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Review

Mutagen Sensitivity: Enhanced Risk Assessment of Squamous Cell Carcinoma

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INTRODUCTION

Squamous cell carcinoma of the head and neck has an incidence of almost 5% of newly diagnosed cancers in North Western European countries and the United States [1]. These carcinomas arise from the mucous membrane of the upper aerodigestive tract and are predominantly found in 50–70-year-old males. Head and neck squamous cell carcinoma (HNSCC) of the oral cavity, larynx and pharynx are the most common. Other sites are not included in this review as it has been suggested that they have a very distinct aetiology and comprise a very heterogeneous group of neoplasms [2].

The treatment of HNSCC is dependent on the tumour site and stage of the disease [3], but commonly consists of surgery, radiotherapy or a combination of both. Especially for more advanced disease involving large tumours (usually with lymph node metastases) the patient survival has not improved significantly during the last decades, despite the development of more sophisticated surgical and radiotherapeutic treatment modalities [4].

In addition to the risk of recurrence and metastases for HNSCC patients with more advanced disease, another important factor which influences survival of patients especially with small tumours is the occurrence of multiple primary tumours (MPT) in the epithelium of the head and neck region, oesophagus and lungs [5]. Patients successfully treated for HNSCC are at relatively high risk (2–3% patients per year) of developing other new tumours in the respiratory and upper digestive tract (RUDT). Such development of multiple malignant epithelial tumours in this area is a major clinical concern. The occurrence of MPT can be explained in the light of "field cancerisation" [6] a concept that postulates that the entire mucous membrane of the RUDT is abnormal and "precancerous", due to a similar exposure to carcinogens, notably tobacco and alcohol. Support for this concept has been provided by several groups using a variety of premalignancy markers [7].

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STRATEGIES TO COMBAT SUBSEQUENT MPT

Two approaches may be pursued to reduce the morbidity and mortality of MPT: early detection and prevention.

Early detection

Until now, early detection has been associated with controversy as to how frequently patients should be followed up and what further investigations should be undertaken at each follow-up visit [8]. New non-invasive methods of early detection are required which can be cost-effectively performed even on large populations. Potential non-invasive methods for early detection involve the measurement of premalignancy markers in exfoliated cells [9, 10].

Primary prevention

In addition to early detection, preventive strategies are essential to reduce the occurrence of MPT. Strict reduction in alcohol intake and tobacco avoidance are crucial. Primary prevention is important, since patients who continue smoking are at much higher risk for the development of MPT than patients who stop [11]. However, after smoking cessation, the chance of developing another tumour only gradually decreases over the course of years, probably because some preneoplastic changes have already occurred. For this reason, and because many patients fail to modify their lifestyles, additional methods to reduce the risk of MPT are desirable. Chemoprevention is such a method.

Chemoprevention

The process of carcinogenesis may be inhibited or aborted through the administration of natural or synthetic compounds. Indications that chemoprevention is feasible originate from epidemiological studies which reveal an increased risk of cancer when certain nutrients or fibres are deficient in the diet [12, 13]. *In vitro* and animal studies have shown that anti-oxidants can be used to protect against carcino-

genic assaults. *N*-acetylcysteine (NAC), a precursor of glutathione, may ameliorate induction of DNA damage through its anti-oxidant/detoxification properties [14, 15]. In addition, inhibition of malignant progression has also been reported [16]. Other nutrients which are considered to have protective effects are vitamins. It has been found in animal studies that vitamin A and its synthetic and natural analogues, the retinoids, such as retinyl palmitate and 13-*cis* retinoic acid (isotretinoin) can inhibit carcinogenesis [17, 18]. It has been suggested that vitamin A may even be effective after transformation of the target cell, probably due to the induction of differentiation [19].

A milestone in the field of HNSCC chemoprevention has been the study by Hong *et al.* [20] who reported that daily treatment with high doses of 13-cis retinoic acid (isotretinoin: 50–100 mg/m^2) was effective in preventing the development of MPT in curatively treated HNSCC patients, even with long-term follow-up [21]. An ongoing chemoprevention trial in Europe to prevent MPT in HNSCC patients is the multicentre EORTC study: Euroscan [22].

Other aspects have to be considered when using chemoprevention. The subjects to whom treatment is offered are usually healthy persons who are cured of their first primary tumour and, for the great majority, treatment is redundant since they will never develop new tumours anyhow. It has to be emphasized that compounds which are selected should have only minimal side-effects and no harmful effects. Antioxidants can have adverse effects in the long term as was discovered by a Finnish chemoprevention trial [23].

By selecting subgroups of HNSCC patients with an increased risk for MPT in the RUDT, chemoprevention can be used more efficiently [24, 25]. However, our knowledge of individual risk factors is insufficient and identification of high risk individuals is essential for both early detection and chemoprevention. The group of patients at risk of MPT is an excellent study group to test these strategies since 2–3% develop a new tumour. Results of these data may reveal important clues for future screening of the whole population.

POPULATION AT RISK OF HNSCC AND MPT

Traditional epidemiology has revealed some important environmentally related risk factors associated with the development of HNSCC. Herpes simplex virus [26] and human papillomavirus [27] have been associated with oral cancer. Exposure to occupational risk factors, such as asbestos [28], organic compounds and coal products [29] have been implicated along with dietary factors [30–32].

The most important risk factors, however, are tobacco smoking [33] and alcohol abuse [34, 35]. The combination of these latter factors has been shown to render relative risks that are more than multiplicative [36, 37]. The majority of people who smoke and drink, however, do not develop tumours, which suggests that an intrinsic susceptibility may also be involved in the carcinogenesis process. This susceptibility is present in a latent fashion and only becomes apparent after carcinogenic exposure. At present, it is acknowledged that between induction of DNA damage and malignant transformation several specific changes have to occur [38]. At any level of this multistep carcinogenic process putative factors can be identified which may be important for cancer susceptibility. For instance, enzymes which are involved in the activation of procarcinogens into active carcinogens may play an important role in the first stage. The cytochrome P-450 (CYP) enzymes have been shown to play a role in this respect [39]. At the level of detoxification, enzymes such as glutathione-S-transferases play a major role and abnormalities have been associated with intrinsic susceptibility to several cancers [40, 41].

In addition to genotypic identification of intrinsic cancer susceptibility, more phenotypically related assays have been developed. One such type of assay is based on a hidden chromosomal instability similar to that found in cancer-prone syndromes such as ataxia telangiectasia (AT). Homozygotes have a 70- to 250-fold risk for developing cancer and the great majority (64%) of these are of the haematopoietic system [42]. The rest are epithelial cancers, including oral cancer [43]. At the cellular level, AT is characterised by an increased spontaneous chromosomal instability . AT heterozygotes (0.5–2.8% of the general population) have an increased risk of developing cancer compared to the normal population, in particular breast carcinomas [44, 45], especially after former exposure to radiation. AT heterozygotes only show chromosomal instability after induction of DNA damage by radiation and radiomimetic double strand break (DSB) inducing drugs like bleomycin and neocarcinostatin [46]. An increased sensitivity to carcinogenic assaults may be the cause of the enhanced susceptibility in AT heterozygotes to cancer. The underlying defect in AT is most likely a defect in DNA processing or DNA damage surveillance [47].

Apart from such cancer-prone syndromes, it is becoming increasingly accepted that in addition to environmental exposure, an intrinsic susceptibility is an important factor in the genesis of common cancers. It has been postulated that a susceptibility to mutagenic insults may exist among some persons as a consequence of minor imperfections in DNA maintenance systems, which in combination with specific exposures lead to an increased cancer risk. To identify such sensitive groups in the population, assays are used which measure the persistence of DNA damage after challenging cells *in vitro* with a relevant model compound.

Chromosomal instability after damage induction in the late S-G2 phase of the cell cycle has been found to be related to cancer susceptibility. Gantt and colleagues claim that high sensitivity to radiation-induced chromosomal instability is related to the development of several types of cancer [48]. Scott and colleagues [49] used the same assay, focusing mainly on the development of breast cancer. For the development of HNSCC a similar association to G2-phase sensitivity has been found. Hsu [50] measured sensitivity to the clastogenic activity (induction of chromatid breaks) of bleomycin (BLM) in peripheral blood lymphocytes of a large number of control persons and cancer patients and referred to this as mutagen sensitivity. Since a clastogenic type of damage was measured the term "mutagen sensitivity" is not entirely correct. However, since this term has become well established in the literature, it is commonly accepted to be used in future communications.

MUTAGEN SENSITIVITY

For the investigation of latent or "hidden" chromosomal instability, the mutagen sensitivity assay is used, as it is reliable and simple. Since a genetic factor should be present

in all normal somatic cells, peripheral blood lymphocytes (PBL) are used for the assay as they can easily be obtained and can be cultured under mitogen stimulation. For the induction of DNA damage, BLM was chosen for its clastogenic properties and for its mechanism of action which resembles that of environmental carcinogens [51]. The final assay developed by Hsu [50] is performed on cultured lymphocytes which are challenged with BLM in the late S-G2 phase of the cell cycle. After harvesting the cells, metaphase spreads are made and the chromosomal damage is examined under a microscope at 1250× magnification. The mean number of chromatid breaks per cell (b/c) in 50–100 metaphases is a measure for the chromosomal instability [52, 53].

Using this mutagen sensitivity assay, a large number of healthy persons and cancer patients (Table 1) have been screened. It was found that in the normal population a large variation exists in mutagen sensitivity [54], showing a normal (Gaussian) distribution. Based on this normal distribution, it appeared that a hypersensitive borderline could be set at a b/c level of 1.0 (mean value of the control persons plus one standard deviation). Theoretically, approximately 16% of the normal population should be at the hypersensitive end of the profile.

A large variation in mutagen sensitivity was shown between the subjects within the various groups (Table 1), reflecting an individual susceptibility to environmental mutagens. The key finding in these investigations was that only cancer patients with tumours that originate from tissues directly exposed to the external environment had an increased mutagen sensitivity, indicating that both a sensitivity phenotype and exposure are important for the development of cancer. For HNSCC and lung cancer mutagen sensitivity was significantly increased compared to control persons. This was even more pronounced in patients who had developed MPT, confirming the hypothesis that a hypersensitivity phenotype can identify patients at risk.

Others have provided additional data supporting this concept of hidden chromosomal instability using slightly different assays [58–60]. In addition, Sanford and colleagues investigated radiosensitivity in skin fibroblasts of control persons and cancer patients [48, 61]. They found an increased sensitivity to irradiation in terms of an increased number of induced chromatid breaks and gaps in individuals predisposed to cancer, such as family members of patients with Wilm's tumour and retinoblastoma.

Whether high mutagen sensitivity has a definite hereditary basis is not yet proven. However, small family studies show an increased mutagen sensitivity in cancer patients from cancer-prone families compared with their first-degree relatives without cancer [62–64].

Implications of mutagen sensitivity

The data on mutagen sensitivity in HNSCC patients underscore the importance of considering inter-individual susceptibility when determining cancer risk. It should be expected that if HNSCC is related to a genetic factor, tumours will also occur in relatively young individuals. Schantz and co-workers found that young adults with HNSCC showed an increased mutagen sensitivity, while men above 50-years, with a minimum of 20 packyears, and without tumours were predominantly (98%) insensitive [65, 66]. This again points towards a different individual genetic susceptibility to carcinogens.

Another implication of the mutagen sensitivity data is the fact that they might be very valuable for the identification of HNSCC patients who are at high risk for the development of MPT. Schantz *et al.* [67] were the first to assess the predictive value of the b/c level in this respect in a small prospective study. They found a relative risk of 4.4 for hypersensitive patients (b/c level >1.0) of developing second primary malignancies as compared to non-sensitive persons. This was later substantiated by enlarging the cohort and extending the duration of follow-up [68].

Recently a large case–control study has been performed in which mutagen sensitivity and exposure to wood dust and smoking were related to risk for lung cancer [57], showing a greater than multiplicative interaction of the combined odds ratios of wood dust exposure, smoking and mutagen sensitivity. This indicates that both exposure to carcinogenic agents and susceptibility to DNA damage determine cancer risk.

It has been postulated that the chromatid breaks induced by bleomycin are not random but occur at specific sites

Table 1. Overview of the b/c score of control persons and several classes of cancer patients

Group (*n*)	Mean b/c* score ± standard deviation	Percentage hypersensitive phenotype†	Reference
Control (335)	0.60 ± 0.35	12	[54]
HNSCC (77)	1.03 ± 0.51	48	
Lung cancer (71)	0.98 ± 0.41	48	
Colon cancer (83)	1.00 ± 0.41	46	
Central nervous system cancer (10)	0.55 ± 0.27	20	[55]
Control (52)	0.77 ± 0.19	14	[56]
HNSCC (50)	0.96 ± 0.31	44	
RUDT (MPT) (20)	1.20 ± 0.47	75	
Lung—male (53)	1.24 ± 0.66	55	[57]
—female (23)	1.00 ± 0.39		

HNSCC, head and neck squamous cell carcinoma; RUDT, respiratory and upper digestive tract; MPT, multiple primary tumours.

*b/c, measurement of chromosomal instability being the mean number of chromatid breaks per cell after challenging lymphocytes with 30 mIU/ml bleomycin.

†Hypersensitive phenotype is defined as a b/c score >1.0.

which may differ among patients with several tumour types [69, 70]. These putative fragile sites are of importance since they may indicate important chromosomal regions in which genes involved in malignant transformation may be located. Cytogenetic studies to compare the breakpoints in the tumours to those in the lymphocytes of the same patients may provide further insight into the role of chromosomal instability in carcinogenesis.

FUTURE PERSPECTIVES

Traditional epidemiology, which focuses on the identification of environmental causes of cancer, is no longer adequate but can be improved when biological markers of susceptibility are included. The need for a multi-disciplinary approach which involves different fields including epidemiology, tumour-biology and clinical medicine is highlighted by the current perception that carcinogenic exposure and genetic susceptibility act in concert to determine cancer risk [71]. Mutagen sensitivity underscores the importance of genetic factors in the development of cancer. It has been shown that risk estimations for HNSCC can dramatically be improved when the mutagen sensitivity phenotype is considered in addition to smoking and alcohol consumption [72]. That mutagen sensitivity plays a role in the development of many environmentally related cancers is widely acknowledged [59, 73]. The small but important differences in mutagen sensitivity are likely to account for increased cancer risk in environmental and occupational exposures.

Mutagen hypersensitivity is most marked in the subset of HNSCC patients with MPT (Table 1). To investigate the potential of the mutagen sensitivity assay to predict which patients are at the highest risk of developing MPT we are currently working on a prospective patient study in a nested case–control approach. Indications that mutagen sensitivity can be used to anticipate MPT in curatively treated HNSCC patients have been reported [67, 68].

Mutagen sensitivity can be used as a biomarker for cancer susceptibility and because it is constitutional, it should be present in all somatic cells of the body. We are currently evaluating a novel, more "true to life " model for the assessment of mutagen sensitivity. Cells of the area of interest, namely oral keratinocytes and fibroblasts of the upper aerodigestive tract, will be tested for their sensitivity to toxic compounds including BLM and cigarette smoke condensate. It should be possible to see whether inter-individual variations in carcinogen susceptibility can also be shown in these cells. This alternative method for testing mutagen sensitivity may enhance the detection of inter-individual differences in sensitivity and will offer the opportunity to investigate more physiologically, interactions between the mucosa and carcinogens.

So far, it has been validated that mutagen sensitivity is a constitutional factor. To prove a hereditary basis of the intrinsic sensitivity to clastogenic compounds, family studies have to be performed. Indications of a distinct hereditary basis for mutagen sensitivity are described in studies in which cancer patients from cancer-prone families were compared to their first degree relatives without cancer [52, 62, 63].

Knowledge of a person's mutagen sensitivity may be valuable for cancer treatment. For breast cancer it has been shown that a hypersensitive phenotype (measured in irradiated fibroblasts) correlates with a better response to radiotherapy [74]. In this respect we may in a future analysis also be able to evaluate whether hypersensitive HNSCC patients have responded better to their radiotherapy compared to nonsensitive patients. Besides a better response of the tumour, it can be postulated that the normal tissue will also suffer more damage in hypersensitive persons. Pollak [75] for instance, speculates that for hypersensitive patients the risk of chemotherapy-induced second malignancies is significantly higher than for the general population. Analysis of the data of the prospective trial of Spitz *et al.* [68] showed no significant differences in the percentage of hypersensitive compared to non-sensitive patients who developed second malignant tumours and had received previous chemotherapy [76].

At the present time, however, mutagen sensitivity is not being used for decisions regarding tumour treatment strategies. Its great future promise probably lies in its value as a biomarker to improve cancer risk assessment [77].

In conclusion, for the investigation of cancer susceptibility, a multi-disciplinary approach is necessary and involves epidemiologists, biologists and physicians. Classical epidemiology using only environmental exposure data is no longer adequate to assess cancer risk and requires the incorporation of specific biomarkers measured in the laboratory [78]. Mutagen sensitivity is a model system which measures a response to genotoxic assaults and reveals a phenotype which reflects susceptibility to cancer, especially in individuals exposed to carcinogens. A major implication of the mutagen sensitivity assay for HNSCC patients is that it may be possible to predict which patients have the highest risk of developing second primary tumours. This subgroup can then be targeted for more frequent and thorough follow-up, intensive behavioural interventions and enrolment in chemoprevention studies.

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