Whole tumor specimens from 24 patients with HNSCC referred for curative surgery were prospectively included. Only patients with tumors > 1.5 cm were included and four patients had both a primary lesion and a lymph node metastasis yielding a total of 28 lesions. The study was approved by the local ethics committee. Figure 1 illustrates the workflow in the histologic processing. All specimens were removed en bloc and sectioned in 2-3 mm consecutive tissue blocks after formalin fixation and embedded in paraffin (Figure 1A-1B). Six tissue blocks from each specimen (two specimens had four and five tumor blocks) were selected randomly and core biopsied in an area with representative tumor tissue assessed from a 4-µm section stained with hematoxylin and eosin (H&E) (Figure 1C-1D). A 3 mm wide core biopsy was taken from each of the 165-selected tumor blocks and used to construct tissue micro array (TMA) blocks (Figure 1E). 4-µm sections were made from the TMA blocks and PD-L1 expression was assessed as percentage of stained tumor cells (TPS) using platform Autostainer Link 48, clone 28-8, pharmDx kit, rabbit monoclonal anti-human, 1 + 200, Dako (Figure 1F-1G). Discordance or concordance in dichotomized PD-L1 positivity was estimated using 1% and 50% as cut off values.

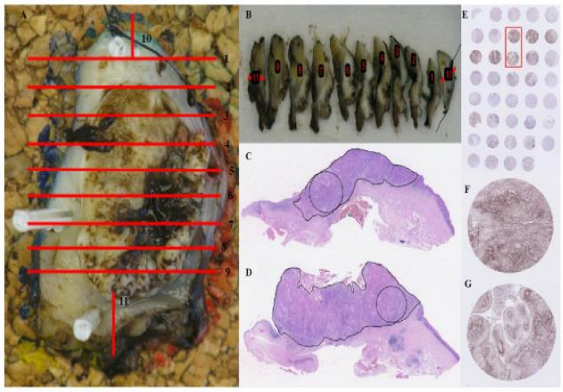

Figure 1. The Figure illustrates the workflow in the study. 1A shows how the lesions were sectioned contiguously in tumor blocks, yielding 11 tumor blocks in this particularly case. Each red line on figure 1A corresponds to the specific tumor block number in figure 2B. Six blocks were selected randomly for further histologic processing. Figure 1C and 1D depicts the 4-µm section stained with hematoxylin and eosin from block 2 and block 8. The black circle indicates from where the 3 mm core biopsy was performed. Figure 1E illustrates a sectioned from a tissue micro array block stained for PD-L1 expression. Figure 1F and 1G are the two cores marked with the red square in 1E and correspond the two cores in shown in 1C and 1D.

Results

Figure 2A depicts the full PD-L1 score from each core biopsy. Using a TPS cut-off value of 1%, 43% of the specimens were concordant in the six biopsies from each lesion. With a 50% cut off the concordance was higher (68%), but most specimens were scored as PD-L1 negative. The scatter plot illustrates that it would be difficult to define a meaningful cut off value for positivity which circumvents the problem of heterogeneity in the tumors. We define the ground truth as the tumor being positive if any of the cores from the tumor specimen is positive. With this definition, the positive predictive value of a single core is identical 100% whereas the negative predictive value of a single negative biopsy (NPV) is 48.6% (CI 41.8–55.4) with 1% cut and NPV = 80.0% (CI 74.7–84.5) with 50% cut off (Figure 2B). There was no correlation between the variance in PD-L1 expression and tumor volume assessed on MRI (spearman rank p=0.61) and heterogeneity in PD-L1 expression was observed both in small and in large tumors. However, on a core biopsy level there was a significant correlation between PD-L1 positivity and percentage of tumor in the core biopsy (p=0.042).

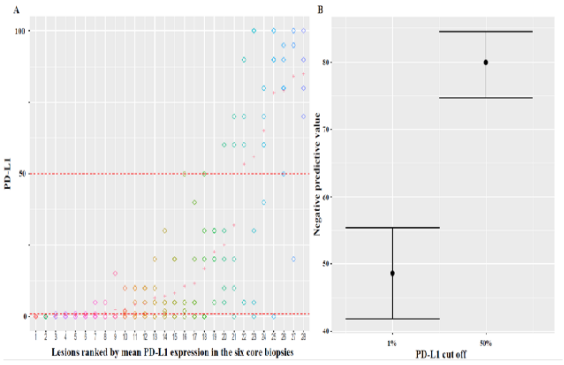

Figure 2. Panel A is a scatter plot of the PD-L1 score in each biopsy. The Y-axis depicts the PD-L1 score from 0-100%. The X-axis depicts the 28 lesions ranked by mean PD-L1 score marked with a red cross. The diamonds depict the actual score from each of the six biopsies. The thickness of the diamond indicates that more than one biopsy had the same score. As an example, in lesion 1 and 2 all biopsies scored 0%. The red dashed lines marks 1% cut off and 50% cut off values. Panel B illustrates the negative predictive value of a single negative core biopsy with confidence interval using a 1% cut off and 50% cut off for positivity. The ground truth is assumed to be positive if any of the tumor biopsies are positive.

Conclusion

Intratumor heterogeneity may contribute to the ambiguous results on PD-L1 status seen in the Checkmate and Keynote studies of HNSCC patients and challenge the use of PD-L1 expression from single tumor biopsies as a predictor for immunotherapy response in HNSCC patients.

PO-107 CAFs secrete factors that sustain cancer stem properties in head and neck squamous carcinoma cells

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Purpose or Objective

Cancer-associated fibroblasts (CAFs) constitute a major component of the tumor microenvironment. Cancer stem cells (CSC) play critical roles in tumor initiation, progression, recurrence, and treatment resistance. There are no reports on the mechanisms by which CAFs regulate CSC biology in the context of head and neck squamous cell carcinomas (HNSCC), which is the central goal of this study.

Material and Methods

A series of functional studies (anchorage-independent growth, tumorsphere formation) were performed in HNSCC cell lines and combined to expression analyses of CSC gene signatures by real-time RT-PCR to assess the effects of conditioned media from CAFs and normal

fibroblasts (NFs) on the CSC phenotype. Further characterization of CAFs and NFs secretomes by mass spectrometry was followed by pharmacologic inhibition of the identified targets.

Results

We demonstrate that in the absence of serum or any other supplements, factors secreted by CAFs but not NFs, robustly enhanced stem cell properties of HNSCC-derived cell lines, including anchorage-independent growth, tumorsphere formation, and CSC marker expression. Modulators of EGF, IGF and PDGF receptor activity were identified between the paracrine cytokines and factors differentially secreted between CAFs and NFs, in a mass spectrometry analysis. Pharmacologic inhibition of EGFR, IGFR and PDGFR significantly reduced CAF-induced tumorsphere formation and anchorage-independent growth suggesting a role of these receptor tyrosine kinases in sustaining the CSC phenotype.

Conclusion

These findings provide novel insights into tumor stroma-CSC communication, and highlight therapeutic targets to effectively block the CAF-enhanced CSC niche signaling circuit to ultimately overcome CSC-mediated disease progression and resistance to therapy.

PO-108 Clinical relevance of Cortactin and Focal Adhesion Kinase as predictors of laryngeal cancer risk

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Purpose or Objective

Amplification of the chromosomal regions 8q23-24 and 11q13 are two of the most recurrent genetic alterations in head and neck squamous cell carcinoma (HNSCC), which have been associated with recurrent and metastatic disease and poor disease outcome. The genes *FAK*/*PTK2* and *CTTN* (formerly *EMS1*) that encode the Focal Adhesion Kinase (FAK) and the actin-binding protein Cortactin (CTTN) have emerged as major candidate genes to respectively drive 8q24- and 11q13-associated aggressive behavior. This study investigates the role of CTTN and FAK in early stages of HNSCC tumorigenesis and their contribution to tumor initiation and acquisition of an invasive phenotype.

Material and Methods

Using a multicenter study CTTN and FAK expression was evaluated by immunohistochemistry in a cohort of 109 patients with laryngeal precancerous lesions, and correlated with clinicopathologic parameters and laryngeal cancer risk. Functional analysis in HNSCC-derived cell lines further contributed to delineate the pathobiological role of CTTN and FAK using both siRNAs and pharmacologic inhibitors.

Results

CTTN and FAK expression was detected in 49 (41%) and 35 (32%) laryngeal dysplasias, respectively. Univariate Cox analysis showed that CTTN and FAK expression robustly and significantly predicted both recurrence risk and laryngeal cancer risk. Patients carrying strong CTTN- or FAK-expressing lesions experienced the highest laryngeal cancer incidence (log-rank $p<0.001$). In marked contrast, histological grading using the new WHO classification did not show a significant role in assessing laryngeal cancer risk in this cohort (Fisher's exact test $p= 1.000$). In multivariate stepwise analysis, FAK expression (HR= 13.91, 95% CI 4.82-40.15; $p<0.001$) and alcohol consumption (HR= 2.22, 95% CI 1.17-4.20; $p= 0.014$) were significant independent predictors of laryngeal cancer development. Targeting FAK by either RNAi or pharmacological inhibitors effectively blocked cell growth, colony formation and invasion into 3D collagen matrices.

Conclusion

These findings demonstrate that CTTN and FAK are robust predictors of laryngeal cancer risk beyond histological grading (current gold standard), thus encouraging their clinical application as complementary markers for risk-stratification. Furthermore, our findings unveil that pharmacological targeting of FAK may constitute a promising therapeutic strategy for HNSCC prevention and treatment.

PO-109 Influence of HPV in the recruitment of macrophages in HNSCC

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Purpose or Objective

Here, we investigated the recruitment of macrophages in HNSCC, studying the influence of HPV on it. Our objective was to expand our clinical cases number in order to validate our previous results and also to distinguish M1 (pro-inflammatory macrophages) and M2 (tumor-associated, "TAMs") macrophages among the macrophages recruited on the tumor site.

Material and Methods

We added 114 new FFPE HNSCC tumors to the 110 we used previously. For these 114 new cases, we determined if there were infected with HPV by performing RTqPCR. The transcriptional activity of the virus in the infected tumors was then assessed by p16 immunostaining. Afterward, the recruitment of macrophages in HNSCC was evaluated by CD68 immunohistochemistry and the distinction between M1 and M2 macrophages was made by double immunofluorescence CD68/CD163. Finally, the results were grouped with those obtained on the first 110 cases and statistically re-evaluated.

Results

A high infiltration of CD68 macrophages inside HNSCC tumors is associated with shorter overall survival (OS, log-rank test, p=0.002) and recurrence-free survival (RFS, p=0.001) of patients. Indeed, the infiltration of CD68 macrophages inside tumors as well as tumor stage, are strong and independent prognostic factors for HNSCC patients (multivariate analyses, OS: p=0.007 and p=0.006 respectively). The recruitment of CD68 macrophages is associated with HPV infection (Chi-square test, p=0.041) and is higher in p16+ HNSCC, both inside the tumors (Mann-Whitney test, p=0.006) and in the stroma around the tumors (p=0.025). It is clear that the recruitment of CD68 macrophages is increased in HPV+p16+ HNSCC tumors (p=0.001) and stroma (p=0.05). Finally, the distinction between M1 (CD68+ CD163-) and M2 (CD68+ CD163+) macrophages in HNSCC demonstrated that 80.2%