Head and neck squamous cells carcinoma, DNMT3B gene and folate pathway: A review

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Abstract

Introduction

The head and neck cancer includes the oral cavity (40%), pharynx (25%) and larynx (15%), and is considered the fifth most common type in the world and is associated with a high mortality rate when diagnosed in advanced stages. The most common histological type (90% of cases) is squamous cell carcinoma. The main risk factors include the consumption of tobacco and alcohol, viral infections and deficiencies or imbalances of vitamins and micronutrients such as folate. Accumulative genetic alterations have been associated with phenotypic progression of SCCHN, resulting in the inactivation of several tumour suppressor genes and activation of oncogenes. DNA methylation is a modification that has multiple functional roles, including control of gene expression, chromatin structure stability and maintenance of genomic stability. DNA methylation is the transfer of methyl groups to position 5 of cytosine residues located in cytosine-guanine dinucleotides (CpG) through reactions catalysed by proteins named DNA methyltransferases (DNMTs). An abnormal methylation may play an important role in the development of several diseases, especially by acting directly on the process of tumorigenesis and/or silencing tumour suppressor genes containing CpG islands in their promoter regions. DNMT3B gene contains single nucleotide polymorphisms (SNPs) that affect gene function. Your overexpression has been associated with inactivation of tumour suppressor genes, suggesting an oncogenic role. The objective was to perform a review of literature on genetic alterations of DNMT3B genes and risk of head and neck cancer.

Conclusion

According to the results of the studies described in the literature in HNSCC and polymorphisms in the DNMT3B gene, it is concluded that it has a difference in genotype frequency for the different ethnic groups in the world.

Introduction

Squamous Cells Carcinoma of Head and Neck

The head and neck cancer includes the oral cavity (40%), pharynx (25%) and larynx (15%), and is currently considered the fifth most common type in the world and is associated with a high mortality rate when diagnosed in advanced stages1,2. The most common histological type (90% of cases) is squamous cell carcinoma. About two thirds of patients with this disease have advanced stage, usually involving regional lymph nodes3.

Despite aggressive treatment approaches and disciplines, the survival rate of five years remains unchanged over the past 40 years and only 30% to 40% of patients achieved this index. The treatment failures occur in the form of recurrent locoregional, which affects approximately 60% of patients usually with distant metastases develop in 15% to 25% of patients, and the appearance of a second primary tumour (another tumour metastasis not derived from the first or no connection with the first tumour)4,5.

The last estimated worldwide number for oral cavity cancer in 2011 was 263,900 new cases and 128,000 deaths (including lip cancer), for nasopharyngeal cancer were 84,400 incident cases and 51,600 deaths in 20086. Another estimate, made by the International Agency for Research on Cancer (Globocan Project) shows that cancer of the head and neck was the 7th in incidence in 2008 and when added the incidence of cancers of the oral cavity including lip, nasopharynx, other regions of the pharynx and larynx total 5.1% of all cancers in the world. In mortality it was the 7th and 6th in prevalence from 2003 to 2008 (Table 1)7.

The main risk factors include the consumption of tobacco and alcohol, viral infections, especially with the Epstein-Barr virus and Human Papillomavirus subtypes 16 and 18 and deficiencies or imbalances of vitamins and micronutrients such as folate, vitamins A, C and E, zinc and selenium8-10.

The most affected group is males with advanced age (average 60 years)10, however, the incidence of cancer at the base of the tongue and tonsils has increased in people aged less than 45 years and this is attributed to the increased prevalence of the HPV virus that contributes to the development of malignancy in developing countries10,11.

Smoking and drinking, when acting together, multiply the risk for this type of cancer, especially for the oral cavity and pharynx, because a cigarette has approximately 4,700 chemical substances and at least 50 of these are carcinogenic and the frequent consumption of alcoholic drink prevents epithelial cells forming the protective barrier against external agents, thus permitting easy entry for the carcinogenic cigarette agents that form adducts of DNA that are not recognized during the process of DNA replication and repair12,13,14.

According to Hashibe et al.12 isolated consumption of alcohol in excess, showed an increased risk of cancer for...
the oropharynx, hypopharynx and larynx in individuals that never smoked. The excessive consumption can affect nutrient absorption by the intestine, causing major nutritional deficiencies and modifying metabolic pathways, such as in the folate pathway, which for example, is responsible for insertion of methyl groups in the metabolic pathway responsible for DNA methylation, purine synthesis and pyrimidines. Therefore, there may be a compromise of genes with a potential role in carcinogenesis as a consequence of abnormal methylation.12, 15,16

Accumulative genetic alterations have been associated with phenotypic progression of SCCHN, resulting in the inactivation of several tumour suppressor genes and activation of oncogenes. Among them, p16INK4A on chromosome 9p and RASSF1A on chromosome 3p which are two important tumour suppressor genes epigenetically inactivated in the early stages of dysplasia from normal mucosa. Over expression of DNA methyltransferase 3B (DNMT3B) was also associated with inactivation of both p16INK4A and RASSF1A, suggesting an oncogenic role of the DNMT3B during tumorigenesis.17,18,19, 20,21 The DNMT3B gene is responsible for de novo methylation, in other words is methylation of DNA regions previously methylated or unmethylated. Correct methylation is important for correct expression of genes. Therefore, normal functioning of DNMT3B is important for correct expression of tumour suppressor genes and oncogenes for control or silencing.

DNA methylation and DNMTs
DNA methylation is a modification that has multiple functional roles, including control of gene expression, chromatin structure stability and maintenance of genomic stability.22,23

DNA methylation is the transfer of methyl groups to position 5 of cytosine residues located in cytosine-guanine dinucleotides (CpG) through reactions catalysed by proteins named DNA methyltransferases (DNMTs).

An abnormal methylation may play an important role in the development of several diseases, especially by acting directly on the process of tumorigenesis and / or silencing tumour suppressor genes containing CpG islands in their promoter regions. Therefore, DNA methylation mediated by DNMTs is considered an important epigenetic mechanism that regulates the stability of the chromosome and gene expression.24 (Figure 1).

The DNA Methyltransferases are divided into two representatives classes: those involved in methylation of DNA hemimethylated ribbons (ribbons of DNA replication process) known as maintenance methylases such as DNMT1, and another group is responsible for most cases of de novo methylation that occurs at sites without any indication of methylation or without the presence of previous methylation, such as the DNMT2, DNMT3A and DNMT3B genes. Methyl radical donors are obtained by diet, mainly by methionine, followed by folate, vitamin B12 and choline.

Studies showed that polymorphisms in the DNMT3B gene may influence the activity of the respective enzyme in DNA methylation and contribute to the pathogenesis of some diseases, especially cancer.21,30,31,32

The objective of this study was to investigate the results of the literature related to SCCHN and DNMT3B gene polymorphisms (-149C>T, -283T>C and -579G>T). We searched the database works of PUBMED for the last 9 years (from 2005).

Discussion
Single nucleotide polymorphisms (SNP) and the development of SCCHN
Single nucleotide polymorphism is the change of a single base in the DNA sequence that can lead to changes in the expression of important genes. There are many publications in the literature related to DNMT3B gene polymorphisms and risk of Squamous Cell Carcinoma of Head and Neck, which show conflicting results.4,6,33,34,35

The DNMT3B gene is located on chromosome 20q11.2, is composed of 23 exons and 22 introns, contains

Figure 1: 1- DHFR – Dihydrofolate reductase; 2- SHMT – Serine hydroxymethyltransferase + B6; 3- MTHFR – Methyltetrahydrofolate reductase + B2; 4- TS – Thymidylate Synthase; 5- MTR – 5-Methyltetrahydrofolate-Homocysteine Methyltransferase + B12; 6- MAT – Methionine Adenosyltransferase; 7- DNMT3B – DNA (cytosine-5-)methyltransferase 3 beta 8- SAHH – 5-Aminyl-L-homocysteine hydrolase.

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single nucleotide polymorphisms (SNPs) that affect gene function. Your overexpression has been associated with inactivation of tumour suppressor genes, suggesting an oncogenic role.

There are three polymorphisms that have been studied in some countries and diseases, especially in the development of cancer in different tissues such as the lung, breast, liver, oesophagus, colorectal, and head and neck. Were found only five articles that evaluated the DNMT3B gene in risk of Squamous Cell Carcinoma of Head and Neck. Many studies focus on two polymorphisms in this gene: in the C46359T (-149 bp from the transcription start region, rs2424913) and G39179T (-579 bp from the transcription start region of exon 1B, rs1569686) in the promoter region. There is still the polymorphism -283T>C (rs6087990) present at the transcription site of exon 1A of the gene.

The polymorphism -149C>T (rs2424913), was correlated with development of carcinomas, colorectal and lung cancers in the U.S., breast, gastric and liver in China and lung in Korea. This polymorphism was associated with an increase of 30% in gene activity in a study performed in the United States in lung cancer with 659 individuals. The presence of the polymorphism was reported to be substantial for promoter activity, resulting in an individual predisposition to some cancers.

The polymorphism -579G>T (rs1569686) was also associated with increased activity of the DNMT3B gene and was correlated with risk of lung cancer in the Asian population. Studies show that the polymorphic allele -579T of this gene reduces the risk for adenocarcinoma, a survey conducted in Korea with 496 patients, and colorectal cancer, a study in China with 445 patients. The study by Lee et al, with 864 patients also showed that the association of wild alleles of -283T>C and -579G>T polymorphisms decreases the risk of lung cancer in the Asian population in relation to polymorphic alleles. The DNMT3B -579G>T polymorphism was not associated with disease development. However, when DNMT3B polymorphisms -149C>T and -579G>T were evaluated together, individuals with both genotype -149TT and -579TT had a higher risk of developing cancer of the head and neck in a study performed in the United States with 1675 individuals, cases and controls.

The exchange of one thymine base by a cytosine at position -283 of the gene DNMT3B is considered as a possible precursor in the process of tumorigenesis showing a 50% reduction of gene activity in an in vitro study. Cancer research showed that the polymorphic variant DNMT3B-283T>C was associated with increased risk for lung cancer in a study with 864 individuals in Korea, however, in colorectal cancer the same polymorphism was not associated with the risk of this disease in a study with 326 individuals in Poland.

In 2007, the study performed with 226 patients with HNSCC and 475 controls in Taiwan, for polymorphism -283T>C only found one patient with homozygous wild genotype in the control group and the polymorphism -579G>T did not find GG (wild) genotype in the same group, showing a frequency between 85% and 89% for polymorphic homozygous genotypes for these SNPs in both groups, cases and controls. As for the polymorphism -149C>T, the C allele was not found in both groups, in other words, all of the patients, as much the case group (226 patients with SCC) as the control group (249 individuals without cancer history) had the polymorphic genotype (TT), where all tests were performed by MALDI-TOF technique.

Another study by the same researcher in Taiwan, in the same gene and polymorphisms, however only in nasopharyngeal cancer (the head and neck region), the same frequencies for these two polymorphisms was reported, however the GG genotype of -579G>T polymorphism was found in the frequency in only 0.8% of the case group, only 2 patients, and again not in the control group. In evaluating the polymorphism -283T>C this time one patient was reported for each group, and 0.4% of 259 cases and 250 controls. And furthermore, the average percentage of each genotype, -283T>C and 579G>T, remained practically the same as the previous job, between 84% and 89% for polymorphic genotype.

In contrast, the study performed in 2008 in southern U.S. (Houston, Texas) in 832 non-Hispanic whites patients with HNSCC and in 843 individuals of control group, the result was different from studies in patients with Asian descendants. According to the author, the results found that genotypic population was as follows: the polymorphism -149C>T had 31.1% of CC, 46.2% of CT and for TT 22.7% for case group and 31.6% of CC, 51.4% of CT and 17.1% of TT for the control group, polymorphism -579G>T had 35.9% of GG, 45.2% of GT and 18.9% of TT for the control group and 36.2% for GG, 47.6% for GT and 16.2% for TT in the control group, the results were homogeneous, and different genotype frequencies were found in Asians. The research for polymorphism -283T>C was not performed in this study.

Table 1: Globocan Project statistics for cancers of head and neck. *Including other regions of the pharynx.

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Incidence</th>
<th>Mortality</th>
<th>5-year prevalence (2003 to 2008)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (%)</td>
<td>Number (%)</td>
<td>Number (%)</td>
</tr>
<tr>
<td>Oral cavity include Lip</td>
<td>263.020</td>
<td>2.1</td>
<td>127.654</td>
</tr>
<tr>
<td><strong>Pharynx</strong></td>
<td>221.063</td>
<td>1.8</td>
<td>147.159</td>
</tr>
<tr>
<td><strong>Larynx</strong></td>
<td>150.677</td>
<td>1.2</td>
<td>81.892</td>
</tr>
</tbody>
</table>

*Including other regions of the pharynx.
On a study conducted in Canada and published in 2012, evaluated were various polymorphisms in genes related to DNA repair, cell cycle control, metabolism of xenobiotics, inflammatory process and immunological growth factor and developing / methylation DNA. In this study, the authors examined whether treatment with Alpha-tocopherol/ beta-caroteno in patients diagnosed with SCC would have a lower chance to not develop a second primary cancer (SPC). 531 patients with squamous cell carcinoma of head and neck that had stages I and II lesions and treated with radiotherapy were analysed, of whom 312 (59%) were alive after five years of diagnostic. The results showed that there was no relationship between the DNMT3B gene and disease-free survival in patients. The polymorphism -149C>T genotype and the frequency of 35% for genotype CC, 47% for CT and 18% for TT, shows a similarity to the Study conducted in the USA. 6

Another study published in 2012 by the same authors and the same group of patients, analysed the influence of various polymorphisms in the chance of developing a secondary primary cancer had the same 531 SCC patients. A total of 111 (21%) had secondary primary cancers, including the lung (45.95%), in the region of the head and neck (11.71%), prostate (10.81%), basal skin (9.01%), colorectal (8.11%) and in other regions: kidney, breast, squamous cell skin, oesophagus, bladder, myeloma, hypothalamus and unspecified (14.4%). The authors evaluated the polymorphism -283T>C and -579G>T, in addition to -149C> T, with the same result as the other study, 35% CC, 47% CT and 18% TT. The genotypic frequencies for the other two most common SNPs of the gene were to -283T>C 36% TT, 49% TC and 15% CC and to -579G>T frequency was 36% GG, 48% GT and 16% TT. According to the author the C-149T polymorphism of the DNMT3B gene was strongly associated with the development of SPC in the analysis of the polymorphic allele TT compared to CC wild. 10 All frequencies of SNPs from cited studies are in table 2.

### Table 2: Genotypes in case numbers and percentages of each research of DNMT3B in SCCHN.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Country</th>
<th>Group</th>
<th>-149C&gt;T</th>
<th>-283T&gt;C</th>
<th>-579G&gt;T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chang et al.</td>
<td>2007</td>
<td>Taiwan</td>
<td>Case</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chang et al.</td>
<td>2008</td>
<td>Taiwan</td>
<td>Case</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Liu et al.</td>
<td>2008</td>
<td>USA</td>
<td>Case</td>
<td>31.1</td>
<td>46.2</td>
<td>22.7</td>
</tr>
<tr>
<td>Azad 1</td>
<td>2011</td>
<td>Canada</td>
<td>Case</td>
<td>35</td>
<td>47</td>
<td>18</td>
</tr>
<tr>
<td>Azad 2</td>
<td>2012</td>
<td>Canada</td>
<td>Case</td>
<td>35</td>
<td>47</td>
<td>18</td>
</tr>
</tbody>
</table>

### Conclusion

According to the results of the studies described in the literature in HNSCC and polymorphisms in the DNMT3B gene, it is concluded that it has a difference in genotype frequency for the different ethnic groups in the world. SNPs of this gene may be related to the increased chance of developing cancer in the head and neck, since there is a higher prevalence of this disease in Asian populations, which may be related to the large number of patients with the presence of polymorphic alleles in the DNMT3B gene.

### References


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All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.

All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

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