

# Clinical Implications of EMT in HNSCC: A Review of the Factors and Pathways at Play

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## Abstract

*Background:* Epithelial–mesenchymal transition (EMT) is effected in squamous cell cancers via activation of the cadherin switch. Activation of the switch results in decreased expression of E-cadherin and increased expression of N-cadherin. This results in loss of cell-cell adhesion and cellular polarity and allows the tumour cells to metastasise.

*Aims & Objectives:* This systematic review was carried out to find the clinical implications of EMT in HNSCC and bring together the molecular, genetic, and epigenetic pathways found to be acting on the cadherin switch.

*Materials & Methods:* An extensive search for relevant papers was made on PubMed, Medline, and Google Search. Only good-quality studies pertaining to epithelial–mesenchymal transition in oral cavity cancers in humans were selected. Furthermore, all selected papers were assessed for their clinical relevance.

*Results:* The cadherin switch is regulated by transcription factors like Snail, SLUG, ZEB1, and ZEB2. It is also acted on by epigenetic modifiers. These transcription factors are regulated by multiple pathways like Wnt/ $\beta$ -catenin, PI3K/AKT, MAPK, etc. These pathways are in turn regulated by molecular and external agents like NNK from tobacco smoke, hypoxia, the DDB2 gene, reactive oxygen species (ROS), and melatonin.

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## Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth-most common malignant tumour in the world. Despite advances in cancer treatment, the mortality rate due to HNSCC is still alarming, and the capacity of invasion/metastasis of this cancer is an important factor associated with its prognosis [1]. Epithelial-mesenchymal transition (EMT) is a biological process where epithelial cells acquire mesenchymal traits. Epithelial cells are characterized by tight cell-cell adhesions and apical-basal polarity, whereas mesenchymal cells are generally elongated in appearance with loose cell-cell interactions, allowing increased cell migration [2].

The process of EMT is divided into three distinct types, i.e., EMT Type 1, 2, and 3. EMT-1 occurs during normal embryogenesis; EMT-2 during wound healing and tissue regeneration; and EMT-3 is associated with cancer progression [3]. At the molecular level, three important mechanisms contribute to the changes in type 3 EMT: a) Downregulation of E-cadherin, b) Changes in the expression of microRNA (miRNA), c) Reorganization of actin and formation of invadopodia [3]. Many studies have been done on EMT pathways in oral cavity carcinoma, but there are few studies about the possible clinical implications.

The aim of our systematic review is to highlight the molecular pathways of EMT and their clinical implications in oral cavity carcinoma.

## Materials and Methods

PRISMA guidelines [4] were followed for this systematic review. An extensive search was done on PubMed, Medline, and Google Search using the search terms “Epithelial mesenchymal transition AND head and neck cancer,” “Epithelial mesenchymal transition,” “head and neck cancer,” and “humans.” The search time was extended up to the 27<sup>th</sup> of August 2023. Studies meeting the following criteria were included:

1. Studies on oral cavity cancers in human subjects
2. Full-text articles in English
3. Studies relating to the clinical implications of EMT

The following types of articles were excluded:

1. Systematic reviews and meta-analyses
2. Articles on animal experiments

All articles were screened for relevance using the title of the paper and the terms “epithelial-mesenchymal transition. Thereafter, two authors reviewed the abstracts to select publications on EMT and human subjects only. Finally, the text of the remaining papers was accessed to ensure that they discussed the clinical implications of EMT. A quality assessment of the selected articles was done using the Newcastle-Ottawa tool [\[5\]](#), and articles of good quality were included. This process is shown in the PRISMA flowchart in Figure 1.

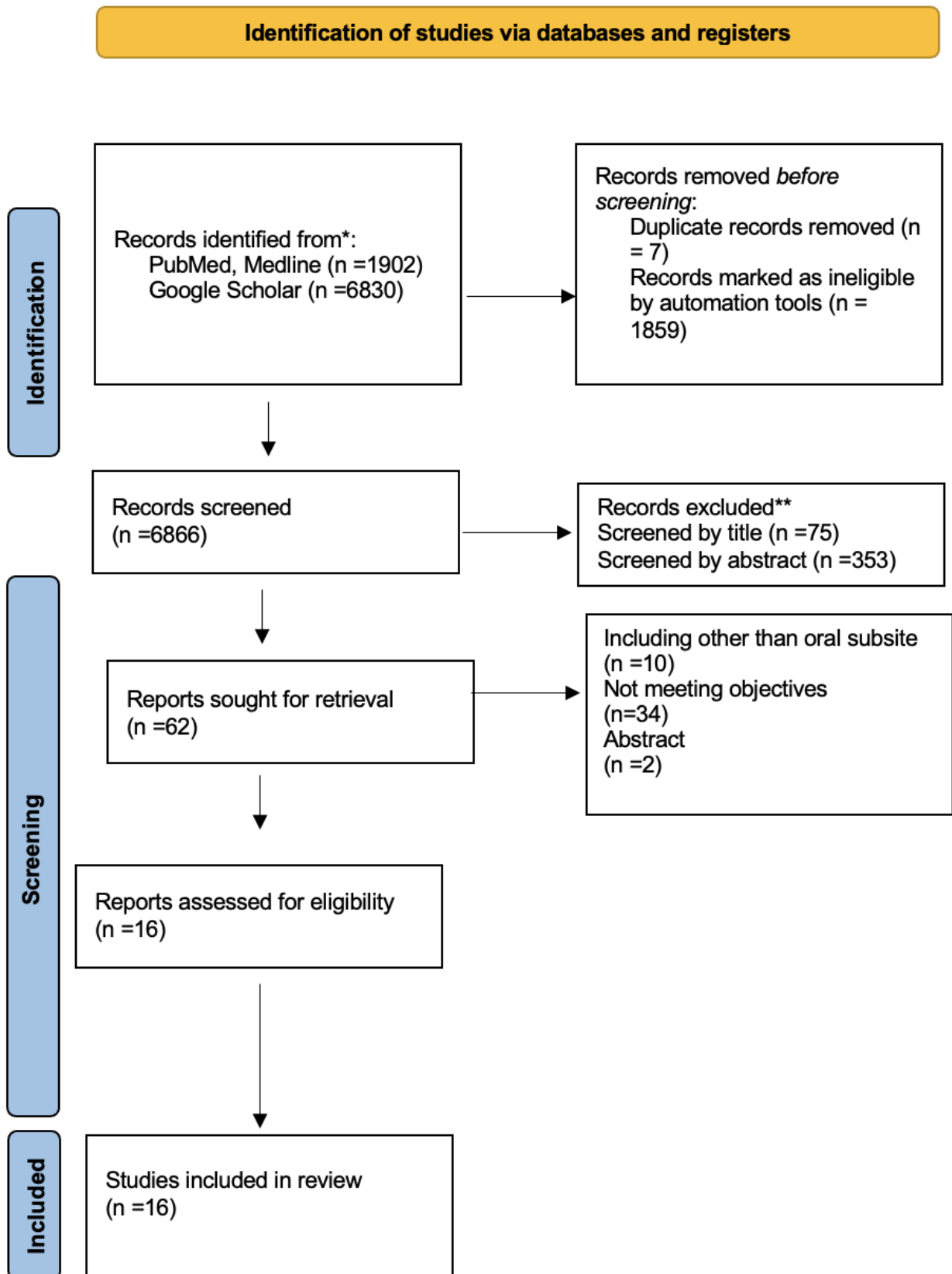


Figure 1. PRISMA FLOW CHART OF ARTICLE SELECTION PROCESS

## Results

1902 articles were found via PubMed and Medline, and 6830 via Google search, giving a total of 8732 articles. Seven were removed because they were duplicates, 75 were screened by title, and 353 by abstract. Finally, full-text retrieval was done on 62 articles. Ten were excluded because they were about sites other than the oral cavity, 33 did not match the objectives of the review, and 2 were only abstracts. Ultimately, 16 articles of good quality, matching the aims and objectives of the review, were included. The details of these studies are listed in Table 1.

**Table 1.** Table showing details of studies included, the molecular pathway examined and result of Quality assessment of each study

Sr.no.	Study(year)	Pathway	Subject	Number	Quality
1	Ali, Mehwish Feroz (2023)	ZAG-2 $\alpha$	Human	120	Good
2	Bommi, Prashant V (2018)	DDB2	Human	28	Good
3	Gudi, Radhika R (2021)	CPAP	Human cancer cell line	-	Good
4	Huynh, Nam Cong-Nhat(2022)	CAFs	Human cancer cell line	-	Good
5	Kim, Sun(2019)	RON	Human	89	Good
6	Liu, Huijuan(2022)	TAB2	Human	57	Good
7	Liu, Rui(2018)	Melatonin & ROS	Human cancer cell line	-	Good
8	Liu, Xue(2019)	ADAR1	Human	61	Good
9	Nieh, S. (2015)	NNK & Tobacco	Human cancer cell line	-	Good
10	Park, Junhee(2019)	CCL28-induced RAR $\beta$	Human	117	Good
11	Rai, K. H. (2019)	N – Cadherin expression	Human	130	Good
12	Wu, Qiuyu(2022)	Chordin like-1	Human	30	Good
13	Yaginuma, Tatsuki(2020)	P130Cas	Human	5	Good
14	Zhang, Hong(2016)	PDGF-D/ PDGFR $\beta$	Human cancer cell line	-	Good
15	Zhang, Y. (2020)	PGK1 & Hypoxia	Human	92	Good
16	Zhou, Xuan(2015)	MALAT1	Human	54	Good

## Discussion

The epithelial-to-mesenchymal transition (EMT) is a process by which epithelial cells lose their cell polarity and cell-cell adhesion, and gain migratory and invasive properties. It typically occurs during tumour progression and correlates with an increase in local invasiveness and metastatic potential of the tumour.

The studies discussed the pathways that lead to metastasis and bone invasion. Some pathways are more important in margin assessment, while others are more important in the diagnosis of less differentiated early tumours. Hence, further discussion has been divided into the important roles played by them.

## Prognosis

EMT has been an indicator of prognosis in carcinomas of many organ systems. Interactions with cancer-associated fibroblasts (CAFs) result in modification of the tumour microenvironment, leading to the formation of partial epithelial – mesenchymal transition (p-EMT) of tumour cells. Partial EMT cells exhibit both epithelial and mesenchymal signatures and play a vital role in tumour progression and metastasis. Huynh et al.<sup>[6]</sup> used a single-set RNA sequence dataset for OSCC cell lines, GSE103322 and GSE164690, from the Gene Expression Omnibus database and were able to identify four subtypes of CAFs fibroblasts and extracellular matrix gene restructuring, which they labelled eCAF. These cells also expressed cancer-associated genes, e.g., SFRP2 and proteoglycan LUM. In metastatic conditions, they detected increased signalling between eCAFs and p-EMT and the WNT and NOTCH signalling pathways. Thus, they have described CAF-mediated tumour microenvironment (TME) modelling, which helps promote invasion and metastasis.

According to Liu H et al.<sup>[7]</sup>, Transforming growth factor  $\beta$ 1-activated kinase 1 binding protein 2 (TAB2) mediates a variety of biological processes through activated nuclear factor  $\kappa$ -light-chain-enhancer of activated B cell (NF- $\kappa$ B) signalling pathways. TAB2 expression was shown to be inversely associated with prognosis. Higher epidermal growth factor receptor (EGFR) signalling can contribute to tumour metastasis and resistance to therapies in oral squamous cell carcinoma (OSCC). Mechanistically, this occurs via the PI3K/AKT pathway, resulting in cell proliferation and inhibition of apoptosis.

Loss of expression of a microtubule/tubulin binding protein, centrosomal protein 4.1-associated protein (CPAP), leads to an increase in EGFR levels and its signalling, hence amplifying the EMT features and invasiveness of OSCC cells<sup>[8]</sup>. Simultaneously, cells that are undergoing EMT express lower levels of EGFR and are less susceptible to EGFR-targeted therapies, leading to chemotherapeutic resistance.

Epithelial cells typically express E-cadherin, whereas N-cadherin is expressed by mesenchymal cells. As the tumour progresses from well-differentiated to poorly-differentiated, there is a progressive increase in N-cadherin expression<sup>[9]</sup>. The authors suggest that N-cadherin expression in OSCC can be considered an accurate marker of EMT.

As per Kim S et al.<sup>[10]</sup>, knockdown of the receptor tyrosine kinase recepteur d'origine nantais (RON) decreases the expression of SLUG, an epithelial-mesenchymal transition (EMT)-related transcription factor, and the phosphorylation of MAPK signalling proteins in human OSCC cells. This blocks cell invasion and migration. This indicates that RON contributes to EMT via the SLUG and MAPK pathways. It provides a theoretical basis for various RON-targeting agents being studied in the therapy of OSCC.

Liu X et al.<sup>[11]</sup> showed that upregulation of ADAR1 may promote OSCC progression by facilitating the maturation of oncogenic miRNAs and EMT. Adenosine deaminases acting on RNA (ADARs) are involved in adenosine-to-inosine (A-to-I) editing, tumorigenesis, and prognosis<sup>[11]</sup>. It is postulated that ADARs, particularly the ADAR-p110 short isoform, are more abundant in OSCC and may be acting by maturation of miRNAs and EMT.

Chemokine (C-C motif) ligand 28 (CCL28)<sup>[12]</sup> leads to induced E-cadherin expression and reduced nuclear localization of  $\beta$ -catenin in OSCC cells with detectable RUNX3 expression levels. This is the result of CCL28-inhibited invasion and

epithelial-mesenchymal transition (EMT) [12]. CCL28 is a chemokine constitutively produced by epithelial cells of various mucosal tissues, but its role in various human cancers is controversial. Under physiologic conditions and during inflammation or infection, it contributes to host mucosal defence. The authors, in their experiment, demonstrate that downregulation of CCL28 in OSCC cells reduces RAR $\beta$  expression, improving their invasive ability.

Damaged-DNA binding protein 2 (DDB2) senses DNA damage and is important in Global Genomic Repair (GG-NER). DDB2 is encoded by the nucleotide excision repair gene and is required for the recognition and removal of UV-light-induced DNA lesions. DDB2 is a critical repressor of EMT-regulatory factors irrespective of cell type or tumour type [13]. The authors have shown that lower DDB2 expression leads to increased expression of pro-EMT transcription factors SNAIL and ZEB1. They suggest that monitoring DDB2 expression may be of therapeutic benefit in patients with advanced OSCC.

Melatonin decreases the migration and invasion of oral cancer cells by the repression of EMT. It is a known potent antioxidant which scavenges a variety of free radicals directly and stimulates the action of antioxidative enzymes indirectly. This requires activation of the ROS-dependent AKT pathway [14]. The key findings of the experiments of Liu et al. are

1. Melatonin suppresses ROS production in oral cancer cells
2. It reduces proliferation and apoptosis escape of OSCC by inactivation of ROS-dependent Akt signalling.
3. It inhibits migration and invasion of OSCC mediated by ROS-reliant Akt signalling
4. It abolishes tube formation by HUVECs induced by ROS-activated Akt and ERK pathways.
5. Melatonin hampers oral cancer tumorigenesis in vivo

Platelet-derived growth factor D (PDGF-D) signalling is crucial during the development and progression of human malignancies through interaction with the receptor of PDGF-D (PDGFR). It has been implicated in the tumorigenesis of breast cancer, colorectal cancer, hepatocellular cancers, head and neck squamous cell cancers, lung cancer, prostate cancer, pancreatic cancer, and renal cell carcinoma. It interacts with its cognate receptor PDGFR and cross-talks with NOTCH-1 and NF- $\kappa$ B pathways to promote EMT and tumour growth. A study by Zhang et al. [15] suggested that the EMT process can also be triggered by the PDGF-D/PDGFR $\beta$  axis in TSCC (tongue SCC) and then be involved in tumour cell invasion via activation of the p38/AKT/ERK/EMT pathway.

Chronic exposure to the carcinogen 4-methylnitrosamino-1-3-pyridyl-1-butanone (NNK), found in tobacco smoke, is involved in the progression of head and neck squamous cell carcinoma. This current study stated that long-term NNK exposure plays a role in HNSCC by increasing anti-apoptosis and therapeutic resistance via the Snail-RKIP signalling pathway [16]. The data also suggests that targeting the  $\alpha$ 7 nicotinic acetylcholine receptor or the Snail pathway may prevent the progression of HNSCC.

Zhou X et al. [17] found that MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) is overexpressed in OSCC tissues compared to normal oral mucosa by real-time PCR. MALAT1 promoted cell migration and invasion via regulating the nuclear translocation of NF- $\kappa$ B and  $\beta$ -catenin, and subsequently EMT in OSCC. It is one of the many long

non-coding RNAs, the abnormal expression of which has been found to contribute to carcinogenesis, metastasis, stem cell differentiation, and resistance to chemotherapy and radiotherapy.

Zinc-2 $\alpha$  glycoprotein is a clinically active protein and plays a role in tumour formation and proliferation. It was first identified as a lipid-mobilising factor and later as a tumour marker in cancer-induced cachexia. Ali MF et al. [18] showed that high expression of Zinc-alpha 2 glycoprotein (ZAG) is associated with early-stage OSCC, smaller tumour size, absence of lymph node involvement, and well-differentiated tumours. The authors suggest that ZAG can be investigated as a reliable biomarker to maintain the epithelial phenotype, predict prognosis, and post-treatment outcomes.

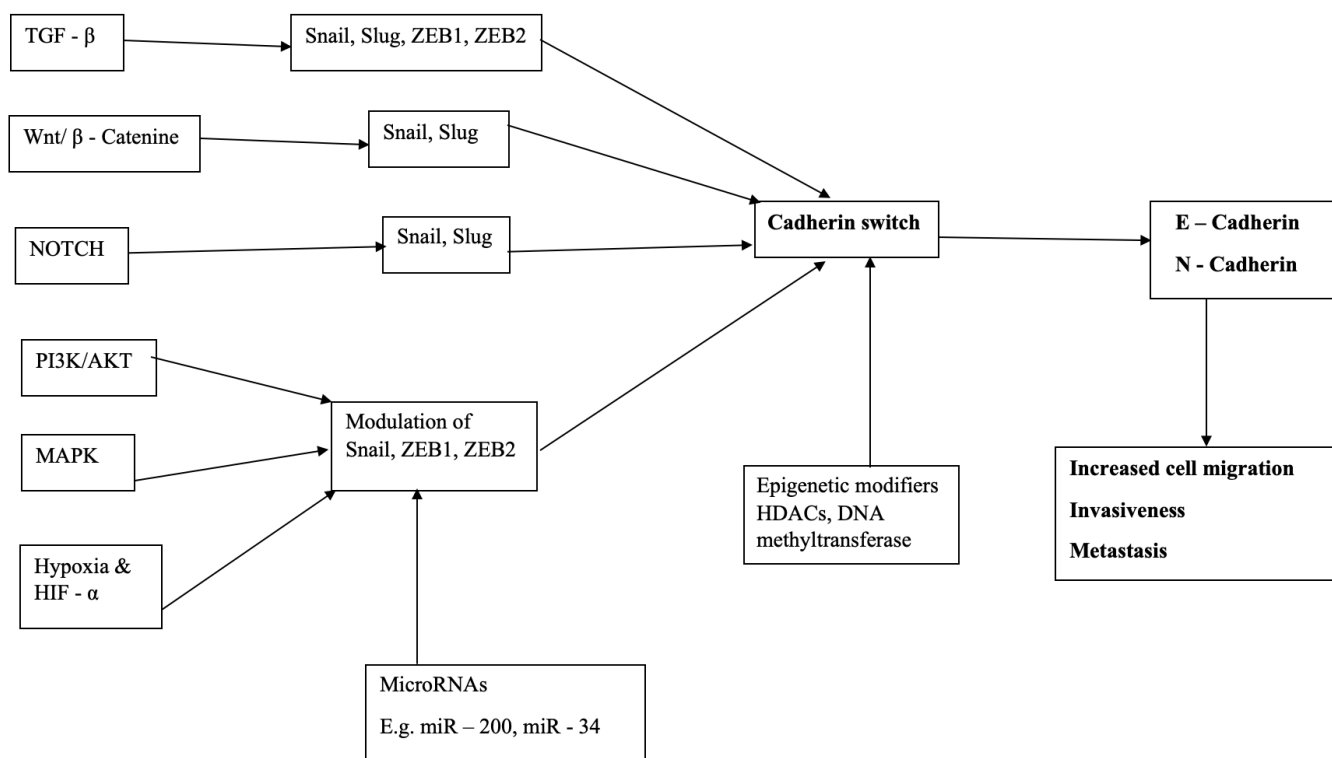
According to Wu Q et al. [19], CHRDL1 was significantly downregulated in OSCC tissues. CHRDL1 knockdown enhanced migration, invasion, adhesion, and EMT. Overexpression of CHRDL1 had the opposite effect. Chordin like-1 is the antagonist of BMP (Bone morphogenetic proteins) proteins belonging to the TGF $\beta$  family, which play an important role in tumour development, progression, and invasion. CHRDL1 has been proven to block BMP-induced increases in breast cancer migration and invasion. With this study, Wu et al. prove the modulatory role of CHRDL1 in oral cavity cancers. Upregulation of CHRDL1 has been found to reverse the epithelial-mesenchymal transition and inhibit metastasis. All of these receptors, markers, and pathways end up acting on the 'cadherin switch'.

The cadherin switch, i.e., the switch from expression of E-cadherin to N-cadherin, plays a central role in reprogramming the cell from an epithelial to a mesenchymal phenotype [20].

The switch is regulated by several key pathways, e.g., transcriptional pathways like the Snail family, ZEB family, and Twist, growth factor signalling pathways like TGF –  $\beta$  [21], Wnt/ $\beta$  – catenin pathway [22], the NOTCH pathway [23], epigenetic changes, and some microRNAs [24][25]. Hypoxia, too, plays an important role in EMT via HIF –  $\alpha$ , a key transcription factor. HIF –  $\alpha$  acts by regulating the expression of genes involved in angiogenesis, erythropoiesis, metabolism, and cell survival while cross-talking with other important pathways like PI3K/AKT and MAPK [26].

A diagrammatic representation of these pathways is shown in Figure 2. Table 2 lists the factors discussed, their putative mode of action, and their effect on EMT. The DDB2 gene and CAF (cancer-associated fibroblasts) both act on the TGF $\beta$  pathway, but CAFs also act on the Wnt and NOTCH pathways. Receptor Tyrosine Kinase (RON) and CHRDL-1 both act on the MAPK pathway, while RON also increases the expression of SLUG, promoting EMT. The Centrosome Protein 4.1-associated protein (CPAP) acts via the EGFR pathway. Reactive oxygen species (ROS), TAB2, PGK1, and PGDF/PGDFR act via the PI3K/AKT pathway, but the PGDF/PGDFR also acts on the NOTCH pathway, which has significant cross-talk with the PI3K/AKT pathway. Melatonin acts by mopping up ROS and suppressing the PI3K/AKT pathway. MALAT1 has been found to act via the Wnt/ $\beta$  – catenin pathway. NNK from tobacco smoke acts on the Snail-RKIP pathway. All the above changes end up modulating the cadherin switch by regulating Snail, SLUG, ZEB1, and ZEB2, transcriptional factors.





**Figure 2.** Schematic diagram of the cadherin switch and the pathways acting on it.

**Table 2.** List of genetic and epigenetic factors discussed in the review, their putative path of action and their effect on EMT

Investigated Factor	Pathway	Result
DDB2 gene	TGFβ	EMT ↘
Centrosomal protein 4.1 – associated Protein(CPAP)	EGFR & phosphoEGFR	EMT ↘
Cancer Associated Fibroblasts (CAF)	Wnt/NOTCH	EMT ↗
Receptor Tyrosine Kinase (RON)	MAPK	EMT ↗
TGFβ activated kinase -1binding protein 2 (TAB2)	PI3K/AKT	EMT ↗
Reactive oxygen species (ROS)	PI3K/AKT	CEMT ↗
Melatonin	ROS & PI3K/AKT	EMT ↘
Adenosine deaminase acting on RNA (ADAR)	MicroRNA	EMT ↗
4-methylnitrosamine-1-3-pyridyl-1-butanone(NNK)	Snail RKIP	EMT ↗
Chordin – like 1 (CHRDL-1)	MAPK	EMT ↘
PGDF & PGDFR	NOTCH & PI3K/AKT	EMT ↗
PGK1	PI3K/AKT	EMT ↗
MALAT1	β - catenin	EMT ↗

## Bone invasion

Bone resection has its functional implications in patients with OSCC. Insights from a study by Park J et al. showed that

downregulation of CCL28, CCR10, or RAR $\beta$  expression was closely related to bone invasion. CCL28 upregulation in OSCC cells can be a new strategy for inhibiting and treating OSCC cell invasion and osteolysis [12]. A study by Yaginuma T et al. concluded that TGF- $\beta$ 1 might enhance the aggressiveness of OSCC, and p130Cas was strongly involved in TGF- $\beta$ 1-induced increase in migration, invasiveness, cell proliferation, and bone invasion. The regulation of p130Cas expression might be a suitable target for the prediction of bone invasion in OSCC patients [27]. These receptors and pathways can be investigated as potential therapeutic targets.

### Margin assessment

Ali et al. found that Zinc- $\alpha$ 2 Glycoprotein staining occurred only in samples of early squamous cell cancers. Positively stained samples were less differentiated, showing that ZAG expression decreased with increased tumour cell proliferation and differentiation. This may also aid a surgeon in achieving tumour-free surgical margins [18].

### Second primary risk assessment

Kim S et al. found that patients with high RON expression tended to have a second primary malignancy more often [10]. This can be used in post-treatment surveillance to screen high-risk patients for second primaries.

### Role of hypoxia

A study by Zhang Y et al. revealed that expression of PGK1 was significantly upregulated 12 and 24 hrs following culture under hypoxic conditions. Furthermore, silencing PGK1 expression in hypoxic conditions also significantly inhibited cell migration and invasion [28].

Hypoxia is common in residual tumours after radiotherapy, which is able to thrive in the absence of a blood supply. HIF- $\alpha$  can be investigated as a potential therapeutic target.

### Tobacco smoke and NNK

A study by Nieh et al. suggests that long-term NNK exposure increases tumour progression via the Snail-RKIP signalling pathway [16]. The authors suggest that the  $\alpha$ -7 nicotinic acetylcholine receptor might be a useful target to prevent progression of squamous cell cancers.

## Conclusions

This systematic review was an attempt to bring together present knowledge about EMT and delve into its clinical implications. Here, an attempt will be made to summarise the possible clinical implications of each paper as suggested by the authors.

Huynh et al. suggest that the p-EMT-related genes found by them can be employed as prognostic markers for HNSCC.

Furthermore, TNFRSF25 and TNFRSF12A could be a good biomarker pair for prognosis and targeting therapies for OSCC and HNSCC.

Liu H et al. suggest that targeting TAB2 may be a promising strategy for the treatment of OSCC, although they admit that the lack of animal experimental data is a serious limitation.

Gudi et al. posit that their study begins to shed light on the molecular mechanisms by which centrosome/MTOC proteins prevent tumorigenesis, but additional studies are needed.

Rai et al. have done a study correlating the expression of N-cadherin with the grade of OSCC and suggest that it may be useful in accurately grading the tumour and as a prognostic factor.

As per Kim et al., the results of their study provide a theoretical basis for the application of RON-targeting agents under investigation.

Liu X et al., through their study, shown that upregulation of ADAR1 promotes OSCC progression and it adds to the current understanding of the molecular mechanisms of ADAR1. They suggest that ADAR1 may be a novel therapeutic target in OSCC.

Park et al. suggest that CCL28, CCR10, and RAR $\beta$  expression levels can be useful markers for the prediction and prognosis of bone invasion. Additionally, CCL28 treatment can be used for preventing osteolysis and bone invasion in OSCC.

Bommi et al. suggest that monitoring DDB2 levels may be of therapeutic interest in patients with advanced HNSCC, but since there are a multitude of signals capable of inducing EMT, a detailed analysis will be required to determine if increased DDB2 expression can prevent EMT.

Rui Liu et al. summarize their paper with the suggestion that melatonin abolishes ROS-promoted growth, metastasis, and angiogenesis in oral cavity cancers. Melatonin, by reducing ROS, may provide promising therapeutic protection against oral cancer.

Zhang et al. state that data from their study suggest that hypoxia can stimulate PGK1 expression and glycolysis while activating oral cancer stem cells and EMT through the AKT pathway, but further details need to be elucidated.

Shin Nieh et al. suggest that theirs is the first study to demonstrate the contribution of long-term NNK exposure to carcinogenesis. They suggest that inhibition of the Snail pathway by use of an NNK inhibitor, e.g., anti-7 $\alpha$  nicotinic acetylcholine receptor, may prevent EMT, alter the properties of cancer stem cells, and prevent the development of cancer resistance.

Zhou X et al. suggest that further investigation into the molecular mechanisms causing dysregulation of MALAT1 expression may provide a better understanding of the progression of OSCC, identify candidate biomarkers for prognosis, and guide the development of therapeutic targets.

Ali MF et al. state that their findings demonstrate a significant expression of ZAG in oral cavity cancers, suggesting its prognostic value, but caution that further investigations are needed to find the precise role of ZAG.

Qiuyu Wu et al. claim that their study reveals the modulatory role and molecular mechanisms of CHRDL-1 in oral cavity cancers and suggest it has possible potential as a molecular therapeutic target.

In a nutshell, there are many genetic, epigenetic, and molecular pathways to activation of the cadherin switch, and most pathways experience significant cross-talk, resulting in a regulatory maze. Some pathways seem more promising as potential therapeutic candidates (e.g., melatonin), while some need further research before they can be considered candidates.

## PRISMA 2020 Main Checklist

Topic	No.	Item	Location where item is reported
<b>TITLE</b>			
<b>Title</b>	1	Identify the report as a systematic review.	Section 1, Page 3, line 63
<b>ABSTRACT</b>			
<b>Abstract</b>	2	See the PRISMA 2020 for Abstracts checklist	
<b>INTRODUCTION</b>			
<b>Rationale</b>	3	Describe the rationale for the review in the context of existing knowledge.	Section 1, Page 3, Lines 63 - 64
<b>Objectives</b>	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Section 1, Page 3, Lines 63 - 64
<b>METHODS</b>			
<b>Eligibility criteria</b>	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Section 2, Page 3, Lines 67- 69
<b>Information sources</b>	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Section 2, Page 3, Lines 67- 69
<b>Search strategy</b>	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Section 2, Page 3, Lines 67- 69
<b>Selection process</b>	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Section 2, Page 3 - 4, Lines 70 - 76
<b>Data collection process</b>	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Section 2, page 4, lines 77 - 80
<b>Data items</b>	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Section 2, Page 3, Lines 67- 69 & Table 1
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	LiSection 2, Page 3, Lines 67- 69 & Table 1ne XX-ZZ
<b>Study risk of bias assessment</b>	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Section 2, page 4, line 81
<b>Effect measures</b>	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the	Literature review only

		synthesis or presentation of results.	
<b>Synthesis methods</b>	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item 5)).	Section 3, page 4, lines 84 - 89
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Literature review only
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Line 89, Table 1
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	No meta-analysis
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	No metaanalysis
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	none
<b>Reporting bias assessment</b>	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Newcastle - Ottawa tool, line 81
<b>Certainty assessment</b>	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	none
<b>RESULTS</b>			
<b>Study selection</b>	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Section 3, page 4, lines 84 - 89
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Prisma Flowchart, Page 4, lines 84 - 89
<b>Study characteristics</b>	17	Cite each included study and present its characteristics.	Table 1
<b>Risk of bias in studies</b>	18	Present assessments of risk of bias for each included study.	Table 1
<b>Results of individual studies</b>	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	none
<b>Results of syntheses</b>	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	none
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	none
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	none
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	none
<b>Reporting biases</b>	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	none
<b>Certainty of evidence</b>	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	none
<b>DISCUSSION</b>			
<b>Discussion</b>	23a	Provide a general interpretation of the results in the context of other evidence.	Section 4 , Pages 4 - 11, lines 99 - 257
	23b	Discuss any limitations of the evidence included in the review.	Pages 12 - 14, Lines 259 - 306
	23c	Discuss any limitations of the review processes used.	Pages 12 - 14, Lines 259 - 306
	23d	Discuss implications of the results for practice, policy, and future research.	Pages 12 - 14, Lines 259 - 306
<b>OTHER INFORMATION</b>			

<b>Registration and protocol</b>	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	not registered
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Protocol not prepared
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	The molecular pathways acting on cadherin switch was added after the articles had been accessed
<b>Support</b>	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	none
<b>Competing interests</b>	26	Declare any competing interests of review authors.	none
<b>Availability of data, code and other materials</b>	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	with corresponding author

## PRISMA Abstract Checklist

Topic	No.	Item	Reported?
<b>TITLE</b>			
<b>Title</b>	1	Identify the report as a systematic review.	No
<b>BACKGROUND</b>			
<b>Objectives</b>	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	No
<b>METHODS</b>			
<b>Eligibility criteria</b>	3	Specify the inclusion and exclusion criteria for the review.	Yes
<b>Information sources</b>	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	No
<b>Risk of bias</b>	5	Specify the methods used to assess risk of bias in the included studies.	Yes
<b>Synthesis of results</b>	6	Specify the methods used to present and synthesize results.	Yes
<b>RESULTS</b>			
<b>Included studies</b>	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	Yes
<b>Synthesis of results</b>	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	Yes
<b>DISCUSSION</b>			
<b>Limitations of evidence</b>	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	Yes
<b>Interpretation</b>	10	Provide a general interpretation of the results and important implications.	Yes
<b>OTHER</b>			
<b>Funding</b>	11	Specify the primary source of funding for the review.	Yes
<b>Registration</b>	12	Provide the register name and registration number.	Yes

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updated guideline for reporting systematic reviews. *MetaArXiv*. 2020, September 14. DOI: 10.31222/osf.io/v7gm2. For more information, visit: [www.prisma-statement.org](http://www.prisma-statement.org)

## Statements and Declarations

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### Conflict of Interests

The authors declare that they have no conflict of interest.

## References

- <sup>^</sup> De Morais EF, Rolim LSA, De Melo Fernandes Almeida DR, De Farias Morais HG, De Souza LB, De Almeida Freitas R. Biological role of epithelial–mesenchymal-transition-inducing transcription factors in head and neck squamous cell carcinoma: A systematic review. *Arch Oral Biol*. 2020 Nov; 119:104904.
- <sup>^</sup> Jayanthi P, Varun B, Selvaraj J. Epithelial–mesenchymal transition in oral squamous cell carcinoma: An insight into molecular mechanisms and clinical implications. *J Oral Maxillofac Pathol*. 2020; 24(1):189.
- <sup>a, b</sup> Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest*. 2009 Jun 1; 119(6):1420–8.
- <sup>^</sup> Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al.. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021 Mar 29;n71.
- <sup>^</sup> Mamikutty R, Aly AS, Marhazlinda J. Selecting Risk of Bias Tools for Observational Studies for a Systematic Review of Anthropometric Measurements and Dental Caries among Children. *Int J Environ Res Public Health*. 2021 Aug 15; 18(16):8623.
- <sup>^</sup> Huynh NCN, Huang TT, Nguyen CTK, Lin FK. Comprehensive Integrated Single-Cell Whole Transcriptome Analysis Revealed the p-EMT Tumor Cells-CAFs Communication in Oral Squamous Cell Carcinoma. *Int J Mol Sci*. 2022 Jun 9; 23(12):6470.
- <sup>^</sup> Liu H, Zhang H, Fan H, Tang S, Weng J. TAB2 Promotes the Biological Functions of Head and Neck Squamous Cell Carcinoma Cells via EMT and PI3K Pathway. *Tu WJ, editor. Dis Markers*. 2022 Aug 8; 2022:1–10.
- <sup>^</sup> Gudi RR, Janakiraman H, Howe PH, Palanisamy V, Vasu C. Loss of CPAP causes sustained EGFR signaling and epithelial-mesenchymal transition in oral cancer. *Oncotarget*. 2021 Apr 13; 12(8):807–22.
- <sup>^</sup> Rai K H, Ahmed J. A Correlative Study of N-Cadherin Expression with Different Grades of Oral Squamous Cell

- Carcinoma Projecting as a Marker of Epithelial to Mesenchymal Transition in Tumor Progression. Asian Pac J Cancer Prev. 2019 Aug 1;20(8):2327–32.*
10. <sup>a, b</sup>Kim S, Lee K, Lee DH, Lee JK, Lim SC, Joo Y, et al.. Receptor tyrosine kinase, RON, promotes tumor progression by regulating EMT and the MAPK signaling pathway in human oral squamous cell carcinoma. *Int J Oncol [Internet]. 2019 Jul 2 [cited 2024 May 27]; Available from: <http://www.spandidos-publications.com/10.3892/ijo.2019.4836>*
  11. <sup>a, b</sup>Liu X, Fu Y, Huang J, Wu M, Zhang Z, Xu R, et al.. ADAR1 promotes the epithelial-to-mesenchymal transition and stem-like cell phenotype of oral cancer by facilitating oncogenic microRNA maturation. *J Exp Clin Cancer Res CR. 2019 Jul 17;38(1):315.*
  12. <sup>a, b, c</sup>Park J, Zhang X, Lee SK, Song NY, Son SH, Kim KR, et al.. CCL28-induced RAR $\beta$  expression inhibits oral squamous cell carcinoma bone invasion. *J Clin Invest. 2019 Nov 4;129(12):5381–99.*
  13. <sup>^</sup>Bommi PV, Ravindran S, Raychaudhuri P, Bagchi S. DDB2 regulates Epithelial-to-Mesenchymal Transition (EMT) in Oral/Head and Neck Squamous Cell Carcinoma. *Oncotarget. 2018 Oct 5;9(78):34708–18.*
  14. <sup>^</sup>Liu R, Wang H li, Deng M jing, Wen X jie, Mo Y yuan, Chen F ming, et al.. Melatonin Inhibits Reactive Oxygen Species-Driven Proliferation, Epithelial-Mesenchymal Transition, and Vasculogenic Mimicry in Oral Cancer. *Oxid Med Cell Longev. 2018;2018:1–13.*
  15. <sup>^</sup>Zhang H, Sun JD, Yan LJ, Zhao XP. PDGF-D/PDGFR $\beta$  promotes tongue squamous carcinoma cell (TSCC) progression via activating p38/AKT/ERK/EMT signal pathway. *Biochem Biophys Res Commun. 2016 Sep 16;478(2):845–51.*
  16. <sup>a, b</sup>Nieh S, Jao S, Yang C, Lin Y, Tseng Y, Liu C, et al.. Regulation of tumor progression via the Snail-RKIP signaling pathway by nicotine exposure in head and neck squamous cell carcinoma. *Head Neck. 2015 Dec;37(12):1712–21.*
  17. <sup>^</sup>Zhou X, Liu S, Cai G, Kong L, Zhang T, Ren Y, et al.. Long Non Coding RNA MALAT1 Promotes Tumor Growth and Metastasis by inducing Epithelial-Mesenchymal Transition in Oral Squamous Cell Carcinoma. *Sci Rep. 2015 Nov 2;5:15972.*
  18. <sup>a, b</sup>Feroz Ali DrM, Hosein DrM, Butt DrS, Siddiqui DrR. Zinc-alpha 2 glycoprotein a diagnostic Biomarker for early stage oral Squamous Cell Carcinoma. *Pak J Med Sci [Internet]. 2023 Jan 25 [cited 2024 May 27];39(2). Available from: <https://www.pjms.org.pk/index.php/pjms/article/view/6488>*
  19. <sup>^</sup>Wu Q, Zheng Z, Zhang J, Piao Z, Xin M, Xiang X, et al.. Chordin-Like 1 Regulates Epithelial-to-Mesenchymal Transition and Metastasis via the MAPK Signaling Pathway in Oral Squamous Cell Carcinoma. *Front Oncol. 2022 Apr 14;12:862751.*
  20. <sup>^</sup>Hazan RB, Qiao R, Keren R, Badano I, Suyama K. Cadherin Switch in Tumor Progression. *Ann N Y Acad Sci. 2004 Apr;1014(1):155–63.*
  21. <sup>^</sup>Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial–mesenchymal transition. *Nat Rev Mol Cell Biol. 2014 Mar;15(3):178–96.*
  22. <sup>^</sup>Clevers H, Nusse R. Wnt/ $\beta$ -Catenin Signaling and Disease. *Cell. 2012 Jun;149(6):1192–205.*
  23. <sup>^</sup>Leong KG, Karsan A. Recent insights into the role of Notch signaling in tumorigenesis. *Blood. 2006 Mar 15;107(6):2223–33.*
  24. <sup>^</sup>Zhao H, Wei J, Sun J. Roles of TGF- $\beta$  signaling pathway in tumor microenvironment and cancer therapy. *Int*



*Immunopharmacol.* 2020 Dec;89(Pt B):107101.

25. ^Lee S, Choi EJ, Cho EJ, Lee YB, Lee JH, Yu SJ, et al.. Inhibition of PI3K/Akt signaling suppresses epithelial-to-mesenchymal transition in hepatocellular carcinoma through the Snail/GSK-3/beta-catenin pathway. *Clin Mol Hepatol.* 2020 Oct 1;26(4):529–39.
26. ^Semenza GL. Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy. *Trends Pharmacol Sci.* 2012 Apr;33(4):207–14.
27. ^Yaginuma T, Gao J, Nagata K, Muroya R, Fei H, Nagano H, et al.. p130Cas induces bone invasion by oral squamous cell carcinoma by regulating tumor epithelial–mesenchymal transition and cell proliferation. *Carcinogenesis.* 2020 Aug 12;41(8):1038–48.
28. ^Zhang Y, Cai H, Liao Y, Zhu Y, Wang F, Hou J. Activation of PGK1 under hypoxic conditions promotes glycolysis and increases stem cell-like properties and the epithelial-mesenchymal transition in oral squamous cell carcinoma cells via the AKT signalling pathway. *Int J Oncol.* 2020 Jun 16;57(3):743–55.