SMGroup

Gene Alterations in Head and Neck Squamous Cell Carcinoma

Kinga Bednarek[1#], Magdalena Kostrzewska-Poczekaj[1#], Krzysztof Szyfter[1,2] and Malgorzata Jarmuz-Szymczak[1,3*]

[1]Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland

[2]Depatment of Audiology and Phoniatry, K. Marcinkowski University of Medical Sciences, Poznan, Poland

[3]Department of Hematology, K. Marcinkowski University of Medical Sciences, Poznan, Poland

[#]*both authors contributed equally to this work*

***Corresponding author:** Malgorzata Jarmuz-Szymczak, Institute of Human Genetics, Polish Academy of Sciences, Department of Cancer Genetics, Department of Hematology, K. Marcinkowski University of Medical Sciences, Poznan, Poland, Email: maljar@man.poznan.pl

Published Date: June 25, 2016

ABSTRACT

HNSCCs are a huge health problem throughout the world. It is the sixth most common cancer worldwide affecting more than 600,000 new patients each year. Only about 50% of patients survive for 5 years period. HNSCCs are a highly heterogeneous group of tumors that arise via one of the two primary carcinogenic pathways. The first is chemical carcinogenesis resulted from exposure to tobacco and alcohol which seems to have a synergistic effect. Alternative pathways are virally induced tumorigenesis (particularly those of the oropharynx).

The studies on genetic background of HNSCC initially were focused on individual variability and sensitivity to carcinogens. Genetic polymorphism of genes processing carcinogens and DNA repair was successfully explored to indicate risk and protective gene variants. Later on an attention has been turned onto genetic regulation of cancer progression. It became clear that

genetic factors regulate the whole process of tumorigenesis. It was shown by discovering a role of oncogenes, tumor suppressor genes and microRNA in the early stage of cancer development, entering into aggressive phase and metastasis, tumor relapse etc. Going to clinic alterations of genetic factors could be used as markers of cancer progression and prognosis. Though, the matter of the articles is being extended from general knowledge on genetic regulation of HNSCC to clinical application.

It is a short review where are described oncogenes, Tumor Suppressor Genes **(TSG)** and microRNAs which structure alteration and abnormal expression level (increased or decreased) could variably contribute to the cancerogenesis process.

Keywords: HNSCC; Oncogenes; TSG; miRNA

INTRODUCTION

Head and Neck Squamous Cell Carcinoma **(HNSCC)** develops mainly in the oral cavity, oropharynx, larynx or hypopharynx. HNSCCs are a huge health problem throughout the world. It is the sixth most common cancer worldwide affecting more than 600,000 new patients each year. Only about 50% of patients survive for 5 years period [1].

HNSCCs are a highly heterogeneous group of tumors that arise via one of the two primary carcinogenic pathways. The first is chemical carcinogenesis resulted from exposure to tobacco and alcohol which seems to have a synergistic effect. Alternative pathway is virally induced tumorigenesis. HPV-negative and HPV-positive HNSCCs represent distinct disease entities; besides various carcinogenic pathways of development also they show differences in incidence, age of arising, site predilection, and prognosis. The incidence of HPV-positive HNSCCs is increasing as compared to HPV-negative tumors and they are more frequent in age below 60 years that it is opposite in HPV-negative, which occur more often above 60 years [2,3]. The predilection site of HPV-positive tumors is oropharynx [4]. Moreover these tumors have a more favorable prognosis than HPV-negative tumors [5].

In this review we describe the most frequent genes, which are altered in HNSCCs. Oncogenes, Tumor Suppressor Genes **(TSG)** and microRNAs are genes which structure alteration and abnormal expression level (increased or decreased) could variably contribute to the cancerogenesis process.

ONCOGENES

According to the basic and most popular definition, oncogenes are mutated or overregulated proto-oncogenes. Normally proto-oncogenes are the group of genes encoding proteins involved in cell division and cell differentiation as well as in anti-apoptotic pathways. All these processes are important for human development, cell survival or apoptotic death and for the preservation of organs and tissues.

When proto-oncogenes mutate to become oncogenes the cell growth is unregulated and transformed, as the cells are no longer capable to respond on normal regulatory signals.

Increased expression of oncogenes leads to accelerated cell division, decreased cell differentiation, inhibition of cell death [6].

In normal cells there are two main ways to keep cell growth under control. The first one is inactivation of the growth pathways and the second one is activation of the apoptotic pathways. Oncogenes are the mutated genes coding proteins which regulate this pathway; hence tumor cells are capable to unlimited division or ignore the apoptotic death signals. The consequence is an increased expression of growth factors, membrane receptors and mediatory molecules as well as overpresentation of proteins involved in apoptosis inhibition [7]. At the cellular level, the mutation in proto-oncogenes is dominant in contrast to tumor suppressor genes. It means that only one mutation in a single allele is sufficient to trigger an oncogenic potential in cells. Dominant nature of proto-oncogenic mutations leads to cancer development. However, it is unlikely that one mutation in one oncogene results in cancer itself. It is noteworthy that one oncogene can increase cell division leading to increased probability of other mutation to occur. An accumulation of oncogene mutations may result in cancer phenotype [8].

Oncogenes are the group of genes directly involved in the formation and progress of the cancer. The main mechanisms leading to activated cell transformation are the mutations, chromosomal translocations and amplification of the proto-oncogene, located in a non-cancerous cell and involved a number of cellular processes. Amplification is frequent an genetic aberration leading to additional copy number of proto-oncogene. Multiple genetic aberrations were found throughout the tumor genomes including additional DNA copy number of 3q, 8q, 5p, 7q, 11q, and 12p [9]. Total number of proto-oncogene is estimated to be over five hundred genes. The classification is taking into account the function of encoded proteins. The proteins coded by oncogenes known also as oncoproteins belong to three mains groups:

- Cell cycle regulatory proteins,
- Proteins involved in apoptosis,
- Other important cell functions, for example: proteins forming the ion channels.

An oncogene activation may be the result of several different types of DNA alterations (described further on), but the most important genes may have different alterations leading to oncogenesis. There are a lot of articles considering oncogenic potential of various genes in context of carcinogenesis. The most important and widely described oncogenes in larynx cancer are: *CCND1, PIK3CA, EGFR* and *MET* [10]. Below we present a brief description of the main function and leading aberrations of these genes observed in HNSCC. The list is not closed and research for the identification of oncogenes and further defining their role in tumor development is still in progress.

CCND1 (Cyclin D1)

The studies based on classical cytogenetic, Fluorescence in Situ Hybridization **(FISH)** and comparative genomic hybridization **(CGH)** has shown frequent amplification of 11q13 chromosomal region in HNSCC. The main established oncogene localized in that region is Cyclin D1 **(*CCND1*)** as one of the first described oncogenes in laryngeal squamous cell carcinoma. Cyclin D1 is a cell cycle regulatory protein that binds Cyclin-Dependent Kinase 4 **(CDK4)** and promotes phosphorylation of the retinoblastoma protein [11]. Retinoblastoma protein phosphorylation is required for progression through the G1-S cell cycle checkpoint [11]. Proper activity of cyclin allows the passage from G1 to S phase of the cell cycle, while the excessive production of the cyclin D1 protein effects in accelerated cell proliferation leading to carcinogenesis [12,13].

In HNSCC, including laryngeal cancers, *CCND1* gene amplification and protein overexpression correlate with poor patient outcome [14-16]. The amplification is the main mechanism of the protein overexpression, but mutations in that gene are also known [17, 18].

Although there is an established driver *CCND1* gene in 11q13 region less is known about other genes localized in this region. There are at least tree oncogene candidates: *FADD, PPFIA1* and *CTTN* potentially involved in head and neck tumor development [19].

PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase)

Genetic alteration involving 3q26 region is one of the most frequent events in HNSCC and the established oncogene situated in this genomic region is *PIK3CA* [20,21].

The protein encoded by this gene represents the catalytic subunit 110 kDa of Phosphatidylinositol 3-kinase **(PI3K).** Kinase PI3K participates in the signal transduction pathway PI3K/AKT and deregulation of this pathway can cause malignant transformation in a variety of tumor cancers. Increased activity of this protein therefore takes part in the activation of AKT kinase. Serine-threonine kinase AKT can phosphorylate further proteins involved in regulation of several cellular processes. As a result it may inhibit apoptosis directly and indirectly through inactivation of apoptotic genes and increase expression of anti-apoptotic genes [22].

Both overegulation of *PIK3CA* gene and downregulation of *PTEN* tumor suppressor gene lead to increased expression of AKT kinase and deregulation of number of cellular processes and in consequence to cancerogenesis process.

PIK3CA amplification has been observed in many tumors. In head and neck cancers a correlation between amplification and overexpression has been shown, but the second mechanism leading to activation this gene are point mutations of the *PIK3CA* gene [20,23,24]. Moreover mutations and deletions of tumor suppressor gene *PTEN*, which is an antagonist of PI3K had also significant role in properly functioning PIK/ACT [22]. Genetic alteration involving 3q26 region is one of the most frequent events in HNSCC and it has been related to disease progression and survival [25,26].

EGFR (Epidermal Growth Factor Receptor)

Oncogenic nature of growth factors receptors was shown in different types of tumors. Gene amplification and overexpression of the growth factors receptors may lead to malignant transformation. The main example of this oncoprotein is Epidermal Growth Factor Receptor **(EGFR)** frequently overexpressed in HNSCC [27]. It has also been shown that *EGFR* gene amplification correlates with upregulation of mRNA and increased protein expression in the oral cavity tumors [28].

The protein encoded by this gene belongs to the ErbB protein family, one of the most widely studied groups of receptor tyrosine kinases. The Epidermal Growth Factor **(EGF)** and its receptor are crucial for the proliferation of squamous cells stimulated by RAS-MAP and PI3K-PTEN-AKT pathways [29]. Both EGF factors and EGF receptors made EGF/EGFR complex which activates cell transduction pathways and nuclear transcription factors. Over-activity of transcription factors leads to overexpression of genes regulated by these factors, increased cell proliferation, invasion and metastasis [30].

MET (met protooncogene - receptor tyrosine kinase)

Another growth factor receptor is encoded by a gene *MET* located in chromosome 7q31. Both gene amplification and mutations leading to overexpression were described in HNSCC [31]. The MET receptor has tyrosine kinase activity and activates RAS-MAP and PI3K-PTEN-AKT pathways inducing cell growth, cell motility and angiogenesis [32]. Activation of the RAS and AKT pathways are also associated with the EGFR protein. These data therefore pointed at a network of molecular relationship between receptor tyrosine kinases in head and neck squamous cell carcinoma.

TUMOR SUPPRESSOR GENES

Tumor Suppressor Genes **(TSG)** are genes normally expressed in cells, required for their proper function. The effects of genes activity are opposite to oncogenes and thus prevent the cancerogenesis. The TSG main roles in normal – non cancer-cells are [33]:

- Cell cycle control – TSGs inhibit cell proliferation by regulation cell cycle checkpoints
- Detection and repair of DNA damage
- Regulation of protein degradation and ubiquitination
- Regulation of ligand-mediated signaling pathways
- Cell specification and differentiation
- Cell migration and tumor angiogenesis

The main mechanisms leading to TSG inactivation are: Loss of Heterozygosity **(LOH),** point mutations, deletions and epigenetic changes of DNA – gene promoter hypermethylation and

interference with microRNA **(miRNA).** All these mechanism may occur in combination of two and even more in one tumor. It was shown, that tumor suppressor genes are recessive, which means that their functions are maintained as long as one copy of the gene remains not affected [34]. It confirms the assumptions of the "two-hit" theory which implies that mutation in both alleles is required to induce the malignant effect [35].

In 1996 Califano et al. had presented the model of cancer progression. It was created on the LOH analysis and shows which regions are frequently lost in particular steps of HNSCC development. The "classic" Califano's model includes 3p, 4q, 6p, 8p, 9p, 8q, 11q, 13q, 14q and 17p deletions [36]. This scheme was supplemented over the years of studies and next alterations observed during the carcinogenesis were added. The main are p14/p16 inactivation and *TP53* mutations, 18q deletion, 10q23 deletion [37], but the numerical loses at 1p, 4, 5q, 6q, 21and allelic loses at 2q, 4p, 5q, 9q, 10q, 15q, 19q are also observed [10].

The cell cycle control regulation depends on the proper function of genes controlling the main restriction points. For several kinds of tumors, including HNSCC, the genes involved in cell cycle regulation that are commonly inactivated are: *RB1, TP53* and *CDKN2A*.

TP53 (tumor protein p53)

The *TP53* gene is crucial for both cell cycle control and apoptosis. It can arrest the cell cycle when DNA is damaged. The occurrence of damage in DNA induces high level of *TP53* expression inducing in turn high level of its product - cellular tumor antigen TP53 which stimulates the expression of p21 protein - the cyclin dependent kinase inhibitor. This protein keeps the cell cycle stopped as long as the damage is not repaired. Lack of *TP53* activity leads to accumulation of mutations and higher genome instability. It was shown, that during the carcinogenesis this gene is often mutated [38]. Majority of the changes are missens mutations localized in DNA binding domain [39]. Simultaneously, deletion or LOH of 17p – the chromosomal region in which *TP53* is localized – occurs in the early step of HNSCC development according to Califano's model of cancer progression, [36,37]. The activity of *TP53* is supported by the *CDKN2A* gene product – p14 **(ARF)**. This protein is responsible for blocking the inhibition of TP53 protein by MDM-2, thus promoting the cell cycle [40].

CDKN2A cyclin-dependent kinase inhibitor 2A

CDKN2A gene is localized in 9p21 region. According to Califano's model of cancer progression, this region is lost in early stages of carcinogenesis. Through the alternative reading frames it encodes two functionally distinct proteins p14 (ARF) and p16 **(INK4a)** both acting as tumor suppressors. As mentioned, p14 role is to protect the TP53 from the MDM-2 dependant inhibition. The p16 (INK4a) is an inhibitor of Cyclin Depentent Kinases **(CDK)** 4 and 6. The suppression of kinases prevents the formation of CDK4/6 - CyclinD complex, essential for phosphorylation of RB -protein product of *RB1* gene [40]. Unphosphorylated RB protein stops the cell proliferation by

inhibition of transition from G1 to S phase of cell cycle. The inactivation of p14 and/or p16 leads to maintenance of the phosphorylated form of the RB protein and proceeding cell cycle.

Although p16 and p14 are products of the same gene, their mechanisms of inactivation may be different. Also, the inactivation does not always occur at the same time for both gene isoforms. It was shown, that methylation of isoform coding the p14 and p16 proteins is more frequent for each gene isoform separately [40]. The gene hypermethylation is second of the more frequent mechanisms of gene inactivation, following the loss of heterozygosity. The alterations in sequence of this gene are rarely observed in HNSCC [41,42].

The function of cell cycle control genes is changed when the cells are infected with Human Papilloma Virus (HPV). Two main oncoproteins encoded by the viral genome are E6 and E7. These proteins bind to TP53 (E6) and RB1 (E7) leading to their inactivation and degradation. As a consequence, the cell cycle is not arrested properly and p53-mediated apoptosis in inhibited. Additionally, for HPV-positive HNSCC patients the *TP53* gene is typically not affected [43]. Taking together – the literature data show, that *CDKNA2* and *TP53* genes are established TSGs mainly in HPV-negative HNSCC while HPV-positive HNSCC are connected with *TP53* and Rb family genes, including *RB1* [10].

SMAD4 (SMAD family member 4) and PTEN (phosphatase and tensin homolog)

Two more TSGs were established for head and neck cancer – *SMAD4* (localized in 18q21) and *PTEN* (10q23) [10]. Both of them are involved in regulation of important cell signaling pathways. The *SMAD4* gene is a key mediator of Transforming Growth Factor Beta **(TGFB)** signaling pathway, crucial for cell growth and differentiation, apoptosis and cellular homeostasis. It was shown, that inactivation of this gene in HNSCC is mainly due to heterozygous and homozygous deletions and rarely mutations leading to early termination codons [44].

PTEN gene is involved in cell cycle regulation and cell survival as it antagonizes the PI3K-AKT/PKB signaling pathway. Two mechanisms of gene inactivation in HNSCC were shown: the loss of chromosome 10 and missense mutations in exons 5, 6, 7, 8 of the gene [45]. The loss of gene or mutation is more frequently observed in HPV-positive HNSCC tumors, and are connected with *PIK3CA* mutations and amplification. It was shown, that mutations of *PIK3CA* and *PTEN* genes are mutually exclusive [23]. The significant relation between *PIK3CA* and *PTEN* with HPV status was shown [43].

NOTCH1 (notch 1)

The gene *NOTCH1* is worth of remark. This gene has an oncogenic and suppressing potential in different types of cancers. As was shown, in T-Acute Lymphoblastic Leukemia (T-ALL) it plays a role of oncogene, as involved in t(7;9) chromosomal translocation. Additionally, two activating mutations of this gene were detected in T-ALL. At least one of these mutations is found in ~55-

60% of human T-ALLs. On the other hand the *Notch1* knockout mice develop the cutaneous squamous cell carcinoma as a result of increased Wnt signaling [46]. As was indicated, in human HNSCC the mutations rather inactivate the *NOTCH1* gene suggesting its involvement in tumor suppressing [47].

microRNA

A separate group of gene inactivating factors are microRNAs. These small non-coding molecules regulate the posttranscriptional gene expression by binding to 3'UTR of mRNA. It results in restraining the gene activity through the mRNA degradation or mRNA inhibition from being translated. As the binding to mRNA is unspecific, one miRNA can regulate more than one gene as well as one gene can by regulated by several microRNAs. In view of the effect induced by the mRNA degradation, microRNAs involved in carcinogenesis can be classified in two main groups: (I) oncomiRNA: microRNA targeting the TSG and thus playing the oncogenic role and (II) tumor suppressor miRNA – miRNA with oncosupressive properties targeting the oncogenes. Moreover, the activity of miRNAs in cancer is connected with its localization in the genome. The miRNAs involved in regulation of TSGs are often amplified resulting in enhanced gene suppression. On the contrary, microRNAs responsible for repressing oncogenes are located in frequently mutated or deleted loci [48]. The involvement of this type of gene expression regulation was shown in HNSCC, indicating the list of oncogenic and tumor suppressor miRNAs. Examples of microRNAs involved in HNSCC derived from review papers are presented in Table 1 [49-52].

Table 1: Examples of microRNAs involved in HNSCC pathogenesis.

microRNAs **up-regulated** in HNSCC (oncomiRNAs)	miR-1290, miR-21-5p, miR-21-3p, *miR-7; miR-16, miR-18a, miR-106b-25* cluster, *miR-130b, miR-142-3p, miR-146a, miR-184, miR-155, miR-31, miR-223,* miR-210, miR-504, miR-10b, miR-181, miR-221 family, miR-30b, miR-29a,c, miR-140-3p
microRNA **down-regulated** in HNSCC (tumor suppressor miRNAs)	miR-100, miR-133a, *miR-125a, miR-125b, miR-200a, miR-375, miR-375, miR-1, miR-99a, miR-125b, miR-143, miR-204, miR-99family, miR-218, miR-212, miR-668, miR-137, miR-124, miR-34a, miR-126, miR-181a, miR-27b, miR-200c, miR-107, miR-101, miR-138*

Described genes alterations in HNSCCs showing the molecular heterogeneity of these diseases. These oncogenes and TSGs are involved in different genetic pathways for example: the TP53 and Retinoblastoma **(RB)** pathways, the Epidermal Growth Factor Receptor **(EGFR)** signalling pathway, the growth inhibitory transforming growth factor-β (TGFβ) pathway, the PI3K–PTEN–AKT pathway. There are a growing number of molecular alterations in HNSCCs. Some of them can be used as potentially prognostic and predictive markers. Comprehension of these biological factors is not such well established as in other cancer but some of them as EGFR are already used in personalized medicine. Therefore understanding the molecular basis of these cancers is very important.

ACKNOWLEDGMENTS

This work was funded by the National Science Centre, number N N403 153140 and 2012/05/N/NZ1/0195.

References

1. Globocan 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012
2. Chaturvedi AK, Engels EA, Anderson WF, Gillison ML. Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. J Clin Oncol Off J Am Soc Clin Oncol. 2008 26: 612-619.
3. Näsman A, Attner P, Hammarstedt L, Du J, Eriksson M, et al. Incidence of human papillomavirus (HPV) positive tonsillar carcinoma in Stockholm, Sweden: an epidemic of viral-induced carcinoma? Int J Cancer. 2009; 125: 362-366.
4. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, et al. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med. 2007; 356: 1944-1956.
5. Ragin CCR, Taioli E Survival of Squamous cell carcinoma of the head and neck in relation to human papillomavirus infection: review and meta-analysis. Int J Cancer. 121:1813-1820.
6. Weinstein IB, Joe AK. Mechanisms of disease: Oncogene addiction--a rationale for molecular targeting in cancer therapy. Nat Clin Pract Oncol. 2006; 3: 448-457.
7. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100: 57-70.
8. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144: 646-674.
9. Patmore HS, Ashman JNE, Stafford ND, Berrieman HK, MacDonald A, et al. Genetic analysis of head and neck squamous cell carcinoma using comparative genomic hybridisation identifies specific aberrations associated with laryngeal origin. Cancer Lett. 2007; 258: 55-62.
10. Leemans CR, Braakhuis BJM, Brakenhoff RH. The molecular biology of head and neck cancer. Nat Rev Cancer. 2011; 11: 9-22.
11. Hunter T, Pines J. Cyclins and cancer. II: Cyclin D and CDK inhibitors come of age. Cell.1994 79: 573-582.
12. Yu Z, Weinberger PM, Haffty BG, Sasaki C, Zerillo C, et al. Cyclin d1 is a valuable prognostic marker in oropharyngeal squamous cell carcinoma. Clin Cancer Res Off J Am Assoc Cancer Res. 2005; 11: 1160-1166.
13. Rudas M, Lehnert M, Huynh A, Jakesz R, Singer C, et al. Cyclin D1 expression in breast cancer patients receiving adjuvant tamoxifen-based therapy. Clin Cancer Res Off J Am Assoc Cancer Res. 2008; 14: 1767-1774.
14. Akervall JA, Michalides RJ, Mineta H, Balm A, Borg A, et al. Amplification of cyclin D1 in squamous cell carcinoma of the head and neck and the prognostic value of chromosomal abnormalities and cyclin D1 over expression. Cancer.1997; 79: 380-389.
15. Bova RJ, Quinn DI, Nankervis JS, Cole IE, Sheridan BF, et al. Cyclin D1 and p16INK4A expression predict reduced survival in carcinoma of the anterior tongue. Clin Cancer Res off J Am Assoc Cancer Res.1999; 5: 2810-2819.
16. Jarmuz M, Grenman R, Golusinski W, Szyfter K. Aberrations of 11q13 in laryngeal squamous cell lines and their prognostic significance. Cancer Genet Cytogenet. 2005; 160: 82-88.
17. Rydzanicz M, Golusinski P, Mielcarek-Kuchta D, Golusinski W, Szyfter K. Cyclin D1 gene (CCND1) polymorphism and the risk of Squamous cell carcinoma of the larynx. Eur Arch Oto-Rhino-Laryngol Off J Eur Fed Oto-Rhino-Laryngol Soc EUFOS Affil Ger Soc Oto-Rhino-Laryngol-Head Neck Surg. 2006; 263:43-48.
18. Izzo JG, Papadimitrakopoulou VA, Liu DD, den Hollander PLC, Babenko IM, et al. Cyclin D1 genotype, response to biochemoprevention, and progression rate to upper aero digestive tract cancer. J Natl Cancer Inst. 2003; 95:198-205.
19. Jarmuz-Szymczak M, Pelinska K, Kostrzewska-Poczekaj M, Bembnista E, Giefing M, et al. Heterogeneity of 11q13 region rearrangements in laryngeal Squamous cell carcinoma analyzed by microarray platforms and fluorescence in situ hybridization. Mol Biol Rep. 2013; 40: 4161-4171.
20. Redon R, Muller D, Caulee K, Wanherdrick K, Abecassis J, et al. A simple specific pattern of chromosomal aberrations at early stages of head and neck Squamous cell carcinomas: PIK3CA but not p63 gene as a likely target of 3q26-qter gains. Cancer Res. 2001; 61:4122-4129.
21. Chen Y-J, Lin S-C, Kao T, Chang C-S, Hong P-S, et al. Genome-wide profiling of oral squamous cell carcinoma. J Pathol. 2004; 204: 326-332.
22. Vanhaesebroeck B, Leevers SJ, Ahmadi K, Timms J, Katso R, et al. Synthesis and function of 3-phosphorylated inositol lipids. Annu Rev Biochem. 2001; 70: 535-602.

23. Murugan AK, Hong NT, Fukui Y, Munirajan AK, Tsuchida N. Oncogenic mutations of the PIK3CA gene in head and neck squamous cell carcinomas. Int J Oncol. 2008; 32:101-111.

24. Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, et al. The mutational landscape of head and neck Squamous cell carcinoma. Science. 2011; 333: 1157-1160.

25. Fenic I, Steger K, Gruber C, Arens C, Woenckhaus J. Analysis of PIK3CA and Akt/protein kinase B in head and neck squamous cell carcinoma. Oncol Rep. 2007: 18: 253-259.

26. Woenckhaus J, Steger K, Sturm K, Münstedt K, Franke FE, et al. Prognostic value of PIK3CA and phosphorylated AKT expression in ovarian cancer. Virchows Arch Int J Pathol. 2007; 450: 387-395.

27. Temam S, Kawaguchi H, El-Naggar AK, Jelinek J, Tang H, et al. Epidermal growth factor receptor copy number alterations correlate with poor clinical outcome in patients with head and neck Squamous cancer. J Clin Oncol Off J Am Soc Clin Oncol. 2007; 25: 2164-2170.

28. Sheu JJ-C, Hua C-H, Wan L, Lin Y-J, Lai M-T, et al. Functional genomics analysis identified epidermal growth factor receptor activation as the most common genetic event in oral squamous cell carcinoma. Cancer Res. 2009; 69: 2568-2576.

29. Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer. 2005; 5: 341-354.

30. Ciardiello F, Tortora G. Epidermal Growth Factor Receptor (EGFR) as a target in cancer therapy: understanding the role of receptor expression and other molecular determinants that could influence the response to anti-EGFR drugs. Eur J Cancer 1990. 2003; 39:1348-1354.

31. Seiwert TY, Jagadeeswaran R, Faoro L, Janamanchi V, Nallasura V, et al. The MET receptor tyrosine kinase is a potential novel therapeutic target for head and neck squamous cell carcinoma. Cancer Res. 2009; 69: 3021-3031.

32. Rossi F, Moschetti R, Caizzi R, Corradini N, Dimitri P. Cytogenetic and molecular characterization of heterochromatin gene models in Drosophila melanogaster. Genetics. 2007; 175: 595-607.

33. Sherr CJ. Principles of tumor suppression. Cell. 2004; 116: 235-246.

34. Comings DE. A general theory of carcinogenesis. Proc Natl Acad Sci U S A. 1973; 70: 3324-3328.

35. Knudson AG. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A. 1971; 68: 820-823.

36. Califano J, van der Riet P, Westra W, Nawroz H, Clayman G, et al. Genetic progression model for head and neck cancer: implications for field cancerization. Cancer Res.1996; 56: 2488-2492.

37. Perez-Ordoñez B, Beauchemin M, Jordan RCK. Molecular biology of squamous cell carcinoma of the head and neck. J Clin Pathol. 2006; 59: 445-453.

38. Somers KD, Merrick MA, Lopez ME, Incognito LS, Schechter GL, et al. Frequent p53 Mutations in Head and Neck Cancer. Cancer Res.1992; 52: 5997-6000.

39. Suh Y, Amelio I, Guerrero Urbano T, Tavassoli M. Clinical update on cancer: molecular oncology of head and neck cancer. Cell Death Dis. 2014; 5: e1018.

40. Weber A, Bellmann U, Bootz F, Wittekind C, Tannapfel A. INK4a-ARF alterations and p53 mutations in primary and consecutive squamous cell carcinoma of the head and neck. Virchows Arch Int J Pathol. 2002; 441:133-142.

41. Ohta S, Uemura H, Matsui Y, Ishiguro H, Fujinami K, et al. Alterations of p16 and p14ARF genes and their 9p21 locus in oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endodontology. 2009; 107: 81-91.

42. Reed AL, Califano J, Cairns P, Westra WH, Jones RM, et al. High Frequency of p16 (CDKN2/MTS-1/INK4A) Inactivation in Head and Neck Squamous Cell Carcinoma. Cancer Res. 1996; 56: 3630-3633.

43. Lechner M, Frampton GM, Fenton T, Feber A, Palmer G, et al. Targeted next-generation sequencing of head and neck squamous cell carcinoma identifies novel genetic alterations in HPV+ and HPV- tumors. Genome Med. 2013; 5: 49.

44. Martin D, Abba MC, Molinolo AA, Vitale-Cross L, Wang Z, et al. The head and neck cancer cell oncogenome: a platform for the development of precision molecular therapies. Oncotarget. 2014; 5: 8906-8923.

45. Poetsch M, Lorenz G, Kleist. Detection of new PTEN/MMAC1 mutations in head and neck squamous cell carcinomas with loss of chromosome 10. Cancer Genet Cytogenet. 2002; 132: 20-24.

46. Roy M, Pear WS, Aster JC. The multifaceted role of Notch in cancer. Curr Opin Genet Dev. 2007; 17: 52-59.

47. Agrawal N, Frederick MJ, Pickering CR, Bettegowda C, Chang K, et al. Exome Sequencing of Head and Neck Squamous Cell Carcinoma Reveals Inactivating Mutations in NOTCH1. Science. 2011; 333: 1154-1157.

48. Iorio MV, Croce CM. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. EMBO Mol Med. 2012; 4: 143-159.

49. Janiszewska J, Szaumkessel M, Szyfter K. microRNAs are important players in head and neck carcinoma: a review. Crit Rev Oncol Hematol. 2013; 88: 716-728.

50. Tu H-F, Lin S-C, Chang K-W. MicroRNA aberrances in head and neck cancer: pathogenetic and clinical significance. Curr Opin Otolaryngol Head Neck Surg. 2013; 21: 104-111.

51. Janiszewska J, Szaumkessel M, Kostrzewska-Poczekaj M, Bednarek K, Paczkowska J, et al. Global miRNA Expression Profiling Identifies miR-1290 as Novel Potential oncomiR in Laryngeal Carcinoma. PloS One. 2015; 10: e0144924.

52. Nohata N, Hanazawa T, Kinoshita T, Okamoto Y, Seki N. MicroRNAs function as tumor suppressors or oncogenes: Aberrant expression of microRNAs in head and neck squamous cell carcinoma. Auris Nasus Larynx. 2013; 40:143-149.