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Genomic analysis to assess disease progression and recurrence in patients with oral squamous cell carcinoma: – a preliminary study

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Abstract

We studied the progression from dysplasia to invasive carcinoma and subsequent second primaries or locoregional recurrences in 11 patients with recurrent squamous cell carcinoma (SCC). Between one and six samples were sequenced/patient. DNA samples were prepared, and libraries multiplexed to between 40 and 80 samples/lane of an Illumina HiSeq 3000 and sequenced with 2 × 100 bp paired end sequencing. Copy number data were generated by CNAnorm (Bioconductor package). Samples of recurrent SCC showed unique patterns of descent when compared with earlier samples from the primary tumour, and three main patterns emerged. In four patients there was convincing evidence that the later lesion was descended directly from cells from the first, and in a further four there were no detectable genomic events between the two lesions. Three patients had some shared events between the early and later lesions, but although there were enough differences to deduce that the two lesions had a shared ancestor, they were not directly descended from each other. We present the patients' characteristics in detail, including the overall survival in each group. There was a distinct genomic pattern after a second episode of SCC in all the groups. A larger study that uses similar methods and a longer duration could provide reliable conclusions with respect to survival. With the use of new techniques, genomic data can be available to clinical teams during the planning of treatment.

Keywords: Oral cancer; Squamous cell carcinoma; Recurrence; Oral dysplasia; DNA sequencing; Genomics

Introduction

Cancer is a genetic disease that develops according to evolutionary principles, and may result from the accumulation of genomic aberrations.[1] Understanding these events will help clinicians to control the progression of disease and guide therapeutic interventions. Squamous cell carcinoma (SCC) of the head and neck is one of the most prevalent cancers in the world and among the main causes of death from cancer.[2] Oral SCC is primarily attributed to the consumption of alcohol and use of tobacco, and local recurrence

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or regional neck node metastases, or both, are important prognostic indicators of survival.[3] The disease also shows considerable heterogeneity.[4,5] Distant metastasis is relatively rare compared with other types of cancer, but the identification of genomic factors that are associated with a high risk of new disease may help clinicians to select patients who will benefit most from specific interventions such as elective neck dissection or adjuvant chemoradiotherapy.

The introduction of next-generation sequencing has enabled research workers to sequence large numbers of genes at a time through fast and relatively inexpensive whole exome and genome sequencing.[6] A considerable amount of data has been generated but as this has often involved tissue from single episodes of disease, it has lacked continuity. Heterogeneity within a tumour and its subclonal structure are poorly understood because studies have used only a single tissue sample/patient, which hinders the analysis of spatial intratumour heterogeneity.[7]

A molecular progression model has previously been described to elucidate the transition from normal mucosa to SCC,[8] but we have looked at the genomic changes in samples of recurrent or second primaries and compared them (in the same patient) with those seen at the initial presentation of dysplasia or oral SCC several years before. We have previously shown extensive clonal variation in spatially-separated samples,[9] and the genomic changes that led from dysplasia to carcinoma in a group of patients with oral SCC, 13 of whom developed the disease again. We have now examined the recent disease in this subgroup and compared the findings. Using low coverage, whole genome sequencing, we examined the genomic copy number for every sample, which allowed us to study the genomic progression from the first presentation to subsequent progession or recurrence. This early work shows what information is available to the clinical team with advancing technology.

Methods

Patients

After a previous study of 200 consecutive patients with oral dysplasia or cancer,[10] we identified 13 for further study. They had initially had an area of dysplasia that progressed to oral SCC or had presented with primary oral SCC (with no history of dysplasia). They had been treated with curative intent before 2010, and had then developed another area of dysplasia or oral SCC several months or years later. All patients gave their informed consent before treatment (ethics REC reference numbers 07/Q1206/30 and 08/H1306/127). Eleven of them produced enough good-quality DNA for sequencing. Their details are shown in Table 1. In total, 28 samples taken after 2010 were sequenced, which is between one and six/patient.

Table 1
Dates of initial and subsequent disease, and details of treatment.

Case No.	Year	Description	Treatment
PG001	2005	LGD	–
PG001	2006	LGD	–
PG001	2008	LGD and HGD	–
PG001	2009	Moderately-differentiated keratinising SCC, T1M0N0	Operation
PG001	2014	LGD and well-differentiated SCC, T2M0N0	Operation/radiotherapy
PG025	2009	HGD and moderately-differentiated, SCC of the lip mucosa	Operation
PG025	2011	Metastatic SCC of the neck	Operation/radiotherapy
PG071	2010	HGD and moderately-differentiated, SCC, T1NM0N0	Operation
PG071	2011	3 HGD	–
PG099	2010	HGD and moderately-differentiated SCC that arose from high-grade surface dysplasia, T1M0N0	Operation, but previous chemoradiotherapy
PG099	2016	Multifocal, early, invasive, poorly-differentiated SCC, T1N0M0	Operation and radiotherapy
PG105	2010	LGD, HGD, and moderately-differentiated SCC, T1 N0 M0	Operation
PG105	2016	Poorly-differentiated SCC, T2N0M0	Operation and radiotherapy
PG109	2010	LGD, HGD, and well-differentiated SCC, T1 N0 M0	Operation only but had previous radiotherapy
PG109	2014	Well-differentiated SCC with verrucous appearance, T1N0M0	Operation
PG113	2010	Poorly-differentiated SCC on tonsil, T2N0M0	Operation
PG113	2014	Moderately-differentiated SCC, T3 N2 Mx	Operation and chemoradiotherapy
PG118	2010	HGD and poorly-differentiated SCC T1N0M0	Operation
PG118	2012	Poorly-differentiated focally keratinising SCC, T4N0M1	Chemotherapy
PG123	2010	LGD, HGD, and poorly-differentiated SCC, T2N2N0M0	Operation
PG123	2011	Metastatic SCC on neck, N4	Operation and chemoradiotherapy
PG156	2011	HGD and poorly-differentiated SCC on tonsil, T4N2CM0)	Excision of tonsil and radiotherapy
PG156	2012	HGD and poorly-differentiated SCC with basaloid features	–
PG196	2008	Well-differentiated SCC, T1N0M0	Operation
PG196	2011	LGD	–
PG196	2015	HGD and moderately-differentiated SCC, T1N0M0	Operation

LGD: low-grade dysplasia; HGD: high-grade dysplasia; SCC: squamous cell carcinoma.

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