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Head and Neck Squamous Cell Carcinoma in the Young: A Spectrum or a Distinct Group? Part 2

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Abstract A recent increase in the number of young patients (often nonsmokers) developing head and neck squamous cell carcinoma (HNSCC) has been documented, however, there remains no clear evidence to support the significance of any single determinant. The typical HNSCC arises as a result of a multistep process and the progression model involving multiple genetic and epigenetic events is thought to be relatively consistent. While the progression model itself may be consistent, detection methods used in genomic studies are variable and all have their limitations. This article reviews changes at a molecular level in the typical HNSCC patient (the over 40 year old male smoker) and compares the profile to that of the young adult with HNSCC. Human papillomavirus infection with high risk types 16 and 18 has widely been reported as one of the prominent mechanisms behind the development of oropharyngeal cancer. A review of recent studies in relation to HPV and HNSCC is undertaken in this article, in an effort to examine the role that HPV plays in the development of HNSCC in young adults.

Keywords Genomic instability · HPV · Comparative genomic hybridization

Head and neck squamous cell carcinoma (HNSCC) is thought to arise as a multistep process and over the years, what has become apparent is the level of consistency seen in these steps [1] (Fig. 1).

With the recent reported increase in young HNSCC cases many of them nonsmokers, the question arises as to whether these cases have the same genetic alterations as seen in the classic progression model. This section will look at the common molecular events in the classic form of HNSCC and compare with those occurring in the young HNSCC patient.

Genetic instability, an important molecular mechanism in head and neck cancers, has been extensively studied and a high level of conformity exists in relation to specific patterns of DNA copy number gains and losses. Consistently, gains at 3, 5, 8, and 11q have been found, along with losses at 3 and 9p [2].

Loss of chromosome region 3p is a common early genetic event in HNSCC [3]. Specific regions of this arm harbour candidate tumour supressor genes including FHIT and RSSF1A. Loss of chromosome region 9p21 appears to be another early event in head and neck squamous neoplasia, and is found in 70–80% of HNSCC [1].

Although many molecular studies of head and neck SCC and oral SCC have been published, there is a paucity of specific studies involving younger patients.

In our study of HNSCC in young adults, we used array comparative genomic hybridization on a cohort of predominantly nonsmoking young adults ($N = 10$) and compared them with a cohort of mostly smoking older adults ($N = 10$) [4]. We used less than 40 years old as the cut-off for young adults. The focus of this was more on tumors of oral cavity rather than oropharyngeal cancers, as it appears these sites differ markedly in their biology and histology. Results from this study showed that when stratified by age the young cohort do not have the genetic alterations that are seen so consistently in older HNSCC. In fact, the mean

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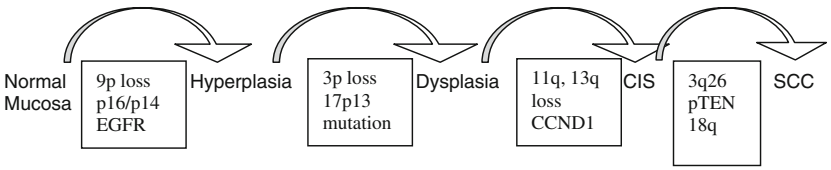

Fig. 1 Progression model for HNSCC carcinogenesis modified from Califano, 1996

number of aberrations in the young nonsmokers was less than 50% of that in the older smokers. In relation to individual targets on the microarray, it was clear that 3p losses were not a frequent event in the young cohort. 3p losses were detected in all of the older cohort, while they were found in only two of the young cohort. Even more striking were the results found at 9p21. The young HNSCCs completely lacked losses in this region, while it remained a consistently altered region in the older cases of HNSCC in the study.

11q13 is a region of interest, as it contains several genes previously shown to be amplified and overexpressed in HNSCC, including CCDN1, EMS 1, FGF3 and FGF4. When examining the copy number gains in this study of young adults with HNSCC, 11q13 copy number gains were detected in the young cohort (60%), although less consistently than in the older cohort (80%) [4]. In relation to smoking status, Koch et al. concluded that 11q13 amplification was significantly more common in smokers. Considering that the young cohort were predominantly nonsmokers in our study, this suggests that 11q13 gain remains a common event in HNSCC irrespective of the causative factor or the age [5].

Overexpression of EGFR can be achieved through regulatory pathway changes, gene structure changes or gene amplification. Ryott et al. noted that EGFR protein expression was found in all 78 of their oral SCC tumours, 72% of which showed high level of expression. 54% of the tumours had high (> or =four gene copies) EGFR gene copy numbers. Studies have shown that a high EGFR gene copy number was significantly more common in nonsmokers, as were EGFR mutations [6]. In our study of genetic alterations in young adults with HNSCC, gains at 7p12 (EGFR region) were detected in 50% of the older group and 50% of the young cohort. Two-thirds of the cases showing EGFR gains were nonsmokers, thus supporting Ryotts findings.

Molecular alterations at the p53 gene have been documented as being the most frequent genetic alteration observed in carcinomas and has been found to be altered in over 70% of HNSCC [7]. Previous p53 studies focusing on young nonsmokers and in fact nonsmokers of any age have found that overexpression of p53 in squamous cell carcinoma is not associated with the classic p53 mutations in exons 5–9 [5]. It has been shown that p53 sequence alterations are decreased in the setting of HPV infection, since there is an alternative means of p53 silencing with the production of E6 [8].

Akt, a downstream mediator of phosphatidylinositol 3-kinase (PI3 K), is a signal transduction protein that plays a central role in tumorigenesis. The tumor suppressor gene PTEN negatively regulates the PI3 K/Akt signaling pathway. In HNSCC, loss of PTEN (10q23.3) can occur in 30% of lesions. In our CGH microarray study, PTEN was found to be deleted five cases (young $N = 2$, old $N = 3$, all smokers). As expected, in line the PTEN loss in these cases, AKT gains were seen. The interesting finding in this study was in relation to the older nonsmokers. All four over 40 year old nonsmokers showed a gain of AKT2, with no corresponding loss of PTEN. This may suggest that dysregulation of the AKT pathway in these nonsmokers is distinct from that of smokers. Further investigation of this signalling network in the context of nonsmokers is warranted.

The CDKN2A gene locus found in chromosome 9p21 encodes two different transcripts, p16 and p14ARF, which are responsible for G1 cell cycle regulation and MDM2 mediated degradation of p53. Even where there are no deletions, p16 is frequently inactivated through methylation resulting in a complete block of gene transcription [9]. In head and neck cancer, the predominant methods of p16 inactivation are methylation and deletion of the gene rather than mutation. Overexpression of $p16^{INK4}$ has also been reported in head and neck cancers, and it is believed that HPV infection, via inactivation of retinoblastoma gene, accounts for these high levels of $p16^{INK4}$ expression [8].

In our CGH microarray study, 9p21 deletion was not a feature found in the young nonsmokers, soTaqMan RT-PCR, methylation specific PCR and immunohistochemistry was used to further profile p16, and it was found that p16 methylation is a more common event in those younger than 40 years in contrast to p16 deletions, which are more common in those older than 40 years. Consequently, it appears that specific modes of inactivation of p16 in HNSCC are related to specific patient risk profiles [10].

It is now recognized that HPV infection plays an important role in the pathogenesis of a unique subset of oropharyngeal HNSCC [8, 10]. While epidemiologic studies can draw an association between HPV seropositivity and oral cancer, it must also be demonstrated that HPV is present and functioning in these infected cells. There are many methods by which HPV can be detected.

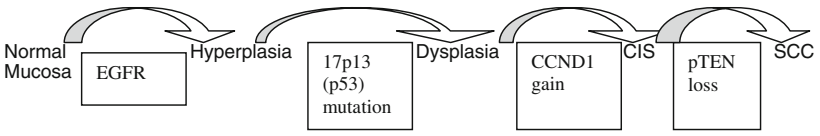

Fig. 2 Progression model of HNSCC in young adult nonsmokers

The commonly used modes of detection include PCR (using either consensus primers or HPV 16 specific primers) for HPV DNA, PCR for E6 mRNA and in situ hybridization for direct visualization of HPV, but every method has its strengths and weaknesses. Our group used nucleic acid sequence based amplification (NASBA) as a robust method for the amplification of E6 and E7 mRNA in 25 HNSCC (including 18 of the cases from the CGH study). Interestingly, HPV 16 messenger RNA was detected exclusively in HNSCC from base of tongue lesions ($N = 4$) and all were males. One of the cases was under 40 years old. Considering the smoking status of these cases and the ages, it seems unlikely that this virus is a primary causative agent of HNSCC in these young adults [10]. Also of note, after detailed study of p16 mRNA expression and p16 immunohistochemistry in this cohort, it was clear that all HPV mRNA positive cases showed p16 overexpression. In relation to copy gain and loss, previous studies have found only occasional chromosomal loss in HPV16 positive cases, suggesting that HPV16 infection is an early event in HNSCC development. The four HPV mRNA positive cases in our study showed the same mean number of copy gains/losses as the average over 40 year old smoking case (mean = 118 total aberrations). This is not surprising considering the positive smoking status of all four cases that were HPV mRNA positive.

Ongoing work by our group involves the use of a chemiluminescence expression array system to study the expression of 31,600 genes in our original 20 samples. Using statistical and data filtering criteria, 131 genes differentially expressed between oral and oropharyngeal cancer in young nonsmokers and old smokers were identified. To date, through follow-up validation (using RT-PCR) of the genes that were found to be differentially expressed in the young nonsmokers, we have found two potential targets of interest: STAT4, which was significantly upregulated in young nonsmokers and CDC6, which was significantly downregulated in this cohort. The study of these is ongoing.

Having reviewed the results from studies focusing on young patients with HNSCC (nonsmokers), we present the current progression model for this patient profile (Fig. 2).

So far, the principal findings in molecular studies of young patients with HNSCC is that HNSCC developing in these patients is markedly different, not in any recognizable phenotypic way, but undoubtedly at a genetic level. The literature to date on this intriguing subset of HNSCC suggests that they are indeed a distinct group rather than a spectrum of the classic disease. It may be helpful for head and neck cancer research groups to collaborate more closely with those from other cancer group, in order to pool resources and subsequent generated data that is specific to the study of young patients. Certainly gastric cancer groups and lung cancer researchers are investigating alterations at a molecular level distinctly related to the young patient.

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