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Review

Molecular events in relapsed oral squamous cell carcinoma: Recurrence vs secondary primary tumor ☆

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SUMMARY

Relapses have a great impact on both the morbidity and mortality rates of oral squamous cell carcinoma (OSCC) patients. Current classification criteria are imprecise and need improvements. Recent advances in understanding of OSCC relapses on a molecular level provide new possibilities to better classify true recurrences and second primary tumors. This review discusses the limitations of the current OSCC relapse classification method and presents possible alternatives to improve this classification based on molecular techniques. Moreover, these molecular techniques add to the further understanding of these lesions and may provide tools for clinical management.

Introduction

Oral squamous cell carcinoma (OSCC) accounts for 24% of malignancies in the upper aero-digestive tract (UADT), being the most common cancer therein [1]. Progresses in diagnosis and management have favorably influenced prognosis, and data of the Surveillance, Epidemiology, and End Results (SEER) program of the National Cancer Institute (NCI) of the USA, indicate that the overall five-year survival rate for patients with SCC of the anterior tongue has increased from 39.6% in 1975 to 64.8% in 2006; for other oral sites a similar increase from 51% in 1975 to 53–58% in 2006 has been recorded [2]. However, the changes in cancer survival rate differ between countries and the improvements in survival are generally not that impressive [3]. For instance, in the Netherlands a moderate improvement of the 5-year relative survival rate was recorded for OSCC, from 56% for cases diagnosed in 1989–94 to 62% in 2007–11 [4].

Relapse is defined as the return of the disease after treatment. It can be classified as tumor recurrence in those cases where tumor cells are not eliminated by treatment and regrow, or second primary tumors (SPTs) when an independent carcinogenetic process is responsible for the development of a new tumor. Both early

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recurrence associated with advanced clinical stage and SPT are adverse prognosticators [5,6].

Relapse in head and neck squamous cell carcinoma (HNSCC) ranges from 16% to 52%, [7–11] and depends on anatomical site, clinical staging and pathological features [12]. With regard to OSCC, as many as 63.6% of the patients may show local relapse, while 13.3% develop locoregional relapse [13]. Local relapses are the main cause (24.4%) of death [11]. Indeed, relapse in the oral cavity is associated with a higher risk of mortality than in other head and neck sites [14–18].

The risk of development of a second primary oral or pharyngeal tumor in patients with OSCC is higher than primary tumor development in the general population [19,20] and increases with time. The likelihood of SPT development, 5 and 10 years following diagnosis, is 33-fold and 36-fold compared to primary tumor diagnosis in the population, respectively. The site of these SPTs is the UADT in almost all cases [19–21].

Early diagnosis of OSCC recurrence and SPTs would be of significance for improving further clinical management [13,19,22]. However, the distinction between OSCC recurrence and SPTs, clinically and even pathologically, is complex. For instance, little is known about factors influencing the time between diagnosis and management of the primary (index) tumor (InT) and relapse, and also about mechanisms involved in this process. Increased understanding of those events would result in a more reliable classification of post-InT morbidity, enabling better comparison of outcome data and accounting for clinical variations. In this context, exploring molecular aspects of the pathogenesis of OSCC recurrence and SPTs is useful. The present review discusses progress towards elucidating the sequential molecular events in OSCC recurrence and SPTs with particular emphasis on relapse classification, limitations of molecular markers and potential clinical use. While undertaking this review, we selected publications with the key words: recurrence, second primary tumor, true recurrence, relapse, field cancerization and field carcinogenesis, combined with the term "oral squamous cell carcinoma".

Shortcomings of current recurrence classification

In 1932, Warren and Gates [23] suggested the following criteria for categorizing OSCC relapses. Tumors deriving from residual disease reflect recurrence, while independent origin accounts for a SPT. In order to clinically characterize a relapse as SPT, the following criteria should be met: (1) clinical appearance indicative of malignancy; (2) manifestation at a distinct site; (3) exclusion of possible metastasis; and (4) occurrence at least 3 years after the InT. A later effort added other criteria: (1) in case of histological similarity between relapse and InT, the former should be characterized as SPT if it is separated from the latter by more than 2 cm of normal epithelium and/or occurs at least 3 years after the initial diagnosis; relapses of different (non-epithelial) histology are regarded as SPTs [24].

Despite the value of these classifications, there is room for improvement that would affect comparative studies, management and prognostication [25,26]. In this vein, the increasing knowledge on molecular alterations involved in OSCC offers interesting possibilities.

Using molecular techniques some investigators had challenged the current classification for relapse in head and neck tumors (Table 1). The applied techniques were based on the premise that specific patterns of DNA mutation could predict tumor clonality. If the same pattern of DNA mutations is observed in both InT and relapse, it is reasonable to infer that they are clonally related tumors, classified as tumor recurrences. On the contrary, the lack of a similar DNA mutation pattern between the lesions would indicate that the relapse has an independent cellular origin and should be classified as a SPT [25].

Gutiérrez et al. [27] studied the genetic profile between laryngopharyngeal tumors and identified discrepancies between genetic and clinical classification of tumor relapses. They employed a molecular approach based on Multiplex Ligation-Based Probe Amplification (MPLA) analyzing 42 specific chromosomal regions that may be involved in HNSCC. Among the cases clinically defined as true recurrence (TR), only 13% were considered clonally related. The clinical criteria seem more accurate for SPTs, since 57% of these were not clonally related. Other authors made similar observations, emphasizing that the molecular analysis of tumor relapses rarely matches the clinical characterization [16,28–31].

On the one hand, these investigations indicated the inconsistency between the molecular characterization of tumor relapses and clinical classification; on the other hand the resolution of the applied techniques is not considered enough to allow definite conclusions. In most studies analysis of microsatellite profiles, *TP53* gene mutations and X chromosome inactivation analysis are used, which provide rather limited genomic information. The variable study design, sample selection and molecular approach also hamper meaningful comparisons. Finally, the existence of intratumoral genetic heterogeneity influences interpretation.

The discussion above indicates the limitations of current classifications of oral OSCC relapse and reveal gaps in knowledge about their biology. Widespread endorsement of a genetic approach may, however, increase understanding of differences in clinical behavior between different types of relapse and will allow more efficient management strategies in turn [26].

Types of cancer recurrences

With InT as a point of reference, OSCC relapse can be classified according to: (1) localization (same site as the InT or distant); (2) time elapsed (less than 6 months since diagnosis of InT, synchronous; more than 6 months, metachronous); and (3) origin (from residual disease or *de novo*) [25,32].

A series of studies provided a sequential comprehension of the carcinogenesis process in the oral cavity. Based on this new knowledge, a basis for a molecular classification of cancer relapse emerged.

In 1953 Slaughter et al. [33] explored pathological features involved in OSCC recurrence and reported that the clinically normal epithelium surrounding a tumor rarely appeared so on histological examination; it often showed dysplasia and occasionally carcinoma *in-situ*. They hypothesized that exposure to carcinogens, affected multiple, topographically independent areas of the oral mucosa, which effect cumulative cellular changes and eventually development of OSCC. They introduced the term "field cancerization" for describing the process of widespread cellular changes in oral mucosa that predispose to cancer development and suggested that widespread squamous epithelial dysplasia may influence the high rate of local recurrence in OSCC.

In later studies cumulative DNA alterations were correlated with progressing histological changes or grades of dysplasia [34]. It was shown that tissues with mild dysplastic changes would show few genetic aberrations in comparison with invasive lesions of malignant architecture and that these aberrations occur in a certain order. However, DNA changes have also been observed in epithelium without obvious changes on routine histological examination and it appears that clinical examination and histological assessment of margin status alone, are not enough to predict recurrence [30,35]. Brandwein-Gensler et al. [36] accordingly reported that recurrences of OSCC occurred even in the absence of involved surgical margins. de Carvalho et al. [37] attempted gene profiling of