. Based on the Euclidean Distance we can create the cluster of genes. Which gene has minimum Euclidean distance from the centre that is the closest gene. From each cluster the gene which is closest to the centroid considered as the influential genes.

Chapter 4:

Results:

For our work there are several steps of calculation done and each steps we have results and which helps us to calculate the further steps of work. From the beta values of methylation of HNSC cancer genes from TCGA data portal a Correlation matrix construct to find the linear relationship between each genes using the Pearson Correlation method equation (1). The Correlation values lies between 1 to -1.

After perform DB-Scan Clustering framework based on the Correlation matrix a cluster graph is generated where the graph show the cluster and outliers.

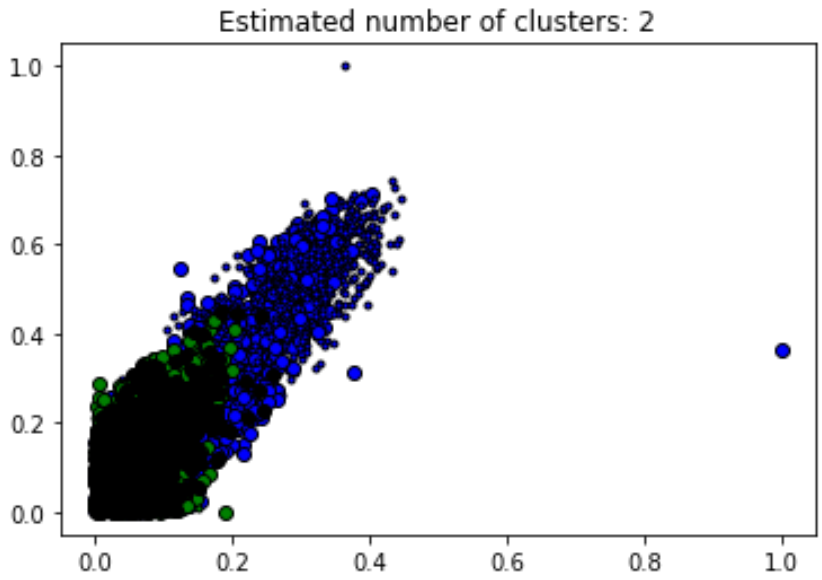

Fig 4.1: DB-Scan Cluster graph.

From Fig 4.1 it shows the cluster graph after performing DB-Scan clustering framework [Equation (2)]. The graph show the two clusters and outliers. The outliers are denoted by black. This outliers contains the rare cancer genes of HNSC. In this graph blue and green points are cluster and adjacent neighbour. Black points are the outside of the clusters, which are the outliers.

From the selected outliers after performing Pearson Correlation K-Means clustering framework [Equation (3)]. The K-Means Cluster shows 5 different clusters by different colour codes.

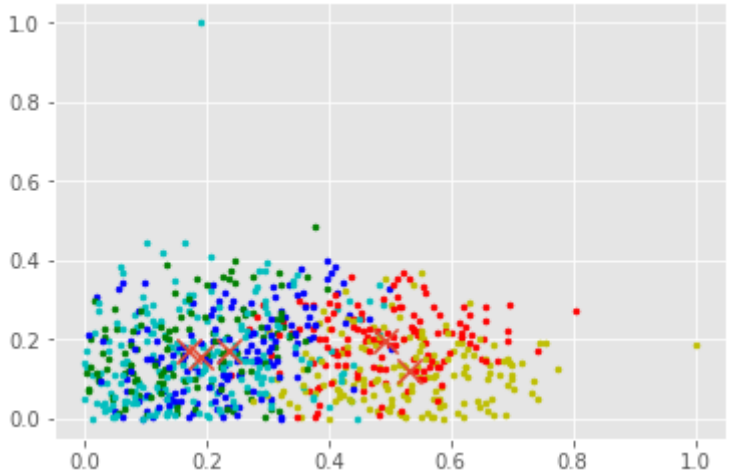

Fig 4.2: K-Means Cluster graph

From Fig 4.2 it shows five different clusters based on the different centroid which are also plotted in the graph by cross mark. Red, yellow, blue, sky, green colours are represent clusrer1, cluster2, cluster3, cluster4 and cluster5 respectively. Based on each cluster and the gene which is most close to the centroid are taken. Those genes are the most influential genes of rare cancer methylation of HNSC. These five genes MGRN1, WWTR1, BAG2, CXCR4, MCL1 are the most influential rare cancer methylation cancer genes of HNSC.

Gene Name	Pathway	Adjusted p-value
MGRN1	Ubiquitin mediated proteolysis	0.006850
WWTR1	Hippo signaling pathway	0.008000
BAG2	Protein processing in endoplasmic reticulum	0.008250
CXCR4	Intestinal immune network for IgA production	0.01491
	Human cytomegalovirus infection	0.01491
	Regulation of actin cytoskeleton	0.01491
	Human immunodeficiency virus 1 infection	0.01491
	Leukocyte transendothelial migration	0.01491
MCL1	MicroRNAs in cancer	0.01770
	Apoptosis	0.01620
	JAK-STAT signaling pathway	0.01620
	PI3K-Akt signaling pathway	0.01770

Table 4.1: KEGG pathway analysis of the rare influential gene

To identify the significant KEGG pathways of five selected co-methylated genes such as MGRN1, WWTR1, BAG2, CXCR4, MCL1 Enrichr tool [51] is used. In Table 4.1 five pathways are reported, which shows active participation of the genes in this disease. Pathways include Ubiquitin mediated proteolysis [52], Hippo signaling pathway [53], Intestinal immune network for IgA production [54] and JAK-STAT signaling pathway [55]. Abnormalities in these pathways often lead to head and neck cancer. Similarly, like KEGG pathway, GO enrichment analysis is performed which is shown in Table 4.2. Some important activities like Regulation of cAMP-mediated signaling, Transcription coactivator activity, Adenyl nucleotide binding, C-C chemokine binding, Death domain binding etc. are found [56-58]. From which it is obvious that selected rare co-methylated genes played an important role in cancer development.

Gene name	Cellular Component	Adjusted p-value
MGRN1	early endosome (GO:0005769)	0.01115
WWTR1	nucleoplasm part(GO:0044451)	0.03095
	nuclear body (GO:0016604)	0.03095
CXCR4	late endosome (GO:0005770)	0.01394
	lytic vacuole (GO:0000323)	0.01394
	early endosome (GO:0005769)	0.01394
	cytoplasmic vesicle (GO:0031410)	0.01394
	lysosome (GO:0005764)	0.02115
MCL1	mitochondrial outer membrane (GO:0005741)	0.01160
	mitochondrion (GO:0005739)	0.05135

Table 4.2: Gene Ontology Cellular Componen

Gene name	Biological Process	Adjusted p-value
MGRN1	negative regulation of cAMP-mediated signaling (GO:0043951)	0.003850
	negative regulation of smoothened signaling pathway (GO:0045879)	0.003850
	negative regulation of G-protein coupled receptor protein signaling pathway (GO:0045744)	0.003850
	endosome to lysosome transport (GO:0008333)	0.004900
	regulation of cAMP-mediated signaling (GO:0043949)	0.003850
WWTR1	negative regulation of catenin import into nucleus (GO:0035414)	0.007529
	regulation of catenin import into nucleus (GO:0035412)	0.007529
	regulation of protein import into nucleus (GO:0042306)	0.007529
	regulation of SMAD protein import into nucleus (GO:0060390)	0.007529
	hippo signaling (GO:0035329)	0.007529
BAG2	regulation of cellular response to stress (GO:0080135)	0.005250
	regulation of cellular response to heat (GO:1900034)	0.005250
CXCR4	myelin maintenance (GO:0043217)	0.003250
	fusion of virus membrane with host plasma membrane (GO:0019064)	0.003250
	positive regulation of glial cell differentiation (GO:0045687)	0.003250
	membrane fusion involved in viral entry into host cell (GO:0039663)	0.003250
	dendritic cell chemotaxis (GO:0002407)	0.003542
MCL1	regulation of oxidative stress-induced neuron intrinsic apoptotic signaling pathway (GO:1903376)	0.004800
	positive regulation of neuron apoptotic process (GO:0043525)	0.004800
	negative regulation of anoikis (GO:2000811)	0.004800
	regulation of anoikis (GO:2000209)	0.004800
	extrinsic apoptotic signaling pathway in absence of ligand (GO:0097192)	0.004800

Table 4.3: Gene Ontology Biological process.

Gene name	Molecular Function	Adjusted p-value
MGRN1	ubiquitin-protein transferase activity (GO:0004842)	0.02090
WWTR1	transcription coactivator activity (GO:0003713)	0.01460
BAG2	adenyl-nucleotide exchange factor activity (GO:0000774)	0.0009000
	adenyl nucleotide binding (GO:0030554)	0.0009000
	ATPase regulator activity (GO:0060590)	0.001950
CXCR4	C-C chemokine binding (GO:0019957)	0.003300
	chemokine binding (GO:0019956)	0.003300
	chemokine receptor activity (GO:0004950)	0.003300
	myosin binding (GO:0017022)	0.005287
	actin binding (GO:0003779)	0.01490
MCL1	macromolecule transmembrane transporter activity (GO:0022884)	0.001487
	death domain binding (GO:0070513)	0.001487
	peptide transmembrane transporter activity (GO:1904680)	0.001487
	protein transmembrane transporter activity (GO:0008320)	0.001487
	protein transporter activity (GO:0008565)	0.005670

Table 4.4: Gene Ontology Molecular process

Chapter 5:

Conclusion and Future Works:

The proposed frame provides rare co-methylation samples from the list of the significantly differential methylated genes. In the subsequent level, selected co-methylated samples have been clustered again. Each cluster consists of genes which are highly co-methylated. Selecting top samples are expected to be influential. However, the literature suggests that the defined way of implication for the selected samples are not well known. Therefore, it can be concluded that the diverse trait of co-methylation is responsible for the enhancement of pathogenicity. Finally, these genomic samples can be utilized for prognostic and therapeutic purposes.

We have analyse the rare cancer genes of HNSC cancer, our future plan is to analyse the rare cancer genes of different cancers. We shall also apply the different clustering methods for analysing for different cancer genes. From the rare cancer genes of HNSC we can extend our work on to find that which gene has higher probability of different HNSC cancer (oral cavity, laryngeal etc.).

Bibliography

1. S. Demokan and N. Dalay, "Role of DNA methylation in head and neck cancer". Clinical Epigenetics; 2(2), 123–150. 2011.

2. J. Cao, J. Zhou, Y. Gao, L. Gu, H. Meng, H. Liu and D. Deng "Methylation of p16 CpG Island associated with malignant progression of oral epithelial dysplasia: a prospective cohort study". Clinical Cancer; 15, 5178–5183. 2009.

3. R.L. Momparler and V. Bovenzi. "DNA methylation and cancer". J Cell Physiology; 183, 145-154. 2000.

4. K. Liu, H. Huang, P. Mukunyadzi, J.Y. Suen, E. Hanna and C.Y. Fan. "Promoter hypermethylation: an important epigenetic mechanism for hMLH1 gene inactivation in head and neck squamous cell carcinoma". Otolaryngol Head Neck Surgery; 126, 548–553, 2002.

5. K. Steinmann, A. Sandner, U. Schagdarsurengin and R.H. Dammann. "Frequent Promoter Hyper methylation of tumor related genes in head and neck squamous cells carcinoma". Oncology Reports; 1519-1526, 2009.

6. W.J. Kong, S. Zhang, C.K. Guo, Y.J. Wang, X. Chen, S.L. Zhang, D. Zhang, Z. Liu and W. Kong. "Effect of methylation-associated silencing of the death-associated protein kinase gene on nasopharyngeal carcinoma". Anticancer Drugs; 17:251–259. 2006.

7. https://www.cancer.gov/types/head-and-neck/head-neck-fact-sheet

8. K. Kozaki, I. Imoto, S. Mogi, K. Omura and J. Inazawa. "Exploration of tumor-suppressive microRNAs silenced by DNA hypermethylation in oral cancer". Cancer Research; 68, 2094–2105. 2008.

9. J.K. Stephen, K. Mei Chen, S. Havard, G. Harris, and M.J. Worsham."Promoter methylation in head and neck tumor genesis". Methods Molecular Biology; 863, 187–206. 2012.

10. J.P. Issa, P.M. Vertino, J. Wu, S. Sazawal, P. Celano, B.D. Nelkin, S.R. Hamilton and S.B. Baylin. "Increased cytosine DNA-methyltransferase activity during colon cancer progression". Journal of the National Cancer Institute; 85, 1235–1240. 1993.

11. S. Maruya, J.P. Issa, R.S. Weber, D.I. Rosenthal, J.C. Haviland, R. Lotan and A.K. El-Naggar. "Differential methylation status of tumor-associated genes in head and neck squamous carcinoma: incidence and potential implications". Clinical Cancer; 10, 3825–3830. 2004.

12. https://www.cancer.net/cancer-types/head-and-neck-cancer/introduction

13. M. Hasegawa, H.H. Nelson, E. Peters, E. Ringstrom, M. Posner and K.T. Kelsey. "Patterns of gene promoter methylation in squamous cell cancer of the head and neck". Oncogene; 21, 4231–4236. 2002.

14. K.T. Yeh, J.G. Chang, T.H. Lin, Y.F. Wang, N. Tien, J.Y. Chang, J.C. Chen and M.C. Shih. "Epigenetic changes of tumor suppressor genes, P15, P16, VHL and P53 in oral cancer". Oncology; 10,659–663.2003.

15. K. Misawa, D. Mochizuki, A. Imai, M. Mima, Y. Misawa and H. Mineta, "Analysis of Site-Specific Methylation of Tumor-Related Genes in Head and Neck Cancer: Potential Utility as Biomarkers for Prognosis". Cancers (Basel); 10(1), 27. 2018.

16. J.K. Stephen, K.M. Chen, V. Shah, S. Havard, A. Kapke, M. Lu, M.S. Benninger, M.J. Worsham "DNA hypermethylation markers of poor outcome in laryngeal cancer". Clinical Epigenetic; 1, 61–69. 201

17. https://www.cancerresearchuk.org/about-cancer/what-is-cancer/genes-dna-and-cancer

18 C.Y. Zhang, L. Mao, L. Li, Z. Tian, X.J. Zhou, Z.Y. Zhang and J. Li. "Promoter methylation as a common mechanism for inactivating E-cadherin in human salivary gland adenoid cystic carcinoma". Cancer; 110:87–95. 2007.

19. P. Yanatatsaneejit, T. Chalermchai, V. Kerekhanjanarong, K. Shotelersuk, P. Supiyaphun, A. Mutirangura and V. Sriuranpong. "Promoter hypermethylation of CCNA1, RARRES1, and HRASLS3 in nasopharyngeal carcinoma". Oral Oncology; 44, 400–406. 2018.

20. K. Grønbaek, C. Hother and P.A. Jones. "Epigenetic changes in cancer". APIMS; 115(10), 1039-1059. 2007.

21. S. Zhang, C. Guo, W. Kong and Z. Liu. "Promoter hypermethylation of DNA repair gene MGMT in laryngeal squamous cell carcinoma". J Huazhong Univ Sci Technolog Medical Science; 26, 101–104. 2006.

22. A.D. Rapidis, N. Givalos and H. Gakiopoulou, et al. "Adenoid cystic carcinoma of the head and neck. Clinicopathological analysis of 23 patients and review of the literature". Oral Oncology; 41, 328– 335. 2005

23. K. Subbalekha, A. Pimkhaokham, P. Pavasant, S. Chindavijak, C. Phokaew, S. Shuangshoti, O. Matangkasombut and A. Mutirangura. "Detection of LINE-1s hypomethylation in oral rinses of oral squamous cell carcinoma patients". Oral Oncology; 45,184–191. 2009.

24. K.W. Lo, Y.S. Tsang, J. Kwong, K.F. To, P.M. Teo and D.P. Huang. "Promoter hypermethylation of the EDNRB gene in nasopharyngeal carcinoma". International Journal of Cancer; 98,651–655, 2002.

25. J. Kwong, K.W. Lo, K.F. To, P.M. Teo, P.J. Johnson and D.P. Huang "Promoter hypermethylation of multiple genes in nasopharyngeal carcinoma". Clinical Cancer; 8, 131–137. 2002.

26. "The Cancer Genome Atlas Network Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature"; 517, 576–582. 2015

27. https://amp.pharm.mssm.edu/Enrichr/

28. A.N. Pullos, R.M. Castilho and C.H. Squarize "HPV Infection of the Head and Neck Region and Its Stem Cells". J. Dental Research; 94, 1532–1543. 2015.

29. A. Argiris, M.V. Karamouzis, D. Raben and R.L. Ferris. "Head and neck cancer". Lancet; 371, 1695–1709. 2008.

30. R.M. Castilho, C.H. Squarize, and L.O. Almeida. "Epigenetic Modifications and Head and Neck Cancer: Implications for Tumor Progression and Resistance to Therapy". International Journal of Molecular Science; 18(7), 1506. 2017. 2017 Jul 12.

31. D.J. Smiraglia, L.T. Smith, J.C. Lang, L.J. Rush, Z. Dai, D.E. Schuller and C. Plass. "Differential targets of CpG island hypermethylation in primary and metastatic head and neck squamous cell carcinoma (HNSCC)". Journal of Medical Genetics; 40:25–33. 2003.

32. K.L. Bennett, W. Lee, E. Lamarre, X. Zhang, R. Seth, J. Scharpf, J. Hunt and C. Eng. "HPV status-independent association of alcohol and tobacco exposure or prior radiation therapy with promoter methylation of FUSSEL18, EBF3, IRX1, and SEPT9, but not SLC5A8, in head and neck squamous cell carcinomas". Genes Chromosomes Cancer; 49,319–326. 2010.

33. S.L. Chan, Y. Cui, A. Hasselt, H. Li, G. Srivastava, H. Jin, K.M. Ng, Y. Wang, K.Y. Lee, G.S. Tsao, S. Zhong, K.D. Robertson, S.Y. Rha, A.T. Chan and Q. Tao." The tumor suppressor Wnt inhibitory factor 1 is frequently methylated in nasopharyngeal and esophageal carcinomas". Laboratory Investigation; 87(7), 644–650. 2007 March 26.

34. A.L. Carvalho, C. Jeronimo, M.M. Kim, R. Henrique, Z. Zhang, M.O. Hoque, S. Chang, M. Brait, C.S. Nayak and W.W. Jiang, et al. "Evaluation of promoter hypermethylation detection in body fluids as a screening/diagnosis tool for head and neck squamous cell carcinoma". Clinical Cancer; 14, 97–107. 2008.

35. Y.W. Chen, S.Y. Kao, H.J. Wang and M.H. Yang "Histone modification patterns correlate with patient outcome in oral squamous cell carcinoma". Cancer; 119, 4259–4267. 2013.

36. J.G. Herman, J. R. Graff, S. Myohanen, B.D. Nelkin and S.B. Baylin. “Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands”. Proceedings of the National Academy of Sciences of the United States of America; 93, 9821–9826. 1996.

37. M.S. Cespedes, M. Esteller, L. Wu, H.N. Danish, G.H. Yoo, W.M. Koch, J. Jen, J. G. Herman and D. Sidransky. “Gene promoter hypermethylation in tumors and serum of head and neck cancer patients”. Cancer; 60, 892–895. 2000.

38. L.P. Webber, V.P. Wagner, M. Curra, P.A. Vargas., L. Meurer, V.C. Carrard, C.H. Squarize, R.M. Castilho and M.D. Martins. “Hypoacetylation of acetyl-histone H3 (H3K9ac) as marker of poor prognosis in oral cancer”. Histopathology; 71(2), 278-286. 2017.

39. M. Hasegawa, H.H. Nelson, E. Peters, E. Ringstrom, M. Posner and K.T. Kelsey. “Patterns of gene promoter methylation in squamous cell cancer of the head and neck”. Oncogene; 21, 4231–4236. 2002.

40. J.G. Herman and S.B. Baylin. ”Promoter-region hypermethylation and gene silencing in human cancer”. Current topics in microbiology and immunology; 249, 35–54. 2000.

41. M.Y. Ahn and J.H. Yoon.“Histone deacetylase 8 as a novel therapeutic target in oral squamous cell carcinoma”. Oncology; 37,540–546. 2007.

42. S.L. Rosas, W. Koch, C.M.G da Costa, L. Wu, J. Califano, W. Westra, J. Jen and D. Sidransky “Promoter hypermethylation patterns of p16, O6-methylguanine-DNA-methyltransferase, and death-associated protein kinase in tumors and saliva of head and neck cancer patients”. Cancer Research; 61, 939–942.2011.

43. H. Marzook, S. Deivendran, R. Kumar and M.R. Pillai. “Role of MTA1 in head and neck cancers”. Cancer Metastasis Reveiws; 33, 953–964. 2014.

44. W.Y. Huang, S.D. Hsu, H.Y. Huang, Y.M. Sun, C.H. Chou, S.L. Weng, and H.D. Huang. “MethHC: a database of DNA methylation and gene expression in human cancer”. Nucleic Acids Research; 43, 856–861. 2015.

45. P.T. Hennessey, M.F. Ochs, W.W. Mydlarz, W. Hsueh, L. Cope, W. Yu and J.A. Califano . "Promoter Methylation in Head and Neck Squamous Cell Carcinoma Cell Lines Is Significantly Different than Methylation in Primary Tumors and Xenografts"; 49(4):319-26. May 26, 2011.

46. W. Sun, D. Zaboli, H. Wang, Y. Liu, D. Arnaoutakis and T. Khan, et al. "Detection of TIMP3 promoter hypermethylation in salivary rinse as an independent predictor of local recurrence-free survival in head and neck cancer". Clinical Cancer Research; 18(4):1082–91. 2012/01/10

47. S.M. Langevin, M. Eliot, R.A. Butler, A. Cheong, X. Zhang, M.D. McClean, D.C. Koestler and K.T. Kelsey. "CpG island methylation profile in non-invasive oral rinse samples is predictive of oral and pharyngeal carcinoma". Clinical Epigenetics; 7,125. 2015.

48. P. Chi, C.D. Allis and G.G. Wang. "Covalent histone modifications—Miswritten, misinterpreted and mis-erased in human cancers". Nature Reviews Cancer; 10, 457–469. 2010.

49. L.M.R.B. Arantes, A.C. de Carvalho, M.E.Melendez, A.L.Carvalho and E.M.Goloni-Bertollo. "Methylation as a biomarker for head and neck cancer". Oral Oncology; 50(6),587-592. 6, June 2014.

50. Y. Lim, C.X. Sun, P. Tran and C. Punyadeera "Salivary epigenetic biomarkers in head and neck squamous cell carcinomas". Biomarkers in Medicine; 10(3), 301–313. 2016.

51. M.V. Kuleshov, M.R. Jones, A.D. Rouillard, , N.F. Fernandez, Q. Duan, Z. Wang, S. Koplev, S.L. Jenkins, K.M. Jagodnik, A. Lachmann, , M.G. McDermott, C.D. Monteiro, G.W. Gundersen and A. Maayan. " Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acid Research"; 44, 90–97. 2016.

52. S. Qie, M. Majumder, K. Mackiewicz, B.V. Howley, Y.K. Peterson, P.H. Howe and V. Palanisamy, J.A. Diehl, "Fbxo4-mediated degradation of Fxr1 suppresses tumorigenesis in head and neck squamous cell carcinoma. Nature Communications"; 8(1534), 1–14 .2017.

53. Y. Wang et.al: “Comprehensive Molecular Characterization of the Hippo Signaling Pathway in Cancer. Cell Reports”; 25(5), 1304–1317. 2018.

54. C. Susal, H. Maier, K. Lorenz and G. Opelz. “Association of IgAantiFab autoantibodies with disease stage in headandneck cancer”. International Journal of Cancer; 57(1), 47–50. 1994.

55. S.J. Thomas, J.A Snowden, M.P. Zeidler and S.J. Danson. “The role of JAK/STAT signalling in the pathogenesis, prognosis and treatment of solid tumours”. British Journal of Cancer; 113(3), 365–371. 2015.

56. L.V. Wehbi and K. Tasken. “Molecular Mechanisms for cAMP-Mediated Immunoregulation in T cells Role of Anchored Protein Kinase A Signaling Units”. Frontiers in Immunology; 7(22), 1–19. 2011.

57. P.J. Sarvaiya, D. Guo, I. Ulasov, P. Gabikian and M.S. Lesniak “Chemokines in tumor progression and metastasis”. Oncotarget; 4(12), 2171–2185. 2013.

58. S. Demoka. “Identification of guanine nucleotide-binding protein -7 as an epigenetically silenced gene in head and neck cancer by gene expression profiling”. International Journal of Oncology; 42(4), 1427–1436. 2013.