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%