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Systematic Review Article

Molecular Mechanisms Linking E-cigarette Exposure to Cancer Metastatic Behaviour: Focus on Epithelial-to-Mesenchymal Transition and Inflammation: A Systematic Review

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Abstract: Electronic cigarette use has been mushrooming globally in recent years, with an estimated 82 million vapers worldwide, including 14.3 million in Southeast Asia. Although e-cigarettes are generally considered a safer alternative to traditional cigarettes, their long-term health effects, particularly on cancer progression and metastasis, remain uncertain. This systematic review evaluated the existing literature to determine whether e-cigarette exposure influences cancer cell metastatic behaviour. A systematic search was conducted using

predefined terms in MEDLINE, PubMed, and Web of Science from database inception to 1st May 2025. The studies must be primary research and investigate the effects of e-cigarette exposure on cancer cell metastasis. Seven eligible studies were selected, assessed for reliability using the SciRAP tool, and synthesised manually. The literature evaluated several aspects of cancer metastasis, including cell invasion, migration and motility, morphological changes, tumour area, growth, proliferation, colonisation or localisation, cell viability, and immunological changes. Evidence synthesis suggests that e-cigarette exposure may promote cancer cell migration; however, these findings are constrained by the small number of studies, methodological limitations, and lack of long-term investigations. Reported effects varied depending on cancer cell type, nicotine presence, and e-liquid flavouring. Current preclinical data indicate e-cigarette exposure may promote cancer metastatic behaviour through epithelial-to-mesenchymal transition (EMT) induction (E-cadherin loss, vimentin upregulation), RAGE-mediated inflammatory signalling, and CCL5/CCR5-dependent monocyte recruitment. However, all seven included studies were rated "not reliable" by SciRAP (methodological quality scores 47–70%), rendering evidence preliminary. Future studies require physiologically relevant direct aerosol exposure systems, cigarette smoke comparators for relative risk assessment, diverse cancer models, and longitudinal clinical investigations to establish the true impact of vaping on cancer metastasis.

Keywords: Epithelial-mesenchymal transition; Vape; Tumor microenvironment; Premature mortality; Tobacco control; Evidence-based policy; Oncology; Public health; Nicotine

1. Introduction

In 2004, the first commercially available electronic cigarette (e-cigarette) was sold in China after being patented in 2003 by Hon Lik, a Chinese pharmacist, as a smoking-cessation product designed to replicate the appearance, sensation, and experience of smoking without the associated toxicities [1,2]. Commonly known as vapes, e-cigarettes are electronic devices consisting of (1) a cartridge filled with e-liquid containing solvents (propylene glycol and vegetable glycerine), flavourings, and in most cases, nicotine, (2) a heating element or atomiser to vaporise the liquid, (3) a mouthpiece for inhalation, and (4) a rechargeable battery [1,3]. With the widespread availability of both electronic devices and e-liquids in physical shops or online stores [3], vaping has rapidly gained traction, particularly among younger populations [4-7]. As of 2021, an estimated 82 million people worldwide were vapers, with 14.3 million residing in Southeast Asia [8]. In Malaysia, according to the Global Adult Tobacco Survey 2023, e-cigarette use among individuals aged 15 and above has surged by

more than 600% over the past 12 years, rising from 0.8% in 2011 to 5.8% in 2023 [9]. This increase was particularly pronounced among young adults aged 15–24, whose prevalence rose from 1.1% in 2011 to 8.6% in 2023 [9].

Due to the absence of combustion—the primary source of many toxicants in tobacco smoke—e-cigarettes are generally considered a less harmful alternative for individuals unwilling or planning to quit smoking [3,10]. However, this potential harm reduction is complicated by the considerable variability and incomplete characterisation of e-liquid composition [11], as well as the diversity of available devices. E-cigarettes are broadly categorised into disposable, closed-system (prefilled cartridges or pods), and open-system (refillable tank or pod) devices [1,12]. Open-system devices are highly customisable, allowing users to select or modify e-liquid formulations with varying nicotine concentrations and flavourings, which creates regulatory challenges and complicates safety assessment [11-13]. Although e-cigarette aerosols generally contain fewer harmful substances than the ~7,000 chemicals found in traditional cigarette smoke [14,15], their long-term health effects remain largely uncertain and warrant further investigation [1,3,11,16]. Preclinical studies have reported that e-cigarettes deliver nicotine and may contain carcinogenic chemicals, raising concerns about their potential role in cancer initiation, progression, and metastasis [17].

Cancer metastasis, the leading cause of cancer-related mortality and morbidity, involves a series of intricate biological processes that enable malignant cells to disseminate beyond the primary tumour site and establish new tumours (secondary and tertiary foci) in distant organs [18,19]. As tumour cell invasion and migration initiate the metastatic cascade [20], understanding the factors that regulate these processes is critical for improving treatment outcomes. A key hallmark of metastasis is epithelial-to-mesenchymal transition (EMT), driven by signalling pathways such as TGF-β, WNT, TNF-α, NF-κB, and Il-6/STAT, and mediated by transcription factors including *SNAIL*, *TWIST*, and *ZEB* [21]. EMT is characterised by loss of E-cadherin, disassembly of adherens junctions, loss of apical-basal polarity, and upregulation of matrix metalloproteinases (MMPs), which degrade the extracellular matrix (ECM) [21].

While the effects of traditional smoking on various cancer metastasis such as enhanced cell invasion, migration and motility, EMT induction, increased cell proliferation and growth, reduced apoptosis, and immunomodulation, have been well-established following extensive studies over the past decades [22-33], the potential role of vaping in influencing cancer cell behaviour, particularly migration and metastasis, remains less clear due to limited investigations.

This systematic review aims to evaluate current evidence regarding the potential effects of vaping on cancer cell metastatic behaviour, with a particular focus on these key molecular drivers of metastasis. By synthesising the available data, this review seeks to provide clarity on vaping's role in cancer progression and identify key areas for future research.

2. Methods

This systematic review was conducted in accordance with the guidelines presented in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement [34]. This review was not prospectively registered in PROSPERO, as it was initiated prior to the decision to submit to a peer-reviewed journal. As quantitative synthesis (meta-analysis) was not feasible due to substantial heterogeneity in study designs, exposure methods, cancer types, and outcomes, findings were integrated through a descriptive narrative synthesis.

2.1. Information Sources and Search Strategy

We systematically searched the following databases for relevant studies published from inception to 1st May 2025: MEDLINE via EBSCOhost, PubMed, and Web of Science. The search terms included "vape," "vaping," "electronic cigarette," "e-cigarette," "migration," "invasion," and "metastasis." Additionally, a manual search was conducted by screening the reference lists of selected papers to ensure that all relevant articles were identified.

2.2. Study Selection

The selection of final articles was conducted in two phases. In the first phase, two reviewers (KL and PEG) independently screened all titles and abstracts retrieved from the systematic search, applying the inclusion and exclusion criteria (Table 1). Duplicate articles and retracted records were removed. In cases of disagreement, consensus on which articles to review in full text was reached through discussion, with a third reviewer (H-LS) consulted when necessary. In the second phase, the reviewers independently assessed the full texts of the selected articles to confirm the final selection. Similarly, any disagreements were resolved through discussion, with a third reviewer (H-LS) involved when needed.

Table 1. Inclusion and exclusion criteria.

	Inclusion	**Exclusion**
Article type	Primary research articles	Case studies; Commentaries; Conference reports; Conference abstracts; Editorials; Letters to the editor; Meta-analyses; Opinions; Press releases; Review articles; Systematic reviews
Article criteria	Measured or reported the effects of vape liquid or aerosol, vaping, or e-cigarettes on cancer migration or metastasis	Did not measure or report cancer migration or metastasis Did not report the use of vape or electronic cigarette Did not report the use of vape liquid or aerosol, but certain chemicals or specific components in vape liquid only
Language	English-language studies	Non-English studies

2.3. Data Extraction

The reviewers extracted the following information from the selected articles: country of study, cancer type and cell line, study design, e-liquid flavour, e-cigarette exposure type and duration, e-liquid or aerosol concentration, research findings on cancer migration and progression, and limitations.

2.4. Study Quality Assessment

The reliability of each included study, both *in vitro* and *in vivo*, was assessed using the web-based Science in Risk Assessment and Policy (SciRAP) tool (http://www.scirap.org/). SciRAP provides structured criteria and guidance for evaluating reporting quality, methodological quality, and relevance. As SciRAP does not include a predefined scheme for categorising study reliability, the methodological quality score of each included study was translated into a reliability category following the approach of Holmer, Zilliacus [35] (Table 2). The SciRAP assessments and reliability categorisations were performed by one reviewer (PEG) and independently checked by a second reviewer (KL), with potential disagreements resolved through discussion.

Table 2. Principles for translating the SciRAP methodological quality score of *in vitro* and *in vivo* studies into reliability categories.

Reliability category	**Principles for reliability categorisation**
1. Reliable without restriction	SciRAP methodological quality score > 80 **and** all key criteria[a] are "Fulfilled" **and** there are no deficiencies in the non-key criteria that might affect study reliability.
2. Reliable with restriction	SciRAP methodological quality score > 65 **and** one or several of the key criteria are "Partially Fulfilled" **or** there are minor deficiencies in the non-key criteria that might affect study reliability.

3. Not reliable	SciRAP methodological quality score < 65 **or** one or several of the key criteria are "Not Fulfilled" **or** there are major deficiencies in the non-key criteria that affect study reliability.
4. Not assignable	Two or more of the key criteria are "Not Reported"[b]

[a] Key criterion are those judged as specifically critical for the reliability of the data in a certain case and are determined *a priori*.

[b] Criteria that were judged as "Not Reported" in SciRAP were not reported or were considered too poorly reported to confidently classify them as "Fulfilled", "Partially Fulfilled", or "Not Fulfilled".

3. Results

3.1. Literature Search

The search identified 496 articles from three databases (Figure 1). After removing duplicates and one retracted record, 286 articles were subsequently screened for suitability based on inclusion and exclusion criteria. Nine full-text articles underwent full-text assessment, of which seven met the eligibility criteria and were included in the review, as the other two articles did not report on cancer migration. The completed PRISMA 2020 Abstract Checklist and PRISMA 2020 Checklist are provided in Supplementary Material (Tables S1 and S2).

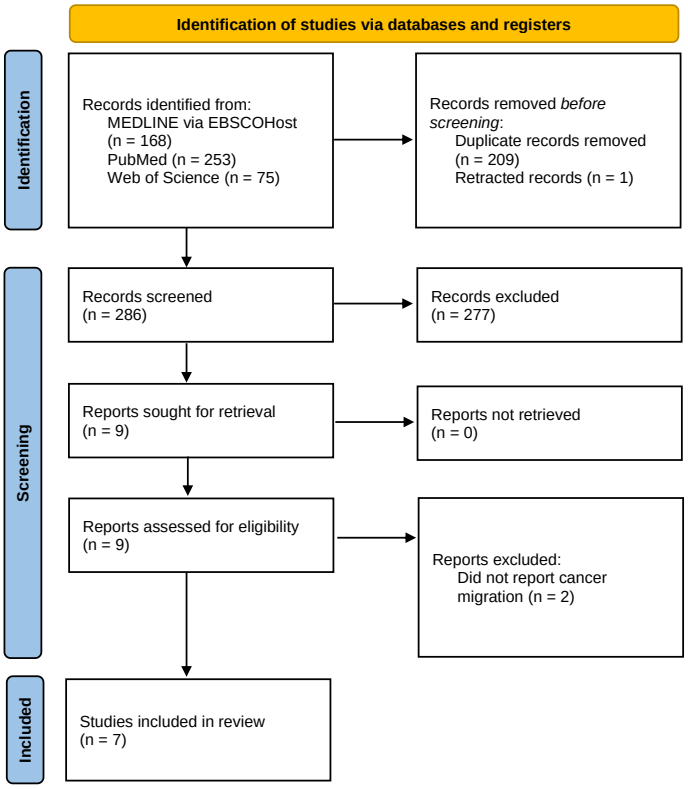


Figure 1. PRISMA flow chart.

Table 3. Study characteristics of the selected articles.

Country	Cancer type	Cancer cell line	Study design	E-liquid flavour	E-cigarette exposure type	E-cigarette exposure duration	E-liquid[a]/e-vapour[b]/EVE[c] concentration	Nicotine content	Study/Ref
US	Lung	A549, H1650	Cell culture	N/R	E-liquid	21 hours, 48 hours	N/R	Source[d]: 15, 16, 18 mg/mL Final[e]: 2 μM (Standardised)	Schaal, Bora-Singhal [36]
US	Lung	A549	Cell culture	Menthol, Tobacco	E-liquid and EVE	3–8 days	E-liquid: 1% dilutions by volume EVE: 6 puffs/1 mL culture medium	Source: 48 mg/mL Final: N/R	Zahedi, Phandthong [37]
US	Breast	MDA-MB-231 LM2	Cell culture, animal model (Four-week-old female NSG mice)	N/R	*In vitro*: EVE *In vivo*: E-vapour	*In vitro*: 24 hours *In vivo*: 2 hours/day, 5 days/week	*In vitro*: 0–0.5% (0–0.4 ng/mL) *In vivo*: N/R	*In vitro*: Source: N/R Final: 80 ng/mL *In vivo*: Source: 24 mg/mL (diluted from 100 mg/mL stock) Final: N/R	Huynh, Huang [38]
US	Breast	EpRAS, 4T07	Cell culture, animal model (Female 5–7 weeks old BALB/c mouse strain)	N/R	*In vitro*: EVE *In vivo*: E-vapour	*In vitro:* 24 hours *In vivo:* 2 hours/day, 5 days/week, for 6 weeks to 7 weeks	*In vitro*: 0.5% *In vivo*: 70 mL/1 puff/min	*In vitro*: Source: 18 mg/mL Final: ~ 2 nM *In vivo*: Source: 24 mg/mL Final: N/R	Pham, Huynh [39]
US	OSCC	Ca9-22, Cal-27	Cell culture	Red Hot, Green Apple	E-liquid	24 hours	2%	Source: Presence/Absence of 6 mg nicotine Final: N/R	Tsai, Hirschi Budge [40]
US	OSCC	Ca9-22, Cal-27	Cell culture	Red Hot, Green Apple	EVE	6 hours	5–50%	Source: Presence/Absence of 6 mg nicotine Final: N/R	Robin, Trudeau [41]
Canada, Brazil	OSCC	Cal-27, HSC3, AT84	Cell culture	Flavoured, unflavoured	E-liquid	24–96 hours	10–0.0001% (v/v) (2 mg/mL, 200 μg/mL, 20 μg/mL, 2 μg/mL, 0.02 μg/mL)	Source: 20 mg/mL (claimed) but 0.73 ± 1 mg/mL (measured) Final: 0.1%, 1% (v/v)	de Lima, Macedo [42]

Abbreviations: OSCC – oral squamous cell carcinoma; NSG – NOD-SCID-Gamma (non-obese diabetic severe combined immunodeficiency gamma); BALB/c – Bagg albino (strain c); N/R – not reported; EVE – e-cigarette vapour extract

[a] E-liquid: the liquid formulation used in e-cigarette devices.
[b] E-vapour: the vapour or aerosol generated from heated e-liquid.
[c] EVE: an extract prepared by passing e-vapour through a liquid (e.g., culture medium) or by condensation.
[d] Source: nicotine concentration in the original commercial e-liquid or prepared e-liquid mixture prior to experimental exposure.
[e] Final: nicotine concentration in the immediate biological exposure environment (e.g., cell culture medium or blood) during the specific experimental conditions.

A549 is a widely used human alveolar basal epithelial cell line and a standard *in vitro* model for lung adenocarcinoma [43]. It harbours an activating KRAS mutation associated with tobacco smoking and has been linked to overexpression of metastasis-related proteins such as RAN, which regulates cell survival, invasion, and EMT [43]. Cal-27 is commonly used in OSCC research due to its reproducibility and invasive characteristics, making it suitable for studying apoptosis, drug response, and tumour progression. However, it has been reported to exhibit adenosquamous characteristics, which may limit its representativeness of all OSCC subtypes [44].

Table 4a. Summary of functional and phenotypic outcomes.

Cancer type	**Cancer cell line**	**Aspect**				**Study**
		Cell invasion/migration/motility	**Tumour growth/proliferation & area**	**Colonisation/localisation**	**Cell viability & apoptosis**	
Lung	A549, H1650	↑	Sphere formation ↑; Self-renewal of CSCs ↑	N/R	N/R	Schaal, Bora-Singhal [36]
Lung	A549	↑	N/R	N/R	N/R	Zahedi, Phandthong [37]
Breast	MDA-MB-231 LM2	↑	Tumour area ↑	Lung colonisation ↑	Apoptosis ↓	Huynh, Huang [38]
Breast	EpRAS, 4T07	↑	Tumour proliferation and area ↑	Lung nodules ↑	Apoptosis ↓	Pham, Huynh [39]
OSCC	Ca9-22, Cal-27	(Ca9-22/Red Hot) ↑; (Ca9-22/Green Apple) ↓; (Cal-27/Red Hot) ↓	N/R	N/R	N/R	Tsai, Hirschi Budge [40]
OSCC	Ca9-22, Cal-27	(Ca9-22/Red Hot with nicotine) ↑; (Ca9-22/Red Hot without nicotine) ↓; (Cal-27/Red Hot) ↓	N/R	N/R	N/R	Robin, Trudeau [41]
OSCC	Cal-27, HSC3, AT84	(Dose-dependent) ↑	Anchorage-independent spheroid growth ↑; Proliferation ↑	N/R	Viability (dose-dependent) ↓	de Lima, Macedo [42]

Abbreviations: OSCC – oral squamous cell carcinoma; ↑ – increased; ↓ – decreased; N/R – not reported; CSCs – cancer stem-like cells

Table 4b. Summary of molecular and immunological markers.

Cancer type	Cancer cell line	Aspect				Study
		EMT markers	**Inflammatory signalling & cytokines**	**RAGE**	**Myeloid Infiltration**	
Lung	A549, H1650	*VIM*, *FN1*, *ZEB1*, *ZEB2* ↑	N/R	N/R	N/R	Schaal, Bora-Singhal [36]
Lung	A549	E-cadherin ↓; VIM ↑; β-catenin translocated to nucleus	N/R	N/R	N/R	Zahedi, Phandthong [37]
Breast	MDA-MB-231 LM2	N/R	N/R	N/R	N/R	Huynh, Huang [38]
Breast	EpRAS, 4T07	N/R	CCL5, VEGF, CX3CL1, CXCL1, CXCL16 ↑	N/R	TAMs ↑	Pham, Huynh [39]
OSCC	Ca9-22, Cal-27	N/R	IL-1α ↑; IL-8, MMP-13 (variable)	↑	N/R	Tsai, Hirschi Budge [40]
OSCC	Ca9-22, Cal-27	N/R	TNF-α (in both cell lines with nicotine) ↑; ERK, JNK, NF-κB (condition-dependent); MMP-9, MMP-13 (variable)	N/R	N/R	Robin, Trudeau [41]
OSCC	Cal-27, HSC3, AT84	E-cadherin ↓; β-catenin ↑	N/R	N/R	N/R	de Lima, Macedo [42]

Abbreviations: OSCC – oral squamous cell carcinoma; EMT – epithelial-to-mesenchymal transition; VIM – vimentin; FN1 – fibronectin 1; ZEB1/2 – zinc finger E-box binding homeobox 1/2; ↑ – increased; ↓ – decreased; N/R – not reported; CCL5 – C-C motif chemokine ligand 5; VEGF – vascular endothelial growth factor; CX3CL1 – C-X3-C motif chemokine ligand 1; CXCL1/16 – C-X-C motif chemokine ligand 1/16; IL-1α/-8 – interleukin-1 alpha/-8; MMP – matrix metalloproteinase; TNF-α – tumour necrosis factor alpha; ERK – extracellular signal-regulated kinase; JNK – c-Jun N-terminal kinase; NF-κB – nuclear factor kappa-light-chain-enhancer of activated B cells; RAGE – receptor for advanced glycation end-products; TAMs – tumour-associated macrophages

Table 5. Summary of the selected articles.

Cancer type	Cancer cell line	Main findings	Limitations	Study
Lung	A549, H1650	Nicotine and e-cigarette extract enhanced CSC self-renewal and induced EMT-associated phenotypes compared to control groups.	Lacks *in vivo* validation; tested single nicotine concentration; relies on e-cigarette extracts; lacks cigarette smoke control.	Schaal, Bora-Singhal [36]
Lung	A549	E-liquids and aerosols induced EMT-like morphological changes (decreased cobblestone cells, increased mesenchymal-like and multinucleated cells, loss of cell adhesion), increased cell motility, upregulated mesenchymal markers (MMP-9, VIM, N-cadherin), reduced E-cadherin, and promoted β-catenin/P120 nuclear translocation.	Low aerosol dose (6 total-puff-equivalents) is significantly below average daily human exposure (~175 puffs); lacks *in vivo* validation; lacks cigarette smoke control; continuous 2D monolayer exposure fails to mimic intermittent human inhalation.	Zahedi, Phandthong [37]
Breast	MDA-MB-231 LM2	E-cigarette exposure increased tumour cell motility *in vitro* and enhanced lung colonisation *in vivo*, with reduced apoptosis but no change in proliferation.	Tail-vein injection model only addresses late-stage colonisation, bypassing initial breast dissemination; immunodeficient (NSG) mice exclude critical immune-mediated mechanisms in humans; lacks cigarette smoke control.	Huynh, Huang [38]
Breast	EpRAS, 4T07	E-cigarette exposure promoted tumour growth and metastasis, increased monocyte or macrophage recruitment through the CCL5/CCR5 axis; enhanced cancer cell migration and monocyte adhesion, and reduced apoptosis.	Murine cell lines in syngeneic mice may not fully recapitulate human gene regulatory networks; specific active components in e-cigarette remain unidentified; lacks cigarette smoke control.	Pham, Huynh [39]
OSCC	Ca9-22, Cal-27	E-cigarette flavouring and nicotine differentially regulated cell invasion and inflammatory marker expression in a flavour-, nicotine-, and cell line-dependent manner (IL-1α, IL-8, MMP-13), with consistent RAGE upregulation.	Lacks *in vivo* validation; relies on direct e-liquid exposure; only male-derived cell lines (gender bias).	Tsai, Hirschi Budge [40]
OSCC	Ca9-22, Cal-27	EVE induced flavour-, nicotine-, and cell line-dependent changes in invasion and inflammatory signalling (TNF-α, NF-κB, ERK, JNK).	Inconsistent findings with Tsai, Hirschi Budge [40] for identical cell lines; lacks *in vivo* validation; relies on extracts; short exposure duration (6 hours); lacks cigarette smoke control.	Robin, Trudeau [41]
OSCC	Cal-27, HSC3, AT84	E-liquid exposure induced EMT-associated changes (downregulation of E-cadherin, upregulation of β-catenin; q-RT-PCR further revealed the overexpression of *VIM*), enhanced migration and invasion, altered cell morphology, and reduced viability, with OSCC cells showing greater resistance than normal oral epithelial cells.	Lacks *in vivo* validation; uses direct e-liquid rather than aerosol; concentrations (0.1–1%) may exceed physiological relevance; lacks cigarette smoke control.	de Lima, Macedo [42]

Abbreviations: OSCC – oral squamous cell carcinoma; CSC – cancer stem-like cell; EMT – epithelial-to-mesenchymal transition; MMP – matrix metalloproteinase; VIM – vimentin; P120 – p120-catenin; CCL5 – C-C motif chemokine ligand 5; CCR5 – C-C chemokine receptor type 5; IL-1α/-8 – interleukin-1 alpha/-8; RAGE – receptor for advanced glycation end-products; EVE – e-cigarette vapour extract; TNF-α – tumour necrosis factor alpha; NF-κB – nuclear factor kappa-light-chain-enhancer of activated B cells; ERK – extracellular signal-regulated kinase; JNK – c-Jun N-terminal kinase; q-RT-PCR – quantitative reverse transcription polymerase chain reaction; NSG – NOD-SCID-Gamma (non-obese diabetic severe combined immunodeficiency gamma)

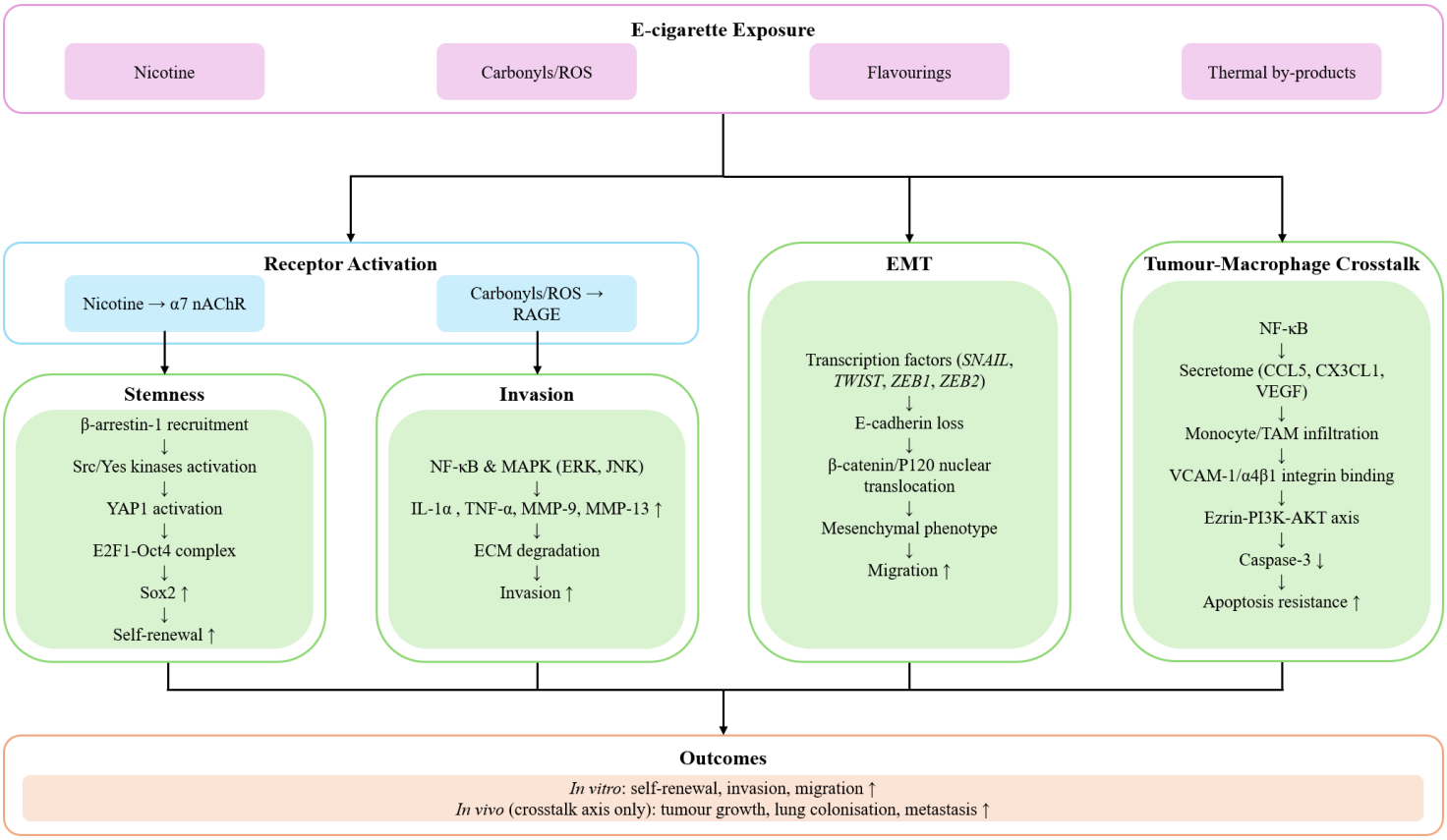


Figure 2. Proposed molecular mechanisms of vaping-induced metastasis synthesised from the seven studies included. E-cigarette constituents activate two receptors with direct experimental support: nicotine via α7 nAChR, and carbonyls/ROS via RAGE. Stemness: Nicotine-mediated α7 nAChR activation recruits β-arrestin-1 and Src/Yes kinases to trigger a YAP1-E2F1-Oct4 complex, inducing Sox2-driven self-renewal [36]. EMT: Transcriptional reprogramming (*SNAIL*, *TWIST*, *ZEB1/2*) promotes the physical dismantling of cell junctions; E-cadherin loss and adherens junction breakdown precede the nuclear translocation of β-catenin and P120 (demonstrated in lung cancer cells [37]; OSCC models showed upregulation of β-catenin protein and mRNA [42]). Invasion: RAGE-driven NF-κB and MAPK (ERK/JNK) signalling orchestrates context-dependent pro-inflammatory surges (e.g., IL-1α) and differential MMP-9/13 production to drive ECM degradation [40,41]. Tumor-Macrophage Crosstalk: NF-κB-driven secretion of an expanded secretome (CCL5, CX3CL1, VEGF) recruits monocytes. Physical binding between tumour-bound VCAM-1 and macrophage α4β1 integrin may recruit the adaptor Ezrin to activate PI3K-AKT signalling, resulting in a reduction in cleaved Caspase-3 and enhanced apoptotic resistance [38,39]. *In vitro* endpoints (self-renewal, migration, invasion) are identified across all axes; *in vivo* systemic outcomes (tumour growth, lung colonisation) were directly measured in breast cancer models [38,39].

Abbreviations: ROS – reactive oxygen species; α7 nAChR – α7 nicotinic acetylcholine receptor; RAGE – receptor for advanced glycation end-products; Src – proto-oncogene tyrosine-protein kinase Src; Yes – YES proto-oncogene 1; YAP1 – Yes-associated protein 1; E2F1 – E2F transcription factor 1; Oct4 – octamer-binding transcription factor 4; Sox2 – SRY-box transcription factor 2; NF-κB – nuclear factor kappa-light-chain-enhancer of activated B cells; MAPK – mitogen-activated protein kinase; ERK – extracellular signal-regulated kinase; JNK – c-Jun N-terminal kinase; IL-1α – interleukin-1 alpha; TNF-α – tumour necrosis factor alpha; MMP – matrix metalloproteinase; ECM – extracellular matrix; EMT – epithelial-to-mesenchymal transition; SNAIL – Snail family transcriptional repressor 1; TWIST – Twist family basic helix-loop-helix transcription factor 1; ZEB1/2 – zinc finger E-box binding homeobox 1/2; P120 – p120-catenin; CCL5 – C-C motif chemokine ligand 5; CX3CL1 – C-X3-C motif chemokine ligand 1; VEGF – vascular endothelial growth factor; TAM – tumour-associated macrophage; VCAM-1 – vascular cell adhesion molecule 1; α4β1 – alpha-4 beta-1 integrin; PI3K – phosphoinositide 3-kinase; AKT – protein kinase B; OSCC – oral squamous cell carcinoma

3.3. Study Reliability Assessment

The reporting quality, methodological quality, and relevance of all seven included studies were assessed using the SciRAP tool [45]. Two of the seven studies included *in vivo* models in addition to *in vitro* methods. For the *in vitro* studies, reporting quality scores ranged from 63.64% to 75% (mean 69.81%), while methodological quality scores ranged from 46.67% to 70% (mean 58.1%). For the *in vivo* studies, the reporting quality was low at 43.10%, whereas methodological quality scores were 62.5% and 59.38%, respectively. Overall, all studies were categorised as "not reliable", either due to methodological quality scores below 65% or because one or more key criteria were not fulfilled (Table 6). Accordingly, all findings presented in this review should be regarded as hypothesis-generating only and interpreted with considerable caution. A post-hoc sensitivity consideration was applied: if a more conservative threshold of >60% methodological quality had been used, one study (de Lima *et al.*, 46.67%) would have been excluded from the evidence synthesis. However, the remaining six studies shared consistent directional signals, particularly increased migration and EMT-associated changes. This suggests the overall pattern of results is not solely driven by the lowest-quality outlier, although the limited number of studies and generally uniformly low methodological quality still constrain confidence in the evidence base.

The *in vitro* studies examined the effects of e-cigarette exposure on cancer metastasis primarily through (i) functional assays (e.g., invasion, migration and wound healing assays), (ii) molecular assays (e.g., immunocytochemistry, immunofluorescence, Western blot, and quantitative reverse-transcription polymerase chain reaction [qRT-PCR] for EMT and immunological markers), and (iii) morphological observations consistent with EMT. While the test systems were well described (cancer type and cancer cell lines), and essential treatment information—including e-cigarette exposure type, duration, and concentration—was specified (Table 3), reporting of e-cigarette products was incomplete in most studies, with insufficient details on source, brand, flavour, device model, and chemical composition. Although all studies reported at least three independent experimental repeats, key experimental design elements, such as controls, were inconsistently reported. One study did not report an untreated control [42]. Only two studies included positive controls: nicotine in one study [36] and cigarette smoke extract in another, although the latter was not used consistently across all assays [40].

Both *in vivo* studies confirmed the inclusion of negative control groups (air-exposed mice) alongside the experimental groups (e-cigarette-exposed mice) [38,39]. They investigated breast cancer metastasis-related endpoints, including tumour progression and metastatic lung

colonisation, supported by immunohistological analyses. Monocyte/tumour-associated macrophage infiltration was assessed in one study [39]. The animal model was clearly specified (species, strain, age, sex), and essential dosing information—including administered concentrations, route of administration, and treatment schedule—was generally well reported (Table 3). Both research groups used the InExpose System (Scientific Respiratory Equipment Inc., Canada) to generate and deliver the e-cigarette aerosols, exposing mice via inhalation in a manner relevant to human exposure. Neither study specified whether the e-liquid was commercially sourced or laboratory-prepared. Only nicotine, propylene glycol, and vegetable glycerine were reported as constituents, without details on the manufacturer and lot number. Furthermore, the housing conditions were poorly described: neither study reported cage materials, bedding, feed, water source, or enrichment, while environmental parameters such as temperature and humidity were only partially described. Allocation methods for animals to treatment groups were not described, and numbers of animals per group were inconsistently reported, thereby reducing reproducibility and increasing the risk of bias in the *in vivo* findings. Funding sources were not disclosed in two studies [38,39], while all included studies declared no competing interests.

Table 6. SciRAP evaluation of reporting and methodological quality, key criteria, and reliability categorisation of included studies: *in vitro* studies (n = 7) and in vivo studies (n = 2).

Study	SciRAP score (%)		Key criteria[a]		Reliability category
	Reporting quality	Methodological quality	Not fulfilled	Not reported	
***In vitro* studies (n = 7)**					
Schaal, Bora-Singhal [36]	63.64	53.33	0	0	Not reliable[b]
Zahedi, Phandthong [37]	70.45	56.67	1	0	Not reliable
Huynh, Huang [38]	72.73	70.00	1	0	Not reliable
Pham, Huynh [39]	75.00	60.00	1	0	Not reliable
Tsai, Hirschi Budge [40]	68.18	63.33	0	0	Not reliable
Robin, Trudeau [41]	68.18	56.67	1	0	Not reliable
de Lima, Macedo [42]	70.45	46.67	1	1	Not reliable
***In vivo* studies (n = 2)**					
Huynh, Huang [38]	43.10	62.50	0	0	Not reliable
Pham, Huynh [39]	43.10	59.38	0	0	Not reliable

[a] Key criteria are those judged as specifically critical for the reliability of the data in a certain case and are determined *a priori*.
[b] Studies were categorised as "Not reliable" if the SciRAP methodological quality score was < 65, if one or more key criteria were not fulfilled, or if there were major deficiencies in the non-key criteria that affected study reliability

3.4. Cell Invasion, Migration, and Motility

In A549 lung cancer cells, the population of motile cells increased after three to four days of exposure to e-liquid (menthol: $p < 0.01$; tobacco: $p < 0.05$) and aerosol (menthol: $p < 0.05$; tobacco: $p = 0.062$). Treated cells exhibited more "directed" and persistent migratory movements, in contrast to the predominantly random Brownian motion as seen in the control group [37], indicating a shift toward enhanced migratory potential that is commonly associated with pro-metastatic cellular phenotypes, including those seen during EMT. In support of these results, wound healing assays showed enhanced migration of A549 cells following e-cigarette treatment [36,37]. Likewise, a 3-fold increase ($p < 0.0001$) in the number of migrated MDA-MB-231 LM2 human breast cancer cells was reported after e-cigarette exposure [38]. The increase in cell migration was also demonstrated in EpRAS ($p < 0.0001$) and 4T07 breast cancer cells ($p < 0.01$) exposed to 0.5% e-cigarette when compared to the negative control [39].

For OSCC cell lines, treatment with 0.1% or 1% nicotine-containing e-liquid resulted in a dose-dependent increase in migration and invasion abilities in Cal-27, HSC3, and AT84 cells, with the strongest effect at 1% ($p < 0.001$) [42]. However, the impact of e-cigarette flavourings and nicotine presence varied across studies when applied to different OSCC cell lines (Table 7).

In Cal-27 cells, Red Hot exposure—either as e-liquid or e-cigarette vapour extract (EVE)—consistently and significantly reduced invasion relative to control, regardless of nicotine presence [40,41]. Tsai, Hirschi Budge [40] reported a decrease following Red Hot e-liquid exposure (1.44-fold without nicotine; 1.3-fold with nicotine), while Robin, Trudeau [41] similarly observed reduced invasion with Red Hot EVE (1.2-fold decrease without nicotine; 2.2-fold decrease with nicotine). However, in the e-liquid condition, nicotine partially attenuated this inhibitory effect by increasing invasion relative to Red Hot alone ($p < 0.002$), although values remained below control levels. Green Apple showed no significant effect on Cal-27 invasion in either study, regardless of nicotine exposure [40,41].

In contrast, Ca9-22 cells displayed opposing and context-dependent responses [40,41]. For Red Hot e-liquid, Tsai, Hirschi Budge [40] reported a significant increase in invasion (3.6-fold without nicotine, which was reduced but remained elevated compared with control upon nicotine exposure (2.3-fold increase). Conversely, Robin, Trudeau [41] observed that nicotine-free Red Hot EVE significantly reduced invasion (1.44-fold decrease), whereas nicotine exposure reversed this effect, resulting in invasion levels exceeding control ($p < 0.0001$). For Green Apple e-liquid, Tsai, Hirschi Budge [40] observed a significant reduction

in invasion both without nicotine (2.7-fold decrease) and with nicotine (1.7-fold decrease), although nicotine partially attenuated the inhibitory effect. However, Green Apple EVE showed no significant effect in Ca9-22 in Robin, Trudeau [41]. The contrasting results between these two studies using identical cell lines and flavours suggest that the exposure method (e-liquid versus EVE) may be an important determinant of biological response.

Overall, e-cigarette flavourings induced cell line-specific effects on invasion that limit generalisability. Cal-27 generally showed reduced invasion following Red Hot exposure, whereas Ca9-22 exhibited variable responses depending on flavouring, nicotine presence, and exposure system. Direct inconsistencies were also observed between studies under comparable conditions, particularly in Ca9-22 cells.

Table 7. Summary of the effects of nicotine and e-cigarette flavours on OSCC cell invasion outcomes relative to control.

OSCC cell line	**Presence of 6 mg nicotine**	**Effect on cell invasion**		**Study**
		E-cigarette flavour		
		Red Hot	**Green Apple**	
Cal-27	Yes	Decreased	No significant effect	Tsai, Hirschi Budge [40]
		Decreased	No significant effect	Robin, Trudeau [41]
	No	Decreased	No significant effect	Tsai, Hirschi Budge [40]
		Decreased	No significant effect	Robin, Trudeau [41]
Ca9-22	Yes	Increased	Decreased	Tsai, Hirschi Budge [40]
		Increased	No significant effect	Robin, Trudeau [41]
	No	Increased	Decreased	Tsai, Hirschi Budge [40]
		Decreased	No significant effect	Robin, Trudeau [41]

Abbreviations: OSCC – oral squamous cell carcinoma

3.5. Cell Morphology and Epithelial-to-Mesenchymal Transition (EMT)

Under normal conditions, A549 lung cancer cells typically grow in tightly adherent "cobblestone" monolayers, with a few large, elongated cells interspersed among them [37]. However, after three to four days of exposure to e-liquid and aerosol treatment, a significant decrease in the cobblestone cells was observed, accompanied by a corresponding increase in

single elongated mesenchymal-like cells and enlarged, often multinucleated cells [37]. In OSCC cell lines (Cal-27, HSC3, and AT84), e-liquid exposure at a non-lethal concentration of 0.1% (v/v) or 0.2 mg/mL for 72 hours also induced significant alterations in cell morphology compared to the control, resulting in cell detachment and a loss of confluency [42]. These changes are consistent with phenotypic features associated with EMT.

At the molecular level, multiple studies reported increased expression of mesenchymal markers following e-cigarette exposure. In A549 cells, immunocytochemistry demonstrated upregulation of matrix metalloproteinases (MMP)-9, vimentin (VIM), and N-cadherin after four to eight days of exposure to menthol- and tobacco- flavoured e-liquid and aerosol treatment [37]. In line with these findings, another study reported that treatment of A549 cells with 2 μM e-cigarette extracts induced expression of mesenchymal markers, including *VIM* and fibronectin (*FN1*), as well as EMT-associated transcription factors *ZEB1* and *ZEB2*, as determined by RT-PCR analysis [36].

In OSCC models, e-liquid exposure similarly increased mesenchymal marker expression, with elevated VIM levels observed in Cal-27 and HSC3 cells at both protein (western blot) and transcript (qRT-PCR) levels [42]. Upregulation of β-catenin (*CTNNB1*) was also reported, further supporting a shift toward a mesenchymal-like phenotype [42].

Conversely, downregulation of the epithelial marker E-cadherin was consistently reported across all three studies following e-cigarette treatment [36,37,42]. In A549 cells, E-cadherin internalisation and perinuclear accumulation indicated loss of cell surface localisation and reduced cell-to-cell adhesion [37]. This was accompanied by the translocation of β-catenin and P120 catenin from the plasma membrane to the cytoplasm and nucleus, further contributing to the destabilisation of cell junctions [37].

Notably, direct assessment of canonical EMT transcription factors was limited, with *ZEB1* and *ZEB2* upregulation reported in one lung cancer study [36]. In the OSCC study by de Lima, Macedo [42], *SNAIL* and *TWIST* were discussed in the context of general EMT biology rather than experimentally measured. Although transcription factor evaluation was restricted, these studies were supported by complementary functional and mechanistic evidence, including migration and invasion assays [36-42], spheroid formation [36,42], as well as pathway-level analyses involving EMT-associated signalling cascades [36,37,39].

3.6. Tumour Area, Growth, Proliferation, Colonisation or Localisation

Among the OSCC studies, only one measured tumour growth and proliferation-related endpoints [42]. OSCC cell lines (Cal-27, HSC3, and AT84) demonstrated differential susceptibility to e-liquid exposure compared with normal oral epithelial cells (HMK and NOE). At low e-liquid concentrations (<1%), OSCC cell lines showed a slight increase in proliferation compared to untreated control cells. In contrast, as e-liquid concentration increased, all cell types exhibited a stepwise decrease in colony counts and survival with more than a twofold reduction at 1% v/v concentration. However, OSCC cells showed comparatively greater resistance to e-liquid-induced cytotoxicity than normal cells [42].

In vitro studies in OSCC and lung cancer models demonstrated increased sphere-forming capacity following e-cigarette exposure. Specifically, de Lima, Macedo [42] reported increased spheroid size and number in OSCC cell lines following e-liquid exposure, indicating enhanced anchorage-independent growth. Similarly, Schaal, Bora-Singhal [36] observed increased sphere formation in lung adenocarcinoma side-population cell lines (A549 and H1650), suggesting that e-cigarette exposure enhanced self-renewal and tumour-initiating potential through the activation of the α7 nAChR-YAP1-Sox2 axis.

In vivo breast cancer models demonstrated that e-cigarette exposure promotes lung metastatic colonisation, although outcomes vary depending on experimental design and the metastatic stage assessed. Pham, Huynh [39], using an orthotopic mammary fat pad injection model in immunocompetent BALB/c mice, reported a significant increase in primary tumour growth, reflected by a reduced doubling time (from 2.27 to 1.24 weeks) and an increase in primary tumour area (from 35.46% to 69.24%), associated with elevated Ki67 expression, suggesting enhanced tumour proliferative activity during metastatic progression. In contrast, Huynh, Huang [38], using a tail-vein injection model in immunodeficient NSG mice, observed no significant changes in gross lung surface nodules between e-cigarette and control groups; however, morphometric analysis revealed a significant increase in lung tumour area (p = 0.0036), indicating enhanced microscopic colonisation. Notably, this occurred without changes in Ki67 expression (p = 0.7953), while reduced apoptosis (cleaved caspase-3) suggests that increased metastatic burden in this model was driven by enhanced tumour cell survival and colonisation rather than proliferative activity.

Taken together, these findings are complementary rather than directly comparable, as Pham, Huynh [39] capture early-to-late metastatic progression, whereas Huynh, Huang [38] isolate late-stage colonisation and tumour cell survival in the absence of early dissemination and immune modulation.

3.7. Cell Viability and Apoptosis

E-cigarette exposure showed limited impact on short-term cell viability *in vitro*, although cancer cells exhibited relatively greater resistance to cytotoxic effects compared to normal cells under certain conditions. *In vitro*, Huynh, Huang [38] reported no significant alteration in the cell number of MDA-MB-231 LM2 human breast cancer cells after 24 hours of exposure to e-cigarette extracts ranging from 0-0.5% (0-0.4 ng/ml). Similarly, de Lima, Macedo [42] observed minimal effects on OSCC cell viability at lower concentrations (1% v/v e-liquid solution) compared to untreated controls, while normal oral epithelium cells exhibited decreased viability ($p < 0.05$). At higher concentrations (10% v/v e-liquid solution), both OSCC and normal cell lines experienced decreased viability ($p < 0.05$), indicating dose-dependent cytotoxicity rather than selective tumour effects [42].

Conversely, *in vivo* breast cancer models reported decreased apoptosis following e-cigarette exposure [38,39]. Huynh, Huang [38] observed a 5-fold lower number of apoptotic breast cancer cells in lung sections of e-cigarette-exposed mice compared to control ($p = 0.0007$), while Pham, Huynh [39] similarly demonstrated reduced apoptosis in both primary and metastatic tumour cells based on cleaved caspase-3 staining. Collectively, these findings suggest that e-cigarette exposure may promote tumour progression by enhancing tumour cell survival through apoptosis suppression in metastatic contexts.

3.8. Immunological Changes

Two studies investigating OSCC cell lines (Ca9-22 and Cal-27) demonstrated complex and context-dependent immunological responses to e-cigarette exposure, influenced by flavouring and nicotine presence [40,41] (Table 8). Among commonly examined markers, tumour necrosis factor (TNF)-α expression was increased in both OSCC cell lines in the presence of nicotine in one study [41], but was below detection in another study [40]. Similarly, MMP-9 expression was either reduced ($p < 0.002$) or unchanged depending on the cell line and flavouring [40]. In contrast, MMP-13 expression was generally reduced or unchanged by flavouring alone but restored or increased upon nicotine addition in certain conditions [40,41].

Divergent responses were also observed in cytokine analysis. In the only OSCC study that examined these markers [40], interleukin (IL)-1α was generally upregulated across both cell lines and flavour conditions, while IL-8 exhibited highly variable responses, including opposite trends between Ca9-22 and Cal-27 cells upon nicotine exposure. In the same study, receptor for advanced glycation end-products (RAGE) expression was consistently increased

across both cell lines and flavour conditions, with further increase when nicotine was added [40].

Additionally, Robin, Trudeau [41] evaluated downstream inflammatory signalling pathways, including nuclear factor kappa B (NF-κB), extracellular signal-regulated kinase (ERK), and N-terminal kinase (JNK). Overall, these pathways exhibited strong cell line- and condition-dependent variability, with no significant differences observed in the absence of nicotine. In contrast, nicotine-containing exposures showed distinct responses between cell lines. In Ca9-22 cells, Green Apple with nicotine led to significant increases in NF-κB, ERK, and JNK, whereas Cal-27 cells showed consistent decreases under the same conditions. Red Hot with nicotine demonstrated a different pattern, characterised by reduced ERK and enhanced JNK activation across both cell lines, while NF-κB responses remained variable [41]. As a follow-up study, these findings further support that e-cigarette-induced inflammatory signalling pathway activation is dependent on cellular context, flavouring and nicotine presence.

In breast cancer models, Pham, Huynh [39] demonstrated that e-cigarette exposure promoted a pro-inflammatory and recruitment tumour microenvironment (TME) through chemokine signalling modulation. In *in vitro*, they observed that e-cigarette exposure had minimal effect on CCL5 mRNA expression but significantly upregulated its receptors, CCR1 and CCR5. At the proteomic level, e-cigarette exposure contributed to the upregulation of CCL5 and secretion of other cytokines involved in immune cell recruitment and metastasis, such as CX3CL1, CXCL1, CXCL16, endostatin, and VEGF from the tumour cells. The study also highlighted that e-cigarette exposure enhanced the direct binding of monocytes to both breast cancer cells, mediated by the VCAM-1/α4β1 integrin axis. Interestingly, an inverse correlation was observed between the number of EpRAS-bound monocytes and the percentage of EpRAS cells undergoing TRAIL-induced apoptosis under e-cigarette exposure [39].

In the *in vivo* analysis using animal models, Pham, Huynh [39] reported that e-cigarette exposure modulated the TME in mice by promoting monocyte and macrophage infiltration via the CCR5/CCL5 axis. Flow cytometry analysis of the EpRAS tumour model identified a significant increase in circulating monocytes in e-cigarette-exposed mice ($p < 0.05$). These monocytes showed upregulation of CCR5 ($p = 0.05$) and CX3CR1 ($p = 0.08$). Notably, e-cigarette exposure led to an increase in F4/80-labelled resident monocytes at primary ($p < 0.05$) and metastatic sites. Similarly, in the 4T07-derived lung metastasis model, a significant

increase in F4/80-labelled macrophage infiltration of over 400% ($p = 0.023$) was observed in e-cigarette-exposed mice compared to the air-exposure group [39].

Table 8. Summary of immunological changes between Ca9-22 and Cal-27 cell lines in two OSCC studies.

Markers	Overall Findings	Evidence
MMP-9	Variable responses depending on cell line and flavour	Robin, Trudeau [41]
MMP-13	Nicotine generally restored or increased MMP-13 expression following flavour exposure	Tsai, Hirschi Budge [40], Robin, Trudeau [41]
TNF- α	Increased with nicotine exposure in both cell lines	Robin, Trudeau [41]
IL-1α	Generally upregulated across cell lines and flavour conditions	Tsai, Hirschi Budge [40]
IL-8	Variable responses depending on cell line and nicotine exposure	Tsai, Hirschi Budge [40]
RAGE	Consistently increased across both cell lines and flavour conditions, with further enhancement by nicotine	Tsai, Hirschi Budge [40]
NF-κB	Cell line-, flavour-, and nicotine-dependent activation	Robin, Trudeau [41]
ERK	Cell line-, flavour-, and nicotine-dependent activation	Robin, Trudeau [41]
JNK	Cell line-, flavour-, and nicotine-dependent activation	Robin, Trudeau [41]

Abbreviations: MMP – matrix metalloproteinase; TNF- α – tumour necrosis factor alpha; IL-1α/-8 – interleukin-1 alpha/-8; RAGE – receptor for advanced glycation end-products; NF-κB – nuclear factor kappa-light-chain-enhancer of activated B cells; ERK – extracellular signal-regulated kinase; JNK – c-Jun N-terminal kinase

Detailed condition-specific responses by cell line, flavour, nicotine status, and exposure conditions are provided in Supplementary Material: Table

4. Discussion

4.1. Summary of Evidence

In this systematic review, we focused on the impact of e-cigarette exposure on cancer metastasis and identified seven studies in total: two on lung cancer, two on breast cancer, and three on OSCC. Notably, only the breast cancer studies involved animal models. Within the specific experimental models examined, the collective findings suggest that e-cigarette exposure may promote cancer progression and metastasis under the tested conditions, through mechanisms such as enhanced cell invasion and migration, EMT induction, increased tumour proliferation and growth, reduced apoptosis, and immunomodulation (Figure 2). The reported effects also appeared to vary depending on the cancer cell line, e-cigarette flavouring, and nicotine presence (Table 7, 8).

However, based on study quality assessment with the SciRAP tool, all included studies were categorised as “not reliable” due to methodological quality scores below 65 or because one or more key criteria were not fulfilled (Table 6). Taken together, the collective evidence from these studies should be considered preliminary. This is due to several limitations, including the small number of eligible studies (n = 7), lack of standardisation in

aerosol generation for *in vivo* studies, use of *in vitro* exposure methods that do not accurately reflect human use (e.g., e-liquid or captured aerosol extracts), potential for over- or underestimation of effects due to non-physiological exposure concentrations and durations, absence of appropriate controls, limited reporting of chemical composition and product diversity, as well as reliance on cell culture and animal models that inadequately reflect human physiology. Consequently, the results from these models cannot be reliably translated to human health risks and should therefore be interpreted with caution.

4.2. Potential Mechanisms Suggested by the Included Preclinical Studies

The included studies in this systematic review propose several biologically plausible mechanisms through which vaping may promote cancer metastasis. One reported observation is that e-cigarette exposure can induce immunological changes, including the upregulation of RAGE and differential expression of MMPs [40], as well as increased TNF-α [41] (Table 8). Notably, Tsai, Hirschi Budge [40] found that RAGE upregulation was consistently increased across both OSCC cell lines (Ca9-22 and Cal-27) and both flavour conditions (Red Hot and Green Apple), with further enhancement when nicotine was added, suggesting RAGE may be a central mediator of e-cigarette-induced effects that warrants further mechanistic investigation. RAGE plays a key role in various immune and inflammatory responses, where its activation and interaction with ligands stimulate multiple signalling pathways, including MMPs, mitogen-activated protein kinases (MAPKs), and NF-κB [46,47]. These events promote the secretion of proinflammatory cytokines such as IL-6, IL-8 and TNF-α, and contribute to tumour proliferation, angiogenesis, apoptosis evasion, EMT induction, and metastasis [46,48-51].

EMT is a key criterion for successful metastasis, enabling tumour cells to attain invasive and stem cell-like characteristics through transdifferentiation [52,53]. EMT activation downregulates E-cadherin, which mediates cell-cell adherence, and upregulates VIM, which facilitates migration and invasion, causing tumour epithelial cells to lose their apical-basal polarity and cell-cell junctions to become mesenchymal cells, gaining invasion and migration capabilities [52]. Herein, e-cigarette exposure increased cell invasion and migration in lung, breast, and OSCC cell lines, with EMT markers and morphological changes observed in lung and OSCC cells [36-42]. Additionally, tumour cells undergoing chronic inflammation and EMT also acquire the ability to evade apoptosis [49,54]. In this review, a reduction in apoptosis was also observed in breast cancer cells, supporting the primary tumour progression and distant metastasis development *in vivo* [38,39].

4.3. Methodological Limitations

Despite suggesting potential links between vaping and cancer metastasis, the current evidence base is constrained by significant methodological weaknesses that limit its translational relevance. One of the concerns is the variability in exposure methods. The included studies employed three common testing tiers for modelling e-cigarette exposure: direct e-liquid exposure, exposure to captured aerosol extracts, and direct aerosol exposure [55]. Among the seven included studies, three used e-liquid [36,40,42], one used captured aerosol extracts [41], one used both e-liquid and captured aerosol extracts [37], and two used captured aerosol extracts and direct aerosol exposure (for *in vivo* studies) [38,39] (Table 3).

Both *in vivo* studies exposed mice to e-cigarette aerosols via inhalation in a manner relevant to human exposure using the InExpose System (Scientific Respiratory Equipment Inc., Canada) [38,39]. Pham, Huynh [39] specified that mice were exposed via whole-body exposure, while Huynh, Huang [38] did not specify the exposure mode. These details are critical because they can influence both the scientific interpretation and ethical relevance of the findings. Nose-only exposure allows precise delivery to the respiratory system but involves restraint that may induce stress and confound outcomes, whereas whole-body exposure is less stressful and aligns better with animal welfare guidelines, though it can introduce variability in the actual dose as the unrestrained animals are exposed via their entire body surface, including potential dermal and oral absorption [56]. While both studies measured urine cotinine to confirm nicotine uptake, the lack of aerosol concentration data limits accurate estimation of the inhaled dose and hampers comparison with human exposure levels. Continuous monitoring of the chamber atmosphere is therefore important to ensure each animal receives a defined exposure and to mitigate variability, as it provides a real-time concentration profile that can be used to determine the stability of gas generation and delivery, or to provide a warning if there is a problem in the generation and/or delivery system that affects the exposure conditions [56].

In addition to these *in vivo* limitations, the *in vitro* studies also present challenges in replicating human exposure accurately. None of the included studies employed direct aerosol exposure; instead, they used e-liquids or captured aerosol extracts, which do not mimic the aerosol actually inhaled by human users, to investigate the effects of e-cigarette exposure on cancer metastasis. While each approach has its practical advantages and drawbacks, whole aerosol testing most closely reflects human exposure without solvent-induced selection bias and captures potential thermal by-products [55,57]. Although technically more demanding and lower in throughput [55], this method provides the most biologically relevant data for

evaluating cancer metastasis and should be prioritised where feasible, especially to enhance the translational value of experimental findings.

In contrast, e-liquid and captured aerosol extracts are easier to produce and assess *in vitro* but have significant methodological limitations. The absence of heating the e-liquid prevents assessment of the thermal by-products that are generated during vaporisation and may impact cancer metastasis. Captured aerosol extracts may fail to fully capture aerosolised chemicals and therefore cannot fully replicate the exposure conditions encountered by human users [55]. Several methods are available to generate the captured extracts [58]. The four studies using captured extracts employed either bubble-through [38,39,41] or condensation methods [37], with cell culture medium as the solvent. Such water-based protic solvents tend to capture predominantly water-soluble constituents, while other potentially harmful, highly lipophilic or volatile molecules may remain uncollected if present [58], resulting in incomplete exposure profiles that weaken the interpretability of cancer metastasis outcomes.

In terms of the exposure concentrations (Table 3), exposing cells to e-liquid concentrations above 1% exceeds physiological relevance [59-61], potentially inducing artificial cytotoxicity or stress responses that do not reflect human exposure conditions, overestimating the impact of e-cigarettes on cancer cells, and compromising the reliability and interpretability of the results. However, diluting them below 1% may limit assay sensitivity to detect biologically meaningful responses [55]. Therefore, for future studies involving e-liquids or captured aerosol extracts, adopting a dose-response design that spans concentrations below and above 1% would help identify biologically relevant thresholds. Additionally, continuous exposures lasting 24 hours or longer do not accurately reflect human use practices, further limiting translational relevance. Consistent with these concerns, Zahedi, Phandthong [37] reported that aerosol exposure in their A549 model was lower than estimated human e-cigarette exposure (6 total-puff-equivalents versus approximately 175 puffs per day), highlighting variability in exposure scaling and complicating direct translation of in vitro findings to real-world use.

As a result, findings from extract- or e-liquid-based exposures carry a higher risk of underestimating or misrepresenting the true effects of e-cigarette aerosol on cancer metastasis, and therefore should be interpreted with caution and given less evidential weight. To address these limitations and improve robustness, Bishop, Miazzi [55] proposed a multi-assay and multi-matrix approach, in which diverse testing matrices (e-liquids, captured aerosol extracts, and whole aerosols) are combined with multiple biological endpoints. This strategy enables a more comprehensive evaluation of potential risks and mechanisms, better

captures complex interactions and thermal breakdown products, and improves the representativeness of experimental models for real-world e-cigarette exposures.

A further methodological consideration relates to the types of endpoints used to assess cancer metastasis. All included *in vitro* studies incorporated at least one functional assay—such as invasion, migration, or wound healing assays—to directly measure the metastatic potential of tumour cells under e-cigarette exposure. Many studies also employed molecular assays, including immunocytochemistry, immunofluorescence, Western blot, and qRT-PCR for EMT- or immunology-related markers, as additional validation. While these molecular endpoints can provide mechanistic insights, some techniques—particularly qRT-PCR—may not be the most suitable for determining if metastasis occurs. qRT-PCR is highly sensitive and can detect mRNA changes that are not necessarily biologically relevant, and it only reflects transcript levels, which do not always correlate with protein abundance or functional activity [62]. Therefore, while molecular data provide valuable mechanistic insights, functional assays offer the more definitive evidence for actual metastatic potential in this evidence base [63].

Another major methodological limitation that undermines the conclusions of these studies is the lack of appropriate controls. Despite cigarette smoke being a well-established inducer of these immunological changes [27,32] and EMT-driven invasion [22,25,26], only one study included cigarette smoke extracts as a positive control, and not across all *in vitro* assays [40]. Most studies relied solely on negative (untreated) controls, which prevents direct comparison and validation of the experimental effects observed with e-cigarette exposure. Similarly, the *in vivo* studies did not include positive control groups (cigarette smoke-exposed mice). Consequently, the studies cannot conclusively demonstrate that e-cigarette exposure produces similar effects to cigarette smoke, as the absence of an appropriate positive control means the observed outcomes may not be specifically attributable to e-cigarette exposure. The omission of cigarette smoke comparators represents a major gap, as it prevents any assessment of relative metastatic risk between e-cigarettes and combustible tobacco—a comparison that is essential for informing public health policy and harm reduction messaging. Regulatory and clinical guidance currently relies on the assumption that e-cigarettes are less harmful than combustible tobacco; however, without empirical head-to-head data in preclinical metastasis models, this assumption cannot be substantiated or refuted. Future studies should systematically include matched cigarette smoke extract or whole smoke exposure arms using standardised protocols (e.g., ISO or Health Canada Intense smoking regimens) to enable direct comparisons across molecular endpoints including EMT markers, RAGE expression, and immune cell recruitment.

4.4. Heterogeneity and Generalisability

The generalisability of the current evidence is further constrained by profound heterogeneity and a lack of representativeness across geographical, biological, and methodological dimensions. First, the intellectual origin of the research is confined to North and South America (six studies from the US and one from Canada and Brazil) (Table 3). This geographical concentration creates a critical gap, as regions with high vaping prevalence, such as Europe (20.1 million vapers), Western Pacific (16.0 million vapers), South-East Asia (14.3 million vapers), Eastern Mediterranean (9.2 million vapers), and Africa (5.6 million vapers) [8], are not represented in the current evidence base. These regions differ in regulations and market preferences, which are likely to result in significantly different e-cigarette constituents [64]. As a result, the global relevance of the evidence is undermined.

Second, the studies assessed only a narrow range of e-cigarette brands, flavours, and cancer types, limiting their ability to capture the diversity of real-world products and pathological conditions (Table 3). This limited scope is particularly significant for flavourings, as variation in flavour profiles (e.g., candy, fruit, and menthol) and their specific flavouring agents used across brands contributes substantially to chemical diversity and may introduce carcinogenic compounds such as aldehydes (e.g., formaldehyde and acrolein) [65-67]. However, only three of the seven selected studies specified flavour or examined its potential relationship to metastatic outcomes [37,40,41].

This limited scope and inadequate characterisation led to inconsistent and inconclusive findings. Preclinical studies indicate that variables such as flavourings and nicotine can produce variable biological effects across cancer cell lines (Table 7 and 8). For instance, while five studies reported that e-cigarette exposure with nicotine demonstrated positive observations in cancer cell invasion, migration, and motility [36-39,42], two OSCC studies found that the influence of nicotine on cell invasion varied in a cell-type- and flavour-dependent manner [40,41]. This context dependency is further highlighted by the observations that identical e-liquids (Red Hot, 8Ohm1, Inc., and Green Apple, Daze Mfg.) containing the same nicotine concentration (6 mg) produced discrepancies in cell invasion and inflammatory marker expression in the same two OSCC cell lines (Ca9-22 and Cal-27) [40,41]. Variations in responses are likely due to cell-specific differences in growth patterns and behaviours [27,68] and varying exposure parameters (Table 3), underscoring that a narrow experimental focus is inadequate for drawing generalisable conclusions. An unexpected finding was the apparent reduction in cell invasion following nicotine addition under specific conditions (e.g., Red Hot-treated Cal-27 cells in Robin, Trudeau [41] and Ca9-22 cells in Tsai, Hirschi Budge [40]),

highlighting the need for mechanistic studies to better understand nicotine-flavour interactions.

Furthermore, the chemical complexity of e-cigarettes and insufficient reporting of their composition complicate the interpretation of the findings, as well as limit meaningful cross-study comparisons. E-liquids and aerosols contain a wide range of compounds, including carbonyls, heavy metals, nicotine derivatives, polycyclic aromatic hydrocarbons, and other potentially oncogenic chemicals generated by pyrolysis [69,70]. Consequently, the findings from this review suggest that the biological effects are unlikely to be attributable to nicotine alone but instead probably arise from complex interactions among multiple e-cigarette constituents. Although nicotine is well-documented to promote tumourigenic processes such as cell proliferation, EMT, and metastasis in human cancer cell lines [71,72], its specific role within the complex and variable mixture of e-cigarette exposure remains difficult to determine and may be modulated by other e-liquid constituents.

4.5. Translational Relevance and Remaining Gaps

The translational relevance of the current evidence is also hampered by its exclusive reliance on preclinical models, which inadequately reflect human pathophysiology. All seven selected studies were preclinical, with only two incorporating *in vivo* models (Table 3). Most relied on two-dimensional (2D) cell cultures, which do not represent the complex TME, including cell-cell interactions, extracellular matrix, and immune components vital for tumourigenesis and metastasis [73-78]. Consequently, findings from these conventional monolayer approaches may not accurately reflect the impact of vaping on cancer migration in a physiological context. While three-dimensional (3D) cell culture approaches, used in two studies (sphere formation assay [36] and spheroid cell culture technique [42]), better mimic the TME and provide a bridge toward *in vivo* conditions [74,75,79], they cannot replicate the complex interactions between organs and physiological systems in whole organisms, particularly the intricate, multicellular, and multistep interactions within the immune system and TME [80-82].

Similarly, although *in vivo* experiments offer more accurate and pathologically relevant results than *in vitro* systems [63], they also present significant limitations for translational research. The tail vein injection approach used by Huynh, Huang [38] primarily assessed lung colonisation, representing the later stage of cancer metastasis without addressing the initial spread from the breast. In addition, although Huynh, Huang [38] used a human breast cancer cell line, the use of a severely immunodeficient mouse model limited the study of immune cell involvement that are critical in human cancer progression. By

contrast, Pham, Huynh [38] employed murine cell lines to maintain the immunocompetent system and integrated orthotopic tumour models alongside immune cell analysis. However, the use of murine systems introduces its own translational constraints.

While mouse models share high homology with humans [83] and can mimic biological characteristics of human cancer cells' occurrence, development, and metastasis [84]. However, notable differences in transcriptional regulation in cancer between mice and humans exist, particularly in transcription factors such as MYC, p53, p63, and p73, which show limited conservation [85]. For example, only about 5% of total mouse p53 binding peaks are conserved in the corresponding human genomic regions, with just 86 of nearly a thousand direct target genes shared due to significant differences in their gene regulatory networks [85].

Furthermore, in the reviewed studies, the cell-derived xenograft model used by Huynh, Huang [38] may lose tumour heterogeneity over time due to continuous *in vitro* adaptation [76], while the syngeneic mouse model used by Pham, Huynh [39] fails to fully capture the complexity and heterogeneity of human tumours [76]. Thus, despite their value in initial screening and in offering mechanistic insights, these preclinical models leave a substantial gap between experimental findings and established human pathology.

Collectively, these shortcomings, including methodological constraints, narrow biological and representative scope, and reliance on preclinical models, suggest that the current evidence remains preliminary. Existing studies provide biological plausibility for potential cancer metastatic behaviours associated with e-cigarette exposure; however, these findings cannot yet be considered proven clinical risks. Critically, most studies lacked detailed cigarette smoke control, restricting the assessment of relative risk compared to traditional smoking, which is essential for guiding public health communication and policy decisions [86]. This gap is further widened by the real-world prevalence of dual use. The difficulty in isolating the effects of e-cigarettes from those of prior or concurrent combustible tobacco use complicates both the interpretation of existing human data and the design of preclinical studies that accurately reflect population-level exposure [87,88]. Although a recent prospective cohort study did report an association between e-cigarette use and higher odds of lung metastasis from non-lung primary cancers, human data remain scarce and far from conclusive [89]. Together, current preclinical and early epidemiological evidence raises a level of concern, but it remains insufficient to prove definitive clinical risk, highlighting the critical need for rigorous longitudinal studies to determine the true impact of vaping on cancer progression.

4.6. Future Directions

To bridge the significant translational gap identified in this review, a multifaceted research strategy is urgently needed. To begin with, the scope of investigation should be expanded to better reflect real-world exposure. Preclinical studies should systematically evaluate a broad spectrum of e-cigarette brands and flavours against diverse cancer types, coupled with rigorous chemical characterisation of e-cigarette constituents collected through different methods [58].

Moreover, advanced preclinical models should be employed to more accurately recapitulate human pathophysiology and assess the safety profile of vaping. This includes the use of 3D microfluidic models integrated with bioprinting and computational modelling to dynamically mimic the TME conditions [90]. In parallel, *in vivo* experiments should utilise more translational models, such as patient-derived xenografts, humanised mouse models [85], and other animal models with genome and molecular similarities to humans, including canines and tree shrews [83,84], to better capture human-relevant tumour biology.

Ultimately, it is critical to address the current lack of long-term human evidence. Although direct clinical research on vaping and cancer migration is challenging due to safety and ethical concerns [91], observational studies can be performed to track and longitudinally monitor vaping behaviours and cancer outcomes in the populations [92,93]. Data on cancer patients, including questionnaire surveys, patient-reported outcomes, and medical records [94], along with information on their vaping history or ongoing vaping lifestyle, can be collected from the public, such as hospitals, clinics, or registries, to analyse their cancer progression. Additionally, advanced artificial intelligence techniques, including machine learning and deep learning, can integrate patient and laboratory data to improve the efficacy of cancer metastasis diagnosis and prediction [95,96]. By identifying complex data patterns, these technologies may also help in understanding the impact of vaping on cancer metastasis by accurately recognising the carcinogens [97]. Notably, integrative multi-omics feature selection approaches, such as Support Vector Machine – Recursive Feature Elimination (SVM-RFE) coupled with deep learning classifiers, have shown strong performance in identifying informative biomarkers from combined genomic, epigenomic, and transcriptomic lung cancer datasets [98], and could similarly be applied to uncover multi-omic signatures underlying vaping-associated lung cancer metastasis.

5. Conclusion

This systematic review identified seven preclinical studies examining the effects of e-cigarette exposure on cancer progression and metastasis. Based on the SciRAP assessment, all seven studies were rated as "not reliable" (methodological quality scores: 47-70%), primarily due to the use of non-physiological exposure models (e-liquid rather than aerosol), the lack of cigarette smoke controls, and the narrow scope of e-cigarette products evaluated. Despite these limitations, consistent molecular signals emerge suggesting that e-cigarette exposure may promote cancer metastasis through three primary biological axes: EMT induction (E-cadherin loss, vimentin upregulation), RAGE-driven inflammatory pathways, and monocyte recruitment through the CCL5/CCR5 axis. However, contradictory flavour- and cell line-specific effects in OSCC models preclude generalisable conclusions. To advance this field, further studies should: 1) employ direct aerosol exposure systems to accurately replicate human inhalation; 2) transition to more physiologically relevant preclinical models that better recapitulate the human TME; 3) include combustible cigarette smoke controls to enable essential relative risk assessment; 4) standardise reporting of e-liquid chemical composition, device characteristics, and exposure parameters; 5) and incorporate longitudinal clinical studies with well-characterised vaping histories to establish true clinical risk. Until such rigorous evidence is available, current findings remain preliminary and hypothesis-generating, and claims regarding the safety or metastatic risk of e-cigarettes remain unsubstantiated.

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