SQUAMOUS CELL CARCINOMA OF THE ORAL TONGUE IN YOUNG NON-SMOKERS IS GENOMICALLY SIMILAR TO TUMORS IN OLDER PATIENTS WITH A HISTORY OF SMOKING

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Background: Epidemiological studies have identified increasing incidence of squamous cell carcinoma of the oral tongue (SCCOT) in younger patients. The causes for this increasing incidence are unknown. These tumors often occur in non-smoking women and are not HPV-related. This is in contrast to older SCCOT patients who are usually male smokers. We hypothesize that this epidemiologically distinct disease will also be genomically distinct, especially with respect to alterations caused by tobacco smoke.

Patients and Methods: Tumor DNA from 16 young tongue (<45yo, non-smoker) and 28 old tongue SCC patients was subjected to whole exome sequencing and SNP array analysis. Mutations and copy number alterations were identified and compared. A comparable cohort of patients from the TCGA project was used for validation.

Results: Mutation and copy number alteration frequencies were similar between young and old SCCOT patients. TP53 mutations showed a trend toward increased mutation frequency in the young tongue cohort. These findings were validated in the TCGA cohort. The types of base changes observed in the young tongue cohort were similar to those in the old tongue cohort, and distinctly different from those in HPV+ tumors. Comparisons with lung adenocarcinoma, lung SCC, and bladder cancer cohorts demonstrate that the genomic effects of smoking are tumor site specific. Smoking has only a minor impact on the types of mutations observed in SCCOT.

Conclusions: Tumors from young SCCOT patients appear genomically similar to those of older SCCOT patients, and the cause for the increasing incidence of young SCCOT remains unknown. Tobacco smoke was expected to dramatically impact the types of mutations observed in SCCOT, but did not. Additionally, the spectrum of mutations in SCCOT is distinct from that in lung cancer for both smokers and non-smokers. This suggests that the functional impact of smoking on carcinogenesis in SCCOT may be different from that in lung cancer.
CHROMOSOME INSTABILITY IN TUMOR RESECTION MARGINS OF ORAL CANCERS IS A PREDICTOR OF LOCAL RECURRENCE.

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Purpose. Despite considerable efforts to improve adequate ablation in head and neck cancer, local recurrence rates have not decreased significantly. Possible explanations are remains of malignant cells, known as minimal residual disease, or premalignant cells in the resection margins, which progress but are not detected by the current histo-pathologic examinations(1). Furthermore, histopathology is subjected to inter- and intra-observer variability, especially in premalignant (dysplastic) lesions, which even decreases reliability(2).

Chromosome instability (CIN) detection in histologically tumor-free margins with and without dysplasia might be a suitable method to detect these progressive premalignant cells(3), which can develop into an invasive carcinoma even after 10 years(4,5). Fluorescence in situ hybridization is a fast (results within one day), cheap, and efficient method for the detection of CIN in a single tissue section, and is easily implementable in daily practice.

Material and method. 40 patients with oral squamous cell carcinoma (OSCC) radically treated with surgery alone between 1994 and 2003 were included in the study. All resection margins (n=234) were histopathologically tumor free (> 5mm) and follow-up was documented for at least 5 years. The presence of CIN was indicated by nuclear detection of imbalances and/or polyploidization for chromosomes 1 and 7 in all resection margins. P53 expression was analyzed by immunohistochemistry.

Results. FISH analysis could be performed in 33 out of 40 patients. Of the 33 patients, 17 exhibited CIN in at least one resection margin. Of these patients, 9 developed a recurrence within 5 years. Of the 16 patients without CIN only 2 developed a recurrence. The relation between CIN and recurrence was significant (p=0.013). Kaplan Meyer analysis showed a significantly worse progression-free survival in patients with CIN compared with patients without CIN (p=0.027). Neither p53 overexpression nor histopathology were significant predictors of recurrence.

Conclusion. CIN detection using FISH for chromosomes 1 and 7 in resection margins of OSCCs, even though histologically tumor-free, is a suitable method for daily practice which has the potential to improve the identification of patients at risk for developing a local recurrence and to indicate additional treatment.


S207 SERPINE1 AND ASMA EXPRESSION AT THE INVASIVE FRONT PREDICT ECS AND OUTCOME IN ORAL SQUAMOUS CELL CARCINOMA

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Introduction

Extracapsular spread (ECS) from metastatic cervical lymph nodes is the single most prognostic clinical variable for recurrence and death in oral squamous cell carcinoma (OSCC). The absence of good diagnostic tools for ECS means that definitive diagnosis, essential for stratification of patients for therapy, is only possible after exhaustive, post-surgical histological examination. A promising tumour stromal biomarker, alpha smooth muscle actin (ASMA), has shown greater prognostic value than ECS, but its correlation with ECS is not well-defined.

Methods

102 patients treated with primary surgery for OSCC were included in this study. MRI scans were reassessed to determine diagnostic accuracy. SERPINE1 and HEXIM1, identified from whole genome microarray expression data, were assessed together with ASMA by immunohistochemistry (IHC) on a case-matched tissue microarray. A site-specific approach, with analysis of the tumour centre and the advancing-front separately, was used to determine prognostic capability of these genes using Kaplan-Meier (KM) survival analysis with a log-rank (Mantel-Cox) test. Significance of differences between IHC scores with pathological staging and nodal/ECS status was determined using Chi-square analysis.

Results

MRI (n=88) showed very poor sensitivity for the detection of nodal metastasis (56%) and ECS (7%). IHC (n=102) indicated that both SERPINE1 and ASMA expression at the tumour-advancing front was highly significantly associated with nodal/ECS status (p<0.001). HEXIM1 protein was ubiquitously expressed and non discriminatory. Both separately and in combination, SERPINE1 and ASMA were superior to MRI for the detection of ECS (sensitivity; SERPINE1: 95%; ASMA: 82%; combination: 81%). ECS was associated with expression of either or both proteins in all cases. ASMA+/SERPINE1+ expression in combination was highly significantly associated with adverse outcomes (p<0.001).

Conclusion

Our findings suggest that a combination of ASMA and SERPINE1 IHC offers promise in the search for biomarkers that can be used to stratify therapeutic approaches in OSCC.
A SURPRISING CROSS-SPECIES CONSERVATION IN THE GENOMIC LANDSCAPE OF MOUSE AND HUMAN ORAL CANCER IDENTIFIES A TRANSCRIPTIONAL SIGNATURE PREDICTING METASTATIC DISEASE

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Background: Improved understanding of the molecular basis underlying oral squamous cell carcinoma (OSCC) aggressive growth has significant clinical implications because many afflicted patients undergo unnecessary prophylactic cervical lymphadenectomy for therapy and staging. Despite the critical prognostic value of metastatic regional lymphadenopathy, there is an absence of an evidence-based approach to identify nodal disease in clinically node negative patients at initial presentation. In addition, there is a paucity of data on the underpinnings of metastatic disease.

Methods: Next-generation sequencing (NGS) and expression microarrays were performed on carcinogen-induced murine OSCCs with indolent or metastatic growth to define the molecular basis of aggressive growth. Unsupervised hierarchical clustering and significance analysis of microarrays (SAM) was performed to define a mouse metastasis signature. Taqman qRT-PCR was used to confirm selected gene expression differences. Weighted voting of mouse signature genes was performed on human OSCC from the University of Washington/Fred Hutchinson Cancer Research Center (UW/FHCRC, 97 HPV negative patients) and Kaplan-Meier analysis was used to stratify outcomes. Gene Set Enrichment Analysis (GSEA) was used to compare the mouse signature on human OSCC datasets from UW/FHCRC and from The Cancer Genome Atlas effort (TCGA, 134 patients). Using an iterative GSEA approach on UW/FHCRC, TCGA and a third dataset from MD Anderson (71 patients), a common metastasis signature was defined. Outcomes were then assessed on the MD Anderson dataset by multivariate Cox proportional hazards modeling and Kaplan-Meier analysis. A Taqman qRT-PCR assay was then developed and used to stratify an independent set of 31 OSCC patients with respect to metastatic lymphadenopathy using a support vector machine (SVM) learning algorithm.

Results: NGS revealed conservation of human driver pathway mutations in mouse OSCC including in TP53, MAPK, PI3K, NOTCH, JAK/STAT and FAT1-4. Moreover, comparative analysis between TCGA and mouse samples defined novel putative cancer genes. Expression microarrays delineated a 478-transcript aggressiveness signature including previously undescribed candidate metastasis drivers. Strikingly, we identified this mouse signature in three independent human OSCC datasets comprising 302 patients and defined a 118-transcript common signature predictive of clinical outcomes. From this signature, we created a qRT-PCR based assay that stratified OSCC patients with 93.5% accuracy.

Conclusions: These data demonstrate surprising cross-species genomic conservation that led us to define a genetic assay that can confidently risk stratify patients for neck surgery and prognosis has potential biologic implications for human oral squamous cell cancer.
BACTERIAL RICHNESS IS REDUCED IN ORAL CAVITY BIOFILMS OF CHRONIC USERS OF ALCOHOL AND TOBACCO: POSSIBLE IMPLICATIONS FOR ORAL CANCER DEVELOPMENT

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The chronic use of alcohol and tobacco is one of the major risk factors for the development of oral squamous cell carcinomas (OSCCs). This study delves into the impact that these substances may have on oral microbiome biofilms, which may impact the later development of this malignancy. For that, we used swabs to sample the oral biofilm of 9 individuals who were non-users of tobacco or alcohol and 7 chronic users of both. DNA was extracted and the V1 region of the 16s rRNA gene was PCR amplified sequenced, generating 3.7 million high quality reads. DNA sequences were clustered and OTUs were assigned using the Ribosomal Database Project and Qime. We found no differences in species diversity and evenness between both groups. However, we found a significant decrease in species richness in smokers and drinkers, mainly caused by a reduction in members of the eubacterial phyla Proteobacteria and Fusobacteria. Neisseria abundance was significantly decreased in smokers and drinkers. Samples from smokers and drinkers clustered closer together than to controls and had a significant reduction in inter-group dissimilarity distances, indicating this to be a more homogenous. Chronic users of alcohol and tobacco have a significantly reduced bacterial richness, which could lead to a reduction in inter-group variability, and apparently turns the oral biofilm of smokers and drinkers into a more homogenous microenvironment in terms of bacterial communities. This may include the elimination of beneficial bacteria and/or a higher frequency of acetaldehyde-producing bacteria, an ethanol-derived carcinogen that has been associated with OSCC development.
Metastatic oral squamous cell carcinoma (OSCC) is often associated with recurrent gene abnormalities at specific chromosomal loci. Here, we utilized array comparative genomic hybridization (aCGH) and genome-wide screening to identify genes potentially contributing to progression to metastasis. Metastatic and non-metastatic OSCC were microdissected and DNA hybridization was performed. We identified predominant amplifications of chromosomal regions that encompass the Rab genes 5, 7 and 11 (3p24-p22, 3q21.3 and 8p11-12, respectively) in metastatic OSCC. The expression of these Rab GTPases was confirmed by qRT-PCR and immunohistochemistry in OSCC tissues from a unique cohort of patients followed up to ten years. Rab5, Rab7 and Rab11 presented significant overexpression in OSCC cases of advanced stages (80.8%, 90.4%, and 55.8%, respectively). Moreover, co-overexpression of these Rabs within the same tissue was associated with worst survival (log-rank test, P=0.006). In preclinical OSCC models, we demonstrated that these Rab members regulate cell invasion via activation of focal adhesion turnover, namely accelerated focal adhesion disassembly, which is essential for early cancer cell locomotion and invasion. Together these results provide insights into the role of Rab gene amplifications in OSCC progression and support their potential utility as therapeutic targets.
Introduction

Adverse clinical prognosticators in oral squamous cell carcinoma (OSCC) include cervical lymph node metastasis with extracapsular spread (ECS) and distant metastatic (DM) disease. These late manifestations of the metastatic cascade are intimately related to the interactions between tumour cells and the microenvironment. Primary cell culture derived cell lines may offer advantages over multiple-passaged cell lines in exploring the interactions between tumour cells and stromal cells in metastatic disease, but success in producing such cell lines in terms of the clinical determinants of metastasis, ECS and DM has not been described.

Methods

The explant technique was used with cell-type specific media to isolate 35 fibroblast and 15 keratinocyte single-cell populations from 40 OSCC biopsy tissues. Successful outcomes in primary cell culture defined by successful subculture were correlated with nodal/DM status using Chi-square analysis. Vimentin immunofluorescence and Western Blotting was used to confirm mesenchymal origin of fibroblast populations. Organotypic invasion assays were utilised to determine the invasive phenotype of keratinocyte cell lines.

Results

A significant association was found between nodal status and successful generation of finite keratinocyte cell lines (5/19 N–; 3/4 N+ECS–; 7/11 N+ECS+: p=0.04)). Two out of 13 cell lines grew for >10 passages and both were associated with ECS and DM (p<0.001) and were invasive in organotypic models when co-cultured with corresponding primary fibroblasts. Morphological differences were seen between fibroblasts cultured from ECS- and ECS+ disease with larger fibroblast populations showing increased Vimentin expression associated with ECS+ disease.

Discussion

Success in primary cell culture appears to be related to the aggressiveness of the originating tumour. Both of the continuous cell lines were invasive and phenotypic observations in fibroblasts could suggest a more activated microenvironment associated with ECS. These new head and neck cancer cell lines may offer insights into mechanism of ECS and DM.
NEOADJUVANT BEVACIZUMAB IMPROVES TUMOR SPECIFIC UPTAKE OF CETUXIMAB IN PRECLINICAL ORAL CAVITY SQUAMOUS CELL CARCINOMA STUDIES

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Background: Among epidermal growth factor receptor (EGFR) therapies, monoclonal antibodies such as cetuximab remain first-line. However, the response to antibody therapy is limited, even in patients with confirmed EGFR overexpression and lack of mutation. One possible explanation is that the antibody is unreliably delivered to the tumor. It stands to reason, then, that interventions to alter the tumor microenvironment may improve therapeutic delivery. In this study, we hypothesize that bevacizumab, an antibody to vascular endothelial growth factor (VEGF), can normalize tumor vasculature and improve cetuximab uptake in SCC tumors. To measure tumor delivery we labeled cetuximab with a near-infrared probe, IRDye800CW.

Methods: Athymic nude mice were subcutaneously implanted with OSC-19 (oral cavity SCC) in the left flank. Mice were imaged daily for 14 days after intravenous tail vein injections of the respective imaging agents were administered with two near-infrared imaging systems: LUNA (Novadaq) and the Pearl (LICOR). To measure uptake of cetuximab-IRDye800, we defined Tumor-Specific Uptake (TSU) as tumor fluorescence divided by background fluorescence. For the study, six antibody-probe regimen were administered: 200µg IgG-IRDye800CW (Control Group), 200µg cetuximab-IRDye800CW (Cmab Only Group), 200µg bevacizumab-IRDye800CW (Bmab Only Group), 100µg cetuximab-IRDye800CW + 100µg bevacizumab-IRDye800CW (Simultaneous Group). There were two groups that received pretreatment with bevacizumab, one that received 100µg bevacizumab-IRDye800CW at Day 0 then 100µg cetuximab-IRDye800CW at Day 3 (Delayed Group), and one group that received 100 µg unlabeled bevacizumab at Day -3 then 200µg cetuximab-IRDye800CW at Day 0 (Neoadjuvant Group). Ex-vivo tumor imaging and histology were performed at Day 14. Microvasculature and pericyte density were evaluated by immunohistochemistry to confirm vessel normalization.

Results: Within the single-agent groups, the Cmab Only tumor-specific uptake (TSU) was significantly higher than Bmab Only at Day 13 (8.6 vs 2.8, p<0.001). The Simultaneous group (5.0 vs Cmab Only 8.6, p=0.001), which provided the same amount of dye as the single-agent groups through two distinct mechanisms (anti-EGFR and anti-VEGF), demonstrated no synergistic effect when administered simultaneously. However, by simply postponing the cetuximab component, the Delayed group TSU was significantly higher than the Simultaneous group (7.9 vs 5.0, p=0.008). This difference can be attributed to improved antibody uptake measured as a slower decline in tumor fluorescence (-6.4% vs Simultaneous -11.5% per day, respectively). Finally, our Neoadjuvant group, designed to isolate the changes in microenvironment caused by bevacizumab treatment, demonstrated the highest TSU (11.8, ANOVA post-hoc p<0.004 vs all other groups). This increase was predominantly mediated by reduced efflux of antibody post-administration (-6.8% per day). Background antibody uptake and decline was similar across all groups. Taken together, neoadjuvant bevacizumab demonstrates improved TSU, secondary to enhanced intratumoral uptake of cetuximab.

Conclusions: Anti-EGFR antibody demonstrates superior tumor penetrance compared to bevacizumab targeting the VEGF ligand. Although co-administration of bevacizumab with cetuximab demonstrated no synergistic benefit, pretreatment with bevacizumab improved TSU reflecting the increase in tumor-specific uptake in preclinical oral cavity squamous cell cancer models.