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E-Cigarettes Promote Macrophage-Tumor Cells Crosstalk: Focus on Breast Carcinoma Progression and Lung Metastasis

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Abstract

Recurrence and metastasis are the foremost causes of morbidity and mortality for breast cancer (BC). Recent studies have highlighted the critical role of the tumor microenvironment, in particular, because it is related to tumor-associated macrophages (TAMs), in metastasis of BC. TAMs are mainly derived from macrophages that are recruited by C-C motif chemokine ligand 5, which are secreted by cancer cells and cancer-related stromal cells. Although E-cigarettes (E-cigs) were originally proposed as a healthy substitute for conventional cigarette smoking, clinical and experimental evidence has highlighted the potentially lethal effects of this alternative. Several studies have illustrated the immune or macrophage activation and DNA damaging effects of E-cigs. However, the potentially pivotal role of TAM-BC crosstalk during BC progression and metastasis for E-cig vaping has not been explored. This review discussed the significant effect that E-cig use had on the BC tumor microenvironment, which ultimately led to enhanced tumor malignancy and metastasis, with an emphasis on the extent that E-cig uses had on the crosstalk between cancer and immune cells, as well as the potential underlying mechanisms that drive this aggressive phenotype of BC. This review advances our understanding of this matter and provides scientific evidence that could highlight risks associated with vaping and suggest a potential intervention for the treatment of aggressive BCs that present an increased risk of metastasis.

Keywords

Breast cancer; E-cigarette; Infiltrated macrophage; Lung metastasis; Tumor crosstalk

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Author contributions

Kien Pham and He Wang gathered ideas and wrote the paper. Sam DeFina contributed to editing work. All authors have made a significant contribution to this study and have approved the final manuscript.

Conflict of interest

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

Introduction

Among women in the US, breast cancer (BC) is the most commonly diagnosed malignancy and is the second leading cause of cancer-related death, after lung cancer.[1,2] Currently, recurrence and metastasis are the primary causes of morbidity and mortality for BC. In 2016, the Surveillance, Epidemiology, and End Results Program estimated that there were 246,660 new BC cases, which accounted for 14% of all new cancer cases. In addition, although BC is typically limited to women, the Centers for Disease Control and Prevention reported 2,000 new cases of BC among men and 400 deaths in 2013.[3] A study that observed a cohort of 89,835 women, with an average follow-up of 22.1 years, reported that BC was associated with duration, intensity, and cumulative exposure to cigarette smoking (CS).[4] Despite the well-established epidemiological relationship, the underlying mechanisms that link smoking and BC remain incomplete.[5] Recent mechanism studies implicated that the tumor microenvironment, in particular, tumor-associated macrophages (TAMs) constitute approximately 50% of cells in BC bulk, had a pivotal role in tumor progression and metastasis of BC. TAMs are mainly derived from macrophages that are recruited by cytokines, which are secreted by BC cells and cancer-infiltrated macrophages. These cytokines include macrophage colony-stimulating factor (CSF1), C-C motif chemokine ligand 2 (CCL2), and C-C motif chemokine ligand 5 (CCL5).[6] TAM infiltration combined with increased levels of CCL2 have been linked to poor prognosis and are associated with BC metastasis.[7] TAM secretes cytokines that include CCL18, matrix metalloproteinases (MMPs), and vascular endothelial growth factor A (VEGF-A), which stimulate tumor migration, angiogenesis, and metastasis.[8] It has been suggested that CS promotes M2 polarization of macrophages;[9] however, currently, no literature directly addresses the effects of smoking on TAM or BC-TAM crosstalk. This review aims to discuss the significant effect that E-cig use has on the BC tumor microenvironment, which ultimately leads to enhanced tumor malignancy and metastasis, with an emphasis on the impact that E-cig use has on the crosstalk between cancer and immune cells, as well as the potential underlying mechanisms that drive this aggressive phenotype of BC. This will advance our understanding of this matter and will provide scientific evidence with this is required to highlight the risks associated with vaping and suggests a potential intervention for the treatment of aggressive BCs that present an increased risk of metastasis.

CS, E-cigs, and breast cancer

In the US, it has been estimated that 16% of all adults are classified as smokers,[10,11] and therefore, CS persists as the largest preventable cause of death.[2,12] In 2009, the Canadian Expert Panel on Tobacco Smoke and Breast Cancer Risk reported that the association between smoking and BC was consistent with causality.[13] A modest increase in BC prevalence was observed with every additional 20 pack-years, and the hazard ratio increased, which was dependent on the quantity, duration, and initial age of CS.[14] In one study Pierce *et al.* found that BC patients that have a history of smoking (>a 30 pack-year history) were at increased risk (+54%) of overall BC mortality compared with nonsmoking patients.[15] Despite the well-established epidemiological relationship and a significant amount of data, the basic mechanisms that link smoking and BC remain unknown.

Cigarette combustion from smoking has been reported to generate thousands of agents that result in increased motility and epithelial-mesenchymal transition of BC cells, following exposure.[16-19]

In mice, lung metastasis occurred following mammary fat pad injection with cigarette smoke extract (CSE)-treated MCF-7 BC cells; however, no metastasis was identified among mice that received untreated MCF-7 cells.[19] Nicotine, the primary addictive component of cigarettes, has been reported to disrupt cellular metabolic processes, compromise genomic integrity, and accelerate the proliferation of transformed cells.[20] Nicotine operates by activating nicotinic acetylcholine receptors, which stimulate several signaling pathways that exacerbate tumorigenic effects, such as MEK/ERK and Sox2 via Yap1-E2F1 axis in non-small cell lung cancers.[21,22] Clinical presentation of the resulting smoking-related DNA adducts in epithelial cells has been found within the breast milk of smokers.[23,24]

During the last decade, E-cigs were introduced and marketed as a healthy alternative to CS.[25] Since their introduction into the US in 2007, E-cigs have maintained widespread acceptance among smokers and nonsmokers, in particular, among young people.[26] Despite their potential benefit as a reduced-harm product, E-cigs have been reported to pose potential risks. Of note, E-cig exposure has been demonstrated to induce the emergence of mutagenic O^6-methyldeoxyguanosines and γ-hydroxy-1, N^2-propano-deoxyguanosines in lung and bladder-derived DNA in mice.[27,28] In addition, E-cig vapor condensate (EVC) promotes a significant increase in the production of tumor necrosis factor alpha (TNF-α), MMP 9, and IL-6 in cultured alveolar macrophages,[29] and additionally suppresses cellular antioxidant defense within cultured epithelial cells.[30] In addition, chronic vaping induces protease release from users pulmonary immune cells, which compounds its toxicity.[31,32] However, the effect of E-cigs on BC metastasis and its underlying mechanism(s) have not been studied. E-cig liquid is available in a variety of flavors and contains vehicle solvents, such as propylene glycol and vegetable glycerin, in addition to nicotine.[33] Of note, the aerosolization of E-cig liquid from vaping alters its chemical composition, which results in >250 different compounds in the inhaled E-liquid vapor.[34] Emerging evidence, which includes previous research conducted by our group, suggests that E-cig use results in immune cell activation and systemic inflammation. However, the impact of vaping on the development and metastasis of BC remains unknown. The existing literature on lung cancer suggests a substantial booster effect of E-cigs on phase-I carcinogen-bioactivating enzymes, which increase the production of reactive oxygen species (ROS), as well as DNA oxidation, which produces 8-hydroxy-2′-deoxyguanosine in rat models.[35] In addition, E-cigs have been reported to suppress cellular antioxidant defenses, which results in significant DNA damage in pulmonary epithelial cells.[12,36]

E-cig, immunity, and macrophage activation

Increasing evidence suggests that E-cigs alter innate and acquired immunity. E-cig vapor induces allergy-based asthma inflammatory responses[37] and increases susceptibility to viral infection in mouse lung cells.[38,39] In humans, airway epithelial cell exposure to E-cig aerosols promotes the secretion of inflammatory cytokines IL-6 and IL-8.[22] Exposure of alveolar macrophages (AMs) to a sublethal 0.5% EVC or nicotine-free ECVC (nfECVC)

increases ROS production approximately 50-fold and significantly inhibits phagocytosis. Secretion of IL-6, TNF-α, IL-8, CCL2, and MMP-9 is significantly upregulated in AMs following EVC challenge.[13] In cultured Kupffer cells it was observed that exposure to EVC or tobacco promoted the production of oxidative stress, a robust inflammatory response, and subsequent cytokine release.[40] In addition, it was observed in a cohort of 16 healthy human volunteers that increased endothelial progenitor cells, as well as E-selectin positive microvesicles in circulation, were induced by only 10 puffs of E-cig vapor.[11] However, it is important to consider that immune altering effects of E-cig use might be location and macrophage differentiation-dependent. Recently, a study showed that after continuous exposure of E-cig for 1 month, mouse lung AMs showed unaltered M2 markers and substantially decreased M1-associated markers that included IL-1β, TNFα, CD80, NOS2, CD86, and toll-like receptor (TLR)-7.[41] Functionally, in response to polyinosinic:polycytidylic acid stimulation, E-cig exposed AMs exhibited decreased expression of interferon (INF) response factor 7, a principal transcription factor in the regulation of type 1 IFN production in the presence of viral stimuli. In agreement with the activation of systematic inflammation and immune cells, a our unpublished study demonstrated that E-cig vapor exposure over 4 months amplified $CCR2^+$ and activated (IA^+) subsets in Ly6C high and Ly6C low macrophages, as well as $CCR5^+$ subsets in Ly6C high macrophages in the circulation of $ApoE^{-/-}$ mice. In addition, E-cig vapor exposure increased $TLR9^+$ monocytes in the circulation of normal chow-fed $ApoE^{-/-}$ mice. *In vitro* EVC treatment upregulates expression of IL-6 and CCL2 in cultured RAW264.7 murine macrophages, as well as in MM6 human macrophages (unpublished). Furthermore, in addition to our results, the current literature confirms the significant phenotypic alterations among circulating monocytes and resident macrophages that are caused by E-cig use.

TAM, BC metastasis, and BC-TAM crosstalk

Under physiological conditions, the microenvironment of any organ is predominantly tumor-suppressive; however, in the presence of chronic inflammation alterations to the microenvironment could encourage a tumor-promoting microenvironment (TME).[42] This type of inflammatory microenvironments are composed of modified extracellular matrices (ECMs), immune cells, and stromal cells, which include TAMs. Therefore, the pivotal functions of the TME in immunosuppression, tumor progression, and metastasis are apparent.[43] This is further evidenced by the strong association between TAM infiltration and poor prognosis and metastasis in BC.[44] In addition, in BC xenograft mouse models, a reduction in tumor growth rate and metastasis, combined with decreased TAM infiltration, was observed when they were treated with macrophage-targeting chemotherapy (trabectedin) or CSF1 inhibitors, which highlighted a potential therapeutic opportunity.[45,46] TAMs compose ≤50% of the total cell population in situ[47] and are primarily derived from cytokine-recruited macrophages that are secreted by TME stromal cells and cancer cells, and include CCL2, CCL5, and CSF1.[48] CSF1-mediated development of recruited macrophages result in nonpolarized (M0) macrophages,[49] which display significant plastic characteristics that allow for self-alteration of their phenotypes in response to various environmental stimuli. The developed TAMs could be categorized across a functional spectrum, where M1-like and M2-like macrophages constitute opposing extrema of the continuum.[50,51] M1-

like macrophage stimulation is induced by type 1 T helper cell cytokines and functions in an antitumor manner by secreting reactive nitrogen, oxygen intermediates, and pro-inflammatory cytokines.[52,53] The stimulation of M2-like macrophages is mediated by type 2 T helper cell cytokines and shows protumor characteristics. Under different conditions, M2-like macrophages further differentiate into three subtypes that function at every stage of the metastatic cascade, these subtypes consist of M2a, M2b, and M2c.[54] In general, the BC microenvironment is composed of TAMs mainly related to the M2-like phenotype.[49]

Crosstalk between TAMs and BC cells exhibits an auto-regulatory loop. TNF-α produced by both cell types increases TAM expression of CCL8 and SIGLEC1; and CCL8, then stimulates macrophage recruitment, BC invasion, and BC cell secretion of CSF1.[55] Further, TAMs regulate a number of metastatic processes, which includes blood vessel intravasation, local invasion, extravasation at distant sites, and metastatic cell growth.[46,56-58] This type of effect is seen in the CCL2-CCR2 signaling pathway between BC cell and TAMs that promote the initial recruitment of inflammatory macrophages to the premetastatic niche, where these macrophages evolve into metastasis-associated macrophages (MAMs). MAM-derived VEGF-A promotes tumor cell extravasation and seeding.[42] In addition, CCL2-CCR2 signaling activates CCL3-CCR1 signaling in MAMs, which support MAM accumulation at the metastatic site,[59] which enhances signaling pathway-mediated metastatic cell growth via the FMS-like tyrosine kinase 1-focal adhesion kinase (FAK1)-CSF1 and CSF1-C-ets-2-microRNAs pathways in macrophages.[60-64] In addition to their effects on cell growth, TAMs suppress the function and infiltration of antitumor $CD8^+$ T cells into the tumor microenvironment, which conferred treatment resistance in BC xenograft mouse models.[65-67]

VCAM-1 and cancer metastasis

In BC, the aberrantly expressed immunoglobulin (Ig)-like adhesion molecule, VCAM-1, contains seven extracellular Ig domains, which allows it to bind to its counter-receptor, the α4β1 integrin.[68,69] Further analyses of leukocyte subpopulations within metastatic lung lesions indicated that TAMs expressed the highest levels of α4 integrins. The majority of preliminary research on TAM-BC crosstalk has centered on various soluble factors that are secreted by TAMs and tumor cells.[5,55] However, recent studies have shown that the depletion and inhibition of the further recruitment of macrophages into the lungs significantly impede metastasis, although this is not mechanistically understood.[6,58] However, a recent investigation of VCAM-1 presented a potential mechanism,[54] where tumor cells that enter the lung parenchyma are immediately engulfed by macrophages, which is probably due to the induction of innate immune response. Then, due to the proximity that macrophages and tumor cells reside in, interactions between VCAM-1 and the α4 integrins are promoted, which recruit Ezrin, a cytoplasmic adaptor protein that links the actin cytoskeleton to the cytoplasmic tail of VCAM-1, and ultimately results in tyrosine phosphorylation of Ezrin.[70] On activation, Ezrin functions as an adaptor that binds PI3K and its downstream mediator, AKT, which triggers AKT-mediated cell survival signaling.[54] Then, α4 integrin-expressing TAMs induce favorable microenvironment conditions that are conducive to an increased presence of VCAM-1 that express BC cells, which are present in the lungs as well as loci of primary tumor infiltration.

CCL5/CCR1/CCR5 axis in tumor-TAM crosstalk and cancer progression

CCL5 facilitates lymphocyte and macrophage infiltration in a number of different cancers, including BC.[71-73] CCL5 binds to either one of its macrophage-bound receptors, CCR1 or CCR5; therefore, initiating AKT signaling to recruit and repolarize TAMs. In a transplantable model of BC the dual CCR1/CCR5 antagonist, Met-CCL5, impeded tumor growth and inhibits the migration of macrophages and lymphocytes into 410.4 tumors, which potentially implicated CCL5 in the facilitation of tumor-promoting macrophage or lymphocyte infiltration.[70,72] In addition, in murine PDX models of human malignant phyllodes tumors, the CCL5-CCR5 axis that was blocked by the FDA-proved CCR5 inhibitor, maraviroc, suppressed macrophage recruitment to the tumor, significantly retarding tumor growth.[74] Although CCL5-CCR5 expression in healthy breast epithelial duct cells, in general, is limited, its expression by breast tumor cells at primary tumor sites is substantially upregulated with higher levels of CCL5 expression, which is representative of a more aggressive disease course.[75] In animal studies, CCL5 functions as an essential component of the crosstalk between BC and tumor microenvironment cells by: (1) modifying the balance of various leukocytes, in situ, via enhanced recruitment of deleterious TAMs and the suppression of antitumor T cell activity; (2) amplifying metastatic processes; and (3) intensifying migratory and invasion-related properties of BCs.[70,76]

E-cigs, BC metastasis and BC-TAM crosstalk

Emerging experimental and epidemiological findings suggest that CS amplifies lung metastasis of BC.[16,77,78] These studies highlighted that CS was an exacerbating factor in uncontrolled cell proliferation and invasiveness of breast tumor cell lines, the pathogenesis of a subpopulation of $CD44^{+}/CD49f^{+}$ tumor stem cells, and the enhancement of metastasis from the primary injection site.[27] To highlight the critical role of crosstalk between BC and TAM, which could be enhanced following CS/E-cig treatment, our labs have recently shown that E-cig exposure substantially increased BC cell growth in mammary fat pad tumor and metastatic lung colonization. This was supported by immunohistochemical staining that was conducted following E-cig exposure that highlighted an increase in TAM infiltration activity and decreased BC apoptosis, but increased cell proliferation indices. Further, *in vitro* studies have shown that the upregulation of protein expression for CCL5 and VCAM-1 occurs, in addition to various other protumorigenic factors in BC cells, following exposure to E-cig vapor. Mechanistically, coculture systems have demonstrated in EVCs and macrophages stimulated by CCL5/CCR1/CCR5 that axis-mediated BC cell growth and migration were independent of one another. During metastasis, E-cig exposure facilitated BC cell survival via VCAM-1/integrin $\alpha_4\beta1$-mediated direct interaction with infiltrated macrophages. These findings, for the first time, illustrate the deleterious effects of E-cig use in BC growth and metastasis. In addition, this review highlights the essential role of TAMs, via CCL5 and VCAM-1 pathways, in E-cig induced acceleration of BC tumor development.[82]

Future direction

A recent survey into vaping usage found that 2.1 million middle school and high school students reported using E-cigarettes in 2017, this population more than doubled the

following year, and reached 4.9 million students in 2018.[79] The continuous increase in the popularity of vaping, in particular, among teenagers, raises concerns within the public health community. Preliminary evidence on the effects of E-cig exposure revealed it increased the potential for addiction, the development of the adolescent brain, and therefore, had a direct negative impact on overall health;[80,81] however, its contribution to cancer has rarely been reported. In a recent study that used animal models and cell-based systems, the extent that E-cig exposure affected BC progression and lung metastasis, which supports how the potential underlying mechanisms in the process were altered.[82] In this hypothesis, E-cig use drives the infiltration of monocytes into tumor areas, in the primary tumor and lung-colonized tumors, via CCR5 upregulation on the surface of TAMs and VCAM-1 on BC cells, respectively. The CCL5-CCR1/CCR5 axis maintains crosstalk between BC cells and TAMs and VCAM-1 upregulation increases the binding of TAMs and BC cells during infiltration, which enhances the survival rate of metastatic BC cells during lung colonization. In addition, TAMs trigger the secretion of CCL5, which is derived from BC cells and assists in the migration of BC cells to the lungs, and Met-CCL5 inhibitors effectively prevent the contribution of CCL5 to BC cell migration. Furthermore, other cytokines, such as CXCL5/10/16, MMPs, Osteopontin, Proliferin, VEGF, and TNFα are secreted from BC cells after E-cig treatment, which prompts tumor progression and metastasis (Fig. 1).

Conclusions

In conclusion, this hypothesis improves our understanding of the critical role of TAMs, via CCL5 and VCAM-1 pathways, in E-cig promoted BC tumor development. Because of the complexity of the tumor microenvironment and of the mechanism that drives BC malignancy, further questions need to be explored. The findings of this review raise more questions about the role of tumor-TAM crosstalk in cancer progression and metastasis, such as is the CCL5/CCR1/CCR5 axis the only pathway mediated by this crosstalk? What about other smoking-related cancers including lung cancer, head, and neck cancer? The answers to these questions could lead to new therapeutic opportunities to combat cancer progression and metastasis.

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Abbreviations:

BC	breast cancer
TAM	tumor-associated macrophages
CCL5	C-C motif chemokine ligand 5
CCR1/3	chemokine receptor 1/3
VCAM-1	vascular cell adhesion molecule 1
E-cig	E-cigarettes

CS	cigarette smoking
MMPs	matrix metalloproteinases
VEGF	vascular endothelial growth factor
TNFα	tumor necrosis factor α
EVC	E-cigarette vapor condesate

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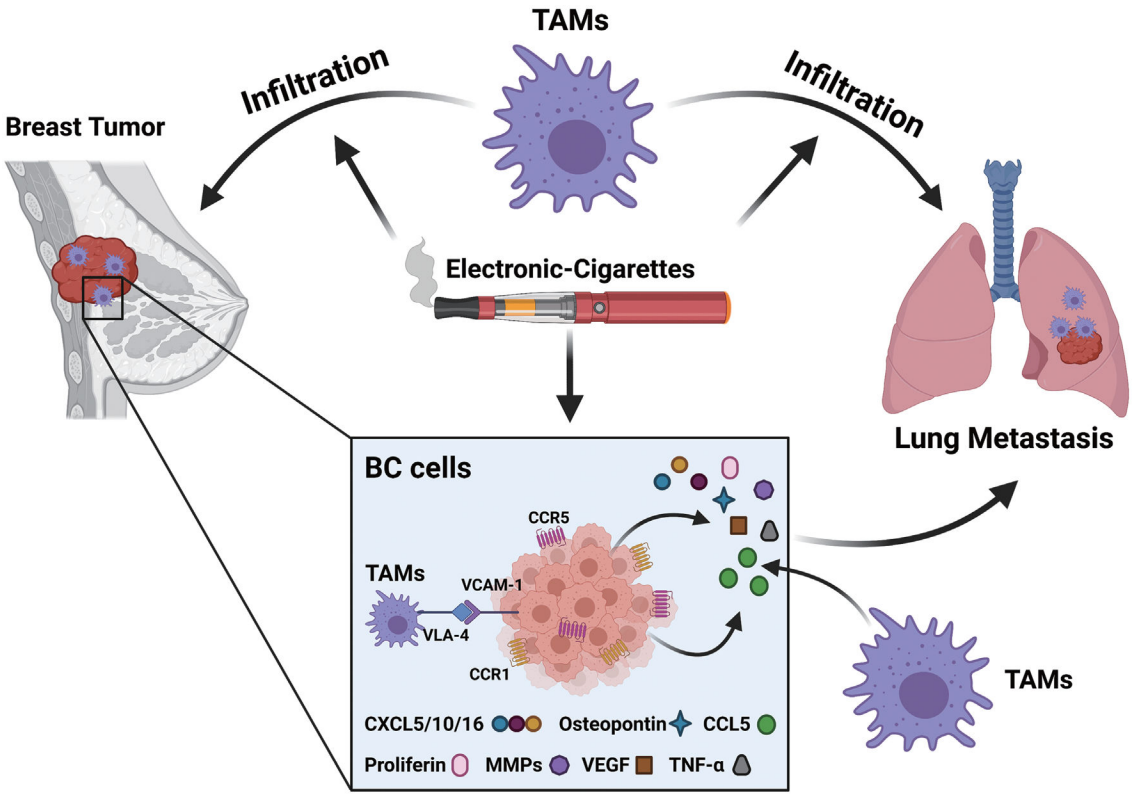

Fig. 1. The schematic mechanisms of E-cigarettes (E-cig) that promotes breast cancer (BC) growth and lung metastasis.

E-cig treatment drives the infiltration of monocytes into tumor areas in primary tumor and lung colonized tumor via CCR5 upregulation on tumor associated macrophage (TAMs) surface and VCAM-1 on BC cells. CCL5/CCR1/CCR5 axis maintains the crosstalk between BC cells and TAMs and VCAM-1 upregulation increases the binding of TAMs and BC cells during infiltration and enhances the survival rate of metastatic BC cells during lung. In addition, TAMs trigger the secretion of CCL5 derived from BC cells (dashed arrow), and assist the migration of BC cells to the lungs. Met-CCL5 inhibitor effectively prevents the contribution of CCL5 to BC cell migration. Furthermore, other cytokines, such as CXCL5/10/16, MMPs, Osteopontin, Proliferin, VEGF, and TNFα are secreted from BC cells after E-cig treatment, which prompts tumor progression and metastasis.