Matrisomal Gene Panel for Head and Neck Cancer

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Squamous cell carcinoma of the head and neck (SCCHN) is common worldwide and related to several risk factors including smoking, alcohol consumption, poor dentition and human papillomavirus (HPV) infection. Different etiological factors may influence the tumor microenvironment and play a role in dictating response to therapeutics. Here, we sought to investigate whether an early-stage SCCHN-specific prognostic matrisome-derived gene signature could be identified for HPV-negative SCCHN patients (n = 168), by applying a bioinformatics pipeline to the publicly available SCCHN-TCGA dataset. We identified six matrisome-derived genes with high association with prognostic outcomes in SCCHN. A six-gene risk score, the SCCHN TMI (SCCHN-tumor matrisome index: composed of MASP1, EGFL6, SFRP5, SPP1, MMP8 and P4HA1) was constructed and used to stratify patients into risk groups. Using machine learning-based deconvolution methods, we found that the risk groups were characterized by a differing abundance of infiltrating immune cells.

Keywords: extracellular matrix ; head and neck cancer ; bioinformatics ; TCGA ; HPV ; prognostic biomarker

1. Introduction

SCCHN comprises a heterogeneous group of tumors arising from squamous cells lining different anatomic sites within the upper aerodigestive tract such as the nasal cavity, paranasal sinuses, lips, oral cavity, oropharynx, hypopharynx or larynx [1][2]. The global incidence rates have increased by 36.5% between 2005 and 2015 [3]. SCCHN predominantly affects people above 50 years old, with incidence rates higher among men than women [4]. Tobacco and alcohol exposure constitutes the major risk factors for the development of such cancers [5][6]. Human papillomavirus (HPV) is an important etiologic factor of SCCHN [7]. Aside from etiology, both tumor staging and pathological features have prognostic value [8]. The presence of metastases and aggressive pathological features such as extranodal extension (ENE), perineural invasion (PNI) or lymphovascular invasion (LVI) are prognostic factors for reduced survival [9][10][11]. Subsite specific etiological factors and associated tumor and microenvironment differences may influence the clinical outcomes of existing modalities of treatment [12][13]. Recent advancements in high throughput molecular profiling have also added new prognostic markers [14][15] that relate to the biology of the specific anatomical subsite of interest, as a consequence of their specific underlying molecular pathways [16]. This could account for the inherent heterogeneity of this malignancy and aid prognostication and possibly treatment selection and intervention.

Epithelial malignancies have been extensively investigated at the genomic and epigenomic levels focusing primarily on tumor cells. Recent evidence increasingly highlights the importance of the tumor microenvironment (TME) in cancer growth, progression and metastasis [17][18]. Therefore, a deeper understanding of the role of the cancer-associated extracellular matrix (ECM) components might help to identify new diagnostic and prognostic tools. In 2015, Naba, A. et al. [19] identified a list of 1068 human ECM genes encoding ECM and ECM-associated proteins and presented omics data indicating their roles in development, homeostasis and disease.

Bioinformatic approaches are powerful tools that enable whole-genome investigation of the abnormalities exhibited by cancer tissues from large groups of patients. Hence, in this study, using a series of recently developed web-based tools and open-source software, a bioinformatic-based study on a transcriptomic dataset publicly available in the “The Cancer Genome Atlas (TCGA)” [20] database was conducted. First, based on recent studies [21][22], we hypothesized that dysregulated matrisomal components could have a common association with patient survival, irrespective of the subsite of origin of the SCCHN. Specifically, transcripts that were associated with survival in HPV-negative and non-metastatic node-negative tumors, were examined to minimize confounding by treatment, stage, and etiology. Next, a novel prognostic signature is defined, the SCCHN-tumor matrisome index (SCCHN TMI), assessed its prognostic ability across independent datasets and its association with immune cell infiltration.

2. Current Insights

The discovery of reliable prognostic biomarkers capable of identifying patients with a higher risk of unfavorable survival outcomes is needed in order to better define patients who might require further adjuvant treatment after surgical resection. The SCCHN TMI gene panel, which was constructed by focusing only on ECM molecules, holds potential clinical as well as biological significance. In an HPV negative subset, where the overall prognosis is poor, the SCCHN TMI was able to predict overall survival (OS), disease-specific survival (DSS), and disease-free interval (DFI). A high SCCHN TMI score was an unfavorable prognostic factor for all the analyzed endpoints. Gene ontology (GO), KEGG pathway enrichment and signaling network analyses revealed that the six SCCHN TMI genes are mostly associated with signaling networks involved in cancer-related transcriptional dysregulation and two important pathways: IL-17 and TNF. The former has already been shown to negatively correlate with the overall survival of head and neck cancer patients [23]. TNF-α, which is found in the TME, is secreted by macrophages, lymphocytes and natural killer (NK) cells and mediates the production of proinflammatory factors that elicit tumor growth and recently emerged as a promising cancer therapy target [24][25].

Patients in the high-risk group exhibited a proinflammatory phenotype enriched with macrophages (M0 and M2 phenotypes). Tumor-associated macrophages (TAMs) are a key component of the SCCHN tumor microenvironment as they have specific roles in regulating the immune response to cancer (refer to Evrard et al. [26] for a detailed review). Moreover, TAMs have been shown to affect cell proliferation, vascularization, stromal formation and dissolution [27]. The results are coherent with the previous literature that highlighted the proinflammatory and tumor-promoting role of TAMs in SCCHN [28][29][30][31]. Two recent meta-analyses [28][29] found that increased densities of TAMs in the TME, particularly M2-like, correlate with poor clinicopathologic markers in SCCHN. A recent study by Tekin et al. [32] showed that M0 macrophages harbor anti-tumorigenic activities, which seem to be mediated by TNF-α which is associated with M0 macrophage-induced cell death in pancreatic cancer. Furthermore, an increasing abundance of infiltrated M0 macrophages was associated with poorer outcomes in breast cancer [33].

Patients in the SCCHN TMI low-risk group exhibited an increased abundance of CD8+ and follicular helper T cells as well as activated dendritic cells. These results are consistent with prior reports which showed significantly better survival outcomes [34][35][36] in SCCHN patients with a higher abundance of infiltrative lymphocytes. Moreover, a recent work by Cillo et al. [37] found that T follicular helper cells are associated with longer progression-free survival in SCCHN patients and that the activation of dendritic cells could improve antitumor T cell responses. Hence, the SCCHN TMI might have important implications for prognosis and further adjuvant treatment decisions, as low-risk scores are associated with high levels of infiltration of antitumor T cells and low levels of infiltration of tumor-promoting TAMs. These provide potential points of therapeutic intervention that need to be validated prospectively in clinical trials of specific inhibitors.

The single-cell RNA-seq analysis of the SCCHN TMI genes revealed that four of these genes (MASP1, EGFL6, SPP1, and P4HA1) are expressed in subpopulations of fibroblasts, macrophages, T cells and in tumor cells. It is noteworthy that two genes, SPP1 and P4H41, exhibited the highest association with immune cells. Specifically, they are highly expressed in both T cells and macrophages, hence they could play an important role in linking the SCCHN risk score with the different patterns of infiltrative immune cells in the SCCHN's TME. Two genes (SFRP5 and MMP8) were lowly expressed in this dataset. In the study by Puram et al. [38], the authors analyzed 5902 single cells from 18 patients with tumors of the oral cavity, which is one of the subsites of SCCHN. Therefore, the low expression of SFRP5 and MMP8 in this dataset might be, to some extent, attributed to the small cohort of patients included in the analysis and the presence of only one SCCHN subsite.

Using machine learning, high computational classification accuracy between the risk groups was achieved in the data collected using different platforms (RNA-seq and microarrays), despite the small sample size of the validation dataset. This has important clinical implications as it demonstrates the robustness of the SCCHN TMI in stratifying HPV-negative, node-negative patients. Finally, statistically different expression levels were found for each SCCHN TMI gene between the two risk groups. As the SCCHN TMI comprises a small number of genes, their expression levels could be quantified using RT-PCR directly on postoperative specimens to conduct prospective validation studies.

3. Conclusions

In conclusion, the identified SCCHN TMI gene signature represents a genomic tool that could potentially enable a better understanding of the molecular mechanisms associated with the interaction between the tumor and its microenvironment. Lastly, the SCCHN TMI could enhance patient stratification progression and selection and aid personalized intervention.

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