Available online at www.sciencedirect.com
ScienceDirect
British Journal of Oral and Maxillofacial Surgery 46 (2008) 370–375
BRITISH Journal of Oral and Maxillofacial Surgery
www.bjoms.com

Predicting recurrence after oral precancer treatment: Use of cell cycle analysis

P.J. Thomson [a], O. Hamadah [a,*], M.L. Goodson [a], N. Cragg [b], C. Booth [b]

[a] *Oral & MaxilloFacial Surgery, School of Dental Sciences, Newcastle University, UK*
[b] *Epistem Ltd, Incubator Building, Grafton Street, Manchester, UK*

Accepted 2 January 2008
Available online 20 February 2008

Abstract

Prediction of the behaviour of oral precancerous lesions (OPLs) is unreliable in clinical practice. The aim of this study was to analyse the efficacy of cell cyclin markers A and B1, and the proliferative marker Ki67, in predicting clinical outcome for patients with OPLs. A cohort of previously-treated patients with single OPLs were retrieved from the MaxilloFacial Dysplasia database and reviewed. All had dysplastic lesions excised by laser and were followed up for 5 years post-treatment. Outcome was determined as no recurrence or further disease. Excision specimens were re-examined immunohistochemically and labelling indices (LIs) for cyclin A, B1 and Ki67 determined. Forty patients, aged between 31 and 91 years, were recruited. There were no differences in age or sex. OPLs were predominantly leukoplakias on the floor of mouth or ventro-lateral tongue (65%), most of which exhibited moderate or severe dysplasia. Cyclin A LIs ranged from 3.9% to 31.3%, B1 0 to 28.3% and Ki67 3.5% to 54.5%. Using median LIs as 'cut off points' (12% cyclins; 22% Ki67) Kaplan-Meier survival analysis showed a significant risk of further progression of disease in patients with OPL LIs exceeding median values (Cyclin A $p = 0.02$, Cyclin B1 $p = 0.01$, Ki67 $p = 0.025$). By combining analysis of both Cyclin A and B LI, the significance of the difference was increased ($p < 0.01$). Cell cycle analysis is effective in identifying patients at risk of further progression of disease following treatment of OPLs. Multi-centre, longitudinal trials are needed to assess the precise role of cell cycle markers in their management.

Keywords: Oral Precancer; Dysplasia; Recurrence; Cell Cycle; Cyclin A; Cyclin B1

Introduction

Oral squamous cell carcinoma (SCC) remains a lethal disease despite advances in diagnosis and multidisciplinary management. Of particular concern is the rising incidence of oral cancer, especially in younger patients and women.[1] Further progress in clinical management requires predictive techniques to identify individual patients and lesions at risk of development of cancer and treatment protocols to intervene at the earliest possible stages of carcinogenesis.[2–4]

Many cancers are preceded by clinically detectable, morphologically altered, precancerous lesions. Initial phenotypic changes are recognised microscopically as epithelial dysplasia, a varying presence of cellular atypia and tissue disorganisation, that risks malignant transformation. This histopathological grading, however, is highly subjective and the behaviour of individual lesions remains unpredictable.[5] Quoted transformation rates to malignancy vary from 6% to 36%, so that accurate and reproducible prediction of carcinogenic change or the clinical progression of individual precancers remains elusive.[6–10]

Although the clinical behaviour and treatment protocols for oral precancers remain controversial,[2,11,12] we have previously shown the efficacy of interventional laser surgery

* Corresponding author. Oral & MaxilloFacial Surgery, School of Dental Sciences, Framlington Place, Newcastle upon Tyne, NE2 4BW, UK. Tel.: +44 191 222 8290; fax: +44 191 222 6137.
E-mail addresses: peter.thomson@ncl.ac.uk (P.J. Thomson), omar.hamadah@ncl.ac.uk (O. Hamadah), michaela.goodson@hotmail.com (M.L. Goodson), n.cragg@epistem.co.uk (N. Cragg), c.booth@epistem.co.uk (C. Booth).

0266-4356/$ – see front matter © 2008 The British Association of Oral and Maxillofacial Surgeons. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.bjoms.2008.01.003

as both a diagnostic and therapeutic management tool.[3] We have also shown increased epithelial proliferative activity in increasingly dysplastic tissue,[2,13] and it is now recognised that alterations in genes that regulate cell division, cell cycle progression, and DNA synthesis and repair are fundamental to carcinogenesis.

Normal cell proliferation requires a precise and complex sequence of events that regulate passage through the various phases of the cell cycle; such movement and timing is controlled by cyclins. The aim of this study was to analyse the efficacy of markers of the cell cycle as predictive agents during interventional management of oral premalignant disease. Three markers were used: Cyclin A (a cell cycle protein synthesised during S phase and required for progression through S, G2 and then into mitosis), Cyclin B1 (a marker of G2 activity and subsequently mitosis) and Ki67 (a marker of general proliferative activity).

Methods

Following ethical approval and informed patient consent, a cohort of previously treated oral precancer patients was randomly selected from the oncology database of the Newcastle University Department of Oral & MaxilloFacial Surgery and invited to take part in the study.

Inclusion criteria required a first presentation of a single precancerous lesion, with no previous oral dysplastic or neoplastic lesions, nor previous treatment. All lesions were confirmed histologically as dysplastic, and all patients underwent a standardised interventional management protocol comprising identification and education regarding risk factors, laser excision of OPLs by one surgeon and follow up for at least 5 years postoperatively with documented clinical outcome. Outcome was classified as 'no recurrence' or 'further-disease development'; the latter included recurrence of the lesion at the same site or development of new lesions at distinct intraoral sites.

Formal histopathological examination was made of archive, paraffin-embedded, haematoxylin-eosin-stained sections. Dysplasia was graded as mild (atypia confined to the basal third of epithelium), moderate (involving up to two-thirds), or severe (greater than two-thirds) while carcinoma-in-situ was characterised as severe tissue dysmaturation extending to an intact basement membrane. All histological diagnoses were made by specialist oral and head and neck pathologists, who worked to standardised criteria.

Immunohistochemical detection was by a standard avidin-biotin-peroxidase technique after microwave oven antigen unmasking in 10 mmol citrate buffer (pH 6.0). The primary antibodies used were cyclin A (6E6; Novocastra, UK) with 1:50 dilution, cyclin B1 (7A9; Novacastra, UK) 1/15 dilution, and Ki67 (MIB-1; Dako, Denmark) 1/50 dilution, with goat antimouse IgG as the secondary antibody.

Tissue samples were analysed with a computer-assisted microscope system that functioned by superimposing the computer screen on to the microscope image (Zeiss Axiohome TM interactive microscope).[2,14] The base of five successive epithelial ridges or five representative fields of view in confirmed pathological tissue, depending upon tissue type and organisation, were defined for each sample and displayed on both computer screen and microscope simultaneously. Labelling indices for cyclin A, B1, and Ki67 were calculated by counting the number of positive immunoreactive cells amongst 1000 cells. All samples were scored by one research worker to avoid interobserver variability.

Statistical analysis was performed using the SPSS V.12 software package (SPSS Inc; Chicago, IL, USA). Correlations between the expression of cyclin A, B1, and Ki67 and between their respective LIs and clinicopathological features were examined using Spearman's rank correlation test. A correlation coefficient greater than 0.4 or less than −0.4 was considered strongly positive or negative, respectively. Kaplan Meier analysis using the log-rank Chi square was done to investigate the use of immunohistochemical markers (median LIs acting as cut-off points) in predicting recurrent disease after OPL treatment. Probabilities of less than 0.05 were accepted as significant.

Results

Forty patients (28 men, 12 women) with an age range from 31 to 91 years (mean age 59.7 years) were recruited. All patients were smokers and drank alcohol. Most patients (65%) presented with leukoplakia, with 78% of cases arising in the floor of mouth and ventral lateral tongue; 95% of lesions had either moderate or severe dysplasia histopathologically. Clinicopathological details of the 40 oral precancerous lesions are summarised in Table 1; all excision margins were clear of dysplasia after laser surgery.

No patients developed squamous cell carcinoma (SCC) during the 5 year follow up period; 24 patients had no recurrence after laser excision, while 16 developed further disease, 7 at new intra oral sites. No relation was found between outcome and clinical appearance of lesions, nor their anatomical site of origin. Further disease, however, was seen more often in patients who had severely dysplastic lesions excised ($p = 0.001$; Fig. 1).

Table 2 lists mean (SD) LI for cyclin A, B1 and Ki67; mean values for cyclin A and B1 were similar (14% and 12%, respectively), but much higher for Ki67 (25.%). There was a strong correlation seen amongst all three markers (Spearman's correlation coefficient $p < 0.001$, Table 3). No significant relation between LI and clinical appearance or anatomical site, although increasing LI were observed with increasing dysplasia (Fig. 2). Significant increases were found between moderate and severe dysplasia for cyclin A ($p = 0.004$; ANOVA) and Ki67 ($p = 0.028$; ANOVA).