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Review

Molecular techniques and genetic alterations in head and neck cancer

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SUMMARY

It is well known that cellular DNA alterations can lead to the formation of cancer, and there has been much discovery in the pathways involved in the development of head and neck squamous cell carcinoma (HNSCC). With novel genome-wide molecular assays, our ability to detect these abnormalities has increased. We now have a better understanding of the molecular complexity of HNSCC, but there is still much research to be done. In this review, we discuss the well described genetic alterations and touch on the newer findings, as well as some of the future directions of head and neck cancer research.

Introduction

The genetic alterations associated with head and neck squamous cell carcinoma (HNSCC) are numerous and include a variety of different pathways. The accumulation and selection of these aberrant pathways can sometimes be due to random chance, but more commonly, they are due to a lifetime of environmental exposure, such as tobacco and alcohol. Thus, the opportunity for DNA damage is high, and there is often a multistep accumulation of genetic events that leads to the development of HNSCC.

The fundamental and simplified concept of the genetic basis behind cancer is the overexpression of oncogenes and/or the silencing of tumor suppressor genes. However, the field of molecular biology has gone well beyond simply looking for areas of deletion, mutation, or amplification. This review will cover many of the known genetic alterations in HNSCC and attempt to compartmentalize them based mostly on molecular techniques. It is important to note, however, that there are many different ways to assess a particular gene's importance in carcinogenesis. While much progress has been made in our understanding of the genetic basis of HNSCC, it is clear that we have only begun to understand the complex interactions between the gene products and pathways, the transcriptional regulation of these genes, and the post-translational modifications that occur. We will try to simplify what we do know while highlighting some of the newer, promising areas of research.

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Cytogenetics and loss of heterozygosity

The cytogenetic analysis of cells has evolved from the gross visual analysis of chromosomes arrested in metaphase, to a detailed high-resolution map of the regions of chromosomal gain, loss, and translocation. Spectral karyotyping adds the use of chromophores specific to each chromosome to provide contrast, in addition to the normal banding pattern seen in metaphase. Comparative genomic hybridization is a technique whereby normal and tumor DNA is labeled and hybridized to normal metaphase chromosomes. The fluorescence pattern can then be analyzed for increased or decreased intensity. Fluorescent in situ hybridization (FISH) employs sequence specific probes that are detected after hybridization, thus allowing for detection of particular genes of interest, as well as a direct visualization of copy number per cell.

These techniques have all been applied in HNSCC, and have shown rather consistent results. Some investigators have also elected to use the techniques in parallel in order to confirm their results. The common regions of chromosomal loss reported are at 1p, 3p, 4p, 5q, 8p, 10p, 11q, 13q, and 18q, with gains at 1q, 3q, 5p, 7q, 8q, 9q, 11q, 12p, 14q, and 15q.[1–5] A recent study looked at DNA ploidy using FISH and found that there was a significantly higher number of non-diploid cells in malignant lesions versus premalignant cells.[6]

Single nucleotide polymorphisms (SNPs) are areas scattered around the genome that have altered DNA sequences that may not lead to an amino acid alteration, or altered sequences that do not seem to have any adverse effect in 'normal' individuals. These SNPs may be markers for disease predisposition, or may be used to genetically identify patients, as they tend to cluster with ethnic background. SNPs located in the DNA repair genes may be a marker for HNSCC development, and this has been suggested in many studies.[7–10] These polymorphisms have also been detected in

enzymes thought to detoxify environmental toxins and certain patterns may confer higher or lower risks for HNSCC.[11–15] The Pro/Pro genotype of the *TP53* codon 72 polymorphism was found to be associated with an increased risk of oral cancer in non-smokers and associated with a worse prognosis.[16] SNPs can also be used as a surrogate representation of other particular genes of interest, providing information about loss or gain of that genomic region.

Microsatellites are tandem nucleotide repeats generally located within non-coding areas of the genome. They can have variable length and have been mapped to specific chromosomal regions, allowing for detection of adjacent genes of interest. Using simple PCR based techniques, one can identify if there is loss of genetic material, represented by complete deletion, or loss, of one allele (also known as loss of heterozygosity or LOH). This technique was the basis of the HNSCC progression model, indicating that there is a relatively common pattern of DNA allelic loss as one progresses from the premalignant to the malignant stage.[17] Presumably, a tumor suppressor gene (TSG) may be in the area of loss and thus would make the host more susceptible to dysfunction of this gene, leading to the development of cancer. LOH has some direct clinical implications in terms of prognosis and will be discussed further in another article.

Microarrays

Broadly, microarray technology involves the miniaturization of DNA sequence hybridization onto microscopic surfaces, which can then be read by a precise laser, able to detect and interpret the signal of these minute fluorophores. These microarrays can be used to detect DNA or RNA and have evolved to incorporate nearly the entire known human genome in a single experiment.

CGH or SNP arrays have the ability to finely map regions of chromosomal gain or loss, much more precisely than the conventional molecular techniques. CGH arrays have been used to profile HNSCC samples,[18,19] and one study identified a novel putative oncogene, *LRP12* using this technique.[20] SNP array has also been used in HNSCC and has drawn attention to loss of chromosome 6q.[21] CGH analysis has also been used to help predict differences in chemoradiosensitivity, as demonstrated by a recent publication where there was a clear distinction in genetic profile between the chemoradiosensitive and resistant patient groups.[22]

While CGH or SNP arrays focus on the DNA alterations, expression microarrays examine the mRNA component of samples. Early experiments focused on creating gene expression lists that differentiated tumor from normal,[23,24] or even from premalignant disease.[25]

More recent analyses have chosen to focus on particular subgroups and to determine what the expression differences are that contribute to the different behavior or characteristics between them. For example, one could develop a gene expression panel to differentiate those samples which presented with nodal metastasis. One group found a panel of 158 genes that differentiated metastatic versus non-metastatic tumors,[26] and another group found a 160 gene set.[27] These gene products can then be further investigated to determine whether they have true functional roles in the development of metastasis. One study used microarrays focused on angiogenesis and metastatic pathways to look at predicting locoregional failure in patients undergoing primary chemoradiotherapy. The investigators found that *MDM2* and *erbB2* expression were independent negative predictors of locoregional disease-free survival.[28] Another similar study looked at expression arrays comparing HNSCC cell lines that exhibited relative radioresistance and radiosensitivity: 167 genes were detected, and of these, 25 were mapped as cancer related genes involved with growth, proliferation, apoptosis, and adhesion.[29] One recent study, profiled 59 HNSCCs and found that there was a cluster of overexpression in hypoxia-regulated genes, which was an independent prognostic factor for recurrence-free survival.[30]

There is no question that microarray technology has greatly improved our ability to identify meaningful targets and to condense years of work into one experiment. The analysis of such large amounts of data is an entire field of study unto itself, and in the future, integrating the data from DNA and mRNA arrays will be an especially powerful tool in identifying the key elements involved in HNSCC.

Tumor suppressor genes

The natural cell cycle includes programmed cell death, or apoptosis. Cells that are able to sidestep this process, or gain a selective growth advantage over the other cells have the potential to be come cancers. TSGs are those genes that normally function to maintain this growth homeostasis. They regulate the cell cycle, apoptosis, cell adhesion, and DNA repair. Aberrations such as deletions or mutations in these genes can lead to unchecked cell division and cancer formation.

TP53, a gene involved in apoptosis and cell cycle regulation, is one of the earliest TSGs discovered in cancer and found to be one of the most pervasive across all tumor types.[31] It was initially thought to be an oncogene, as there was aberrant overexpression of mutated forms of *TP53*, but it was subsequently shown that these forms were not functional.[32] Indeed, it was the silencing of the gene most often through various point mutations that led to tumor formation, and this is the case as well for premalignant disease[33] and HNSCC.[34] Complete knockout of the *TP53* gene in a mouse model led to the development of metastatic squamous cell carcinoma in 100% of the animals, whereas the heterozygous mice developed tumors much later.[35] While *TP53* mutations are a rather complex set to study, a large-scale, recent analysis shows that disruptive *TP53* mutations (those which lead to a large degree of alteration of the protein structure) were associated with reduced survival in HNSCC patients.[36] The *TP53* story becomes even more intricate as the *MDM* class of oncogenes, specifically *MDM2* and now potentially *MDM4*, have been shown to inhibit *TP53* in the absence of *TP53* mutation.[37] Overall, *TP53* continues to be a major focus of study in HNSCC, particularly now that there is evidence that it has a role in predicting patient outcome and response to therapy.[38–43]

The 9p21 locus has been a topic of study in HNSCC, as there is a high incidence of LOH and cytogenetic abnormality at this site. The *CDKN2a* locus codes for both *p16* and *p14(ARF),* which are putative TSGs that regulate the cell cycle and stabilize *TP53* through *MDM2* respectively. Despite the uniformly low expression of these TSGs in HNSCC, there has been a lack of concomitant genetic alterations, such as mutation or LOH. The explanation is most likely that this locus is under epigenetic control, such as promoter hypermethylation, which serves to silence the gene.[44,45]

There have been numerous other putative TSGs involved in HNSCC. More recently, the deleted in colorectal cancer (*DCC*) gene located at 18q21 has been under study for its involvement in HNSCC as a conditional TSG which requires binding to netrin-1 for its growth effects.[46,47] High resolution CGH array analysis of HNSCC also helped to detect loss of the *FAT* gene at 4q35. *FAT* is thought to play a role in cell-cell adhesion within the cadherin family.[48] 8-oxoguanine DNA glycosylase 1 (*OGG1*) is a DNA repair enzyme whose reduction in function has been shown to be a risk factor in lung cancer and head and neck cancer. Interestingly, *OGG* function returned to baseline after successful treatment of the patients' HNSCC.[49]

Our concept of tumorigenesis has not changed in that the role of TSGs continues to be studied in carcinogenesis. It is likely that there are a myriad of TSGs that are silenced in HNSCC, but the pattern of gene silencing may be quite different between patients. Identifying the relevant genes is an important aspect to our understanding of the development of cancer, and in many cases seems to have prognostic as well as therapeutic implications.

Oncogenes

The identification of oncogenes has been somewhat more limited due to technical aspects of molecular biology – it is easier to identify a mutation or loss of DNA, than it is to identify the meaningful overexpression of a gene. It makes intuitive sense that the aberrant overexpression of those genes that are useful in promoting the growth, survival, and spread of cells could lead to the development of a cancerous cell, but the degree and timing of overexpression needed is less evident.

One of the more studied oncogenes in HNSCC is the epidermal growth factor receptor (*EGFR*). It has been shown to be overexpressed in a majority of HNSCC tumors[50] and has been linked to improved survival.[51] There are many therapeutic implications of EGFR, as it works through the tyrosine kinase cascade. This gene will be further discussed in this issue.[73]

The *ΔNp63-α* gene is a homolog to *TP53* and has been shown to be overexpressed in HNSCC with oncogenic effects.[52] Detailed mechanistic studies have suggested that it leads to the accumulation of β-catenin, a transcription factor that may play a role in cell adhesion and in the *Wnt* signaling pathway. A more recent study showed that *ΔNp63-α* inhibited *p73*, a TSG related to *TP53*, which is also involved in the *bcl-2* mediated apoptotic pathway.[53]

Matrix metalloproteinases (*MMPs*) are a family of genes thought to be involved in cell adhesion, proliferation, and migration. *MMP7* gene expression was associated with a risk of early HNSCC.[54] In an early study, *MMP2* was found to be related to lymph node metastasis,[55,56] and a subsequent study confirmed that *MMP9* and other *MMPs* were related to infiltrative growth and lymph node involvement.[56,57] In an oral SCC cell lines, *MMP3* appeared to be necessary for anchorage-independent growth.[58]

Often times, the cytogenetic analyses will focus researchers to particular amplified regions of the cancer genome to identify putative oncogenes. For example, 11q13 amplification has consistently been shown to be present in HNSCC, which has sparked many investigations of the possible targets located at this locus. Recent CGH-array based analysis isolated a 1.7 Mb region with 13 possible genes. The investigators finally settled on the *Fas*-associated death domain as an important gene of interest, that may also be linked to taxol sensitivity.[59] Other possible oncogenes involved in HSNCC include *c-Jun* NH2-terminal kinases (*JNK*), which are involved in T-cell differentiation and apoptosis.[60] Integrated arrays have looked at combining CGH array with expression to match those areas of relative gain to increased expression. Some novel targets on chromosome 7p14 have been identified, including *NT5C3*, *ANLN*, and *INHBA*.[61]

As our molecular techniques advance into more detailed mapping of the genome, our ability to identify oncogenes and TSGs has become more refined as well. As with TSGs, the range of oncogenes being identified is quite broad, and the often interrelated mechanisms by which they work are becoming more complex.

Mitochondrial mutations

Mitochondria are certainly mysterious elements within the cellular structure, as they are self-contained organelles with a separate genome and even possess a unique genetic code. While they are known to play a role in cellular respiration and the generation of ATP, their role in carcinogenesis has only recently come to light.

There is an accumulation of mitochondrial mutations in HSNCC, as evidenced by the increased mitochondrial DNA content seen as one progresses from premalignant to malignant head and neck lesions.[62] One study found that point mutations in the ATP subunit 6 may have an antiapoptotic effect in cancer cell lines.[63] A more direct mechanistic link between cancer formation and mitochondria involve the accumulation of succinate (part of the tricarboxylic acid cycle), which inhibits the degradation if hypoxia-inducible factor(*HIF*)-1alpha, an angiogenic factor activated by tumor hypoxia.[64] Direct sequence analysis of HNSCC samples showed that mitochondrial mutation was a frequent event and also confirmed the accumulation of *HIF-1alpha* in cell lines.[65]

The role of mitochondrial mutations in carcinogenesis will continue to evolve, and they are an attractive target as markers of disease, as there is an abundance of mitochondrial DNA present in every cell. Furthermore, there may be an expanded array of possible therapeutic interventions given their unique role in cellular function.

Other genetic alterations

There are many other newer avenues of research that have emerged, and their impact in HNSCC remains to be seen.

Proteomics involves the high-throughput analysis of proteins within samples. Such assays have been used in HNSCC samples and have shown promising results.[66–69] The patterns detected and the particular proteins identified can be used to characterize the tumors, as well as provide insights into the mechanisms involved in carcinogenesis. There is much more that needs to be done in terms of the bioinformatic analysis of these assays and in the proper identification of the protein peaks. Once there is a better understanding of the data produced by proteomic studies, it opens the door to a broader array of serum analyses whereby the diagnosis, response to therapy, and recurrence might be detected by a simple blood test.

The cancer stem cell hypothesis proposes that there are subsets of cancer cells which have the ability to self-renew, and it is these cells that are the main progenitor cells that fuel carcinogenesis. A recent study sorted HNSCC cells based on the cell surface marker CD44 found that the CD44(+) population had the ability to give rise to new tumors in vivo, and could be passaged and differentiate in vitro.[70] While this experiment has not yet been replicated in HNSCC, it is an intriguing concept in that most experimental molecular biology assumes that there is a clonal population of tumor cells, all sharing the same genetic alterations. The cancer stem cell hypothesis would argue that eliminating the subpopulation of progenitor cells would be much more efficient in treating tumors. Thus, there are widespread clinical and research implications, making it a very exciting area of study.

MicroRNA (mir) molecules are small ~22 nucleotide, non-coding RNA molecules that have been shown to regulate post-transcriptional gene expression.[71] These mirs are thought to be involved in a host of cellular processes including differentiation, apoptosis, and proliferation. MicroRNA arrays are now being performed on HNSCC, but the interpretation of the results is still at its early phase.[72] The interactions of the mirs can be difficult to predict, and each one may have several putative targets. However, there is no question that they play a large role in the regulation of gene transcription, and they are also a powerful experimental tool in studying the effects of gene silencing in vitro or in vivo.

Conclusion

The field of molecular biology has been growing exponentially, with the global expansion of our understanding of cancer biology in general, and the introduction of newer molecular techniques. Our understanding of the fundamental mechanisms behind HNSCC has improved, but this has also led to a greater appreciation for the complexity of this disease process. The lab research led to some clinical applications as discussed in a subsequent article,[74] but there is much more that needs to be understood before we are able to make a significant impact on the treatment of patients.

Conflict of interest statement

None of the authors have any actual or potential conflict of interest including any financial, personal or other relationships with people or organizations that could inappropriately influence (bias) this work.

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References

1. Jin C, Jin Y, Wennerberg J, Annertz K, Enoksson J, Mertens F. Cytogenetic abnormalities in 106 oral squamous cell carcinomas. *Cancer Genet Cytogenet* 2006;**164**(1):44–53.
2. Patmore HS, Ashman JN, Stafford ND, Berrieman HK, MacDonald A, Greenman J, 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**(1):55–62.
3. Singh B, Gogineni S, Goberdhan A, Sacks P, Shaha A, Shah J, et al. Spectral karyotyping analysis of head and neck squamous cell carcinoma. *Laryngoscope* 2001;**111**(9):1545–50.
4. Singh B, Gogineni SK, Sacks PG, Shaha AR, Shah JP, Stoffel A, et al. Molecular cytogenetic characterization of head and neck squamous cell carcinoma and refinement of 3q amplification. *Cancer Res* 2001;**61**(11):4506–13.
5. Uchida K, Oga A, Okafuji M, Mihara M, Kawauchi S, Furuya T, et al. Molecular cytogenetic analysis of oral squamous cell carcinomas by comparative genomic hybridization, spectral karyotyping, and fluorescence in situ hybridization. *Cancer Genet Cytogenet* 2006;**167**(2):109–16.
6. Hirshberg A, Yarom N, Amariglio N, Yahalom R, Adam I, Stanchescu R, et al. Detection of non-diploid cells in premalignant and malignant oral lesions using combined morphological and FISH analysis - a new method for early detection of suspicious oral lesions. *Cancer Lett* 2007;**253**(2):282–90.
7. An J, Liu Z, Hu Z, Li G, Wang LE, Sturgis EM, et al. Potentially functional single nucleotide polymorphisms in the core nucleotide excision repair genes and risk of squamous cell carcinoma of the head and neck. *Cancer Epidemiol Biomarkers Prev* 2007;**16**(8):1633–8.
8. Li C, Hu Z, Lu J, Liu Z, Wang LE, El-Naggar AK, et al. Genetic polymorphisms in DNA base-excision repair genes ADPRT, XRCC1, and APE1 and the risk of squamous cell carcinoma of the head and neck. *Cancer* 2007;**110**(4):867–75.
9. Majumder M, Sikdar N, Ghosh S, Roy B. Polymorphisms at XPD and XRCC1 DNA repair loci and increased risk of oral leukoplakia and cancer among NAT2 slow acetylators. *Int J Cancer* 2007;**120**(10):2148–56.
10. Wang Y, Spitz MR, Lee JJ, Huang M, Lippman SM, Wu X. Nucleotide excision repair pathway genes and oral premalignant lesions. *Clin Cancer Res* 2007;**13**(12):3753–8.
11. Anantharaman D, Chaubal PM, Kannan S, Bhisey RA, Mahimkar MB. Susceptibility to oral cancer by genetic polymorphisms at CYP1A1, GSTM1 and GSTT1 loci among Indians: tobacco exposure as a risk modulator. *Carcinogenesis* 2007;**28**(7):1455–62.
12. Datta S, Majumder M, Biswas NK, Sikdar N, Roy B. Increased risk of oral cancer in relation to common Indian mitochondrial polymorphisms and Autosomal GSTP1 locus. *Cancer* 2007;**110**(9):1991–9.
13. Li D, Wang LE, Chang P, El-Naggar AK, Sturgis EM, Wei Q. In vitro benzo[a]pyrene diol epoxide-induced DNA adducts and risk of squamous cell carcinoma of head and neck. *Cancer Res* 2007;**67**(12):5628–34.
14. Singh M, Shah PP, Singh AP, Ruwali M, Mathur N, Pant MC, et al. Association of genetic polymorphisms in glutathione S-transferases and susceptibility to head and neck cancer. *Mutat Res* 2008;**638**(1-2):184–94.
15. Suzen HS, Guvenc G, Turanli M, Comert E, Duydu Y, Elhan A. The role of GSTM1 and GSTT1 polymorphisms in head and neck cancer risk. *Oncol Res* 2007;**16**(9):423–9.
16. Kuroda Y, Nakao H, Ikemura K, Katoh T. Association between the TP53 codon72 polymorphism and oral cancer risk and prognosis. *Oral Oncol* 2007;**43**(10):1043–8.
17. Califano J, van der Riet P, Westra W, Nawroz H, Clayman G, Piantadosi S, et al. Genetic progression model for head and neck cancer: implications for field cancerization. *Cancer Res* 1996;**56**(11):2488–92.
18. Baldwin C, Garnis C, Zhang L, Rosin MP, Lam WL. Multiple microalterations detected at high frequency in oral cancer. *Cancer Res* 2005;**65**(17):7561–7.
19. Sparano A, Quesnelle KM, Kumar MS, Wang Y, Sylvester AJ, Feldman M, et al. Genome-wide profiling of oral squamous cell carcinoma by array-based comparative genomic hybridization. *Laryngoscope* 2006;**116**(5):735–41.
20. Garnis C, Coe BP, Zhang L, Rosin MP, Lam WL. Overexpression of LRP12, a gene contained within an 8q22 amplicon identified by high-resolution array CGH analysis of oral squamous cell carcinomas. *Oncogene* 2004;**23**(14):2582–6.
21. Tong BC, Dhir K, Ha PK, Westra WH, Alter BP, Sidransky D, et al. Use of single nucleotide polymorphism arrays to identify a novel region of loss on chromosome 6q in squamous cell carcinomas of the oral cavity. *Head Neck* 2004;**26**(4):345–52.
22. van den Broek GB, Wreesmann VB, van den Brekel MW, Rasch CR, Balm AJ, Rao PH. Genetic abnormalities associated with chemoradiation resistance of head and neck squamous cell carcinoma. *Clin Cancer Res* 2007;**13**(15 Pt 1):4386–91.
23. Gottschlich S, Ambrosch P, Cordes C, Gorogh T, Schreiber S, Hasler R. Gene expression profiling of head and neck squamous cell carcinoma using cDNA microarrays. *Int J Oncol* 2006;**29**(3):605–13.
24. Whipple ME, Mendez E, Farwell DG, Agoff SN, Chen C. A genomic predictor of oral squamous cell carcinoma. *Laryngoscope* 2004;**114**(8):1346–54.
25. Ha PK, Benoit NE, Yochem R, Sciubba J, Zahurak M, Sidransky D, et al. A transcriptional progression model for head and neck cancer. *Clin Cancer Res* 2003;**9**(8):3058–64.
26. Carinci F, Arcelli D, Lo Muzio L, Francioso F, Valentini D, Evangelisti R, et al. Molecular classification of nodal metastasis in primary larynx squamous cell carcinoma. *Trans Res* 2007;**150**(4):233–45.
27. Mendez E, Fan W, Choi P, Agoff SN, Whipple M, Farwell DG, et al. Tumor-specific genetic expression profile of metastatic oral squamous cell carcinoma. *Head Neck* 2007;**29**(9):803–14.
28. Ganly I, Talbot S, Carlson D, Viale A, Maghami E, Osman I, et al. Identification of angiogenesis/metastases genes predicting chemoradiotherapy response in patients with laryngopharyngeal carcinoma. *J Clin Oncol* 2007;**25**(11):1369–76.
29. Ishigami T, Uzawa K, Higo M, Nomura H, Saito K, Kato Y, et al. Genes and molecular pathways related to radioresistance of oral squamous cell carcinoma cells. *Int J Cancer* 2007;**120**(10):2262–70.
30. Winter SC, Buffa FM, Silva P, Miller C, Valentine HR, Turley H, et al. Relation of a hypoxia metagene derived from head and neck cancer to prognosis of multiple cancers. *Cancer Res* 2007;**67**(7):3441–9.
31. Olivier M, Eeles R, Hollstein M, Khan MA, Harris CC, Hainaut P. The IARC TP53 database: news online mutation analysis and recommendations to users. *Hum Mutat* 2002;**19**(6):607–14.
32. Sherr CJ. Principles of tumor suppression. *Cell* 2004;**116**(2):235–46.
33. Boyle JO, Hakim J, Koch W, van der Riet P, Hruban RH, Roa RA, et al. The incidence of p53 mutations increases with progression of head and neck cancer. *Cancer Res* 1993;**53**(19):4477–80.
34. Somers KD, Merrick MA, Lopez ME, Incognito LS, Schechter GL, Casey G. Frequent p53 mutations in head and neck cancer. *Cancer Res* 1992;**52**(21):5997–6000.
35. Ku TK, Nguyen DC, Karaman M, Gill P, Hacia JG, Crowe DL. Loss of p53 expression correlates with metastatic phenotype and transcriptional profile in a new mouse model of head and neck cancer. *Mol Cancer Res* 2007;**5**(4):351–62.
36. Poeta ML, Manola J, Goldwasser MA, Forastiere A, Benoit N, Califano JA, et al. TP53 mutations and survival in squamous-cell carcinoma of the head and neck. *New Engl J Med* 2007;**357**(25):2552–61.
37. Valentin-Vega YA, Barboza JA, Chau GP, El-Naggar AK, Lozano G. High levels of the p53 inhibitor MDM4 in head and neck squamous carcinomas. *Hum Pathol* 2007;**38**(10):1553–62.
38. Bradford CR, Zhu S, Ogawa H, Ogawa T, Ubell M, Narayan A, et al. P53 mutation correlates with cisplatin sensitivity in head and neck squamous cell carcinoma lines. *Head Neck* 2003;**25**(8):654–61.
39. Cabelguenne A, Blons H, de Waziers I, Carnot F, Houllier AM, Soussi T, et al. p53 alterations predict tumor response to neoadjuvant chemotherapy in head and neck squamous cell carcinoma: a prospective series. *J Clin Oncol* 2000;**18**(7):1465–73.
40. Koch WM, Brennan JA, Zahurak M, Goodman SN, Westra WH, Schwab D, et al. p53 mutation and locoregional treatment failure in head and neck squamous cell carcinoma. *J Nat Cancer Inst* 1996;**88**(21):1580–6.
41. Mineta H, Borg A, Dictor M, Wahlberg P, Akervall J, Wennerberg J. p53 mutation, but not p53 overexpression, correlates with survival in head and neck squamous cell carcinoma. *Br J Cancer* 1998;**78**(8):1084–90.

42. Temam S, Flahault A, Perie S, Monceaux G, Coulet F, Callard P, et al. p53 gene status as a predictor of tumor response to induction chemotherapy of patients with locoregionally advanced squamous cell carcinomas of the head and neck. *J Clin Oncol* 2000;**18**(2):385–94.
43. Friedman J, Nottingham L, Duggal P, Pernas FG, Yan B, Yang XP, et al. Deficient TP53 expression, function, and cisplatin sensitivity are restored by quinacrine in head and neck cancer. *Clin Cancer Res* 2007;**13**(22 Pt 1):6568–78.
44. Ai L, Stephenson KK, Ling W, Zuo C, Mukunyadzi P, Suen JY, et al. The p16 (CDKN2a/INK4a) tumor-suppressor gene in head and neck squamous cell carcinoma: a promoter methylation and protein expression study in 100 cases. *Mod Pathol* 2003;**16**(9):944–50.
45. Reed AL, Califano J, Cairns P, Westra WH, Jones RM, Koch W, et al. High frequency of p16 (CDKN2/MTS-1/INK4A) inactivation in head and neck squamous cell carcinoma. *Cancer Res* 1996;**56**(16):3630–3.
46. Carvalho AL, Chuang A, Jiang WW, Lee J, Begum S, Poeta L, et al. Deleted in colorectal cancer is a putative conditional tumor-suppressor gene inactivated by promoter hypermethylation in head and neck squamous cell carcinoma. *Cancer Res* 2006;**66**(19):9401–7.
47. Venugopalan M, Wood TF, Wilczynski SP, Sen S, Peters J, Ma GC, et al. Loss of heterozygosity in squamous cell carcinomas of the head and neck defines a tumor suppressor gene region on 11q13. *Cancer Genet Cytogenet* 1998;**104**(2):124–32.
48. Nakaya K, Yamagata HD, Arita N, Nakashiro KI, Nose M, Miki T, et al. Identification of homozygous deletions of tumor suppressor gene FAT in oral cancer using CGH-array. *Oncogene* 2007;**26**(36):5300–8.
49. Paz-Elizur T, Ben-Yosef R, Elinger D, Vexler A, Krupsky M, Berrebi A, et al. Reduced repair of the oxidative 8-oxoguanine DNA damage and risk of head and neck cancer. *Cancer Res* 2006;**66**(24):11683–9.
50. Ishitoya J, Toriyama M, Oguchi N, Kitamura K, Ohshima M, Asano K, et al. Gene amplification and overexpression of EGF receptor in squamous cell carcinomas of the head and neck. *Br J Cancer* 1989;**59**(4):559–62.
51. Rubin Grandis J, Melhem MF, Gooding WE, Day R, Holst VA, Wagener MM, et al. Levels of TGF-alpha and EGFR protein in head and neck squamous cell carcinoma and patient survival. *J Nat Cancer Inst* 1998;**90**(11):824–32.
52. Hibi K, Trink B, Patturajan M, Westra WH, Caballero OL, Hill DE, et al. AIS is an oncogene amplified in squamous cell carcinoma. *Proc Nat Acad Sci USA* 2000;**97**(10):5462–7.
53. Rocco JW, Leong CO, Kuperwasser N, DeYoung MP, Ellisen LW. p63 mediates survival in squamous cell carcinoma by suppression of p73-dependent apoptosis. *Cancer Cell* 2006;**9**(1):45–56.
54. Vairaktaris E, Serefoglou Z, Yapijakis C, Vylliotis A, Nkenke E, Derka S, et al. High gene expression of matrix metalloproteinase-7 is associated with early stages of oral cancer. *Anticancer Res* 2007;**27**(4B):2493–8.
55. Kusukawa J, Sasaguri Y, Shima I, Kameyama T, Morimatsu M. Expression of matrix metalloproteinase-2 related to lymph node metastasis of oral squamous cell carcinoma A clinicopathologic study. *Am J Clin Pathol* 1993;**99**(1):18–23.
56. P OC, Rhys-Evans PH, Eccles SA. Expression of matrix metalloproteinases and their inhibitors correlates with invasion and metastasis in squamous cell carcinoma of the head and neck. *Arch Otolaryngol Head Neck Surg* 2001;**127**(7):813–20.
57. Franchi A, Santucci M, Masini E, Sardi I, Paglierani M, Gallo O. Expression of matrix metalloproteinase 1, matrix metalloproteinase 2, and matrix metalloproteinase 9 in carcinoma of the head and neck. *Cancer* 2002;**95**(9):1902–10.
58. Liu SY, Liu YC, Huang WT, Huang GC, Su HJ, Lin MH. Requirement of MMP-3 in anchorage-independent growth of oral squamous cell carcinomas. *J Oral Pathol Med* 2007;**36**(7):430–5.
59. Gibcus JH, Menkema L, Mastik MF, Hermsen MA, de Bock GH, van Velthuysen ML, et al. Amplicon mapping and expression profiling identify the Fas-associated death domain gene as a new driver in the 11q13. 3 amplicon in laryngeal/pharyngeal cancer. *Clin Cancer Res* 2007;**13**(21):6257–66.
60. Gross ND, Boyle JO, Du B, Kekatpure VD, Lantowski A, Thaler HT, et al. Inhibition of Jun NH2-terminal kinases suppresses the growth of experimental head and neck squamous cell carcinoma. *Clin Cancer Res* 2007;**13**(19):5910–7.
61. Shimizu S, Seki N, Sugimoto T, Horiguchi S, Tanzawa H, Hanazawa T, et al. Identification of molecular targets in head and neck squamous cell carcinomas based on genome-wide gene expression profiling. *Oncol Rep* 2007;**18**(6):1489–97.
62. Kim MM, Clinger JD, Masayesva BG, Ha PK, Zahurak ML, Westra WH, et al. Mitochondrial DNA quantity increases with histopathologic grade in premalignant and malignant head and neck lesions. *Clin Cancer Res* 2004;**10**(24):8512–5.
63. Shidara Y, Yamagata K, Kanamori T, Nakano K, Kwong JQ, Manfredi G, et al. Positive contribution of pathogenic mutations in the mitochondrial genome to the promotion of cancer by prevention from apoptosis. *Cancer Res* 2005;**65**(5):1655–63.
64. Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, et al. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. *Cancer Cell* 2005;**7**(1):77–85.
65. Zhou S, Kachhap S, Sun W, Wu G, Chuang A, Poeta L, et al. Frequency and phenotypic implications of mitochondrial DNA mutations in human squamous cell cancers of the head and neck. *Proc Nat Acad Sci USA* 2007;**104**(18):7540–5.
66. He QY, Chen J, Kung HF, Yuen AP, Chiu JF. Identification of tumor-associated proteins in oral tongue squamous cell carcinoma by proteomics. *Proteomics* 2004;**4**(1):271–8.
67. Koike H, Uzawa K, Nakashima D, Shimada K, Kato Y, Higo M, et al. Identification of differentially expressed proteins in oral squamous cell carcinoma using a global proteomic approach. *Int J Oncol* 2005;**27**(1):59–67.
68. Roesch-Ely M, Nees M, Karsai S, Ruess A, Bogumil R, Warnken U, et al. Proteomic analysis reveals successive aberrations in protein expression from healthy mucosa to invasive head and neck cancer. *Oncogene* 2007;**26**(1):54–64.
69. Sewell DA, Yuan CX, Robertson E. Proteomic signatures in laryngeal squamous cell carcinoma. *ORL J Otorhinolaryngol Relat Spec* 2007;**69**(2):77–84.
70. Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proc Nat Acad Sci USA* 2007;**104**(3):973–8.
71. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993;**75**(5):843–54.
72. Tran N, McLean T, Zhang X, Zhao CJ, Thomson JM, O'Brien C, et al. MicroRNA expression profiles in head and neck cancer cell lines. *Biochem Biophys Res Commun* 2007;**358**(1):12–7.
73. Lorch JH, Posner MR, Wirth LJ, Haddad RI. Seeking alternative biological therapies: The future of targeted molecular treatment. *Oral Oncol* 2009;**45**:449–55.
74. Glazer CA, Chang SS, Ha PK, Califano JA. Applying the molecular biology and epigenetics of head and neck cancer in everyday clinical practice. *Oral Oncol* 2009;**45**:442–8.