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REVIEW ARTICLE

Chromosomal aberrations in squamous cell carcinomas of the upper aerodigestive tract: biologic insights and clinical opportunities

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Oncogenesis results from a progressive accumulation of genetic aberrations consequent to a complex interplay between carcinogenic factors and innate infidelity of DNA surveillance mechanisms. Although the development of genetic aberrations is random, those conferring survival advantages are selected for in a Darwinian manner, thus allowing continuous adaptation to selection pressures. Chromosomal aberrations are a prominent manifestation of genetic damage, which can be closely linked with tumor behavior and outcome as exemplified by curative treatment of chronic myelogenous leukemia resulting from targeting the *BCR-ABL* translocation. In the case of head and neck squamous cell carcinomas (HNSCC), chromosomal changes are detectable at all stages of tumor development, providing excellent opportunities for genomic prognostication and therapy. Several studies have shown that the overall genomic profile of HNSCC is highly consistent, but individual tumors vary significantly in their complement of genetic alterations, thereby confounding clinical correlation. The application of modern genetic and bioinformatic analytic approaches has facilitated the identification of critical genomic changes in HNSCC, many of which have been linked to clinical outcome. These genetic aberrations represent excellent targets for novel therapeutics, but require validation. The initiation of phase III trials evaluating the therapeutic utility of genetic aberrations suggests a promising future for genome-based treatment of HNSCC.

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Introduction

Modern genetic theory is rooted in Gregor Mendel's work demonstrating that heredity is passed on in discrete units. The content and localization of the hereditary units remained obscure until Miescher's identification of structures that he termed nuclein in white blood cells in 1869. Ultimately, the observations of Sutton (1) that the segregation patterns of chromosome in meiosis matches the Mendelian inheritance and the realization that human diseases (alkaptonuria) is inherited according to Mendelian rules, established Mendelian genetic theory (1–3). Wilhelm Johannsen (4) coined the term gene to describe the Mendelian unit of inheritance and more then a century later, Martin and his colleagues showed that the wrinkled seed characteristic in Mendel's experiments was due to a transposon-like insertion in a gene encoding starch-branding enzyme (4). Extending from this work is the realization that human disease results from genetic aberrations, with cancer being the prototypic process.

Cancer develops when normal cells acquire specific changes in their genetic information that allow them to overcome normal growth regulatory mechanisms, invade surrounding structures and spread to distant anatomic sites (5). Cancer causing genetic changes invoke increased activity of genes that induce cell growth (proto-oncogenes), surrounding blood vessel ingrowth (angiogenesis), cellular dissociation from the environment and cellular migration (proto-oncogenes), and inactivation of genes that limit these processes or promote programmed cell death (tumor suppressor genes) (6). Activation of proto-oncogenes may be acquired through gene dosage increase (as a result of chromosomal gain or genetic amplification), genetic rearrangement (translocation) or changes in single nucleotides in the blueprint of the gene (activating point mutations) (7). On the contrary, tumor suppressor genes are inactivated through loss of genetic information (genetic deletion), inactivating mutations in the genetic code (i.e. missense/nonsense mutations) and blockage in

production of proteins (i.e. promotor hypermethylation) (8, 9).

Normal cells may be born with mutations inherited through the germ line (10). More importantly, all cells acquire a significant number of somatic genetic mutations over the course of their lifetime (5). This as a consequence of the physiologic imbalance between inherent errors in DNA replication and exposure to mutagenic influences on the one hand, and the fidelity of intrinsic DNA repair mechanisms on the other hand (7, 10). Based on the interplay of these processes, mutagenesis is a random process and the chance of inheriting oncogenic mutations is linearly related to the extent of the mutational burden (10). Accordingly, cancer develops at a higher rate in the setting of certain inherited mutational syndromes, increased exposure to mutagenic influences or diminished activity of DNA repair (10, 11). Nonetheless, it has been under debate whether the physiologic mutation rate is sufficient to cause the large number of genetic mutations that are found in cancer cells ($>12\,000$ individual mutations) (12–14). Studies of colorectal cancer have fueled this debate (15). Early in the course of their development, colorectal cancers may increase their chances of acquiring critical oncogenic mutations through inactivation of pathways maintaining genomic stability (16, 17). An important form of genetic instability in colorectal cancers involves chromosomal instability (CIN) (18). CIN may be a result of acquired defects in DNA repair, mitotic spindle formation, and chromosome segregation (19–21). CIN increases genetic heterogeneity within the cellular population thereby paving the way for perpetual Darwinian selection and clonal outgrowth of cells with the fittest genomic content (16, 22). This feature explains the continuous adaptation to inhibiting influences (selective pressures) that characterizes malignant behavior. The 'clonal selection' theory is the root for deciphering the 'genetic barcode' of human cancers and exploiting the information for therapeutic benefit (23, 24). The clinical relevance of chromosomal aberrations in cancer is best exemplified by the therapeutic efficacy of recently identified agents targeting the Philadelphia (Ph)-chromosome in chronic myeloid leukemia (25–27). The Ph-chromosome represents a reciprocal translocation between chromosomes 9 and 22 [t(9;22)(q34;q11.2) resulting in a fusion of the tyrosine kinase proto-oncogene c-ABL (chromosome 9q34) with the serine/threonine kinase gene *BCR* (chromosome 22q11.2) (28). The chimeric protein product features strong oncogenicity through its constitutive tyrosine kinase activity (29, 30). Forty years after identification of the Ph-chromosome, treatment with tyrosine kinase inhibitors directed offers a potential curative treatment for patients with CML (31). This experience serves as the prototypic endpoint for genetic screening strategies in human cancers including head and neck squamous cell carcinoma (HNSCC). In this review, we will focus in on the pathologic relevance of chromosomal aberrations occurring in HNSCC and outline how they can be exploited for therapeutic benefit.

Identification of chromosomal aberrations in human cancer

Traditional analysis of chromosomal aberrations in cancer cells has relied on microscopic evaluation of Giemsa-stained chromosomal-banding patterns in cultured tumor metaphase cells – i.e. conventional cytogenetic karyotyping (CCK) (3). These efforts have revealed a number of key reciprocal chromosomal translocations beyond the *BCR-ABL* translocation, such as the *RET/PTC* rearrangement in papillary thyroid carcinoma (32), the *PAX8/PPARγ* rearrangement in follicular thyroid carcinoma (33, 34) and several recurrent translocations in soft tissue sarcomas as well as several numerical alterations in both hematogenic malignancies and solid tumors (35). However, due to the low resolution of classical-banding techniques, the difficulty of culturing solid tumors, and the cytogenetic complexity of most neoplasms, the majority of chromosomal alterations in cancer remained obscure (36). The evolution of polymerase chain reaction (PCR)-based genomic evaluation techniques and fluorescence *in situ* hybridization (FISH) has facilitated high-resolution chromosomal analysis (without the need for culturing) and contributed significantly to the analysis of solid tumors (Table 1) (36, 37). PCR-based allelotyping analysis [loss of heterozygosity (LOH) analysis] through comparison of the numerical balance between the maternally and paternally inherited alleles of a given locus (normally 1:1), demonstrates that the vast majority of human tumors are characterized by allelic imbalance that may involve large chromosomal regions of all chromosomal arms (38). Although LOH of a given locus is traditionally viewed as evidence for the presence of a putative tumor suppressor gene deletion, a variety of genetic events can give rise to LOH such as chromosomal gain, gene amplification, mitotic recombination, break-induced replication, and gene conversion (39). As LOH analysis alone is unable to distinguish between these events, actual identification of target genes based on LOH has been limited (40). This shortcoming has been resolved with the improvement of FISH-based analytic techniques. Comparative genomic hybridization (CGH; based on competitive hybridization of equal amounts of differentially labeled tumor and normal reference DNA onto normal metaphase chromosomes; Table 1) and spectral karyotyping (SKY; visualization of individual tumor metaphase chromosomes through hybridization with chromosome-specific painting probes; Table 1), both well-established exponents of this development, have contributed significantly to the understanding of the genetic basis of solid tumors with over 1500 published reports utilizing these techniques in the present literature (41, 42). Based on CGH and SKY analyses, gene amplifications associated with cisplatin resistance of germ cell tumors (43), resistance of prostate cancer to endocrine therapy (44), and resistance of colon carcinoma cells to ecteinascidin 743 have been identified (45). Recently, the resolution and speed of genome-wide screening has been improved significantly by the introduction of microarray-based analysis (array CGH, cDNA microarray analysis, tissue microarray analysis)

Table 1 Advantages and limitations of commonly used genome-wide screening techniques

Technique	*Advantages*	*Limitations*
CCK	Overview of complete karyotype including structural and numerical chromosomal changes	Limited resolution unable to identify origins of marker chromosomes, and double minute chromosomes. Need for tissue culture
LOH	High-resolution detection of allelic imbalance Feasible on archival DNA	No true genome-wide screen. Requirement for matched normal reference Does not identify origins of allelic imbalance (i.e. gain, loss, amplification)
FISH	Highly sensitive and specific gene mapping and detection of numerical and structural changes in metaphase chromosomes and interphase nuclei (i.e. feasible on archival tissue)	No true genome-wide screen Prior knowledge of aberrations/loci required
CGH	Genome-wide detection and mapping of chromosomal gains, loosen, and amplifications Requires only small amount (2 μg) of (archival) DNA No need for tissue culture or matched normal reference DNA	Limited resolution (<5 to 10 Mb) Unable to detect balanced chromosomal changes/structural aberrations
SKY	High resolution overview of complete tumor karyotype including structural and numerical aberrations	Does not detect inversions and subtle deletions
cDNA micrarray	Simultaneous appraisal of expression levels of large number ($>10\,000$) of genes in one experiment	Does not detect expression levels of unknown genes or foreign sequences Typically needs matched normal reference
Array CGH	Simultaneous detection of gene copy number of large number of genes without need for culturing, matched reference DNA or fresh frozen tissue	Does not detect structural aberrations or DNA copy number of unknown genes or foreign sequences (HPV, EBV viral integrations)
SAGE	Genome-wide, quantitative evaluation of gene expression, detects expression of all expressed genes within cell	Very laborious
Digital karyotyping	High resolution, quantitative, genome-wide appraisal of DNA copy number Detects foreign sequences (HPV, EBV viral integrations)	Very laborious, small (1000 bp) amplifications or deletions not detected
Quantitative PCR	Highly sensitive, quantitative evaluation of genetic copy number without need for culturing, fresh frozen tissue or matched reference DNA	No true genome wide, unbiased evaluation

PCR, polymerase chain reaction; CGH, comparative genomic hybridization; SKY, spectral karyotyping; EBV, Epstein–Barr virus; CCK, conventional cytogenetic karyotyping; LOH, loss of heterozygosity; FISH, fluorescence *in situ* hybridization.

and high-throughput PCR-based analysis such as digital karyotyping (Table 1) (46, 47). It is quite evident that the accuracy of genomic analysis is improved significantly by using multiple analytic techniques in concert. Using this approach, Barlund et al. associated ribosomal protein S6 kinase activation with poor prognosis in breast cancer, providing a paradigm for high-throughput identification of disease markers and treatment targets (48). Recently, we applied this approach to the analysis of papillary thyroid cancer, identifying MUC1 activation as an independent prognosticator of outcome in these tumors (49). Although techniques for statistical analysis of genomic data remain in evolution (50), it is clear that these are a key adjunct for accurate interpretation and combination of genetic screening results.

Recurrent chromosomal abnormalities in HNSCC

The HNSCC constitutes an excellent model to study the role of chromosomal aberrations in cancer initiation and progression for several reasons. First, HNSCC develops from normal aerodigestive mucosa progressing through a series of histologically identifiable pre-malignant stages (51). This cascade is initiated and promoted by a complex interplay between established mutagens including tobacco, alcohol, and human papilloma virus (HPV) exposure (51). Secondly, HNSCC is associated with an inherited genetic syndrome, namely Fanconi anemia, predisposing affected patients to CIN, and SCC development (52–54). Thirdly, studies have shown that HNSCCs are characterized by a high rate of aneuploidy including a recurrent pattern of both structural and numerical aberrations (55, 56). The complexity of aberrations in HNSCC is evident from genomic screening studies showing possible involvement of virtually every chromosome in these tumors (57, 58). Nonetheless, the core group of abnormalities is highly consistent between tumors, suggesting they are likely to play an important role in HNSCC pathogenesis and progression (57).

The LOH studies of HNSCC demonstrate that chromosomal arms most frequently affected by allelic imbalance include 1q, 3p, 3q, 4q, 6p, 8p, 9p, 11q, 13q, 14q, 17p, 19q, 22q (59, 60). These LOH events predominantly reflect genetic gains, deletions, and amplifications as suggested by CGH studies showing a high rate of copy number aberrations in the LOH-affected chromosomal regions (57, 58) (Fig. 1). Given that gains, losses, and amplifications mainly result from structural chromosomal aberrations in HNSCC [as evident from SKY studies (56, 61); Fig. 1], it is likely that they are caused by chromosomal double-strand breakage and fusion events (39). These findings suggest that dysfunction of the double-strand repair-recombination machinery

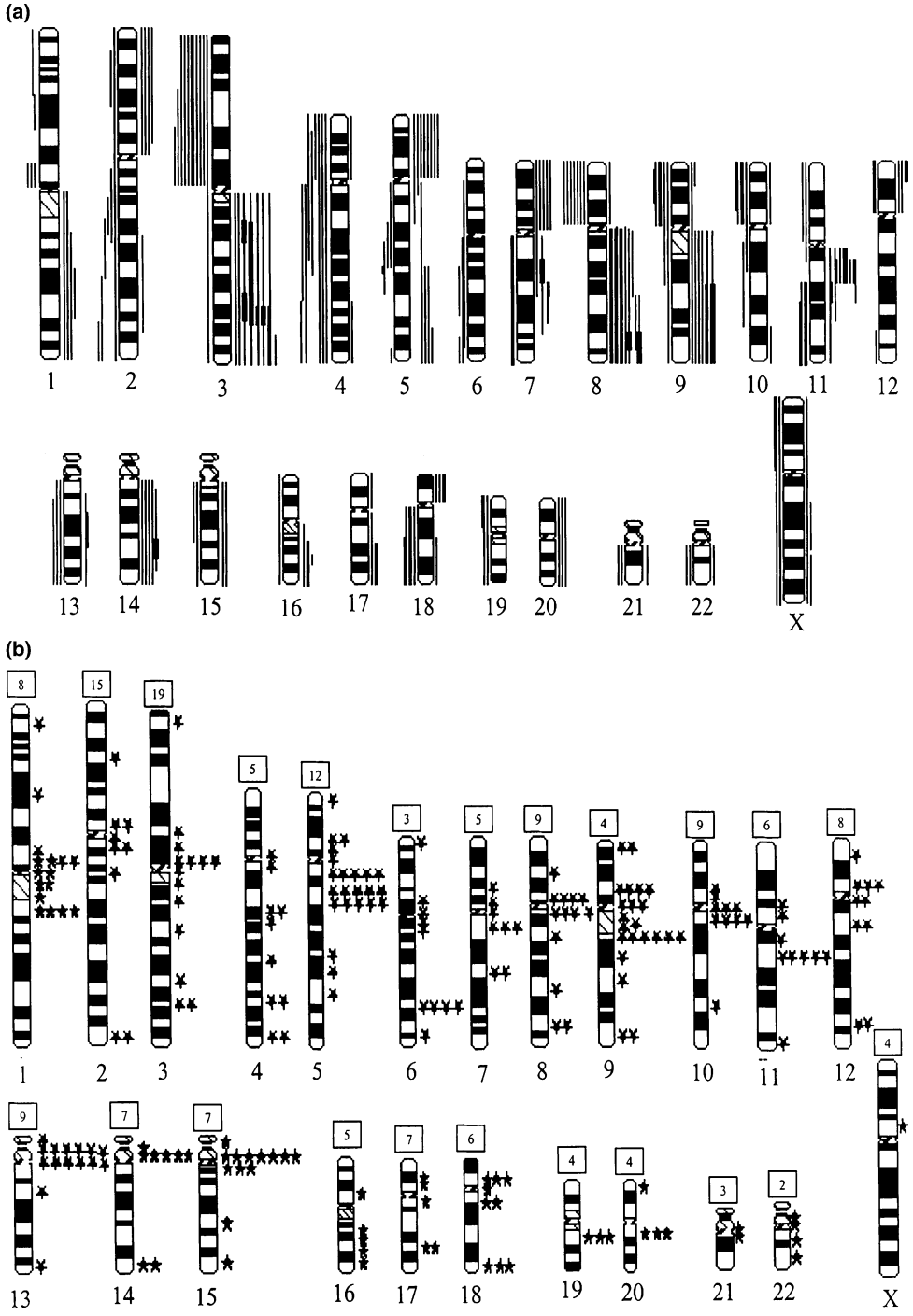

Figure 1 Ideogram showing DNA copy number changes identified by comparative genomic hybridization (CGH) in head and neck cancer cell lines ($n = 12$). Thin vertical lines on either side of the ideogram indicate losses (left) and gains (right) of the chromosomal region. The chromosomal regions of the high-level amplification are shown by thick lines (right). (b) Ideogram showing all of the breakpoints noted in the cell lines identified by spectral karyotyping (SKY; inverted 4′,6′-diamidino-2-phenylindole hydrochloride (DAPI)). The number of breakpoints in each chromosome that were identified by SKY but could not be precisely assigned to a chromosomal band are noted in the box on top of the chromosome.

contributes significantly to HNSCC pathogenesis, as has been implicated for colorectal cancers (39).

The high level of aneuploidy in HNSCC suggests the presence of inherent CIN – i.e. cellular vulnerability to acquire chromosomal alterations (12). Studies investigating the onset of CIN in HNSCC suggest that it occurs early in tumorigenesis, as a significant number of chromosomal aberrations (for example, LOH at chromosomes 3p and 9p) are identifiable by LOH in the earliest stages of neoplastic progression – i.e. mucosal hyperplasia (62, 63). Based on this observation, Califano et al. analyzed the presence of LOH events along different histopathologic stages of progression to HNSCC, showing a stepwise increase in genomic complexity/aneuploidy

from benign squamous hyperplasia to dysplasia, carcinoma *in situ* and finally invasive carcinoma (63). Moreover, the authors associated individual genomic aberrations with specific time-points in malignant progression leading to the development of a HNSCC genetic progression model, similar to that proposed by Vogelstein for colorectal cancer (64). These findings have as been confirmed and refined using a variety of different analytic techniques, including CGH and microarray-based studies (65–67). The clinical implications of deciphering the genetic progression model are clear, including prediction of risk for malignant progression and identification of molecular diagnostic and therapeutic targets [as reviewed recently (51, 68)]. As an example, Mao et al. demonstrated that pre-malignant lesions containing 3p14 or 9p21 alterations have a significantly higher likelihood of evolving into HNSCC (33%) compared to pre-malignant lesions without these changes (6%) (69). The application of genetic knowledge for potential clinical benefit can be further exemplified by: (i) the correlation between the presence of genetically abnormal cells in histologically benign mucosa within the surgical margins of HNSCC resection specimens and a higher risk for recurrence (70); (ii) the localization of unknown primary HNSCC (71); and (iii) the detection of tumor cells in saliva and circulating blood stream from patients with HNSCC (72, 73). These studies foreshadow the introduction of molecular blood tests for HNSCC detection and molecular margin assessment as an aid to surgical decision-making, once the findings are validated and analytic techniques optimized.

Genetic heterogeneity of HNSCC: implications for prognostication

Although HNSCC as a whole are characterized by a highly recurrent pattern of chromosomal aberrations, individual tumors vary significantly in the complement of individual aberrations. The variances allow an opportunity for segregation patients with HNSCC into genomically defined groups. For example, based on CGH analysis, Huang et al. suggested HNSCC could be categorized into at least five different genetic subgroups, a finding that was supported by the results of karyotyping analysis by Hoglund et al. (55, 74). The precise implication of the genetic categorizations remains to be defined but may reflect the pathogenetic influences underlying HNSCC development (75). For example, comparison of CGH findings in HNSCCs occurring in patients with Fanconi anemia and a matched cohort of sporadic HNSCCs from the general population showed a dramatic difference in the pattern of chromosomal aberrations (D. I. Kutler, P. H. Rao, V. B. Wreesmann, A. Goberdhan, I. Ngai, A. G. Huvos, O. Levran, K. Pujara, R. Diotti, A. D. Auerbach and B. Singh, personal communication). Specifically, Fanconi anemia-associated HNSCC were characterized by a high rate of deletions of the chromosomal regions 9q, 10q, and 22q, which are rarely detected in sporadic HNSCC (76). The higher prevalence of HPV in HNSCC from the Fanconi anemia population may be an explanation for the observed differences, as HPV-positive HNSCC feature a genetic profile different from HPV-negative HNSCC (77, 78). Additional evidence implicating a carcinogen-specific pattern of aberrations in HNSCC is derived from the divergent genomic profile of HNSCC occurring in non-smokers compared with those in heavy smokers, similar to that observed in SCCs of the lung (76, 79–83). However, the considerable overlap of carcinogenic exposures within individual cases and differences in carcinogen sensitivity between individual patients makes the establishment of a carcinogenic cause specific pattern of genomic aberrations difficult (76).

The observed genomic heterogeneity in HNSCC may also be used to predict clinical behavior. Several recent studies suggest that chromosomal aberrations may be superior predictors of treatment response and disease outcome compared to classical clinicopathologic factors, with relative risk ratios exceeding those of tumor node metastasis (TNM) staging and other clinical variables by up to 20-fold (57, 58). A variety of individual chromosomal aberrations have been associated with dismal outcome of HNSCC, involving 3p (84, 85), 3q (86), 8p21–23 (85, 87), 9p21 (85, 88), 10q (89), 11q13 (90), 11q23 (91), 14q (92), 18q (93), and 22q (94). Interestingly, some of these genomic abnormalities (3p, 3q26, 9p21) represent early events in the HNSCC progression model (63, 67) suggesting that the course of HNSCC may be determined early in its development. A key limitation in deciphering the clinical significance of genomic data revolves around issues pertaining to multiple testing and confounding variables. Few studies have applied rigorous statistical analysis to overcome the analytic issues. Bockmuhl et al. recently identified gains/amplifications at 3q26 and 11q13 and deletions of 8p21–22 as independent outcome predictors after controlling CGH data for multiplicity of testing in 113 surgically resected HNSCC (58). In addition, Ashman et al. illustrated the independent prognostic significance for deletion of chromosome 22 in a panel of 45 HNSCC (95). However, each of these reports contains statistical limitations. To address these limitations, we developed a novel statistical approach applicable to the analysis of complex chromosomal data by combining bioinformatics and statistical methods. We applied this approach to the analysis of CGH profiles of 82 HNSCC, identifying independent prognostic significance for overrepresentations of the chromosomal regions 11q13 and 12q24 and deletions of 5q11–15, 6q14–21, and 21q11–21 (57). In this regard, the 6q14 deletion is of particular interest because it was recently shown to harbor a locus involved in HNSCC cell survival (96). It is likely that the accuracy of genetic prediction will increase with the development of bioinformatic approaches toward the analysis of increasingly complex data from high-resolution genomic screening data. This is evident from three recent studies demonstrating the ability to predict the development of HNSCC recurrence based on a combination of high-resolution molecular profiling and rigorous statistical scrutiny (97–99). In addition, the identification of molecular prognostic factors will be critically dependent on assembly of homogeneous study

populations as the molecular profile of HNSCC is known to be influenced by multiple clinicopathologic variables that may obscure survival correlations.

Candidate genes driving selection for chromosomal aberrations: implications for treatment

As cancer development and progression is primarily caused by genomic aberrations, genes whose expression is directly influenced by such aberrations may be biologically relevant and attractive targets of innovative therapeutic interventions (48, 49, 100). Analogous to the clinical implications of the Ph-chromosome in leukemia, the consistent presence of chromosomal aberrations in HNSCC suggests multiple targeting opportunities. However, unlike translocation events, identification of individual genes driving selection for chromosomal deletions and gains has been difficult to decipher, as regions of chromosomal amplification can be as large

Table 2 Candidate genes proposed as targets of chromosomal aberrations identified in human squamous cell carcinomas

Chromosomal location	*Gene*	*Authors*	*Evidence*
1q32	ATF3	Pimkhaokham (2000)	Amplified/overexpressed in ESCC
1q32	CENPF	Pimkhaokham (2000)	Amplified/overexpressed in ESCC
3p14	FHIT	Ohta (1996)	Deleted/underexpressed in HNSCC
3p21	RASSF1A	Hogg (2002)	Deleted/hypermethylated in HNSCC
3q25.3	*Cyclin L*	Redon et al. (103)	Amplified/overexpressed in HNSCC
3q25.3	TIPARP	Katoh (2003)	Amplified/overexpressed in HNSCC
3q26	TERC	Yokoi (2003)	Amplified/overexpressed in LSCC
3q26	EIF-4G1	Cromer (2003)	Amplified/overexpressed in HNSCC
3q26	DVL3	Cromer (2003)	Amplified/overexpressed in HNSCC
3q26	PIK3CA	Singh et al. (86)	Amplified/overexpressed in HNSCC
3q26	SCCRO	Singh (2003)	Amplified/overexpressed in HNSCC
3q27	AIS	Hibi (2000)	Amplified/overexpressed in HNSCC
5p13	SKP2	Yokoi (2002); Zhu (2004)	Amplified/overexpressed in LSCC/ESCC
5q21	APC	Kok (2002)	Mutated/deleted in HNSCC
7p11	EGFR	Shimizu (1980)	Amplified/overexpressed in HNSCC
7q22	EPHB4	Cromer (2003)	Amplified/overexpressed in HNSCC
7q22	MCM7	Cromer (2003)	Amplified/overexpressed in HNSCC
7q22–31	MPP11	Resto (2000)	Amplified/overexpressed in HNSCC
8p21–22	TRAIL-R2	Pai (1998); Lee et al. (92)	Mutated/deleted in HNSCC
8p23	PCM1	Garnis (2003)	Deleted in HNSCC
8p23	CSMD1	Sun (2001)	Homozygous deletions in HNSCC
8q24	PTK2	Agochiya (1999)	Amplified/overexpressed in HNSCC
8q22	LRP12	Garnis (2003)	Amplified/overexpressed in HNSCC
9p21	CDKN2A	Kamb (1994)	Deleted/underexpressed in HNSCC
9p23–24	GASC1	Yang (2000)	Amplified/overexpressed in ESCC
10q23	PTEN	Okami et al. (109)	Homozygous deletions in HNSCC
10q25	MCH3	Soung (2003)	Mutated/deleted in HNSCC
11q13	BRMS1	Cromer (2003)	Amplified/overexpressed in HNSCC
11q13	SART1	Cromer (2003)	Amplified/overexpressed in HNSCC
11q13	*Cyclin D1*	Inaba (1992)	Amplified/overexpressed in HNSCC
11q13	TAOS1	Huang et al. (74)	Amplified/overexpressed in HNSCC
11q13	GST-pi	Cullen et al. (119)	Amplified/overexpressed in CDDP-resistant HNSCC
11q13	EMS1	Schuuring (1998)	Amplified/overexpressed in HNSCC
11q21–23	cIAP1	Imoto (2001)	Amplified/overexpressed in ESCC
13q14	DICE1	Li (2003)	Mutated/deleted in ESCC
13q33–34	ING1	Gunduz (2000)	Mutated/deleted in HNSCC
14q12–13	BAZ1A	Yasui (2001)	Amplified/overexpressed in ESCC
14q12–13	SRP54	Yasui (2001)	Amplified/overexpressed in ESCC
14q12–13	NFKBIA	Yasui (2001)	Amplified/overexpressed in ESCC
14q12–13	MBIP	Yasui (2001)	Amplified/overexpressed in ESCC
14q12–13	HNF3	Yasui (2001)	Amplified/overexpressed in ESCC
14q12–13	AA9918651	Yasui (2001)	Amplified/overexpressed in ESCC
14q12–13	AA167732	Yasui (2001)	Amplified/overexpressed in ESCC
16q23–24	WWOX	Kuroki (2002)	Mutated/deleted in ESCC
18p11.3	YES1	Nakakuki (2002)	Amplified/overexpressed in ESCC
18p11.3	TYMS	Nakakuki (2002)	Amplified/overexpressed in ESCC
18p11.3	HEC	Nakakuki (2002)	Amplified/overexpressed in ESCC
18p11.3	TGIF	Nakakuki (2002)	Amplified/overexpressed in ESCC
18q21.1	DPC4	Hahn and Weinberg (8)	Deleted/underexpressed in HNSCC
20q	AIB1	Fujita (2003)	Amplified/overexpressed in ESCC
20q	BTA	Fujita (2003)	Amplified/overexpressed in ESCC
20q	DcR3	Fujita (2003)	Amplified/overexpressed in ESCC
20q	E2F1	Fujita (2003)	Amplified/overexpressed in ESCC

HNSCC, head and neck squamous cell carcinoma; ESCC, esophageal squamous cell carcinoma; LSCC, lung squamous cell carcinoma.

as 1 Mb in size or more and several genes within the amplified region may be overexpressed but have no clinical or biologic significance (101–103). Accordingly, validation of individual targets becomes complicated and is often non-conclusive. Nonetheless, a growing number of genes that putatively drive selection for chromosomal alterations in HNSCC have been identified. Well established examples include *FHIT* (3p) (104), *PIK3CA* (3q26.3) (102, 105), *EGFR* (7p12) (106), *MYC* (8q24) (107), CDKN2A (9p21) (108), *PTEN* (10q23) (109), *Cyclin D1* (11q13) (107), and *p53* (17p13) (110) whose oncogenic role is supported by functional studies (Table 2). As an example, constitutive activation of *PIK3CA* in HNSCC through 3q26.3 amplification results in resistance to p53-induced apoptosis and consequent immortalization, malignant progression, and treatment resistance (102). Recent studies report several novel genes whose expression is dysregulated in HNSCC that are located within the altered chromosomal regions. These genes are important subjects of future functional validation studies (Table 2). Additional candidate genes for chromosomal alterations in HNSCC may include genes driving selection for chromosomal aberrations occurring in SCCs with a common etiology such as those occurring in the anogenital tract, esophagus, and lung (Table 2).

Once identified, therapeutic targeting of genes driving selection for chromosomal aberrations is an obvious next objective of molecular-therapeutic efforts. For example, recent studies used an adenovirus (ONYX-015) selectively replicating in p53-deficient HNSCC tumor cells showing early (phase I and II) clinical efficacy (111–113). Similarly, efforts are underway to target oncogenes in HNSCC. Antisense *Cyclin D1* (11q13 amplification) treatment has been shown to inhibit HNSCC xenograft proliferation and induces apoptosis leading to tumor shrinkage in mice and is currently being considered for human trials (114, 115). We previously identified a novel oncogene within the 3q26.3 amplicon, named SCR-related oncogene (*SCCRO*; G. Reddy, P. O-charoenrat, I. Sarkaria, B. Singh, personal communication), overexpression through amplification of which is associated with poor outcome of HNSCC (116, 117). In mouse xenograft models of HNSCC, RNAi treatment of *SCCRO* transfected xenografts result in a therapeutic benefit, suggesting a key role for this gene in HNSCC tumorigenesis (E. Maghami, K. Patel and B. Singh, personal communication). Perhaps, the most promising subjects of therapeutic targeting efforts are chromosomal aberrations induced by selection pressures such as chemotherapy. For example, Wang et al. showed that resistance of metastatic colon cancer to 5-fluorouracil (5-FU) treatment is mediated through amplification of thymidylate synthase, a cellular target of 5-FU (118). Likewise, it was recently shown that resistance of HNSCC to cisplatin is associated with overexpression through amplification of *glutathione S-transferase pi* (11q13), a gene involved in detoxification of many xenobiotic substances through conjugation to glutathione (119). At present, the most prominent exponents of the molecular generation are the small molecule tyrosine kinase inhibitors such as ZD1839 ('Iressa') (120), which target aberrant EGFR receptor signaling commonly present in cancer and have demonstrated clinical efficacy in the treatment of SCRs of both the upper and lower aerodigestive tract (121–123). Recently, it was shown that lung tumors sensitive to ZD1839 may be identifiable by the presence of activating mutations in the EGFR gene, suggesting that the clinical efficacy of this agent in HNSCC may be increased significantly as well (124, 125). However, the overall effectiveness of single gene targeting, in the genetically heterogeneous tumor environment that is developmentally multifactorial, has been disappointing. Accordingly, several efforts (including several multi-institutional phase III trials) are underway to combine molecular and conventional therapies, showing promising results (Table 3).

Conclusions

The application of modern genetic analytic techniques has led to the identification of an array of genetic

Table 3 Molecular therapeutics in phase II and III clinical trials at the NCI (http://www.cancer.gov/clinicaltrials)

Phase III randomized study of Ad5CMV-p53 gene therapy (INGN 201) vs. methotrexate in patients with refractory squamous cell carcinoma of the head and neck (INTROGEN-T301)[a]
Phase III randomized study of cisplatin and fluorouracil with or without Ad5CMV-p53 gene therapy (INGN 201) in patients with unresectable recurrent squamous cell carcinoma of the head and neck (INTROGEN-T302)[a]
Phase III study of Zd1839 (Iressa^{TM}: Astrazeneca, Wilmington, DE, USA) vs. methotrexate for previously treated patients with squamous cell carcinoma of the head and neck (ZD1839IL/0704)[a]
Phase I/II study of erlotinib and docetaxel in patients with locally advanced, metastatic, or recurrent squamous cell carcinoma of the head and neck (OSU-02H0084)[a]
Phase I/II randomized study of bevacizumab and erlotinib in patients with recurrent or metastatic head and neck cancer (UCCRC-11956A)[a]
Phase I/II study of erlotinib and cisplatin in patients with recurrent or metastatic squamous cell cancer of the head and neck (PMH-PHL-002)[a]
Phase II study of flavopiridol in patients with recurrent or metastatic squamous cell carcinoma of the head and neck (NCI-00-C-0128)[a]
Phase II study of gefitinib in patients with recurrent and/or metastatic squamous cell cancer of the head and neck (UUMC-8429-01)[a]
Phase II randomized pilot study of adjuvant celecoxib in patients with early stage head and neck cancer or non-small cell lung cancer (NU-02V2)[a]
Phase II study of perifosine in patients with recurrent or metastatic squamous cell head and neck cancer (UCCRC-12198A)[a]
An open-label phase II study of lonafarnib in patients with recurrent squamous cell carcinoma of the head and neck (P02530)[a]
Phase II study of OSI-774/cisplatin/taxotere in head and neck squamous cell cancer (ID02-668)[a]

[a]Trial IDs between brackets.

aberrations in HNSCC many of which have been correlated with tumor progression, aggressive behavior, and clinical outcome of HNSCC. Several gene targets of genetic aberrations have been identified and successfully targeted to form therapeutic benefit. Given the findings to date, it is likely that the clinical utility of genetic aberrations will allow improved diagnostics, prognostication, and therapeutics in patients with HNSCC.

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