Clinical Management of Head and Neck Cancer Cases: Role of Pharmacogenetics of CYP2 and GSTs

Munindra Ruwali\textsuperscript{a} Ankur Dhawan\textsuperscript{b,c} Mohan C. Pant\textsuperscript{b} Qamar Rahman\textsuperscript{c} S.M. Paul Khurana\textsuperscript{a} Devendra Parmar\textsuperscript{d}

\textsuperscript{a}Amity Institute of Biotechnology, Amity University Haryana, Manesar, India; \textsuperscript{b}Department of Radiotherapy, King George’s Medical University, Lucknow, India; \textsuperscript{c}Amity University, Lucknow Campus, Lucknow, India; \textsuperscript{d}Developmental Toxicology Division, CSIR-Indian Institute of Toxicology Research, Lucknow, India

\textbf{Introduction}

The term head and neck squamous cell carcinoma (HNSCC) is used to describe a wide range of malignant tumors originating in the upper aerodigestive tract including the oral cavity, larynx, pharynx, and nasopharynx. The majority (90%) of head and neck cancers are squamous cell carcinomas arising from the epithelial membranes (mucous lining) of these regions [1], due to which they have many common features relating to their etiology and classification. Throughout the world, the annual incidence of head and neck cancer is more than 550,000 cases [2]. The main treatment options are surgery, radiotherapy (RT), and chemotherapy. However, the type of treatment used depends on the site and stage of the disease as well as on the patient’s overall health status. In the case of locally advanced head and neck cancer, chemoradiotherapy (CRT) is an important option, while patients with advanced (metastatic) or recurrent disease have limited treatment options. Although chemotherapy has been in use for many years, patients with advanced (metastatic) or recurrent head and neck cancer still have a poor prognosis, with a median survival of 6–10 months [3]. Of the various treatment options, RT is the most prevalent mode of treatment used in nearly 75% of all head and neck cancer patients with either curative or palliative intent. A multimodality approach uses RT along with other treatment modalities available for the clinical management of HNSCC followed by a description of the contribution of genetic variations to chemotherapeutic toxicity and response. Furthermore, studies addressing the association of genetic variants of drug-metabolizing enzymes with treatment response in head and neck cancer are also discussed.

\textbf{Keywords}

Head and neck squamous cell carcinoma · Radiotherapy · Chemotherapy · Chemoradiotherapy · Treatment response · Pharmacogenomics

\textbf{Summary}

Head and neck squamous cell carcinoma (HNSCC) describes a wide range of malignant tumors which originate in the upper aerodigestive tract and have a multifactorial origin involving both genetic and lifestyle risk factors. The clinical management of head and neck cancer involves surgery, radiotherapy, and chemotherapy. With the advances in treatment strategies for HNSCC, newer targeted therapies are adding to the progress already achieved in the multimodality management of patients although the problems of differences in drug response and adverse drug reactions are still grave concerns. Cancer pharmacogenomics has fast emerged as a new and promising field for the early identification of genetic markers that can predict drug response or toxicity. This could greatly help in identifying genetic markers useful for the selection of optimal drugs, dose, and treatment duration on an individual basis resulting in improved drug efficacy and decreased toxicity. This review focuses on the various treatment modalities available for the clinical management of HNSCC followed by a description of the contribution of genetic variations to chemotherapeutic toxicity and response. Furthermore, studies addressing the association of genetic variants of drug-metabolizing enzymes with treatment response in head and neck cancer are also discussed.

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Munindra Ruwali and Ankur Dhawan have contributed equally to the manuscript.
Clinical Management of Head and Neck Cancer

Radiation Therapy for HNSCC Treatment

RT designs have seen significant changes over the past few years. There has been a transformation from use of two-dimensional (2D) to three-dimensional (3D) images with increasingly complex computer algorithms [6]. Two-dimensional radiation therapy (2DRT) includes a single beam from 1–4 directions with the radiation fields designed on 2D fluoroscopic simulation images. However, lower survival rates and significant adverse effects of 2DRT led to the widespread adoption and application of conformal RT methods. In conformal RT, cytotoxic radiation beams are 'shaped' to cover the tumor volume including a surrounding tissue margin. The Internal Commission on Radiation Units and Measurements (ICRU) has introduced terms such as gross tumor volume (GTV) which deals with identification of gross disease by physical examination and imaging, clinical target volume (CTV) which includes the GTV and potential areas at risk of developing disease, and planning target volume (PTV) which is an expansion of the CTV by a small margin (a few mm) to adjust for variables such as movement of the patient and the organ [7]. Various modalities of conformal external beam photon-based RT include three-dimensional conformal radiation therapy (3DRT), intensity-modulated radiation therapy (IMRT), stereotactic body radiation therapy (SBRT), and charged particle-based conformal external beam therapy such as proton beam radiotherapy (PBRT) [6, 8, 9]. The protocol for 3DRT includes 60–70 gray (Gy) delivered in 25–40 fractions (usually 1.8–2 Gy) delivered over a period of 5–10 weeks.

IMRT represents an advancement from 3DRT delivering a high dose of ionizing radiation conformally to the target volume while causing minimal damage to the surrounding healthy tissues. This modality typically includes a total dose of 60–70 Gy delivered in 25–40 fractions over a period of 5–10 weeks. With greater control of beam intensity and shape of radiation, this modality causes considerably lower xerostomia with substantially increased quality of life [6, 8]. In an effort to reduce the number of treatment sessions for the patients while delivering large doses of radiation, SBRT was developed. In this modality, the tumor is located using several computed tomography imaging techniques in 4 dimensions. This modality generally comprises a total dose of 60 Gy at > 10 Gy per fraction in 5 or fewer fractions, and can deliver very high doses above 100 gray equivalent (GyE) [9]. Another modality, PBRT, has the edge over photon therapy due to the lack of an 'exit dose', allowing physicians to deliver high energy conformal doses to the tumor volume with almost negligible harm to healthy tissue.

Chemoradiotherapeutic Approaches for HNSCC Treatment

Chemoradiotherapeutic Approaches for HNSCC Treatment

Another significant approach in the treatment of HNSCC has been concurrent CRT. For the treatment of patients with resectable and/or inoperable locally advanced head and neck cancers, RT alone was the traditional single treatment which yielded poor results. This led to the development of concurrent CRT [10–12, 16, 17]. The initial agents that were tried in combination with RT included methotrexate, hydroxyurea, 5FU, and bleomycin; however, each of these drugs increased mucositis and stomatitis which worsened the condition of the patients when compared to RT alone. However, when cisplatin was tried with RT, it did not induce mucositis and did not increase the local toxicity of RT. As a result, the complete response rate obtained with concurrent cisplatin and RT (single daily fraction) in patients with locally advanced head and neck cancer ranged from 65 to 70% [11, 12, 16, 17]. However, the addition of other agents such as 5FU or taxanes to cisplatin concomitant with RT did not improve the response rate and instead increased local side effects especially mucositis [12, 16].

The other important agent investigated for use in CRT was carboplatin. Carboplatin was used in a weekly schedule concurrent with RT in patients with head and neck cancers, and the complete response rate reported in phase II studies was in the range of 65–70% which was similar to that reported with cisplatin plus RT [11, 12], Mitomycin C, taxanes, and gemcitabine were also tested for radiopotentiation. Mitomycin C showed improved local control but no differences in overall survival [12] while taxanes alone or with other agents given concurrently with RT had a complete response rate of approximately 65% [12] although local side effects such as mucositis remained problematic. Gemcitabine plus RT also exhibited a high complete response rate, but local toxicities such as pharyngeal scarring and stenosis increased [12].
Role of Genetic Polymorphisms in Determining Treatment Outcome: Cancer Pharmacogenomics

Pharmacogenomics deals with the study of the association of inter-individual genetic variation with drug response or toxicity [18]. A recent development has been the evolution of pharmacogenomics into ‘pharmacogenomics’, signaling a shift of focus from individual candidate genes to genome-wide association studies. Such studies are based on a case-control approach wherein genetic markers or mutations across the genome are compared between persons affected by a complex disease or drug-response phenotype and those who are not [19]. Cancer pharmacogenomics has fast emerged for the identification of pharmacogenomic markers that predict drug response or toxicity, since chemotherapeutic drugs usually have narrow therapeutic indices resulting in potentially life-threatening toxicity or non-response to treatment [20]. Identifying genetic markers useful in the selection of optimal drugs, dose, and treatment duration on an individual basis could result in improved drug efficacy and decreased toxicity (fig. 1).

Cancer pharmacogenomics is significantly different from the pharmacogenomics of other complex diseases. First, there is the involvement of 2 genomes in cancer pharmacogenomics: the germline genome of the patient and the somatic genome of the tumor. Inter-individual inherited genetic differences are due to variations in germline genome while variations in tumor genome due to accumulation of acquired somatic mutations play an important role in inconsistent responses seen in patients treated with chemotherapy. The role of somatic mutations is best exemplified by those seen in the tyrosine kinase domain of the epidermal growth factor receptor gene that is associated with response to gefitinib in non-small-cell lung cancer patients [21, 22]. Other examples include HER2 overexpression or amplification in patients with breast cancer and the response of these tumors to trastuzumab [23, 24], and the action of PLX4032 which is a specific inhibitor that targets the mutant activated serine-threonine protein kinase encoded by BRAF. PLX4032 prolongs survival in patients carrying the mutation, and this finding was based on the discovery of a BRAF mutation through the sequencing of a large number of kinase genes in tumors [25, 26]. Apart from somatic mutations, germline genetic variations play a major role in the inter-individual differences in drug metabolism. The other problems associated with cancer pharmacogenomics include the difficulty of performing human studies, administering toxic chemotherapy drugs to healthy individuals, and the involvement of several genes in determining treatment outcome which makes it difficult to pinpoint the role of each individual locus. A possible solution to these problems is to perform a large clinical study to identify the markers followed by validation in a large cohort, although this approach is complicated by the huge expenses involved in clinical studies, consistent drug use and dosage, and concomitant medications or alternative therapies [27, 28].

The role of pharmacogenomics in the effective management of head and neck cancer assumes greater significance in the era of The Cancer Genome Atlas (TCGA) which is a comprehensive and coordinated effort to increase the pace of understanding of the molecular basis of cancer through the application of genome analysis technologies, including large-scale genome sequencing. A recent study published by The Cancer Genome Atlas Network [29] reports a comprehensive genomic characterization of HNSCC. The consortium profiled 279 HNSCC to provide a comprehensive landscape of somatic genomic alterations. The study reported helical domain mutations of the oncogene PIK3CA, novel alterations involving loss of TRAF3, and amplification of the cell cycle gene E2F1. HNSCC associated with smoking had almost universal loss-of-function TP53 mutations and CDKN2A inactivation with frequent copy number alterations including amplification of 3q26/28 and 11q13/22. Oral cancer tumors exhibiting better clinical outcome displayed activating mutations of HRAS or PIK3CA coupled with inactivating mutations of CASP8, NOTCH1, and TP53. Other subgroups of HNSCC contained loss-of-function alterations of the chromatin modifier NSD1, WNT pathway genes AJUBA and FAT1, and activation of oxidative stress factor NFE2L2, particularly in laryngeal tumors.

The clinical application of pharmacogenomics needs to be accelerated in order to translate the laboratory findings into clinical settings. There are several important issues which need to be addressed to achieve this goal. First, there is a need to establish clinical utility of genetic testing so that reimbursement for routine use of pharmacogenetic testing becomes feasible; second, simple clinical formulations should be developed to help physicians interpret and use genetic data; third, mass awareness should be raised amongst all healthcare professionals about clinical genomics; fourth, rapid and cost-effective methods for comprehensive high-throughput pharmacogenomic genotyping must be developed which will address the problems of time, delay, and cost, as physicians will receive genetic information about a patient from a single cost-effective test for many pharmacogenomic variants before prescribing any drug; and lastly, efforts are needed to create comprehensive databases that help physicians search for the impact of specific genetic variants on relevant drugs. Despite these challenges and shortcomings, there has been quite a success in identifying the genetic markers that are associated with differences in response to

**Fig. 1.** a Inter-individual variations in drug response due to genetic variations in drug-metabolizing enzymes. b Drug response can be increased by genetic screening followed by pharmacogenetic evaluation.
chemotherapy. The following section discusses some of these findings and their implications in designing a suitable chemotherapeutic regimen to reduce the incidence of adverse drug reactions and inter-individual differences in response to various chemotherapeutic drugs.

### Table 1. Treatment response in cases of head and neck squamous cell carcinoma (HNSCC) with variant genotypes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotypes</th>
<th>Cases, n (%)</th>
<th>Responders, n (%)</th>
<th>Non-responders, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2A6</td>
<td>*1A/*1A</td>
<td>130 (52.0)</td>
<td>90 (69.2)</td>
<td>40 (30.8)</td>
</tr>
<tr>
<td></td>
<td><em>1A/non</em>1A+non<em>1A/non</em>1A</td>
<td>105 (42.0)</td>
<td>45 (42.8)</td>
<td>60 (57.2)</td>
</tr>
<tr>
<td></td>
<td>1A/*4C+/1B/*4C+/*4C/*4C</td>
<td>15 (6.0)</td>
<td>04 (26.6)</td>
<td>11 (73.4)</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>CYP2C9*1</td>
<td>148 (37.8)</td>
<td>108 (73.0)</td>
<td>40 (27.0)</td>
</tr>
<tr>
<td></td>
<td>CYP2C9*2</td>
<td>142 (36.5)</td>
<td>54 (38.0)</td>
<td>88 (62.0)</td>
</tr>
<tr>
<td></td>
<td>CYP2C9*3</td>
<td>100 (25.6)</td>
<td>36 (35.6)</td>
<td>64 (64.4)</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>CYP2C19*1 (wild-type (Wt))</td>
<td>100 (66.7)</td>
<td>71 (71.0)</td>
<td>29 (29.0)</td>
</tr>
<tr>
<td></td>
<td>CYP2C19*2 (poor metabolizer (PM))</td>
<td>39 (26.0)</td>
<td>10 (26.0)</td>
<td>29 (74.0)</td>
</tr>
<tr>
<td></td>
<td>CYP2C19*3 (PM)</td>
<td>8 (5.3)</td>
<td>4 (50.0)</td>
<td>4 (50.0)</td>
</tr>
<tr>
<td></td>
<td>CYP2C19*2 and *3 (PM)</td>
<td>3 (2.0)</td>
<td>0 (00)</td>
<td>3 (100.0)</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>CYP2D6*1</td>
<td>105 (36.2)</td>
<td>76 (72.4)</td>
<td>29 (27.6)</td>
</tr>
<tr>
<td></td>
<td>CYP2D6*4</td>
<td>106 (36.6)</td>
<td>35 (33.0)</td>
<td>71 (67.0)</td>
</tr>
<tr>
<td></td>
<td>CYP2D6*10</td>
<td>79 (27.2)</td>
<td>26 (32.9)</td>
<td>53 (67.1)</td>
</tr>
<tr>
<td></td>
<td>CYP2D6*/4/*10</td>
<td>62 (21.4)</td>
<td>12 (19.4)</td>
<td>50 (80.6)</td>
</tr>
<tr>
<td></td>
<td>CYP2D6*/2C9*/2C19*2</td>
<td>113 (39.5)</td>
<td>35 (31.0)</td>
<td>78 (69.0)</td>
</tr>
<tr>
<td></td>
<td>CYP2D6*/10/2C9*/2C19*2</td>
<td>108 (37.2)</td>
<td>31 (28.7)</td>
<td>77 (71.3)</td>
</tr>
<tr>
<td>GST</td>
<td>[38] positive (+)</td>
<td>171 (57.0)</td>
<td>81 (47.4)</td>
<td>90 (52.6)</td>
</tr>
<tr>
<td></td>
<td>null (–)</td>
<td>129 (43.0)</td>
<td>70 (54.3)</td>
<td>59 (45.7)</td>
</tr>
<tr>
<td>GST1</td>
<td>positive (+)</td>
<td>223 (74.3)</td>
<td>103 (46.2)</td>
<td>120 (53.8)</td>
</tr>
<tr>
<td></td>
<td>null (–)</td>
<td>77 (25.7)</td>
<td>45 (58.4)</td>
<td>32 (41.6)</td>
</tr>
<tr>
<td>GSTM1 and GSTT1</td>
<td>both positive</td>
<td>135 (45.0)</td>
<td>61 (45.2)</td>
<td>74 (54.8)</td>
</tr>
<tr>
<td></td>
<td>either null</td>
<td>105 (35.0)</td>
<td>60 (57.1)</td>
<td>45 (42.9)</td>
</tr>
<tr>
<td></td>
<td>both null</td>
<td>60 (20.0)</td>
<td>40 (66.7)</td>
<td>20 (33.3)</td>
</tr>
<tr>
<td>GSTP1</td>
<td>Ile/Ile</td>
<td>210 (70.0)</td>
<td>59 (28.1)</td>
<td>151 (71.9)</td>
</tr>
<tr>
<td></td>
<td>Ile/Val+Val/Val</td>
<td>90 (30.0)</td>
<td>47 (52.2)</td>
<td>43 (47.8)</td>
</tr>
</tbody>
</table>

### Association of Genetic Variants of Drug Metabolizing Enzymes with Treatment Response in Head and Neck Cancer

The association of genetic variants of some of the phase I and phase II xenobiotic metabolizing enzymes with treatment response in head and neck cancer has been investigated by our group (table 1). In our studies, cases were subjected to 3 cycles of neoadjuvant chemotherapy (NACT) before RT or concurrent CRT. Each cycle of NACT consisted of cisplatin (50 mg/day) days 1–3 and 5FU (1 g/day) days 1–3 and was administered once every 3 weeks. CRT included administration of 50 mg of cisplatin once every week for 7 weeks along with 70 Gy of RT (200 centigray or 2 Gy/fraction, depending on tumor size) daily for 7 weeks. A study from our laboratory reported that the treatment response in the patients with variant genotypes of CYP2C19 (CYP2C19*2 and CYP2C19*3) was poor, particularly in those with a CYP2C19*2 genotype [30]. A significantly higher number of non-responders was associated with the variant genotypes of CYP2C19 (74% with CYP2C19*2 and 50% with CYP2C19*3).

Another important drug-metabolizing enzyme, CYP2D6, is reported to be involved in the metabolism of anti-cancer drugs such as cyclophosphamide, tamoxifen, and gefitinib [31, 32]. Another study from our laboratory found a prevalence of non-responders among cases with poor metabolizer (PM) genotypes of CYP2D6, which could possibly be attributed to the overall poor drug metabolizing capacity of the PMs [33]. Furthermore, Sailaja et al. [34] have shown that leukemia patients with PM genotypes of CYP2D6 did not respond to the treatment of imatinib leading to drug resistance and poor prognosis. Accumulation of the drug was found to occur in cases with PM genotypes of CYP2D6. Another interesting observation in our study was a much higher prevalence of non-responders among cases with a combination of PM genotypes of CYP2D6, CYP2C9*2, and CYP2C19*2, suggesting that the interaction of CYP2D6 with CYP2C9 or CYP2C19 or both may have a synergistic role in modulating treatment response. Although these enzymes may not have a direct role in the metabolism of cisplatin...
(except CYP2C9 to a small extent), these drug-metabolizing cytochromes P450 are involved in the metabolism of several of the supportive care drugs used in chemotherapy.

Another recent study by Yadav et al. [35] also reported poor treatment response in cases with PM genotype of CYP2C9, which was possibly due to decreased availability of metabolites in PMs. Studies have also been carried out to examine the effect of genetic variations on the metabolism of chemotherapeutic drugs such as cyclophosphamide and ifosfamide. Another study from our laboratory further evaluated the role of CYP2A6 in modulating the treatment outcome in HNSCC cases [36]. The treatment response was poor, particularly in cases with at least 1 deletion allele of CYP2A6. Furthermore, we also investigated the role of genetic variations in phase II xenobiotic metabolizing enzymes, namely glutathione S-transferases (GSTs), in modifying the treatment response in HNSCC cases. Cases deficient in GSTM1 or GSTT1 exhibited superior treatment response when compared to those with wild-type genotype, which could be due to higher levels of circulating reactive oxygen species (ROS) in these cases owing to the deficient detoxification of these reactive intermediates. It has been suggested that RT and chemotherapy exert their anticancer effects by generating ROS and their byproducts which are generally detoxified by GSTs, superoxide dismutases, catalases, and glutathione peroxidases [37].

For GSTP1, cases of HNSCC with variant genotypes of GSTP1 exhibited a superior treatment response with a higher number of responders [38]. GSTP1 is involved in platinum resistance as demonstrated by in vitro studies showing its significant participation in detoxification of platinum compounds used in chemotherapy [39]. Increased treatment response in cases with variant genotypes of GSTP1 could thus be attributed to a lowered detoxification of platinum compounds and subsequent increase in bioavailability and efficacy of platinum-based compounds in the treatment of HNSCC.

**Effect of Genetic Variants on Metabolism of Chemotherapeutic Drugs**

Genetic variants have an influence on the metabolism of most of the commonly used chemotherapeutic drugs. A lowering of CYP2C19 activity could have an impact on the efficacy and toxicity of chemotherapeutic agents and other drugs used in standard oncology since PMs of CYP2C19 exhibit little response to the respective chemotherapy compared to the normal genotype [40, 41]. Reduced activity of CYP2C19 also reduces the metabolic activation of chemotherapeutic drugs like cyclophosphamide and ifosfamide with subsequent lowering of the toxicity risk but worsening of the therapeutic response [42]. Another drug, omeprazole, metabolized by CYP2C19 to its inactive metabolite, 5-hydroxomeprazole, results in superior acid suppression and higher cure rates in persons who are CYP2C19 PMs while ultrarapid metabolizers have a risk of therapeutic failure [43]. In vitro studies demonstrated that wild-type CYP2C9 was more efficient than the mutant allele CYP2C9*3 in cyclophosphamide 4-hydroxylation and ifosfamide 4-hydroxylation [44]. Another example of a genetic variant influencing the metabolism of chemotherapeutic drugs includes that of CYP2A6 which catalyzes the bioactivation of tegafur, a prodrug of 5FU. A decrease in CYP2A6 activity due to reduced activity alleles could have an impact on the efficacy of tegafur [45]. There have also been clinical studies that showed lowered GST activity resulting in an increase in efficacy of chemotherapeutic drugs like 5FU, cisplatin, and paclitaxel [46] while high plasmatic GSTP1 levels could confer protection of cisplatin DNA adduct formation and consequently decrease the antitumor activity of platinum-based compounds [47].

In conclusion, the availability of combination chemotherapy and RT regimens for HNSCC treatment is faced with the problem of inter-individual differences in drug response and adverse drug reactions. These inter-individual differences are mainly due to differences in the genotype of the patients, which has led to the need for better information to individualize cancer therapy based on genotype. Pharmacogenetics and pharmacogenomics have emerged as important tools to search for answers surrounding the hereditary basis for inter-individual differences in drug response. This will help in clinical decision making regarding treatment strategies, with the goal of avoiding adverse drug reactions while achieving the best drug response. Future progress in this area will likely require a combination of patient cohort studies as well as in vitro studies, and the effective implementation of pharmacogenomic findings into clinical practice to facilitate effective treatment of HNSCC.

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