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INFLUÊNCIA DA RIGIDEZ DA MATRIZ EXTRACELULAR E DA EXPRESSÃO DE YAP/TAZ NO PROGNÓSTICO DE PACIENTES COM CARCINOMA ESPINOCELULAR ORAL

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2026

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Tese apresentada como requisito parcial para a obtenção do título de Doutor em Odontologia, área de concentração Patologia Bucal, ao Programa de Pós-Graduação em Odontologia da Faculdade de Odontologia da Universidade Federal do Rio Grande do Sul.

Orientador: Prof. Dr. Marcelo Lazzaron Lamers

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Tese aprovada para obtenção do título de Doutor no Programa de Pós-Graduação em Odontologia da Universidade Federal do Rio Grande do Sul pela banca examinadora formada por:

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Há decisões que não se medem apenas pela distância geográfica, mas pela transformação que provocam. Deixar Sergipe, minha origem, minha referência de mundo, foi uma dessas decisões. Saí do menor estado do país levando comigo os valores aprendidos em casa, a simplicidade das minhas raízes, o compromisso com o estudo como instrumento de mudança.

Minha trajetória começou na Universidade Federal de Sergipe. Foi lá que aprendi a pensar com método, a questionar com responsabilidade e a enxergar na Patologia Bucal não apenas uma especialidade, mas um campo de compromisso científico. Ali nasceu o desejo de ir além. Não por insatisfação, mas por inquietação. Eu queria me testar. Queria sair do lugar onde tudo era familiar e descobrir até onde eu poderia chegar.

Decidir vir para a UFRGS foi, talvez, uma das escolhas mais difíceis que já fiz. Havia a vontade de ampliar horizontes, de submeter minhas ideias a um ambiente mais exigente, de crescer em um contexto que me desafiasse diariamente. Mas desejar é diferente de partir. Partir exigiu renúncia. Exigiu deixar o convívio cotidiano da minha família, abrir mão da proximidade dos amigos, me adaptar a outra cultura, a outro ritmo, a outro clima...

Viver longe de casa me ensinou sobre ausência e maturidade. Houve dias de entusiasmo e descoberta, mas também de solidão. Aprendi a lidar com a saudade como parte do processo. Aprendi que amadurecer, às vezes, significa sustentar escolhas mesmo quando elas doem. Ainda assim, foi nesse cenário que compreendi, com mais clareza, o sentido da formação acadêmica: rigor, responsabilidade e perseverança.

Esta tese é resultado de estudo, disciplina e método, mas também é fruto de escolhas difíceis. É a consolidação de um percurso que começou muito antes da UFRGS... começou na educação que recebi dos meus pais, no incentivo constante das minhas irmãs, na confiança daqueles que sempre acreditaram que eu poderia crescer.

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Ao Prof. Dr. Marcelo Lamers, que foi mais do que um orientador. Com exigência e rigor científico, ajudou a moldar meu pensamento crítico e a consolidar minha identidade como pesquisador. Sua confiança foi decisiva nos momentos de dúvida, e seu acolhimento tornou possível transformar a distância de casa em um espaço de pertencimento acadêmico.

À UFRGS, que deixou de ser apenas uma instituição de excelência para se tornar um lugar de transformação pessoal e profissional. Aqui fui desafiado, cobrado, formado. Aqui cresci. Aos professores que elevaram meu rigor científico e aos colegas que tornaram o caminho mais leve, meu reconhecimento sincero.

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A todos que caminharam comigo, meu profundo e sincero agradecimento.

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Table 1 - Search strategies in the databases.

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LISTA DE ABREVIATURAS E SIGLAS

Antecedentes e Justificativa

Carcinoma espinocelular oral - CEC oral

Instituto Nacional de Câncer - INCA

Papilomavírus humano - HPV

International Agency for Research on Cancer - IARC

Espécies reativas de oxigênio - ROS

Radiação ultravioleta - UV

Nicotinamida Adenina Dinucleotídeo - NADH

Flavina Adenina Dinucleotídeo - FAD

Transição epitélio-mesênquima - EMT

Transição mesênquima-epitélio - MET

Matriz extracelular - MEC

Quinase de adesão focal - FAK

Metaloproteinases de matriz - MMPs

Lisil oxidase - LOX

Fibroblastos associados ao câncer - CAFs

Microscopia de força atômica - AFM

Elastografia por ondas de cisalhamento - SWE

Elastografia por ressonância magnética - MRE

Microambiente tumoral - MT

Fator de crescimento endotelial vascular - VEGF

Linker of Nucleoskeleton and Cytoskeleton - LINC

Yes-associated protein - YAP

Transcriptional coactivator with PDZ-binding motif - TAZ

Fator de crescimento do tecido conjuntivo – CTGF

Artigo científico 1

Oral Squamous Cell Carcinoma - OSCC

Extracellular matrix - ECM

Lysyl oxidase - LOX

Focal adhesion kinase - FAK

Epithelial-mesenchymal transition - EMT

Preferred Reporting Items for Systematic Reviews and Meta-Analyses - PRISMA
Health Sciences Descriptors - DeCS
Medical Subject Headings - MeSH
Embase Subject Headings - Emtree
Confidence intervals - CI
Carcinoma in situ - CIS
Overall survival - OS
Recurrence-free survival - RFS
GMP-AMP synthase – cGAS

Artigo científico 2
Oral Squamous Cell Carcinoma - OSCC
Systematic Reviews and Meta-Analyses - PRISMA
Hazard Ratio - HR
Overall Survival - OS
Extracellular matrix - ECM
Epithelial–mesenchymal transition - EMT
Transcriptional coactivator with PDZ-binding motif - TAZ
Health Sciences Descriptors - DeCS
Medical Subject Headings - MeSH
Embase Subject Headings - Emtree
Comprehensive Meta-Analysis - CMA
Disease-Free Survival - DFS
Recurrence Free Survival - RFS
Immunohistochemistry - IHC

RESUMO

O carcinoma espinocelular oral (CEC oral) é a neoplasia maligna mais prevalente da cavidade oral, sendo responsável por altas taxas de morbidade e mortalidade, especialmente em decorrência do diagnóstico em estágios avançados. Apesar dos avanços terapêuticos das últimas décadas, a taxa de sobrevida global em cinco anos permanece em torno de 50%, o que evidencia a necessidade de identificar novos biomarcadores prognósticos capazes de orientar estratégias terapêuticas mais precisas. O microambiente tumoral (MT), e em particular a matriz extracelular (MEC), tem emergido como um regulador ativo da progressão neoplásica, exercendo influência sobre as células tumorais por meio de sinais bioquímicos, mas também por meio de suas propriedades mecânicas. O enrijecimento progressivo da MEC, promovido pela deposição e reticulação excessiva de colágeno mediadas pelos fibroblastos associados ao câncer, ativa vias de mecanotransdução que favorecem a invasão tumoral, a transição epitélio-mesênquima e a resistência terapêutica. Nesse contexto, as proteínas YAP e TAZ, efetores transcricionais da via Hippo e sensores diretos da rigidez matricial, têm sido identificadas como mediadores centrais da resposta oncogênica aos estímulos mecânicos, ativando programas transcricionais que promovem proliferação, sobrevivência e disseminação metastática. A presente tese investiga, por meio de revisões sistemáticas da literatura com meta-análise e de análise histológica quantitativa e organizacional do teor de colágeno estromal em amostras de CEC oral, a influência da rigidez da MEC e da atividade de YAP/TAZ no prognóstico dessa neoplasia. Os resultados obtidos indicam que o CEC oral apresenta maior rigidez quando comparado à mucosa normal ($d = 5.28$; 95% CI: 2.61–7.94; $Z = 3.88$; $p < 0.001$), a qual pode ativar vias de mecanotransdução, como YAP/TAZ e contribuir para pior sobrevida global (YAP: HR = 1.918; 95% CI: 1.036 – 3.549; TAZ: 2.845 95% CI: 1.682 – 4.811). Dessa forma, os resultados obtidos contribuem para a compreensão do papel do microambiente mecânico na biologia do CEC oral e posicionam a rigidez da MEC e a atividade de YAP/TAZ como biomarcadores prognósticos com potencial aplicação clínica na estratificação de risco e no desenvolvimento de novas abordagens terapêuticas dirigidas ao MT.

Palavras-chave: Carcinoma Espinocelular Oral; Matriz extracelular; Rigidez da Matriz Extracelular; Colágeno; Mecanotransdução; YAP; TAZ; Via Hippo; Prognóstico; Microambiente tumoral.

ABSTRACT

Oral squamous cell carcinoma (OSCC) is the most prevalent malignant neoplasm of the oral cavity and is associated with high morbidity and mortality rates, largely due to diagnosis at advanced stages. Despite therapeutic advances over recent decades, the overall five-year survival rate remains around 50%, highlighting the need to identify novel prognostic biomarkers capable of guiding more precise therapeutic strategies. The tumor microenvironment (TME), particularly the extracellular matrix (ECM), has emerged as an active regulator of neoplastic progression, influencing tumor cells not only through biochemical signaling but also via its mechanical properties. Progressive ECM stiffening, driven by excessive collagen deposition and crosslinking mediated by cancer-associated fibroblasts, activates mechanotransduction pathways that promote tumor invasion, epithelial–mesenchymal transition, and therapeutic resistance. In this context, the proteins YAP and TAZ—transcriptional co-activators of the Hippo pathway and direct sensors of matrix stiffness—have been identified as central mediators of the oncogenic response to mechanical cues, activating transcriptional programs that support proliferation, survival, and metastatic dissemination. This thesis investigates, through systematic literature reviews with meta-analysis and through quantitative and organizational histological analysis of stromal collagen content in OSCC samples, the influence of ECM stiffness and YAP/TAZ activity on the prognosis of this neoplasm. The results indicate that OSCC exhibits greater stiffness compared to normal mucosa ($d = 5.28$; 95% CI: 2.61–7.94; $Z = 3.88$; $p < 0.001$), which may activate mechanotransduction pathways such as YAP/TAZ and contribute to poorer overall survival (YAP: HR = 1.918; 95% CI: 1.036–3.549; TAZ: HR = 2.845; 95% CI: 1.682–4.811). Overall, these findings contribute to understanding the role of the mechanical microenvironment in OSCC biology and position ECM stiffness and YAP/TAZ activity as prognostic biomarkers with potential clinical application in risk stratification and in the development of novel therapeutic approaches targeting the tumor microenvironment.

Keywords: Oral Squamous Cell Carcinoma; Extracellular Matrix; Extracellular Matrix Stiffness; Collagen; Mechanotransduction; YAP; TAZ; Hippo Pathway; Prognosis; Tumor Microenvironment.

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1 ANTECEDENTES E JUSTIFICATIVA

O câncer é uma condição complexa caracterizada pela transformação de células normais em células malignas, impulsionada por mutações genéticas e alterações epigenéticas que desregulam mecanismos fundamentais de controle celular (HANAHAN; WEINBERG, 2022). O conhecimento sobre a biologia do câncer avançou significativamente nas últimas décadas, revelando que essa transformação envolve, além da proliferação celular descontrolada, profundas alterações no MT e nas interações célula-célula e célula-matriz (LAMBERT; BHATT; BHATT, 2024).

De acordo com os dados mais recentes do GLOBOCAN, a incidência global de câncer em 2022 foi de aproximadamente 19,98 milhões de casos novos, com 9,74 milhões de óbitos registrados no mesmo período (BRAY *et al*., 2023). O aumento progressivo da incidência tem sido atribuído, em parte, ao envelhecimento e ao crescimento populacional, enquanto fatores comportamentais e ambientais, como tabagismo, consumo de álcool, dietas inadequadas, sedentarismo e exposição a carcinógenos, respondem por parcela significativa dos casos potencialmente evitáveis (WILD *et al*., 2020).

O impacto econômico do câncer é expressivo e crescente. Estima-se que os custos globais relacionados à doença possam ultrapassar 1,8 trilhão de dólares anuais até 2030 (WHO, 2022). Embora estimativas globais isoladas específicas para o CEC oral não estejam disponíveis, evidências demonstram o peso econômico dessa neoplasia em diferentes sistemas de saúde. Por exemplo, na Austrália o custo total do carcinoma espinocelular oral (CEC oral), incluindo despesas com saúde e perdas de produtividade, foi estimado em aproximadamente 1,4 bilhões de dólares em 2019 (GAMA *et al*., 2025). Esse ônus é desproporcionalmente elevado em países de baixa e média renda, onde limitações no acesso a diagnóstico precoce, radioterapia e terapias sistêmicas avançadas resultam em maior mortalidade e menor sobrevida em comparação a países de alta renda (GINSBURG *et al*., 2020).

A magnitude epidemiológica e econômica do câncer justifica sua posição central nas pesquisas biomédicas contemporâneas. A complexidade biológica da doença, marcada pela heterogeneidade tumoral, plasticidade celular e influência do microambiente, torna indispensável a investigação contínua de seus mecanismos subjacentes (HANAHAN; WEINBERG, 2022). Nesse contexto, o avanço no entendimento dos processos oncogênicos tem pavimentado o caminho para abordagens terapêuticas mais precisas, com menor toxicidade sistêmica, alinhadas

aos princípios da medicina de precisão e à necessidade de redução da carga global do câncer (TSIMBERIDOU *et al*., 2015).

1.1 Carcinoma espinocelular oral

O CEC oral é o tipo mais comum de câncer da cavidade oral, originando-se das células escamosas do epitélio oral (TAN *et al*., 2023; FATIMA *et al*., 2024). Essa neoplasia pode acometer diversas regiões, incluindo lábios, língua, assoalho bucal, mucosa jugal, gengiva, palato duro e mole, e trígono retromolar (KUNHABDULLA *et al*., 2025). É caracterizado por comportamento biológico agressivo, com marcada capacidade de invasão local e alto potencial de metástase para linfonodos regionais (BERNASCONI *et al*., 2023; ALQUTUB *et al*., 2024). Um dos principais desafios clínicos dessa neoplasia reside no diagnóstico tardio: estima-se que aproximadamente 60% dos casos sejam diagnosticados nos estágios III ou IV, quando as opções terapêuticas são mais limitadas e o prognóstico é significativamente desfavorável (WARNAKULASURIYA; KERR, 2018; JOHNSON *et al*., 2020; MENDOZA *et al*., 2021).

1.2 Aspectos epidemiológicos

O CEC oral figura entre os dez tipos de câncer mais incidentes no mundo, com aproximadamente 389.846 casos novos e 188.438 óbitos registrados em 2022 (BRAY *et al*., 2023). Apesar dos avanços terapêuticos das últimas décadas, a taxa de sobrevida global em cinco anos permanece em torno de 50%, um índice que pouco se alterou nas últimas três décadas e que reflete os desafios inerentes ao diagnóstico tardio e à heterogeneidade biológica da doença (JOHNSON *et al*., 2020). Esse cenário é agravado pelo fato de que a maioria dos casos é diagnosticada nos estágios III e IV, quando a doença já apresenta invasão local avançada ou comprometimento linfonodal regional, reduzindo significativamente as possibilidades terapêuticas e as chances de cura (WARNAKULASURIYA; KERR, 2018).

As taxas de incidência e mortalidade do CEC oral variam consideravelmente entre as regiões do mundo, refletindo diferenças na prevalência de fatores de risco comportamentais, no acesso a cuidados de saúde e nos níveis de conscientização pública (GHANEM *et al*., 2025). A Ásia concentra a maior parte dos casos, respondendo por aproximadamente 66,3% da incidência global, padrão associado, em parte, ao uso disseminado de tabaco sem fumaça e à mastigação de noz de areca

nessa região (BRAY *et al*., 2023). No Brasil, o câncer de boca representa um relevante problema de saúde pública. O Instituto Nacional de Câncer (INCA) estimou para o biênio 2023-2024 cerca de 15.100 casos novos anuais, sendo o país um dos com maior incidência da neoplasia na América Latina (INCA, 2022).

O CEC oral apresenta incidência significativamente maior no sexo masculino, que representa aproximadamente 70% dos casos diagnosticados, com taxas de mortalidade proporcionalmente mais elevadas, cerca de 67% dos óbitos ocorrem em homens (BRAY *et al*., 2023). Essa predominância masculina tem sido historicamente associada à maior exposição aos principais fatores de risco, como tabaco e álcool (CHEN *et al*., 2023; ELORANTA *et al*., 2024). No entanto, dados recentes apontam para uma tendência de aumento da incidência em mulheres e em pacientes mais jovens, particularmente em casos associados à infecção pelo papilomavírus humano (HPV), o que tem modificado o perfil epidemiológico clássico da doença (GHANEM *et al*., 2025).

1.3 Etiologia e fatores de risco

O CEC é uma neoplasia multifatorial, com uma série de agentes ambientais, comportamentais e biológicos associados ao seu desenvolvimento. Dentre os fatores mais prevalentes, o tabagismo e o consumo excessivo de álcool são amplamente reconhecidos na literatura como os principais fatores de risco para a doença, com um efeito conjunto sinérgico que aumenta consideravelmente a probabilidade de seu surgimento (IARC, 2022; FATIMA *et al*., 2024).

O tabaco é um dos agentes carcinogênicos mais bem documentados no CEC oral, com evidências que indicam que o uso de cigarro pode danificar diretamente a estrutura do DNA das células da mucosa oral (CHEN *et al*., 2023). Substâncias presentes no tabaco, como as nitrosaminas, entre elas a N’-nitrosonornicotina, são capazes de induzir alterações genéticas, incluindo a metilação do DNA, o que contribui para a transformação maligna das células epiteliais (STANFILL *et al*., 2023). Além disso, a exposição prolongada ao calor gerado pelo cigarro pode causar alterações estruturais na mucosa bucal, promovendo um ambiente propício para o desenvolvimento de lesões precursoras e câncer (GHANEM *et al*., 2025). Estudos sugerem que o risco de desenvolvimento de CEC oral em fumantes aumenta proporcionalmente à duração e intensidade do consumo, com fumantes pesados

apresentando risco até 10 vezes maior do que não fumantes (MEHANNA *et al*., 2013; WYSS *et al*., 2013).

Embora o álcool seja classificado pela IARC como carcinógeno do Grupo 1, a magnitude do seu risco isolado para o CEC oral é menor do que a observada para o tabaco (IARC, 2024). No entanto, sabe-se que o álcool, especialmente quando consumido em grandes quantidades, pode agir como um facilitador da carcinogênese, uma vez que aumenta a permeabilidade da mucosa oral, tornando-a mais suscetível à penetração de carcinógenos presentes no tabaco (NCI, 2021; CHEN *et al*., 2023). Além disso, o álcool promove alterações no metabolismo celular, induzindo a produção de espécies reativas de oxigênio (ROS) e alterando a expressão de microRNAs envolvidos na progressão tumoral (GAPSTUR *et al*., 2023). O acetaldeído, principal metabólito do álcool, é um agente genotóxico bem estabelecido que pode interagir diretamente com o DNA, prejudicando a capacidade das células de reparar lesões genéticas, o que favorece a evolução para malignidade (GAPSTUR *et al*., 2023).

A interação entre o tabaco e o álcool apresenta um efeito sinérgico, onde o risco de CEC oral em indivíduos que consomem ambos os produtos são consideravelmente maiores do que as somas dos riscos individuais (JUN *et al*., 2024). Estudos sugerem que o uso concomitante desses fatores resulta em um aumento exponencial no risco de desenvolvimento do câncer oral, devido ao potencial de ambos os agentes em modificar o ciclo celular e gerar lesões genéticas (HOES *et al*., 2021). Além disso, os efeitos sistêmicos do álcool, como lesões hepáticas, podem comprometer a desintoxicação e o sistema imunológico, exacerbando os danos causados pelo tabaco e outras substâncias carcinogênicas (GAPSTUR *et al*., 2023).

Outro importante fator para o desenvolvimento do CEC oral, especialmente no que diz respeito ao câncer de lábio, é a exposição à radiação ultravioleta (UV) (THAVAROOL *et al*., 2019). A exposição crônica à radiação UV, sem a proteção adequada, induz danos ao DNA das células da mucosa oral, principalmente por meio da geração de ROS, que causam mutações e instabilidade genômica (KIM *et al*., 2025). O impacto da radiação UV é particularmente relevante para populações com alta exposição ao sol, como aqueles que vivem em regiões tropicais ou subtropicais, onde a incidência de câncer de lábio tem mostrado importante aumento (GHANEM *et al*., 2025).

Além desses fatores amplamente reconhecidos, outros elementos têm sido cada vez mais discutidos na literatura científica como potenciais contribuintes para o desenvolvimento do CEC oral (SHARMA *et al*., 2024). A infecção por HPV, especialmente os tipos 16 e 18, tem sido fortemente associada ao câncer de orofaringe, e embora sua associação com o CEC oral não seja tão forte, estudos indicam que a infecção por HPV pode contribuir para a progressão de lesões orais, principalmente em pacientes mais jovens (SHARMA *et al*., 2024). A alteração do ciclo celular promovida pelo HPV, que inativa genes supressores de tumor como p53 e pRb, pode facilitar o desenvolvimento de malignidades orais, particularmente em indivíduos imunocomprometidos ou com histórico de múltiplos fatores de risco (CUI *et al*., 2025).

Fatores genéticos também modulam a susceptibilidade individual ao CEC oral, com polimorfismos em genes envolvidos no metabolismo de carcinógenos, como CYP1A1 e GSTT1, e na resposta ao dano ao DNA podendo aumentar o risco em indivíduos expostos a agentes ambientais (BARROSO *et al*., 2025). Adicionalmente, a presença de lesões potencialmente malignas, como leucoplasia e eritroplasia, representam condições que aumentam o risco de transformação maligna e que devem ser consideradas no contexto da vigilância clínica (WARNAKULASURIYA, 2018).

1.4 Características clínicas

As características clínicas do CEC oral variam dependendo da localização da lesão e do estágio da doença. A língua é o local mais comumente afetado, seguida pela mucosa labial, gengiva, palato e mucosa alveolar, embora o CEC oral possa surgir em qualquer área da cavidade bucal (CHAMOLI *et al*., 2021). A manifestação inicial da doença frequentemente se dá por alterações mucosas que podem ser detectadas durante o exame clínico, sendo as mais comuns as leucoplasias (placas brancas), eritroplasias (lesões vermelhas) ou lesões mistas (leucoeritroplasias) (AGUIRRE-URIZAR *et al*., 2021).

Essas alterações pré-cancerígenas são classificadas como desordens potencialmente malignas, devido ao risco variável de transformação em carcinoma. A leucoplasia, uma das condições mais comuns, é definida como uma placa branca que não pode ser caracterizada como outra condição benigna (AGUIRRE-URIZAR *et al*., 2021). Apesar de muitas leucoplasias serem assintomáticas, estudos sugerem que o risco anual de transformação maligna dessas lesões varia de 1% a 3%, dependendo de fatores como localização e histopatologia (PIMENTA-BARROS *et al*., 2024). Entre

as leucoplasias, destaca-se a leucoplasia verrucosa proliferativa, uma forma de alto risco que se apresenta com uma superfície rugosa e de crescimento lenta, frequentemente associada a um risco aumentado de malignização (STAINES; ROGERS, 2024). Essa variante é caracterizada por placas brancas irregulares, com aspectos não homogêneos, sendo considerada uma lesão de alto risco para a transformação maligna (STAINES; ROGERS, 2024).

As eritroplasias, por sua vez, são lesões vermelhas que, embora menos comuns que as leucoplasias, possuem um risco significativamente maior de se transformarem em CEC oral. Essas lesões são frequentemente diagnosticadas com displasia epitelial ou até carcinoma *in situ* (LORENZO-POUSO *et al*., 2022). Ao contrário das leucoplasias, as eritroplasias podem apresentar uma superfície mais granulosa ou áspera, e, embora geralmente sejam assintomáticas, alguns pacientes podem se queixar de dor ou sensação de queimação (LORENZO-POUSO *et al*., 2022).

Além das características iniciais, as lesões de CEC oral podem adotar dois padrões principais de crescimento: exofítico e endofítico. O padrão exofítico é caracterizado por uma formação nodular que pode ter uma superfície ulcerada, com bordas irregulares e endurecidas, típica de um tumor que se projeta para fora da mucosa (ELTOHAMI; SULEIMAN, 2023). Este tipo de crescimento é frequentemente observado em estágios mais avançados, quando o tumor já ultrapassou a camada basal do epitélio e começou a invadir as camadas mais profundas (ELTOHAMI; SULEIMAN, 2023). Por outro lado, o padrão endofítico é observado quando a lesão assume uma forma deprimida, ulcerada e irregular, com bordas bem definidas que refletem a invasão do tumor nos tecidos subjacentes. Esse é o aspecto clássico das lesões de CEC oral, especialmente em estágios mais invasivos, quando a ulceração não cicatriza, permanecendo ativa por longos períodos e frequentemente associada a sintomas como dor e sangramento (ELTOHAMI; SULEIMAN, 2023). As lesões endofíticas podem ser particularmente dolorosas e dificultar a alimentação e a fala, o que impacta significativamente a qualidade de vida dos pacientes (ELTOHAMI; SULEIMAN, 2023).

Em lesões localizadas nos lábios, o CEC oral frequentemente se apresenta como uma úlcera endurecida com crostas, o que pode ser confundido com outras condições benignas. Além disso, é comum a presença de linfadenopatia cervical, indicando disseminação local do câncer (WANG *et al*., 2026). Embora a maioria das

lesões orais iniciais sejam assintomáticas, em estágios mais avançados, os pacientes podem relatar sintomas como disfagia, dor persistente e faringite (TAN *et al*., 2023).

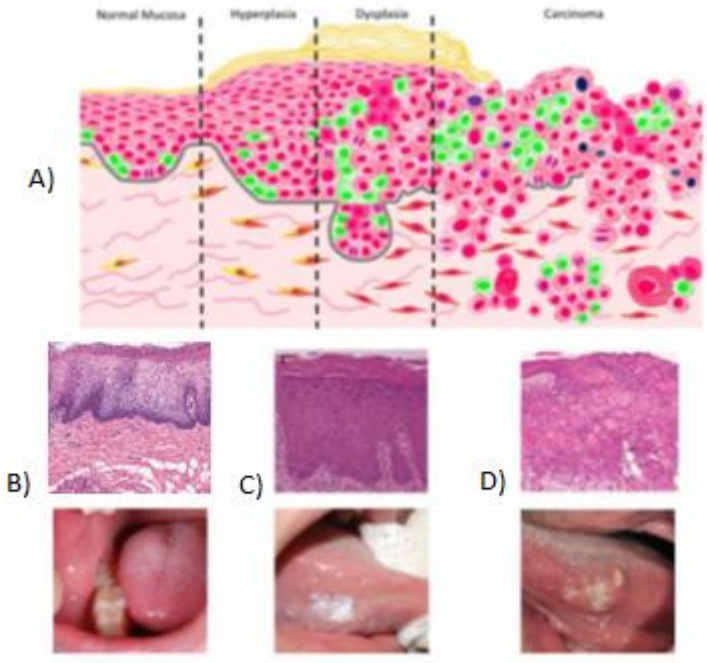


Figura 1 - Progressão de CEC oral (A) esquema do processo de tumorigênese demonstrando regiões macias e duras do tumor. Histologia e imagens anatômicas de regiões de tecido normal (B), displásico (C) e CEC Oral (D).

1.5 Características histopatológicas

O CEC oral é caracterizado por uma série de alterações histológicas que refletem sua natureza invasiva e agressiva (AHMED *et al*., 2019). Microscopicamente, o CEC oral se apresenta com um padrão de invasão em que o epitélio maligno penetra o tecido conjuntivo subjacente, formando ilhas e cordões celulares que se estendem até os vasos sanguíneos e canais linfáticos (AHMED *et al*., 2019). Essa invasão em estruturas vasculares é um dos principais mecanismos que facilita a disseminação do tumor para outras áreas do corpo (COSTA *et al*., 2022).

A ulceração é um dos achados mais comuns, indicando uma doença em estágio mais avançado. Esse padrão de ulceração resulta de uma combinação de necrose tumoral e destruição da mucosa (AHMED *et al*., 2019). Além disso, as alterações citológicas incluem um aumento significativo na hipercromasia, que é indicativo de uma maior quantidade de DNA nas células tumorais, bem como pleomorfismo celular e nuclear, acantose, disceratose e mitoses frequentes, que são

típicas de tumores com alta taxa de proliferação (AHMED *et al*., 2019). A presença de necrose e inflamação crônica nas lâminas histológicas também são características marcantes, refletindo a agressividade e o estresse do MT (AHMED *et al*., 2019).

O CEC é classificado histologicamente em quatro graus de diferenciação, com base nas características celulares e no padrão de invasão. Carcinomas de grau I apresentam alta ceratinização, com células bem diferenciadas que possuem um padrão de invasão bem definido e discretamente infiltrado por células linfoplasmocitárias. Esse grau é associado a uma menor agressividade e melhor prognóstico (EL-NAGGAR *et al*., 2022). O grau II, por sua vez, é caracterizado por uma ceratinização moderada e um aumento no pleomorfismo nuclear. A invasão tumoral ocorre em forma de cordões ou bandas de células, com um infiltrado linfoplasmocitário mais pronunciado (ALMANGUSH *et al*., 2021; EL-NAGGAR *et al*., 2022).

No grau III, o CEC apresenta pouca ceratinização e abundante pleomorfismo nuclear, com um padrão de invasão formado por pequenos grupos ou cordões de células malignas. A quantidade de células inflamatórias diminui, e o tumor começa a se tornar mais agressivo (ALMANGUSH *et al*., 2020). O grau IV, por sua vez, é o mais indiferenciado, com ausência de ceratinização e alto pleomorfismo nuclear. O padrão de invasão é caracterizado por dissociação celular disseminada, onde as células tumorais se dispersam em pequenos grupos ou como células individuais. Este grau é frequentemente associado à ausência de infiltrado linfoplasmocitário, indicando um câncer altamente agressivo, com maior potencial de metastatização (RODRIGUES *et al*., 2022).

A OMS classifica o CEC oral com base no nível de diferenciação celular, que descreve a aparência das células tumorais em relação às células normais. CECs orais bem diferenciados são compostos por células escamosas dispostas em ilhas, com a formação de pérolas de queratina, estruturas características da queratinização. Estas células têm núcleos picnóticos e acidófilas, o que significa que mantêm uma aparência similar ao epitélio escamoso normal (EL-NAGGAR *et al*., 2022). Nos CECs orais moderadamente diferenciados, observa-se um aumento do pleomorfismo celular, com núcleos de tamanhos e formas variadas. As ilhas de células epiteliais são atípicas e frequentemente invadem o estroma, com a presença de mitoses frequentes e pontes intercelulares raras. Esse grau é um indicador de maior agressividade, embora ainda exista uma certa preservação da arquitetura celular (RODRIGUES *et al*., 2022). Já

nos CECs orais pouco diferenciados, as células malignas são predominantemente imaturas, com mitoses atípicas e um padrão arquitetural completamente desorganizado, sem qualquer semelhança com o epitélio escamoso normal. Esse grau está intimamente relacionado a um pior prognóstico e a uma maior capacidade de infiltração e metástase, o que dificulta a resposta terapêutica (ALMANGUSH *et al*., 2020).

Essas características histológicas permitem classificar o CEC oral, oferecendo informações sobre a agressividade do tumor, sua capacidade de invasão e o risco de metástase. O grau de diferenciação celular, assim como o padrão de invasão e a presença de inflamação, são fatores que determinam o prognóstico e guiam as estratégias terapêuticas (DOLENS *et al.*, 2021).

1.6 Diagnóstico

O diagnóstico precoce do CEC oral é um importante fator para melhorar o prognóstico dos pacientes, uma vez que, quando detectado em estágios iniciais, as chances de tratamento bem-sucedido são significativamente maiores (ALMANGUSH *et al*., 2020). A biópsia ainda é considerada o padrão-ouro para o diagnóstico definitivo do CEC oral, permitindo uma análise histopatológica detalhada que ajuda a determinar o grau de diferenciação celular e a extensão da invasão tumoral (PENTENERO *et al*., 2020). Durante o procedimento, amostras do tecido suspeito são coletadas e processadas para examinar características como hipercromasia, pleomorfismo nuclear e a presença de mitoses atípicas, típicas desse tipo de carcinoma (BOXBERG *et al*., 2019).

No entanto, embora a biópsia seja altamente eficaz, ela apresenta algumas limitações técnicas (LIMA *et al*., 2021). O procedimento pode ser difícil em lesões grandes ou de localização difícil, onde o profissional precisa selecionar cuidadosamente áreas representativas para evitar diagnósticos errôneos (SEOANE *et al*., 2021). Além disso, as lesões de CEC oral podem apresentar heterogeneidade histológica, com diferentes áreas exibindo padrões variados de diferenciação e agressividade, o que pode complicar a interpretação (ALMANGUSH *et al*., 2020).

Nos últimos anos, novas técnicas diagnósticas têm ganhado relevância, com destaque para a biópsia líquida (RAPADO-GONZÁLEZ *et al*., 2020). Este método não invasivo oferece uma abordagem promissora para o diagnóstico e monitoramento do CEC oral, sendo utilizado para a detecção de células tumorais circulantes, DNA e RNA

tumoral, proteínas e exossomos presentes em fluidos biológicos como saliva, sangue e urina (KONG; BIRKELAND, 2021; PATEL *et al*., 2021). A biópsia líquida tem se mostrado particularmente útil na identificação precoce de CEC oral, mesmo em estágios iniciais, e pode ajudar a monitorar a resposta ao tratamento e a detecção de recidivas (LOUSADA-FERNANDEZ *et al*., 2022).

Embora a biópsia líquida apresente um grande potencial, ela ainda não substitui a biópsia tradicional, sendo indicada como um complemento (LOUSADA-FERNANDEZ *et al*., 2022). Em casos onde não há lesões clinicamente evidentes, como nas fases iniciais do câncer ou em áreas de difícil acesso, a biópsia líquida pode ser uma alternativa útil, especialmente quando combinada com a análise genômica, fornecendo informações valiosas sobre a assinatura molecular do tumor (KONG; BIRKELAND, 2021). Assim, a combinação dessas abordagens pode oferecer um diagnóstico mais completo e menos invasivo, aprimorando a detecção e o acompanhamento do CEC oral de forma eficiente (RAPADO-GONZÁLEZ *et al*., 2020).

Além disso, o uso de tecnologias não invasivas, como a autofluorescência tem sido utilizadas como métodos de detecção precoce (LIMA *et al*., 2021). A autofluorescência explora a alteração na concentração de fluoróforos endógenos, como Nicotinamida Adenina Dinucleotídeo (NADH) e Flavina Adenina Dinucleotídeo (FAD), nos tecidos tumorais, permitindo a detecção de áreas alteradas da mucosa bucal sem agentes adicionais (LIMA *et al*., 2021). Paralelamente, a ultrassonografia intraoral de alta frequência tem surgido como ferramenta complementar na detecção e estadiamento precoce (CAPRIOLI *et al*., 2022). A ultrassonografia permite a avaliação da profundidade de invasão e da espessura tumoral, parâmetros que influenciam diretamente a estratificação do risco de metástases linfonodais e o prognóstico do paciente. Em estudos clínicos, a medida de profundidade de invasão por ultrassonografia mostrou correlação significativa com a profundidade de invasão histológica, reforçando sua utilidade na avaliação pré-operatória (CAPRIOLI *et al*., 2022).

1.7 Alterações genéticas e epigenéticas envolvidas na progressão tumoral

Uma vez estabelecida, a progressão do CEC oral é um processo multifásico e dinâmico, no qual diversas alterações genéticas e epigenéticas contribuem para a transformação maligna, invasão local e metástase (LEEMANS *et al*., 2018). Dentre os eventos biológicos centrais nesse processo, destaca-se a transição epitélio-

mesênquima (EMT), fenômeno pelo qual células epiteliais perdem suas características de adesão e adquirem propriedades mesenquimatosas, tornando-se mais móveis e invasivas (THIERY *et al*., 2009).

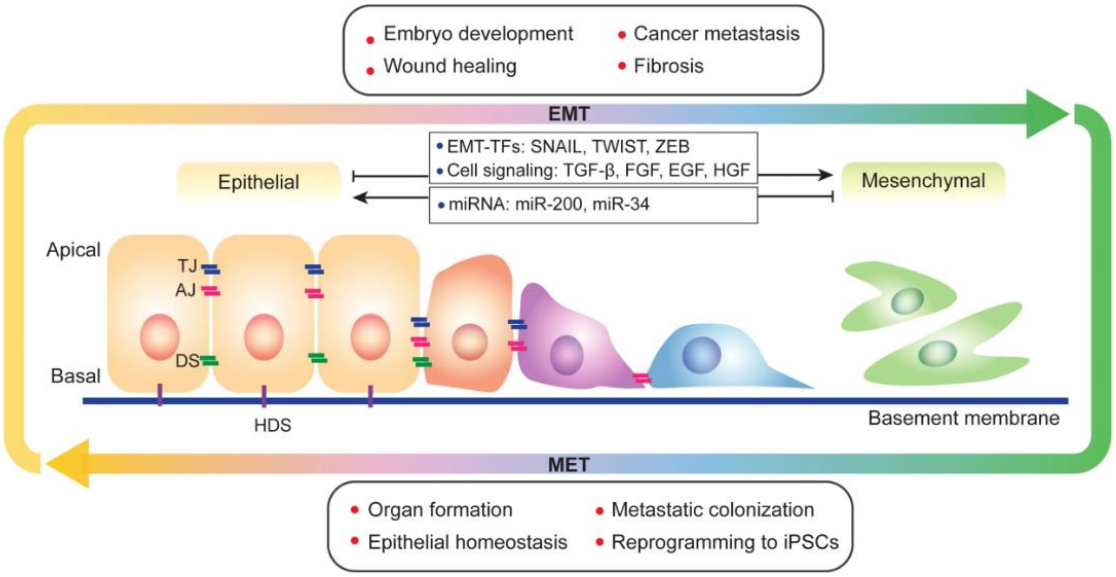


Figura 2 - Visão geral da EMT e da transição mesênquima-epitélio (MET). A EMT e seu processo reverso, a MET, participam tanto de eventos fisiológicos quanto patológicos e ocorrem de maneira dinâmica, compondo um espectro fenotípico intermediário entre os estados epitelial e mesenquimal. Durante a EMT, células epiteliais perdem progressivamente a polaridade apical-basal e reduzem as interações célula-célula e célula-matriz extracelular, adquirindo características associadas a maior plasticidade e potencial migratório. Esse processo é controlado por múltiplos mecanismos regulatórios, incluindo fatores de transcrição específicos, vias de sinalização intracelular e modificações epigenéticas. TJ: junções de oclusão (tight junctions); AJ: junções aderentes (adherens junctions); DS: desmossomos; HDS: hemidesmossomos (LAI *et al*., 2020).

A indução da EMT envolve uma série de modificações genéticas e epigenéticas que alteram a expressão de várias proteínas envolvidas na adesão celular (LAMOUILLE; XU; DERYNCK, 2014). Um aspecto central desse processo é a redução da expressão de E-caderina, uma glicoproteína essencial para a adesão intercelular nas células epiteliais (BERX; VAN ROY, 2009). Essa perda é mediada por fatores de transcrição como Snail, Twist e Zeb, que desempenham um papel crítico na regulação negativa do gene da E-caderina (MITTAL, 2018). Além disso, há um aumento na

expressão de N-caderina e vimentina, proteínas associadas a características mesenquimatosas, que favorecem a migração celular e a invasão tumoral (DONGRE *et al*., 2019).

Recentemente, estudos mostraram que essas alterações fenotípicas podem ser amplificadas por alterações epigenéticas, como a metilação do DNA, que atua como um regulador adicional na progressão do câncer (MESGARI *et al*., 2023). A metilação do promotor da E-caderina é uma modificação epigenética observada em vários tipos de câncer, incluindo o CEC, levando à sua desregulação e à promoção da invasão celular (HUANG *et al*., 2023). Além disso, a modulação de microRNAs (miRNAs) tem se mostrado um mecanismo importante na regulação da EMT, onde miRNAs como o miR-200 e miR-34b/c podem regular a expressão de genes críticos para a adesão celular e a migração, afetando diretamente a capacidade invasiva das células tumorais (LU; KANG, 2019).

As alterações genéticas que favorecem a progressão tumoral no CEC oral também incluem a mutação de genes supressores de tumor, como o p53, o p16 e a pRb, cujas disfunções promovem a perda de controle do ciclo celular, facilitando a proliferação descontrolada das células tumorais (LEEMANS; SNIJDERS; BRAKENHOFF, 2018). O p53 é frequentemente mutado no CEC oral, o que compromete a capacidade das células de reparar danos no DNA e de induzir a apoptose em resposta a danos genéticos (FARAH, 2021). A perda da função do p16, regulador negativo da quinase dependente de ciclina 4/6 (CDK4/6), também é comumente observada no CEC oral, permitindo que as células escapem dos mecanismos de checkpoint celular e continuem a se dividir (LEEMANS; SNIJDERS; BRAKENHOFF, 2018).

O impacto das alterações genéticas e epigenéticas na progressão tumoral do CEC oral vai além da invasão local, contribuindo também para a formação de metástases (JOHNSON *et al*., 2020). A migração de células tumorais para linfonodos regionais e, posteriormente, para outros órgãos é facilitada por um complexo de interações moleculares que envolvem a perda de adesão celular, a remodelação da MEC e a indução de características mesenquimatosas (LU; KANG, 2019).

1.8 Matriz extracelular

A MEC é uma rede tridimensional e dinâmica de macromoléculas que preenche o espaço intersticial dos tecidos e constitui o principal componente não celular do MT (THEOCHARIS *et al*., 2016; PRAKASH; SHAKED, 2024). A MEC é reconhecida como um regulador ativo e multifuncional do comportamento celular, capaz de modular proliferação, sobrevivência, migração, diferenciação e apoptose por meio de sinais bioquímicos e biofísicos integrados (PICKUP; MOUW; WEAVER, 2014; YUAN *et al*., 2023).

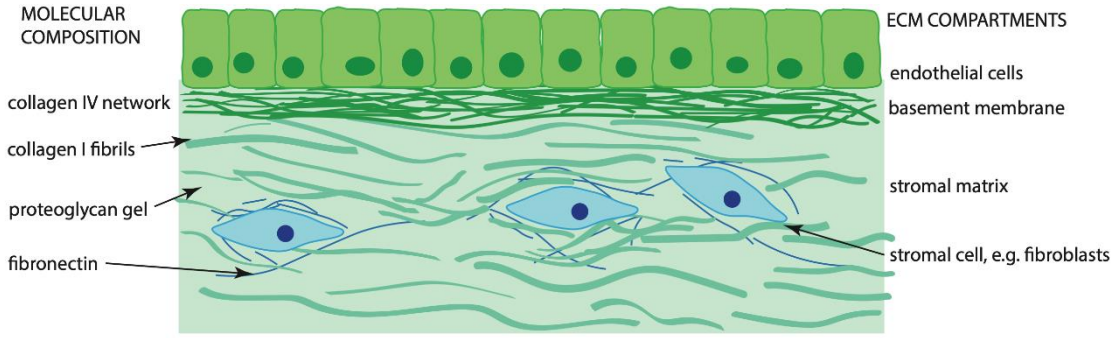


Figura 3 – Representação esquemática da MEC e seus compartimentos. (GOLDBERG *et al*., 2018).

A MEC é constituída por aproximadamente 300 moléculas distintas, organizadas em duas grandes categorias: proteínas fibrosas e proteoglicanos/glicosaminoglicanas (PRAKASH; SHAKED, 2024). As proteínas fibrosas incluem os colágenos, a fibronectina, a laminina, a elastina e a tenascina-C, e são responsáveis pela integridade estrutural, elasticidade e tensão mecânica do tecido (THEOCHARIS *et al*., 2016). O colágeno é o componente mais abundante da MEC e seus diferentes tipos conferem propriedades mecânicas e sinalizadoras específicas a cada tecido (YUAN *et al*., 2023). Em particular, os colágenos do tipo I e III predominam na matriz intersticial, enquanto o colágeno tipo IV é o principal constituinte da membrana basal, que separa o epitélio do estroma subjacente (PRAKASH; SHAKED, 2024).

Os proteoglicanos e as glicosaminoglicanas (GAGs) constituem o segundo grande grupo de componentes da MEC (KARAMANOS *et al*., 2021). Por meio de suas longas cadeias de dissacarídeos com carga negativa, incluindo ácido hialurônico, condroitina sulfato, heparina sulfato e queratina sulfato, esses componentes têm

capacidade de sequestrar água e cátions, conferindo à MEC suas propriedades viscoelásticas e sua função de reservatório de fatores de crescimento como TGF-β, FGF e VEGF (THEOCHARIS *et al*., 2016; WINKLER *et al*., 2020). Essa capacidade de sequestro e liberação controlada de fatores de crescimento é particularmente relevante no contexto tumoral, onde a MEC atua como um depósito de moléculas pró-oncogênicas que podem ser liberadas mediante degradação enzimática (YUAN *et al*., 2023).

Além de seu papel estrutural, a MEC desempenha funções regulatórias críticas na homeostase tecidual normal (FRANTZ; STEWART; WEAVER, 2010). Por meio de receptores transmembrana como as integrinas, dímeros de subunidades α e β que medeiam a adesão célula-MEC, a MEC transmite sinais bioquímicos e mecânicos para o interior celular, ativando cascatas de sinalização que regulam o citoesqueleto, a expressão gênica e o comportamento celular (YUAN *et al*., 2023). A ativação das integrinas desencadeia a fosforilação da quinase de adesão focal (FAK) e da quinase Src, culminando na ativação de vias como ERK/MAPK, PI3K/AKT e RhoA/ROCK, que regulam migração, sobrevivência e proliferação celular (THEOCHARIS *et al*., 2016). Adicionalmente, a MEC regula a polaridade epitelial e atua como barreira física à invasão em condições fisiológicas normais, barreira essa que é progressivamente subvertida durante a carcinogênese (PICKUP; MOUW; WEAVER, 2014).

A MEC é altamente dinâmica, sendo continuamente depositada, remodelada e degradada por células residentes para manutenção da homeostase tecidual (WINKLER *et al*., 2020). Os principais reguladores dessa remodelação são as metaloproteinases de matriz (MMPs), endopeptidases dependentes de zinco que degradam componentes específicos da MEC, particularmente MMP-1, MMP-2 e MMP-9, que são amplamente expressas no CEC oral e estão associadas à invasão tumoral e à metástase linfonodal (YUAN *et al*., 2023). Em contrapartida, as enzimas da família lisil oxidase (LOX e suas isoformas LOXL1-4) catalisam a reticulação covalente de fibras de colágeno e elastina, promovendo o enrijecimento progressivo da MEC (PRAKASH; SHAKED, 2024).

No contexto do câncer, a MEC sofre alterações composicionais e estruturais que a distinguem da matriz de tecidos normais (PICKUP; MOUW; WEAVER, 2014). Há excessiva deposição de colágeno tipo I e fibronectina, degradação seletiva de componentes da membrana basal, reorganização das fibras colágenas em padrões linearizados e perpendiculares à frente de invasão tumoral, e aumento das

reticulações mediadas por LOX (WINKLER *et al*., 2020). Essas modificações alteram tanto as propriedades bioquímicas quanto as mecânicas da MEC tumoral, criando um ambiente que favorece ativamente a progressão neoplásica. No CEC oral, estudos demonstraram que os fibroblastos associados ao câncer (CAFs) são os principais produtores de LOX no estroma tumoral, e que a inibição dessa enzima compromete a invasão e a progressão tumoral por reduzir a rigidez matricial (ZHANG *et al*., 2021).

1.9 Propriedades mecânicas da matriz extracelular

As propriedades mecânicas da MEC são determinantes do comportamento celular tanto em condições fisiológicas quanto no contexto da progressão tumoral (PICKUP; MOUW; WEAVER, 2014; KAI; DRAIN; WEAVER, 2019). A rigidez da MEC é quantificada pelo módulo de Young ou módulo elástico, expresso em pascal (Pa) ou quilopascal (kPa), e representa a principal grandeza biofísica utilizada para caracterizar o microambiente mecânico ao redor das células (SLEEBOOM *et al*., 2024). Essa grandeza varia entre os tecidos: o parênquima cerebral apresenta rigidez inferior a 1 kPa, tecidos epiteliais normais situam-se na faixa de 1 a 5 kPa, enquanto tumores sólidos podem alcançar 20 a 50 kPa ou mais, valores que chegam a ser dez vezes superiores aos do tecido adjacente não neoplásico (SLEEBOOM *et al*., 2024).

A rigidez da MEC é determinada principalmente pela composição, densidade, organização espacial e grau de reticulação das fibras que a constituem, em especial o colágeno e a elastina (WINKLER *et al*., 2020). A reticulação do colágeno é mediada primariamente pela família das LOX, enzimas secretadas por fibroblastos e CAFs que catalisam a oxidação de resíduos de lisina e hidroxilisina, formando ligações covalentes intra e intermoleculares nas fibras colágenas (YUAN *et al*., 2023). A superexpressão de LOX e de sua isoforma LOXL2 no MT do CEC oral está associada ao enrijecimento da MEC, à progressão tumoral e ao pior prognóstico clínico, e sua inibição tem demonstrado capacidade de reverter parcialmente o fenótipo invasivo das células tumorais (COX, 2021). Além da reticulação enzimática, o alinhamento e a linearização das fibras colágenas em direção perpendicular à frente de invasão tumoral também contribuem para o aumento da rigidez local e facilitam a migração das células neoplásicas (WINKLER *et al*., 2020).

Além da rigidez propriamente dita, a MEC apresenta comportamento viscoelástico, respondendo à forças mecânicas de maneira dependente do tempo, comportando-se simultaneamente como um sólido elástico e um fluido viscoso

(PRAKASH; SHAKED, 2024). A viscoelasticidade é conferida principalmente pelos proteoglicanos e pelo ácido hialurônico, que formam estruturas do tipo gel capazes de absorver e redistribuir energia mecânica (KARAMANOS *et al*., 2021). Essa propriedade tem implicações diretas sobre a adesão e migração celular: células tumorais em ambientes altamente viscoelásticos demonstram padrões migratórios distintos daqueles observados em substratos puramente elásticos, com implicações para a invasão tumoral (PRAKASH; SHAKED, 2024).

A mensuração da rigidez da MEC pode ser realizada por diferentes abordagens. A microscopia de força atômica (AFM) é considerada o padrão ouro para medições *ex vivo* e *in vitro*, permitindo a caracterização das propriedades mecânicas com resolução nanométrica em amostras teciduais e matrizes reconstituídas (STYLIANOU; LEKKA; STYLIANOPOULOS, 2019). Para avaliação clínica *in vivo*, a elastografia por ondas de cisalhamento (SWE) e a elastografia por ressonância magnética (MRE) permitem a estimativa não invasiva da rigidez tecidual em pacientes, com potencial aplicação diagnóstica e prognóstica (SLEEBOOM *et al*., 2024). No contexto de análise histológica, a coloração com Picrosirius sob luz polarizada é amplamente utilizada para a avaliação semiquantitativa e quantitativa do teor e da organização do colágeno na MEC tumoral, permitindo discriminar fibras colágenas espessas e densamente reticuladas (birrefringência avermelhada/alaranjada) de fibras finas e imaturas (birrefringência esverdeada) (WINKLER *et al*., 2020).

1.10 Microambiente tumoral

O MT desempenha papel central na evolução e progressão do CEC oral (JOHNSON *et al*., 2020). Trata-se de um sistema dinâmico e heterogêneo, composto por células tumorais e não tumorais, incluindo fibroblastos associados ao câncer, células do sistema imune, células endoteliais e componentes da matriz extracelular, integradas por uma rede complexa de moléculas de sinalização, citocinas e fatores de crescimento (HANAHAN, 2022; YANG *et al*., 2023). A interação contínua entre esses elementos cria condições que favorecem a proliferação celular descontrolada, a evasão imunológica, a resistência terapêutica e a formação de metástases, tornando o MT um determinante crítico do comportamento biológico e do prognóstico do CEC oral (BARROSO *et al*., 2025).

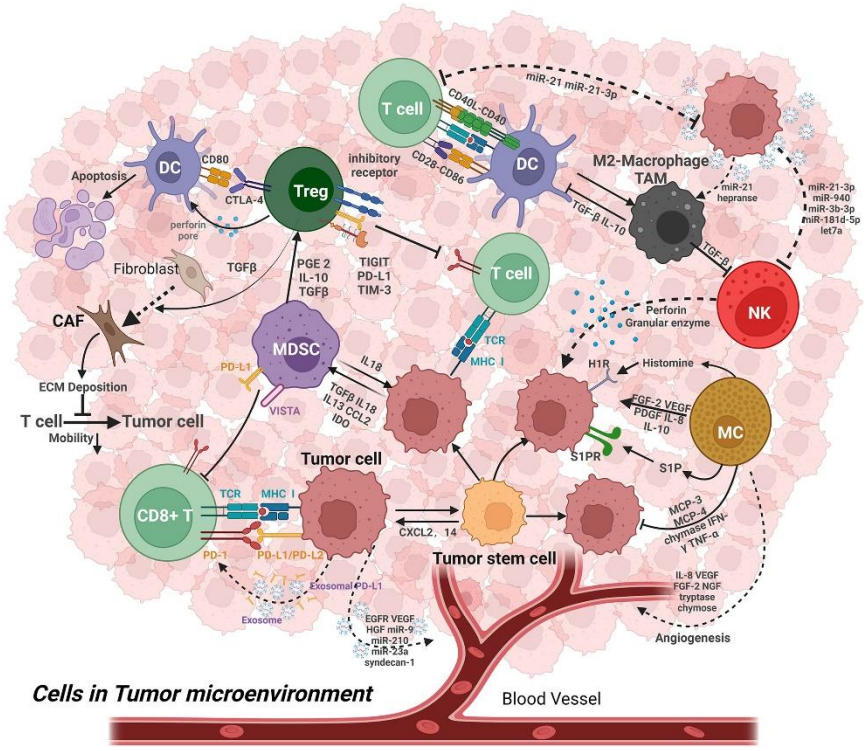


Figura 4 – Representação esquemática do MT. Esquema das interações entre células tumorais, células-tronco tumorais e componentes do sistema imune (T CD8+, Treg, NK, DC, TAM, MDSC, CAF e mastócitos). Destacam-se mecanismos de imunossupressão, sinalização por citocinas e checkpoints imunológicos, além de processos como angiogênese e remodelamento da matriz que favorecem a progressão tumoral. (WANG *et al*., 2023).

A angiogênese representa um dos processos mais relevantes mediados pelo MT. Células endoteliais, estimuladas por fatores pró-angiogênicos secretados pelas células tumorais e pelos demais componentes do microambiente, formam novos vasos sanguíneos que suprem o tumor com oxigênio e nutrientes e facilitam a eliminação de metabólitos (CARMELIET; JAIN, 2011). O fator de crescimento endotelial vascular (VEGF) é o principal mediador desse processo e pode ser induzido por hipóxia tumoral, por fibroblastos ativados e por células imunes polarizadas presentes no MT (APTE; CHEN; FERRARA, 2019). Além de sustentar o crescimento tumoral, a neovascularização fornece uma rota preferencial para a disseminação hematogênica das células malignas, tornando-se um fator determinante na progressão metastática do CEC oral (WINKLER *et al*., 2020).

A inflamação crônica associada ao tumor constitui outro aspecto fundamental do MT (ALMEIDA *et al*., 2019). Embora a resposta inflamatória fisiológica tenha função protetora, no contexto neoplásico ela frequentemente é subvertida para favorecer a progressão tumoral (GRETEN; GRIVENNIKOV, 2019). Os macrófagos associados ao tumor exemplificam essa dualidade: quando polarizados para o fenótipo M1, exercem função antitumoral por meio da produção de citocinas pró-inflamatórias e da ativação de respostas citotóxicas (MANTOVANI *et al*., 2017). Contudo, no MT do CEC oral, predomina a polarização para o fenótipo M2, caracterizado pela secreção de IL-10, TGF-β e fatores pró-angiogênicos, que promovem imunossupressão local, remodelação da MEC e facilitam a invasão tumoral (CHAURASIA *et al*., 2025). Essa reprogramação funcional dos macrófagos tem sido associada a pior prognóstico em pacientes com CEC oral, reforçando o papel do MT como modulador da agressividade tumoral (PETRUZZI *et al*., 2017). Dados do nosso laboratório demonstram que o ambiente inflamatório, em particular com a predominância de macrófagos M2 pode favorecer a invasão e metástase do CEC oral (ALVES *et al*., 2021).

Os CAFs representam um dos componentes mais ativos e relevantes do MT no CEC oral. Ativados por sinais provenientes das células tumorais, especialmente TGF-β, PDGF e proteínas do estresse oxidativo, os CAFs assumem um fenótipo contrátil e secretório que difere substancialmente do fibroblasto quiescente normal (SAHAI *et al*., 2020). Por meio da produção exacerbada de colágeno tipos I e III, fibronectina e enzimas de reticulação como a LOX, os CAFs promovem a remodelação e o enrijecimento progressivo da MEC, alterando suas propriedades biomecânicas de forma a favorecer a migração e invasão das células tumorais (WANG *et al*., 2021). Além disso, os CAFs secretam quimiocinas como CXCL1 e CCL2, além de fatores de crescimento que estimulam a proliferação, a sobrevivência e a resistência terapêutica das células neoplásicas, estabelecendo um ciclo de retroalimentação que sustenta e amplifica a progressão tumoral (HUANG *et al*., 2022).

A MEC constitui um componente estrutural e sinalizador essencial do MT. Além de fornecer suporte físico às células, a MEC regula ativamente a comunicação intercelular por meio de proteínas de adesão, fatores de crescimento sequestrados em sua estrutura e gradientes de rigidez mecânica (COX, 2021). As integrinas, receptores transmembrana que medeiam a interação entre as células tumorais e os componentes da MEC, são fundamentais nesse processo, pois a sua ativação

desencadeia cascatas de sinalização intracelular que regulam proliferação, sobrevivência, migração e invasão (COOPER; GIANCOTTI, 2019).

Nesse contexto, a progressão para metástases, que é responsável por mais de 90% das mortes por câncer, está intimamente relacionada à dinâmica do MT (FARES *et al*., 2020). O processo metastático envolve várias etapas, incluindo a invasão local, a disseminação sistêmica e a recolonização ectópica, todas facilitadas pela sinalização parácrina dentro do microambiente (ROMA-RODRIGUES *et al*., 2019). A expressão de moléculas como o VEGF e o TGF-β em células do MT contribui para a formação de novos vasos e para a modulação do comportamento das células tumorais, criando um nicho permissivo para a chegada das células malignas a outros órgãos (SHI *et al*., 2022).

Diante disso, fatores derivados do MT podem ser responsáveis pela resistência a tratamentos antineoplásicos (CHEN; SONG, 2019). A presença de CAFs, células endoteliais e células imunes imunossupressoras cria um ambiente protetor que atenua a eficácia de terapias convencionais, como quimioterapia e radioterapia, e de terapias-alvo (ROMA-RODRIGUES *et al*., 2019). A secreção de citocinas e fatores de crescimento pelo MT, aliada às alterações biomecânicas da MEC, pode ampliar a plasticidade fenotípica das células tumorais e enriquecer subpopulações com características de células-tronco, conferindo maior resistência ao tratamento (PLAKS; KONG; WERB, 2015). Assim, compreender a complexidade do MT, incluindo o papel da rigidez da MEC e das vias de mecanotransdução, é, portanto, fundamental para o desenvolvimento de estratégias terapêuticas mais eficazes no manejo do CEC oral (KAI; DRAIN; WEAVER, 2019).

1.11 Mecanotransdução

A mecanotransdução é definida como o processo pelo qual as células convertem estímulos mecânicos do microambiente extracelular em respostas bioquímicas intracelulares (DI *et al*., 2023). Trata-se de um processo contínuo, bidirecional e altamente dinâmico, no qual as células respondem aos sinais mecânicos recebidos, remodelam ativamente a MEC ao seu redor e geram novos estímulos que sustentam o sistema (SARASWATHIBHATLA; INDANA; CHAUDHURI, 2023). As principais forças mecânicas que atuam sobre as células incluem a rigidez da MEC, a pressão hidrostática intersticial, as forças de cisalhamento de fluidos, a tensão de

tração e a compressão, sendo a rigidez da MEC o estímulo mais estudado no contexto da carcinogênese (DI *et al*., 2023; SLEEBOOM *et al*., 2024).

O processo de mecanotransdução se inicia na interface célula-MEC, onde as integrinas atuam como os principais receptores mecânicos, conectando fisicamente os componentes da MEC ao citoesqueleto intracelular (YUAN *et al*., 2023). Ao se ligarem a ligantes da MEC como fibronectina, colágeno e laminina, as integrinas se agrupam e recrutam proteínas de adesão focal, formando complexos multiproteicos denominados adesões focais. Nesses sítios, proteínas como a FAK, a quinase Src, a talina, a vinculina e a paxilina são fosforiladas e ativadas, atuando como transdutores primários do sinal mecânico (SARASWATHIBHATLA; INDANA; CHAUDHURI, 2023). A magnitude e a organização dessas adesões focais são proporcionais à rigidez da MEC, uma vez que em substratos rígidos, as adesões focais são maiores, mais maduras e mais ativas do ponto de vista sinalizador (DI *et al*., 2023).

A partir das adesões focais, o sinal mecânico é transmitido ao interior celular por duas vias complementares. Na via bioquímica, a ativação de FAK e Src desencadeia cascatas de fosforilação que ativam as vias PI3K/AKT, ERK/MAPK, RhoA/ROCK e NF-κB, regulando proliferação, sobrevivência, migração e inflamação (YUAN *et al*., 2023). Na via biofísica, as forças mecânicas são transmitidas ao núcleo por meio do citoesqueleto de actina, que se conecta ao envoltório nuclear pelo complexo LINC (Linker of Nucleoskeleton and Cytoskeleton), composto pelas proteínas nesprinas e SUN (SARASWATHIBHATLA; INDANA; CHAUDHURI, 2023). Essa transmissão direta de força ao núcleo promove deformação do envoltório nuclear, abertura de poros nucleares e remodelação da cromatina, alterações que modificam a acessibilidade de genes e regulam a expressão gênica em resposta a sinais puramente mecânicos (DI *et al*., 2023).

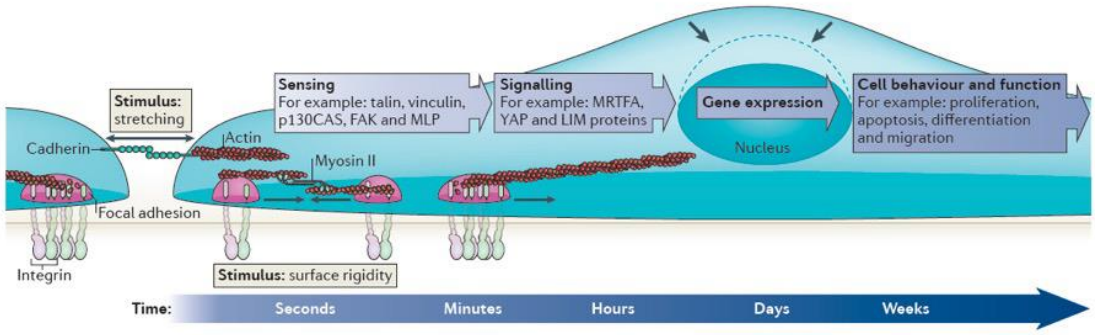


Figura 5 – Mecanotransdução celular. Esquema da conversão de estímulos mecânicos, como estiramento e rigidez da matriz, em sinais intracelulares via integrinas, adesões focais e citoesqueleto de actina. Esses sinais modulam a expressão gênica e determinam respostas celulares como proliferação, diferenciação, migração e apoptose ao longo do tempo. (ISKRATSCH *et al*., 2014).

A GTPase RhoA e sua quinase efetora ROCK desempenham papel central na transdução de sinais mecânicos. Em resposta à rigidez da MEC, RhoA é ativada e promove a polimerização de filamentos de actina-F e a formação de fibras de estresse, estruturas contráteis que aumentam a tensão intracelular e amplificam os sinais mecânicos provenientes do microambiente (YUAN *et al*., 2023). Além das integrinas e do citoesqueleto, canais iônicos mecanossensíveis representam uma via paralela e complementar de mecanotransdução. O canal Piezo1, ativado por forças de estiramento e deformação da membrana plasmática, promove influxo de Ca^{2+} que ativa diversas quinases intracelulares e modulam a expressão de genes associados à invasão e à resistência terapêutica (SLEEBOOM *et al*., 2024). O canal TRPV4 também responde à rigidez da MEC e tem sido associado à progressão tumoral em carcinomas epiteliais. A ativação desses canais em ambientes de elevada rigidez reforça e amplifica as cascatas de sinalização iniciadas pelas integrinas, integrando múltiplas vias mecanotransdutórias em uma resposta celular coerente (DI *et al*., 2023; SLEEBOOM *et al*., 2024).

No contexto do câncer, a mecanotransdução exerce efeitos pleiotrópicos que contribuem para praticamente todas as marcas da doença descritas por Hanahan e Weinberg (HANAHAN, 2022). A rigidez crescente da MEC tumoral ativa vias de sinalização que promovem proliferação celular, resistência à apoptose, angiogênese, indução da EMT e potencial metastático (YUAN *et al*., 2023). A tensão mecânica também modula o microambiente imune, induzindo a polarização de macrófagos para o fenótipo M2 imunossupressor e promovendo a expressão de PD-L1 nas células

tumorais, o que dificulta a resposta imune antitumoral (SLEEBOOM *et al*., 2024). No CEC oral, a rigidez elevada da MEC, determinada em grande parte pelo acúmulo e reticulação de colágeno mediados por CAFs, ativa mecanotransdutores que favorecem a invasão local, a disseminação linfonodal e a resistência ao tratamento (DI *et al*., 2023). Dados do nosso laboratório demonstraram que a exposição prolongada de células de CEC oral a um microambiente de alta rigidez induz a aquisição de fenótipo mesenquimal migratório, com regulação positiva de miosina II e marcadores de EMT, e que esse fenótipo persiste mesmo após a redução da rigidez da MEC, configurando uma "memória mecânica" cuja manutenção depende da sinalização por FAK e AKT e que pode ser necessária para a disseminação do CEC oral (MOON *et al*., 2023).

Dentre os múltiplos efetores moleculares ativados pela mecanotransdução, as proteínas YAP (Yes-associated protein) e TAZ (transcriptional coactivator with PDZ-binding motif) surgem como os principais integradores dos sinais mecânicos em respostas transcricionais pró-tumorais (ZANCONATO; CORDENONSI; PICCOLO, 2019; CUNNINGHAM; HANSEN, 2022). Em MEC rígidas, o aumento da tensão do citoesqueleto de actina promove a translocação nuclear de YAP/TAZ, onde ativam programas transcricionais oncogênicos por meio da interação com fatores de transcrição da família TEAD (PANCIERA *et al*., 2017; MOYA; HALDER, 2019; PICCOLO *et al*., 2023). Em matrizes macias, YAP/TAZ ficam retidos no citoplasma e subsequentemente degradados. Essa sensibilidade à rigidez posiciona YAP/TAZ como interruptores moleculares da mecanotransdução oncogênica, cuja biologia será detalhada na seção seguinte (PANCIERA *et al*., 2017; PICCOLO *et al*., 2023).

1.12 Via Hippo

A via Hippo é uma cascata de sinalização evolutivamente conservada, inicialmente identificada em Drosophila melanogaster como um regulador fundamental do tamanho de órgãos e da proliferação celular (CUNNINGHAM; HANSEN, 2022). O nome da via deriva do gene hippo, cuja perda de função em moscas resulta em hiperplasia tecidual com aparência característica de hipopótamo, evidência inicial de sua função supressora do crescimento (ZANCONATO; CORDENONSI; PICCOLO, 2019). Em mamíferos, a via Hippo é altamente conservada e controla a homeostase tecidual por meio da regulação do equilíbrio entre proliferação e apoptose, tendo seus componentes principais identificados como

homólogos funcionais dos genes originalmente descritos em Drosophila (CUNNINGHAM; HANSEN, 2022). A desregulação da via Hippo foi identificada como uma das alterações de sinalização mais frequentes em cânceres humanos, posicionando-a entre as vias oncogênicas de maior relevância clínica (HARVEY; TANG, 2025).

Do ponto de vista molecular, a via Hippo canônica é organizada em duas estruturas funcionais: o módulo upstream de quinases, responsável pela integração de sinais extra e intracelulares; e o módulo downstream de efetores transcricionais, responsável pela execução das respostas biológicas (MOYA; HALDER, 2019; KIM *et al*., 2025). No módulo upstream, as quinases MST1 e MST2 (homólogos mamíferos de Hippo) formam complexo com a proteína adaptadora SAV1 e fosforilam as quinases LATS1 e LATS2 em resíduos de treonina específicos, Thr1079 em LATS1 e Thr1041 em LATS2, com participação das proteínas cofatoras MOB1A e MOB1B (CUNNINGHAM; HANSEN, 2022). Uma vez ativadas, LATS1/2 fosforilam os efetores transcricionais YAP em Ser127 e TAZ em Ser89, promovendo o reconhecimento dessas proteínas pela proteína adaptadora 14-3-3, que as retém no citoplasma e direciona sua ubiquitinação e degradação proteossomal (MA *et al*., 2019; KIM *et al*., 2025). Nesse estado, denominado via Hippo ativa, a sinalização pró-proliferativa de YAP/TAZ é suprimida, mantendo a homeostase e o controle do tamanho tecidual (KIM *et al*., 2025).

Quando a via Hippo está inativa, seja por sinalização upstream reduzida, por alterações genéticas nos componentes da cascata ou por sinais mecânicos do microambiente, YAP e TAZ não são fosforilados, escapam da retenção citoplasmática e translocam para o núcleo (AMANO *et al*., 2024). No núcleo, YAP/TAZ não se ligam diretamente ao DNA, mas atuam como coativadores transcricionais ao interagir com fatores de transcrição da família TEAD (TEAD1–4), formando um complexo transcricional que ativa um programa gênico pró-oncogênico abrangente (CUNNINGHAM; HANSEN, 2022). Os principais alvos transcricionais de YAP/TAZ-TEAD incluem CTGF (fator de crescimento do tecido conjuntivo), CYR61 (proteína rica em cisteína e angiogênica 61), ANKRD1, genes reguladores do ciclo celular como CCND1 e MYC, genes anti-apoptóticos como BCL-xL, além de mediadores da EMT e da angiogênese (POBBATI; HONG, 2013; ZANCONATO; CORDENONSI; PICCOLO, 2019). Em conjunto, esses alvos genéricos promovem proliferação, sobrevivência,

invasão, formação de metástases e resistência a tratamentos antineoplásicos (PANCIERA *et al*., 2017).

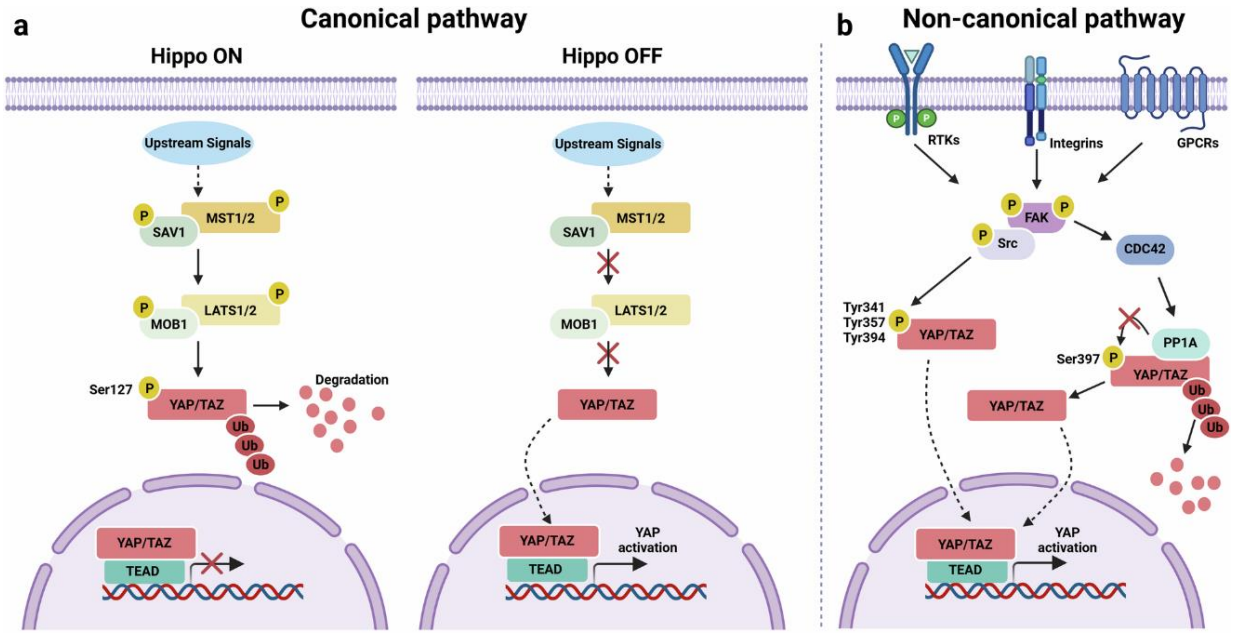


Figura 6 – Ativação e inativação da via Hippo canônica e não canônica. A) A ativação da via Hippo leva à fosforilação dos coativadores transcricionais YAP/TAZ, impedindo assim a sua entrada no núcleo e consequentemente a sua atividade. Por outro lado, com a inativação da via Hippo, YAP/TAZ não são fosforiladas, permitindo a sua entrada no núcleo, ligação aos fatores de transcrição TEAD e a indução da expressão gênica. B) A via não canônica promove a ativação de YAP/TAZ independentemente da inativação da via Hippo, como a sinalização FAK/Src mediada por integrinas (KIM *et al*., 2025).

Um aspecto de crescente importância na biologia de YAP/TAZ é sua regulação não canônica por sinais mecânicos, independentemente da cascata clássica MST-LATS (PANCIERA *et al*., 2017; CUNNINGHAM; HANSEN, 2022). Evidências recentes demonstram que YAP e TAZ funcionam como sensores diretos da rigidez da MEC: em matrizes rígidas, a elevada tensão do citoesqueleto de actina-F, mediada pelo eixo RhoA/ROCK, inibe a atividade de LATS1/2, resultando em hipofosforilação de YAP/TAZ e sua translocação nuclear constitutiva (ZANCONATO; CORDENONSI; PICCOLO, 2019). Por outro lado, em matrizes macias, a actina assume conformação globular (G-actina), o que favorece a atividade de LATS1/2 e a consequente fosforilação e retenção citoplasmática de YAP/TAZ (PANCIERA *et al*., 2017;

CUNNINGHAM; HANSEN, 2022). Essa regulação mecanossensível é mediada pelas integrinas e pelos complexos de adesão focal, no qual a ligação de integrinas à MEC rígida ativa FAK e Src, que por sua vez inibem LATS1/2 por meio do eixo FAK-SRC-PI3K-PDK1, liberando YAP/TAZ para translocação nuclear independentemente da sinalização MST (ZANCONATO; CORDENONSI; PICCOLO, 2019). Essa via não canônica é particularmente relevante no contexto do CEC oral, onde a rigidez crescente da MEC tumoral pode manter YAP/TAZ em estado nuclear constitutivamente ativo mesmo na ausência de mutações nos componentes canônicos da via Hippo (FARAJI *et al*., 2022).

Além disso, YAP e TAZ integram sinais provenientes de múltiplas vias oncogênicas além da mecanotransdução. A ativação de receptores acoplados à proteína G (GPCRs) pelo fator de ativação plaquetária e pela esfingosina-1-fosfato estimula RhoA e inibe LATS1/2, ativando YAP/TAZ independentemente de estímulos mecânicos (MOYA; HALDER, 2019). A via Wnt/β-catenina, a sinalização por Mitch e a ativação de receptores de tirosina quinase como EGFR também convergem sobre YAP/TAZ, amplificando sua atividade em tumores com co-ativação dessas vias (MOYA; HALDER, 2019; MA *et al*., 2019). YAP/TAZ modulam a expressão de TGF-β e estabelecem um circuito de *feedback* com os CAFs do MT, no qual YAP/TAZ ativos nas células tumorais estimulam a produção de TGF-β, que ativa CAFs; esses, por sua vez, depositam colágeno e aumentam a rigidez da MEC, o que retroativamente mantém YAP/TAZ em estado nuclear nas células tumorais (CALVO *et al*., 2013; ZANCONATO; CORDENONSI; PICCOLO, 2019). Esse ciclo de retroalimentação entre mecanotransdução, CAFs, rigidez da MEC e atividade de YAP/TAZ é um dos importantes mecanismos para a manutenção do fenótipo invasivo (PICCOLO *et al*., 2023).

No contexto do CEC oral, a expressão nuclear aumentada de YAP foi identificada desde lesões potencialmente malignas, como leucoplasia e eritroplasia, sendo progressivamente mais intensa em carcinomas invasivos, o que sugere que a desregulação de YAP/TAZ ocorre precocemente e pode contribuir para a iniciação tumoral (AMANO *et al*., 2024). Estudos de imunohistoquímica em amostras de CEC oral demonstraram que a expressão nuclear elevada de YAP se associa a pior grau histológico, maior estadiamento TNM, maior frequência de metástase linfonodal regional e menor sobrevida livre de doença e sobrevida específica por câncer (AMANO *et al*., 2024; COSTA *et al*., 2022). A expressão de YAP nos CAFs do estroma

tumoral também foi associada de forma independente a pior prognóstico, sugerindo que a atividade de YAP/TAZ no compartimento estromal, e não apenas no tumor propriamente dito, contribui para a agressividade do CEC oral (AMANO *et al*., 2024). Em modelos celulares, a inibição de YAP/TAZ por silenciamento gênico ou pelo inibidor farmacológico verteporfin compromete a proliferação, a migração e a resistência à cisplatina em linhagens de CEC oral, validando esses efetores como alvos terapêuticos relevantes (COSTA *et al*., 2022).

Do ponto de vista imunológico, YAP/TAZ exercem funções imunossupressoras no MT que contribuem para a evasão imune (PAN *et al*., 2019). A ativação de YAP/TAZ nas células tumorais promove a expressão de PD-L1, o recrutamento de células T regulatórias e a polarização de macrófagos para o fenótipo M2, criando um ambiente que inibe a resposta citotóxica antitumoral e compromete a eficácia das terapias de *checkpoint* imunológico (KIM *et al*., 2025). Esse papel de YAP/TAZ na imunossupressão representa uma dimensão adicional de sua oncogenicidade, particularmente relevante em um cenário no qual as terapias anti-PD-1/PD-L1 estão sendo avaliadas no tratamento do CEC recorrente e metastático (ZHANG *et al*., 2021).

No que se refere ao potencial terapêutico, a inibição farmacológica da via YAP/TAZ representa uma estratégia promissora e ativa no campo da oncologia (CUNNINGHAM; HANSEN, 2022). O verteporfin, aprovado para uso oftalmológico, inibe a interação YAP-TEAD e demonstrou atividade antitumoral em modelos pré-clínicos de CEC oral (COSTA *et al*., 2022). Diante disso, a compreensão do papel da rigidez da MEC na ativação de YAP/TAZ e de suas consequências prognósticas no CEC oral constitui o eixo central da investigação apresentada nos artigos que compõem esta tese.

2 OBJETIVOS

2.1 Objetivo geral

- Investigar a influência da rigidez da matriz extracelular e da atividade das proteínas YAP/TAZ no prognóstico de pacientes com CEC oral, por meio de revisões sistemáticas da literatura e de análise histológica quantitativa do teor de colágeno estromal.

2.2 Objetivos específicos

- Avaliar, por meio de revisão sistemática da literatura e meta-análise, o papel da rigidez da MEC na progressão tumoral e no prognóstico de pacientes com CEC oral.
- Investigar, por meio de revisão sistemática da literatura e meta-análise, a influência da superexpressão e da ativação nuclear de YAP/TAZ na progressão tumoral e no prognóstico de pacientes com CEC oral.
- Quantificar o teor de colágeno estromal em amostras histológicas de CEC oral por meio de coloração com Picrosirius
- Correlacionar os grupos de alto e baixo teor de colágeno com o grau de diferenciação histopatológica com dados clinicopatológicos dos pacientes.

3 ARTIGO CIENTÍFICO 1

Artigo científico submetido à revista *Critical Reviews in Oncology/Hematology*
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The role of extracellular matrix stiffness in the progression and prognosis of oral squamous cell carcinoma: a systematic review and meta-analysis

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ABSTRACT

Aim: To critically evaluate the influence of extracellular matrix (ECM) stiffness on tumor progression, prognosis, and clinical implications in patients with oral squamous cell carcinoma (OSCC). **Materials and Methods**: This systematic review followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines, and a protocol was registered in the PROSPERO database. Five databases (PubMed, Scopus, Embase, Web of Science, and OATD) were searched without restrictions on publication year or language. Studies that evaluated ECM stiffness in OSCC, either as an assessment method or as a prognostic factor, were included. **Results**: Eight studies involving 436 patients were included. Elastography was the most frequent measurement method, employed in four studies. Mean tumor thickness ranged from 3.0 to 4.4 mm. Extracellular matrix stiffness was expressed in

various units of measurement (e.g., 37.7 gf, 23.5 kPa) and was consistently higher in tumor tissue compared to normal mucosa in all studies. Three studies demonstrated a significant correlation between stiffness and tumor thickness. One study identified predictive cut-off values for lymph node metastasis and reported that high matrix stiffness was an independent predictor of worse overall survival (HR=4.022; 95%CI: 1.294-12.495; p=0.016) and recurrence-free survival (HR=5.680; 95%CI: 1.146-28.143; p=0.033). **Conclusion**: Extracellular matrix stiffness influences the progression and prognosis of patients with OSCC and may represent a clinically relevant biomechanical biomarker for early detection, prognostic stratification, and therapeutic guidance.

Keywords: Head and Neck Squamous Cell Carcinoma, Extracellular Matrix, Biomechanical Phenomena, Prognosis, Elasticity Imaging Techniques, Biomarkers, OSCC.

INTRODUCTION

Oral Squamous Cell Carcinoma (OSCC) is one of the most prevalent malignancies of the head and neck and is considered a major global public health concern[1,2,3]. In 2020, approximately 377,713 new cases were reported worldwide according to GLOBOCAN data, with projections indicating a 40% increase in incidence by 2040[4]. Early detection is critical for improving prognosis and reducing morbidity and mortality[5,6,7]. However, over 60% of OSCC cases are diagnosed at advanced stages[8], when the 5-year survival rate is substantially reduced[4,8]. This delayed diagnosis largely stems from the subtle nature of early clinical presentation.

A clinically relevant hallmark of malignant transformation in solid tumors is increased tissue stiffness[9]. This mechanical alteration is intrinsically associated with extracellular matrix (ECM) remodeling, whereby the tumor microenvironment becomes significantly stiffer than normal tissues, primarily due to increased cellular density, intense fibrosis, and qualitative and quantitative changes in ECM composition[9,10,11].

Histologically, ECM stiffness is driven by excessive collagen deposition and crosslinking mediated by enzymes such as lysyl oxidase (LOX), a phenomenon known as desmoplasia[11,12]. Increased stiffness does not merely represent a passive clinical sign but rather acts as an active modulator of tumor cell behavior through mechanotransduction[13,14]. In this process, mechanical signals induced by ECM stiffness are converted into intracellular biochemical responses, activating pathways

such as FAK (focal adhesion kinase) and promoting invasion, cell migration, and epithelial-mesenchymal transition (EMT)[12,13,14].

For objective and non-invasive assessment of these mechanical properties of tumor stiffness, elastography methods have been employed, providing quantitative data on tissue elasticity[12,15]. The clinical utility of these methods is well established in medicine, particularly in breast, thyroid, and hepatobiliary oncology, having demonstrated high diagnostic potential in differentiating between malignant and benign lesions[16,17,18]. This success is based on the ability of these techniques to quantitatively map the elevated stiffness characteristic of malignant tissues[15,16].

Despite the growing recognition of stiffness as a tumor biomarker and the promising results of assessment methods in other cancer types, its systematic application in the context of OSCC remains incipient[19,20]. ECM stiffness is linked to mechanisms of tumor progression, such as the induction of an invasive-dormant subpopulation in response to mechanical stress[20,21], suggesting a crucial role for these methods not only in diagnosis but also in predicting lymph node metastasis[22].

Considering that stiffness measurement can aid in early detection, provide prognostic information, and guide therapeutic decisions[23,24], it is essential to systematically consolidate the available evidence. In this context, the present systematic review aims to critically evaluate the influence of ECM stiffness on tumor progression, prognosis, and clinical implications in patients with OSCC.

MATERIAL AND METHODS

Protocol and registration

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[25] statement and Cochrane guidelines[26]. The review protocol was registered in the PROSPERO database CRD420251156429.

Study design

This is a systematic review designed to answer the following guiding PEO question: “Does extracellular matrix stiffness (***Exposure***) influence the progression and prognosis (***Outcomes***) of patients with oral squamous cell carcinoma (***Population***)?”

Elebility criteria

Studies evaluating extracellular matrix stiffness in oral squamous cell carcinoma, either as an assessment method or as a prognostic factor, were included without restrictions on year, language, or publication status (including in press).

The following studies were excluded: Studies not related to the topic; Studies focused on other types of cancer without specific data for oral cancer; Review articles, editorials, letters to the editor, and conference abstracts without complete data; Studies evaluating only the composition of the extracellular matrix without analyzing stiffness; Studies in which oral cancer data could not be isolated from other head and neck cancers; Animal studies; In vitro studies.

Sources of information and search

Two reviewers (IFPL and ACTS) independently searched electronic databases. Descriptors were identified using DeCS (Health Sciences Descriptors), MeSH (Medical Subject Headings), and Emtree (Embase Subject Headings), and the Boolean operators “AND” and “OR” were used to optimize the search strategy (Table 1). The results were exported to EndNote Basic/Online (Thomson Reuters, Toronto, Canada), and duplicates were removed.

Study selection

The selection of studies was conducted in three distinct stages. In the first stage, two reviewers (IFPL and ACTS) systematically screened the titles of all retrieved records. Articles with titles relevant to the objective of this systematic review were selected for the second stage. In the second stage, the same reviewers screened the abstracts and applied the inclusion and exclusion criteria. Studies without available abstracts were evaluated in the third stage. In this final stage, the full texts of the articles were assessed. When disagreements arose regarding a study’s eligibility, a third reviewer (MLL) was consulted to make a final decision. Studies that did not meet the criteria were recorded separately, with the reasons for exclusion clearly documented.

Process of data collection and extraction

Two authors (IFPL and ACTS) performed data extraction using specific spreadsheets designed for this purpose. The following information was extracted from

the eligible studies: author and year, sample size (n), sex, age (years), tumor location, tumor type, tumor stage, follow-up, stiffness measurement method, stiffness classification, tumor thickness (mean), quantitative stiffness parameters (mean ± SD), histopathological correlations, progression factors, and prognostic outcomes.

As a calibration exercise, the reviewers (IFPL and ACTS) discussed the eligibility criteria and applied them to a random sample of 20% of the retrieved studies to assess inter-rater agreement. After reaching an adequate level of agreement (κ = 0.83–0.87), the reviewers independently extracted data from all studies. Any discrepancies were resolved by consensus and supervision of a third reviewer (MLL). Authors of studies with insufficient data or missing information necessary for summarization and comparison were contacted via email to request additional details.

Risk of individual Bias of the studies

The risk of bias for individual studies was independently assessed by two authors (IFPL and ACTS) using the "*Joanna Briggs Institute Critical Appraisal Checklist for Diagnostic Test Accuracy Studies*" and "*The Joanna Briggs Institute Critical Appraisal Checklist for Cohort Studies*". The risk of bias was classified as **"High"** when the study achieved up to 49% of "yes" responses, ***"Moderate"*** when it ranged from 50% to 69%, and ***"Low"*** when it exceeded 70% (Table 3).

Data Integration and Statistical Evaluation

Data from the five of eight included studies were integrated and analyzed using Comprehensive Meta-Analysis (CMA) software, Version 4 (Biostat, Inc., Englewood, NJ, USA; available at meta-analysis.com). The primary outcome measure consisted of continuous data, specifically assessing the standardized mean difference in extracellular matrix stiffness between oral control (normal oral mucosa) and oral squamous cell carcinoma (OSCC) samples.

Effect sizes were calculated and presented as standardized mean differences (Cohen's d) with their respective 95% confidence intervals (CI). Forest plots were generated to visually represent the standardized mean differences and 95% CI for each individual study, along with the pooled summary effect estimate. A distribution plot of true effects was also constructed to illustrate the overall pattern of effect sizes across studies.

Statistical heterogeneity among the included studies was objectively evaluated using the I^2 statistic. The I^2 statistic quantifies the proportion of overall variation among studies attributable to genuine heterogeneity rather than random variation. Interpretation of I^2 values followed established criteria: 0% to 40% indicated negligible heterogeneity, 30% to 60% denoted moderate heterogeneity, 50% to 90% signified substantial heterogeneity, and 75% to 100% represented considerable heterogeneity. Given the expected variability in meta-analyses of clinical studies and the heterogeneity in stiffness measurement methods across studies (including elastography, manual devices, and histological quantification), a random-effects model using the DerSimonian-Laird method was employed for all meta-analyses. This model assumes that the true effect size varies among studies, accounting for both within-study sampling error and between-study heterogeneity. The pooled summary effect measure was the standardized mean difference, computed with its 95% confidence interval.

Publication bias was assessed through both visual and quantitative methods. Funnel plots and precision plots were generated to visually identify potential publication bias by plotting the precision of each study against it corresponding standardized mean difference. Asymmetry in the funnel plot, particularly the absence of small studies exhibiting negative effects, may suggest the presence of publication bias. Additionally, Egger's regression intercept test and Begg's rank correlation test were employed to statistically assess funnel plot asymmetry, contingent upon the number of studies included and deemed appropriate for such analysis.

RESULTS

Selection of studies

The search of PubMed, Web of Science, Embase, Scopus, and OATD databases resulted in 5,480 articles. After removing duplicates, 3,995 records remained for title screening. Of these, 180 were considered potentially eligible for abstract review. After abstract screening, 23 articles were selected for full-text assessment. In addition, all references of the selected manuscripts (a total of 634 references) were carefully analyzed to identify articles not captured by the main search strategy, resulting in the inclusion of 2 additional articles. Thus, 25 articles were assessed in full text. After excluding 17 articles for various reasons, 8 studies were ultimately included in this systematic review (Figure 1).

Study characteristics

The publication dates of the articles[27-34] ranged from 1998 to 2025, and all studies[27-34] were conducted in patients (Table 2). The total sample comprised 436 patients across all studies[27-34], with individual study samples ranging from 2 to 127 patients. The age of the participants ranged from 20 to 91 years, with mean ages varying from 45.2 to 60.2 years when reported. One study[30] additionally included carcinoma *in situ* (CIS), and one study[34] included a mixed sample of malignant and benign oral lesions. The tongue, particularly the lateral border, was the most commonly investigated anatomical location, being the exclusive focus in five studies[28-32]. One study[27] included multiple oral sites (tongue, floor of mouth, and buccal mucosa), and one study did not specify the exact oral location[34].

Baseline tumor stage was reported in five[28-30,32,33] of the eight studies. Two studies included T1 and T2 tumors, one study[28] also included T3 tumors, one study[32] reported pathological staging (pT1 and pT2), and one study[33] included the full spectrum from T1 to T4. Nodal status was specified in two studies[29,30], with Ogura et al. (2000)[29] reporting N0 to N2 stages and Shingaki et al. (2017)[30] including only N0 patients. Only three studies[29,30,33] reported follow-up periods: Shingaki et al. (2017)[30] followed patients for 1 year, Ogura et al. (2000)[29] for 2 years, and Jingyuan et al. (2023)[33] for 3 years.

Regarding stiffness measurement methods, the most common approach was strain elastography, employed in four studies[30-32,34]. These studies used intraoral ultrasonography with transducer frequencies ranging from 7 to 15 MHz, applying manual compression with acoustic coupling gel or polymer gel to assess tissue elasticity. Three studies[27-29] utilized a manual hardness-measuring device developed by the authors, consisting of a handheld probe (2 mm diameter) pressed into the mucosa to a depth of 2 mm, with load measurement via a strain gauge. One study[33] employed an indirect method, using Masson's trichrome histological staining to semi-quantify collagen fiber content as a surrogate marker for tissue stiffness.

Stiffness classification varied across studies. Three studies[27-29] used quantitative measurements expressed in grams[28-29] or gamma-force[27]. Three studies[30,32,34] employed elastography-derived metrics, including strain ratio and Young's modulus. One study[31] expressed results as percentage strain values. One study[33] used a semi-quantitative scoring system (0-4) based on the percentage of

collagen fibers visualized in tumor stroma, with a binary classification of low stiffness (score <3) versus high stiffness (score ≥3). One study[34] explicitly stated the use of arbitrary values to test diagnostic accuracy, which significantly limits the validity of its findings.

Risk of Bias of studies

Only one study[33] presented a low risk of bias, while seven studies were classified as having a moderate risk of bias. No study was judged to have a high risk of bias (Table 3). Question 5 was considered not applicable for one study[31], as the objective was to characterize and evaluate quantitative strain without defining or applying a diagnostic threshold (cutoff point) to classify the lesions.

Individual results of the studies

Of the 8[27-34] included studies, only three[28,30,32] reported mean tumor thickness, ranging from 3.0 to 4.4 mm (Table 4). Stiffness parameters were assessed using diverse units and methodologies across studies, being expressed in gamma-force[27], grams[28,29], kPa[34], percentage strain[31], and semiquantitative scores[30,33]. One study[31] reported complete absence of strain (0%) in tumors, while another[33] classified stiffness based on collagen content (>50% indicating high stiffness).

One study[27] reported a mean stiffness of 37.7 gf for OSCC, which was 2.3 to 19.1 times harder than normal mucosa (4.8 gf), demonstrating significant differences ($p<0.001$). Another study[28] found an overall mean consistency of 22.1±8.0 g, with values varying significantly according to T-stage: T1 tumors measured 15.4±4.0 g (n=17), T2 tumors 24.8±5.7 g (n=21), and T3 tumors 30.3±9.5 g (n=7), with T2 and T3 being significantly firmer than T1 ($p<0.001$). In a subsequent study[29], tumors with pathologically negative nodes (PN-) measured 16.8±6.3 g (n=36), while those with regional metastasis measured 24.2±5.2 g (n=7) and those with regional recurrence measured 23.5±5.9 g (n=10), both significantly firmer than PN- tumors ($p<0.05$).

Studies employing strain elastography reported strain ratios (SR)[32,34], Young's modulus values[34], or percentage strain[31]. Mean strain ratios ranged from 4.8±0.9 to 9.7±9.8 for OSCC across studies[32,34], compared to benign lesions with values of 1.9±0.5[34]. Young's modulus for malignant lesions was 23.5±4.3 kPa compared to 8.2±2.1 kPa for benign lesions ($p<0.001$)[34]. One preliminary study[31] reported

percentage strain values demonstrating complete absence of strain (0.000%) in tumor tissue.

Qualitative and semi-quantitative scoring systems were employed in two studies[30,33]. One study[30] utilized a four-grade elasticity scoring system based on color-coded elastography images, where scores ranged from 1 (very soft, predominantly green-red appearance) to 4 (very hard, predominantly blue appearance). In this study, all carcinomas (n=15) scored 3 (n=8, 53%) or 4 (n=7, 47%), while all non-malignant lesions (n=2) scored 1 or 2, achieving perfect diagnostic separation (100% sensitivity and specificity). Another study[33] employed Masson's trichrome staining to semi-quantify collagen fiber content in tumor stroma as an indirect measure of matrix stiffness, using a scoring system from 0 to 4 based on the percentage of collagen fibers: 0 (<10%), 1 (11-30%), 2 (31-50%), 3 (51-80%), and 4 (>80%).

Six studies[28-30,32-34] investigated histopathological correlations in relation to tumor stiffness, of which three studies[28,29,32] demonstrated significant positive correlations between OSCC stiffness and tumor thickness.

Only one study[33] reported long-term survival outcomes, in which patients with high matrix stiffness (Masson score ≥3) had significantly shorter overall survival (OS) and recurrence-free survival (RFS) compared to those with low stiffness (score <3) (log-rank test, $p<0.001$ for OS and $p=0.047$ for RFS). Multivariate Cox regression analysis, adjusting for other clinical and pathological variables, identified high matrix stiffness as an independent risk factor for OS (HR=4.022, 95%CI: 1.294-12.495, $p=0.016$) and RFS (HR=5.680, 95%CI: 1.146-28.143, $p=0.033$). The remaining seven studies[27-32,34] did not assess or report survival outcomes.

Meta-Analysis Results

A meta-analysis of five studies comprising 307 patients was conducted to assess the standardized mean difference in extracellular matrix stiffness between oral control (normal oral mucosa) and oral squamous cell carcinoma (OSCC) samples. The included studies were published between 1998 and 2025, with individual study samples ranging from 32 to 127 participants. The pooled analysis revealed a statistically significant and substantial increase in ECM stiffness in OSCC tissue compared to normal oral mucosa, with a standardized mean difference (Cohen's d) of 5.28 (95% CI: 2.61–7.94; $Z = 3.88$; $p < 0.001$). The five studies with available

quantitative data demonstrated consistent findings favoring OSCC tissue stiffness (Figure 2).

In our meta-analytic calculations, the standardized mean difference for Babkair et al. (2025) was 4.53 (95% CI: 3.79–5.27; Z = 11.99; p < 0.001) based on a sample of 50 control and 50 OSCC specimens. Jingyuan et al. (2023) yielded the largest effect size among the included studies, with a standardized mean difference of 13.54 (95% CI: 12.34–14.74; Z = 22.06; p < 0.001) derived from a substantially larger sample of 127 control and 127 OSCC participants. For Ogura et al. (1998), the calculated standardized mean difference was 3.52 (95% CI: 2.95–4.09; Z = 12.12; p < 0.001) across 80 control and 45 OSCC samples. The analysis of Ogura et al. (2000) yielded a standardized mean difference of 2.65 (95% CI: 2.02–3.28; Z = 8.20; p < 0.001) in a sample of 36 control and 36 OSCC specimens. For Sato et al. (1995), the standardized mean difference was calculated as 2.38 (95% CI: 1.74–3.02; Z = 7.29; p < 0.001) in 32 control and 32 OSCC samples (Figure 2A). All individual study estimates demonstrated statistical significance at the p < 0.001 level, with confidence intervals that did not cross zero, indicating evidence of increased stiffness in OSCC tissue.

Substantial heterogeneity was observed among the included studies, as evidenced by an I^2 statistic of 75% to 100%, indicating that the majority of variation in effect sizes was attributable to genuine differences between studies rather than random sampling error. This heterogeneity was anticipated and likely reflects the diversity in stiffness measurement methodologies employed across studies, including elastography (the most frequent method, used in four studies), manual devices, and histological quantification approaches. The heterogeneity also reflects variations in study design, patient populations, tumor characteristics (including differences in tumor thickness ranging from 3.0 to 4.4 mm), and anatomical locations (with the tongue, particularly the lateral border, being the most commonly investigated site). Given this substantial heterogeneity, a random-effects model using the DerSimonian-Laird method was appropriately employed for the meta-analysis, which accounts for both within-study sampling error and between-study heterogeneity. The 95% prediction interval for the true effect ranged from −5.25 to 15.81, reflecting the considerable variability in effect sizes across studies while still indicating that the true effect in future studies is likely to favor OSCC tissue stiffness (Figure 2B).

Publication bias was systematically evaluated through both visual and quantitative methods (Figure 3). Funnel plot analysis revealed a relatively symmetric

distribution of studies around the pooled effect estimate, with studies distributed across various levels of precision (standard error) (Figure 3A). The precision plot, which plots precision (1/standard error) against standardized mean difference, similarly demonstrated a symmetric pattern without evidence of systematic asymmetry that would suggest preferential publication of studies with larger or smaller effect sizes (Figure 3B). Quantitative assessment of funnel plot asymmetry using Egger's regression intercept test and Begg's rank correlation test did not reveal statistically significant asymmetry, suggesting minimal evidence of publication bias in the included studies. The absence of small studies with negative or null effects further supports the conclusion that publication bias is unlikely to substantially influence the overall meta-analytic findings.

DISCUSSION

Increased tissue stiffness is a characteristic property of tissues and organs undergoing morphofunctional alterations[35]. Recent studies have evaluated tumor stiffness as a diagnostic tool and as a method for predicting metastatic potential. This systematic review synthesized evidence from eight studies involving patients with OSCC, demonstrating that extracellular matrix stiffness represents a consistent and clinically significant biomechanical marker of malignant transformation in the oral cavity. The meta-analysis revealed that tumor tissue exhibits substantially higher stiffness compared to normal oral mucosa and functions as a predictor of worse overall survival and recurrence-free survival outcomes.

OSCC is a tumor of malignant epithelial origin that, in most cases, presents clinically as a hardened solid mass[9], which is closely related to qualitative and quantitative alterations in ECM components[9,10]. As a way to measure such stiffness, studies[27,34] have been developed for this purpose. Among the most evaluated methods, employed in four studies[30-32,34] of the present systematic review, is elastography. This is an approach that visualizes and quantifies tissue elasticity through ultrasound evaluation of differential displacement under stress or by shear waves and mechanical waves[10]. This approach enables the acquisition of quantitative metrics (strain ratio, Young's modulus) that facilitate objective comparison between patients and tissues through measurement of variations in biomechanical properties[10].

In this context, elastography has been extensively applied to evaluate stiffness in various malignancies, including breast tumors[9], liver[36,37], pancreas[38,39], and prostate

cancers[40,41]. Supporting the validity of this measurement method, Shingaki et al.[30] demonstrated difference between malignant (elasticity scores 3–4) and benign (scores 1–2) oral lesions, while Shibata et al.[32] correlated strain ratios with histopathological collagen content, thereby validating the biological basis of elastography measurements. However, it is important to acknowledge that elastography requires specialized equipment, technical expertise, and careful attention to compression uniformity to ensure reproducible and reliable results[9].

Nevertheless, the heterogeneity of stiffness measurement approaches employed in the included studies, such as manual mechanical devices[27-29], strain elastography[30-32,34], and histological quantification of collagen content[33], reflects the evolving landscape of biomechanical tissue characterization for tumor analyses. Manual hardness-measuring devices, evaluated in three studies[27,28,29,] provided objective quantification of tissue resistance to deformation through controlled probe displacement and load measurement. Although operator-dependent and requiring direct tissue contact, these devices demonstrated good reproducibility[27,28,29]. The simplicity and low cost of these devices may make them particularly suitable for resource-limited settings or for quantifying intraoperative palpation findings[27,28,29]. On the other hand, histological quantification of collagen fibers through Masson's trichrome staining, as employed in one study[33], provides an indirect but biologically grounded assessment of matrix stiffness[33]. However, the semi-quantitative nature of histological scoring may not capture continuous biological variation, and post-fixation processing may alter tissue mechanical properties, limiting direct assessment for in vivo stiffness[42].

The quantitative stiffness values for OSCC in the present systematic review ranged from 15–40 g in manual measurements, 4.8–9.7 in strain ratios, and 23.5 kPa in Young's modulus. In comparison, studies of breast carcinomas demonstrate mean Young's modulus values ranging from 80–120 kPa measured by shear wave elastography[43]. A recent study reported mean hardness values of 189.5 kPa for breast tumors[44], substantially higher than those observed in OSCC. This difference can be attributed to the composition and organization of the extracellular matrix in breast carcinomas, which exhibit extensive desmoplasia with considerable deposition of type I collagen organized in parallel linear bundles[45]. In contrast, although OSCC demonstrates significant collagen deposition, the fibrillar architecture tends to be more disorganized and multidirectional, particularly at invasive margins[46].

Concurrently, studies[47] have demonstrated mean hardness exceeding 100 kPa in pancreatic tumors, which is also significantly higher than that found in OSCC in the present systematic review. Similarly, liver tumors, specifically hepatocellular carcinoma[48], demonstrated stiffness of 55 kPa, while in studies[49] that evaluated prostate tumors, values of 91.6-131.8 kPa were reported. Multiple factors appear to be involved in the differences found, such as variable stroma-tumor proportion[50], specific extracellular matrix composition of each tumor[51,52], and methodological heterogeneity found in different forms of stiffness measurement.

On the other hand, cervical squamous cell carcinomas, which share similar histological and etiological characteristics with OSCC, exhibit a mean stiffness of 5.27 kPa when evaluated with magnetic resonance elastography[53]. Additionally, esophageal squamous cell carcinomas demonstrate strain ratios by ultrasound elastography of 1±0.9 (range 0.3 to 2.9)[54], resembling the values reported for tongue cancer. This consistency across squamous cell carcinomas of mucosal origin suggests that the biomechanical phenotype of malignant squamous epithelium may be relatively conserved, driven by common mechanisms of stromal remodeling, collagen deposition, and mechanical stress responses inherent to squamous carcinogenesis[55].

When considering the relationship between stiffness and the degree of tumor invasion across sites, it is observed that pancreatic and breast carcinomas, which frequently exhibit early systemic dissemination, demonstrate very high absolute stiffness[56,57]. In contrast, basal cell carcinomas of the skin, known for their high invasion capacity but low metastatic potential, show moderately elevated stiffness compared to normal dermis[58]. OSCC occupies an intermediate position, with significant locoregional invasiveness and cervical lymph node metastasis, but less frequent distant dissemination than breast or pancreatic cancers[59]. The finding from one study[29] in the present systematic review that tumors exceeding 30.1 g showed 100% lymph node involvement supports an effect whereby high stiffness correlates with invasive capacity, potentially mediated through mechanotransduction pathways that promote epithelial-mesenchymal transition, matrix metalloproteinase expression, and migratory phenotypes[60,61].

Thus, understanding how physical properties of the extracellular matrix are transformed into biological responses is fundamental to comprehending the mechanisms that influence tumor progression[60]. The focal adhesion kinase (FAK) pathway and Hippo-YAP/TAZ signaling are currently considered the main transducers

of mechanical signals, with matrix stiffness promoting FAK phosphorylation at focal adhesions and nuclear translocation of YAP/TAZ, driving expression of pro-proliferative and pro-invasive genes[62]. Based on this, one study[33] from the present systematic review demonstrated that mechanical stress related to stiffness causes nuclear envelope rupture and DNA damage, which is sensed by cyclic GMP-AMP synthase (cGAS), triggering sequential phosphorylation of STING (stimulator of interferon genes), TBK1, and IRF3, creating dormant cell populations and favoring epithelial-mesenchymal transition, fundamental for tumor progression.

Despite limitations in the studies involved, such as heterogeneity in stiffness measurement methods and differences in protocols used, the findings of this systematic review have very important clinical implications. The substantial heterogeneity observed in this meta-analysis ($I^2 = 98.61\%$) reflects the methodological diversity of stiffness measurement approaches employed in the included studies, including manual mechanical devices[27-29] strain elastography[30-32,34] and histological quantification of collagen content[33]. While this heterogeneity demonstrates the robustness of findings across diverse measurement techniques, it limits direct comparison of absolute stiffness values. Standardization of measurement protocols and reporting formats would facilitate future meta-analyses and clinical translation. Variations in tumor characteristics (thickness: 3.0–4.4 mm) and anatomical locations (predominantly tongue) may also contribute to effect size heterogeneity. The tongue, particularly the lateral border, represents the most common OSCC site and may exhibit distinct biomechanical properties compared to other oral sites. Future research should systematically investigate these variables to identify potential moderators of effect size.

Despite that, this systematic review and meta-analysis provides robust evidence that extracellular matrix stiffness is substantially and significantly elevated in oral squamous cell carcinoma compared to normal oral mucosa ($d = 5.28$; 95% CI: 2.61–7.94; $p < 0.001$). The consistency of this finding across diverse measurement methodologies and study populations, combined with the absence of significant publication bias, supports the biological significance of ECM stiffness as a fundamental characteristic of oral malignancy. The universal elevation of tumor tissue stiffness across all included studies, coupled with documented correlations between ECM stiffness and clinically relevant parameters (tumor thickness, lymph node metastasis, and survival outcomes), establishes ECM stiffness as a robust and clinically meaningful biomechanical biomarker in OSCC.

The integration of biomechanical assessment into clinical practice could enhance diagnostic accuracy, improve prognostic stratification, and facilitate more personalized therapeutic guidance. However, clinical translation requires standardization of measurement protocols, prospective validation studies with adequate sample sizes, and systematic investigation of the mechanistic relationships linking ECM stiffness to tumor progression and clinical outcomes. Ultimately, the integration of ECM stiffness assessment into comprehensive diagnostic and prognostic frameworks could contribute to earlier detection, improved risk stratification, and more effective and personalized management of patients with OSCC.

CONCLUSION

Tumor tissue in OSCC exhibited higher stiffness than normal oral mucosa in all studies evaluated, with significant correlations with tumor thickness, lymph node metastasis, and survival outcomes. Therefore, extracellular matrix stiffness demonstrates consistent influence on the progression and prognosis of patients with OSCC and may represent a clinically relevant biomechanical biomarker in OSCC, with potential to improve early detection, prognostic stratification, and therapeutic guidance.

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Table 1 - Search strategies in the databases.

Databases	*Search strategies (September, 2025)*	*Results*
PubMed http://www.ncbi.nlm.nih.gov/pubmed	("stiffness" OR "extracellular matrix" OR "matrix stiffness") AND (“oral cancer” OR “head and neck cancer”)	397
Scopus https://www.scopus.com/	("stiffness" OR "extracellular matrix" OR "matrix stiffness") AND (“oral cancer” OR “head and neck cancer”)	847
Embase https://www.embase.com/	('stiffness'/exp OR 'stiffness' OR 'extracellular matrix'/exp OR 'extracellular matrix' OR 'matrix stiffness'/exp OR 'matrix stiffness') AND ('oral cancer'/exp OR 'oral cancer' OR 'head and neck cancer'/exp OR 'head and neck cancer')	3,735
Web Of Science http://apps.webofknowledge.com/	("stiffness" OR "extracellular matrix" OR "matrix stiffness") AND (“oral cancer” OR “head and neck cancer”)	466
OATD https://oatd.org/	("stiffness" OR "extracellular matrix" OR "matrix stiffness") AND (“oral cancer” OR “head and neck cancer”)	35
TOTAL		5,480

Table 2 - Summary of the main characteristics of the eligible studies.

Author and year	*Sample (n)*	*Sex*	*Age (mean)*	*Tumor location*	*Type of tumor*	*Tumor stage*	*Follow-up*	*Stiffness measurement method*	*Stiffness classification*	*Control values*
Sato et al., 1995[27]	32	--	--	Oral mucosa (Tongue, floor of mouth, buccal mucosa)	OSCC	--	No	Manual hardness measuring device (2mm probe, 2mm depth, load measurement)	Quantitative (gf)	Normal mucosa
Ogura et al., 1998[28]	45	31 (male) 14 (female)	26-85 (55.5)	Tongue	OSCC	T1, T2 and T3	No	Manual hardness measuring device (2mm probe, 2mm depth, strain gauge)	Quantitative (g)	Contralateral normal tongue
Ogura et al., 2000[29]	53	34 (male) 19 (female)	23-85 (56.1)	Tongue	OSCC	T1, T2, N0 and N1-N2	2 years	Manual hardness measuring device (same method)	Quantitative (g)	Contralateral normal tongue
Shingaki et al., 2017[30]	17	11 (male) 6 (female)	38-85 (--)	Tongue	OSCC and CIS	cT1-T2, N0	1 year	Strain elastography (intraoral probe, 7-13 MHz, manual compression, acoustic coupling gel)	Elasticity score 1-4*	Normal tongue (adjacent tissue)
Ogura et al., 2018[31]	2	2 (male)	50-59 (--)	Tongue	OSCC	--	No	Strain elastography (intraoral ultrasonography, Aplio 300, 14 MHz, manual compression)	Quantitative; Strain (%)	--
Shibata et al., 2021[32]	62	28 (male) 34 (female)	28-91 (60.2)	Tongue	OSCC	pT1 and pT2	No	Strain elastography (intraoral, Aplio 500, 7-14 MHz hockey-stick probe, manual compression)	SR and semi-quantitative histology (%CFC, %CCC, %TIICs)	Normal tongue
Jingyuan et al., 2023[33]	127	--	>60 and <60 (--)	Oral cavity	OSCC	T1-T4; N0-N+	3 years	Masson's trichrome staining of collagen fibers in tumor stroma	Semi-quantitative score; Percentage of collagen fibers in the tumor stroma classified into scores	Tumor adjacent epithelium
Babkair, 2025[34]	50	30 (male) 20 (female)	20-70 (45.2)	Oral lesions (suspicious)	Several	--	No	Ultrasound elastography (7-15 MHz linear transducer)	SR and Young's Modulus (kPa)	Normal tissue

-- Not informed by the author; OSCC: oral squamous cell carcinoma; CIS: carcinoma in situ.; *Grade 1: (very soft; high elasticity): lesion appeared predominantly green to red (70% of total lesion area), with a few small blue areas; Grade 2 (moderately soft; moderately high elasticity): more green areas than blue areas, but not predominant (50–70% of total lesion area); Grade 3 (moderately hard; moderately low elasticity): more blue areas than green areas in the lesion, but not predominant; Grade 4 (very hard; low elasticity): predominantly blue areas in the lesion with a few small green areas; g: gama-force; g:gram; kPa: kilopascal.

Table 3 - Risk of bias assessed by the Joanna Briggs Institute Critical Appraisal Tools for use in JBI Systematic Reviews for Diagnostic Test Accuracy Studies and Cohort.

Type of Studie	Authors	Q.1	Q.2	Q.3	Q.4	Q.5	Q.6	Q.7	Q.8	Q.9	Q.10	Q.11	% yes/risk
Accuracy	*Shingaki et al., 2016*[30]	X	√	X	U	X	√	U	√	√	√	--	50% Moderate
	Ogura et al., 2018[31]	X	√	U	U	NA	√	U	√	√	√	--	50 % Moderate
	Babkair, 2025[34]	U	√	√	√	X	√	U	U	√	√	--	60% Moderate
	Ogura et al., 1998[28]	U	X	√	U	√	√	U	√	√	√	--	60% Moderate
	Ogura et al., 2000[29]	U	√	√	U	X	√	U	√	√	√	--	60% Moderate
	Shibata et al., 2022[32]	X	√	√	U	X	√	U	√	√	√	--	60% Moderate
	Sato et al., 1995[27]	U	X	√	U	X	√	√	√	√	√	--	60% Moderate
Cohort	*Jingyuan et al., 2023*[33]	√	√	√	√	√	√	√	U	U	U	√	72% Low

Diagnostic Test Accuracy Studies - Q1. Was a consecutive or random sample of patients enrolled?; Q2. Was a case control design avoided?; Q.3 Did the study avoid inappropriate exclusions?; Q.4 Were the index test results interpreted without knowledge of the results of the reference standard?; Q.5 If a threshold was used, was it pre-specified?; Q.6 Is the reference standard likely to correctly classify the target condition?; Q.7 Were the reference standard results interpreted without knowledge of the results of the index test?; Q.8 Was there an appropriate interval between index test and reference standard?; Q.9 Did all patients receive the same reference standard?; Q.10 Were all patients included in the analysis?

Cohort Studies - Q.1 Were the two groups similar and recruited from the same population?; Q.2 Were the exposures measured similarly to assign people to both exposed and unexposed groups?; Q.3 Was the exposure measured in a valid and reliable way?; Q.4 Were confounding factors identified?; Q.5 Were strategies to deal with confounding factors stated?; Q.6 Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?; Q.7 Were the outcomes measured in a valid and reliable way?; Q.8 Was the follow up time reported and sufficient to be long enough for outcomes to occur?; Q.9 Was follow up complete, and if not, were the reasons to loss to follow up described and explored?; Q.10 Were strategies to address incomplete follow up utilized?; Q.11 Was appropriate statistical analysis used? √ - yes; X - No; U – Unclear; NA – Not Applicable.

Table 4 - Summary of the main results of the eligible studies.

Author and year	*Tumor thickness (average)**	*Stiffness parameters of tumor (Mean ± SD)*	*Stiffness values of control*	*Histopathological correlations*	*Progression factors*	*Survival outcomes*	*Key prognosis findings*
Sato et al., 199527	--	37,7 gf	4,8 gf	OSCC was 2.3-19.1 times harder than normal	--	No	OSCC 2.3-19.1x harder than normal mucosa
Ogura et al., 1998[28]	3,0mm	22,1 ± 8,0 g	5.1 ± 0.8 g	OSCC consistency correlated positively and significantly with tumor thickness (r=0,954, p<0.001)	T2 (24,8 ± 5,7 g) and T3 (30,3 ± 9,5 g) were significantly firmer than T1 (15,4 ± 4,0 g) (p<0.001)	No	Consistency strongly correlates with tumor thickness (r=0.954)
Ogura et al., 2000[29]	--	(N0) PN(-) 16.6 ± 6.2 (N0) PN(+) 23.2 ± 5.6 24.2 ± 5.2 (Regional metastasis) 23.5 ± 5.9 (Regional recurrence)	5.0 ± 0.8 g	The consistency of tongue carcinoma was significantly correlated with tumor thickness (r=0.954, p<0.001)	≤15.0 g → 100% PN(-) = 0% metastasis ≥30.1 g → 100% PN(+) = 100% metastasis	--	Consistency predicts cervical metastasis; Can guide selective neck dissection decisions
Shingaki et al., 2017[30]	OSCC: 3,0 mm Non-malignancy: 3,5mm	Score 3: n=8 (53%) Score 4: n=7 (47%)	Non-malignancy Score 1: n=1 Score 2: n=1	--	all N0 cases	--	Elastography can differentiate malignant from benign lesions with 100% accuracy
Ogura et al., 2018[31]	--	Patient 1: Tumor: 0.000% Patient 2: Tumor: 0.000%	Patient 1: Normal: 1.468% Patient 2: Normal: 1.007%	--	--	No	Preliminary proof of concept (n=2)
Shibata et al., 2021[32]	4.4 ± 2.6 mm	SR: 9.7 ± 9.8 Histological parameters: %CFC: 31.1±7.8% (10.6-57.1%) %CCC: 38.4±11.3% (10.2-67.9%) %TIICs: 19.9±8.9% (2.3-48.3%)	--	%CFC: 31.1±7.8% (10.6-57.1%) %CCC: 38.4±11.3% (10.2-67.9%) %TIICs: 19.9±8.9% (2.3-48.3%) Positive correlation: Log (SR) vs %CFC (rs=0.379, p=0.024); Negative correlation: %CFC vs %TIICs (rs=-0.318, p=0.012)	Positive correlation between Stiffness vs. Depth of Invasion (rs = 0.250, p=0.049)	--	Stiffness correlates with collagen contente; %CFC inversely correlates with %TIICs
Jingyuan et al., 2023[33]	--	Low stiffness (<3): 50.3% High stiffness (≥3): 49.7%	--	Negative correlation with Ki67 (p<0.001);	High stiffness: T2-T4 Low stiffness: T1; High stiffness: N+ (p<0.001)	OS: HR 4.022 (p=0.016) RFS: HR 5.680 (p=0.033)	High stiffness = poor prognosis; Independent predictor of survival and recurrence
Babkair, 2025[34]	--	YM: 23,5 ± 4,3 kPa SR: 4,8 ± 0,9	Benign (n=15): YM: 8.2±2.1 kPa SR: 1.9±0.5	Increased cell density, fibrosis and alteration of the extracellular matrix	--	--	--

**Distance from the surface of the tumor to the invasive front of the tumor; gf: gama-force; -- Not informed by the author; OSCC: squamous cell carcinoma; CIS: carcinoma in situ; CFC: Collagen Fiber Content; SR: Strain Ratio; OS: Overall survival; RFS: Recurrence Free Survival; YM: Young's Modulus.*

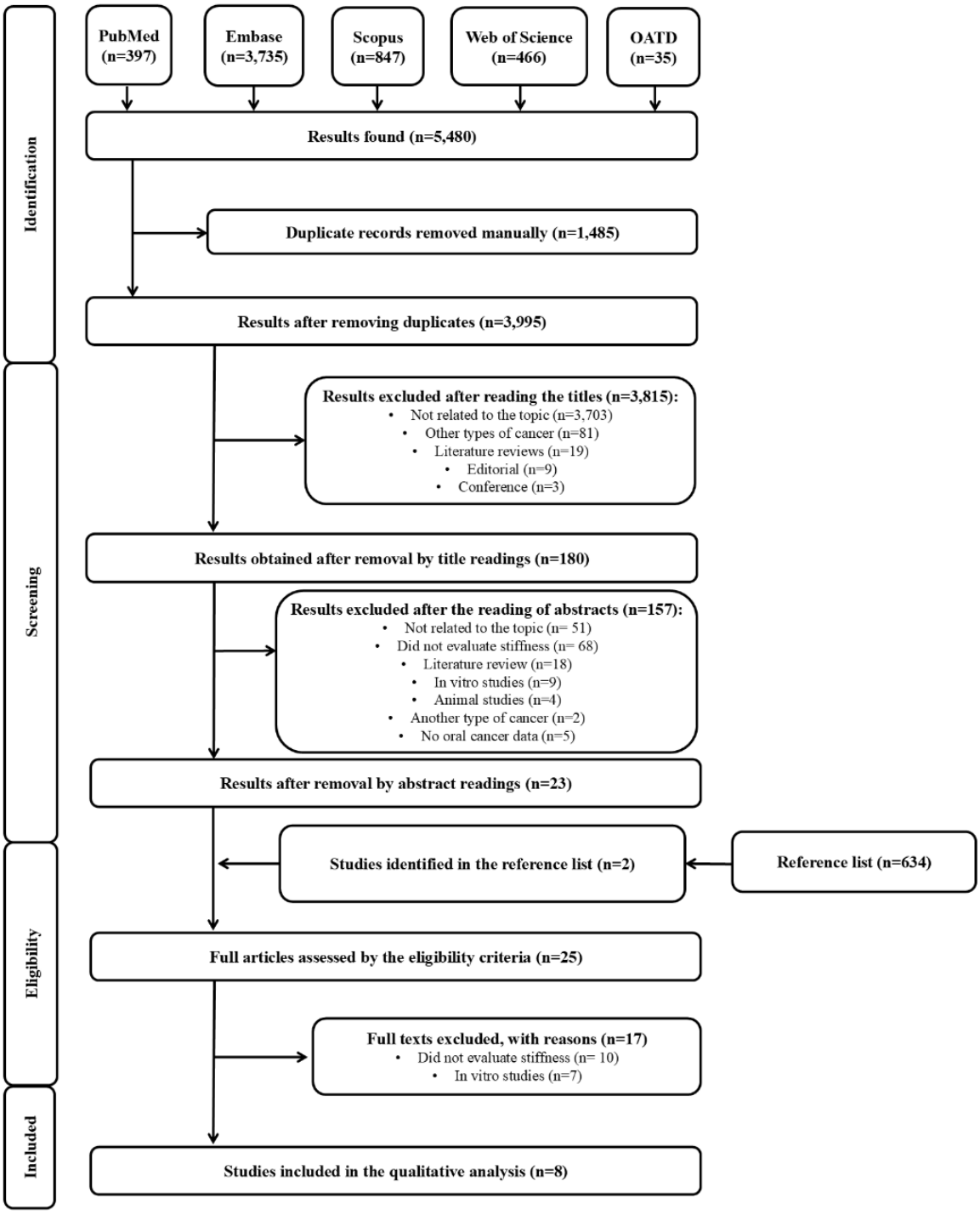


Figure 1. PRISMA Flow Diagram for Systematic Review and Meta-Analysis of Extracellular Matrix Stiffness in Oral Squamous Cell Carcinoma.

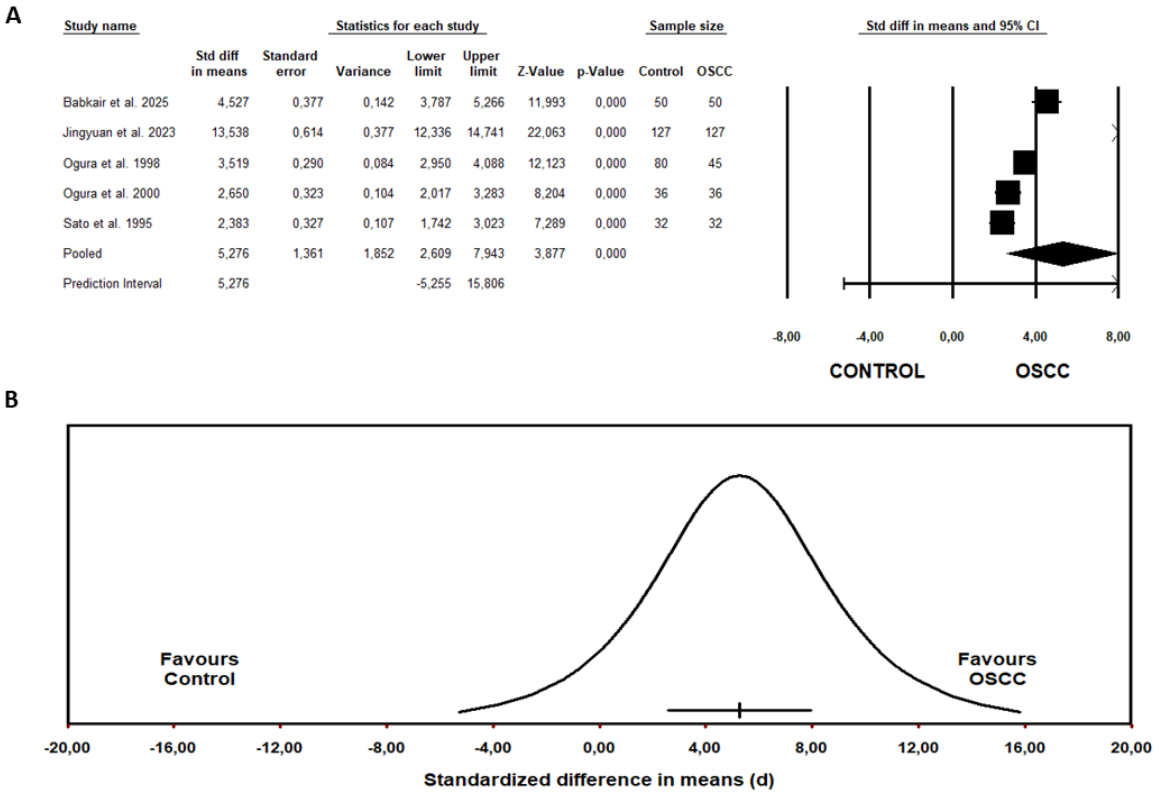


Figure 2. Forest Plot and Distribution of True Effects for Extracellular Matrix Stiffness in Oral Squamous Cell Carcinoma versus Normal Oral Mucosa. (A) Forest plot with standardized mean differences (Cohen's d) with 95% confidence intervals for five quantitative studies comparing extracellular matrix stiffness between oral squamous cell carcinoma (OSCC) and normal oral control samples. Individual study estimates are represented by squares, with horizontal lines indicating 95% confidence intervals. The pooled effect estimate (random-effects model, DerSimonian-Laird method) is shown as a diamond, indicating a standardized mean difference of 5.28 (95% CI: 2.61–7.94; $p < 0.001$). The prediction interval (−5.26 to 15.81) reflects the range of true effects expected in future studies. The vertical reference line at zero indicates no difference between groups. All individual studies and the pooled estimate favor OSCC tissue stiffness (right side of zero). (B) Distribution plot of true effects illustrating the probability density of standardized mean differences across studies. The bell-shaped curve represents the distribution of true effect sizes, with the majority of effects concentrated in the positive range (favoring OSCC), indicating substantial and consistent elevation of extracellular matrix stiffness in oral squamous cell carcinoma tissue. The x-axis ranges from −20.00 to 20.00 standardized difference units, with directional indicators showing "Favours Control" (left) and "Favours OSCC" (right). The

distribution demonstrates the substantial heterogeneity among studies (I^2 = 98.61%) while maintaining consistency in the directional effect.

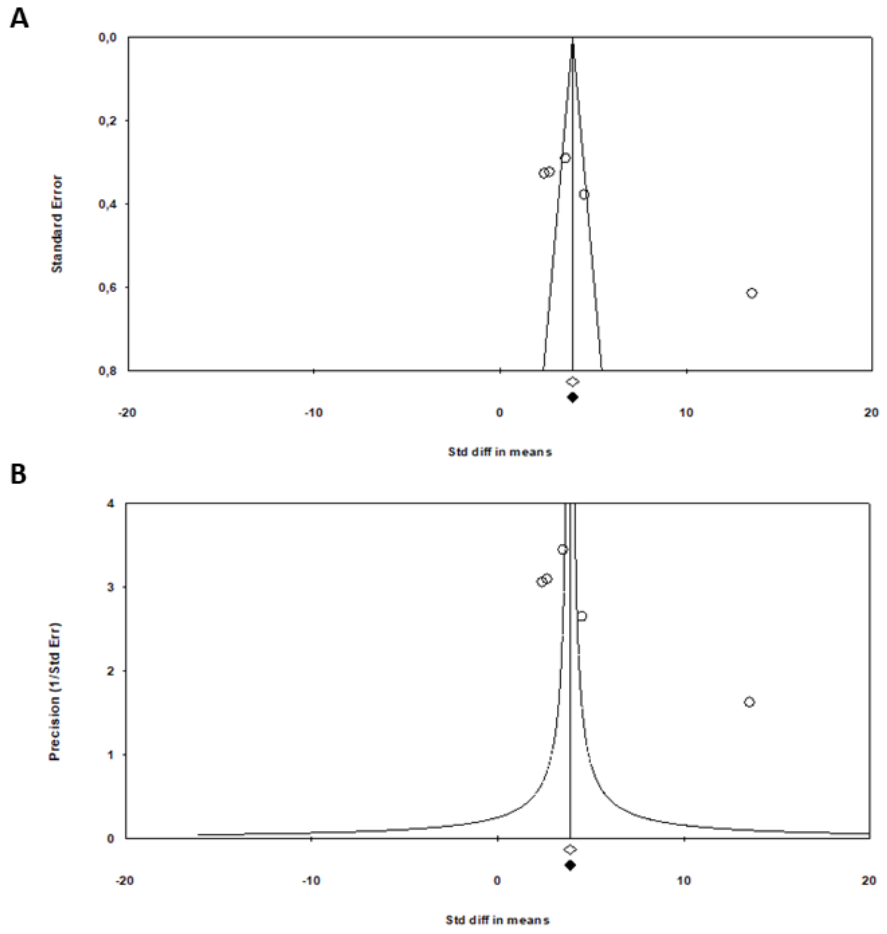


Figure 3. Funnel Plot and Precision Plot for Assessment of Publication Bias. (A) Funnel plot displaying the relationship between standardized mean differences (x-axis: "Std diff in means," ranging from −20 to 20) and standard error (y-axis, ranging from 0 to ~3.5). Each circle represents an individual study, with the central diamond indicating the pooled effect estimate (d = 5.28). The funnel-shaped contour lines represent the expected 95% confidence region for studies assuming no publication bias. The symmetric distribution of studies around the pooled effect, with no apparent clustering of small studies on one side, suggests minimal publication bias. Studies are distributed across various levels of precision, with larger studies (lower standard error) positioned toward the apex of the funnel and smaller studies (higher standard error) distributed more broadly at the base. (B) Precision plot presenting the inverse relationship between precision (y-axis: "Precision [1/Std Err]," ranging from 0 to ~10) and standardized mean differences (x-axis: "Std diff in means," ranging from −20 to 20).

Each circle represents an individual study, with the central diamond indicating the pooled effect estimate. The funnel-shaped contour lines define the expected symmetric distribution region under the assumption of no publication bias. The symmetric arrangement of data points around the pooled effect, without evidence of asymmetric clustering, indicates absence of preferential publication of studies with larger or smaller effect sizes. Higher precision values (upper portion) represent larger studies with smaller standard errors, while lower precision values represent smaller studies with larger standard errors. Quantitative tests (Egger's regression intercept test and Begg's rank correlation test) confirmed the absence of statistically significant funnel plot asymmetry ($p > 0.05$), supporting the conclusion that publication bias is unlikely to substantially influence the meta-analytic findings.

4 ARTIGO CIENTÍFICO 2

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The prognostic significance of Hippo pathway biomarkers in oral squamous cell carcinoma: a systematic review and meta-anylisis

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ABSTRACT

Objective: This systematic review and meta-analysis evaluated the prognostic value of Hippo signaling pathway biomarkers in oral squamous cell carcinoma (OSCC). **Methods**: Following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, 17 studies (1,194 patients) were identified through electronic databases. The relationship between immunohistochemical expression of Hippo effectors (primarily YAP and TAZ) and clinicopathological outcomes was assessed. Meta-analyses using random-effects models estimated pooled Hazard Ratio (HR) for Overall Survival (OS). **Results**: High, predominantly nuclear, expression of YAP and TAZ significantly correlated with aggressive features, including advanced clinical stage, lymph node metastasis, and higher histological grade. Quantitative synthesis revealed that YAP overexpression was associated with poorer OS (pooled HR: 1.918; 95% CI: 1.036–3.549; p=0.038). TAZ overexpression similarly predicted worse OS (pooled HR: 2.845; 95% CI: 1.682–4.811; p=0.0001). Although substantial

heterogeneity ($I^2 > 80\%$) was detected due to methodological variations in staining protocols, the direction of the prognostic effect remained consistently adverse across cohorts. **Conclusion**: Dysregulation of the Hippo pathway, evidenced by YAP/TAZ nuclear accumulation, serves as a robust indicator of tumor aggressiveness and poor survival in OSCC. Integrating these biomarkers into clinical practice could significantly refine risk stratification and personalized therapeutic strategies.

Keywords: mouth neoplasms, oral squamous cell carcinoma, Hippo Signaling Pathway, YAP1 protein, WW Domain-Containing Transcription Factor TAZ, prognosis

HIGHLIGHTS

- Comprehensive meta-analysis of Hippo biomarkers involving 1,194 patients with OSCC.
- Nuclear YAP and TAZ overexpression significantly correlates with poor OS.
- High levels of Hippo effectors are linked to lymph node metastasis and advanced stages.
- Quantitative synthesis identifies YAP and TAZ as robust independent prognostic factors.
- Hippo pathway dysregulation represents a valuable target for OSCC risk stratification.

INTRODUCTION

With origin from the stratified squamous epithelium that lines the oral mucosa, OSCC is the most prevalent malignant neoplasm of the oral cavity[1]. OSCC is characterized by its high aggressiveness and significant capacity for local invasion, in addition to its propensity for regional lymphatic dissemination[2,3]. Furthermore, OSCC presents a cervical metastasis rate that can reach 40% of cases at diagnosis[2,3]. Despite advances in therapeutic modalities, including surgery, radiotherapy, and chemotherapy, OSCC remains associated with an unfavorable prognosis, with a five-year OS rate below 50% in recent decades[4,5]. The significant morbidity related to treatment, which often results in functional and aesthetic impairment, together with the high rate of regional recurrence (20–40%), reinforces the need for a better understanding of the molecular mechanisms underlying oral carcinogenesis[6,7].

OSCC represents a global public health challenge, ranking among the ten most common malignant neoplasms today, with more than 377,000 new cases and

approximately 177,000 deaths recorded every year[8,9]. The incidence shows significant geographic variation, with particularly high rates in South and Southeast Asia, where it accounts for up to 40% of all malignant neoplasms, in contrast to rates below 4% in developed regions such as North America and Western Europe[10,11]. The main challenges include late diagnosis, observed in approximately 60% of cases that present at advanced stages (III–IV), therapeutic resistance, the absence of reliable predictive biomarkers for risk stratification, and the limited effectiveness of available targeted therapies[12,13,14].

Recent studies have shown that tumor progression is influenced by several factors, including mechanical signals originating from the tumor microenvironment[15,16]. Neoplastic cells reside in a dynamic microenvironment characterized by altered stiffness of the extracellular matrix (ECM), compressive forces, and ECM remodeling, factors that influence cell behavior through specific signaling pathways[17,18]. Mechanotransduction has been implicated in the activation of pro-oncogenic transcriptional programs, epithelial–mesenchymal transition (EMT), and maintenance of cancer stem cells, processes that are fundamental to tumor progression and metastasis[19,20,21]. In this context, the Hippo signaling pathway appears to be the main mediator of mechanotransduction in cells[22,23]. The nuclear effectors of this pathway, the proteins YAP and TAZ (Transcriptional coactivator with PDZ-binding motif), function as transcriptional coactivators that, when dysregulated, promote aberrant expression of genes involved in cell proliferation, survival, and migration[24,25].

Despite the importance of the Hippo pathway in the process of oral carcinogenesis, there is still a gap in knowledge regarding the real prognostic value of the expression of its molecular components in OSCC[26,27]. Therefore, the present systematic review aims to critically analyze the available evidence in the literature regarding the immunohistochemical expression of Hippo pathway biomarkers (YAP, TAZ, MST1/2, LATS1/2, SAV1) in human tumor tissue of OSCC and to evaluate their correlation with clinicopathological parameters and prognostic outcomes.

MATERIAL AND METHODS

Protocol and registration

This systematic review was conducted following the PRISMA[28] statement and Cochrane guidelines[29]. The review protocol was registered in the PROSPERO database CRD420251231277.

Study design

This is a systematic review designed to answer the following guiding PECO question: “In patients with oral squamous cell carcinoma (Population), is high expression of HIPPO pathway biomarkers (Exposure), compared with low expression (Comparison), associated with worse tumor progression or clinical prognosis (Outcome)?”

Elebility criteria

Studies that evaluated the expression of Hippo pathway biomarkers (such as YAP, TAZ, MST1/2, LATS1/2, SAV1, and other related molecules) in OSCC and assessed tumor progression or clinical outcomes, with no restrictions on year, language, or publication status (including those in press), were included.

The following studies were excluded: studies focused on other cancer types without specific data for OSCC; review articles, editorials, letters to the editor, or conference abstracts without complete data; studies evaluating only molecular aspects of the Hippo pathway without analysis of expression in tumor tissue or without correlation with prognosis; studies in which OSCC data could not be dissociated from other head and neck cancers; in vitro studies; and animal studies.

Sources of information and search

Two reviewers (IFPL and PGR) independently searched electronic databases. Descriptors were identified using DeCS (Health Sciences Descriptors), MeSH (Medical Subject Headings), and Emtree (Embase Subject Headings), and the Boolean operators “AND” and “OR” were used to optimize the search strategy (Table 1). The results were exported to EndNote Basic/Online (Thomson Reuters, Toronto, Canada), and duplicates were removed.

Study selection

The selection of studies was conducted in three distinct stages. In the first stage, two reviewers (IFPL and PGR) systematically screened the titles of all retrieved records. Articles with titles relevant to the objective of this systematic review were selected for the second stage. In the second stage, the same reviewers screened the abstracts and applied the inclusion and exclusion criteria. Studies without available abstracts were evaluated in the third stage. In this final stage, the full texts of the articles were assessed. When disagreements arose regarding a study's eligibility, a third reviewer (MLL) was consulted to make a final decision. Studies that did not meet the criteria were recorded separately, with the reasons for exclusion clearly documented.

Data collection and extraction

Two authors (IFPL and PGR) performed data extraction using specific spreadsheets designed for this purpose. The following information was extracted from the eligible studies: author and year, sample size (n), sex, age (mean), tumor location, typr of tumor, biomarker, analysis method, prognosis and survival analysis, expression of biomarker, subcellular location, clinicopathological correlation, survival analysis and prognosis analysis.

As a calibration exercise, the reviewers (IFPL and PGR) discussed the eligibility criteria and applied them to a random sample of 20% of the retrieved studies to assess inter-rater agreement. After reaching an adequate level of agreement ($\kappa = 0.81–0.84$), the reviewers independently extracted data from all studies. Any discrepancies were resolved by consensus and supervision of a third reviewer (MLL). Authors of studies with insufficient data or missing information necessary for summarization and comparison were contacted via email to request additional details.

Risk of bias of individual studies

The risk of bias for individual studies was independently assessed by two authors (IFPL and PGR) using the "*Joanna Briggs Institute Critical Appraisal Checklist for Analytical Cross Sectional Studies*" and "*The Joanna Briggs Institute Critical Appraisal Checklist for Cohort Studies*"[29]. The risk of bias was classified as **"High"** when the study achieved up to 49% of "yes" responses, ***"Moderate"*** when it ranged from 50% to 69%, and ***"Low"*** when it exceeded 70% (Table 3).

Statistical analysis

Data from eleven included studies were integrated and analyzed using Comprehensive Meta-Analysis (CMA) software, Version 4 (Biostat, Inc., Englewood, NJ, USA; available at meta-analysis.com).

The prognostic value of YAP and TAZ expression was evaluated by pooling HR and their 95% Confidence Intervals (CI). HRs were extracted directly from the articles or estimated from Kaplan-Meier survival curves using the software Webplotdigitizer Version 5 (Rohatgi, A. (2025). WebPlotDigitizer: Version 5.2. https://automeris.io), when necessary.

Heterogeneity among studies was assessed using the Chi-square test and quantified by the I^2 statistic. An I^2 value greater than 50% was considered indicative of substantial heterogeneity. Consequently, a random-effects model (DerSimonian and Laird method) was employed to calculate the pooled HR to account for the variation in study populations and methodologies (specifically IHC protocols). Publication bias was evaluated through visual inspection of funnel plots for asymmetry. A p-value of <0.05 was considered statistically significant.

RESULTS

Selection of studies

The search of the PubMed, Web of Science, Embase, Scopus, and OATD databases yielded 780 articles. After removal of duplicates, 523 records remained for title screening, of which 108 were considered potentially eligible for abstract review. Following abstract screening, 19 articles were selected for full-text assessment. In addition, all references cited in the selected manuscripts (a total of 822 references) were carefully examined to identify potential studies not captured by the primary search strategy. Thus, the full texts of 19 articles were evaluated. After the exclusion of 2 articles, one for not presenting OSCC data separately for analysis[30], and another due to incomplete data for YAP/TAZ analysis[31]. 17 studies were finally included in this systematic review (Figure 1).

Study characteristics

The 17 included articles were published between 2013 and 2026, and all studies were conducted in patients with OSCC[32–48]. The total sample comprised 1,194

patients, with individual sample sizes ranging from 9 to 130 participants. Where reported, there was a predominance of males in most studies, whereas only one study[46] described a slight predominance of females. The mean age of participants ranged approximately from 50.76 to 68.21 years, while other studies reported age only in categorical form, such as <50 vs. ≥50 years[37] or ≤60 vs. >60 years[40].

With regard to the anatomical location of the tumors, the tongue was the most frequently investigated site, being the exclusive focus in four studies[32,37,39,44] and included, in others, within samples comprising multiple intraoral sites[32–41,43–48]. Other sites evaluated included the oral mucosa, floor of the mouth, gingiva, retromolar region, maxillary and mandibular alveolar ridge, hard palate, lip, retrocommissural region, and the oral cavity more broadly. In all studies[32–48], the lesions evaluated were malignant and classified as OSCC, with no benign lesions or other histological tumor types being included.

Regarding Hippo pathway biomarkers, most studies focused on the core effectors YAP/YAP1[33,35–41,43–48] and TAZ[32–35,40,43–45], assessed either individually or in combination. Some studies additionally evaluated markers that are upstream, downstream, or functionally related to the Hippo pathway, such as p-YAP1[40], MST1[46], MST2[46], LATS1[46], LATS2[46], PIEZO1[43], GPR39[41], SOX2[42] and Kank1[37], thereby exploring broader molecular panels. In all studies[32–48], biomarker expression was analyzed by immunohistochemistry on tumor samples, allowing quantification of expression in neoplastic tissue and subsequent stratification of patients into high- versus low-expression groups for prognostic purposes.

With respect to prognosis and survival analysis, 13[32,34–41,44–47] of the 17 studies constructed Kaplan–Meier survival curves to compare outcomes between patients with high and low expression of Hippo pathway biomarkers, and six of these[32,34,35,44,46,47] further complemented this approach with Cox proportional hazards regression models to estimate the independent prognostic impact after adjustment for clinicopathological variables. In the remaining studies[33,42,43,48], prognosis was inferred indirectly through correlations between biomarker expression and adverse clinicopathological features (such as more advanced tumor stage or higher histological grade) or evidence of lesion progression, without the formal presentation of survival curves.

Risk of Bias of studies

Using the Joanna Briggs Institute Critical Appraisal Tools for cohort and analytical cross-sectional studies[29], 16 of the 17 articles were judged to have a low risk of bias, while one cohort study[45] was classified as having a moderate risk of bias (63.63% “yes” responses). No study was rated as high risk of bias (Table 3). All three analytical cross-sectional studies achieved 75% “yes” ratings but did not identify confounding factors or describe strategies to address them (Q5 and Q6). In the cohort studies, most domains related to exposure and outcome measurement and statistical analysis were adequately fulfilled; however, confounding factors (Q5) and issues concerning completeness of follow-up and management of losses (Q9 and Q10) were frequently rated as unclear, which may limit the internal validity of some estimates.

Individual results of the studies

All 17 included studies analyzed the expression of Hippo pathway biomarkers, with most reporting high expression of YAP/YAP1[32–33,35–39,41,43–48] and/or TAZ[32,34,35,42] in OSCC cases, particularly when compared with normal oral mucosa or potentially malignant lesions. Three studies[33,44,45] did not report TAZ expression data. One study[46] described reduced expression or loss of inhibitory components of the pathway (such as MST1/2 and LATS1/2), as well as the co-expression of functionally related markers, including PIEZO1[43], GPR39[41], SOX2[42] and Kank1[37]. Regarding subcellular localization, YAP/YAP1 and TAZ expression was predominantly nuclear or nucleo-cytoplasmic, and this was reported in 13 studies[32–36,38,39,41–43,46–48]. Only four studies[37,40,44,45] did not specify the subcellular localization of the staining.

With regard to clinicopathological correlations, 15[32–33,36–43,44–48] of the 17 studies provided at least one association between Hippo pathway biomarker expression and features of tumor aggressiveness, whereas one study[44] did not report clinicopathological data and another focused solely on the correlation between YAP and TAZ expression, without clinical outcomes[35]. Overall, high expression of YAP/YAP1 and/or TAZ, particularly with nuclear localization, was associated with larger tumor size[34,41,47], more advanced clinical and/or pathological stage[32,33,37,41,45,47], higher histological grade[32,33,36,47,48] (with greater expression in moderately or poorly differentiated tumors), and, most notably, with lymph node involvement[34,38–43,45] and metastasis[39–43,45,46]. In addition, one study[41] related Hippo pathway biomarker expression to tumor recurrence, while another described an association with EMT

features[46]. Hippo pathway–related markers, such as GPR39, SOX2, and TAZ/SOX2 coexpression, were also associated, in two studies[41,42], with metastasis or other adverse prognostic factors.

With respect to survival analysis, 12 of the 17 studies[32,34–36,38–41,44–47] performed some type of time-to-event analysis, generally using Kaplan–Meier curves to compare OSCC patients with high versus low expression of Hippo pathway biomarkers, whereas 5 studies[33,37,42,43,48] did not report or did not conduct survival analysis. Among the studies[32,34–36,38–41,44–47] that evaluated these outcomes, most showed that high expression of YAP/YAP1 and/or TAZ, particularly when nuclear, was associated with poorer OS[32,34,38–41,44–47] and/or shorter disease-free or recurrence-free survival[32,34–36,39,46], frequently with statistically significant differences between groups. Conversely, 4 studies[35,38,46,47] did not find a significant association between certain expression patterns and survival, especially when cytoplasmic fractions of YAP, the isolated expression of some upstream components, or TAZ expression without further stratification were analyzed.

Finally, in multivariable prognostic analysis, six studies[32,34,35,44,46,47] applied Cox regression models with hazard ratio estimates to test whether Hippo pathway biomarkers constituted independent risk factors after adjustment for classical clinicopathological variables (such as age, sex, tumor stage, histological grade, and nodal status). In three of these studies[32,34,35], high expression of YAP/YAP1 and/or TAZ remained associated with worse OS and/or DFS after adjustment, with hazard ratios indicating a clinically relevant increase in the risk of death or recurrence (HR=4.351; 95% CI: 1.477–12.819; p=0.008 for high TAZ expression[32]). Although Hippo pathway biomarkers were included in Cox models in other studies[44,46,47], isolated YAP1 expression did not emerge as an independent predictor of OS or DFS, despite its association with more aggressive histopathological features in univariable analyses. In addition, several studies[36,38-41] reported an association between Hippo pathway activation (or combined expression profiles, such as YAP/TAZ-activated gene signatures) and worse prognosis only in univariable or qualitative analyses, without presentation of Cox models.

In the remaining 11 studies, the assessment of the prognostic impact of Hippo pathway biomarkers was limited. Six studies[36,38–41,45] analyzed only Kaplan–Meier curves, without Cox models, generally showing worse OS, Disease-Free Survival (DFS), or Recurrence Free Survival (RFS) in patients with high expression or nuclear

localization of YAP/YAP1 and/or TAZ. The other five studies[33,37,42,43,48] did not perform survival analysis, restricting their evaluation to correlations between biomarker expression and clinicopathological variables (such as stage, grade, metastases, and EMT) without time-to-event outcomes.

Quantitative synthesis

Analysis of YAP

The meta-analysis of YAP expression and OS revealed a pooled HR of 1.918 (95% CI: 1.036 – 3.549) using a random-effects model. This statistically significant association (p=0.038) indicates that high YAP expression is significantly correlated with a poorer prognosis, suggesting an increased risk of mortality in patients with oral squamous cell carcinoma. The pooled effect size, was positioned entirely to the right of the null effect line (HR=1), confirming an adverse prognostic role for YAP overexpression (fig. 2).

Substantial statistical heterogeneity was observed across the included studies. The I^2 statistic was 82.4%, indicating a high level of inconsistency. This was further supported by a significant Chi-square test (Q=39.06, p<0.001). The between-study variance, Tau^2, was estimated at 0.588. Despite this high heterogeneity, the direction of the effect consistently pointed towards an increased risk associated with high YAP expression across most primary studies, suggesting that the variability primarily lies in the magnitude rather than the direction of the prognostic effect. This consistency in effect direction supports the aggregation of data, albeit with appropriate caution.

The funnel plot for YAP revealed a noticeable asymmetry, characterized by a scarcity of small studies with negative or non-significant findings in the lower-left quadrant. This pattern suggests the potential presence small-study effects, where smaller studies with positive or statistically significant results are more likely to be published. However, the overall effect size (HR>2.0) remained robust, indicating a strong prognostic impact of YAP despite these potential biases (fig. 3). The interpretation of funnel plot asymmetry should be made with caution, particularly when the number of included studies is limited.

Analysis of TAZ

For the TAZ biomarker, the meta-analysis yielded a pooled HR of 2.845 (95% CI: 1.682 – 4.811) for OS, calculated using a random-effects model. This result was

statistically significant (p = 0.0001), indicating that elevated TAZ expression is significantly associated with a worse prognosis. The confidence interval for the pooled HR did not include unity, confirming that high TAZ expression confers an increased risk of adverse outcomes, thereby favoring the control group (low TAZ expression) in terms of survival (fig. 4).

Significant heterogeneity was detected among the studies evaluating TAZ. The I2 statistic was 81.3%, signifying a high degree of inconsistency. The Chi-square test (Q=21.436, p<0.001) further confirmed this substantial variability. The estimated between-study variance, Tau2, was 0.593, reflecting the dispersion of true effects across studies. The presence of high heterogeneity necessitated the use of a random-effects model to provide a more conservative estimate of the pooled effect and its confidence interval.

The assessment of publication bias for TAZ was challenging due to the limited number of included studies (n=3) and the high observed heterogeneity (I2=81.3%). In such scenarios, the interpretation of funnel plot asymmetry can be unreliable, as scatter may be primarily attributable to true heterogeneity rather than publication bias (fig. 5) Furthermore, statistical tests for funnel plot asymmetry, such as Egger's test, are typically underpowered with fewer than 10 studies, making their application less informative. While publication bias cannot be definitively ruled out, the results should be interpreted with caution, acknowledging the inherent limitations imposed by the small sample size of included studies. The observed scatter in the funnel plot is likely influenced by the high heterogeneity due to the small number of studies included as well as their size.

DISCUSSION

OSCC remains an important global health problem, with projections of a 30–40% increase in incidence and mortality by 2040[49]. In this context, there is an urgent need to develop and validate more accurate diagnostic and prognostic approaches, and several authors have emphasized the role of prognostic biomarkers to improve outcome prediction in OSCC and head and neck squamous cell carcinoma beyond conventional staging[50]. The identification of robust prognostic biomarkers capable of refining risk stratification beyond the traditional TNM system therefore represents a key strategy to improve patient survival[51]. In this regard, the present systematic review identified a consistent pattern of high, particularly nuclear, expression of YAP/TAZ and

related markers, which was strongly associated with adverse clinicopathological features and with significantly poorer overall and disease-free survival.

In recent years, mechanobiology has emerged as a fundamental theme in cancer biology, demonstrating how cells sense and integrate mechanical cues, such as ECM stiffness, cell shape, shear stress and cell–cell crowding, to regulate proliferation, survival and differentiation[52]. Within this context, YAP and TAZ have been studied as key mechanotransducers that translate these physical stimuli into transcriptional programs, acting downstream of both the canonical Hippo kinase cascade (MST1/2–LATS1/2) and Hippo-independent cytoskeletal and adhesion signals[53]. When the Hippo pathway is active, LATS1/2 phosphorylate YAP/TAZ, promoting their cytoplasmic retention and degradation. However, when Hippo signaling is inhibited, or when cells experience a stiff ECM, high mechanical tension and reduced contact inhibition, YAP/TAZ translocate to the nucleus and cooperate with TEAD and other transcription factors to activate genes involved in proliferation, survival, stemness, EMT and metabolic reprogramming[54]. This mechanotransduction axis has been implicated in tumor initiation, progression and therapy resistance, making the Hippo–YAP/TAZ system an important conceptual framework for understanding OSCC behavior[55].

In this context, the expression patterns of Hippo pathway effectors observed in OSCC appear to reflect a logic in which mechanical and oncogenic signals converge on YAP/TAZ activation. In our systematic review, most studies reported high expression of YAP/YAP1 and/or TAZ in OSCC, predominantly with nuclear or nucleocytoplasmic staining. In addition, the data also suggested attenuation of upstream inhibitory components, such as low MST1/LATS1/LATS2 expression alongside YAP1 positivity, or regulatory events consistent with increased YAP/TAZ activity, such as LATS2 downregulation (Table 4). This OSCC pattern is aligned with evidence that YAP/TAZ hyperactivation is frequent in solid tumors and contributes to proliferation, EMT, invasion, and metastasis[56]. Likewise, studies on hepatocellular carcinoma[57] and renal cancers[58] have associated YAP/TAZ overexpression/nuclear expression with advanced disease and poor prognosis, and recent clinical work has even proposed YAP-based prognostic models to refine risk prediction beyond standard parameters.

On the other hand, in breast cancer, the literature[59] indicates that YAP may be associated with poor prognosis in certain subtypes, whereas in ER-positive tumors this

relationship may differ or even be reversed, suggesting that the clinical impact of YAP/TAZ is not uniform and may depend on the biological context of the subtype, lineage-specific transcriptional architecture, and treatment-imposed selective pressures. To some extent, cytosolic YAP may exert tumor-suppressive effects in specific contexts by restraining oncogenic signaling, such as Wnt/β-catenin, whereas nuclear YAP/TAZ generally drive pro-tumorigenic transcriptional programs[60]. In this context, our OSCC findings, predominant YAP/TAZ overexpression with concomitant signals of weakened upstream inhibition, fit the prevailing oncogenic model of Hippo pathway deregulation.

In addition to the level of YAP/TAZ expression, their subcellular localization also has biological and clinical relevance. In general, activation of the Hippo pathway culminates in the phosphorylation of YAP/TAZ by MST1/2 and, especially, LATS1/2, which favors interactions with regulatory proteins and promotes their cytoplasmic retention/degradation, being associated with a functionally inactive state. However, nuclear accumulation of YAP/TAZ indicates relative dephosphorylation and TEAD-mediated transcriptional activation, with the potential induction of pro-oncogenic programs[33,61]. Similarly, in renal[63,64] and hepatocellular tumors[65], alterations in polarity and cell-adhesion proteins have been implicated in facilitating YAP/TAZ nuclear translocation and enhancing invasive and metastatic phenotypes. In renal carcinoma, studies[64] indicate that YAP/TAZ activation (including nuclear enrichment of TAZ) is associated with higher stage/grade and poorer survival, and that polarity regulators such as Par3 may act as pro-metastatic cofactors via the YAP/TAZ axis. In hepatocellular carcinoma[65], loss of apical polarity determinants such as MPP5/PALS1 correlates with nuclear accumulation of YAP/TAZ and worse outcomes. In the present review, nuclear or nucleocytoplasmic staining of YAP/YAP1 and TAZ was the dominant pattern in OSCC, reported in most studies, and this pattern was linked to parameters of aggressiveness and poorer outcomes, consistent with observations in other anatomical sites.

Despite the consistent direction of effect, our meta-analysis revealed substantial statistical heterogeneity among the included studies for both YAP (I^2 = 82.4%) and TAZ (I^2 = 81.3%). This high level of heterogeneity, while common in meta-analyses of biomarker studies, necessitates careful interpretation. Several factors likely contribute to this variability. Differences in immunohistochemical (IHC) staining protocols, including antibody clones, dilution, and detection systems, can significantly impact the

assessment of protein expression. Moreover, the lack of standardized cut-off values for defining “high” versus “low” expression across studies introduces considerable variability. Some studies used median values, others used quartiles, and some employed receiver operating characteristic (ROC) curve analysis, leading to diverse thresholds. Furthermore, variations in patient populations (e.g., geographical origin, ethnicity), tumor characteristics (e.g., stage, differentiation grade, anatomical subsite), and follow-up durations among the primary studies could also contribute to the observed heterogeneity. While the high I^2 value suggests that a substantial proportion of the observed variance is due to true differences in effect sizes rather than random error, the consistent direction of the pooled effect (HR>1) across most studies indicates that the overall conclusion regarding the prognostic role of YAP and TAZ remains robust.

Regarding the heterogenity, the analysis of the funnel plot for YAP suggested some asymmetry, with a potential underrepresentation of small studies reporting non-significant or negative findings. This “small-study effect” could lead to an overestimation of the true effect size. For TAZ, the limited number of studies (n=3) restricts the power of visual or statistical tests for publication bias, and the observed scatter in the funnel plot is more likely attributable to the high heterogeneity rather than clear evidence of bias. While these potential biases are acknowledged limitations, the magnitude and statistical significance of the pooled HRs for both YAP and TAZ, particularly for YAP, suggest that their prognostic utility is unlikely to be entirely spurious.

This meta-analysis, while comprehensive, is subject to several limitations. Firstly, the high degree of heterogeneity observed for both YAP and TAZ, primarily due to methodological differences in biomarker assessment and diverse patient cohorts, warrants cautious interpretation. Although a random-effects model was employed to account for this variability, residual confounding cannot be entirely excluded. Secondly, the relatively small number of studies included for TAZ (n=3) limits the robustness of its pooled estimate and the power to detect publication bias. Thirdly, the reliance on immunohistochemistry for biomarker assessment, while common, introduces inherent variability and subjectivity. Future studies should consider more quantitative methods, such as qRT-PCR or Western blot, or explore the prognostic value of YAP/TAZ activation status rather than just total protein expression.

In this context, investigating clinicopathological correlations is essential, as it is through these analyses that risk stratification and a deeper understanding of tumor progression become possible[49]. Recent reviews[49] emphasize that integrating biomarkers with histopathological parameters is important to improve prognostic assessment and guide therapeutic decisions. In this regard, the results of the present systematic review show that 15 of the 17 studies associated YAP/YAP1 and/or TAZ with features of aggressiveness, particularly advanced stage, higher histological grade, and lymph node involvement, with stronger associations generally observed when expression was nuclear, a pattern that is corroborated in other solid tumors. Recent studies[66] in basal-like/triple-negative breast cancer have established YAP/TAZ as axes directly implicated in progression and metastasis, aligning with our finding that YAP/TAZ activation accompanies an aggressive phenotype. Similarly, clinical studies in colorectal cancer[67] have shown associations between high YAP expression and larger tumor size, invasion, and lymph node metastasis, as well as prognostic impact in specific subgroups, corroborating the results observed in OSCC in this review.

In contrast, in ER-positive breast tumors, YAP activation may interfere with estrogen receptor–driven transcriptional programs and exhibit a more complex behavior, sometimes not reproducing the same prognostic pattern observed in mesenchymal tumors[68]. This may be explained by the fact that the clinical effects of YAP/TAZ depend on crosstalk with lineage-specific networks, microenvironmental states, and therapy-imposed selective pressures, which modulate how molecular activation translates into measurable phenotypic aggressiveness[69]. Thus, evaluating YAP/TAZ in the clinicopathological context helps integrate mechanotransduction, tumor plasticity, and metastatic capacity into a clinically applicable risk framework, supporting the relevance of YAP/TAZ as candidate biomarkers of progression and as potential therapeutic targets in high-risk subgroups[69].

Survival analysis represents a fundamental step in translating this biological evidence into prognostic utility, allowing evaluation of whether YAP/TAZ activation effectively translates into an increased risk of death and/or recurrence[55]. In the present systematic review, 12 of the 17 studies performed time-to-event analyses, and six applied Cox regression. In most studies, high expression of YAP/YAP1 and/or TAZ, particularly when nuclear, was associated with poorer OS and/or shorter DFS/RFS, and in at least three studies[32,34,35] the effect remained independent after adjustment for

clinicopathological variables (for example, high TAZ: HR=4.351; 95% CI: 1.477–12.819; p=0.008).

Corroborating these findings, clinical studies in colorectal cancer[70] indicate that YAP/TAZ coexpression/activation is associated with proliferation, metastasis, and worse survival, with evidence of relevant prognostic value in human cohorts. In pancreatic ductal adenocarcinoma, studies[71] describe YAP/TAZ as mediators of progression and resistance, frequently associated with unfavorable prognosis in clinical series and syntheses of the field. Similarly, clinical evidence[72] indicates that YAP activation is linked to more aggressive gastric cancer subtypes and poorer outcomes, reinforcing the view of YAP/TAZ as amplifiers of tumor progression in digestive tract epithelia. In non-small cell lung cancer, focused reviews[73] on YAP/TAZ also describe associations with adverse clinical evolution and suggest potential prognostic/predictive utility in specific therapeutic contexts. Nevertheless, it is important to emphasize that not all cohorts across different tumors demonstrate prognostic independence for YAP/TAZ, suggesting a context-dependent role influenced by molecular subtype, microenvironment, and co-activated pathways.

Thus, the data from the present systematic review reinforce that Hippo pathway deregulation and YAP/TAZ activation, particularly with a nuclear pattern, constitute a biologically and clinically relevant axis in OSCC progression, being consistently associated with tumor aggressiveness and adverse outcomes. By integrating evidence on expression levels, subcellular localization, clinicopathological correlations, and survival analyses, this review consolidates YAP/TAZ as important candidate prognostic biomarkers, with the potential to complement and refine TNM-based risk stratification.

The findings from our meta-analysis position YAP and TAZ as promising prognostic biomarkers in OSCC. Their ability to independently predict poorer OS suggests their potential utility in risk stratification for OSCC patients. In a clinical setting, identifying patients with high YAP/TAZ expression could help tailor more aggressive treatment strategies, including intensified adjuvant therapy or closer surveillance. For instance, patients with early-stage OSCC but high YAP/TAZ expression might benefit from more rigorous follow-up or consideration for systemic therapies typically reserved for advanced disease. Conversely, patients with low expression might be spared unnecessary aggressive treatments. However, for these biomarkers to be adopted into routine clinical practice, standardization of IHC protocols

and definition of robust, clinically validated cut-off values are imperative. The development of a standardized scoring system would enhance reproducibility and comparability across different institutions.

To enable this translation into clinical practice, the development of additional multicenter prospective studies is essential. In parallel, mechanistic approaches using 3D models, organoids, and co-culture systems may elucidate how matrix stiffness, inflammation, the oral microbiota, and therapeutic pressure converge to modulate the Hippo–YAP/TAZ axis within the tumor microenvironment. Finally, incorporation of YAP/TAZ into immunohistochemical panels, together with the development of therapies that interfere with mechanotransduction and Hippo signaling, may pave the way for more precise identification of high-risk patients and for interventions targeting central biological vulnerabilities in OSCC.

CONCLUSION

Overexpression of Hippo pathway biomarkers, particularly YAP/YAP1 and/or TAZ, is associated with greater tumor aggressiveness and progression and poorer survival in patients with OSCC.

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Table 1 - Search strategies in the databases.

Databases	Search strategies (September, 2025)	Results
PubMed http://www.ncbi.nlm.nih.gov/pubmed	("Hippo" OR "YAP" OR "TAZ" OR "mechanotransduction" OR "mechanosignaling") AND ("Mouth Neoplasms" OR "head and neck cancer" OR "Oral cancer" OR "Oral squamous cell carcinoma" OR "Oral cavity neoplasms" OR "Mouth neoplasms") AND ("Prognosis" OR "Survival" OR "Survival rate" OR "Recurrence" OR "Disease progression")	99
Scopus https://www.scopus.com/	("Hippo" OR "YAP" OR "TAZ" OR "mechanotransduction" OR "mechanosignaling") AND ("Mouth Neoplasms" OR "head and neck cancer" OR "Oral cancer" OR "Oral squamous cell carcinoma" OR "Oral cavity neoplasms" OR "Mouth neoplasms") AND ("Prognosis" OR "Survival" OR "Survival rate" OR "Recurrence" OR "Disease progression")	110
Embase https://www.embase.com/	('hippo' OR 'yap' OR 'taz' OR 'mechanotransduction'/exp OR 'mechanotransduction' OR 'mechanosignaling'/exp OR 'mechanosignaling') AND ('head and neck cancer'/exp OR 'head and neck cancer' OR 'oral cancer'/exp OR 'oral cancer' OR 'oral squamous cell carcinoma'/exp OR 'oral squamous cell carcinoma' OR 'oral cavity neoplasms' OR 'mouth neoplasms'/exp OR 'mouth neoplasms') AND ('prognosis'/exp OR 'prognosis' OR 'survival'/exp OR 'survival' OR 'survival rate'/exp OR 'survival rate' OR 'recurrence'/exp OR 'recurrence' OR 'disease progression'/exp OR 'disease progression')	474
Web Of Science http://apps.webofknowledge.com/	("Hippo" OR "YAP" OR "TAZ" OR "mechanotransduction" OR "mechanosignaling") AND ("Mouth Neoplasms" OR "head and neck cancer" OR "Oral cancer" OR "Oral squamous cell carcinoma" OR "Oral cavity neoplasms" OR "Mouth neoplasms") AND ("Prognosis" OR "Survival" OR "Survival rate" OR "Recurrence" OR "Disease progression")	93
OATD https://oatd.org/	("Hippo" OR "YAP" OR "TAZ" OR "mechanotransduction" OR "mechanosignaling") AND ("Mouth Neoplasms" OR "head and neck cancer" OR "Oral cancer" OR "Oral squamous cell carcinoma" OR "Oral cavity neoplasms" OR "Mouth neoplasms") AND ("Prognosis" OR "Survival" OR "Survival rate" OR "Recurrence" OR "Disease progression")	4
TOTAL		780

Table 2 - Summary of the main characteristics of the eligible studies.

Author and year	*Sample (n)*	*Sex*	*Age (mean)*	*Tumor location*	*Type of tumor*	*Biomarker*	*Analysis method*	*Prognosis and survival analysis*
Wei et al., 2013[32]	52	28 (male) 24 (female)	53.5	Tongue	OSCC	TAZ	IHC	Kaplan-Meier and Cox regression model of proportional hazards
Hiemer et al., 2015[33]	10	--	--	Tongue and floor of the mouth	OSCC	YAP (YAP1) and TAZ (WWTR1)	IHC	The prognosis was inferred by correlating it with advanced tumor grade and stage
Li et al., 2015[34]	111	--	--	Tongue, oral mucosa, floor of the mouth,and gingiva	OSCC	TAZ	IHC	Kaplan-Meier and Cox regression model of proportional hazards
Szelachowska et al., 2019[35]	127	102 (male) 25 (female)	--	Tongue and floor of the mouth	OSCC	YAP (YAP1) and TAZ (WWTR1)	IHC	Kaplan-Meier and Cox regression model of proportional hazards
Ono et al., 2019[36]	80	--	--	Oral cavity	OSCC	YAP	IHC	Kaplan-Meier
Fan et al., 2019[37]	32	--	<50 (n=15) >50 (n=17)	Tongue	OSCC	YAP and Kank1	IHC	Kaplan-Meier
Qi et al., 2019[38]	68	45 (male) 23 (female)	55.5	Tongue, oral mucosa, retromolar region, gingiva and floor of the mouth	OSCC	YAP	IHC	Kaplan-Meier
Omori et al., 2020[39]	86	--	--	Tongue	OSCC	YAP (YAP1)	IHC	Kaplan-Meier
Peng et al., 2020[40]	56	34 (male) 22 (female)	≤60 (n=20) >60 (n=36)	Tongue and oral cavity	OSCC	YAP1, p-YAP1, TAZ and p-TAZ	IHC	Kaplan-Meier
Jiang et al., 2020[41]	122	--	--	Alveolar crest, tongue, oral mucosa, floor of the mouth, hard palate, lip, oral cavity	OSCC	GPR39 and YAP	IHC	Kaplan-Meier
Stasikowska-Kanicka et al., 2021[42]	86	53 (male) 33 (female)	63.77 (M+) 68.21 (M-)	Floor of the mouth	OSCC	TAZ, SOX2	IHC	Clinical-pathological correlation of M+ and M-
Hasegawa et al., 2021[43]	45	--	--	Oral cavity	OSCC	YAP, TAZ and PIEZO1	IHC	Clinical-pathological correlation
Yang et al., 2023[44]	129	69 (male) 60 (female)	--	Tongue	OSCC	YAP and TAZ	IHC	Kaplan-Meier and Cox regression model of proportional hazards
Tsai et al., 2024[45]	21	--	--	Oral cavity	OSCC	YAP and TAZ	IHC	Kaplan-Meier
Amano et al., 2024[46]	130	60 (male) 70 (female)	63.9	Tongue, gingiva, floor of the mouth and oral mucosa	OSCC	MST1, MST2, LATS1, LATS2 and YAP1	IHC	Kaplan-Meier and Cox regression model of proportional hazards
Gupta et al., 2025[47]	30	25 (male) 5 (female)	50.76	Oral mucosa, lip, floor of the mouth, tongue, alveolus maxillary, alveolus mandibular and retrocommissural region	OSCC	YAP	IHC	Kaplan-Meier and Cox regression model of proportional hazards
Nandhakumar et al., 2026[48]	9	7 (male) 2 (female)	57.34	Tongue, oral mucosa and gingiva	OSCC	YAP1	IHC	Correlation between lesion expression and progression

-- Not informed by the author; OSCC: Oral Squamous Cell Carcinoma; YAP: Yes-Associated Protein; TAZ: Transcriptional coactivator with PDZ-binding motif; IHC: Immunohistochemistry

Table 3 - Risk of bias assessed by the Joanna Briggs Institute Critical Appraisal Tools for use in JBI Systematic Reviews for Cohort studies and Analytical Cross Sectional Studies.

Type of Studie	Authors	Q.1	Q.2	Q.3	Q.4	Q.5	Q.6	Q.7	Q.8				% yes/risk
Analytical Cross Sectional Studies	*Peng et al., 2020*[40]	√	√	√	√	X	X	√	√				75% Low
	Stasikowska-Kanicka et al., 2021[42]	√	√	√	√	X	X	√	√				75% Low
	Nandhakumar et al., 2026[48]	√	√	√	√	U	X	√	√				75% Low
Type of Studie	**Authors**	**Q.1**	**Q.2**	**Q.3**	**Q.4**	**Q.5**	**Q.6**	**Q.7**	**Q.8**	**Q.9**	**Q.10**	**Q.11**	**% yes/risk**
Cohort	*Wei et al., 2013*[32]	√	√	√	√	√	√	√	√	U	U	√	81,81% Low
	Hiemer et al., 2015[33]	√	√	√	√	√	√	√	√	U	U	√	81,81% Low
	Li et al., 2015[34]	√	√	√	√	U	√	√	√	U	U	√	72,72% Low
	Fan et al., 2019[37]	√	√	√	√	U	√	√	√	U	U	√	72,72% Low
	Ono et al., 2019[36]	√	√	√	√	U	√	√	√	U	U	√	72,72% Low
	Szelachowska et al., 2019[35]	√	√	√	√	U	√	√	√	U	U	√	72,72% Low
	Qi et al., 2019[38]	√	√	√	√	√	√	√	√	U	U	√	81,81% Low
	Omori et al., 2020[39]	√	√	√	√	√	√	√	√	U	U	√	81,81% Low
	Jiang et al., 2020[41]	√	√	√	√	U	√	√	√	U	U	√	72,72% Low
	Hasegawa et al., 2021[43]	√	√	√	√	U	√	√	√	U	U	√	72,72% Low
	Yang et al., 2023[44]	√	√	√	√	√	√	√	√	U	U	√	81,81% Low
	Amano et al., 2024[46]	√	√	√	√	√	√	√	√	U	U	√	81,81% Low
	Tsai et al., 2024[45]	√	√	√	√	U	√	√	U	U	U	√	63,63% Moderate
	Gupta et al., 2025[47]	√	√	√	√	√	√	√	√	U	U	√	81,81% Low

Analytical Cross Sectional Studies - Q1. Were the criteria for inclusion in the sample clearly defined?; Q2. Were the study subjects and the setting described in detail?; Q3. Was the exposure measured in a valid and reliable way?; Q4. Were objective, standard criteria used for measurement of the condition?; Q5. Were confounding factors identified?; Q6. Were strategies to deal with confounding factors stated?; Q7. Were the outcomes measured in a valid and reliable way?; Q8. Was appropriate statistical analysis used?

Cohort Studies - Q1 Were the two groups similar and recruited from the same population?; Q2 Were the exposures measured similarly to assign people to both exposed and unexposed groups?; Q3 Was the exposure measured in a valid and reliable way?; Q4 Were confounding factors identified?; Q5 Were strategies to deal with confounding factors stated?; Q6 Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?; Q7 Were the outcomes measured in a valid and reliable way?; Q8 Was the follow up time reported and sufficient to be long enough for outcomes to occur?; Q9 Was follow up complete, and if not, were the reasons to loss to follow up described and explored?; Q10 Were strategies to address incomplete follow up utilized?; Q11 Was appropriate statistical analysis used? √ - yes; X - No; U – Unclear; NA – Not Applicable.

Table 4 - Summary of the main results of the eligible studies.

Author and year	*Expression of biomarker*	*Subcellular location*	*Clinicopathological correlation*	*Survival analysis*	*Prognosis analysis*
Wei et al., 2013[32]	High: 30 cases Low: 22 cases	Cytoplasm and nucleus	High TAZ expression associated with T3–T4 (p=0.033); High TAZ expression associated with Grades II–III (p=0.026) and III-IV (p=0.013)	OS = 0.020 DSF = 0.019 5-year OS = 50%	TAZ Expression (High) HR: 4.351 (95% CI, 1.477–12.819; p=0.008)
Hiemer et al., 2015[33]	YAP High: 4 cases TAZ not informed	YAP: nucleus TAZ: not analyzed	Poorly differentiated tumors showed high levels of YAP protein*	Not analyzed	The YAP/TAZ-activated gene signature correlated significantly with high tumor grade and stage (GSEA and ASSIGN analysis), remaining enriched until Stage IV
Li et al., 2015[34]	High: 56 cases	Cytoplasm and nucleus	Positive correlation for tumor size, pathological grade, and cervical lymph node metastasis (p<0.05)	OS = 0.0008 DSF = 0.0017	Cox regression model of proportional hazards: OS = 0.003 DSF = 0.011
Szelachowska et al., 2019[35]	YAP 104 cases (>0%) 23 cases (=0%) TAZ 94 cases (>0%) 33 cases (=0%)	YAP: cytoplasm TAZ: nucleus	Weak negative correlation between YAP and TAZ expression in tumor cells (rs = −0.220; p=0.012)	YAP 5-year DSF = 0.011 TAZ Not significant	YAP Unfavorable independent prognostic factor for DFS HR: 0.360; p=0.009 TAZ Not significant
Ono et al., 2019[36]	High: 43 cases	Cytoplasm and nucleus	High YAP expression was more frequent in moderately (46.6%) and poorly (40.0%) differentiated OSCC, compared to well-differentiated OSCC (16.6%)	DSF = 0.047	It is suggested that high YAP expression is a marker that contributes to predicting a worse prognosis after surgery.
Fan et al., 2019[37]	YAP protein expression was significantly higher in OSCC tissue than in normal tissue*	--	YAP was negatively correlated with Kank1 expression in OSCC. Kank1 was associated with clinical stage (p=0.030) and poor prognosis.	--	High YAP expression is associated with tumor progression and is inferred as an indicator of a worse prognosis.
Qi et al., 2019[38]	YAP protein expression was significantly higher in OSCC tissue than in normal tissue*	Nucleus (majority) and cytoplasm	Nuclear YAP expression correlated positively with cervical lymph node metastasis (Z=−3.624; p<0.001) Cytoplasmatic YAP not significant (Z=−1.330; p=0.138)	Nuclear YAP 5-year OS (p=0.008) Cytoplasmatic YAP Not significant (p=0.209)	Nuclear YAP can be used as a potential marker for tumor invasiveness.
Omori et al., 2020[39]	High: 14 cases	Nucleus	YAP1 correlated with lymph node metastasis*	OS (p<0.001) RFS (p=0.003)	Patients with high YAP1 expression had significantly reduced survival compared to those with low expression.
Peng et al., 2020[40]	LATS2 was significantly downregulated (p<0.001); LATS2, YAP1, and p-YAP1 were decreased in tumor tissue; miR-31 was markedly increased (p<0.001).	--	Low levels of circ_0000140 (which induces LATS2) were significantly correlated with greater lymph node metastasis (p=0.015)	Lower expressions of circ_0000140 (indicating high YAP/TAZ activity) were associated with worse overall survival (p<0.001)	High YAP/TAZ activity, mediated by LATS2 suppression, is associated with tumor progression and metastasis.
Jiang et al., 2020[41]	YAP High: 37 cases out of 88 analyzed GPR39 High: 41 cases out of 103 analyzed	YAP Nucleus GPR39 Cytoplasm and membrane	YAP Positive correlation with tumor size, clinical stage, recurrence status, and lymph node metastasis status*	YAP Patients with high YAP nuclear staining showed noticeably worse survival (p=0.0025)	YAP High YAP activity correlated with worse prognosis GPR39 High expression associated with poor survival

Stasikowska-Kanicka et al., 2021[42]	High: 44 cases M+: 30 cases M-: 14 cases	Nucleus	TAZ immunoexpression was significantly increased in OSCC M+ compared to OSCC M– (p<0.001)	--	Significant association between TAZ/SOX2 coexpression and the presence of metastases (p<0.007)
Hasegawa et al., 2021[43]	<u>YAP</u> Positive: 43 cases <u>PIEZO1</u> Positive: 45 cases	<u>YAP</u> Nucleus and cytoplasm <u>PIEZO1</u> Cytoplasm	<u>YAP</u> Metastasis+ (n=5) Metastasis- (n=25) <u>PIEZO1</u> Metastasis+ (n=4) Metastasis- (n=16) There was no significant correlation with lymph node metastasis	--	--
Yang et al., 2023[44]	High expression of YAP*; TAZ expression was not reported.	--	--	YAP/TAZ is associated with a poor prognosis*	YAP/TAZ is associated with a poor prognosis*
Tsai et al., 2024[45]	YAP expression was high (upregulated) in all six platinum-resistant patient samples*; TAZ not reported	--	High YAP expression was significantly correlated with higher staging and nodal statuses*	Significant correlation between YAP protein levels and patient survival rates*	YAP overexpression is associated with the development of oral cancer*
Amano et al., 2024[46]	<u>High expression</u> in OSCC grade 3 YAP1 (100%) MST2 (100%) MST1, LATS1,LATS2 low expression	YAP1: nucleus and cytoplasm MST1, MST2, LATS1, LATS2: cytoplasm	YAP1 expression correlated significantly with EMT characteristics and MST2 with aggressive prognosis factors	5-year OS <u>YAP High</u> 77,7% Isolated expression of YAP1 and MST2 was not significantly associated with DFS or OS	5-year DFS <u>YAP High</u> 49,1% High YAP1 expression alone did not correlate with OS or DFS HR: 1.5948 (95% CI: 0.5946–4.2773) p=0.3538
Gupta et al., 2025[47]	High: 17 cases	Nucleus and cytoplasm	YAP expression correlated significantly with tumour size (p=0.034), clinical stage (p=0.034), histopathological differentiation (p=0.001); Nodal status: not significantly (p=0.141)	There was no significant difference in survival rate between the high and low YAP expression groups (p = 0.65)	The isolated expression of YAP did not serve as an independent predictor of OS
Nandhakumar et al., 2026[48]	High: 9 cases	Nucleus and cytoplasm	An increasing trend in mean YAP1 staining intensity was observed with the degree of tumor dedifferentiation; There was a significant association between YAP1 staining intensity and progression	Unrealized	The progressive increase in YAP1 expression and its nuclear localization correlate with lesion severity and tumor aggressiveness*

-- Not informed by the author; OSCC: Oral Squamous Cell Carcinoma; YAP: Yes-Associated Protein; TAZ: Transcriptional coactivator with PDZ-binding motif; OS: Overall Survival; DFS: Disease-Free Survival; *:no numerical data

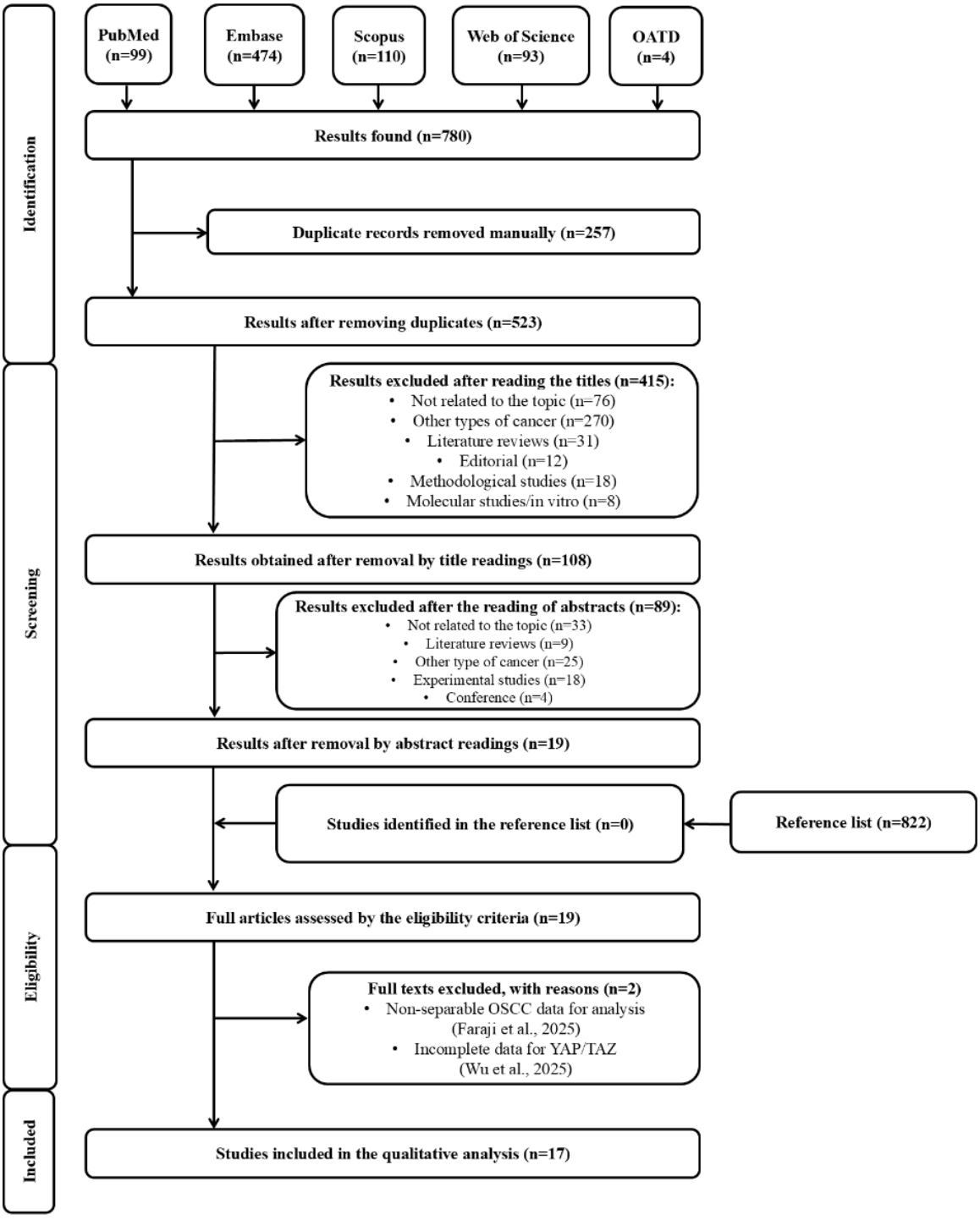


Figure 1. PRISMA Flow Diagram for Systematic Review and Meta-Analysis of the prognostic significance of Hippo pathway biomarkers in oral squamous cell carcinoma.

Group by Subgroup within study	Study name	Subgroup within study	Statistics for each study				
			Hazard ratio	Lower limit	Upper limit	Z-Value	p-Value
Disease Free Survival	Amano et al., 2024	Disease Free Survival	1,595	0,595	4,277	0,927	0,354
Disease Free Survival	Omori et al., 2020	Disease Free Survival	2,970	1,391	6,343	2,812	0,005
Disease Free Survival	Ono et al., 2019	Disease Free Survival	3,030	0,857	10,708	1,721	0,085
Disease Free Survival	Szelachowska et al., 2019	Disease Free Survival	0,360	0,166	0,780	-2,590	0,010
Disease Free Survival	Pooled		1,457	0,489	4,342	0,675	0,499
Disease Free Survival	Prediction Interval		1,457	0,010	203,306		
Overall Survival	Amano et al., 2024	Overall Survival	0,563	0,108	2,929	-0,682	0,495
Overall Survival	Gupta et al., 2025	Overall Survival	0,910	0,550	1,506	-0,367	0,714
Overall Survival	Jiang et al., 2020	Overall Survival	2,250	1,350	3,750	3,111	0,002
Overall Survival	Omori et al., 2020	Overall Survival	5,470	2,880	10,390	5,192	0,000
Overall Survival	Ono et al., 2019	Overall Survival	3,640	1,351	9,807	2,555	0,011
Overall Survival	Qi et al., 2019	Overall Survival	2,530	1,381	4,634	3,006	0,003
Overall Survival	Szelachowska et al., 2019	Overall Survival	0,569	0,300	1,078	-1,730	0,084
Overall Survival	Tsai et al., 2024	Overall Survival	6,130	1,221	30,765	2,203	0,028
Overall Survival	Pooled		1,918	1,036	3,549	2,073	0,038
Overall Survival	Prediction Interval		1,918	0,252	14,570		
Overall	Pooled		1,795	1,050	3,068	2,138	0,033
Overall	Prediction Interval		1,795	0,281	11,459		

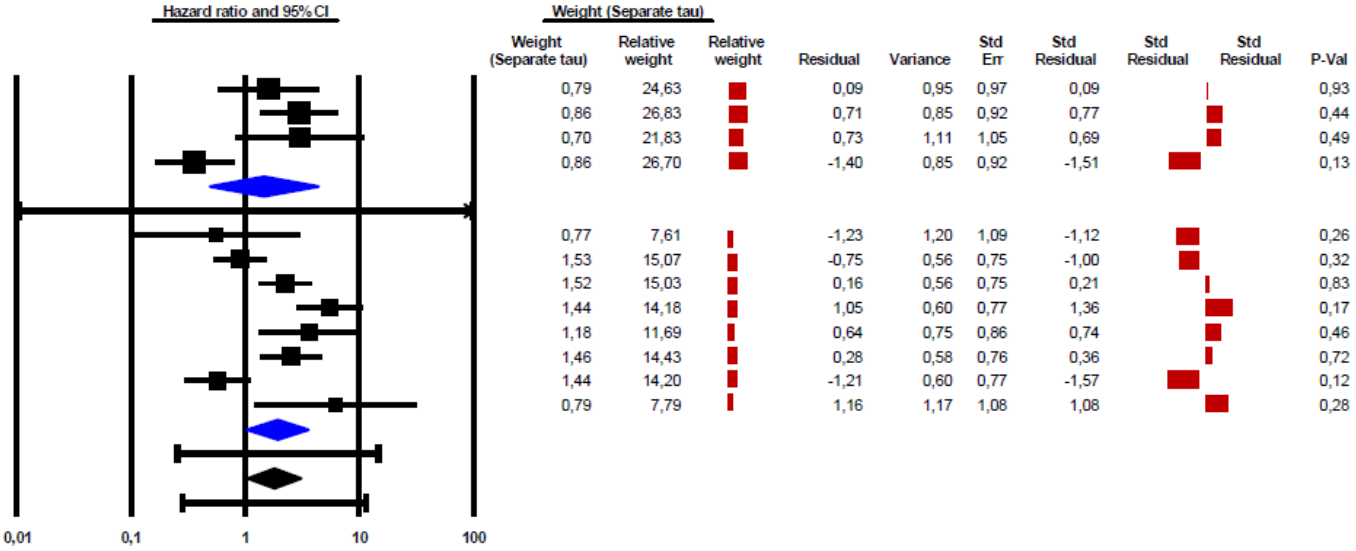


Figure 2. The prognostic value of YAP immunohistochemical expression in oral squamous cell carcinoma (OSCC). (A) Association between high nuclear YAP expression and Overall Survival (OS). (B) Association between high nuclear YAP expression and Disease-Free Survival (DFS). Note: The squares represent the hazard ratio (HR) for each individual study, with the size proportional to the study's weight in the meta-analysis. Horizontal lines indicate the 95% confidence interval (95% CI). The diamond at the bottom represents the pooled HR. An HR value greater than 1 indicates that high YAP expression is significantly associated with poor survival outcomes.

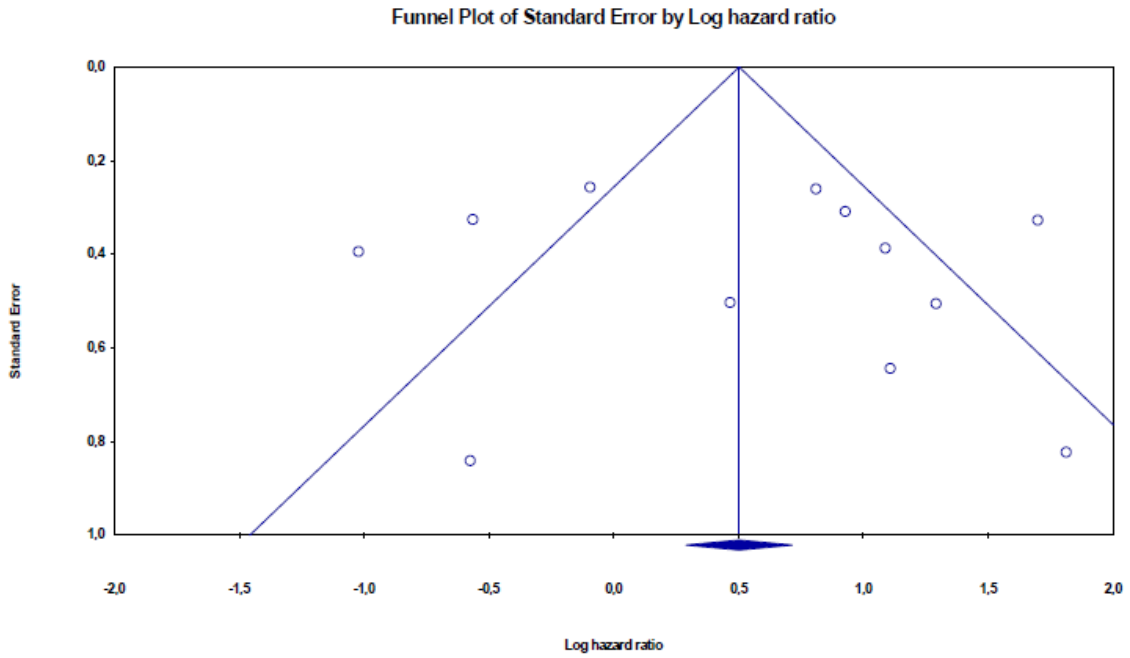


Figure 3. Funnel plot assessing potential publication bias and heterogenity in the meta-analysis of YAP expression. This scatter plot illustrates the distribution of the included studies regarding YAP expression and survival outcomes. The horizontal axis represents the effect size (Log Hazard Ratio), while the vertical axis indicates the standard error (SE) of the estimate.

Group by Subgroup within study	Study name	Subgroup within study	Statistics for each study				
			Hazard ratio	Lower limit	Upper limit	Z-Value	p-Value
Disease Free Survival	Li et al., 2015	Disease Free Survival	2,218	1,174	4,190	2,455	0,014
Disease Free Survival	Szelachowska et al., 2019	Disease Free Survival	0,680	0,386	1,198	-1,335	0,182
Disease Free Survival	Wei et al., 2013	Disease Free Survival	6,680	2,342	19,055	3,551	0,000
Disease Free Survival	Pooled		2,025	0,609	6,727	1,152	0,250
Disease Free Survival	Prediction Interval		2,025	0,000	5219984,890		
Overall Survival	Li et al., 2015	Overall Survival	2,494	1,367	4,551	2,979	0,003
Overall Survival	Wei et al., 2013	Overall Survival	4,351	1,477	12,818	2,667	0,008
Overall Survival	Pooled		2,845	1,682	4,811	3,900	0,000
Overall Survival	Prediction Interval						
Overall	Pooled		2,694	1,664	4,359	4,034	0,000
Overall	Prediction Interval		2,694	0,206	35,300		

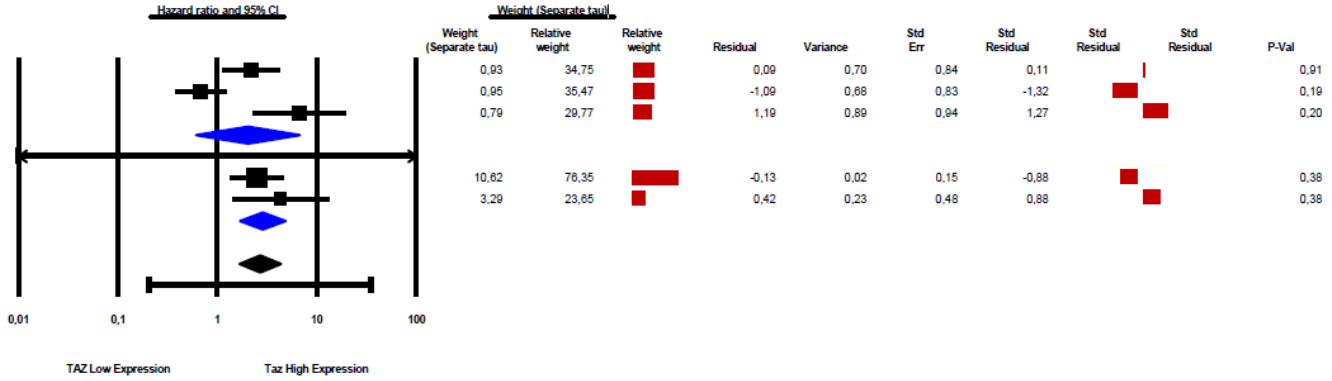


Figure 4. Forest plots depicting the prognostic significance of TAZ expression in oral squamous cell carcinoma (OSCC). (A) Meta-analysis of the association between high TAZ expression and Overall Survival (OS). (B) Meta-analysis of the association between high TAZ expression and Disease-Free Survival (DFS). Note: Individual study effect sizes are represented by squares, with horizontal lines indicating 95% confidence intervals (CI). The diamond represents the pooled Hazard Ratio (HR), where an HR > 1 signifies that TAZ overexpression is a risk factor for poor survival outcomes.

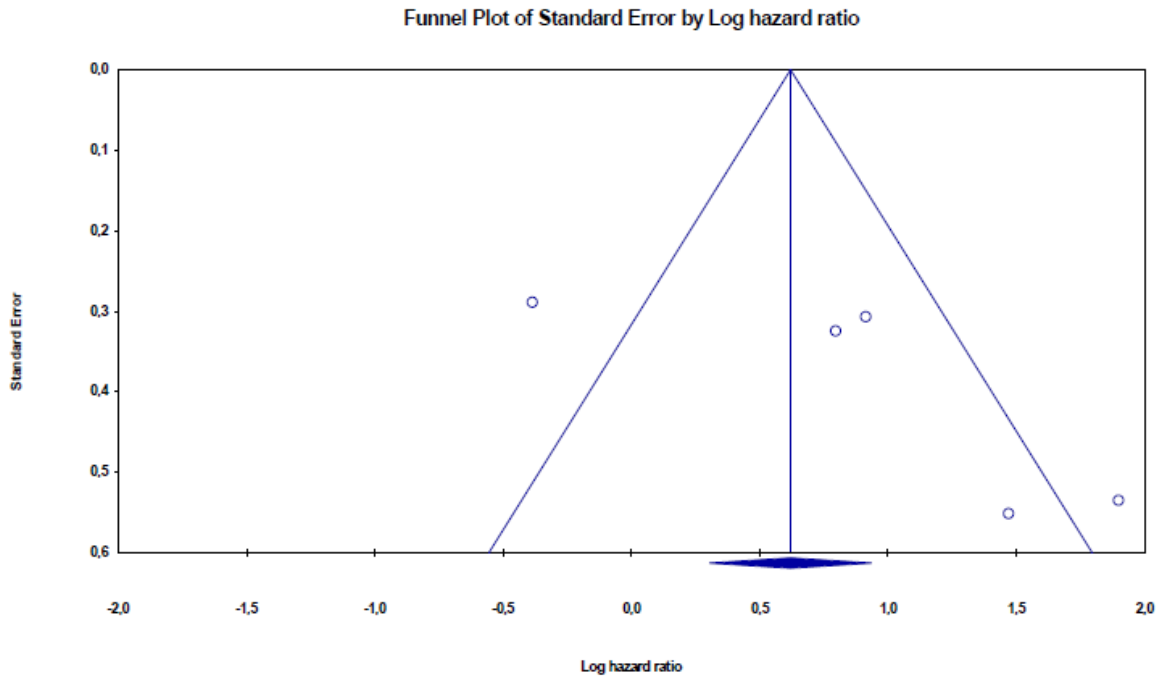


Figure 5. Funnel plot for the evaluation of publication bias in studies investigating TAZ expression. The scatter plot displays individual studies included in the meta-analysis of TAZ survival outcomes. The x-axis represents the effect size (Log Hazard Ratio), and the y-axis represents the precision of the study (Standard Error). The plot symmetry is used to assess the presence of potential publication bias or small-study effects across the analyzed studies.

5 CONSIDERAÇÕES FINAIS

O CEC oral permanece associado a prognóstico desfavorável a despeito dos avanços terapêuticos, em parte porque os sistemas de estadiamento convencionais não capturam a heterogeneidade biológica do MT, dimensão que, conforme evidenciado pelas revisões sistemáticas conduzidas neste trabalho, é determinada de forma crítica pela rigidez e pela organização da MEC e pela ativação dos efetores mecanotransdutores YAP e TAZ. O estudo experimental demonstrou que o CEC oral exibe dois fenótipos estromais morfometricamente distintos, com forte acoplamento entre o teor de colágeno e o grau de alinhamento fibrilar ($r = 0,910$; $p < 0,001$), e que o fenótipo de alto teor de colágeno associou-se significativamente à maior frequência de metástase linfonodal regional (OR = 5,630; $p = 0,033$), sugerindo que o estroma desmoplásico maduro e direcional configura um microambiente permissivo à disseminação tumoral por mecanismos de contato e mecanotransdução mediada por integrinas e YAP/TAZ.

Assim, os três componentes desta tese demonstram que a remodelação da MEC é participante ativa do processo de progressão tumoral e sua caracterização morfométrica agrega informação biologicamente relevante e potencialmente prognóstica ao CEC oral. As limitações inerentes ao delineamento retrospectivo e ao tamanho amostral demandam confirmação em estudos prospectivos com maior casuística, dados clínicos completos, análises de sobrevida e incorporação de imunohistoquímica para marcadores de mecanotransdução, como FAK fosforilada, integrinas β1 e YAP/TAZ, cuja expressão diferencial nos dois fenótipos estromais identificados representa a hipótese central a ser investigada em continuidade a este trabalho.

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