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Research Article

Cancer Epidemiology, Biomarkers & Prevention

Changes in Epidermal Growth Factor Receptor Gene Copy Number during Oral Carcinogenesis

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is a global healthcare problem associated with poor clinical outcomes. Early detection is key to improving patient survival. OSCC may be preceded by clinically recognizable lesions, termed oral potentially malignant disorders (OPMD). As histologic assessment of OPMD does not accurately predict their clinical behavior, biomarkers are required to detect cases at risk of malignant transformation. Epidermal growth factor receptor gene copy number (EGFR GCN) is a validated biomarker in lung non–small cell carcinoma. We examined EGFR GCN in OPMD and OSCC to determine its potential as a biomarker in oral carcinogenesis.

Methods: EGFR GCN was examined by *in situ* hybridization (ISH) in biopsies from 78 patients with OPMD and 92 patients with early-stage (stages I and II) OSCC. EGFR ISH signals were scored by two pathologists and a category assigned by consensus. The data were correlated with patient demographics and clinical outcomes.

Results: OPMD with abnormal EGFR GCN were more likely to undergo malignant transformation than diploid cases. EGFR genomic gain was detected in a quarter of early-stage OSCC, but did not correlate with clinical outcomes.

Conclusion: These data suggest that abnormal EGFR GCN has clinical utility as a biomarker for the detection of OPMD destined to undergo malignant transformation. Prospective studies are required to verify this finding. It remains to be determined if EGFR GCN could be used to select patients for EGFR-targeted therapies.

Impact: Abnormal EGFR GCN is a potential biomarker for identifying OPMD that are at risk of malignant transformation. *Cancer Epidemiol Biomarkers Prev; 25(6); 927–35. ©2016 AACR.*

Introduction

Oral squamous cell carcinoma (OSCC) is a major healthcare problem and is associated with poor clinical outcomes. Approximately 50% of patients diagnosed with OSCC die prematurely as a consequence of the disease (1, 2). Outcomes for patients with OSCC may be improved if the disease is identified in its earliest stages (3). OSCC formation occurs through the stepwise accumulation of genetic damage (4, 5). OSCC may be preceded by clinically recognizable lesions termed oral potentially malignant disorders (OPMD; ref. 6). However, the histologic features of OPMD do not reliably predict their clinical behavior (7, 8). There is consequently a need to develop biomarkers that enhance prognostication and direct treatment (9).

EGFR gene copy number (GCN) is used in the prognostication of non–small cell lung carcinoma (10, 11) and the prediction of its response to EGFR-targeted chemotherapeutic agents (12). The potential of EGFR as a biomarker in OSCC was first highlighted in the early 1990s (13). EGFR is a cell surface tyrosine kinase receptor, one of four proteins in the ErbB family, and is expressed in most epithelial tissues (14). Binding of growth factors (e.g., EGF and TGFα) to the extracellular domain induces a conformational change in the internal receptor (15, 16). Subsequent phosphorylation of intracellular substrates triggers a myriad of downstream signaling cascades (17). In OSCC, these contribute to an increase in cell proliferation, angiogenesis, invasion, and metastasis, which are the hallmarks of cancer (18, 19).

EGFR genomic gain is associated with poor clinical outcomes in OSCC (20–22). The prevalence of EGFR genomic gain in OSCC ranges from 9% to 56% (23–26) and is more frequent in stage III/IV disease, suggesting that EGFR genomic gain is a late event in oral carcinogenesis. By contrast, data from two OPMD studies show that cases with low polysomy are more likely to progress to OSCC (27, 28). These data suggest that EGFR GCN starts to increase in the early stages of oral carcinogenesis and raise the possibility that it could be used as a biomarker of malignant transformation. However, both studies were limited by small sample sizes and analysis of tissue microarrays rather than whole sections. Furthermore, low polysomy is not

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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regarded as EGFR genomic gain in the criteria currently validated for interpretation of non–small cell lung carcinoma as only high polysomy/clustered EGFR GCN signals are reported to correlate significantly with clinical outcome and response to EGFR-targeted therapy (10, 11). Consequently, the biologic significance of EGFR low polysomy is uncertain, particularly given the complexity of the EGFR signaling pathway (29, 30).

The aims of this study were as follows:

- To determine the frequency of EGFR GCN abnormalities in patients with OPMD and early-stage OSCC.
- To correlate EGFR GCN abnormalities with clinicopathologic data and patients' clinical outcomes.
- To determine EGFR protein expression in OPMD and early-stage OSCC in order to gauge the likely functional significance of EGFR GCN changes.

Materials and Methods

Patients

Cases of OPMD that did not transform to OSCC were identified from a group of patients attending a hospital-based OPMD clinic. These cases had a minimum of 24 months' follow-up.

Cases of OPMD that underwent malignant transformation were identified using a systematic search of the electronic archives using SNOMED (Systematized Nomenclature for Human Medicine) codes. The search spanned a 12-year period (1997–2009). The subsequent OSCC was also identified and retrieved for analysis. Clinical follow-up data were obtained from medical records.

Consecutive local cases of early-stage (pStage I/II) OSCC were identified by searching hospital databases and latterly the DAHNO (DAta on Head and Neck Oncology) UK database. The search spanned an 8-year period (2000–2008).

Cases with the following characteristics were excluded: (i) previous upper aerodigestive tract cancer; (ii) previous radiotherapy to the head and neck region; (iii) index lesions arising on the lip or in the oropharynx; (iv) <24 months' follow-up; (v) <6 months between index OPMD biopsy and OSCC diagnosis; (vi) proliferative verrucous leukoplakia; (vii) nondysplastic OPMD diagnosed with specific clinicopathologic entities, e.g., chronic hyperplastic candidosis and lichen planus.

For each case, patient demographic data (sex, age at first biopsy) and mucosal subsite of the OPMD/OSCC were recorded. For OPMD, the clinical outcome (i.e., whether or not the lesion underwent malignant transformation to OSCC) was recorded. For OPMD that underwent malignant transformation, time from diagnosis of OPMD to developing OSCC was calculated. For early-stage OSCC, the histologic grade of differentiation (Broders' classification) was determined, and clinical outcomes (disease-free survival, overall survival) were calculated.

Pathology methods

Hematoxylin and eosin (H&E)–stained sections and formalin-fixed paraffin-embedded tissue blocks were retrieved for each case to confirm the presence of disease and adequacy of material for subsequent analysis. For OPMD, epithelial dysplasia was graded independently by two pathologists (M. Robinson and P. Sloan) using a binary system (low-grade vs. high-grade; refs. 7, 8). Discordant cases were reviewed, and a grade was assigned by consensus.

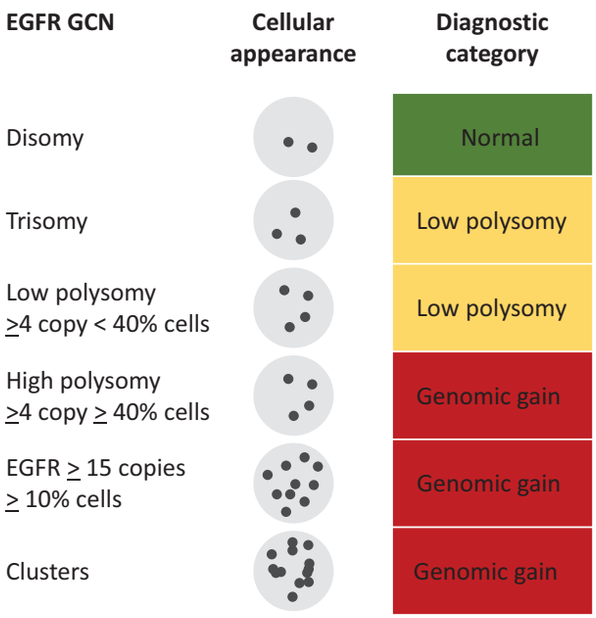

Figure 1.
Interpretation of dual EGFR gene and chromosome 7 ISH signal. Adapted from "Interpretation Guide, Ventana Inform EGFR DNA Probe: DNA Probe Staining of Non-Small-Cell Lung Carcinoma" (31). (Used with manufacturer's permission. Full copyright © 2015 Ventana Medical Systems, Inc.)

EGFR *in situ* hybridization

EGFR GCN was assessed by a dual-color *in situ* hybridization (ISH) technique using proprietary reagents (INFORM EGFR-Chromosome 7 dual-color assay; Ventana Medical Systems Inc). This detects the EGFR gene (using silver ISH, seen as black nuclear dots) and chromosome 7 centromeres (using Ultraview Alkaline Phosphatase Red ISH, seen as red nuclear dots) on the same section. Sections (4 μm) were stained using the Ventana Benchmark Autostainer according to the manufacturer's instructions. Negative controls (with DNA probes omitted) were performed for each staining batch.

Dual-stained ISH sections were examined by two pathologists (T. Bates and M. Robinson), and a category was assigned by consensus. According to the predominant nuclear signal, each case was assigned to one of the six categories described and validated by the manufacturers for the interpretation of non–small cell lung carcinoma (Fig. 1; ref. 31). Dividing cells and overlapping cells were not assessed. During analyses, the six descriptive categories were reduced to three groups for comparison: normal, low polysomy, and genomic gain (Fig. 1) and also analyzed in a binary classification: normal versus abnormal EGFR GCN.

EGFR immunohistochemistry

EGFR protein expression was detected using a proprietary antibody (anti-EGFR 5B7 clone; Ventana Medical Systems Inc). Sections (4 μm) were stained using a Ventana Benchmark Autostainer according to the manufacturer's instructions. Morphologically normal epithelium provided an internal control for each section. Negative controls (primary antibody omitted) were performed for each staining batch.

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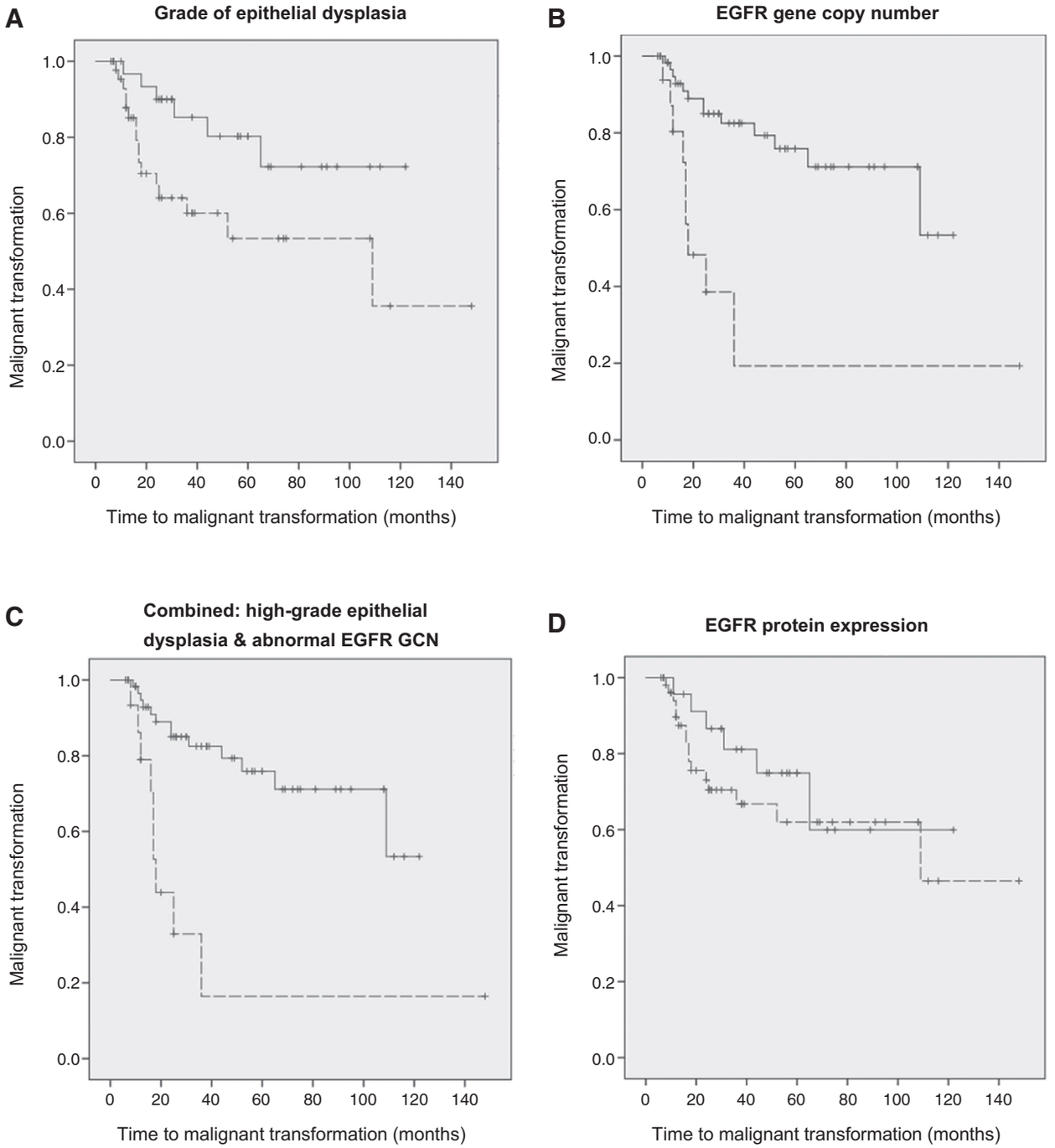

Figure 2.
Kaplan–Meier time-to-event analysis showing malignant transformation in OPMD stratified according to grade of epithelial dysplasia, EGFR GCN, and EGFR protein expression. Solid line: (A) low-grade epithelial dysplasia; (B) normal EGFR GCN; (C) low-grade epithelial dysplasia and normal EGFR GCN combined; (D) low EGFR protein expression. Broken line: (A) high-grade epithelial dysplasia; (B) abnormal EGFR GCN; (C) high-grade epithelial dysplasia and abnormal EGFR GCN combined; (D) high EGFR protein expression. A, there was a significant correlation between high-grade epithelial dysplasia and malignant transformation ($P < 0.05$, χ^2 value = 4.974, 1 d.f.). B, there was a significant correlation between abnormal EGFR GCN and malignant transformation ($P < 0.0001$; χ^2 value = 13.929, 1 d.f.). C, a similar correlation was identified when epithelial dysplasia and EGFR GCN categories were combined ($P < 0.0001$; χ^2 value = 16.069, 1 d.f.). D, there was no correlation between EGFR protein expression and malignant transformation ($P = 0.356$).

EGFR-stained slides and corresponding H&E sections were scanned using the Aperio Scanscope platform (x400 magnification). Files were uploaded to and analyzed using the Aperio Spectrum image analysis system (Spectrum Version 11.1.0.751; Aperio Technologies, Inc.). H&E sections were used to map areas of normal epithelium, epithelial dysplasia,

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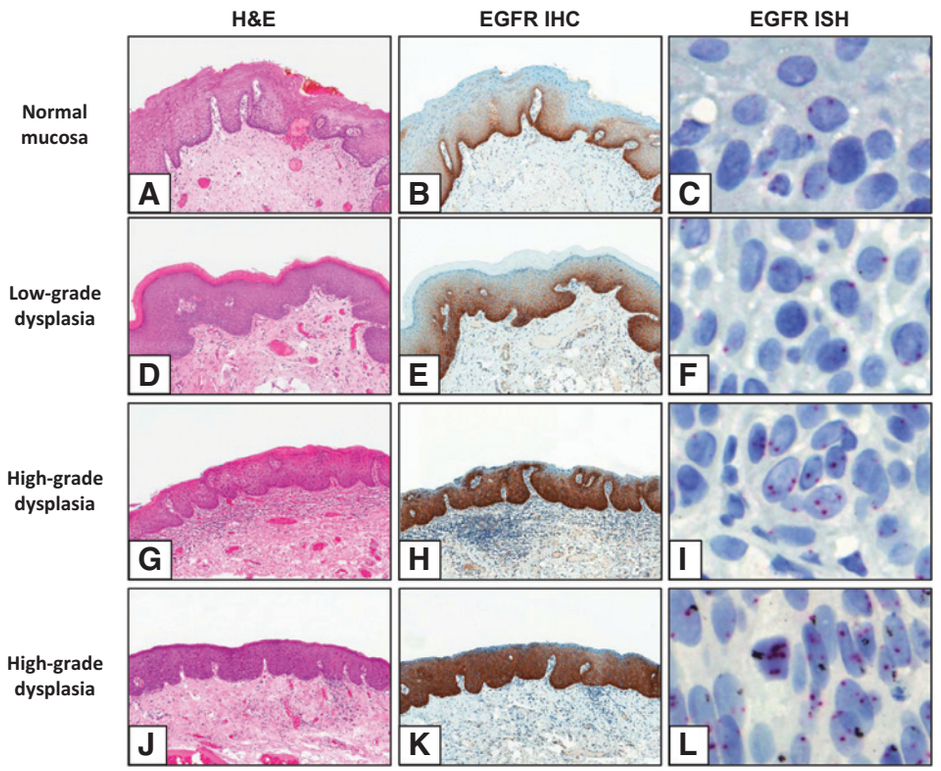

Figure 3.
EGFR protein expression and EGFR ISH for normal mucosa and OPMD with low-grade and high-grade epithelial dysplasia. A, normal mucosa. B, in normal squamous epithelium, EGFR protein is expressed most strongly in the basal and parabasal layers, but is lost toward the surface. C, nuclei of keratinocytes show disomy by ISH. D, OPMD with low-grade epithelial dysplasia. E, EGFR protein expression is increased in low-grade epithelial dysplasia relative to normal squamous epithelium. Expression is most noticeably stronger in the prickle layer. F, however, nuclei of keratinocytes still show disomy by ISH. G, OPMD with high-grade epithelial dysplasia. H, EGFR protein expression is increased in high-grade epithelial dysplasia relative to both normal squamous epithelium (B) and low-grade epithelial dysplasia (E). Expression is strong throughout the full thickness of the epithelium. I, in this example of high-grade epithelial dysplasia, nuclei of keratinocytes show an abnormal signal, low polysomy, by ISH. J, OPMD with high-grade epithelial dysplasia. K, there is strong full-thickness expression of EGFR protein. L, this example of high-grade epithelial dysplasia shows a clustered nuclear signal by ISH. H&E and EGFR IHC, ×100 magnification; EGFR ISH, ×400 original magnification.

and OSCC on corresponding EGFR-stained section. Representative areas were annotated and analyzed using the Aperio cellular algorithm. The algorithm generated data for a range of parameters, including the number of cells analyzed, the proportion of positive cells, and the proportion of strongly positive cells. Data were collated in an Excel file prior to statistical analysis.

Statistical analysis

Statistical analysis was performed using SPSS for Windows (version 21.0; SPSS Inc.). Following a test of normality, parametric data were analyzed using one-way ANOVA/independent sample *t* tests, and nonparametric data using Kruskal–Wallis/Mann–Whitney *U* tests. A Bonferroni correction was applied to multiple comparisons. Time-to-event analyses were plotted using Kaplan–Meier curves and assessed using log-rank (Mantel–Cox) calculations. Receiver operator curves (ROC) were generated by plotting true-positive rates against the false-positive rates. Prior to analysis, cases were classified into binary groups depending on the variable of interest (e.g., high/low-grade epithelial dysplasia; normal/abnormal EGFR GCN; high/low EGFR protein expression; i.e., above or below mean proportion of positive cells for the normal epithelium). Ordinal data were analyzed using Pearson's χ^2 test. Results were considered significant at $P < 0.05$.

Ethical approval

The study had a favorable ethical opinion from the National Research Ethics Service (NRES) Committee North East, Sunderland (REC reference: 11/NE/0118).

Results

Patient characteristics and clinical outcomes

A total of 78 OPMD and 92 OSCC cases satisfied the study inclusion criteria. Mean ages for these two groups were 58.6 (range, 30–94) and 61.8 years old (range, 33–93), respectively. Both had a male predominance (overall M:F, 1.54:1). Clinical outcomes and other characteristics are summarized in Supplementary Table S1 (see Supplementary Data). There was no correlation between the clinical outcome of OPMD/OSCC and either patient demographics (age, sex) or mucosal subsite (data not shown).

For OPMD, the histologic grade of epithelial dysplasia showed a significant correlation with clinical outcome. Cases with high-grade epithelial dysplasia were more likely to

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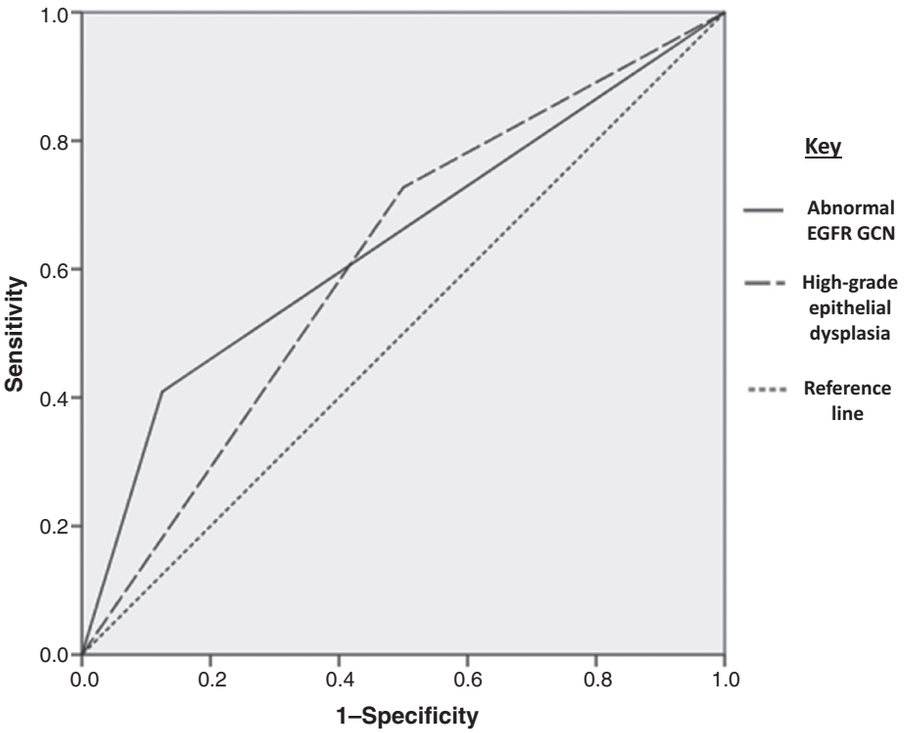

Figure 4.
ROC analysis of malignant transformation for high-grade epithelial dysplasia and abnormal EGFR GCN. Solid line: abnormal EGFR GCN. Broken line: high-grade epithelial dysplasia. Dotted line: reference line. The table beneath the chart summarizes the differences between the two curves. The greater the area beneath the curve, the greater the predictive reliability of the marker. The area beneath the curve for abnormal EGFR GCN was greater than the area for high-grade epithelial dysplasia. This indicates that abnormal EGFR GCN was more reliably predictive of malignant transformation than high-grade epithelial dysplasia. This is further borne out by comparison of the asymptotic significance of the two tests: only abnormal EGFR GCN is significant at $P < 0.05$.

Test variable	Area beneath curve	Standard error	Asymptotic significance	Asymptotic 95% confidence interval
High-grade epithelial dysplasia	0.61	0.069	$P > 0.10$	0.48–0.75
Abnormal EGFR GCN	0.64	0.074	$P < 0.05$*	0.50–0.79

undergo malignant transformation than cases with low-grade epithelial dysplasia ($P < 0.05$; Fig. 2A).

EGFR ISH

Nuclei in normal epithelium adjacent to OPMD or OSCC consistently showed disomy, the normal EGFR ISH signal (Fig. 3C).

OPMD

Low polysomy was detected in 15 OPMD cases (Fig. 3I). Eight of these cases underwent malignant transformation. One OPMD case displayed clustered signals consistent with EGFR genomic gain (Fig. 3L). This case underwent malignant transformation after 17 months.

For statistical analysis, the 15 OPMD with low polysomy were combined with the one case of EGFR genomic gain to form a single "abnormal EGFR GCN" group ($n = 16$). Kaplan–Meier time-to-event analysis demonstrated a statistically significant correlation between abnormal EGFR GCN and malignant transformation ($P < 0.0001$; Fig. 2B). Comparison using ROC analysis confirmed that abnormal EGFR GCN was a more reliable predictor of malignant transformation than high-grade epithelial dysplasia (Fig. 4). A combined category (cases with both abnormal EGFR GCN and high-grade epithelial dysplasia) showed similar Kaplan–Meier curves to EGFR GCN alone (Fig. 2C), and the ROC profile was identical (data not shown).

OSCC arising from OPMD cases

Twenty-two OPMD cases underwent malignant transformation to OSCC. Biopsy material was available for 21 of these cases. EGFR genomic gain was detected in nearly one-quarter of the associated OSCC (5 cases, 24.0%). One-third of the associated OSCC showed low polysomy (7 cases, 33.3%). The associated OSCC generally either maintained the low polysomy of the OPMD, or showed progression to EGFR genomic gain. The EGFR GCN categories of the transforming OPMD and associated OSCC are shown in Fig. 5.

Early-stage OSCC

EGFR genomic gain was identified in 23 (24.7%) early-stage OSCC (11 showed high polysomy and 12 showed clusters). EGFR genomic gain was associated with a reduction in mean

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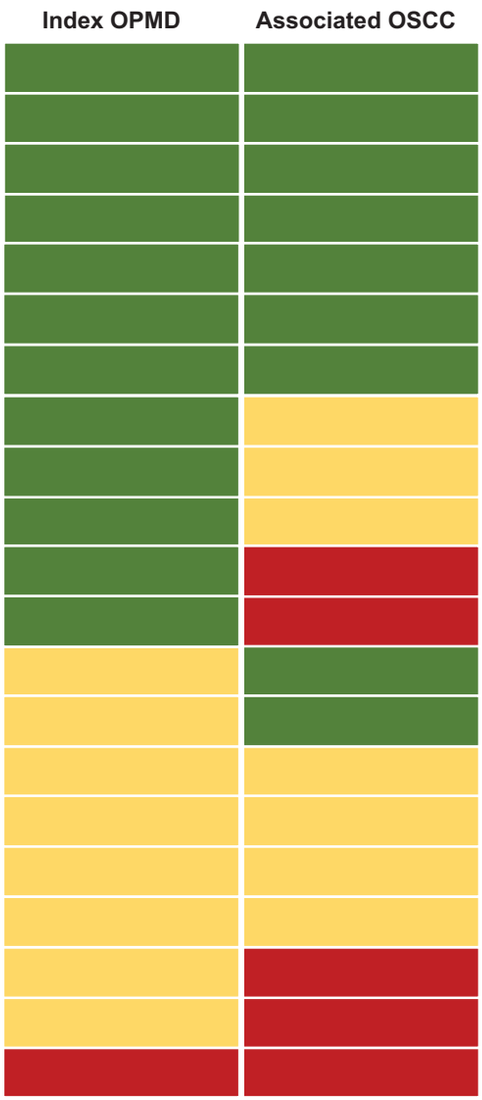

Figure 5.
EGFR GCN of OPMD that underwent malignant transformation and their associated OSCC. Green, normal GCN; amber, low polysomy; and red, genomic gain.

overall survival time (50.2 months vs. 57.7 months for cases with no genomic gain) and disease-free survival (45.6 months vs. 47.7 months for cases with no genomic gain); however, neither trend was statistically significant ($P = 0.201$ and $P = 0.472$, respectively). EGFR genomic gain did not correlate with tumor grade, recurrence, or lymph node metastasis (data not shown).

EGFR protein expression

OPMD. Areas of epithelial dysplasia showed significantly higher mean EGFR protein expression than normal epithelium (Fig. 6). There was also a correlation between EGFR protein expression and grade of epithelial dysplasia: OPMD with high-grade epithelial dysplasia had significantly higher levels of EGFR-positive cells than OPMD with low-grade epithelial dysplasia (Fig. 6). However, the level of EGFR protein expression did not correlate with malignant transformation (Figs. 2D and 6).

OSCC arising from OPMD cases. The OSCC associated with the transformed group of OPMD showed significantly higher mean EGFR protein expression than normal epithelium ($P < 0.0001$; independent t test).

Early-stage OSCC. Early-stage OSCC had significantly higher mean EGFR protein expression than normal epithelium (Fig. 6); however, EGFR expression did not correlate with tumor grade, stage, or clinical outcome (data not shown).

Correlation between EGFR GCN and protein expression

EGFR protein expression was significantly higher in OPMD with an abnormal EGFR GCN than cases with normal EGFR GCN (abnormal EGFR GCN mean, 49.9%, SD, 12.1 vs. normal EGFR GCN mean, 29.3%, SD, 15.7; $P < 0.0001$). EGFR protein expression was significantly higher in OSCC with EGFR genomic gain relative to cases with no genomic gain (EGFR genomic gain mean, 51.2%, SD, 21.9 vs. no genomic gain mean, 35.9%, SD, 22.5; $P < 0.01$).

Discussion

There is a continuing need for biomarkers that refine morphologic diagnoses and inform clinical decisions for patients with OPMD and OSCC (9). EGFR GCN is used in the prognostication of non–small cell lung carcinoma (10, 11) and to predict response to EGFR-targeted chemotherapeutic agents (12). Over recent years, EGFR GCN has emerged as a potential biomarker for OPMD and OSCC (30, 32). However, the prevalence and clinical significance of EGFR GCN abnormalities in OPMD and OSCC are not well defined (20, 21, 33, 34). Furthermore, it is unclear how criteria validated for interpretation of EGFR GCN signals in non–small cell lung carcinoma should be applied to oral cancer. It is well documented that detection of high-risk human papillomavirus (HPV) by p16 immunohistochemistry (IHC) and ISH is significant in the prognostication of oropharyngeal squamous cell carcinoma (35–37). The current study was restricted to the examination of potentially malignant disorders/squamous cell carcinoma of the oral cavity and excluded oropharyngeal subsites. Consequently, we would only expect a very small number of cases to harbor oncogenic HPV infection, less than 5% (38, 39). HPV status was therefore unlikely to influence the results of our study.

One-fifth of OPMD in the present study showed an abnormal EGFR GCN, but only one case showed evidence of EGFR genomic gain according to the criteria validated for non–small cell lung cancer. This is consistent with data from studies of EGFR GCN in OSCC, which indicate that EGFR genomic gain is a late event in oral carcinogenesis (23–25). It is striking, however, that the majority of OPMD with low polysomy progressed to OSCC. This finding is consistent with two recent studies that suggest low polysomy is an early feature of OPMD destined to undergo malignant transformation, one which heralds EGFR genomic gain later in oral carcinogenesis (27, 28). Both studies used FISH rather than the chromogenic ISH (CISH) technique used in the current study. Benchekroun and colleagues (27) studied EGFR FISH in a subset of 49 OPMD, applying a definition of FISH positivity that

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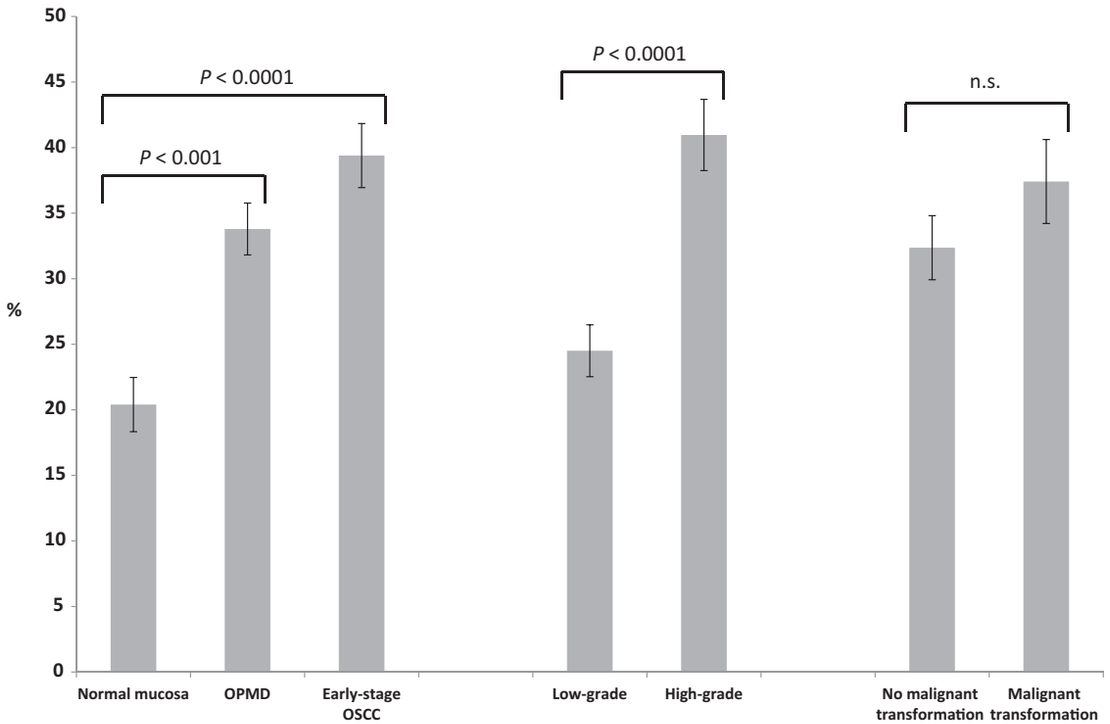

Figure 6.
Comparison of EGFR protein expression for normal epithelium, OPMD, and early stage OSCC. Areas of epithelial dysplasia and early-stage OSCC had significantly higher EGFR expression than normal epithelium ($P < 0.001$ and $P < 0.0001$, respectively). OPMD with high-grade epithelial dysplasia had significantly higher levels of EGFR than OPMD with low-grade epithelial dysplasia ($P < 0.0001$). There were no significant differences in the EGFR protein expression of OPMD that underwent malignant transformation and those which did not ($P > 0.05$). The error bars show the standard error of the mean.

encompassed all EGFR GCN abnormalities, including trisomy and low polysomy. While only one case showed evidence of EGFR genomic gain, a further 41% of cases showed FISH positivity using their modified classification. FISH-positive OPMD had significantly higher rates of malignant transformation compared with diploid cases. A recent study of 20 OPMD by Poh and colleagues (28) also supports the application of a lower threshold for classifying EGFR GCN as abnormal: although only one case showed EGFR genomic gain, any increase in EGFR GCN was strongly associated with an increased risk of malignant transformation, irrespective of whether the EGFR GCN increase was low or high; increased EGFR GCN was also associated with a reduced time to malignant transformation (28). Together, these studies suggest that EGFR GCN may have some clinical utility in the risk management of OPMD, but is not sufficiently predictive to be used as a standalone biomarker.

Although the frequency of EGFR mutations documented in OSCC is low (40–42), it is a limitation of the current study that neither EGFR mutation status nor downstream EGFR targets were evaluated. It is possible that EGFR GCN represents a "surrogate" marker for other genetic and molecular abnormalities and simply reflects chromosomal instability; nevertheless, the positive correlation between EGFR GCN and protein overexpression suggests that increased EGFR GCN may be functionally relevant. Data from the group of OSCC that transformed from OPMD provide some evidence to support this hypothesis: the majority of these OSCC either maintained the abnormal EGFR GCN of the index OPMD or progressed to EGFR genomic gain, suggesting that EGFR genetic abnormalities accumulate during oral carcinogenesis. Interestingly, however, two cases of OPMD with abnormal EGFR GCN produced OSCC with a normal EGFR ISH signal. This may reflect clonal evolution of carcinoma from malignant cells with normal EGFR GCN; alternatively, it may simply represent tumor heterogeneity and the consequent limitations of sampling.

A quarter of OSCC in the present study showed EGFR genomic gain. This finding was consistent across both the early-stage and transformed OSCC groups. It is higher than the 9% rate reported in a tissue-microarray study by Rössle and colleagues (24). This earlier study also focused on early-stage (stage I/II) OSCC; however, it was limited by assessment of 0.6 mm diameter tissue cores. It is our experience that the EGFR ISH pattern in OSCC is heterogeneous; tissue microarray sampling may therefore not correlate with measurements taken from whole sections. Notwithstanding these issues, however, the proportion of cases with EGFR genomic gain in the current study is toward the lower end of the range of values reported to date (range, 9% to 56%; refs. 23–26).

Our study did not identify a significant correlation between EGFR genomic gain and clinical outcome in OSCC, which is similar to two recent studies (24, 43). By contrast, Temam and colleagues (20) reported a 9% 5-year survival rate for patients with EGFR genomic gain compared with 71% 5-year survival rate for patients with no genomic gain. Although the study used quantitative real-time PCR, its findings have been corroborated by other studies using FISH (21, 23). This apparent discrepancy may reflect the inclusion of late-stage OSCC in these previous studies;

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our study differed in its focus on the transition from OPMD to OSCC and assessment of early-stage OSCC.

Our data confirm that EGFR protein expression is increased in the majority of OPMD and OSCC (44–46). The ubiquity of EGFR overexpression highlights a likely important role in oral carcinogenesis, but limits its clinical utility as a biomarker for stratifying patient management. In OPMD, EGFR protein expression was less predictive of clinical outcome than grade of epithelial dysplasia. It is possible that increased EGFR protein expression represents a bystander change, reflecting but not driving tumor progression, which may account for the lack of correlation with disease-specific clinical outcomes (29, 30, 47).

There is evidence to suggest that EGFR GCN may help to predict the response of head and neck cancers to EGFR-targeted agents. For example, EGFR genomic gain has been shown to predict which patients have an increased likelihood of response to erlotinib therapy (33). The present study was not designed to investigate response to EGFR-targeted agents or other clinical interventions. None of the patients received EGFR-targeted therapy, and the OPMD group was heterogeneous, including cases managed by surveillance and laser excision (48). Despite these limitations, our data support the view that a subgroup of OPMD and OSCC harbor EGFR GCN abnormalities and have increased EGFR protein expression; however, whether these lesions have a differential response to EGFR-targeted agents or other therapies remains to be tested.

Conclusion

This study highlights the potential clinical utility of EGFR GCN assessment for predicting malignant transformation in OPMD. EGFR GCN abnormalities are more reliably predictive of malignant transformation than the histologic grade of epithelial dysplasia. EGFR genomic gain is present in a quarter of early-stage OSCC, but does not correlate with their clinical outcomes. OSCC derived from OPMD generally either maintained the abnormal EGFR GCN of the index OPMD, or progressed to EGFR genomic gain. This suggests that, in a subset of cases, EGFR has an oncogenic function during oral carcinogenesis. Further studies are required to verify these findings and to determine whether EGFR GCN predicts the response of OPMD and OSCC to EGFR-targeted therapies.

Disclosure of Potential Conflicts of Interest

M. Robinson is a consultant/advisory board member for Leica Biosystems Ltd. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: T. Bates, S. Thavaraj, P. Sloan, M. Robinson
Development of methodology: T. Bates, S. Thavaraj, M. Robinson
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T. Bates, M. Kennedy, A. Diajil, M. Goodson, P. Thomson, P. Sloan, M. Robinson
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Bates, S. Thavaraj, P. Sloan, R. Kist, M. Robinson
Writing, review, and/or revision of the manuscript: T. Bates, M. Kennedy, P. Thomson, S. Thavaraj, P. Sloan, R. Kist, M. Robinson
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T. Bates, E. Doran, H. Farrimond, M. Robinson
Study supervision: P. Sloan, R. Kist, M. Robinson

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