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Review

Multistep laryngeal carcinogenesis helps our understanding of the field cancerisation phenomenon: a review

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Abstract

In this paper, we try to briefly review the most recent knowledge on head and neck cancer, and especially multistep laryngeal carcinogenesis, and to simply explain how this has modified our understanding of field cancerisation phenomenon. Experimental studies, made possible by the recent evolution of microdissection systems, have demonstrated that the 'spatial progression' of the histopathological phenotype in the surroundings of malignant or premalignant head and neck lesions correlates with molecular progression. Such a 'spatial progression' can be hypothesised to reflect temporal progression. The field cancerisation process has been divided into three phases, each with its own histological and molecular characteristics. Each of these phases may have clinical implications: detection and monitoring of fields may help cancer prevention (molecular epidemiology), early detection of recurrence (or, more exactly, of second field tumours (SFTs)) (molecular diagnostics) and prognostic prediction after treatment. This model appears plausible, especially in explaining the development of multiple primary tumours (MPTs) in adjacent head and neck mucosal regions, with peculiar clinical and prognostic implications: These tumours can be defined as multiple field tumours (SFTs). However, the model, in our opinion, does not convincingly explain the development of second primary tumours (SPTs) at more distant sites, such as the lung, colon and prostate.

Keywords: Larynx; Head and neck; Cancer; Multistep carcinogenesis; Field cancerisation

1. Multistep carcinogenesis

It is now generally accepted that solid primary tumours result from a multistep process of accumulated genetic alterations. At least four–six events involving oncogenes and tumour suppressor genes appear to be necessary for tumour development. In the past few years, a model for the initiation and progression of colorectal cancer has become a paradigm for other human solid tumours, including those of the brain and bladder [1–6]. Tumours of the head and neck region and, more particularly, of the larynx, have been less extensively studied, but several presumably important molecular alterations, have been described. Like colorectal cancer, laryngeal squamous cell carcinomas (LSCC) is thought to progress through a series of well-defined clinical and histopathological stages. Current theories on tumour progression have focused on the emergence of clonal populations of cells that undergo successive genetic alterations, producing a malignant phenotype with a selective growth advantage [1].

It is well known that the development of LSCC is closely associated with exposure to tobacco and alcohol. In recent years, the molecular changes and the sequence of the events induced by these agents have begun to be elucidated, although the overall genetic and molecular basis of LSCC remains ill defined.

An useful approach for the study of LSCC carcinogenesis derived from the observation that a spatial sequence of histological phenotypes, molecular alterations and genetic events is detectable in the surroundings of

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the squamous cell carcinomas (SCCs) with a 'spatial progression' from normal mucosa, to the various degrees of dysplasia, to carcinoma *in situ*, and finally to invasive carcinoma. In fact, histological sections from head and neck squamous cell carcinoma (HNSCC) resections were demonstrated to exhibit an apparently contiguous and continuous transition from normal to hyperplastic to dysplastic epithelium, to low grade carcinoma and to high-grade carcinoma. Experimental studies, made possible by the recent evolution of microdissection systems, have demonstrated that such progression in the histopathological phenotype correlates with the genetic progression of HNSCC in a series of patients and among adjacent histopathologically distinct areas in the same patient [6,7]. So once contiguous tissue regions are identified, specific genetic events purported to be important for tumorigenesis can be spatially correlated with specific downstream phenotypic consequences. Such 'spatial progression' can be hypothesised to reflect temporal progression. Using this approach, it has become possible in recent years to clarify some aspects of the temporal sequence of HNSCC progression. This has helped both in further understanding the molecular mechanisms underlying carcinogenesis and in the planning of the clinical use of various molecular markers.

1.1. Cytogenetic progression

In a series of experimental papers [6–8], Califano and colleagues applied such an approach to the study of the cytogenetics of HNSCC, associating an increasing number of chromosomal alterations to the various steps of histological progression towards cancer. By the use of polymerase chain reaction (PCR)-based microsatellite marker analysis, studies were performed on precancerous and malignant lesions of the head and neck region, demonstrating a spatial progression and an analogue progression 'over time' and confirming the 'historical' value of tissues surrounding the SCCs [6,7]. The most frequently altered chromosomal regions in HNSCC that they observed were 9p21 which contains the *p*16 gene, 11q13 which contains the *CCND1* locus, 17p13 where the *p*53 gene is located, 3p with at least three putative tumour suppressor loci, 13q21, 6p, 8 (Fig. 1). Certain genetic events (9p21 loss of heterozygosity (LOH), 3p LOH, and 17p13 LOH) tended to occur earlier on the progression pathway. 9p21 and 3p14 were already known to be effective risk markers for oral cancer [9,10]. Such chromosomal alterations have been demonstrated to precede the development of malignancy by several years. Other cytogenetic events, such as those involving 13q21, 11q13, 8, are usually late-occurring, but may also occur early in the time course of carcinogenesis. A preliminary but not unequivocal, schematic of the temporal sequence has been drawn by these Authors.

However, for a complete definition of HNSCC progression, this useful cytogenetic information needs to be integrated with data about single-gene, epigenetic, translational and post-translational alterations.

1.2. Key genes in multistep progression

Previously published studies demonstrated that histological progression was marked by increasing genetic instability [11], increasingly deregulated proliferation [12], and increasingly abnormal activation of key regulatory molecules such as epidermal growth factor (EGF) receptor and telomerase catalytic subunit [13,14] (Fig. 1).

Among the most frequent and relevant cellular changes in laryngeal carcinogenesis are those involving *p*53, cyclin D1 (*CCND*1), *p*16 and EGFR (Fig. 1). Studies using informative tissue specimens of cancer and preneoplastic lesions and surrounding mucosa tried to define the temporal patterns of such molecular alterations.

The nuclear phosphoprotein *p*53, one of the most studied molecular markers in HNSCC, is involved in a continuously increasing number of key cell functions such as gene transcription, DNA synthesis and repair, cell cycle coordination and apoptosis. It has been defined as 'the guardian of the genome' [15] because of its primary importance in coordinating the cell response to DNA damage (by inducing cell cycle arrest and/or apoptosis) and thus in protecting cells against somatic mutations. Disruption, or at least perturbation, of *p*53 function is presumably present in virtually all head and neck cancers. *p*53 point mutations or deletions (also by LOH at 17p13) are frequent. *p*53 inactivation by other cellular proteins such as mdm2 [16] or by the Human Papilloma Virus (HPV) E6 oncoprotein [17] may represent alternative, but functionally comparable, pathways leading to loss of *p*53 function. For some authors, in HNSCC [6,18] as in many other tumour types, *p*53 inactivation occurs in the transition from the preinvasive to the invasive state. On the other hand, increasing experimental evidence suggests that in epithelial cells of the upper aerodigestive tract, loss of *p*53 function occurs in earlier phases of tumorigenesis [6,7,14,19,20], when this might cause an uncoupling of DNA damage and growth inhibition and promote successive genetic hits through an increased genomic instability. *p*53 mutation has been hypothesised to be the earliest event in the development of a genetically altered field, identifying an area of clonally related cells with a malignant potential [9].

Cyclin D1 is a member of the cyclin family of regulatory proteins involved in cell cycle progression, which interacts with cyclin-dependent kinases. Cyclin D1 gene (*CCND*1) amplification and overexpression were studied in HNSCC patients at the same time in tumoral and

peritumoral tissues [21]. When *CCND*1 amplification is observed, in cancerous and precancerous lesions, cyclin D1 is always overexpressed. In turn, cyclin D1 overexpression, frequently present in cancers, but also in the earliest premalignant lesions, does not determine, but always anticipates, gene amplification, which is probably a more stable, non-reversible, alteration in tumour cells. At present, we can hypothesise that in the early phases of tumorigenesis, altered *p*53 gene function [22–24], and *CCND*1 gene overexpression [22,24,25] increase genetic instability and promote further genetic and chromosomal alterations such as *CCND*1 amplification [21], which is considered by some authors key for the ultimate transforming event by the selection of a malignant subclone from a genetically altered field [9] (Fig. 1).

The definition of a genetic progression model for head and neck cancer has several important implications. From a cognitive viewpoint, identification of tumour suppressor genes and proto-oncogenes may be critical for an understanding of the biological initiation and progression of head and neck cancer. Moreover, acquiring information about the time course of a single molecular alteration may guide us in their clinical use for molecular epidemiology, diagnostics or characterisation of laryngeal cancer (Fig. 1). The earliest

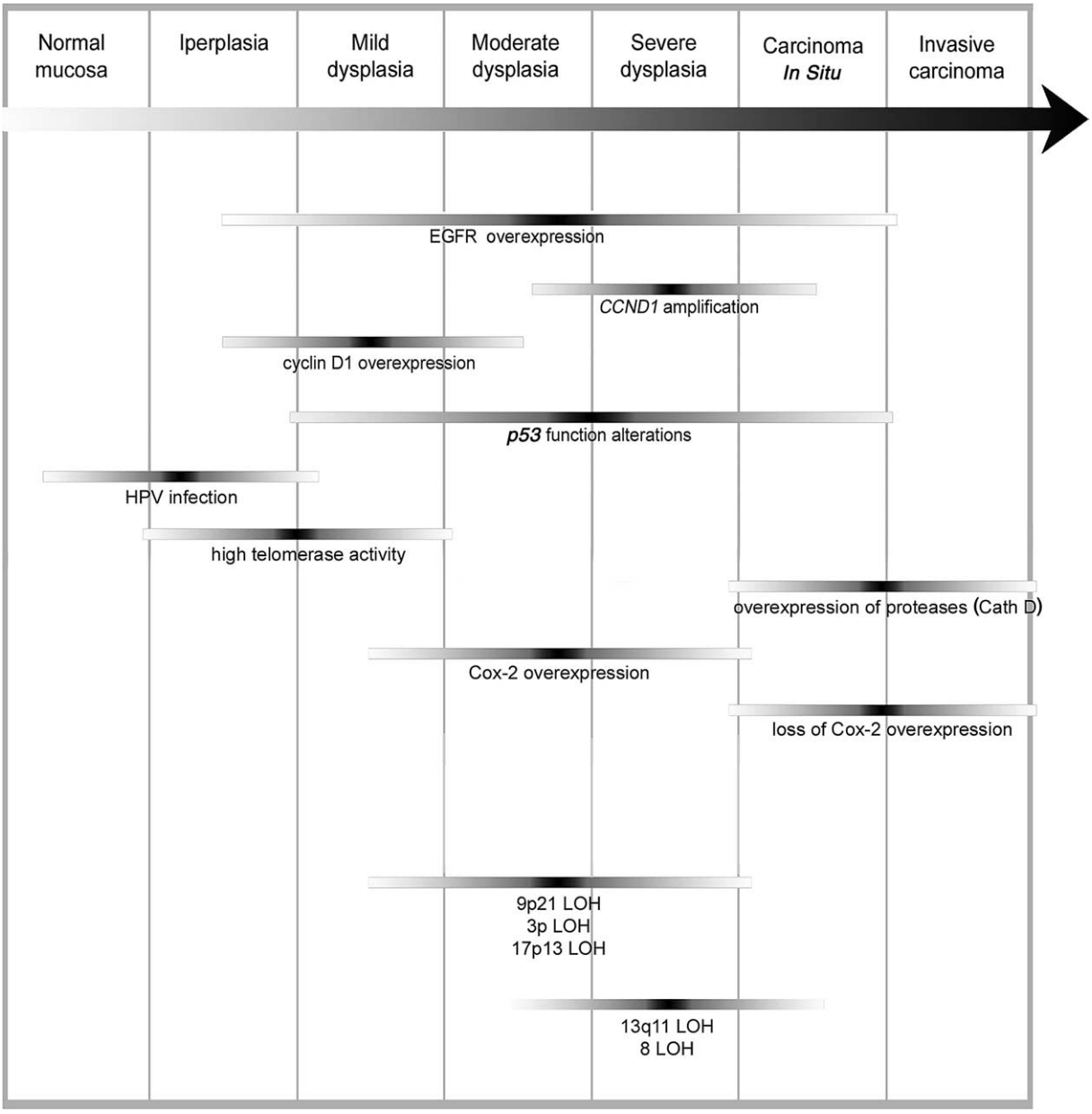

Fig. 1. A grey scale in the corresponding bars represents the most probable times of onset of various molecular events during laryngeal carcinogenesis. LOH, loss of heterozygosity; HPV, Human Papilloma Virus.

alterations could be used to identify genetically altered fields, the latest ones could be searched in SCCs for molecular characterisation; an alteration specifically occurring in the passage from dysplasia to invasive cancer could then be used for molecular diagnostics. Furthermore, the determination of the genetic status of a primary tumour and of the tissues surrounding the invasive cancer may have prognostic significance for tumour recurrence. For example, the presence of transforming clonal events in the surrounding normal epithelium at the time of cancer resection may predict late local recurrence (or, more exactly, the development of second field tumours (SFTs)) in some patients.

The acquisition of an unequivocal model for HNSCC progression might prove to be impossible because of the extreme heterogeneity of these tumours on a clinical (different sites and risk factors involved), histopathological and, most of all, biological level (variable molecular alterations and timing, see Fig. 1). To overcome such obstacles, it might be necessary to identify homogeneous subsets of laryngeal cancers, and HPV-positive cancers could be one such subset [26–28], with similar characteristics on a molecular and clinical level and preceded by constant, typical, carcinogenic steps.

2. Field cancerisation

All laryngeal cancer patients are at a significantly elevated risk of developing second primary tumours (SPTs). In an attempt to explain carcinogenesis of multiple neoplasms and the development of multiple premalignant lesions in the upper aerodigestive tract (and in particular in the oral cavity) Slaughter [29] elaborated the theory of "field cancerisation". This hypothesis proposes that long-term carcinogenic exposure (e.g., from tobacco use and/or alcohol consumption) results in "condemned mucosa" containing many mutated cells, from which multifocal independently arising (polyclonal) tumours develop. This theory has been widely accepted and has been the basis for chemoprevention trials in patients with premalignant lesions and/or previously treated HNSCC. In recent years, doubts have been raised about the classical explanation of field cancerisation, and a monoclonal origin has been hypothesised. Recent acquisitions about multistep carcinogenesis have allowed the delineation of a new model for the clinical phenomenon of field cancerisation [6,7,9]. Areas of histopathological abnormality surrounding malignant and premalignant lesions, with an

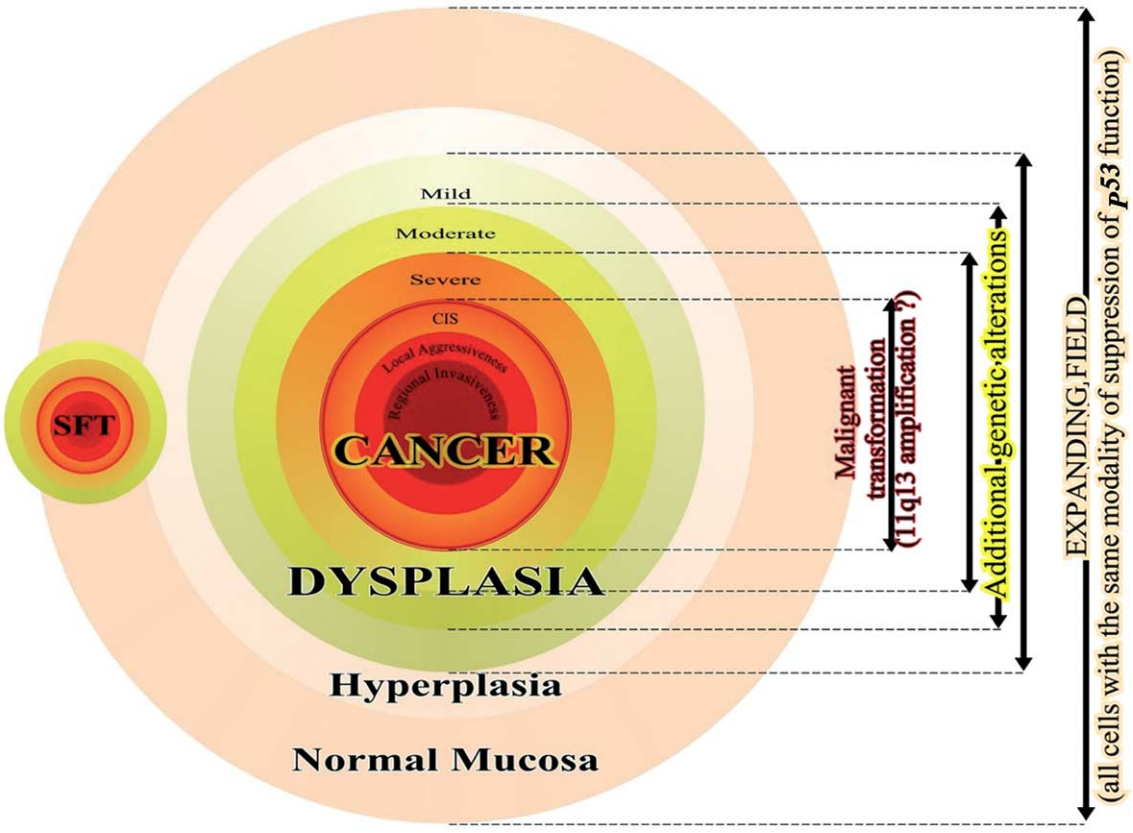

Fig. 2. A scheme of spatial and temporal progression in an expanding field; development of a second field tumour (SFT).

extension of several centimetres, have been described, within which a 'spatial progression' can be evidenced. These are proposed to generally be derived from a common single progenitor clone. Subsequent genetic events produce genetic divergence and different phenotypic alterations, resulting in a variety of histopathologically diverse regions in a local anatomical area and in the selection of various subclones: the malignant ones are naturally those with the higher growth advantage (Fig. 2). Successive genetic events in other subclones could be associated with the development of multiple primary tumours (MPTS). Therefore, such a hypothesis proposes a clonal origin for premalignant cells with successive lateral migration, over years or even decades, to adjacent mucosal areas, so that MPTs would not be monoclonal, but clonally-related. The field cancerisation process has been divided into three phases each with its own histological and molecular characteristics [9]. In the initial phase, a stem cell acquires genetic alterations and forms a 'patch', a clonal unit of altered daughter cells; it could be recognised on the basis of a mutation in the *p*53 gene. The conversion of a patch into an 'expanding field' is the next critical step which requires additional genetic alterations which confer a growth advantage to one or more subclones and allow them to proliferate and to displace the normal mucosa. An expanding field, usually not detectable by routine diagnostic techniques, can reach dimensions of more than 7 cm in diameter. In an expanding field, clonal divergence can lead to the development of several different 'malignant tumours' (third phase) over years. In the selection of the malignant clone, a role has been postulated for *CCND*1 amplification in 11q13, a frequent molecular alteration in laryngeal cancer [30].

This model has clinical implications: the detection and monitoring of fields that may help cancer prevention (molecular epidemiology), the early detection of recurrence (or, more exactly, of SFTs) (molecular diagnostics) and the prediction of local relapse after treatment. It might also suggest a higher risk of local recurrence and/or MFTs after treatment for elderly HNSCC patients who have a long history of exposure to environmental carcinogens, in which the expanding field is presumably larger because of the longer time before the malignant transformation.

This model appears plausible, especially in explaining the development of MPTs in adjacent head and neck mucosal regions. These are also called multiple field tumours (MFTs) and have peculiar clinical and prognostic implications. However, the model does not convincingly explain the development of SPTs at more distant sites such as lung, colon, and prostate. We think that genetic predisposition, especially polymorphisms of the tobacco detoxifying enzymes [31] and acquired risk factors should be accurately evaluated to study the aetiopathogenesis of SPTs of the upper aerodigestive tract (including lung). For other distant sites, different risk factors should also be evaluated. The most frequent, non-aerodigestive malignant tumours arise in the colon and prostate [32]. For those in the colon, a common likely risk factor is hypofolataemia [33] and thus factor has also recently been reported to increase the risk for HNSCC [34]. As for prostate malignancies, a role for steroid hormone pathways can be hypothesised, especially in relation with laryngeal cancer, which has a markedly higher incidence in males. Quercetin and tamoxifen have been demonstrated to exert a dose-dependent inhibition of cell growth in laryngeal cancer cell lines, probably interacting with Type II oestrogen binding sites, that are expressed in laryngeal cancer [35]. Furthermore, the expression of methyl-*p*-hydroxyphenyllactate esterase (MeHPLAase), an enzyme involved in oestrogen pathways, has been demonstrated to correlate with a longer relapse-free and overall survival [36] in primary laryngeal SCCs.

References

1. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990, **61**(5), 759–767.
2. Vogelstein B, Fearon ER, Hamilton SR, *et al*. Genetic alterations during colorectal-tumor development. *New Engl J Med* 1988, **319**(9), 525–532.
3. Sidransky D, Mikkelsen T, Schwechheimer K, Rosenblum ML, Cavanee W, Vogelstein B. Clonal expansion of p53 mutant cells is associated with brain tumour progression. *Nature* 1992, **355**(6363), 846–847.
4. Simoneau AR, Jones PA. Bladder cancer: the molecular progression to invasive disease. *World J Urol* 1994, **12**(2), 89–95.
5. Dalbagni G, Presti J, Reuter V, Fair WR, Cordon-Cardo C. Genetic alterations in bladder cancer. *Lancet* 1993, **342**(8869), 469–471.
6. Califano J, van der Riet P, Westra W, *et al*. Genetic progression model for head and neck cancer: implications for field cancerisation. *Cancer Res* 1996, **56**(11), 2488–2492.
7. Califano J, Westra WH, Meininger G, Corio R, Koch WM, Sidransky D. Genetic progression and clonal relationship of recurrent premalignant head and neck lesions. *Clin Cancer Res* 2000, **6**(2), 347–352.
8. Califano J, Westra WH, Koch W, *et al*. Unknown primary head and neck squamous cell carcinoma: molecular identification of the site of origin. *J Natl Cancer Inst* 1999, **91**(7), 599–604.
9. Braakhuis BJ, Tabor MP, Kummer JA, Leemans CR, Brakenhoff RH. A genetic explanation of Slaughter's concept of field cancerization: evidence and clinical implications. *Cancer Res* 2003, **63**(8), 1727–1730.
10. Mao L, Lee JS, Fan YH, Ro JY, Batsakis JG, Lippman S, *et al*. Frequent microsatellite alterations at chromosomes 9p21 and 3p14 in oral premalignant lesions and their value in cancer risk assessment. *Nat Med* 1996, **2**(6), 682–685.
11. Voravud N, Shin DM, Ro JY, Lee JS, Hong WK, Hittelman WN. Increased polysomies of chromosomes 7 and 17 during head and neck multistage tumorigenesis. *Cancer Res* 1993, **53**(12), 2874–2883.
12. Shin DM, Voravud N, Ro JY, Lee JS, Hong WK, Hittelman WN. Sequential increases in proliferating cell nuclear antigen expression

in head and neck tumorigenesis: a potential biomarker. *J Natl Cancer Inst* 1993, **85**(12), 971–978.
13. Shin DM, Ro JY, Hong WK, Hittelman WN. Dysregulation of epidermal growth factor receptor expression in premalignant lesions during head and neck tumorigenesis. *Cancer Res* 1994, **54**(12), 3153–3159.
14. Hohaus S, Cavallo S, Bellacosa A, *et al*. Telomerase activity in human laryngeal squamous cell carcinomas. *Clin Cancer Res* 1996, **2**(11), 1895–1900.
15. Lane DP. Cancer. p53, guardian of the genome. *Nature* 1992, **358**(6381), 15–16.
16. Osman I, Sherman E, Singh B, *et al*. Alteration of p53 pathway in squamous cell carcinoma of the head and neck: impact on treatment outcome in patients treated with larynx preservation intent. *J Clin Oncol* 2002, **20**(13), 2980–2987.
17. Scheffner M, Huibregtse JM, Vierstra RD, Howley PM. The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. *Cell* 1993, **75**(3), 495–505.
18. Boyle JO, Hakim J, Koch W, *et al*. The incidence of p53 mutations increases with progression of head and neck cancer. *Cancer Res* 1993, **53**(19), 4477–4480.
19. Gallo O, Santucci M, Franchi A. Cumulative prognostic value of p16/CDKN2 and p53 oncoprotein expression in premalignant laryngeal lesions. *J Natl Cancer Inst* 1997, **89**(15), 1161–1163.
20. Homann N, Nees M, Conradt C, *et al*. Overexpression of p53 in tumor-distant epithelia of head and neck cancer patients is associated with an increased incidence of second primary carcinoma. *Clin Cancer Res* 2001, **7**(2), 290–296.
21. Izzo JG, Papadimitrakopoulou VA, Li XQ, *et al*. Dysregulated cyclin D1 expression early in head and neck tumorigenesis: *in vivo* evidence for an association with subsequent gene amplification. *Oncogene* 1998, **17**(18), 2313–2322.
22. Tainsky MA, Bischoff FZ, Strong LC. Genomic instability due to germline p53 mutations drives preneoplastic progression toward cancer in human cells. *Cancer Metastasis Rev* 1995, **14**(1), 43–48.
23. Shin DM, Ro JY, Shaw T, Hong WK, Hittelman WN. p53 expression and genetic instability in head and neck tumorigenesis. *Proc Am Ass Cancer Res* 1994, **35**, 944.
24. Roh HJ, Shin DM, Lee JS, *et al*. Visualization of the timing of gene amplification during multistep head and neck tumorigenesis. *Cancer Res* 2000, **60**(22), 6496–6502.
25. Tlsty TD, White A, Sanchez J. Suppression of gene amplification in human cell hybrids. *Science* 1992, **255**(5050), 1425–1427.
26. Almadori G, Cadoni G, Cattani P, *et al*. Detection of human papillomavirus DNA in laryngeal squamous cell carcinoma by polymerase chain reaction. *Eur J Cancer* 1996, **32A**(5), 783–788.
27. Cattani P, Hohaus S, Bellacosa A, *et al*. Association between cyclin D1 (*CCND1*) gene amplification and human papillomavirus infection in human laryngeal squamous cell carcinoma. *Clin Cancer Res* 1998, **4**(11), 2585–2589.
28. Almadori G, Cadoni G, Cattani P, *et al*. Human papillomavirus infection and epidermal growth factor receptor expression in primary laryngeal squamous cell carcinoma. *Clin Cancer Res* 2001, **7**(12), 3988–3993.
29. Slaughter DP, Southwick HW, Smejkal W. 'Field cancerization' in oral stratified epithelium: clinical implications of multicentric origin. *Cancer (Phila)* 1953, **6**, 963–968.
30. Bellacosa A, Almadori G, Cavallo S, *et al*. Cyclin D1 gene amplification in human laryngeal squamous cell carcinomas: prognostic significance and clinical implications. *Clin Cancer Res* 1996, **2**(1), 175–180.
31. Zheng Z, Park JY, Guillemette C, Schantz SP, Lazarus P. Tobacco carcinogen-detoxifying enzyme UGT1A7 and its association with orolaryngeal cancer risk. *J Natl Cancer Inst* 2001, **93**(18), 1411–1418.
32. Narayana A, Vaughan AT, Fisher SG, Reddy SP. Second primary tumors in laryngeal cancer: results of long-term follow-up. *Int J Radiat Oncol Biol Phys* 1998, **42**(3), 557–562.
33. Giovannucci E, Rimm EB, Ascherio A, Stampfer MJ, Colditz GA, Willett WC. Alcohol, low-methionine–low-folate diets, and risk of colon cancer in men. *J Natl Cancer Inst* 1995, **87**(4), 265–273.
34. Almadori G, Bussu F, Galli J, *et al*. Serum folate and homocysteine levels in head and neck squamous cell carcinoma. *Cancer* 2002, **94**(4), 1006–1011.
35. Ferrandina G, Almadori G, Maggiano N, *et al*. Growth-inhibitory effect of tamoxifen and quercetin and presence of type II estrogen binding sites in human laryngeal cancer cell lines and primary laryngeal tumors. *Int J Cancer* 1998, **77**(5), 747–754.
36. Maurizi M, Ferrandina G, Almadori G, *et al*. Prognostic significance of methyl-p-hydroxy-phenyllactate-esterase activity in laryngeal squamous cell carcinoma. *Br J Cancer* 1998, **77**(8), 1253–1259.