Biomedicine & Pharmacotherapy 161 (2023) 114502

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Dissecting the functions of cancer-associated fibroblasts to therapeutically target head and neck cancer microenvironment

Llara Prieto-Fernández [a,b,c], Irene Montoro-Jiménez [a,b,c], Beatriz de Luxan-Delgado [a,b], María Otero-Rosales [a,b], Juan P. Rodrigo [a,b,c], Fernando Calvo [d], Juana M. García-Pedrero [a,b,c,*], Saúl Álvarez-Teijeiro [a,b,c,*]

[a] Department of Otolaryngology, Hospital Universitario Central de Asturias and Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), University of Oviedo, Oviedo, Spain
[b] Instituto Universitario de Oncología del Principado de Asturias (IUOPA), University of Oviedo, Oviedo, Spain
[c] CIBERONC, Instituto de Salud Carlos III, Madrid, Spain
[d] Instituto de Biomedicina y Biotecnología de Cantabria (Consejo Superior de Investigaciones Científicas, Universidad de Cantabria), Santander, Spain

ARTICLE INFO

Keywords:
Head and neck cancer
Tumor microenvironment
Cancer hallmarks
Fibroblast
CAFs
CAFs-based therapies

ABSTRACT

Head and neck cancers (HNC) are a diverse group of aggressive malignancies with high morbidity and mortality, leading to almost half-million deaths annually worldwide. A better understanding of the molecular processes governing tumor formation and progression is crucial to improve current diagnostic and prognostic tools as well as to develop more personalized treatment strategies. Tumors are highly complex and heterogeneous structures in which growth and dissemination is not only governed by the cancer cells intrinsic mechanisms, but also by the surrounding tumor microenvironment (TME). Cancer-associated fibroblasts (CAFs) emerge as predominant TME components and key players in the generation of permissive conditions that ultimately impact in tumor progression and metastatic dissemination. Although CAFs were initially considered a consequence of tumor development, it is now well established that they actively contribute to numerous cancer hallmarks i.e., tumor cell growth, migration and invasion, cancer cell stemness, angiogenesis, metabolic reprograming, inflammation, and immune system modulation. In this scenario, therapeutic strategies targeting CAF functions could potentially have a major impact in cancer therapeutics, providing avenues for new treatment options or for improving efficacy in established approaches. This review is focused on thoroughly dissecting existing evidences supporting the contribution of CAFs in HNC biology with an emphasis on current knowledge of the key molecules and pathways involved in CAF-tumor crosstalk, and their potential as novel biomarkers and/or therapeutic targets to effectively interfere the tumor-stroma crosstalk for HNC patients benefit. involved in CAF-tumor crosstalk, and

Abbreviations: HNC, Head and Neck Cancer; TME, Tumor Microenvironment; CAFs, Cancer Associated Fibroblasts; HNSCC, Head and Neck Squamous Cell Carcinoma; ECM, Extracellular Matrix; NFs, Normal Fibroblasts; IL-1β, Interleukin 1β; IRAK, Interleukin-1 receptor associated kinase; NF-kβ, Nuclear factor kappa-light-chain-enhancer of activated B cells; IL-6, Interleukin 6; COX-2, Cyclooxygenase 2; mPGES-1, microsomal PGE-synthase-1; PGE2, prostaglandin E2; PGI2, prostaglandin I2; HGF, Hepatic Growth Factor; MAPK, mitogen-activated protein kinases; STAT3, Signal Transducer and Activator of Transcription 3; BDNF, Brain-derived neurotrophic factor; TrkB, tropomyosin-like kinase B receptor; STC2, Stanniocalcin 2; SERPINE1, Serpin peptidase inhibitor type 1; FNDC1, Fibronectin type III domain-containing 1; CDKN1A, cyclin-dependent kinase inhibitor 1 A; KGF, Keratinocyte growth factor; SDF-1, Stromal Cell-derived Factor-1; TNF- α, Tumor necrosis factor α; RASSF2, Ras association domain family member 2; PARP-4, Poly (ADP-ribose) polymerase 4; OSCC, Oral squamous cell carcinoma; MMPs, Matrix-metalloproteinases; TGF-1β, Tumor growth factor 1β; OPN, Osteopontin; HAS2, Hyaluronan synthase 2; MT1-MMP, membrane type 1-matrix metalloproteinase; SCC, Squamous cell carcinoma; SFK, Scr family kinases; CSCs, Cancer stem cells; CBPE, Carboxypeptidase E; PDGFD, Platelet-derived growth factor; FBLN3, EGF-containing fibulin-like extracellular matrix protein-1; IGFBP5, Insulin-like growth factor binding protein-5; IGFBP7, Insulin-like growth factor binding protein-7; EMT, Endothelial to mesenchymal transition; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; Oxphos, Oxidative phosphorylation; ATP, Adenine-triphosphate; Cav-1, Caveolin-1; MCT-4, Monocarboxylate transporter 4; TMVs, Tumoral microvesicles; ITGB2, Integrin β2; HIF, Hypoxia-inducible factor; MTDH, Metadherin; TAMs, Tumor-associated macrophages; Tregs, Regulatory T cells; POSTN, Periostin; FGF, Fibroblast growth factor; BMP-4, Bone morphogenetic protein 4; HUVECs, human umbilical vein endothelial cells; sEV, small extracellular vesicles; PLCg2, phospholipase C gamma 2; α-SMA, α -Smooth-muscle actin; FAP, Fibroblast Activation Protein.

* Correspondence to: Instituto de Investigación Sanitaria del principado de Asturias (ISPA), Oviedo, Spain.
E-mail addresses: juanagp.finba@gmail.com (J.M. García-Pedrero), saul.teijeiro@gmail.com (S. Álvarez-Teijeiro).

https://doi.org/10.1016/j.biopha.2023.114502
Received 30 December 2022; Received in revised form 22 February 2023; Accepted 7 March 2023
Available online 14 March 2023

their potential as novel biomarkers and/or therapeutic targets to effec- tively interfere the tumor-stroma crosstalk for HNC patients benefit.

1. Introduction

Head and neck cancers (HNC) are a heterogeneous group of diseases arising from the mucosal surfaces of the oral and nasal cavity, oropharynx, hypopharynx, and larynx, showing also marked differences in progression, treatment and prognosis. Overall, HNC harbor multiple and very diverse genetic and molecular alterations caused by carcinogenic exposure. Around 95% of HNC are squamous cell carcinomas (HNSCC), which represent the sixth leading cancer by incidence worldwide, with approximately 887,000 new cases diagnosed each year and over 450,000 estimated deaths [1–3]. Currently, the standard treatment strategies for HNC patients include surgery, chemotherapy, based on cisplatin, and/or radiotherapy. There are only few molecular therapeutic strategies approved by the FDA: anti-EGFR antibody cetuximab [4] and nivolumab and pembrolizumab (anti-PD-1/PD-L1 antibodies) for the treatment of patients with recurrent or metastatic disease [5]. However, despite advancements in local control and overall quality-of-life achieved with the use of different combination treatment strategies, the survival rates for HNC patients have only modestly improved over the last decades.

Cancer was originally considered as a disease caused by transformed cells that acquire uncontrolled proliferation, limitless replication, and invasive potential. Nevertheless, it has now become clear that tumor growth is not only supported by the cancer cells themselves but also the surrounding microenvironment. Therefore, in-depth understanding of the tumor progression process should involve a deeper characterization and knowledge of the functional roles of both tumor and stromal cells. The tumor stroma is composed of a variety of cell types including cancer-associated fibroblasts (CAFs), endothelial cells, mesenchymal stem cells, innate and adaptive immune cells and also the extracellular matrix (ECM), collectively constituting the so-called tumor microenvironment (TME) [6,7]. Normal tissue homeostasis relies on a well-established and controlled communication among all cell types; however, in cancer, the crosstalk with the environment goes awry with important consequences. This crosstalk is bidirectional, since one cellular component influences the other and vice versa, in a mutual molecular and metabolic reprogramming, affecting tumor cell growth, stemness, epithelial to mesenchymal transition (EMT), migration and invasion, immune system modulation, inflammation and angiogenesis, ultimately having a major impact on patient prognosis and disease outcome [8–10]. On this basis, TME-based therapies may offer new opportunities for cancer treatment.

CAFs are the majority TME component, found in aberrantly high numbers in HNC, which mainly differ from the normal fibroblasts (NFs) by their permanent activated phenotype [11,12]. NFs are mostly active during embryonic development, whereas they usually remain quiescent in adult normal tissues and only activate in response to inflammation, fibrosis or during wound healing [13]. By contrast, CAFs exhibit a characteristic activated phenotype, leading to the assumption of tumors as “wounds that do not heal” [14,15]. Diverse markers have been reported and used to characterize the presence and activation status of CAFs, revealing their heterogeneity and functional diversity with a wide range of tumor-promoting or suppressing functions [16]. Moreover, the presence of CAFs in the TME has been associated with a poor prognosis in various types of carcinomas, including HNC [17–21]. According to the clinical and biological relevant role of CAFs, commonly shared by HNC and other solid cancers with CAF-rich environments, the development of CAF-targeted therapies poses a promising strategy to improve patient treatment and outcome. To date, there is still rather limited information on the specific roles of CAFs in solid tumors, making difficult the development and safe implementation of CAF-based cancer treatment strategies [22].

In this review, we intend to thoroughly examine the existing functional and mechanistic evidences supporting the contribution of CAFs to major cancer hallmarks and HNC progression. A special emphasis is devoted on current knowledge of the key molecules and pathways involved in CAF-tumor crosstalk, and their potential as novel CAF-based biomarkers and/or therapeutic strategies to improve patient treatment and care.

2. CAF involvement in HNC proliferation

Abnormal and unrestraint proliferation is the most fundamental feature of cancer cells. Normal tissues exert a tightly controlled production and release of molecules that trigger signals to promote cell proliferation or cell death, thus ensuring the cell number required for the maintenance of normal tissue homeostasis. By contrast, cancer cells ignore these control mechanisms and acquire the ability to sustain chronic proliferation, cell death resistance and unlimited replicative potential. In addition, cancer cells also produce multiple factors that regulate the surrounding microenvironment, altering its behavior to support cancer cell proliferation. Specifically, CAFs contribute to cancer cell proliferation, mainly through the secretion of ECM proteins, growth factors, and inflammatory ligands (cytokines and chemokines) that mediate interactions between fibroblasts, ECM, and cancer cells, thereby playing a key role in HNC development and progression [23]. In this regard, CAFs can promote tumor growth by different ways (Fig. 1), for instance: i) secreting pro-tumorigenic factors; ii) releasing microRNAs into exosomes and ii) modifying the ECM components and density.

During the last decades, several CAF-secreted molecules have been identified, having a great impact on HNC growth and progression. Among them, increased IL-6 levels have been associated with a higher incidence of second primary tumors, and suggested as a possible predictive biomarker for recurrence and survival in HNC patients [24]. The Interleukin IL-1β produced by cancer cells is assimilated by CAFs, thereby triggering Interleukin-1 receptor-associated kinase-1 (IRAK) phosphorylation and, subsequently, nuclear translocation of NFκBα and induction of the IL1β-regulated genes IL-6 and COX-2 [24]. In turn, IL-6 and COX-2 may directly influence cell proliferation and metastasis by decreasing E-cadherin expression in tumor cells [24]. Another study in HNC has reported that conditioned media from fibroblasts co-cultured with HNC cells enhanced cell proliferation by increasing the fraction of cells in S and G2/M phase. Proteomic analysis revealed that tumor cells release IL-1, which in turn induces the expression of COX-2 and microsomal PGE-synthase-1 (mPGES-1) in fibroblasts leading to the release of prostaglandin E2 (PGE2), a recognized inducer of cell proliferation [25,26].

In addition, CAFs may promote cell proliferation through the secretion of hepatic growth factor (HGF) which has the ability to bind to the tyrosine kinase c-MET and activate signaling pathways like MAPK, RAS, PI3K and STAT3, thereby favoring cell growth and survival [27–29]. Brain-derived neurotrophic factor (BDNF) secreted by CAFs specifically interacts with tropomyosin-like kinase B receptor (TrkB), and drives epithelial-mesenchymal transition (EMT) in tumor cells. Notably, inhibition of CAF-derived BDNF decreased tumor growth in vivo in models of HNC and other carcinoma types such as pancreas, lung, colon and prostate or neuroblastoma and multiple myeloma [30]. Currently, other CAF-secreted molecules identified for their role in cell proliferation and HNC progression are: Stanniocalcin 2 (STC2), Serpin peptidase inhibitor type 1 (SERPINE1), Integrin α6 (that interacts with CDKN1A altering cell cycle progression) [31], Activin A [32], Keratinocyte growth factor (KGF) [33], Stromal-derived factor 1 (SDF-1) [24, 34], and Tumor necrosis factor alpha (TNF-α) [24].

Several microRNAs have also been described as important players in the tumor-stroma communication in different cancers. Thus, miRNAs can be secreted into exosomes and act as paracrine signaling factors to control several proliferation pathways. In the HNC context, miR-7 has been found upregulated in CAFs compared to NFs. Exogenous expression of miR-7 induced the conversion of fibroblasts into CAFs, whereas miR-7 inhibition caused the opposite effect. Mechanistically, miR-7 overexpression in fibroblasts down-regulated Ras association domain family member 2 (RASSF2) and decreased the secretion of PAR-4 (a protein that acts as a tumor suppressor and apoptosis inducer) from CAFs, which was accompanied by increased growth and migration of cancer cells in co-culture experiments [35]. It has also been reported that miR-34a-5p binds to AXL and suppresses the proliferation of OSCC cells. miR-34a-5p levels are found downregulated in CAFs-derived exosomes compared to NFs, thus contributing to OSCC growth and progression [36]. Alike miRNAs, long non-coding RNAs (lncRNA) can also be secreted into exosomes, among which LOC400221 released from fibroblasts, has been reported to induce fibroblast reprogramming to CAF via IL-33 to support OSCC growth [37]. Our group reported that miR-196a/b dysregulation causes pleiotropic effects in HNC cell lines and CAFs leading to the inhibition or activation of cell proliferation and migration depending on the cellular context. Besides, miR-196a/b have been found to downregulate specific gene families such as the HOX family involved in the regulation of several proliferation-related signaling pathways [38]. On the other hand, CAFs also regulate cancer cell proliferation through the secretion of different ECM components, such as collagen, among others. A high expression of COL11A1 was found to enhance the proliferation, migration and invasion of HNC cells and CAFs [39,40]. Besides, COL11A1 has been associated with the progression of different carcinomas [41]. High expression of COL3A1 and COL8A1 by CAFs has been associated with proliferative phenotypes and poor prognosis in HNC and OSCC, respectively [42,43].

Overall, the TME and specifically CAFs have a profound impact on HNC growth by providing additional sources of proliferative signals in the form of soluble factors, ECM components and metabolites. Currently, chemotherapy and radiotherapy remain the major treatment options for HNC patients, which are based on targeting high-proliferating cancer cells. However, these therapies also have important side-effects in normal tissues with high proliferative rates. According to the herein presented evidences, CAFs sustain tumor cell proliferation by multiple different mechanisms, as such CAF-based therapeutic strategies could potentially be useful to control tumor outgrowth and to improve the effectiveness and tolerability of current treatments while reducing their side-effects. Based on the existing literature, strategies aimed at targeting some CAF-secreted factors, such as IL-6 or IL-1 could be effective to block tumor growth supportive signals driven by CAFs. Conventional chemotherapy and radiotherapy are based on targeting hyper-proliferative features of cancer cells, hence their combination with CAF-based therapies could enhance tumor sensitivity and treatment efficacy using lower and better tolerable doses with minimal side-effects.

3. CAFs contribution to HNC migration and invasion

One of the crucial steps in tumor progression is the ability of cancer cells to invade, firstly the surrounding tissues, and thereafter to disseminate to distant organs. In this regard, HNC is primarily a loco-regional disease commonly characterized by frequent cervical lymph node metastases (considered the worst prognostic factor), detected in over 50% of patients at diagnosis. Distant metastases remain relatively low compared to other cancers (around 20%); however, they profoundly aggravate patient prognosis [44].

Carcinogenesis is a complex process that evolves through several changes not only restricted to epithelial cells, but also affecting the surrounding stroma. This phenomenon, so-called stromagenesis, appears to occur coupled to the tumorigenesis process generating subsets of pro-tumorigenic fibroblasts [45]. In this sense, cancer cells modulate stroma behavior to create a permissive microenvironment that enables tumor progression and invasion [46]. It is becoming increasingly clear that TME plays a very important role in the regulation of cancer cell migration and invasion, participating at various different levels either in the promotion or inhibition of tumor progression and metastatic

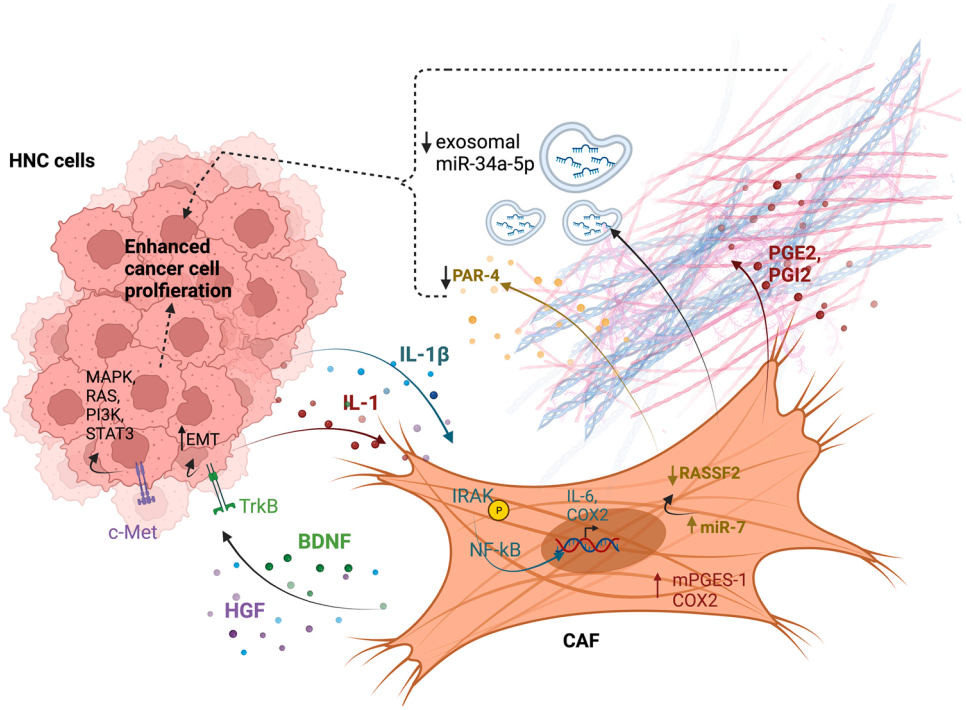

Fig. 1. Representative scheme of the main proliferation signaling pathways induced by CAFs in HNC. Molecules interconnected in the same pathways are depicted using same colors (black excluded). Further details of the crosstalk between CAFs and HNC cells are summarized in Table 1.

spreading (Fig. 2).

3.1. Paracrine modulation

CAFs may regulate tumor invasion through the secretion of a large variety of molecules such as chemokines, cytokines, growth factors, ECM components and matrix-metalloproteinases (MMPs) to modulate the invasive potential of tumor cells. Nowadays, several molecules involved in CAF-HNC crosstalk have been identified. In co-culture experiments, CCL7 secretion by CAFs has been reported to stimulate the migration and invasion of OSCC cells, which was neutralized by using a CCL7 antibody [47]. In addition, CCL11 secretion by CAFs also promote invasive and migration abilities of HNC cells as well as EMT induction [48]. The crosstalk between CAFs and OSCC cells also led to elevated CXCL1 in the TME, enhancing the migration and invasion potential of cancer cells [49]. Besides, IL-1β secretion by CAFs into the HNC TME induced CCL22 expression by NF-κB activation, thereby enhancing cell proliferation, migration and invasion [50]. IL-33 has also been reported as a critical mediator of CAF-induced invasiveness in HNC models, which increased cell migration and invasion through EMT induction [51]. Likewise, stromal transforming growth factor-β1 (TGF-β1) has also been reported to activate podoplanin expression in tumor cells, leading to more invasive phenotypes [52]. Moreover, CAF-secreted IL-6 has been described as a major upstream molecule triggering osteopontin overexpression in HNC, which caused increased growth, migration and invasion of cancer cells in vitro, and it was correlated with poor prognosis in HNC patients [53]. These data evidence that CAFs may exert an important regulatory role in tumor invasion and dissemination through a plethora of secreted factors.

3.2. ECM remodeling

The ECM is another TME component that can regulate cancer invasion. Changes in ECM composition and density alter its stiffness, consequently modifying tumor behavior. CAFs play a key role regulating ECM stiffness by synthetizing or degrading various principal components such as collagens, fibronectin or proteoglycans. They also contribute to matrix stiffening through the mechanical contraction of actomyosin [54]. Fibronectin is produced by CAFs around carcinomas, presumably in response to soluble factors released by tumor cells to the TME, and its expression has been inversely correlated with the tumor grading and invasive potential [55]. Furthermore, a study conducted by Gopal and co-workers showed that fibronectin released by CAFs is an independent predictor of unfavorable prognosis in HNC [56]. It has also been demonstrated both in vitro and in vivo that collagen expression by CAFs is increased compared with NFs, and able to enhance the migration and invasion of HNC cells [39,43]. Syndecan-1, a member of the proteoglycans family that binds several ECM components, is highly expressed during keratinocyte differentiation while markedly diminished in squamous cell carcinomas development, and proposed as a prognostic tool in HNC [57]. Thus, ECM components are important factors that affect the invasive behavior of HNC cells.

Besides, CAFs are the main producers of MMPs in the TME and hence play a crucial role in ECM degradation and remodeling, helping the tumor to create paths and a more permissive ECM that favors local invasion. Co-cultures of HNC cells and CAFs showed that CAF-derived MMPs resulted in a more active ECM degradation, whereas individually each cell type failed to produce this effect. Also, the specific involvement of CAF-MMPs in matrix degradation, and subsequently in tumor invasion, was evidenced using the MMP inducer EMMPRIN [58]. Increased expression of hyaluronan synthase 2 (HAS2) has been detected in CAFs compared to paired NFs, and significantly correlated with advanced clinical stages and cervical lymph node metastasis. Moreover, HAS2 overexpression has been linked to high MMP-1 and low TIMP1 levels, and enhanced invasiveness of OSCC cells [59]. CAF-secreted MMP-2 reduced keratinocyte cohesion and concomitantly increased epithelial invasion into collagen gels in a TGF-β-dependent manner, promoting more aggressive oral cancer phenotypes [60]. Furthermore, MMP-2 has also been revealed as a key player in HNC metastasis. It is secreted by CAFs in its inactive form, and cancer cells generate the active MMP-2 form through membrane type 1-matrix metalloproteinase (MT1-MMP) [61]. Co-culture experiments showed that direct contact between CAFs and HNC cells activates MMP-2 and MMP-9 secretion and modulates HNC invasive behavior by the CXCL12/CXCR4 pathway [62]. MMP-3 expression in co-cultured HNC cells and CAFs has also been involved in ECM remodeling and cancer cell invasion [63]. MMP-13, expressed by tumor cells and CAFs in squamous cell carcinomas, has been correlated with high invasive capacity of cancer cells both in vitro and in vivo [64]. These data reflect the great impact of CAFs on the regulation of MMP production and activity in the HNC TME.

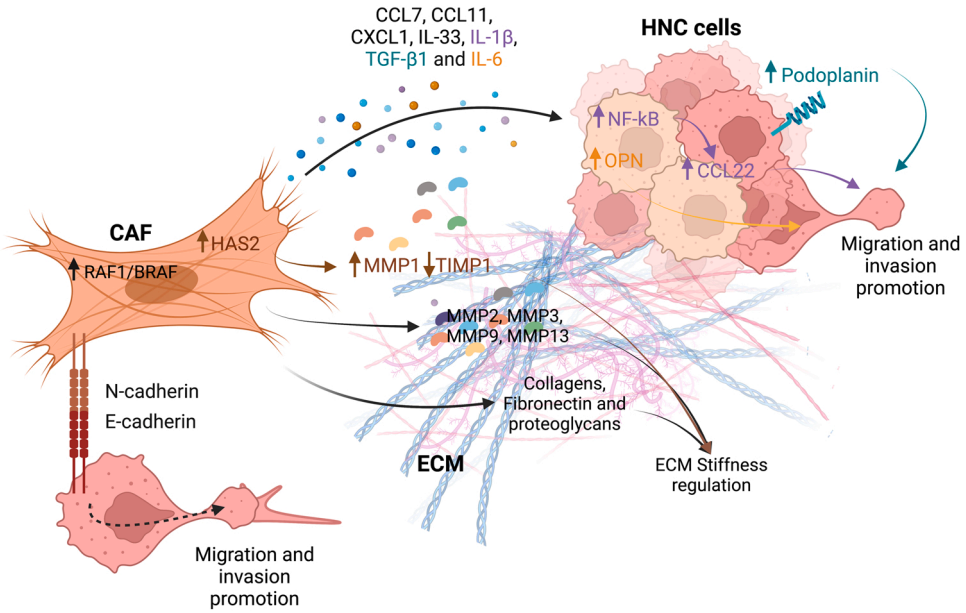

Fig. 2. Schematic overview of the main migration and invasion pathways induced by CAFs in HNC. Molecules interconnected in the same pathways are depicted using same colors (black excluded). Further details of the crosstalk between CAFs and HNC cells are summarized in Table 1.

Interestingly, Gaggioli et al. demonstrated that CAFs support local cancer cell invasion generating tracks in the ECM that are used by squamous cell carcinoma (SCC) cells, thus uncovering CAFs as the leading cells driving SCC invasion [65]. Later studies further contributed to delineate several mechanisms involved in this process, such as the heterotypic physical interaction between N-cadherin on CAF membrane and E-cadherin on cancer cell membrane, enabling cell adhesion, migration and invasion [66].

Anti-invasive cancer therapies were classically designed and aimed to only affect cancer cells, based on targeting major invasion-related signaling pathways altered in cancers. In the last decades, several anti-metastatic drugs targeting/blocking the activity of proteins involved in cancer cell migration or invasion have been developed and tested in preclinical and clinical HNC settings. Dasatinib (BMS-354825) and saracatinib (AZD0530) were developed to broadly inhibit Src family kinases (SFK) signaling, due to its critical role in cancer cell motility and invadopodia formation [67]. Initial in vitro studies demonstrated the promising effects of these SFK inhibitors, potently impairing invasiveness of HNC cell lines [68]. Nevertheless, a subsequent phase II trial evidenced no clinical benefit of saracatinib or dasatinib as single agents in patients with advanced HNC [69,70]. Beyond, our group uncovered striking deleterious effects of dasatinib and saracatinib acting as stemness promoters in HNC models [71]. Similarly, the Abl family inhibitor imatinib also failed to demonstrate a clinical benefit for HNC patients [72], and a phase II clinical trial with the anti-EGFR monoclonal antibody cetuximab only showed partial response in a small subset of HNC patients [73,74]. Interestingly, recent results from our group have unveiled that HNC-secreted factors drive exacerbated invasion of stromal fibroblasts in both CAFs and NFs to a similar extent, showing their high plasticity to be corrupted by the tumor [75]. In-depth mechanistic characterization using phosphoproteomics revealed 11 targetable kinases (MKK7, MKK4, ASK1, RAF1, BRAF, ARAF, COT, PDK1, RSK2 and AKT1) potentially involved in HNC-driven fibroblast invasion. Further functional validation using pharmacologic inhibitors demonstrated that RAF/B-Raf inhibition with sorafenib was the most effective drug to block tumor-promoted invasion of CAFs and NFs. These data unprecedentedly uncover sorafenib as a promising drug to effectively target exacerbated invasive properties of CAFs and to prevent HNC invasion and dissemination. This should be of great importance since tumor recurrences and distant metastasis are major causes of mortality in HNC.

On the other hand, results from clinical trials such as dasatinib that showed no clinical benefit are somehow relevant to highlight that preclinical HNC models based only on tumor cells fail to resemble tumor complexity and clinical behavior. Given the prominent role of CAFs as drivers of tumor invasion and aggressive phenotypes in HNC, they emerge as critical players within the TME that should be considered to develop better HNC models and disease-relevant platforms for testing drugs or novel combination treatments aimed at targeting/blocking tumor invasion and spreading, and ultimately improving HNC patient's life expectancy.

4. CAF modulation of HNC stemness

Tumors are complex structures where not all malignant cells are functionally equivalent but hierarchically organized. Tumor heterogeneity lies in intrinsic features, including genetic, epigenetic and biological properties of cancer cells, and extrinsic features affected by TME characteristics that jointly contribute to tumor progression [9]. In this sense, the role of cancer stem cells (CSCs) is of paramount importance, as these subpopulations of cancer cells are considered to drive tumor initiation and to maintain tumor heterogeneity by their self-renewal and differentiation capacities [9]. CSCs are also characterized by their capacity to develop innate resistance to therapy, due to the low replication, expression of drug export systems, induction of angiogenesis, EMT, hypoxia resistance and immune escape. Consequently, they are also closely linked to relapse and metastatic spread [76].

Nowadays, it is well-accepted that CAFs are a key cellular component to maintain the CSC niche. CAFs co-evolve with the tumor and promote CSC features through paracrine crosstalk. As such, CAFs play an active role in cancer progression, by regulating CSC properties in various ways (Fig. 3): i) acting directly on CSC subpopulations to promote self-renewal; ii) re-inducing a stem cell phenotype in more differentiated tumor cells (reprogramming); and iii) inducing autocrine signaling loops in tumor cells to sustain their stem cell-like potential [77].

Specifically, several publications have demonstrated CAF contribution to sustain CSC properties in HNC. A recent paper reported that periostin released by CAFs promoted stemness through PTK7–Wnt/β–Catenin pathway, as well as tumor initiation and progression in vivo [78]. It has also been described that CAFs enhanced CSC properties in OSCC, as reflected by increased expression of the pluripotency transcription factors Sox2, Oct4 and Nanog and the membrane proteins CD44 and CD105, leading to increased proliferation, reduced apoptosis, enhanced cell motility and chemotherapy resistance [79]. Le and co-workers described a paracrine signaling loop between cancer cells and CAFs involving Wnt pathway (a well-known CSC promoter), capable of regulating CSC characteristics, tumorsphere formation and invasiveness in vitro. Furthermore, the Wnt inhibitors OMP-18R5 and OMP-54F28 reduced tumor growth of HNC patient-derived xenografts [80]. In addition, CAF-secreted IL-6 has been shown to enhance survival and self-renewal of CSC populations [81].

CAFs have also been involved in triggering mechanisms responsible for switching HNC cells from a non-CSC to a CSC state. Our group investigated the paracrine crosstalk between CAFs and HNC CSCs. For that purpose, the effect of conditioned media (CM) from CAFs or NFs was studied on CSC properties, analyzing tumorsphere formation, anchorage-independent growth and the expression of CSC markers [82]. Results revealed that CM from CAFs alone (without serum or any supplement) greatly enhanced tumorsphere formation in HNC cell lines, whereas NF-CM was much more inefficient. This was concomitantly accompanied by increased expression of CSC markers (specially, NANOG, ABCG2, CD44 and SOX2), which were also more robustly induced by CAF-CM. In addition, MS/MS analysis of CAF/NF secretomes was accomplished to identify the repertoire of CAF-secreted proteins and related signaling transduction programs responsible for sustaining the HNC CSC phenotype [82]. Among the most highly up-regulated proteins identified were Carboxypeptidase E (CBPE), platelet-derived growth factor D (PDGFD), EGF-containing fibulin-like extracellular matrix protein-1 (FBLN3), insulin-like growth factor binding protein-5 (IGFBP5) and insulin-like growth factor binding protein-7 (IGFBP7). Noteworthy, pharmacological targeting of the related signaling pathways to the growth factors EGF, IGF and PDGF dramatically reduced tumorsphere-forming capability and anchorage-independent growth [82]. According to these findings, new drugs targeting these proteins or downstream signaling pathways may emerge as a good strategy for designing more efficacious treatments that eradicate the HNC CSC niche.

Similarly, other studies have described a CSC-promoting role of CAFs in other cancer types, thus reflecting that this is a common characteristic in different cancers. Donnarumma and colleagues reported that CAFs promoted breast cancer progression by enhancing stemness, EMT phenotype, and anchorage-independent growth [83]. Mechanistically, CAFs were found to secret ADAM10-rich exosomes to promote cell motility and activate RhoA and Notch signaling in several cancer cell lines [84]. In addition, primary colon CAFs released HGF that induced nuclear translocation of ß-catenin in tumor cells and a stem cell-like transcription profile [85]. In prostate cancer, tumor cells released IL-6 leading to fibroblast activation and MMP secretion, which in turn elicited an EMT phenotype in cancer cells, as well as increased tumor growth and development of spontaneous metastases. CAF-induced EMT in prostate carcinoma cells was accompanied by increased expression of CSC markers, and enhanced tumorsphere formation and self-renewal [86]. Therefore, the TME, and specifically CAFs, play an essential role

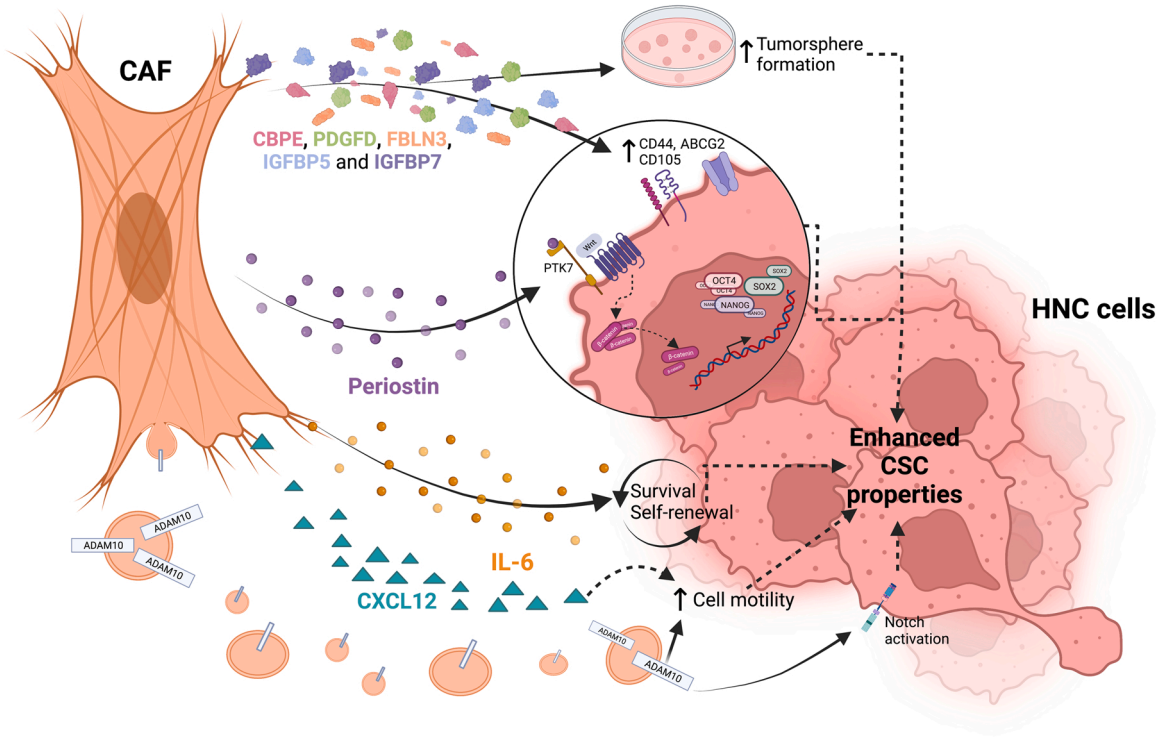

Fig. 3. Representative illustration of the main molecules and signaling pathways involved in CAF-enhanced CSC features in HNC. Molecules interconnected in the same pathways are depicted using same colors (black excluded). Further details of the crosstalk between CAFs and HNC cells are summarized in Table 1.

in CSC promotion and maintenance, acting through various key signaling pathways related to stemness, such as Wnt, Notch, NFκB, SOX2, NANOG, CXCL12/CXCR4 and VEGF/VEGFR pathways among others [77,87].

Altogether, these findings reflect the high plasticity of cancer cell subpopulations to switch between CSC and non-CSC phenotypes, showing that epithelial cells may dedifferentiate, thus entering back into the stem cell pool. For all these reasons, therapies aimed at targeting CSCs within the tumor will not be curative if the CSCs pool can be continuously regenerated from plastic non-CSCs, capable of dedifferentiating and re-entering the CSC state. The identification of key molecules/signals responsible for mediating this conversion is indispensable to select the most appropriate drugs, or combinations of them, to efficiently eliminate CSC populations or attenuate their ability to survive conventional cytotoxic therapies, and to ultimately reduce the risk of metastatic outgrowth and tumor relapse. Therefore, understanding the precise role of the TME components and more specifically CAFs in sustaining the CSC niche will certainly allow the design and development of drugs to effectively eliminate the CSC pool, and to improve clinical outcomes in HNC patients. In this regard, we highlight that drugs, specifically targeting EGF, IGF and PDGF signaling pathways, emerge as excellent strategies to block functionally CAF-enhanced stemness and tumorsphere forming ability, to consequently reduce the CSC pool in HNC.

5. CAF involvement in HNC cell metabolism

In the last two decades, reprogrammed cancer metabolism has attracted enormous interest as a topic for drug discovery, emerging as a next generation cancer hallmark [88]. However, metabolic-targeted therapies are as of yet not clinically available for HNC patients. Mitochondrial respiration via oxidative phosphorylation (oxphos) and glycolysis are the main pathways that cells use to produce ATP. In order to satisfy energetic and anabolic demands derived from continuous tumor proliferation and growth, malignant cells must increase nutrient uptake and energy production. Consequently, the cellular metabolic program is altered, shifting from an oxygen-based towards a glycolytic metabolism, even in the presence of sufficient oxygen supply [88]. This phenotype switch to an anaerobic metabolism is called the "Warburg effect".

Even though cancer metabolism traditionally was focused on an homogeneous glycolytic metabolism of malignant cells without considering the surrounding stroma crosstalk, the growing importance of TME, and particularly CAFs, prompted to recognize their role supporting and even promoting the classic cancer hallmarks via the production of metabolites [89]. This concept was termed "reverse Warburg effect" and involves how the tumors cells force stromal cells to undergo glycolysis, and to produce and release metabolites, such as pyruvate and lactate, as energy suppliers that cancer cells will use as fuel source [90,91]. This phenomenon is normally characterized by the upregulation of monocarboxylate transporters (MCT; MCT4 in lactate exporting cells and MCT1 in receiver cells) and the loss of caveolin-1 (Cav-1) expression in CAFs that impairs mitochondrial function, and therefore triggers aerobic glycolysis [92]. Despite growing evidence of the metabolic crosstalk between cancer cells and CAFs and its impact on the progression of different cancers, the role of CAF metabolism is still poorly understood in HNC. Considering that HNC tumors are dysplastic with up to 80% fibroblasts [93], to deepen understanding of the metabolic role of CAFs in this tumor type could be crucial to improve current HNC treatments and patients' survival.

There is increasing literature describing the importance of the crosstalk between cancer cells and normal fibroblasts in the metabolic reprogramming that drives CAFs development (Fig. 4). It has been reported a metabolic rewiring of normal surrounding fibroblasts driven by OSCC cells in co-cultured cells, where normal fibroblasts exhibited downregulation of caveolin 1 (Cav-1), overexpression of lactate exporter MCT-4 and enhanced aerobic glycolysis, thereby leading to increased release of L-lactate, hypoxia-induced oxidative stress and mitophagy, and hence displaying a CAF phenotype. These metabolically corrupted fibroblasts establish direct and indirect contact with the OSCC cells to

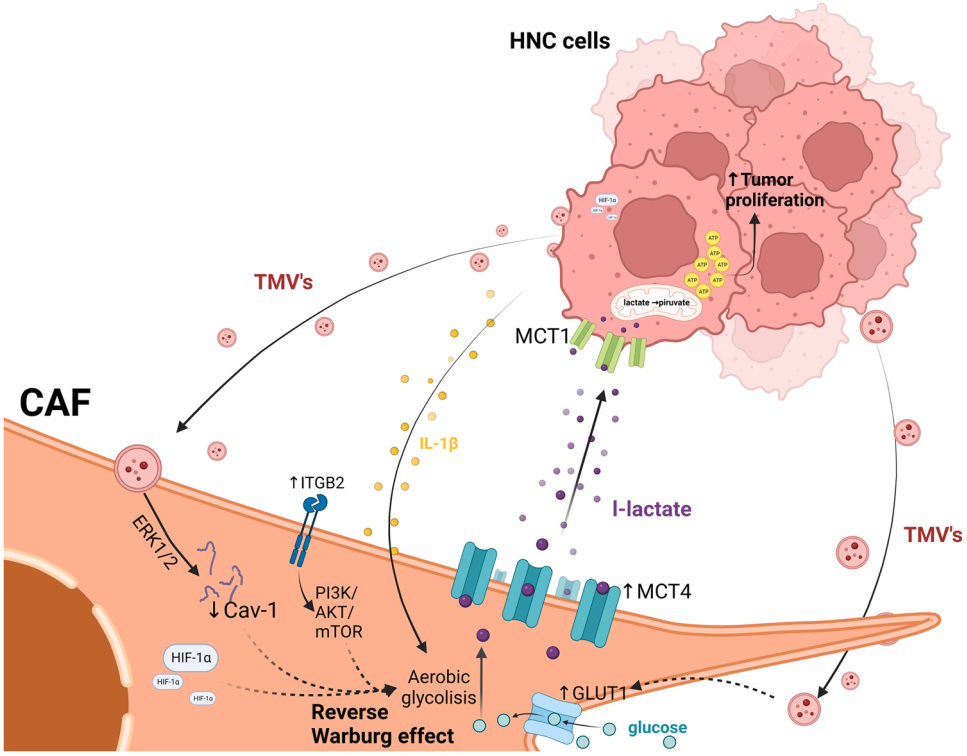

Fig. 4. Representation of the main molecules and pathways involved in the metabolic crosstalk between CAFs and HNC cells. Molecules interconnected in the same pathways are depicted using same colors (black excluded). Further details of the crosstalk between CAFs and HNC cells are summarized in Table 1.

export mitochondria towards OSCC cells, indicating that the TME can be reprogrammed for the benefit of cancer cells [94]. Likewise, normal fibroblasts undergo metabolic reprogramming towards a CAF phenotype, when incubated in the presence of tumor microvesicles (TMVs) [95]. TMVs function as glycometabolic reprogramming mediators inducing the degradation of stromal Cav-1 through ERK1/2 pathway activation, thereby driving a switch from mitochondrial oxidation to aerobic glycolysis in primary fibroblasts that ultimately induce glucose absorption and lactate production [95]. In addition, it has also been reported that the metabolic switch driven by CAFs in OSCC is mediated by upregulation of integrin beta 2 (ITGB2) [91]. ITGB2 expression by CAFs enhanced PI3K/AKT/mTOR-mediated glycolysis and lactate release that OSCC cells absorb and metabolize, boosting mitochondria activity and therefore promoting tumor proliferation. Interestingly, this proliferative boost mediated by ITGB2 was effectively inhibited by targeting the OXPHOS system using metformin (mitochondrial complex I inhibitor) [91]. IL-1β has also been demonstrated to regulate the stromal-epithelial lactate shuttle in HNC. Accordingly, IL-1β plays a key role in the reciprocal metabolic reprogramming between stroma and epithelium, revealing that an enhanced expression of this cytokine in tumor cells leads to upregulated glycolysis and lactate release by CAFs, accelerating cancer cell proliferation [96].

On the other hand, Curry and co-workers have proposed a metabolic compartmentalization model for HNC, which was divided into three different metabolic compartments: 1) highly proliferative, mitochondrial-rich and oxphos-dependent population of epithelial cells, 2) non-proliferative and mitochondrial-poor well-differentiated carcinoma cells, and 3) non-proliferative and mitochondrial-poor MCT4-positive CAFs, which present high oxidative stress and produce and release high lactate and ketone body amounts that fuel proliferative carcinoma cells, and also HNC aggressiveness [97]. Furthermore, Kumar and co-workers described that the metabolic symbiosis between HNC malignant cells and CAFs triggers a mutual and reciprocal feeding between both cell types to boost cell proliferation, migration and tumor progression. Notably, this can be reversed inhibiting the c-Met/FGFR signaling using the inhibitors PF-02341066 (c-Met inhibitor) and AZD-4547 (FGFR inhibitor) [93].

Besides, cancer cells can also adapt their metabolism according to the mechanical signals coming from ECM, promoting tumor invasion and survival pathways. In this sense, tumors niche stiffness plays an important role in the metabolic crosstalk between CAFs and HNC cells, since matrix stiffening is able to mechano-activate the glucose and glutamine metabolism in both HNC cells and CAFs, coordinating non-essential amino acid flux in the tumor niche to sustain tumors growth and malignancy [98].

Another important regulator of cancer metabolism is hypoxia, since the oxygen levels in the tumors have a profound impact in the metabolic response of cells. To adapt to the lack of oxygen, cancer cells activate the hypoxia-inducible factor isoforms: HIF-1α, mainly during an acute hypoxia, and HIF-2α, being the dominant isoform during chronic hypoxia [99]. HIF-1α activation regulates the transcription of many glycolytic genes that lead cells towards Warburg effect [100]. Specifically, the multifaceted oncogene metadherin (MTDH) plays an important role in the regulation of HNC metastasis, and glycolysis under hypoxic conditions via an HIF-1α-MTDH loop [101]. In addition, hypoxia has a great participation in the crosstalk between CAFs and cancer cells, as HIF-1α triggers the shift to glycolytic-based metabolism in both cell types, while HIF-2α plays a part in the oxphos-dependent metabolism in cancer cells [102].

Notably, evidences have been reported in breast cancer to demonstrate striking side-effects of conventional chemotherapy treatment on normal fibroblasts promoting metabolic and phenotypic conversion into CAFs This leads to a highly glycolytic, autophagic and pro-inflammatory microenvironment that activates stemness (Sonic hedgehog/GLI signaling), antioxidant response and interferon-mediated signaling in adjacent breast cancer cells. These effects could also extend to adjacent normal epithelial cells triggering tumorigenesis. These findings merit to be investigated in HNC [103].

In summary, metabolism is a crucial mechanism involved in HNC pathogenesis, and it also plays an important role in the communication

between cancer cells and the surrounding TME. Nevertheless, cancer metabolism is a complex and dynamic process, where slight changes may have a great impact, disrupting the desired equilibrium. On this matter, a good approach to develop new drugs and to address the current challenges could be to target the molecules/receptors that trigger the metabolic wiring in CAFs, such as IL-1β, to halt CAFs conversion and the supportive feeding of cancer cells. Nevertheless, as of yet there are no available therapies aimed at disrupting cancer metabolism or the tumor-stroma crosstalk, it is necessary to further investigate this process to be able to introduce new therapies to treat HNC patients, minimizing/avoiding negative side effects.

6. Immunomodulatory role of CAFs

The main function of immune system is the protection of the body against illness and infections, and it also plays a crucial role in the surveillance and control of emerging tumors, since some cells of the immune system can recognize cancer cells as abnormal and kill them. Unfortunately, tumors are capable of evading the immune system's response [104]. CAFs have also emerged as essential factors in the modulation of immune system, supporting cancer progression through the generation of an immunosuppressive environment [105] (Fig. 5). Nonetheless, the specific immune modulatory functions of CAFs are still poorly understood. There are studies showing that a high abundance of CAFs correlates with immune exclusion and immunotherapy failure [106], while others reported that the absence of CAFs is associated with a lower immune infiltration [107]. One explanation for the different observations in CAFs behavior could be due to differences in their activation status, the presence of distinct CAF subpopulations coexisting in the TME as well as their interaction with the rest of the tumor-stroma components. During recent years, several reports have already described that different subpopulations of CAFs coexist in HNC [108]. In addition, Obradovic and co-workers reported the existence of different CAF subpopulations and demonstrated their functional importance modulating the immunoregulatory milieu of HNC. Beyond, they identified some CAFs subtypes as useful biomarkers to predict resistance to nivolumab [109].

Specifically, several mechanisms have been described demonstrating the immunomodulatory role of CAFs in HNC. One of them is related to the generation of fibrosis, characterized by a strong cross-linked ECM acting as a physical barrier, which impairs immune cell infiltration thereby facilitating immune escape [110]. This has been associated with more aggressive phenotypes in lung cancer [111], melanoma [112], breast cancer [113] or pancreatic cancer [114]. In the context of HNC, Derakhshandeh et al. showed that Semaphorin 4D produced by tumor cells promotes collagen formation in CAFs, which results in a more fibrotic environment hence making immune cell infiltration more difficult [115]. Taking this into account, the ECM stiffness could be considered a potential therapeutic target in order to increase tissue permeability, and to consequently improve immune cell penetration, ultimately leading to cancer cell death.

Another way for CAFs to contribute to the generation of an immunosuppressive environment is through the education of tumor-associated macrophages (TAMs). A study by Takahashi and co-workers showed that the infiltration of CAFs in OSCC samples is associated with the presence of TAMs, which also correlated with lymph node involvement, lymphatic invasion and vascular invasion. These authors also demonstrated that TAM stimulation by CAFs, ultimately leads to the suppression of T cell proliferation [116]. In addition, CAF-secreted IL-6 induces monocyte differentiation into macrophages instead of antigen presenting dendritic cells, hence controlling the antigen-presenting cell development [117].

It has also been reported that CAFs modify the activity of regulatory T cells (Tregs), which are negative regulators of the immune response,

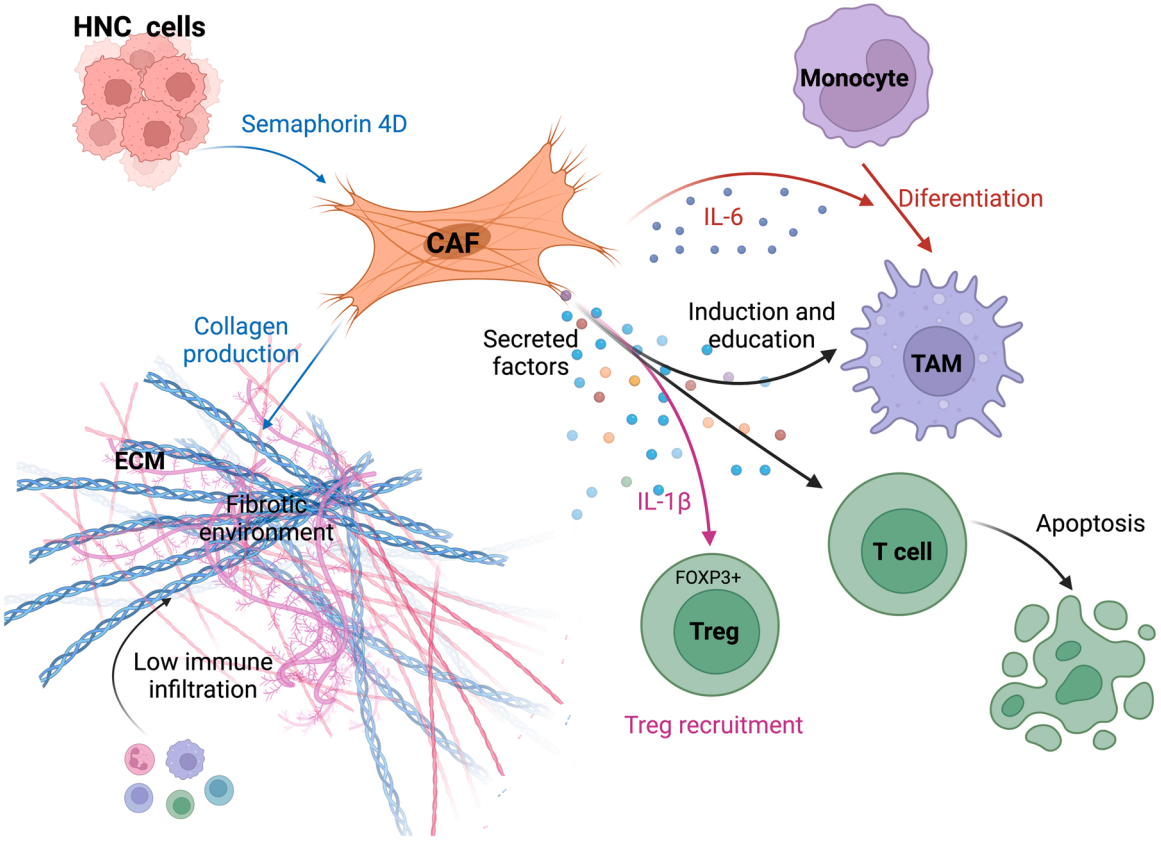

Fig. 5. Schematic illustration showing the main immunomodulatory pathways induced by CAFs in HNC. Molecules interconnected in the same pathways are depicted using same colors (black excluded). Further details of CAF-HNC cell crosstalk are summarized in Table 1.

and thus Tregs activation has been linked to immunosuppression in cancers [118]. Accordingly, it has been found a correlation between CAFs and Treg activation. Schipmann et al. demonstrated that the percentage of FOXP3^{+} cells (a well-known Treg marker) increased from 75% to nearly 100% when SCC cells were co-cultured with fibroblasts [119]. Tregs are recruited to the tumor site by tumor cells expressing CCL22, and its expression is promoted by CAF-secreted IL-1β, demonstrating that crosstalk between both cell types generates an immunosuppressive environment. CCL22 activation by CAFs can be blocked with a NF-κB inhibitor, indicating the involvement of NF-κB in this process [50]. Furthermore, CAFs are able to modify Treg activation themselves, as observed in co-cultures of peripheral blood mononuclear cells (which are immune cell precursors) with supernatants from CAFs or NFs, showing a higher presence of Tregs using CAFs [120]. Besides, CAFs secretomes also have a deleterious effect on T cells increasing their apoptosis compared to NF supernatants [120].

During the last decade, there has been an exponential increase in the publications showing the importance of cell-surface inhibitory molecules present on tumor-infiltrating lymphocytes in cancer, leading to revolutionary advances in immunotherapy. This has definitively changed cancer treatment landscape with the incorporation of new molecules and antibodies targeting these inhibitory receptors. Although many of them have been approved by the FDA in several types of cancer, unfortunately, only 15–20% of HNC patients benefit from immunotherapies anti-PD-1/PD-L1 with nivolumab and pembrolizumab [121]. In summary, the evidences presented in this section emphasize the crucial role of CAFs generating an immunosuppressive environment in HNC. Taking into consideration that solid tumors evolve in fibroblast-rich environments, CAF-based therapies pose as a promising strategy to counteract immunosuppression and reach successful anti-tumor immunity. These immunomodulatory properties of CAFs seem to be a common feature in HNC and other cancers, pinpointing them as a possible immunotherapeutic tool to develop combinatory strategies aimed at improving the clinical efficacy of current anti-PD1/PD-L1 immunotherapies, which is still rather limited in HNC patients treated with nivolumab and pembrolizumab. Moreover, the relationship between CAFs presence and the activation status of macrophages is becoming more evident. Accordingly, novel therapies aimed to modulate their interaction to enhance the immune response in the tumor microenvironment could bring great benefits for HNC patients.

7. Role of CAFs in angiogenesis

Angiogenesis consists in the formation of new vessels from the pre-existent vasculature. It is active during morphogenesis and developmental processes, but absent in adulthood under physiological conditions, except during wound healing. Tumors, like normal tissues, require a constant supply of nutrients and oxygen, as well as to eliminate carbon dioxide and metabolic waste. To this purpose, tumors promote angiogenesis, thereby causing that the normally quiescent vasculature may continually sprout new vessels to sustain tumor outgrowth [88].

The role of fibroblasts in angiogenesis has been well-studied during wound healing processes, in which fibroblasts are activated and contribute to blood vessel formation and tissue repair. However, in pathological conditions such as cancer, aberrant wound repair leads to fibrosis and desmoplasia [122]. Specifically, CAFs can stimulate

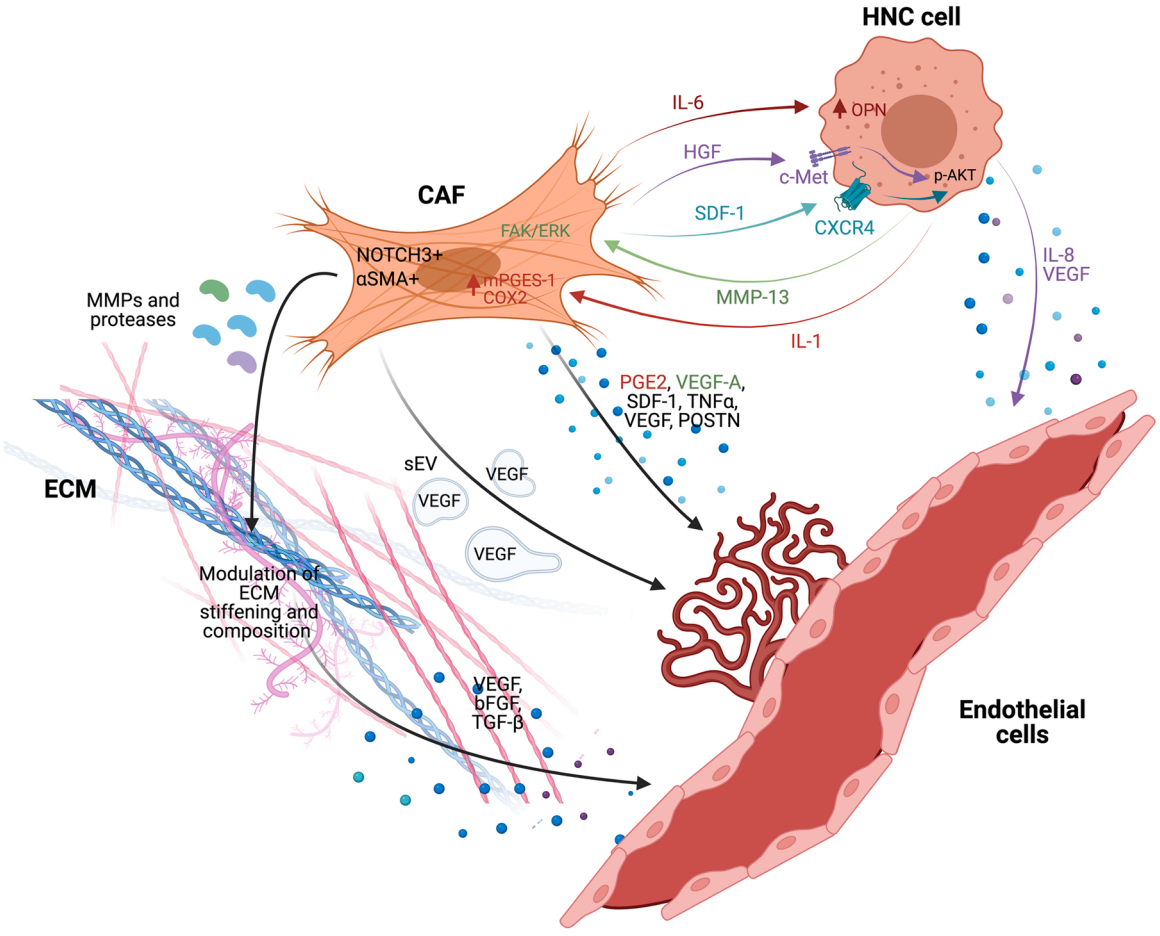

Fig. 6. Schematic overview of the main angiogenesis pathways induced by CAFs in HNC. Molecules interconnected in the same pathways are depicted using same colors (black excluded). Further details of CAF-HNC cells crosstalk are summarized in Table 1.

proangiogenic mechanisms by secreting a wide range of molecules such as periostin (POSTN), osteopontin (OPN), prostaglandin E2 (PGE2), hepatocyte growth factor (HGF), stromal cell-derived factor 1 (SDF-1), bone morphogenetic protein 4 (BMP-4), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF2), and transforming growth factor β (TGF-β) [11,31]. Moreover, CAFs may indirectly promote tumor angiogenesis by modulating ECM composition and biomechanical properties, and also by secreting proteases that can release the above-mentioned proangiogenic factors present in the ECM [123,124] (Fig. 6).

In OSCC, it has been reported that cancer cells stimulate CAFs to induce the expression of Notch Receptor 3 (NOTCH3), being NOTCH3 + CAFs able to induce in vitro tube formation in human umbilical vein endothelial cells (HUVECs), and to subsequently increase blood vessel density. In addition, OSCC samples harboring NOTCH3 + CAFs display a higher blood vessel density than the negative subgroup [125]. In addition, periostin (POSTN) secreted by CAFs has emerged as a protein involved in cellular adhesion, EMT and angiogenesis by activating αVβ3/FAK pathway, which resulted in VEGFR2 upregulation in endothelial cells. This protein has been detected overexpressed in several malignancies including HNC, where periostin was also found to promote lymphangiogenesis [126,127]. Interestingly, Qin et al. demonstrated that CAFs were the main source of POSTN in HNC tumors, which suggests that CAFs could paracrinally induce angiogenesis in HNC via POSTN secretion [128]. Moreover, OSCC patients harboring POSTN-positive tumors exhibited a higher blood vessel density than negative cases, and also POSTN enhanced capillary formation in vitro [129].

Osteopontin (OPN) is another protein involved in the regulation of various angiogenic molecules, found to be upregulated in both tumor tissue samples and plasma from HNC patients, as well as HNC-derived cell lines [53]. CAF-secreted IL-6 enhanced OPN expression in HNC cells, thereby promoting tumors growth through integrin αVβ3 and NF-kB activation in a mouse xenograft model. Moreover, OPN has been detected overexpressed in HNC cells, and CAFs showed higher expression levels than NFs. Interestingly, OPN expression was induced in NF by co-culture with tumors cells [53]. The interaction between HNC cells and fibroblasts induced PGE2 biosynthesis, which was accompanied by increased expression of mPGES-1 and COX2 in fibroblasts. Thus, conditioned media (CM) from fibroblasts, previously treated with CM from cancer cells, induced the formation of capillary-like structures in HUVEC cells via PGE2 secretion [25].

Matrix metalloproteinases (MMPs) have also emerged as important players in the angiogenesis process. Matrix metalloproteinase 13 (MMP-13) secreted by HNC cells promoted in vitro capillary formation in HUVECs. MMP-13 activates the focal adhesion kinase (FAK) and extracellular signal-regulated kinase (ERK), which in turn increased VEGF-A secretion by fibroblasts. Remarkably, this role was further supported by clinical data, thus showing a correlation between MMP-13 and the number of blood vessels in HNC patients [130]. Moreover, OSCC samples with higher α-SMA expression displayed a concomitantly higher micro-vessel density and peritumoral lymphatic vessel density [131]. In line with all these data, Mendes et al. found that CM from fibroblasts promoted angiogenesis in immortalized endothelial cells [132].

It is noteworthy to mention that VEGF, one of the most important proangiogenic factors, has been reported as a major contributor of the CAF-induced angiogenesis in HNC. As such, VEGF expression has been correlated with a higher microvessel density in maxillary sinus SCC patients [133], and its expression was upregulated in OSCC cells and CAFs via CAF-secreted IL-6 [134]. Similar results have been reported in ESCC, where interaction between cancer cells and fibroblasts was crucial for angiogenesis. Cancer cells induced CAF activation mainly via TGF-β secretion, which in turn increased VEGF secretion from CAFs and the subsequent vasculature formation [135]. Besides, it has also been described in OSCC, that VEGF bound into CAF-secreted small extracellular vesicles (sEV) induced VEGFR2/AKT/ERK signaling in HUVECs, and hence angiogenesis in vitro and in vivo [136].

SDF-1 has also been described as a critical proangiogenic factor in HNC. Thus, Zhou and coworkers demonstrated that CAF-secreted SDF-1 enhanced in vitro tube formation in HUVECs. Mechanistically, they found that OSCC cells secrete TNF-α that may act directly to induce angiogenesis, but also indirectly promoting the transformation of NFs into CAFs, which increases SDF-1 secretion and angiogenesis [137]. Moreover, SDF-1/CXCR4 pathway has also been found to contribute to angiogenesis in laryngeal squamous cell carcinoma (LSCC). SDF-1 binds to CXCR4 in tumors cells what triggers Akt phosphorylation and IL-8 secretion, acting as a proangiogenic factor in LSCC [138]. Given the role of this pathway in tumor angiogenesis, and that SDF-1 secretion by CAFs is widely reported in different HNC types [139–141], these observations reflect that CAFs are a major SDF-1 source within the TME, and key modulators of angiogenesis in HNC.

Some growth factors such as HGF and FGF2 have also been reported as angiogenesis regulators. HNC-derived fibroblasts secrete HGF that interacts with c-Met in tumor cells. This interaction results in Akt phosphorylation and stimulates tumor secretion of IL-8 and VEGF, and subsequently angiogenesis [28]. Findings by Dong and co-workers further support the relevance of this paracrine interplay in angiogenesis, since HNC cell lines treated with HGF also produced the proangiogenic factors IL-8 and VEGF [142]. Moreover, HGF and c-Met are frequently found upregulated in HNC specimens compared to normal mucosa, which reinforces the importance of this pathway in this cancer type [28]. In addition, HNC tissue specimens showed high expression of FGF2, a pro-angiogenic growth factor, as well as FGFR1 and FGFR2 expression in tumors cells, vasculature and fibroblasts [143]. Furthermore, another study revealed the upregulation of several angiogenic genes in bevacizumab-resistant HNC tumors using a mouse xenograft model [144]. Among these factors are phospholipase C gamma-2 (PLCg2), Frizzled 4 (FZD4), C-X3-C motif chemokine 1 (CX3CL1) and C-C motif chemokine ligand 5 (CCL5), known to activate ERK. In this model, ERK activation led to FGF2 upregulation, which acts mainly at endothelial level, modulating angiogenesis and also contributing to bevacizumab resistance [144].

Great effort has been devoted in translating antiangiogenic strategies into useful clinical therapies. Few examples of these approaches are drugs designed against VEGFR such as axitinib [145] and sorafenib [146,147], and also combinatorial therapies targeting VEGFR and EGFR with pazopanib and cetuximab, respectively [148], or targeting VEGF and FGFR with bevacizumab and PD173074, respectively [144], or treatments combining the anti-VEGF agent bevacizumab with platinum-based chemotherapy [149]. Despite the numerous clinical trials performed to test anti-angiogenic therapies for HNC, they have shown to date none or very limited success, as well as a high toxicity. These data suggest that it is necessary a deeper understanding of CAFs activation and angiogenesis induction to design better anti-angiogenic treatments. Evidences strongly support CAFs as a major source of proangiogenic factors. Therefore, disruption of tumor-CAFs interplay and the production/secretion of pro-angiogenic factors by CAFs may settle the basis for the development of better combinatorial treatments, avoiding potential CAF-mediated mechanisms of resistance to anti-angiogenic therapeutic agents.

8. Perspectives of potential CAF-based therapies in HNC

Currently, surgery, chemotherapy (based on cisplatin) and/or radiotherapy represent the baseline treatment options for most HNC patients. In addition, nowadays the only FDA-approved targeted therapies for HNC are cetuximab (anti-EGFR), nivolumab and pembrolizumab (anti-PD-1/PD-L1), which unfortunately have demonstrated rather limited clinical benefit. As major pitfalls, most HNC patients usually develop resistance to both radio/chemotherapy, and recurrent relapses are also a highly common issue. Elucidation of the TME landscape and related aberrant signaling pathways could bring valuable

insights into the molecular pathogenesis of this disease and to open up new avenues for treatment.

Mounting functional and mechanistic evidences herein discussed support the contribution of CAFs to major cancer hallmarks in HNC, which are tumors with characteristic CAF-rich environments. Accordingly, the development of CAF-targeted therapies poses a promising strategy to improve patient treatment and outcome. In this review, several promising actionable molecules and signaling pathways have been identified that could serve as the basis for drug development. Among them, CAF-secreted IL-6 and IL-β1 have been demonstrated to contribute broadly to enhance the proliferation, invasiveness and stemness of HNC cells as well as to regulate tumor metabolism pathways and anti-tumor immune response, thus making them perfect targets for depletion and/or pharmacologic blockade of their receptors, to block/prevent these protumoral actions. Similarly, COX-2 and mPGES1 have also been reported to regulate proliferation, metabolism activity and angiogenesis in HNC, pointing them as promising therapeutic targets to improve current treatment options for HNC. Likewise, PDGF, EGF and IGF are involved in proliferation, metabolism and angiogenesis; however, they also play important roles in the normal tissue homeostasis, making extremely difficult to develop targeted drugs for these molecules or their receptors without considerable side effects.

Table 1
Role of CAFs molecular mechanisms involved in HNC cell biology.

CAFs in	Events in CAFs		Downstream effect/molecular pathways involved	References
HNC proliferation	Nuclear translocation of NFκBα and induction of IL-6 and COX2		E-cadherin expression downregulated in tumor cells. Increased IL-6 levels associated with recurrences	[26]
	Induction of COX2 and mPGEs-1		Release of PGE2 and PGI2 to ECM enhancing cancer cell proliferation	[27]
	Secretion of	HGF	Activates MAPK, Ras, PI3K and STAT3 pathways in tumor cells through c-Met signaling	[28]
		BDNF	Interacts with TrkB and promotes EMT in tumor cells. Inhibition blocks in vitro tumor growth	[31]
		STC2, FNDC1, SERPINE1, Integrin α6, Activin A, KGF, SDF-1 or TNF-α	Increased HNC proliferation and progression	[21],[26],[32],[33],[34],[35]
		miR-34a-5p-devoid exosomes	Less binding to AXL and enhance OSCC progression	[37]
		Exosomal LOC400221	Conversion of fibroblasts into CAFs via IL-33 and OSCC growth	[38]
	miR-7 upregulation		Conversion of fibroblasts into CAFs. Decreased PAR-4 (TSG, apoptosis inducer) secretion and increased tumor cell growth and migration	[36]
	miR-196a/b upregulation		Pleiotropic proliferative effects in HNSCC cells and CAFs. Downregulate HOX genes.	[39]
	High expression of COL11A1, COL3A1 and COL8A1		Enhanced proliferation in HNSCC cells. Poor prognosis in HNSCC/OSCC	[40],[42],[43],[44]
HNC invasion	Secretion of	CCL7, CXCL1	Stimulate migration and invasion	[48],[50]
		CCL11, IL-33	Promote invasion and migration. EMT induction	[49],[52]
		IL-1β	CCL22 expression by NF-kβ activation. Cell proliferation, migration and invasion	[51]
		IL-6	Osteopontin overexpression causing increased growth, migration and invasion of cancer cells	[55]
		MMP2, MMP9	Modulates HNC invasive behavior by CXCL12/CXCR4 pathway	[62],[63],[64]
	Fibronectin, Collagen, MMP3, MMP13 expression		Enhance the migration and invasion of HNC cells	[40],[44],[57],[58],[65],[66]
	HAS2 expression		linked to high MMP-1 and low TIMP1 levels, and enhanced invasiveness of OSCC cells	[61]
	TGF-β1 pathway		Induced EMT by activation of the PI3K/AKT and Smad2/3 pathways. Podoplanin expression. Cell migration and invasion.	[53],[54]
HNC stemness	Secretion of	Periostin	Enhanced tumor stemness via PTK7–Wnt/β–Catenin	[83]
		CBPE, PDGFD, FBLN3, IGFBP5 and IGFBP7.	Promoting and sustaining CSC phenotype	[87]
	Increased expression of Sox2, Oct4, Nanog, CD44 and CD105		Increased proliferation, cell motility, chemotherapy resistance and less apoptosis	[82],[84],[92]
	CXCL12/CXCR4 pathway		CSC enhancement	[84]
	Wnt pathway		Tumorsphere formation and invasiveness	[85]
HNC cell metabolism	Downregulation of Cav-1, overexpression of MCT-4, enhanced aerobic glycolysis.		Metabolic rewiring of normal fibroblast by cancer cells in their own benefit	[98]
	ITGB2 expression		Enhanced PI3K/AKT/mTOR-mediated glycolysis and lactate release. Tumor proliferation	[95]
	HIF-1α expression		Shift to glycolytic-based metabolism	[108]
HNC immune response	Collagen production promoted by Semaphorin 4D		ECM stiffness impairing immune infiltration and facilitates immune scape.	[115],[120]
	Education and stimulation of TAMs		Suppression of T cell proliferation	[121]
	Secretion of IL-6		Monocyte differentiation into macrophages instead of DC	[123]
	Co-culture increased FOXP3 + cells		Increased FOXP3 + cells	[122],[125]
	Secretion of IL-1β		Tregs' recruital by CCL22-expressing cancer cells	[51]
HNC angiogenesis	NOTCH3 expression induced by cancer cells		Induce tube formation in HUVECs	[130]
	Tumor-induced secretion of PGE2		Induced capillary-like structures in HUVECs	[27]
	Tumor-derived MMP13 induced VEGFA secretion		Promotion of in vitro capillary formation in HUVECs	[135]
	VEGF in CAFs-secreted sEV		Induction of angiogenesis by VEGFR2/AKT/ERK signaling	[141]
	Secretion of	IL-6	Upregulation of VEGF expression in OSCC cells and CAFs	[139]
		Periostin	Activation AlfaVbeta3/FAK pathway and VEGFR2 upregulation	[131],[132],[133]
		SDF-1	Tube formation in HUVECs Binding to CXCR4 causing Akt phosphorylation and IL-8 (proangiogenic factor) secretion in cancer cells	[142],[143]
		HGF	Akt posphorilation and IL-8 and VEGF secretion	[29]

On the other hand, CAF-based strategies specifically aimed at targeting pro-invasive phenotypes could be particularly promising for HNC treatment. Noteworthy, using phosphoproteomics approaches, we have recently identified several promising actionable kinases responsible for tumor-promoted invasion of stromal fibroblasts (both CAFs and NFs): MKK7, MKK4, MEKK6, ASK1, RAF1, COT, BRAF, ARAF, PDK1, RSK2 and AKT1. Further pharmacologic inhibition of these kinases or related signaling pathways revealed sorafenib (RAF1/BRAF inhibitor) as an effective drug to block exacerbated pro-invasive fibroblast clusters. Since CAFs are actively involved in driving HNC invasion and ECM remodeling, the failure of current anti-invasive therapies together with CAF-driven pro-invasive phenotypes encourage and strongly support the potential CAF-based therapies as promising strategies to effectively prevent tumor invasion and dissemination. In combination with chemotherapy/radiotherapy, sorafenib could potentially emerge as a valuable anti-invasive strategy to prevent HNC cell dissemination and to reduce tumor recurrences and distant metastasis, which are major causes of mortality in HNC patients. Despite the numerous promising molecules and signaling pathways discussed in this review with potential to develop new therapeutic strategies, there is an important unmet need yet to be addressed in HNC treatment, whichis the lack of reliable predictive biomarkers to guide therapeutic choices. Therefore, future efforts should be focused on the improvement and implementation of precision medicine, helping clinicians to select a personalized treatment strategy according to patient characteristics, based on the specific molecular profile of the tumor and the surrounding TME.

9. Conclusions

According to the herein reviewed data, it is very clear that CAFs play an active role in HNC progression, thereby regulating a wide range of signaling pathways and critical cellular processes for tumor cell biology (Table 1). Remarkably, HNC evolve in fibroblast-rich environments in which CAF-tumor interplay contribute to most cancer hallmarks. Therefore, CAF-based targeted therapies emerge promising for the treatment of this disease, having still a high mortality and scarce molecular-targeted therapeutic options. Since CAF-based therapies are primarily aimed to target CAFs functions but not to kill cancer cells, it should be reasonable to propose combination strategies targeting CAFs with others that efficiently eradicate the tumor mass to reach the highest clinical efficacy and patient benefit.

According to the evidences presented in this review, it is worth to highlight that CAF-targeted therapies pose particularly useful to control tumor spreading and metastasis, since it has been demonstrated that CAFs act as leading cells driving HNC tumor invasion. Interestingly, pharmacologic RAF/B-Raf inhibition with sorafenib has demonstrated to robustly and effectively abrogate exacerbated CAF invasion driven by HNC-secreted factors [75]. It is indeed obvious that such strategies aimed at preventing metastatic dissemination should be applied before metastasis occur to achieve the intended biological impact and optimal clinical benefit. In addition, the immunomodulatory role of CAFs also poses highly beneficial to develop combination treatments that augment the rather limited efficacy of current anti-PD1/PD-L1 immunotherapies in HNC as well as other solid tumors.

Nevertheless, there are still a number of unsolved questions that are critical before CAF-based therapies could be successfully and safely implemented into clinical practice. There is nowadays high controversy regarding the origin of CAFs, markers specificity, phenotypes, or functions for the different subtypes. Regarding their origin, it is still unclear which cell type/lineage transform into CAFs and whether the original precursors are responsible for the final CAF phenotypes. CAF-specific markers are still lacking, some canonical markers such as FAP or alpha-SMA have been described to distinguish between normal fibroblast and CAFs; however, the identification of a single marker may never come to fruition due to the high CAFs heterogeneity. Likely, a combination of markers will be necessary to jointly provide functional insights of CAF subtypes and their clinical relevance.

It has been reported that solid tumors like HNC harbor high CAFs heterogeneity making necessary a spatio-temporal profiling of CAF subtypes that should be precisely linked to specific functions and clinical relevance. Current state-of-art single cell technologies and spatial transcriptomics are bringing tremendous advancements in pursuing this goal. Given that CAFs exhibit a high plasticity and are probably able to change into different phenotypes depending on the spatial-temporal characteristics of the TME, this technology could be extremely helpful to comprehend the spatial organization of CAF subpopulations according to their intrinsic features or their distinct distribution pattern (i.e. intratumoral or peritumoral CAFs). These studies will certainly allow us to integrate and fully depict the complexity of tumor-stroma communication dynamics to apply this knowledge to design more effective treatment strategies targeting both the tumor and the surrounding TME.

Considering the intricate interplay of CAFs and tumor cells, as well as with other components of the TME, anti-CAFs therapies should be designed to avoid direct CAF ablation. Instead, targeting more specifically CAF functions or to shift the ratio of specific CAF subpopulations at certain time points, it might be more suitable to reduce tumor aggressiveness and spreading, or to increase tumors cell sensitivity to current cytotoxic or immunotherapeutic agents, avoiding adverse effects. In this review we have compiled valuable available information on multiple cellular processes and cancer hallmarks regulated by CAFs as well as key related signaling pathways that are susceptible to be therapeutically targeted. It is conceivable that further detailed investigations will lead to the identification of key molecules and signaling pathways in the CAF-HNC tumor circuit that could emerge as excellent actionable targets to specifically block the pro-tumoral effects of CAFs. Beyond, the combination of mutational tumor status and additional molecular/stromal features will allow to improve patient stratification for personalized treatment decisions.

Funding

This study was supported by the Instituto de Salud Carlos III (ISCIII) through the project grants PI19/00560, PI22/00167 and CIBERONC (CB16/12/00390) and co-funded by the European Union, FINBA-ISPA (2021–047-INTRAMURAL NOV-ALTES; sponsored by Janssen), the Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Fundación Bancaria Cajastur-IUOPA, Ayudas para Grupos de Investigación de Organismos del Principado de Asturias 2021–2023 (IDI/2021/000079) and the FEDER Funding Program from the European Union. SAT is recipient of a Sara Borrell postdoctoral fellowship from ISCIII (CD20/00006). LPF is recipient of an FPU-PhD fellowship from the Spanish Ministry of Education (FPU20/01588), and IMJ recipient of a Severo Ochoa predoctoral fellowship from the Principado de Asturias (BP20–152).

CRediT authorship contribution statement

Llara Prieto-Fernandez, Irene Montoro-Jiménez, Beatriz de Luxan-Delgado and María Otero-Rosales: Writing - Original Draft and Visualization; **Juan P. Rodrigo and Fernando Calvo:** Writing - Review & Editing; **Juana M. García-Pedrero:** Supervision, Funding acquisition and Writing - Review & Editing; **Saúl Álvarez-Teijeiro:** Conceptualization, Visualization, Formal analysis and Writing - Original Draft and Supervision.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Images were created using Biorender.com.

Consent for publication

All listed authors have approved the manuscript before submission, including the names and order of authors.

References

[1] C.R. Leemans, B.J.M. Braakhuis, R.H. Brakenhoff, The molecular biology of head and neck cancer, Nat. Rev. Cancer 11 (2011) 9–22, https://doi.org/10.1038/nrc2982.
[2] F. Kamangar, G.M. Dores, W.F. Anderson, Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world, J. Clin. Oncol. 24 (2006) 2137–2150, https://doi.org/10.1200/JCO.2005.05.2308.
[3] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, Ca. Cancer J. Clin. 68 (2018) 394–424, https://doi.org/10.3322/caac.21492.
[4] M. James, A. Bonner, M. Paul, Harari Jordi Giralt, Dong Nozar Azarnia, M. Shin, B. Roger, Cohen Christopher, U. Jones, Sur Ranjan, David Raben, Jacek Jassem, Ove Roger, S. Merrill, Kies, Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck, N. Engl. J. Med. 354 (2006) 567–578.
[5] J. Moreira, A. Tobias, M.P. O'Brien, M. Agulnik, Targeted therapy in head and neck cancer: an update on current clinical developments in epidermal growth factor receptor-targeted therapy and immunotherapies, Drugs (2017), https://doi.org/10.1007/s40265-017-0734-0.
[6] S. Koontongkaew, The tumor microenvironment contribution to development, growth, invasion and metastasis of head and neck squamous cell carcinomas, J. Cancer 4 (2013) 66–83, https://doi.org/10.7150/jca.5112.
[7] D. Hanahan, L.M. Coussens, Accessories to the crime: functions of cells recruited to the tumor microenvironment, Cancer Cell 21 (2012) 309–322, https://doi.org/10.1016/j.ccr.2012.02.022.
[8] J.A. Joyce, J.W. Pollard, Microenvironmental regulation of metastasis, Nat. Rev. Cancer 9 (2009) 239–252, https://doi.org/10.1038/nrc2618.
[9] A. Albini, A. Bruno, C. Gallo, G. Pajardi, D.M. Noonan, K. Dallaglio, Cancer stem cells and the tumor microenvironment: interplay in tumor heterogeneity, Connect. Tissue Res. 56 (2015) 414–425, https://doi.org/10.3109/03008207.2015.1066780.
[10] M.E. Fiori, S. Di Franco, L. Villanova, P. Bianca, G. Stassi, R. De Maria, Cancer-associated fibroblasts as abettors of tumor progression at the crossroads of EMT and therapy resistance, Mol. Cancer 18 (2019) 70, https://doi.org/10.1186/s12943-019-0994-2.
[11] G. Leef, S.M. Thomas, Molecular communication between tumor-associated fibroblasts and head and neck squamous cell carcinoma, Oral. Oncol. 49 (2013) 381–386, https://doi.org/10.1016/j.oraloncology.2012.12.014.
[12] D.F. Quail, J.A. Joyce, Microenvironmental regulation of tumor progression and metastasis, Nat. Med. 19 (2013) 1423–1437, https://doi.org/10.1038/nm.3394.
[13] D.W. Powell, P.A. Adegboyega, J.F. Di Mari, R.C. Mifflin, Epithelial cells and their neighbors: I. Role of intestinal myofibroblasts in development, repair, and cancer, Am. J. Physiol. - Gastrointest. Liver Physiol. 289 (2005) G2–G7, https://doi.org/10.1152/ajpgi.00075.2005.
[14] R. Kalluri, M. Zeisberg, Fibroblasts in cancer, Nat. Rev. Cancer 6 (2006) 392–401, https://doi.org/10.1038/nrc1877.
[15] O.E. Franco, A.K. Shaw, D.W. Strand, S.W. Hayward, Cancer associated fibroblasts in cancer pathogenesis, Semin. Cell Dev. Biol. 21 (2010) 33–39, https://doi.org/10.1016/j.semcdb.2009.10.010.
[16] E. Sahai, I. Astsaturov, E. Cukierman, D.G. DeNardo, M. Egeblad, R.M. Evans, D. Fearon, F.R. Greten, S.R. Hingorani, T. Hunter, R.O. Hynes, R.K. Jain, T. Janowitz, C. Jorgensen, A.C. Kimmelman, M.G. Kolonin, R.G. Maki, R. S. Powers, E. Puré, D.C. Ramirez, R. Scherz-Shouval, M.H. Sherman, S. Stewart, T. D. Tlsty, D.A. Tuveson, F.M. Watt, V. Weaver, A.T. Weeraratna, Z. Werb, A framework for advancing our understanding of cancer-associated fibroblasts, Nat. Rev. Cancer 20 (2020) 174–186, https://doi.org/10.1038/s41568-019-0238-1.
[17] M. Vered, A. Dobriyan, D. Dayan, R. Yahalom, Y.P. Talmi, L. Bedrin, I. Barshack, S. Taicher, Tumor-host histopathologic variables, stromal myofibroblasts and risk score, are significantly associated with recurrent disease in tongue cancer, Cancer Sci. 101 (2010) 274–280, https://doi.org/10.1111/j.1349-7006.2009.01357.x.
[18] P.S. Carneiro Lúcio, A. Leite Cavalcanti, P. Muniz Alves, G. Pina Godoy, C.F. Weege Nonaka, Myofibroblasts and their relationship with oral squamous cell carcinoma, Braz. J. Otorhinolaryngol. 79 (2013) 112–118, https://doi.org/10.5935/1808-8694.20130019.
[19] D. Marsh, K. Suchak, K.A. Moutasim, S. Vallath, C. Hopper, W. Jerjes, T. Upile, N. Kalavrezos, S.M. Violette, P.H. Weinreb, K.A. Chester, J.S. Chana, J. F. Marshall, I.R. Hart, A.K. Hackshaw, K. Piper, G.J. Thomas, Stromal features are predictive of disease mortality in oral cancer patients, J. Pathol. 223 (2011) 470–481, https://doi.org/10.1002/path.2830.
[20] Y.C. Ko, T.Y. Lai, S.C. Hsu, F.H. Wang, S.Y. Su, Y.L. Chen, M.L. Tsai, C.C. Wu, J. R. Hsiao, J.Y. Chang, Y.M. Wu, D.R. Robinson, C.Y. Lin, S.F. Lin, Index of cancer-associated fibroblasts is superior to the epithelial–mesenchymal transition score in prognosis prediction, Cancers (Basel) 12 (2020) 1718, https://doi.org/10.3390/cancers12071718.
[21] I.O. Bello, M. Vered, D. Dayan, A. Dobriyan, R. Yahalom, K. Alanen, P. Nieminen, S. Kantola, E. Läärä, T. Salo, Cancer-associated fibroblasts, a parameter of the tumor microenvironment, overcomes carcinoma-associated parameters in the prognosis of patients with mobile tongue cancer, Oral. Oncol. 47 (2011) 33–38, https://doi.org/10.1016/j.oraloncology.2010.10.013.
[22] M. Shimoda, K.T. Mellody, A. Orimo, Carcinoma-associated fibroblasts are a rate-limiting determinant for tumour progression, Semin. Cell Dev. Biol. 21 (2010) 19–25, https://doi.org/10.1016/j.semcdb.2009.10.002.
[23] J. Dudás, A. Fullár, A. Romani, C. Pritz, I. Kovalszky, V. Hans Schartinger, G. Mathias Sprinzl, H. Riechelmann, Curcumin targets fibroblast-tumor cell interactions in oral squamous cell carcinoma, Exp. Cell Res. 319 (2013) 800–809, https://doi.org/10.1016/j.yexcr.2012.12.001.
[24] J. Dudás, A. Fullár, M. Bitsche, V. Schartinger, I. Kovalszky, G.M. Sprinzl, H. Riechelmann, Tumor-produced, active Interleukin-1 β regulates gene expression in carcinoma-associated fibroblasts, Exp. Cell Res. 317 (2011) 2222–2229, https://doi.org/10.1016/j.yexcr.2011.05.023.
[25] S. Alcolea, R. Antón, M. Camacho, M. Soler, A. Alfranca, F.X. Avilés-Jurado, J. M. Redondo, M. Quer, X. León, L. Vila, Interaction between head and neck squamous cell carcinoma cells and fibroblasts in the biosynthesis of PGE 2, J. Lipid Res. 53 (2012) 630–642, https://doi.org/10.1194/jlr.M019695.
[26] D. Wang, R.N. Dubois, Prostaglandins and cancer, Gut 55 (2006) 115–122, https://doi.org/10.1136/gut.2004.047100.
[27] T.Y. Seiwert, R. Jagadeeswaran, L. Faoro, V. Janamanchi, V. Nallasura, M. El Dinali, S. YaIa, R. Kanteti, E.E.W. Cohen, M.W. Ungen, L. Martin, S. Krishnaswamy, A. Klein-Szanto, J.G. Christensen, E.E. Vokes, R. Salgia, The MET receptor tyrosine kinase is a potential novel therapeutic target for head and neck squamous cell carcinoma, Cancer Res. 69 (2009) 3021–3031, https://doi.org/10.1158/0008-5472.CAN-08-2881.
[28] L.M. Knowles, L.P. Stabile, A.M. Egloff, M.E. Rothstein, S.M. Thomas, C. T. Gubish, E.C. Lerner, R.R. Seethala, S. Suzuki, K.M. Quesnelle, S. Morgan, R. L. Ferris, J.R. Grandis, J.M. Siegfried, HGF and c-Met participate in paracrine tumorigenic pathways in head and neck squamous cell cancer, Clin. Cancer Res 15 (2009) 3740–3750, https://doi.org/10.1158/1078-0432.CCR-08-3252.
[29] P. Gao, C. Li, Z. Chang, X. Wang, M. Xuan, Carcinoma associated fibroblasts derived from oral squamous cell carcinoma promote lymphangiogenesis via c-Met/PI3K/AKt in vitro, Oncol. Lett. 15 (2018) 331–337, https://doi.org/10.3892/ol.2017.7301.
[30] J. Dudás, M. Bitsche, V. Schartinger, C. Falkeis, G.M. Sprinzl, H. Riechelmann, Fibroblasts produce brain-derived neurotrophic factor and induce mesenchymal transition of oral tumor cells, Oral. Oncol. 47 (2011) 98–103, https://doi.org/10.1016/j.oraloncology.2010.11.002.
[31] J.M. Curry, J. Sprandio, D. Cognetti, A. Luginbuhl, V. Bar-Ad, E. Pribitkin, M. Tuluc, Tumor microenvironment in head and neck squamous cell carcinoma, Semin. Oncol. 41 (2014) 217–234, https://doi.org/10.1053/j.seminoncol.2014.03.003.
[32] L.M. Sobral, A. Bufalino, M.A. Lopes, E. Graner, T. Salo, R.D. Coletta, Myofibroblasts in the stroma of oral cancer promote tumorigenesis via secretion of activin A, Oral. Oncol. 47 (2011) 840–846, https://doi.org/10.1016/j.oraloncology.2011.06.011.
[33] J. Lin, C. Liu, L. Ge, Q. Gao, X. He, Y. Liu, S. Li, M. Zhou, Q. Chen, H. Zhou, Carcinoma-associated fibroblasts promotes the proliferation of a lingual carcinoma cell line by secreting keratinocyte growth factor, Tumor Biol. 32 (2011) 597–602, https://doi.org/10.1007/s13277-011-0158-5.
[34] A. Orimo, P.B. Gupta, D.C. Sgroi, F. Arenzana-Seisdedos, T. Delaunay, R. Naeem, V.J. Carey, A.L. Richardson, R.A. Weinberg, Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion, Cell (2005), https://doi.org/10.1016/j.cell.2005.02.034.
[35] Z. Shen, X. Qin, M. Yan, R. Li, G. Chen, J. Zhang, W. Chen, Cancer-associated fibroblasts promote cancer cell growth through a miR-7-RASSF2-PAR-4 axis in the tumor microenvironment, Oncotarget 8 (2017) 1290–1303, https://doi.org/10.18632/oncotarget.13609.
[36] Y. yin Li, Y. wei Tao, S. Gao, P. Li, J. mao Zheng, S. en Zhang, J. Liang, Y. Zhang, Cancer-associated fibroblasts contribute to oral cancer cells proliferation and metastasis via exosome-mediated paracrine miR-34a-5p, EBioMedicine 36 (2018) 209–220, https://doi.org/10.1016/j.ebiom.2018.09.006.
[37] L. Ding, J. Ren, D. Zhang, Y. Li, X. Huang, Q. Hu, H. Wang, Y. Song, Y. Ni, Y. Hou, A novel stromal lncRNA signature reprograms fibroblasts to promote the growth of oral squamous cell carcinoma via LncRNA-CAF/interleukin-33, Carcinogenesis 39 (2018) 397–406, https://doi.org/10.1093/carcin/bgy006.
[38] S. Álvarez-Teijeiro, S.T. Menéndez, M.Á. Villaronga, J.P. Rodrigo, L. Manterola, L. De Villalaín, J.C. De Vicente, L. Alonso-Durán, M.P. Fernández, C.H. Lawrie, J. M. García-Pedrero, Dysregulation of miR-196b in head and neck cancers leads to pleiotropic effects in the tumor cells and surrounding stromal fibroblasts, Sci. Rep. 7 (2017) 17785, https://doi.org/10.1038/s41598-017-18138-8.
[39] J.C. Sok, J.A. Lee, S. Dasari, S. Joyce, S.C. Contrucci, A.M. Egloff, B.K. Trevelline, R. Joshi, N. Kumari, J.R. Grandis, S.M. Thomas, Collagen type XI α1 facilitates head and neck squamous cell cancer growth and invasion, Br. J. Cancer 109 (2013) 3049–3056, https://doi.org/10.1038/bjc.2013.624.
[40] C.E. Schmalbach, D.B. Chepeha, T.J. Giordano, M.A. Rubin, T.N. Teknos, C. R. Bradford, G.T. Wolf, R. Kuick, D.E. Misek, D.K. Trask, S. Hanash, Molecular Profiling and the Identification of Genes Associated with Metastatic Oral Cavity/

Pharynx Squamous Cell Carcinoma, Arch. Otolaryngol. - Head. Neck Surg. 130 (2004) 295–302, https://doi.org/10.1001/archotol.130.3.295.
[41] F. Vázquez-Villa, M. García-Ocaña, J.A. Galván, J. García-Martínez, C. García-Pravia, P. Menéndez-Rodríguez, C.G. del Rey, L. Barneo-Serra, J.R. de los Toyos, COL11A1/(pro)collagen 11A1 expression is a remarkable biomarker of human invasive carcinoma-associated stromal cells and carcinoma progression, Tumor Biol. 36 (2015) 2213–2222, https://doi.org/10.1007/s13277-015-3295-4.
[42] K. Kumari, S. Ghosh, S. Patil, D. Augustine, S. Samudrala Venkatesiah, R.S. Rao, Expression of type III collagen correlates with poor prognosis in oral squamous cell carcinoma, J. Investig. Clin. Dent. 8 (2017), https://doi.org/10.1111/jicd.12253.
[43] S.L. Lai, M.L. Tan, R.J. Hollows, M. Robinson, M. Ibrahim, S. Margielewska, E. K. Parkinson, A. Ramanathan, R.B. Zain, H. Mehanna, R.J. Spruce, W. Wei, I. Chung, P.G. Murray, L.F. Yap, I.C. Paterson, Collagen induces a more proliferative, migratory and chemoresistant phenotype in head and neck cancer via DDR1, Cancers (Basel) 11 (2019) 1766, https://doi.org/10.3390/cancers11111766.
[44] D.E. Johnson, B. Burtness, C.R. Leemans, V.W.Y. Lui, J.E. Bauman, J.R. Grandis, Head and neck squamous cell carcinoma, Nat. Rev. Dis. Prim. 6 (2020) 92, https://doi.org/10.1038/s41572-020-00224-3.
[45] D.A. Beacham, E. Cukierman, Stromagenesis: The changing face of fibroblastic microenvironments during tumor progression, Semin. Cancer Biol. 15 (2005) 329–341, https://doi.org/10.1016/j.semcancer.2005.05.003.
[46] N.A. Bhowmick, E.G. Neilson, H.L. Moses, Stromal fibroblasts in cancer initiation and progression, Nature 432 (2004) 332–337, https://doi.org/10.1038/nature03096.
[47] D.W. Jung, Z.M. Che, J. Kim, K. Kim, K.Y. Kim, D. Williams, J. Kim, Tumor-stromal crosstalk in invasion of oral squamous cell carcinoma: A pivotal role of CCL7, Int. J. Cancer 127 (2010) 332–344, https://doi.org/10.1002/ijc.25060.
[48] H. Li, J. Zhang, S.W. Chen, L. lu Liu, L. Li, F. Gao, S.M. Zhuang, L. ping Wang, Y. Li, M. Song, Cancer-associated fibroblasts provide a suitable microenvironment for tumor development and progression in oral tongue squamous cancer, J. Transl. Med 13 (2015) 198, https://doi.org/10.1186/s12967-015-0551-8.
[49] L.Y. Wei, J.J. Lee, C.Y. Yeh, C.J. Yang, S.H. Kok, J.Y. Ko, F.C. Tsai, J.S. Chia, Reciprocal activation of cancer-associated fibroblasts and oral squamous carcinoma cells through CXCL1, Oral. Oncol. 88 (2019) 115–123, https://doi.org/10.1016/j.oraloncology.2018.11.002.
[50] Y.H. Huang, C.Y. Chang, Y.Z. Kuo, W.Y. Fang, H.Y. Kao, S.T. Tsai, L.W. Wu, Cancer-associated fibroblast-derived interleukin-1β activates protumor C-C motif chemokine ligand 22 signaling in head and neck cancer, Cancer Sci. 110 (2019) 2783–2793, https://doi.org/10.1111/cas.14135.
[51] S.F. Chen, S. Nieh, S.W. Jao, M.Z. Wu, C.L. Liu, Y.C. Chang, Y.S. Lin, The paracrine effect of cancer-associated fibroblast-induced interleukin-33 regulates the invasiveness of head and neck squamous cell carcinoma, J. Pathol. 231 (2013) 180–189, https://doi.org/10.1002/path.4226.
[52] Y.S. Hwang, K.K. Park, W.Y. Chung, Stromal transforming growth factor-beta 1 is crucial for reinforcing the invasive potential of low invasive cancer, Arch. Oral. Biol. 59 (2014) 687–694, https://doi.org/10.1016/j.archoralbio.2014.03.017.
[53] X. Qin, M. Yan, X. Wang, Q. Xu, X. Wang, X. Zhu, J. Shi, Z. Li, J. Zhang, W. Chen, Cancer-associated Fibroblast-derived IL-6 promotes head and neck cancer progression via the osteopontin-NF-kappa B signaling pathway, Theranostics 8 (2018) 921–940, https://doi.org/10.7150/thno.22182.
[54] G.S. Karagiannis, T. Poutahidis, S.E. Erdman, R. Kirsch, R.H. Riddell, E. P. Diamandis, Cancer-associated fibroblasts drive the progression of metastasis through both paracrine and mechanical pressure on cancer tissue, Mol. Cancer Res 10 (2012) 1403–1418, https://doi.org/10.1158/1541-7786.MCR-12-0307.
[55] L. Moro, M. Colombi, M.P.M. Tosatti, S. Barlati, Study of fibronectin mRNA in human laryngeal and ectocervical carcinomas by IN SITU hybridization and image analysis, Int. J. Cancer 51 (1992) 692–697, https://doi.org/10.1002/ijc.2910510505.
[56] S. Gopal, L. Veracini, D. Grall, C. Butori, S. Schaub, S. Audebert, L. Camoin, E. Baudelet, A. Adwanska, S. Beghelli-De La Forest Divonne, S.M. Violette, P. H. Weinreb, S. Rekima, M. Ilie, A. Sudaka, P. Hofman, E. Van Obberghen-Schilling, Fibronectin-guided migration of carcinoma collectives, Nat. Commun. 8 (2017) 14105, https://doi.org/10.1038/ncomms14105.
[57] P. Inki, H. Joensuu, R. Grénman, P. Klemi, M. Jalkanen, Association between syndecan-1 expression and clinical outcome in squamous cell carcinoma of the head and neck, Br. J. Cancer 70 (1994) 319–323, https://doi.org/10.1038/bjc.1994.300.
[58] E.L. Rosenthal, W. Zhang, M. Talbert, K.P. Raisch, G.E. Peters, Extracellular matrix metalloprotease inducer-expressing head and neck squamous cell carcinoma cells promote fibroblast-mediated type I collagen degradation in vitro, Mol. Cancer Res 3 (2005) 195–202, https://doi.org/10.1158/1541-7786.mcr-04-0203.
[59] Z. Zhang, D. Tao, P. Zhang, X. Liu, Y. Zhang, J. Cheng, H. Yuan, L. Liu, H. Jiang, Hyaluronan synthase 2 expressed by cancer-associated fibroblasts promotes oral cancer invasion, J. Exp. Clin. Cancer Res 35 (2016) 181, https://doi.org/10.1186/s13046-016-0458-0.
[60] Y. Hassona, N. Cirillo, K. Heesom, E.K. Parkinson, S.S. Prime, Senescent cancer-associated fibroblasts secrete active MMP-2 that promotes keratinocyte dis-cohesion and invasion, Br. J. Cancer 111 (2014) 1230–1237, https://doi.org/10.1038/bjc.2014.438.
[61] Y. Tokumaru, M. Fujii, Y. Otani, K. Kameyama, Y. Imanishi, N. Igarashi, J. Kanzaki, Activation of matrix metalloproteinase-2 in head and neck squamous cell carcinoma: Studies of clinical samples and in vitro cell lines co-cultured with fibroblasts, Cancer Lett. 150 (2000) 15–21, https://doi.org/10.1016/S0304-3835(99)00371-7.
[62] S. Koontongkaew, P. Amornphimoltham, P. Monthanpisut, T. Saensuk, M. Leelakriangsak, Fibroblasts and extracellular matrix differently modulate MMP activation by primary and metastatic head and neck cancer cells, Med. Oncol. 29 (2012) 690–703, https://doi.org/10.1007/s12032-011-9871-6.
[63] B. Horváth, H. Hegyesi, P. Nagy, A. Falus, Z. Schaff, Expression of Ets-1 transcription factor in human head and neck squamous cell carcinoma and effect of histamine on metastatic potential of invasive tumor through the regulation of expression of Ets-1 and matrix metalloproteinase-3, Head. Neck 27 (2005) 585–596, https://doi.org/10.1002/hed.20188.
[64] N. Johansson, K. Airola, R. Grénman, A.L. Kariniemi, U. Saarialho-Kere, V. M. Kähäri, Expression of collagenase-3 (matrix metalloproteinase-13) in squamous cell carcinomas of the head and neck, Am. J. Pathol. 151 (1997) 499–508.
[65] C. Gaggioli, S. Hooper, C. Hidalgo-Carcedo, R. Grosse, J.F. Marshall, K. Harrington, E. Sahai, Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells, Nat. Cell Biol. 9 (2007) 1392–1400, https://doi.org/10.1038/ncb1658.
[66] A. Labernadie, T. Kato, A. Brugués, X. Serra-Picamal, S. Derzsi, E. Arwert, A. Weston, V. González-Tarragó, A. Elosegui-Artola, L. Albertazzi, J. Alcaraz, P. Roca-Cusachs, E. Sahai, X. Trepat, A mechanically active heterotypic E-cadherin/N-cadherin adhesion enables fibroblasts to drive cancer cell invasion, Nat. Cell Biol. 19 (2017) 224–237, https://doi.org/10.1038/ncb3478.
[67] L.C. Kelley, A.G. Ammer, K.E. Hayes, K.H. Martin, K. Machida, L. Jia, B.J. Mayer, S.A. Weed, Oncogenic Src requires a wild-type counterpart to regulate invadopodia maturation, J. Cell Sci. 123 (2010) 3923–3932, https://doi.org/10.1242/jcs.075200.
[68] A. Gatesman Ammer, L.C. Kelley, K.E. Hayes, J.V. Evans, L. Ann Lopez-Skinner, K. H. Martin, B. Frederick, B.L. Rothschild, D. Raben, P. Elvin, T.P. Green, S. A. Weed, Saracatinib impairs head and neck squamous cell carcinoma invasion by disrupting invadopodia function, J. Cancer Sci. Ther. 1 (2009) 52–61, https://doi.org/10.4172/1948-5956.1000009.
[69] D. Quandt, E. Fiedler, D. Boettcher, W.C. Marsch, B. Seliger, B7-H4 expression in human melanoma: Its association with patients' survival and antitumor immune response, Clin. Cancer Res 17 (2011) 3100–3111, https://doi.org/10.1158/1078-0432.CCR-10-2268.
[70] M.G. Fury, S. Baxi, R. Shen, K.W. Kelly, B.L. Lipson, D. Carlson, H. Stambuk, S. Haque, D.G. Pfister, Phase II study of saracatinib (AZD0530) for patients with recurrent or metastatic head and neck squamous cell carcinoma (HNSCC), Anticancer Res 31 (2011) 249–253.
[71] F. Hermida-Prado, M.Á. Villaronga, R. Granda-Díaz, N. Del-Río-Ibisate, L. Santos, M.A. Hermosilla, P. Oro, E. Allonca, J. Agorreta, I. Garmendia, J. Tornín, J. Perez-Escuredo, R. Fuente, L.M. Montuenga, F. Morís, J.P. Rodrigo, R. Rodríguez, J. M. García-Pedrero, The SRC inhibitor dasatinib induces stem cell-like properties in head and neck cancer cells that are effectively counteracted by the mithralog EC-8042, J. Clin. Med 8 (2019) 1157, https://doi.org/10.3390/jcm8081157.
[72] A.S. Tsao, S. Liu, J. Fujimoto, I.I. Wistuba, J.J. Lee, E.M. Marom, C. Charnsangavej, F.V. Fossella, H.T. Tran, G.R. Blumenschein, V. Papadimitrakopoulou, M.S. Kies, W.K. Hong, D.J. Stewart, Phase II trials of imatinib mesylate and docetaxel in patients with metastatic non-small cell lung cancer and head and neck squamous cell carcinoma, J. Thorac. Oncol. 6 (2011) 2104–2111, https://doi.org/10.1097/JTO.0b013e31822e7256.
[73] M.G. Fury, E. Sherman, D. Lisa, N. Agarwal, K. Algazy, B. Brockstein, C. Langer, D. Lim, R. Mehra, S.K. Rajan, S. Korte, B. Lipson, F. Yunus, T. Tanvetyanon, S. Smith-Marrone, K. Ng, H. Xiao, S. Haque, D.G. Pfister, A randomized phase II study of cetuximab every 2 weeks at either 500 or 750 mg/m2 for patients with recurrent or metastatic head and neck squamous cell cancer, JNCCN J. Natl. Compr. Cancer Netw. 10 (2012) 1391–1398, https://doi.org/10.6004/jnccn.2012.0144.
[74] M. Merlano, E. Russi, M. Benasso, R. Corvò, I. Colantonio, R. Vigna-Taglianti, V. Vigo, A. Bacigalupo, G. Numico, N. Crosetto, M. Gasco, C. Lo Nigro, R. Vitiello, S. Violante, O. Garrone, Cisplatin-based chemoradiation plus cetuximab in locally advanced head and neck cancer: A phase II clinical study, Ann. Oncol. 22 (2011) 712–717, https://doi.org/10.1093/annonc/mdq412.
[75] L. Prieto-Fernandez, M. de los, A. Villaronga, F. Hermida-Prado, M. Hijazi, I. Montoro-Jimenez, M. Pevida, S. Llames, J.P. Rodrigo, P. Cutillas, F. Calvo, J. M. Garcia-Pedrero, S. Alvarez-Teijeiro, Driving role of head and neck cancer cell secretome on the invasion of stromal fibroblasts: Mechanistic insights by phosphoproteomics, Biomed. Pharmacother. 158 (2023), 114176, https://doi.org/10.1016/j.biopha.2022.114176.
[76] P. Valent, D. Bonnet, R. De Maria, T. Lapidot, M. Copland, J.V. Melo, C. Chomienne, F. Ishikawa, J.J. Schuringa, G. Stassi, B. Huntly, H. Herrmann, J. Soulier, A. Roesch, G.J. Schuurhuis, S. Wöhrer, M. Arock, J. Zuber, S. Cerny-Reiterer, H.E. Johnsen, M. Andreeff, C. Eaves, Cancer stem cell definitions and terminology: The devil is in the details, Nat. Rev. Cancer 12 (2012) 767–775, https://doi.org/10.1038/nrc3368.
[77] K. Chen, Y.H. Huang, J.L. Chen, Understanding and targeting cancer stem cells: therapeutic implications and challenges, Acta Pharmacol. Sin. 34 (2013) 732–740, https://doi.org/10.1038/aps.2013.27.
[78] B. Yu, K. Wu, X. Wang, J. Zhang, L. Wang, Y. Jiang, X. Zhu, W. Chen, M. Yan, Periostin secreted by cancer-associated fibroblasts promotes cancer stemness in head and neck cancer by activating protein tyrosine kinase 7, Cell Death Dis. 9 (2018) 1082, https://doi.org/10.1038/s41419-018-1116-6.
[79] X. Li, W. Bu, L. Meng, X. Liu, S. Wang, L. Jiang, M. Ren, Y. Fan, H. Sun, CXCL12/CXCR4 pathway orchestrates CSC-like properties by CAF recruited tumor

associated macrophage in OSCC, Exp. Cell Res. 378 (2019) 131–138, https://doi.org/10.1016/j.yexcr.2019.03.013.
[80] P.N. Le, S.B. Keysar, B. Miller, J.R. Eagles, T.S. Chimed, J. Reisinger, K.E. Gomez, C. Nieto, B.C. Jackson, H.L. Somerset, J.J. Morton, X.J. Wang, A. Jimeno, Wnt signaling dynamics in head and neck squamous cell cancer tumor-stroma interactions, Mol. Carcinog. 58 (2019) 398–410, https://doi.org/10.1002/mc.22937.
[81] S. Krishnamurthy, K.A. Warner, Z. Dong, A. Imai, C. Nör, B.B. Ward, J.I. Helman, R.S. Taichman, E.L. Bellile, L.K. McCauley, P.J. Polverini, M.E. Prince, M. S. Wicha, J.E. Nör, Endothelial interleukin-6 defines the tumorigenic potential of primary human cancer stem cells, Stem Cells 32 (2014) 2845–2857, https://doi.org/10.1002/stem.1793.
[82] S. Álvarez-Teijeiro, C. García-Inclán, M.Á. Villaronga, P. Casado, F. Hermida-Prado, R. Granda-Díaz, J.P. Rodrigo, F. Calvo, N. Del-Río-ibisate, A. Gandarillas, F. Morís, M. Hermsen, P. Cutillas, J.M. García-Pedrero, Factors secreted by cancer-associated fibroblasts that sustain cancer stem properties in head and neck squamous carcinoma cells as potential therapeutic targets, Cancers (Basel) 10 (2018) 334, https://doi.org/10.3390/cancers10090334.
[83] E. Donnarumma, D. Fiore, M. Nappa, G. Roscigno, A. Adamo, M. Iaboni, V. Russo, A. Affinito, I. Puoti, C. Quintavalle, A. Rienzo, S. Piscuoglio, R. Thomas, G. Condorelli, Cancer-associated fibroblasts release exosomal microRNAs that dictate an aggressive phenotype in breast cancer, Oncotarget 8 (2017) 19592–19608, https://doi.org/10.18632/oncotarget.14752.
[84] M. Shimoda, S. Principe, H.W. Jackson, V. Luga, H. Fang, S.D. Molyneux, Y. W. Shao, A. Aiken, P.D. Waterhouse, C. Karamboulas, F.M. Hess, T. Ohtsuka, Y. Okada, L. Ailles, A. Ludwig, J.L. Wrana, T. Kislinger, R. Khokha, Loss of the Timp gene family is sufficient for the acquisition of the CAF-like cell state, Nat. Cell Biol. 16 (2014) 889–901, https://doi.org/10.1038/ncb3021.
[85] L. Vermeulen, F. De Sousa, E. Melo, M. Van Der Heijden, K. Cameron, J.H. De Jong, T. Borovski, J.B. Tuynman, M. Todaro, C. Merz, H. Rodermond, M. R. Sprick, K. Kemper, D.J. Richel, G. Stassi, J.P. Medema, Wnt activity defines colon cancer stem cells and is regulated by the microenvironment, Nat. Cell Biol. 12 (2010) 468–476, https://doi.org/10.1038/ncb2048.
[86] E. Giannoni, F. Bianchini, L. Masieri, S. Serni, E. Torre, L. Calorini, P. Chiarugi, Reciprocal activation of prostate cancer cells and cancer-associated fibroblasts stimulates epithelial-mesenchymal transition and cancer stemness, Cancer Res 70 (2010) 6945–6956, https://doi.org/10.1158/0008-5472.CAN-10-0785.
[87] C.L. Chaffer, I. Brueckmann, C. Scheel, A.J. Kaestli, P.A. Wiggins, L.O. Rodrigues, M. Brooks, F. Reinhardt, Y. Suc, K. Polyak, L.M. Arendt, C. Kuperwasser, B. Bierie, R.A. Weinberg, Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state, Proc. Natl. Acad. Sci. U. S. A 108 (2011) 7950–7955, https://doi.org/10.1073/pnas.1102454108.
[88] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: The next generation, Cell 144 (2011) 646–674, https://doi.org/10.1016/j.cell.2011.02.013.
[89] U.E. Martinez-Outschoorn, M.P. Lisanti, F. Sotgia, Catabolic cancer-associated fibroblasts transfer energy and biomass to anabolic cancer cells, fueling tumor growth, Semin. Cancer Biol. 25 (2014) 47–60, https://doi.org/10.1016/j.semcancer.2014.01.005.
[90] S. Pavlides, D. Whitaker-Menezes, R. Castello-Cros, N. Flomenberg, A. K. Witkiewicz, P.G. Frank, M.C. Casimiro, C. Wang, P. Fortina, S. Addya, R. G. Pestell, U.E. Martinez-Outschoorn, F. Sotgia, M.P. Lisanti, The reverse Warburg effect: Aerobic glycolysis in cancer associated fibroblasts and the tumor stroma, Cell Cycle 8 (2009) 3984–4001, https://doi.org/10.4161/cc.8.23.10238.
[91] X. Zhang, Y. Dong, M. Zhao, L. Ding, X. Yang, Y. Jing, Y. Song, S. Chen, Q. Hu, Y. Ni, ITGB2-mediated metabolic switch in CAFs promotes OSCC proliferation by oxidation of NADH in mitochondrial oxidative phosphorylation system, Theranostics 10 (2020) 12044–12059, https://doi.org/10.7150/thno.47901.
[92] M. Custódio, A. Biddle, M. Tavassoli, Portrait of a CAF: The story of cancer-associated fibroblasts in head and neck cancer, Oral. Oncol. 110 (2020), 104972, https://doi.org/10.1016/j.oraloncology.2020.104972.
[93] D. Kumar, J. New, V. Vishwakarma, R. Joshi, J. Enders, F. Lin, S. Dasari, W. R. Gutierrez, G. Leef, S. Ponnurangam, H. Chavan, L. Ganaden, M.M. Thornton, H. Dai, O. Tawfik, J. Straub, Y. Shnayder, K. Kakarala, T.T. Tsue, D.A. Girod, B. Van Houten, S. Anant, P. Krishnamurthy, S.M. Thomas, Cancer-associated fibroblasts drive glycolysis in a targetable signaling loop implicated in head and neck squamous cell carcinoma progression, Cancer Res 78 (2018) 3769–3782, https://doi.org/10.1158/0008-5472.CAN-17-1076.
[94] Z. Zhang, Z. Gao, S. Rajthala, D. Sapkota, H. Dongre, H. Parajuli, S. Suliman, R. Das, L. Li, L.A. Bindoff, D.E. Costea, X. Liang, Metabolic reprogramming of normal oral fibroblasts correlated with increased glycolytic metabolism of oral squamous cell carcinoma and precedes their activation into carcinoma associated fibroblasts, Cell. Mol. Life Sci. 77 (2020) 1115–1133, https://doi.org/10.1007/s00018-019-03209-y.
[95] E. Jiang, Z. Xu, M. Wang, T. Yan, C. Huang, X. Zhou, Q. Liu, L. Wang, Y. Chen, H. Wang, K. Liu, Z. Shao, Z. Shang, Tumoral microvesicle–activated glycometabolic reprogramming in fibroblasts promotes the progression of oral squamous cell carcinoma, FASEB J. 33 (2019) 5690–5703, https://doi.org/10.1096/fj.201802226R.
[96] J. Wu, Y. Hong, T. Wu, J. Wang, X. Chen, Z. Wang, B. Cheng, J. Xia, Stromal-epithelial lactate shuttle induced by tumor-derived interleukin-1β promotes cell proliferation in oral squamous cell carcinoma, Int. J. Mol. Med 41 (2018) 687–696, https://doi.org/10.3892/ijmm.2017.3267.
[97] J.M. Curry, M. Tuluc, D. Whitaker-Menezes, J.A. Ames, A. Anantharaman, A. Butera, B. Leiby, D. Cognetti, F. Sotgia, M.P. Lisanti, U.E. Martinez-Outschoorn, Cancer metabolism, stemness and tumor recurrence: MCT1 and MCT4 are functional biomarkers of metabolic symbiosis in head and neck cancer, Cell Cycle 12 (2013) 1371–1384, https://doi.org/10.4161/cc.24092.
[98] T. Bertero, W.M. Oldham, E.M. Grasset, I. Bourget, E. Boulter, S. Pisano, P. Hofman, F. Bellvert, G. Meneguzzi, D.V. Bulavin, S. Estrach, C.C. Feral, S. Y. Chan, A. Bozec, C. Gaggioli, Tumor-Stroma Mechanics Coordinate Amino Acid Availability to Sustain Tumor Growth and Malignancy, Cell Metab. 29 (2019) 124–140, https://doi.org/10.1016/j.cmet.2018.09.012.
[99] J.M. Heddleston, Z. Li, J.D. Lathia, S. Bao, A.B. Hjelmeland, J.N. Rich, Hypoxia inducible factors in cancer stem cells, Br. J. Cancer 102 (2010) 789–795, https://doi.org/10.1038/sj.bjc.6605551.
[100] B. Peltanova, M. Raudenska, M. Masarik, Effect of tumor microenvironment on pathogenesis of the head and neck squamous cell carcinoma: A systematic review, Mol. Cancer 18 (2019) 63, https://doi.org/10.1186/s12943-019-0983-5.
[101] G. Zhu, F. Peng, W. Gong, L. She, M. Wei, H. Tan, C. Chen, D. Zhang, G. Li, D. Huang, X. Zhang, Y. Liu, Hypoxia promotes migration/invasion and glycolysis in head and neck squamous cell carcinoma via an HIF-1α-MTDH loop, Oncol. Rep. 38 (2017) 2893–2900, https://doi.org/10.3892/or.2017.5949.
[102] K. Mortezaee, Hypoxia induces core-to-edge transition of progressive tumoral cells: a critical review on differential yet corroborative roles for HIF-1α and HIF-2α, Life Sci. 242 (2020), 117145 https://doi.org/10.1016/j.lfs.2019.117145.
[103] M. Peiris-Pagès, F. Sotgia, M. Lisanti, Chemotherapy induces the cancer-associated fibroblast phenotype, activating paracrine Hedgehog-GLI signalling in breast cancer cells, Oncotarget 6 (2015) 10728–10745, https://doi.org/10.18632/oncotarget.3828.
[104] F. Cavallo, C. De Giovanni, P. Nanni, G. Forni, P.L. Lollini, 2011: The immune hallmarks of cancer, Cancer Immunol. Immunother. 60 (2011) 319–326, https://doi.org/10.1007/s00262-010-0968-0.
[105] A. Elmusrati, J. Wang, C.Y. Wang, Tumor microenvironment and immune evasion in head and neck squamous cell carcinoma, Int. J. Oral. Sci. 13 (2021) 1–11, https://doi.org/10.1038/s41368-021-00131-7.
[106] A. Chakravarthy, L. Khan, N.P. Bensler, P. Bose, D.D.De Carvalho, TGF-β-associated extracellular matrix genes link cancer-associated fibroblasts to immune evasion and immunotherapy failure, Nat. Commun. 9 (2018) 1–10, https://doi.org/10.1038/s41467-018-06654-8.
[107] B.C. Özdemir, T. Pentcheva-Hoang, J.L. Carstens, X. Zheng, C.C. Wu, T. R. Simpson, H. Laklai, H. Sugimoto, C. Kahlert, S.V. Novitskiy, A. DeJesus-Acosta, P. Sharma, P. Heidari, U. Mahmood, L. Chin, H.L. Moses, V.M. Weaver, A. Maitra, J.P. Allison, V.S. LeBleu, R. Kalluri, Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival, Cancer Cell 25 (2014) 719–734, https://doi.org/10.1016/j.ccr.2014.04.005.
[108] S.V. Puram, I. Tirosh, A.S. Parikh, A.P. Patel, K. Yizhak, S. Gillespie, C. Rodman, C.L. Luo, E.A. Mroz, K.S. Emerick, D.G. Deschler, M.A. Varvares, R. Mylvaganam, O. Rozenblatt-Rosen, J.W. Rocco, W.C. Faquin, D.T. Lin, A. Regev, B.E. Bernstein, Single-cell transcriptomic analysis of primary and metastatic tumor ecosystems in head and neck cancer, e24, Cell 171 (2017) 1611–1624, https://doi.org/10.1016/j.cell.2017.10.044.
[109] A. Obradovic, D. Graves, M. Korrer, Y. Wang, S. Roy, A. Naveed, Y. Xu, A. Luginbuhl, J. Curry, M. Gibson, K. Idrees, P. Hurley, P. Jiang, X.S. Liu, R. Uppaluri, C.G. Drake, A. Califano, Y.J. Kim, Immunostimulatory Cancer-Associated Fibroblast Subpopulations Can Predict Immunotherapy Response in Head and Neck Cancer, Clin. Cancer Res 28 (2022) 2094–2109.
[110] D.E. Kuczek, A.M.H. Larsen, M.L. Thorseth, M. Carretta, A. Kalvisa, M.S. Siersbæk, A.M.C. Simões, A. Roslind, L.H. Engelholm, E. Noessner, M. Donia, I.M. Svane, P. T. Straten, L. Grøntved, D.H. Madsen, Collagen density regulates the activity of tumor-infiltrating T cells, J. Immunother. Cancer 7 (2019) 1–15, https://doi.org/10.1186/s40425-019-0556-6.
[111] Y. Geng, Y. Shao, W. He, W. Hu, Y. Xu, J. Chen, C. Wu, J. Jiang, Prognostic role of tumor-infiltrating lymphocytes in lung cancer: A meta-analysis, Cell. Physiol. Biochem. 37 (2015) 1560–1571, https://doi.org/10.1159/000438523.
[112] L. Guerra, T. Odorisio, G. Zambruno, D. Castiglia, Stromal microenvironment in type VII collagen-deficient skin: The ground for squamous cell carcinoma development, Matrix Biol. 63 (2017) 1–10, https://doi.org/10.1016/j.matbio.2017.01.002.
[113] J.A. Tice, E.S. O'Meara, D.L. Weaver, C. Vachon, R. Ballard-Barbash, K. Kerlikowske, Benign breast disease, mammographic breast density, and the risk of breast cancer, J. Natl. Cancer Inst. 105 (2013) 1043–1049, https://doi.org/10.1093/jnci/djt124.
[114] H.O. Lee, S.R. Mullins, J. Franco-Barraza, M. Valianou, E. Cukierman, J.D. Cheng, FAP-overexpressing fibroblasts produce an extracellular matrix that enhances invasive velocity and directionality of pancreatic cancer cells, BMC Cancer 11 (2011) 245, https://doi.org/10.1186/1471-2407-11-245.
[115] R. Derakhshandeh, S. Sanadhya, K.L. Han, H. Chen, O. Goloubeva, T.J. Webb, R. H. Younis, Semaphorin 4D in human head and neck cancer tissue and peripheral blood: A dense fibrotic peri-tumoral stromal phenotype, Oncotarget 9 (2018) 11126–11144, https://doi.org/10.18632/oncotarget.24277.
[116] H. Takahashi, K. Sakakura, T. Kudo, M. Toyoda, K. Kaira, T. Oyama, K. Chikamatsu, Cancer-associated fibroblasts promote an immunosuppressive microenvironment through the induction and accumulation of protumoral macrophages, Oncotarget 8 (2017) 8633–8647, https://doi.org/10.18632/oncotarget.14374.
[117] P. Chomarat, J. Banchereau, J. Davoust, A.K. Palucka, IL-6 switches the differentiation of monocytes from dendritic cells to macrophages, Nat. Immunol. 1 (2000) 510–514, https://doi.org/10.1038/82763.
[118] W.J. Chen, W. Jin, N. Hardegen, K.J. Lei, L. Li, N. Marinos, G. McGrady, S. M. Wahl, Conversion of Peripheral CD4+CD25- Naive T Cells to CD4+CD25+

Regulatory T Cells by TGF-β Induction of Transcription Factor Foxp3, J. Exp. Med 198 (2003) 1875–1886, https://doi.org/10.1084/jem.20030152.
[119] S. Schipmann, K. Wermker, H.J. Schulze, J. Kleinheinz, G. Brunner, Cutaneous and oral squamous cell carcinoma-dual immunosuppression via recruitment of FOXP3+ regulatory T cells and endogenous tumour FOXP3 expression? J. Cranio-Maxillofac. Surg. 42 (2014) 1827–1833, https://doi.org/10.1016/j.jcms.2014.06.022.
[120] H. Takahashi, K. Sakakura, R. Kawabata-Iwakawa, S. Rokudai, M. Toyoda, M. Nishiyama, K. Chikamatsu, Immunosuppressive activity of cancer-associated fibroblasts in head and neck squamous cell carcinoma, Cancer Immunol. Immunother. 64 (2015) 1407–1417, https://doi.org/10.1007/s00262-015-1742-0.
[121] P. Economopoulou, I. Kotsantis, A. Psyrri, Tumor microenvironment and immunotherapy response in head and neck cancer, Cancers (Basel) 12 (2020) 3377, https://doi.org/10.3390/cancers12113377.
[122] R. Kalluri, The biology and function of fibroblasts in cancer, Nat. Rev. Cancer 16 (2016) 582–598, https://doi.org/10.1038/nrc.2016.73.
[123] M. De Palma, D. Biziato, T.V. Petrova, Microenvironmental regulation of tumour angiogenesis, Nat. Rev. Cancer 17 (2017) 457–474, https://doi.org/10.1038/nrc.2017.51.
[124] M. Yamauchi, D.L. Gibbons, C. Zong, J.J. Fradette, N. Bota-Rabassedas, J. M. Kurie, Fibroblast heterogeneity and its impact on extracellular matrix and immune landscape remodeling in cancer, Matrix Biol. 91– 92 (2020) 8–18, https://doi.org/10.1016/j.matbio.2020.05.001.
[125] K. Kayamori, K.I. Katsube, K. Sakamoto, Y. Ohyama, H. Hirai, A. Yukimori, Y. Ohata, T. Akashi, M. Saitoh, K. Harada, H. Harada, A. Yamaguchi, Notch3 is induced in cancer-associated fibroblasts and promotes angiogenesis in oral squamous cell carcinoma, PLoS One 11 (2016), https://doi.org/10.1371/journal.pone.0154112.
[126] G.E. Romanos, K.P. Asnani, D. Hingorani, V.L. Deshmukh, Periostin: role in formation and maintenance of dental tissues, J. Cell. Physiol. 229 (2014) 1–5, https://doi.org/10.1002/jcp.24407.
[127] N. Ohta, A. Ishida, K. Kurakami, Y. Suzuki, S. Kakehata, J. Ono, H. Ikeda, K. Okubo, K. Izuhara, Expressions and roles of periostin in otolaryngological diseases, Allergol. Int. 63 (2014) 171–180, https://doi.org/10.2332/allergolint.13-RAI-0673.
[128] X. Qin, M. Yan, J. Zhang, X. Wang, Z. Shen, Z. Lv, Z. Li, W. Wei, W. Chen, TGFβ3-mediated induction of Periostin facilitates head and neck cancer growth and is associated with metastasis, Sci. Rep. 6 (2016) 20587, https://doi.org/10.1038/srep20587.
[129] B.S.M.S. Siriwardena, Y. Kudo, I. Ogawa, M. Kitagawa, S. Kitajima, H. Hatano, W. M. Tilakaratne, M. Miyauchi, T. Takata, Periostin is frequently overexpressed and enhances invasion and angiogenesis in oral cancer, Br. J. Cancer 95 (2006) 1396–1403, https://doi.org/10.1038/sj.bjc.6603431.
[130] Y. Kudo, S. Iizuka, M. Yoshida, T. Tsunematsu, T. Kondo, A. Subarnbhesaj, E. M. Deraz, S.B.S.M. Siriwardena, H. Tahara, N. Ishimaru, I. Ogawa, T. Takata, Matrix metalloproteinase-13 (MMP-13) directly and indirectly promotes tumor angiogenesis, J. Biol. Chem. 287 (2012) 38716–38728, https://doi.org/10.1074/jbc.M112.373159.
[131] N.N. Lin, P. Wang, D. Zhao, F.J. Zhang, K. Yang, R. Chen, Significance of oral cancer-associated fibroblasts in angiogenesis, lymphangiogenesis, and tumor invasion in oral squamous cell carcinoma, J. Oral. Pathol. Med 46 (2017) 21–30, https://doi.org/10.1111/jop.12452.
[132] J.M.F. Mendes, L. de, F. Valverde, M.T.A. Vidal, B.D. Paredes, P. Coelho, K. J. Allahdadi, R. Della Coletta, B.S. de, F. Souza, C.A.G. Rocha, Effects of igf-1 on proliferation, angiogenesis, tumor stem cell populations and activation of akt and hedgehog pathways in oral squamous cell carcinoma, Int. J. Mol. Sci. 21 (2020) 6487, https://doi.org/10.3390/ijms21186487.
[133] N. Bandoh, T. Hayashi, M. Takahara, K. Kishibe, T. Ogino, A. Katayama, M. Imada, S. Nonaka, Y. Harabuchi, VEGF and bFGF expression and microvessel density of maxillary sinus squamous cell carcinoma in relation to p53 status, spontaneous apoptosis and prognosis, Cancer Lett. 208 (2004) 215–225, https://doi.org/10.1016/j.canlet.2003.11.031.
[134] M. Mirkeshavarz, M. Ganjibakhsh, P. Aminishakib, P. Farzaneh, N. Mahdavi, F. Vakhshiteh, A. Karimi, N.S. Gohari, F. Kamali, M.J. Kharazifard, S.A.S. Fazeli, A. Nasimian, Interleukin-6 secreted by oral cancer- associated fibroblast accelerated VEGF expression in tumor and stroma cells, Cell. Mol. Biol. 63 (2017) 131–136, https://doi.org/10.14715/cmb/2017.63.10.21.
[135] K. Noma, K.S.M. Smalley, M. Lioni, Y. Naomoto, N. Tanaka, W. El-Deiry, A. J. King, H. Nakagawa, M. Herlyn, The Essential Role of Fibroblasts in Esophageal Squamous Cell Carcinoma-Induced Angiogenesis, Gastroenterology 134 (2008) 1981–1993, https://doi.org/10.1053/j.gastro.2008.02.061.
[136] J. Li, X. Liu, S. Zang, J. Zhou, F. Zhang, B. Sun, D. Qi, X. Li, J. Kong, D. Jin, X. Yang, Y. Luo, Y. Lu, B. Lin, W. Niu, T. Liu, Small extracellular vesicle-bound vascular endothelial growth factor secreted by carcinoma-associated fibroblasts promotes angiogenesis in a bevacizumab-resistant manner, Cancer Lett. 492 (2020) 71–83, https://doi.org/10.1016/j.canlet.2020.08.030.
[137] B. Zhou, X.M. Zhuang, Y.Y. Wang, Z.Y. Lin, D.M. Zhang, S. Fan, J.S. Li, W.L. Chen, Tumor necrosis factor α induces myofibroblast differentiation in human tongue cancer and promotes invasiveness and angiogenesis via secretion of stromal cell-derived factor-1, Oral. Oncol. 51 (2015) 1095–1102, https://doi.org/10.1016/j.oraloncology.2015.08.017.
[138] K.C. Li, Y.H. Huang, C.Y. Ho, C.Y. Chu, S.T. Cha, H.H. Tsai, J.Y. Ko, C.C. Chang, C. T. Tan, The role of IL-8 in the SDF-1α/CXCR4-induced angiogenesis of laryngeal and hypopharyngeal squamous cell carcinoma, Oral. Oncol. 48 (2012) 507–515, https://doi.org/10.1016/j.oraloncology.2012.01.006.
[139] T. Ishikawa, K.I. Nakashiro, S. Hara, S.K. Klosek, C. Li, S. Shintani, H. Hamakawa, CXCR4 expression is associated with lymph-node metastasis of oral squamous cell carcinoma, Int. J. Oncol. 28 (2006) 61–66, https://doi.org/10.3892/ijo.28.1.61.
[140] D. Uchida, N.M. Begum, A. Almofti, K.I. Nakashiro, H. Kawamata, Y. Tateishi, H. Hamakawa, H. Yoshida, M. Sato, Possible role of stromal-cell-derived factor-1/CXCR4 signaling on lymph node metastasis of oral squamous cell carcinoma, Exp. Cell Res. 290 (2003) 289–302, https://doi.org/10.1016/S0014-4827(03)00344-6.
[141] B. Zhou, W.L. Chen, Y.Y. Wang, Z.Y. Lin, D.M. Zhang, S. Fan, J.S. Li, A role for cancer-associated fibroblasts in inducing the epithelial-to-mesenchymal transition in human tongue squamous cell carcinoma, J. Oral. Pathol. Med 43 (2014) 585–592, https://doi.org/10.1111/jop.12172.
[142] G. Dong, Z. Chen, Z.Y. Li, N.T. Yeh, C.C. Bancroft, C. Van Waes, Hepatocyte growth factor/scatter factor-induced activation of MEK and PI3K signal pathways contributes to expression of proangiogenic cytokines interleukin-8 and vascular endothelial growth factor in head and neck squamous cell carcinoma, Cancer Res 61 (2001) 5911–5918.
[143] F.R. Dellacono, J. Spiro, R. Eisma, D. Kreutzer, Expression of basic fibroblast growth factor and its receptors by head and neck squamous carcinoma tumor and vascular endothelial cells, Am. J. Surg. 174 (1997) 540–544, https://doi.org/10.1016/S0002-9610(97)00169-4.
[144] R. Gyanchandani, M.V. Ortega Alves, J.N. Myers, S. Kim, A proangiogenic signature is revealed in FGF-mediated bevacizumab-resistant head and neck squamous cell carcinoma, Mol. Cancer Res 11 (2013) 1585–1596, https://doi.org/10.1158/1541-7786.MCR-13-0358.
[145] P.L. Swiecicki, L. Zhao, E. Belile, A.G. Sacco, D.B. Chepeha, I. Dobrosotskaya, M. Spector, A. Shuman, K. Malloy, J. Moyer, E. McKean, S. McLean, G.T. Wolf, A. Eisbruch, M. Prince, C. Bradford, T. Carey, F.P. Worden, A phase II study evaluating axitinib in patients with unresectable, recurrent or metastatic head and neck cancer, Invest. N. Drugs 33 (2015) 1248–1256, https://doi.org/10.1007/s10637-015-0293-8.
[146] C. Elser, L.L. Siu, E. Winquist, M. Agulnik, G.R. Pond, S.F. Chin, P. Francis, R. Cheiken, J. Elting, A. McNabola, D. Wilkie, O. Petrenciuc, E.X. Chen, Phase II trial of sorafenib in patients with recurrent or metastatic squamous cell carcinoma of the head and neck or nasopharyngeal carcinoma, J. Clin. Oncol. 25 (2007) 3766–3773, https://doi.org/10.1200/JCO.2006.10.2871.
[147] S.K. Williamson, J. Moon, C.H. Huang, P.P. Guaglianone, M. LeBlanc, G.T. Wolf, S.G. Urba, Phase II Evaluation of Sorafenib in Advanced and Metastatic Squamous Cell Carcinoma of the Head and Neck: Southwest Oncology Group Study S0420, J. Clin. Oncol. 28 (2010) 3330–3335, https://doi.org/10.1200/JCO.2009.25.6834.
[148] D. Adkins, P. Mehan, J. Ley, M.J. Siegel, B.A. Siegel, F. Dehdashti, X. Jiang, N. N. Salama, K. Trinkaus, P. Oppelt, Pazopanib plus cetuximab in recurrent or metastatic head and neck squamous cell carcinoma: an open-label, phase 1b and expansion study, Lancet Oncol. 19 (2018) 1082–1093, https://doi.org/10.1016/S1470-2045(18)30350-4.
[149] A. Argiris, S. Li, P. Savvides, J.P. Ohr, J. Gilbert, M.A. Levine, A. Chakravarti, M. Haigentz, N.F. Saba, C.V. Ikpeazu, C.J. Schneider, H.A. Pinto, A.A. Forastiere, B. Burtness, Phase III randomized trial of chemotherapy with or without bevacizumab in patients with recurrent or metastatic head and neck cancer, J. Clin. Oncol. 37 (2019) 3266–3274, https://doi.org/10.1200/JCO.19.00555.