Abstract

Objectives: Although TP53 mutations in head and neck squamous cell carcinoma (HNSCC) have been extensively studied, their association with the different subsites in the head and neck region has never been described. Methods: Sanger sequence analysis evaluating exons 4–9 in the TP53 gene was performed on 116 HNSCC patients. The exon location, exact codon and corresponding substitution in relation to the anatomical site (subsite) of the HNSCC were evaluated. Results: We found nonsynonymous TP53 mutations in 70% (81/116) of the patients. In oral cavity carcinomas, most mutations occurred in exon 7 (37%). In oropharyngeal and laryngeal tumors, mutations were mainly found in exons 6 and 7. The most common mutation was located in codon 220, and all of these were an Y220C mutation. Five out of nine (56%) Y220C mutations occurred in oropharyngeal tumors. Additionally, 22% of all mutations observed in oropharyngeal squamous cell carcinoma (OPSCC) consisted of Y220C mutations. Conclusion: In this study, the subsite-related distribution of TP53 mutations underlines the biological diversity between tumors arising from different anatomical regions in the head and neck region. Moreover, the Y220C mutation was by far the most prevalent TP53 mutation in HNSCC and a relative hotspot mutation in the oropharynx.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide. HNSCC is an anatomically heterogeneous group, usually involving the oral cavity, oropharynx, hypopharynx and larynx [1]. The behavior of these tumors originating from different anatomical locations differs and they show variations in gene profile [2].

Major risk factors of HNSCC are excessive alcohol consumption, tobacco use and infection with human papillomavirus (HPV), in particular HPV type 16 [3, 4]. Currently, HPV-positive HNSCC is considered a distinct molecular and clinical entity compared to HPV-negative HNSCC [5, 6]. In both HPV-positive and HPV-negative tumors, inactivation of the tumor suppressor gene TP53 is an important pathogenetic event in the carcinogenesis
TP53 is pivotal in cell cycle regulation, apoptosis, DNA replication and repair, as illustrated by the fact that aberrations in this gene are observed in the majority of malignancies [7–9]. When TP53 is inactivated, it can lose its tumor-suppressive properties and thereby endorse cell growth and improve the survival of oncogenic cells. Consequences of TP53 inactivation are tumor formation, increased genetic instability, proliferation and metastasis [8, 10]. Additionally, some mutations show a gain-of-function resulting in oncogenic effects [11, 12]. The biology of TP53 is complex and mutations of this gene have an impact in nearly all of the cancer hallmarks proposed by Hanahan and Weinberg [13].

Two different mechanisms can cause TP53 inactivation in HNSCC and seem to be mutually exclusive [14]. The first mechanism is p53 inactivation by HPV in HPV-positive HNSCC (mostly OPSCC). These tumors contain the E6 protein that binds and inactivates p53 [1, 15, 16]. Therefore, HPV-positive tumors are characteristically TP53 wild-type tumors [17]. The second mechanism of TP53 inactivation occurs in HPV-negative HNSCC. These tumors often show TP53 mutations which are associated with and induced by excessive use of tobacco and alcohol [18, 19]. These mutations lead to structural inactivation of the TP53 gene and subsequent loss of the p53 protein. In HNSCC, TP53 shows a mutation rate varying between 60 and 80% [1].

TP53 mutation analysis of multiple tumors is routinely used to discriminate between a metastasis and a second primary tumor as it is considered an early event in carcinogenesis, a non-hotspot mutation (a feature shared by tumor suppressor genes) and it has a high mutation rate (approx. 70%) [20]. TP53 mutation frequencies and profiles of HNSCC have been explored widely, though the anatomical subsites of primary HNSCCs in the context of nonsynonymous mutations of TP53 have not been evaluated before [19, 21]. As carcinogenesis of HNSCC shows site-specific clinical and molecular features, we wondered whether specific TP53 mutations occurred more often at particular subsites. The goal of this study is to investigate the prevalence of nonsynonymous TP53 mutations in HNSCC, their exact codon and corresponding substitution and their relation to the anatomical subsite.

Materials and Methods

Patients
All 116 patients with a histologically confirmed HNSCC and registered TP53 mutation analyses between January 2008 and October 2013 at the University Medical Center Utrecht were retrospectively analyzed. We excluded all patients with wild-type TP53, polymorphisms and deletions or insertions in TP53, which left 81 patients for further analysis. Demographical and clinical data were retrieved from hospital charts. If a clonal relationship based on TP53 mutation could not be ruled out, we used 5 years as a cut-off value for a cure. If a patient had a new tumor after 5 years, we interpreted it as a second primary tumor and extracted the TNM classification and incidence date from that tumor. The history of tobacco and alcohol use was reported. Nonsmokers and nondrinkers were defined as patients who never, rarely (i.e. ≤2 drinks a day) or had stopped smoking/drinking >20 years before being diagnosed with a head and neck tumor. Former drinkers or smokers were defined as patients who quit more than 1 year before being diagnosed with head and neck cancer. Finally, smokers and drinkers were defined as patients with moderate or heavy use of cigarettes (at least 10 pack-years) and alcohol (>2 drinks a day). HPV status was not routinely tested in this cohort. According to Dutch national ethical guidelines, no ethical approval is necessary to use leftover material for scientific purposes. The use of anonymous leftover material for scientific purposes is part of the treatment agreement with patients at the University Medical Center Utrecht [22].

Molecular Analysis
Paraffin-embedded biopsy specimens were analyzed for mutations in exons 4–9 in the TP53 gene. A dedicated head and neck pathologist (S.W.) identified tumor areas on HE slides. Only samples with a tumor percentage of at least 20% were included for analysis of the TP53 mutation. After deparaffinization, tumor and normal tissue were scraped off the unstained slides using a scalpel. DNA was isolated using a DNA sample preparation kit according to the manufacturer’s protocol (Roche 05985536190). To analyze the TP53 mutation, target sequences were amplified by PCR using primer pairs (shown in online suppl. table S1; www.karger.com/doi/10.1159/000369102). PCR products of TP53 were Sanger-sequenced in forward and reverse directions. The sequence products were analyzed on a 3730 DNA Analyzer (Applied Biosystems) using sequence analysis.

Statistical Analysis
Statistical analyses were conducted using IBM SPSS 20.0 statistical software. The clinical and demographic findings were analyzed with respect to TP53 mutations with the use of the Fisher exact test. Overall survival was estimated by means of the Kaplan-Meier method and survival curves were compared by use of the log-rank test. p < 0.05 was considered statistically significant.

Results

Patient Characteristics
The original molecular database including TP53 mutation analysis from January 2008 to October 2013 consisted of 116 patients with an HNSCC. Of these patients, 22 (19%) had wild-type TP53, 13 (11%) had a deletion or insertion of TP53 and 81 (70%) had a nonsynonymous TP53 mutation. These 81 patients, with a total of 94 non-
synonymous TP53 mutations, were enrolled in our study, i.e. 70% of our study population had nonsynonymous mutations in exons 4–9 of the gene TP53. Table 1 summarizes the characteristics of our study group of patients with nonsynonymous TP53 mutations.

The most common primary tumor subsites were the oral cavity (31%), oropharynx (24%) and larynx (18%). Sixty-eight percent of the patients presented with late-stage III or IV cancer, a common feature of head and neck cancer as it is often diagnosed at a late stage. Most patients were current smokers (51/81) or had smoked in the past (14/81). Of the included patients, 53% reported excessive alcohol use at the time of diagnosis and 17% had stopped drinking alcohol. The HPV status was known for 13 patients and was negative in all these cases. We did not investigate the HPV status in the other patients as tumors with wild-type TP53 were excluded.

**Mutation Analysis**

In total, 94 nonsynonymous mutations were detected in 81 patients. Nine patients had 2 primary tumors in the head and neck region with different TP53 mutations, and 4 tumors had tandem mutations of the TP53 gene. Overall, the mutations were most commonly located in exon 5 (n = 20), exon 6 (n = 25), exon 7 (n = 26) and exon 8 (n = 17). The total number of mutations across these exons was distributed equally. The findings concerning TP53 mutations divided by exons distributed over the different subsites in the head and neck region are shown in Table 2. Mutations in tumors arising in the oral cavity mostly occurred in exon 7 (35%), in the oropharynx in exon 6 (30%) and in the larynx in exons 6 (29%) and 7 (29%).

Figure 1 shows the nonsynonymous mutation frequency of all TP53 mutations in our study population. All but 3 mutations were located in the DNA-binding domain and the most common mutation occurred at codon 220 (exon 6). Strikingly, all these mutations (n = 9) were Y220C mutations, replacing tyrosine for cysteine. Except in 1 case, all patients with this mutation were classified as smokers and more than half of these tumors that had this mutation (5/9) were located in the oropharynx. Two patients with this mutation never drank alcohol. Moreover, >20% (5/23) of all the mutations observed in OPSCC were Y220C mutations. There was no association between gender and Y220C mutation frequency in the oropharynx (p = 0.343) or between age and Y220C mutation frequency in this region (p = 0.117). Other relative mutation hotspots occurred in exon 6 at codon 193 and exon 7 at codons 245 and 248. The hotspot mutations in these codons showed a wider variety than in codon 220. In codon 193, the histidine was substituted by either leucine or arginine. In codon 245, glycine was substituted by either serine or aspartic acid as shown in Table 3. In both codons, mutations were equally distributed across the different subsites. Six mutations were reported in codon 248, replacing arginine for tryptophan or glutamine. Half the number of this hotspot mutation occurred in the oral cavity. In addition, 10% of all mutations in the oral cavity occurred in codon 248. No significant associations between the mutations in codon 248 in HNSCC and clinical variables were found (i.e. age, smoking, alcohol use, gender and tumor stage).

| Table 1. Characteristics of the study population |
|-----------------------------------------------|
| Characteristics | |
| **Patient characteristics** | |
| Patients | 81 (100) |
| Tumors | 90 (100) |
| Mean age, years (range) | 62 (40–86) |
| Sex | |
| Male | 51 (63) |
| Female | 30 (37) |
| Smoking historyb | |
| Never | 14 (17) |
| Former | 14 (17) |
| Active smoker | 51 (63) |
| Alcohol useb | |
| Never | 14 (17) |
| Former | 5 (6) |
| <2 units/day | 17 (21) |
| 2–6 units/day | 30 (37) |
| >6 units/day | 13 (16) |
| **Tumor characteristics** | |
| Clinical stagea,b | |
| I | 18 (20) |
| II | 6 (7) |
| III | 20 (23) |
| IV | 44 (48) |
| Subsite of the primary tumor | |
| Oral cavity | 29 (31) |
| Oropharynx | 23 (24) |
| Larynx | 17 (18) |
| Hypopharynx | 10 (11) |
| Esophagus | 13 (14) |
| Unknown | 2 (2) |

All values are n (%) unless otherwise indicated.

a According to the TNM staging system of the American Joint Committee on Cancer.
b In 2 patients, the history of smoking and alcohol consumption and the clinical stage were unknown.
Survival Analyses

The presence of any TP53 mutation was not associated with a significant different overall survival compared to wild-type HNSCC in our cohort [log rank p = 0.344, hazard ratio 1.394 (0.70–2.78) p = 0.346]. The mean survival was 28 months for wild-type TP53 and 26 months for patients with a TP53 mutation. Compared to the patients with wild-type TP53, 9 patients with a Y220C mutation and 6 with a TP53 mutation on codon 248 did not have significantly worse survival [log rank p = 0.469, hazard ratio 0.689 (0.249–1.904) and log rank p = 0.776, hazard ratio 0.804 (0.175–3.69), respectively].

**Table 2.** Number of TP53 mutations distributed over 5 exons and head and neck subsites

| Exon | Oral cavity | Oropharynx | Hypopharynx | Larynx | Esophagus | Unknown | Total |
|------|-------------|------------|-------------|--------|-----------|---------|-------|
| 4    | 0 (0)       | 2 (9)      | 1 (10)      | 1 (5)  | 1 (8)     | 0       | 5     |
| 5    | 7 (24)      | 4 (17)     | 2 (20)      | 3 (18) | 4 (31)    | 0       | 20    |
| 6    | 6 (21)      | 7 (30)     | 2 (20)      | 5 (29) | 5 (38)    | 0       | 25    |
| 7    | 10 (35)     | 6 (26)     | 2 (20)      | 5 (29) | 2 (15)    | 1 (50)  | 26    |
| 8    | 5 (17)      | 4 (18)     | 3 (30)      | 3 (18) | 1 (8)     | 1 (50)  | 17    |
| 9    | 1 (3)       | 0 (0)      | 0 (0)       | 0 (0)  | 0         | 0       | 1     |
| Total| 29 (100)    | 23 (100)   | 10 (100)    | 17 (100)| 13 (100) | 2 (100) | 94 (100) |

**Fig. 1.** TP53 mutations found in HNSCC distributed across anatomical subsites. The structure of the gene is distributed in 6 regions on the X-axis. CT = C terminus; PRR = proline-rich domain; TAD = transcription activation domain; TET = tetramerization domain. Vertical lines represent the frequency at which the mutations were found at each codon. Several relative hotspot mutations were indicated at codons 157, 193, 220, 245, 248 and 286.

**Fig. 2.** Distribution of TP53 mutations in OPSCC. The relative hotspot mutation at this subsite is the Y220C mutation (22%).
Table 3. Substitutions and corresponding codons of the most frequent TP53 mutations

| Exon | Codon | Amino acid substitution | Number of mutations |
|------|-------|-------------------------|---------------------|
| 5    | 157   | valine → phenylalanine  | 4                   |
| 6    | 193   | histidine → leucine     | 3                   |
|      |       | histidine → arginine    | 2                   |
| 7    | 220   | tyrosine → cysteine     | 9                   |
| 245  | glycine → aspartic acid | 3                   |
| 248  | arginine → tryptophan    | 2                   |
| 248  | arginine → glutamine     | 4                   |
| 286  | glutamic acid → lysine   | 3                   |
|      |       | glutamic acid → glycine | 1                   |

Discussion

HNSCCs are a heterogeneous group of cancers presenting as tumors at several anatomical subsites. Each subsite in the head and neck region displays a diverse biological and clinical behavior based on divergent etiological pathways [23]. This heterogeneity has previously been shown on epigenetic, genetic and transcriptomic levels [24–26]. It has been proposed that different tumor types have their own TP53 mutational pattern that represents the agent responsible for this mutation [27]. In our study, the subdivision of HNSCC by anatomic site showed different spectra of TP53 mutations in the various head and neck regions.

In our cohort of 116 HNSCC patients, 81 (70%) had nonsynonymous mutations of TP53. This is in line with current literature that reports a range of 60–80% of TP53 mutations in HNSCC [1]. This variation in results may be due to geographical differences among the studied populations as well as differences in the detection methods used [28–30]. We found a relatively high frequency of TP53 alterations, which may be because most patients in our cohort already had a history of HNSCC and presented with a possible recurrence or metastasis. Subsequent TP53 mutation analysis was carried out to show possible clonal relatedness between the 2 tumors or between the tumor and metastasis. TP53 mutations are related to disease progression and worse survival, therefore more mutations were found in our cohort in comparison with study groups consisting of patients with a primary HNSCC [28, 31]. However, in our retrospective cohort, we could not identify this relationship, probably due to the fact that most of the patients included with wild-type TP53 had a history of HNSCC and TP53 mutation analysis was carried out to identify possible recurrences or metastasis. Independent of the presence of a TP53 mutation, these wild-type tumors were aggressive and had a poor survival.

In our pilot study of 94 nonsynonymous mutations in 81 patients, we observed that most mutations are single amino acid changes, with 97% occurring in the DBD compared to 80–90% in the literature [8]. Our results show the heterogeneity of TP53 mutations across different anatomical subsites. However, we could not demonstrate a significant difference in TP53 mutations and subsite in our cohort, probably due to the small sample size. Nevertheless, in the oral cavity, most mutations occur in exon 7 and the known hotspot mutations in cancer, G245 and R248, occur most often at this subsite. The Y220C mutation turned out to be a relative hotspot mutation in the oropharyngeal region (22%). Other subsites did not show relative hotspot mutations. These results are in accordance with a previous study by Greenblatt et al. [7] that demonstrated differences in A–G spectra of TP53 mutations between the nasopharynx, oral and pharynx/larynx tumors. This heterogeneity across different anatomical subsites could be explained by differences in exposure to carcinogenetic agents, e.g. soluble tobacco-specific N-nitrosamines in oral cancer, HPV in OPSCC, the Epstein-Barr virus in nasopharyngeal tumors and tobacco combustion products in pharyngeal and laryngeal tumors, or else a variation in the activation of carcinogenetic molecular pathways [2, 32].

Our findings are clinically relevant. First of all, one should be careful about interpreting Y220C mutations as a marker for clonal relationship in HNSCC, especially in OPSCC. As mutations in the TP53 gene are considered non-hotspot mutations, the chance is very low that unrelated multiple tumors in the same patient harbor the same mutation. Vice versa, tumors that show an identical Y220C mutation turned out to be a relative hotspot mutation in the oropharyngeal region (22%). Other subsites did not show relative hotspot mutations. These results are in accordance with a previous study by Greenblatt et al. [7] that demonstrated differences in A–G spectra of TP53 mutations between the nasopharynx, oral and pharynx/larynx tumors. This heterogeneity across different anatomical subsites could be explained by differences in exposure to carcinogenetic agents, e.g. soluble tobacco-specific N-nitrosamines in oral cancer, HPV in OPSCC, the Epstein-Barr virus in nasopharyngeal tumors and tobacco combustion products in pharyngeal and laryngeal tumors, or else a variation in the activation of carcinogenetic molecular pathways [2, 32].

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able to identify mutations and alterations in hotspot regions of 50 common mutated oncogenes and tumor suppressor genes, including TP53, PIK3CA and FGFR. This method will more accurately determine clonality among tumors compared to Sanger sequencing and the costs of this technique are comparable. A second clinically relevant finding is that the specific hotspot Y220C mutation in OPSCC observed in this study may become important in the light of personalized cancer therapies. Previous studies showed that HNSCC patients with mutated TP53 tumors have a worse survival rate than patients with TP53 wild-type tumors [28]. Due to the decreased survival associated with mutated, nonfunctional TP53, new treatment strategies to restore TP53 function in HNSCC have become an important research challenge and may pave the road for targeted therapy. Drugs targeting overactivated oncoproteins are already widely used in cancer therapy, and are usually based on inhibiting the function of the consequently activated pathway by targeting the mutated (receptor tyrosine) kinase or its downstream target [36, 37]. The opposite, i.e. targeting inactivated proteins such as those encoded by tumor suppressor genes, is much more challenging, because this requires the restoring of the normal function of a tumor suppressor gene. In addition, not all TP53 mutants have the same effect, with some even showing a degree of wild-type DNA-binding and transcriptional function [8]. It has been shown that the Y220C mutation lowers the melting temperature of the p53 protein, which allows a faster denaturation. Recently, a class of small molecules has been introduced that can resistabilize the Y220C-mutated p53 protein by binding in its mutated pocket, thereby restoring its tumor-suppressive function [38]. In the era of personalized medicine, this specific mutation may guide therapeutic decisions in the future, especially in TP53 Y220C-mutated OPSCC.

In conclusion, the subdivision of HNSCC into anatomical locations shows different patterns of TP53 mutations. The Y220C mutation occurs in 10% of all TP53 mutations in HNSCC. Consequently, caution should be taken with the interpretation of the Y220C mutation in the context of clonally relationship analyses. Moreover, Y220C mutations occur most often in OPSCC. This highlights the biological diversity of tumors in the head and neck region, which can have implications for further studies and more stratified treatment.

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Disclosure Statement

The authors declare that they have no competing interests.
17 Kimple RJ, Smith MA, Blitzer GC, Torres AD, Martin JA, Yang RZ, Peet CR, Lorenz LD, Nickel KP, Klingelhoht AJ, et al: Enhanced radiation sensitivity in HPV-positive head and neck cancer. Cancer Res 2013;73: 4791–4800.

18 Brennan JA, Boyle JO, Koch WM, Goodman SN, Hruby RH, Eby YJ, Couch MJ, Forastiere AA, Sidransky D: Association between cigarette smoking and mutation of the p53 gene in squamous-cell carcinoma of the head and neck. N Engl J Med 1995;332:712–717.

19 Somers KD, Merrick MA, Lopez ME, Incognito LS, Schechter GL, Casey G: Frequent p53 mutations in head and neck cancer. Cancer Res 1992;52:5997–6000.

20 Sidransky D, Hollstein M: Clinical implications of the p53 gene. Annu Rev Med 1996;47: 283–301.

21 Nagai MA, Miracco EC, Yamamoto L, Moura RP, Simpson AJ, Kowalski LP, Brentani RR: TP53 genetic alterations in head-and-neck carcinomas from Brazil. Int J Cancer 1998;76: 13–18.

22 van Diest PJ: No consent should be needed for using leftover body material for scientific purposes. For. BMJ 2002;325:648–651.

23 McMahon S, Chen AY: Head and neck cancer. Cancer Metastasis Rev 2003;22:21–24.

24 Takes RP, Baantenburg de Jong RJ, Schuuring E, Litvinov SV, Hermans J, Van Krieken JH: Differences in expression of oncopgenes and tumor suppressor genes in different sites of head and neck squamous cell. Anticancer Res 1998;18:4793–4800.

25 van Kempen PM, Noorlag R, Brauwnis WW, Stegeman I, Willems SM, Grohman W: Differences in methylation profiles between HPV-positive and HPV-negative oropharynx squamous cell carcinoma: a systematic review. Epigenetics 2014;9:194–203.

26 Lleras RA, Smith RV, Adrien LR, Schlecht NF, Burkh RD, Harris TM, Childs G, Grystowsky MB, Behbin T: Unique DNA methylation loci distinguish anatomic site and HPV status in head and neck squamous cell carcinoma. Clin Cancer Res 2013;19:5444–5455.

27 Lasky T, Silbergeld E: p53 mutations associated with breast, colorectal, liver, lung, and ovarian cancers. Environ Health Perspect 1996;104:1324–1331.

28 Poeta ML, Manola J, Goldwasser MA, Forastiere A, Benoit N, Califano JA, Ridge JA, Goodwin J, Kenady D, Saunders J, et al: TP53 mutations and survival in squamous-cell carcinoma of the head and neck. N Engl J Med 2007;357:2552–2561.

29 Hsieh LL, Wang PF, Chen IH, Liao CT, Wang HM, Chen MC, Chang JT, Cheng AJ: Characteristics of mutations in the p53 gene in oral squamous cell carcinoma associated with betel quid chewing and cigarette smoking in Taiwanese. Carcinogenesis 2001;22:1497–1503.

30 Munirajan AK, Tutsumi-Ishii Y, Mohan-prasad BK, Hirano Y, Munakata N, Shannumgam G, Tsuchida N: p53 gene mutations in oral carcinomas from India. Int J Cancer 1996;66:297–300.

31 Norberg T, Klaar S, Karf G, Nordgren H, Holmberg L, Bergh J: Increased p53 mutation frequency during tumor progression – results from a breast cancer cohort. Cancer Res 2001;61:8317–8321.

32 Koch WM, Lango M, Sewell D, Zahurak M, Sidransky D: Head and neck cancer in non-smokers: a distinct clinical and molecular entity. Laryngoscope 1999;109:1544–1551.

33 Chung KY, Mukhopadhyay T, Kim J, Casson A, Ro JT, Goepfert H, Hong WK, Roth JA: Discordant p53 gene mutations in primary head and neck cancers and corresponding second primary cancers of the upper aerodigestive tract. Cancer Res 1993;53:1676–1683.

34 Kropveld A, Rozemuller EH, Leppers FG, Scheidel KC, de Weger RA, Koole R, Hordijk GJ, Slootweg PJ, Tilanus MG: Sequencing analysis of RNA and DNA of exons 1 through 11 shows p53 gene alterations to be present in almost 100% of head and neck squamous cell cancers. Lab Invest 1999;79:347–353.

35 Mao L: A new marker determining clonal outgrowth. Clin Cancer Res 2002;8:2021–2023.

36 Riemer AB, Zielinski CC: Use of trastuzumab in the therapy of breast cancer. Ther Umsch 2008;65:217–222.

37 Dhomen NS, Mariadason J, Tebbutt N, Scott AM: Therapeutic targeting of the epidermal growth factor receptor in human cancer. Crit Rev Oncog 2012;17:31–50.

38 Basse N, Kaar JL, Settanni G, Joerger AC, Rutherford TJ, Fersht AR: Toward the rational design of p53-stabilizing drugs: probing the surface of the oncogenic Y220C mutant. Chem Biol 2010;17:46–56.