Review Article

The Effects of Cigarette Smoke on Cancer Cells

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Abstract

Smoking is one of the largest preventable risk factors for developing cancer and continued smoking by cancer patients is associated with increased toxicity, recurrence, and mortality. Cigarette smoke (CS) contains thousands of chemicals including many known carcinogens. Most research examines the carcinogenic properties of CS and relatively little work has been done on the effects of CS on cancer cells. Examination of the literature demonstrates that CS induces a more malignant tumor phenotype by increasing proliferation, migration, invasion, angiogenesis, and conferring a pro-survival tumor phenotype. Specific pathways associated with CS exposure are reviewed.

ABBREVIATIONS

CS: Cigarette Smoke; **MCS:** Mainstream Cigarette Smoke; **SCS:** Sidestream Cigarette Smoke; **ETS:** Environmental Tobacco Smoke; **FTC:** Federal Trade Commission; **ISO:** International Organization for Standardization; **PAH:** Polycyclic Aromatic Hydrocarbons; **TSNA:** Tobacco Specific Nitrosamines; **TPM:** Total Particulate Matter; **CSE:** Cigarette Smoke Extract; **WS:** Whole Smoke; **TGF-²:** Transforming- Growth Factor-² ; **ROS:** Reactive Oxygen Species; **SA² -gal:** Senescence-Associated² - Galactosidase; **AHR:** Aryl Hydrocarbon Receptor; **UGT:** UDP-glucuronosyl transferase; **MRP:** Multidrug Resistance-Associated Proteins; **P-gp:** P-glycoproteins; **BaP:** Benzo(a)pyrene; **XRE:** xenobiotic response elements; **ADM:** Adrenomeullin; **Dikk-1:** Dickkopf-1; **LRP-6:** LDL Receptor Protein-6; **Dvl-2:** Dishevelled-2; **EZH2:** Histone-lysine N-Methyltransferase; **SirT1:** Sirtuin1; **MiR:** MicroRNA; **PRC:** Polycomb Repressor Complexes; **COX-2:** Cycloxygenase-2; **CSE-C:** CSE Chloroform Fraction; **CSE-E:** CSE Ethanol Fraction; **DSS:** Dextran Sulfate Sodium; **5-LOX:** 5-Lipoxygenase- Activating Protein; **MMP:** Matrix Metalloproteinase; **LTB4:** Leukotriene B4; **PGE2:** Prostaglandin E2; **ChIP:** Chromatin Immunopercipitation; **EGFR:** Epidermal Growth Factor Receptor; **EGF:** Epidermal Growth Factor; **TGF-±:** Transforming Growth Factor-±; **HB-EGF:** Heparin-Binding EGF-like Growth Factor; **AREG:** Amphiregulin; **ADAM:** A Disintegrin and Metalloproteinase; **NSCLC:** Non-Small Cell Lung Cancer; **PKCµ:** µ- Isoform of Protein Kinase C; **TACE:** Tumor Necrosis Factor-Convertase; **TKI:** Tyrosine Kinase Inhibitor; **AK3:** Adenylate Kinase 3; **MSE:** Mainstream Smoke Extract; **SSE:** Sidestream Smoke Extract; **NOS-2:** Nitric Oxide Synthase-2; **IRF-6:** Interferon Regulatory Factor-6; **LC3B:** Microtubule-Associated Protein Light Chain 3; **MTA1:** Metastasis Tumor Antigen 1; **EMT:** Epithelial to Mesenchymal Transition; **SR-B1:** Scavenger Receptor B1; **lncRNA:** Long Non-coding RNA; **SCAL1:** Smoke and Cancer- Associated lncRNA-1; **NRF2:** Nuclear Factor (erythroid-derived 2)-like 2; **NF-° B:** Nuclear Transcription Factor-° B; **IKK:** I-° B Kinase

INTRODUCTION

Cigarette smoking and other forms of tobacco use are generally considered the largest preventable cause of cancer as well as heart disease, pulmonary disease, and many other diseases [1]. There are 7000 compounds in cigarette smoke (CS) and the primary addictive substance nicotine, leads most people to start smoking prior to age 18 [2].Overwhelming evidence demonstrates that smoking causes a wide spectrum of cancers and significant work has been done to understand fundamental biologic processes associated with tobacco induced carcinogenesis [3]. However, there is proportionately far less data on the clinical effects of smoking in cancer patients. The most recent edition of the Surgeon General's Report presents a convincing case associating continued smoking by cancer patients with increased cancer recurrence, treatment toxicity, risk of second primary cancer, and mortality across virtually all cancer disease sites [1]. The systemic effects of smoking on cancer treatment suggest that common biologic processes may be involved, but there is relatively little data on the effects of smoking on cancer biology. The purpose of this manuscript is to review the known effects of CS on cancer cells.

The composition of cigarette smoke

Cigarette smoke is a complex mixture of aerosolized chemicals and tobacco toxicants, which can be classified according to the location of origin and composition of the tobacco leaf. The burning of cigarettes generates three types of smoke: 1) mainstream

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cigarette smoke (MCS) produced from the cigarette butt and drawn directly into the smokers lungs (active smoking), 2) sidestream cigarette smoke (SCS) produced from the continued cigarette smoldering between puffs (passive smoking), and 3) environmental tobacco smoke (ETS) a combination of MCS and SCS generated within the vicinity of the smoker [4]. The way in which a cigarette is smoked effects the ratio of MCS and SCS generated. There are standardized smoke conditions as defined by the Federal Trade Commission (FTC) and International Organization for Standardization (ISO) of a 35ml puff volume with 2-second duration at a 1-minute puff frequency, but these conditions may not accurately represent current cigarette usage behavior [5].

The different components found in CS are distributed between the particulate and gaseous phases. For example, the aldehydes (formaldehyde, acrolein, and acetaldehyde) are found primarily within the gaseous phase and are associated with chronic pulmonary disease and lung toxicology, while the polycyclic aromatic hydrocarbons (PAH), tobacco specific nitrosamines (TSNA), and metals (arsenic, cadmium, chromium) are found in the particulate phase and are most frequently associated with cancer [6]. Additionally, the tar or particulate phase is rich in long lived radicals including semiquinone, which forms hydroxyl radicals and hydrogen peroxide when it reacts with the superoxide anion. Short lived oxidants such as the superoxide anion and nitric acid are more predominant in the gas phase, but they quickly react to form a highly reactive peroxynitrite [7]. It is believed that under biologic conditions a large amount of redox-cycline occurs within the aqueous portion of CS at the lung-lining fluid over a long period of time [7]. Studies have also observed that when combined, CS and ethanol work synergistically to induce colon tumors because ethanol solubilizes the liposoluble CS components [4]. Furthermore, studies have shown that heating condensates enhance their mutagenicity and they theorize that this occurs by favoring the oxidation of promutagenic moieties into Mutagenic components or by disruption the inhibitors of mutagenicity found within CS condensates [4].

The FTC uses a uniform machine-based test method for measuring tar and nicotine yield in cigarettes. In agreement with efforts in the 1960s to reduce exposure to tobacco toxicants, the FTC mandated that cigarette producers reduce the tar and nicotine content present in CS. This effort changed the composition of cigarettes and reduced the amount of tar and nicotine present in CS from 38 mg and 2.7 mg in 1954 to 12 mg tar and 0.95 mg nicotine respectively [3]. However such efforts have not actually been effective in reducing the exposure to cigarette toxicants because smokers have altered their smoking habits trying to compensate for reduced nicotine levels and achieve the same biologic high/effect. Such measures may include but are not limited to, covering the ventilation holes at the base of the cigarette filter to deliver more free-nicotine and inhaling deeper into the lungs. The net effect of changes in design and smoking habits have actually led to alterations in disease patterns associated with tobacco use. For example, low tar filtered cigarettes that were inhaled more deeply actually led to an increased risk of developing lung cancer with parallel changes in the location and histology of lung cancer from centrally located squamous cell cancers to more peripherally located adenocarcinomas [8].

There is no one standard method employed to generate CS and for the purpose of this review smoke collection methods will be defined as follows: 1) total particulate matter (TPM) – smoke is collected on Cambridge glass fiber filter and eluted into DMSO, 2) cigarette smoke extract (CSE) – smoke is bubbled into a solution (ethanol, chloroform, media, or PBS as indicated), and 3) whole smoke (WS) – smoke is directly exposed to cells or animals using a smoking chamber. Many of the studies utilizing CS try to examine the carcinogenesis of smoking related cancer. Frequently these studies expose immortalized "normal" tissues to various CS preparations to better understand the early mutations and phenotypic changes that contribute to cancer initiation. And an even smaller number of studies choose to examine the effect of whole cigarette smoke exposure on a biologic system rather than characterizing the effect of a single tobacco carcinogen. While such analyses are pivotal in tracking the carcinogenesis of smoking Related cancer formation, they provide limited information as to the effect of tobacco smoke on tumor biology. Very few studies specifically investigate the effect of CS exposure on altering the cellular functions of cancer cells. In this review, we will discuss the effect of CS on cancer progression emphasizing effects on cell proliferation, apoptosis, invasion and metastasis, angiogenesis, and immune modulation.

Cell proliferation

Deregulation normal cell proliferative pathways have been shown to be central to cancer initiation and progression; however, the effect of CS on cell proliferative pathways in cancer cells remains unclear and needs to be further evaluated (Figure 1). Hussain. evaluated the effect of 10-day TPM exposure on A549 and Calu-6 lung cancer cells and found that it increased the tumorigenicity of xenografts in nude mice [9]. More long-term (1 year) TPM exposure studies in A549 cells also demonstrated enhanced tumorigenicity *in vivo* [10]. Further analysis identified that TPM decreased the formation of the Smad3/Smad4 transcription complex induced by transforming-growth factor-β (TGF-β) [10]. This decrease in Smad3/Smad4 complex formation increased cell viability and is attributed to
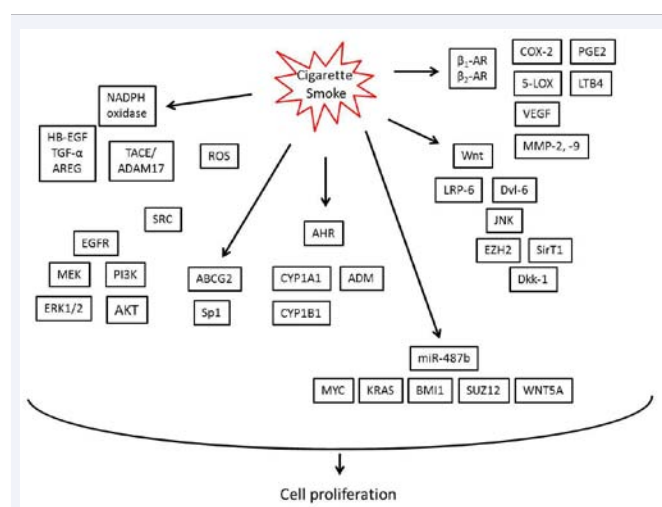

Figure 1 Network of proteins involved in mediating cancer cell invasion and metastasis that are modulated by cigarette smoke exposure.

CS-induced reduction of Smad3 expression [10]. Thus, chronic CS exposure was found to inhibit TGF-β signaling and confer a more malignant and tumorigenic phenotype [10]. Conversely, a study conducted by Tsuji *et al.* provided evidence that CSE bubbled in DMSO induced a senescence phenotype in A549 cells [11]. Specifically, cells acquired a flat and enlarged appearance and exhibited other features characteristic of senescence including increased senescence-associated β -galactosidase (SAβ -gal) activity, increased lipofusion, increased p21CIP1/WAF1/Sdi1, and irreversible Induction of growth arrest [11]. This study also showed that the CSE-induced senescence was mediated in part by reactive oxygen species (ROS) formation [11].

Aryl hydrocarbon receptor

The aryl hydrocarbon receptor (AHR) is a basic helix-loop-helix transcription factor that is involved in maintaining a wide range of homeostatic functions including but not limited to, cell proliferation, gene transcription, cell motility and migration, and inflammation [12]. AHR activation results in the transcription of three types of detoxifying genes: 1) phase I drug metabolizing cytochrome P450 enzymes including CYP1A1, CYP1A2, CYP1B1, CYP2S1, 2) phase II enzymes such as UDP-glucuronosyl transferase (UGT)-1AG6 and several glutathione-S-transferases, and 3) phase III transporters including multidrug resistance-associated proteins (MRPs) and P- glycoproteins (P-gp) [12]. Dysregulation of AHR has been shown to contribute to multiple aspects of cancer including initiation, promotion, and progression [12]. The AHR functions as the primary mediator of xenobiotic metabolism; chemical carcinogens such as those found in CS (including benzo(a)pyrene (BaP) and polycyclic aromatic hydrocarbons (PAH)) act as ligands and directly bind to the receptor [12]. Specifically, exposure of A549 cells to CSE induced activated AHR to bind specific DNA xenobiotic response elements (XREs) and drive expression of both CYP1A1 and adrenomeullin (ADM), a proto-oncogene that acts as growth factor [13]. ADM was also found to mediate CS-induced tumor growth in subcutaneous A549 tumors [13]. Studies performed by Uppstad *et al.* found that TPM exposure induced both CYP1A1 and CYP1B1 in 10 additional lung cancer cell lines [14]. Exposure of lung and esophageal cancer cells to TPM was also found to upregulate the expression of ABCG2, a xenobiotic pump shown to be upregulated by ARH signaling; inhibition of AHR was shown to partially abrogate TPM-induced ABCG2 increased expression [15]. This increase in ABCG2 expression results from activation of Specificity protein 1 (Sp1) sites within the ABCG2 promoter; treatment with mithramycin, a SP1 inhibitor reduced the expression of TPM-induced ABCG2 expression and inhibited cell growth *in vitro* and *in vivo* [15]. TPM-induced ABCG2 expression increased side population of Calu-6 and A549 cancer cells which suggested an increase in population of pluripotent tumor cells [15]. Furthermore, knockdown of TPM-induced ABCG2 expression decreased cell proliferation, clonogenicity, and migration suggesting that ABCG2 may contribute to a more malignant invasive phenotype [15].

Nonconical WNT signaling pathway

The wnt signaling pathway is involved in governing the maintenance, self- renewal and differentiation of mammalian adult tissues [16]. Wnt signaling is also believed to be involved

in maintaining cancer stem cells and different aspects of the pathway have recently been shown to be activated in response to CS exposure. Studies performed by Hussain *et al.* examined the effect of CS on epigenetic changes and found that 10-day TPM exposure of A549 and Calu-6 lung cancer cells increased the tumorigenicity of xenografts in nude mice [9]. Moreover, TPM exposure induced the downregulation of Dickkopf-1 (Dkk-1), a wnt signaling inhibitor that is frequently silenced by methylation in cancer [9]. This Dkk-1 repression was also found to modulate nonconical wnt signaling by increasing T-cell factor reporter activity, increasing cyclin D, and phosphorylating LDL receptor related protein 6 (LRP-6), dishevelled-2 (Dvl-2), and JNK [9]. Hussain *et al.* also observed that the TPM exposure induced the irreversible recruitment of the polycomb machinery to the Dkk-1 promoter and that knockdown of histone-lysine N-methyltransferase (EZH2) and Sirtuin1 (SirT1) abrogated this induced repression [9]. Expanding upon these findings, a study by Xi *et al*. evaluating the effect of CS on altering the microRNA (miR) transcriptome in lung cancer cells found that TPM-induced repression of miR-487b upregulated several target mRNAs involved wnt signaling [17]. Characterization of miR-487b found that it mediated cell signal arrest and senescence in lung cancer cells by directly targeting the following mRNA sequences: 1) BMI1, 2) SUZ12, 3) WNT5A, 4) MYC, and 5) KRAS [17]. Of these mRNA targets, BMI1 and SUZ12 encode for core components of the polycomb repressor complexes (PRC) -1 and -2, respectively, WNT5A is a nonconical Wnt ligand, and MYC and KRAS are involved in cell proliferation [17]. Collectively, these studies provide evidence that CS induced cell proliferation may be governed in part by epigenetic alterations in the Wnt signaling pathway however, the mechanism by which this occurs and its relevance to the maintenance of cancer stem cells needs to be further evaluated.

The COX-2/5-LOX pathway

Several studies have examined the link between cigarette smoking induced upregulation of the arachidonic acid cascade and cancer progression. Li et al examined the role of cycloxygenase-2 (COX-2) and the β1-adrenergic receptors in the pathogenesis of smoking-related esophageal squamous-cell carcinomas and found that exposure of EC109 cells to either chloroform or ethanol fraction of CS (CSE-C and CSE-E, respectively) stimulated cell proliferation [18]. CSE-C was also found to increase mRNA expression of β1- and β1-adrenergic receptors and COX-2 while CSE-E induced increase expression of β1- and β1-adrenergic receptors but did not alter COX-2 expression [18]. Furthermore, they found that β1- and β1-adrenergic receptor antagonists and COX-2 inhibitor eliminated CSE-C induced cell proliferation but not that induced by CSE-E [18]. Thus, these observations suggest that the proliferative action of chloroform-extract in EC109 squamous esophageal cells is mediated through a β1- and β1-adrenergic receptor and COX-2 dependent mechanism while the pathway stimulated by the ethanol- extract of CS needs to be further investigated.

Treatment with WS was found to promote the formation of inflammation- associated adenomas in the colons of mice treated dextran sulfate sodium (DSS) [19]. Specifically, the combination of DSS and WS exposure increased tumor incidence from 12.5%

to 87.5% and these tumors were characterized by increased vascularization and elevated expression of 5-lipoxygenase-activating protein (5-LOX), vascular endothelial growth factor (VEGF), and matrix metalloproteinase-2 (MMP-2) [19]. *In vitro* studies performed in SW1116 colon cancer cells found that exposure to CSE-E similarly induced increased cell proliferation; however, unlike in EC109 cells, CSE-E also dose-dependently increased COX-2 expression [20]. SW1116 cells incubated with ethanol- extract for 18 hours prior to subcutaneous implantation in balb/c nude mice increased tumor growth in comparison with controls and this CSE-E induced effect was decreased by COX-2 inhibition [20]. The combination of ethanol and chloroform CSEs also stimulated the proliferation of SW1116 cells with increased expression of 5-LOX, a cell proliferation promoter and its downstream product leukotriene B4 (LTB4), but with no effect on COX-2 and prostaglandin E2 (PGE2) levels [21,22]. Chromatin immunoprecipitation (ChIP) analysis revealed that the increase in 5-LOX expression and subsequent cell proliferation resulted from a loss of methylation in the CpG dense region at nucleotides 13-121 of the 5-LOX promoter [21]. Additionally, CSE increased the protein levels of the angiogenic signaling molecules VEGF, MMP-2, MMP-9 [22]. Pre- exposure of cells to CSE also induced a 3-fold increase in tumor xenografts and tumors exhibited increased cell proliferation, decreased apoptosis and increased levels of 5-LOX and LTB4, COX-2 and PGE2 [21]. Inhibition of either 5-LOX or COX-2 reduced tumor size and decreased LTB4 and PGE2 levels respectively [21]. Moreover, inhibition of 5- LOX partially blocked CSE induced proliferation and reduced the induced VEGF, MMP- 2, and MMP-9 expression while inhibition of MMP-2 and MMP-9 reduced VEGF but did not alter 5-LOX expression *in vitro* [22]. Collectively, these studies suggest that COX-2 and 5-LOX play a role in mediating CS-induced cell proliferation and tumor growth.

EGFR pathway

The epidermal growth factor receptor (EGFR) is a key component in mediating cell proliferation, survival, and differentiation during development [23]. The deregulation of EGFR is highly studied within the context of cancer biology and there is evidence that EGFR signaling may be modulated by CS exposure. Preliminary examinations found that whole smoke exposure of H292 mucoiepidermoid pulmonary carcinoma cells promoted cell proliferation and that pretreatment with AG1478, an EGFR kinase inhibitor, reduced this effect [24]. Several studies also reported observing an increase in EGFR phosphorylation following CS exposure. Thus suggesting the involvement of the EGFR pathway in mediating CS stimulated cell proliferation in lung cancer cells. There are two schools of thought in how EGFR activation occurs and here we will examine both ligand-dependent and -independent EGFR activation.

Ligand-dependent EGFR activation

Canonical EGFR activation involves the binding of several identified EGFR ligands including: epidermal growth factor (EGF), transforming growth factor-± (TGF-±), heparin-binding EGF-like growth factor (HB-EGF), amphiregulin (AREG), beta- cellulin, epiregulin, and epigen [23]. These ligands remain anchored along the outer surface of the plasma membrane in their "pro-" or inactive form until the cell membrane- anchored metalloprotease

A Disintegrin and Metalloproteinase (ADAM) proteins activate the ligands. This process is known as ligand "shedding" and occurs in response to specific physiologic signals which essentially solubilize the EGFR ligands. These mature ligands can then bind to the EGFR receptor, induce phosphorylation and dimerization of the EGFR subunits, and results in subsequent activation of the Ras/MAPK, PI3K/AKT, and STAT signaling cascades.

Exposure to CS has been shown to induce EGFR activation in non-small cell lung cancer (NSCLC) cell lines, despite there being no known exogenous EGFR ligand present in cigarette smoke [24,25]. The earliest event in the subsequent CS-induced signaling cascade is the generation of *de novo* ROS by membrane bound NADPH oxidase [24]. Generation of ROS then induces phosphorylation of SRC kinase and SRC in turn activates the μ-isoform of protein kinase C (PKCμ) [26]. Next, PKCμ directly interacts with the tumor necrosis factor-convertase (TACE)/ ADAM17 metalloproteinase and phosphorylates it at serine/ threonine residues [26] and TACE/ADAM17 facilitates the cleavage and release of EGFR ligands [24,25]. Studies disagree on which EGFR ligand is shed from the cell membrane, but reports suggest that CS induces HB-EGF [27,28], AREG [24,27,29], and TGF-± [25,27,29] to activate EGFR. Although these studies all used the same H292 lung carcinoma model system, they did not use the same cigarette types or CS exposure preparations (See Table 1). Regardless of which CS- induced ligand binds to EGFR, activation of the receptor triggers the Ras/Raf/MEK/ERK and PI3K/AKT signaling cascades and results in the transcription of several downstream target genes including: MMP-1 [28], IL-8 [27-29] and MUC5AC [25,28,29].

Ligand-independent EGFR activation

There is also evidence that CS oxidative stress stimulates ligand-independent EGFR activation. Studies in A549 lung cancer cells show that oxidative stress from whole CS induced EGFR autophosphorylation at Y1068, Y1173, and Y845, but this activation did not generate receptor subunit dimerization and pretreatment with an EGFR inhibitor did not alter CSE induced EGFR phosphorylation at these sites [30]. The Y845 residue is a specific SRC-dependent phosphorylation site and analysis showed that SRC is recruited to EGFR and phosphorylated at Y416 [30]. SRC is generally not involved in traditional EGF/ EGFR signal transduction thus indicating that the conformation of EGFR induced by CS may be distinct from the one induced by EGF binding [30]. Additional analyses determined that the innate kinase activity was neither necessary nor essential for EGFR to interact with SRC [31]. Despite the failure of EGFR to properly dimerize because it is in the wrong conformation, ERK1/2 and AKT signaling cascades were activated and pretreatment with an EGFR inhibitor did not prevent the observed cascade activation [30,32]. Furthermore exposure of tyrosine kinase inhibitor (TKI) sensitive cells to CS induced treatment resistance and inhibition of SRC restored sensitivity to TKIs [30].

Modulating cell death

Evasion of cell death is an essential hallmark of cancer; however, there are a limited number of studies that evaluate the impact of CS exposure on the ability of cancer cells to evade death related pathways. Chronic TPM exposure in A549 cells showed

Table 1: Referenced methodologies of cigarettes smoke preparation showing EGFR ligand shedding.

Study	CS Prep	Cig Type	Tar (mg/cig)	Nicotine (mg/cig)	EGFR Ligand
Richter et al. [27]	Aqueous CSE	1R4F*	9.2	0.8	TGF-±, AREG, HB-EGF
Lemjabbar et al. [24]	WS	1R4F	9.2	0.8	AREG
Shao et al. [25]	Aqueous CSE	2R1	32.9	2.19	TGF-±
Baginski et al. [29]	Aqueous CSE	1R1	32.9	1.98	TGF-±
Newland et al. [28]	TPM	2R4F	9.2	0.8	HB-EGF

Abbreviations: **CS Prep:** Cigarette Smoke Preparation; **Cig:** Cigarette; **EGFR:** Epidermal Growth Factor Receptor; **CSE:** Cigarette Smoke Extract; **WS:** Whole Smoke; **TPM:** Total Particulate Matter; **TGF-±:** Transforming Growth Factor-±; **AREG:** Amphiregulin; **HB-EGF:** Heparin-binding EG

that the observed decrease in TGF-² induced Smad3/Smad4 complex formation also correlated with a reduction of apoptosis, which resulted from Bcl-2 upregulation [10]. Chronic exposure of SCaBER, a bladder cancer cell line, to CSC vapor altered cell tumor biology in several ways; *in vitro* CSC vapor induces reduced mitochondrial-resistant protein adenylate kinase 3 (AK3) levels, decreased mitochondria membrane potential (”Èm), increased intracellular ROS, and cisplatin resistance [33]. Additionally, 6-month CSC vapor exposure of SCaBERs was found to increase tumorigenicity, induce cisplatin resistance, and elevate Bcl-xL and Bcl-2 protein expression in subcutaneous xenografts in comparison with CSC vapor naïve control tumors [33].

Ratovitski examined the effect of different smoke types on altering cell biology of HNSCC, a head and neck squamous cell carcinoma cell line [34]. This study found that both mainstream smoke extract (MSE) and sidestream smoke extract (SSE) induced the expression of” Np63± and nitric oxide synthase (NOS)-2 through the regulation of interferon regulatory factor (IRF)-6 in the NOS-2 promoter region [34]. Furthermore, MSE induced the cleavage of the autophagic marker microtubule-associated protein light chain 3 (LC3B); knockdown of” Np63±, IRF6, and NOS-2 using small interfering (si)- RNAs was also found to modulate the CS-induced autophagic response [34]. Thus, these observations provide evidence that the regulation of NOS-2 expression by” Np63±/IRF6 interplay governs the induction of autophagy in response to CS exposure in HNSCC cancer cells [34]. Collectively, these data suggest that CS modulates cell death pathways leading to a pro-survival phenotype.

Invasion and metastasis

Several studies have evaluated the effect of CS on mediating invasion and metastasis in cancer cells (Figure 2). One such study found that long-term aqueous CSE exposure conferred a more mesenchymal phenotype to MCF7 cells; breast cancer cells took on a spindly fibroblast-like appearance and exhibited enhanced anchorage independent cell growth and increased migration [35]. Chronic CSE exposed cells implanted into the mammary fat pads of immunodeficient NSG mice formed highly invasive and highly metastatic MCF7 tumors [35]. Aqueous CSE was also found to modulate the expression of metastasis tumor antigen 1 (MTA1), a subunit of the NuRD nuclear remodeling complex thought to be involved in mediating the epithelial to mesenchymal transition (EMT) [36]. Increased MTA1 mRNA and protein expression enhanced the invasiveness of NSCLC cell lines [36].

Exposure of A549 cells to CS impaired the function of Na,K-ATPase, an ATP- dependent pump used to maintain the sodium gradient across membranes [37]. Specifically, CS induced ROS production which reduced Na,K-ATPase pump activity and reduces NaK-±1 levels at the cell surface [37]. The effect of CS-induced decrease in Na,K-ATPase activity on cancer progression needs to be further investigated; however, it is postulated that loss of Na,K-ATPase activity disrupts tight junctions, alters cell polarity and may be involved in early EMT events [37]. Further examination of the effect of CS on the expression of claudin, a central protein component of tight junctions, in lung carcinoma cell lines, demonstrated an early CSE induced increase in the expression of several claudins followed by a subsequent decrease in mRNA levels [38]. These CS- induced changes in claudin expression may be important in lung cancer biology as tight junction dysfunction and claudin alterations can cause decreased cell adhesion, loss of differentiation, uncontrolled cell proliferation, loss of cohesion and invasiveness, all of which contribute to cancer progression [38].

Aqueous CSE was found to induce time and dose-dependent expression of MUC4 mucin in well-differentiated pancreatic cancer cell lines [39]. *In vivo* studies showed that WS exposure of orthotopic pancreatic cancer xenografts increased pancreatic tumor weight and the occurrence of metastasis; furthermore, smoke exposed tumors showed upregulation of MUC4, ±7nAChR, and increased phosphorylation of STAT3 (Y705) [39]. These observations provide evidence that CS increases MUC4 mucin production in pancreatic cancer through the activation of the ±7nAChR/JAK2/STAT3 signaling cascade thereby promoting metastasis [39]. As a whole, data suggest that CS increases migration and invasion leading to increased metastasis.

IMMUNE MODULATION

Evidence demonstrates that CS can induce changes in cancer cell immune responses. Analysis of CS composition has shown that each puff contains 1014-1016 oxidants and that these oxidants can cause prolonged redox-cycling at the lung lining [7]. Vitamin E (±-tocopherol) is an antioxidant found in the alveolar lining fluid of the lung and is responsible for protecting the tissue from oxidant related damage [40]. Studies have shown that exposure to CS interferes with A549 lung cancer cells ability to control tocopherol levels [40]. Specifically, CS induced the degradation and redistribution of Scavenger receptor B1 (SR-B1), the primary receptor involved in regulating tocopherol uptake and decreasing oxidant induced damage [40]. These observations suggest a mechanism by which CS oxidants intrinsically enhance the carcinogenicity of CS by inhibiting the uptake of vitamin E into lung tumors. Oxidative stresses found in CS were also found to induce the expression of a novel long non-coding

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RNA, (lncRNA) termed smoke and cancer-associated lncRNA-1 (SCAL1), in multiple lung cancer cell lines [41]. Additionally, SCAL1 expression is regulated by nuclear factor (erythroid-derived 2)-like 2 (NRF2), a transcription factor activated by chemical and oxidative stresses and known to alter expression of several protective antioxidant genes [41]. Knockdown of SCAL1 with CSE exposure was found to increase oxidative toxicity suggesting that SCAL1 may play a role in mediating the cytoprotective function of NRF2 in response to oxidative stress in the lung [41]. Data also implicates nuclear transcription factor-° B (NF-° B) signaling as a modulator of immune response by CS in cancer models. NF-°B is a ubiquitous transcription factor that exists in the cytoplasm as a heterotrimer (p50, p65 (RelA), c-Rel, p52, and RelB subunits) and is kept inactive as a dimer in the cytoplasm by the I° B inhibitory protein (I° B isoforms: I° B±, I° B^2 , I° B^3 , I° B´, I° Bμ, and Bcl-3). The NF-° B pathway is triggered when an external stimulus interacts with specific receptors and activates the I-° B kinase (IKK) complex made up of two catalytic subunits, IKK± and IKK^2, and one regulatory subunit, IKK^3. Activated IKK phosphorylates I° B and thereby facilitates I° B degradation which in turn enables NF-° B to translocate into the nucleus and induce target gene expression. NF-° B is a ubiquitous transcription factor involved in the regulation of inflammation and is activated by many different stimuli such as the cytokines IL-1 and TNF or extracellular stressors such as H2O2 and CS [42]. Exposure with TPM induced NF-° B activation in multiple cancer cell types including U937 (human histiocytic lymphoma), HeLa (human epithelial adenocarcinoma), Jurkat (human T cell), H1299 (human NSCLC carcinoma), 14B and 1483 (human head and neck squamous cell carcinoma) [43]. Activation of NF-° B by TPM exposure was dependent on IKK± mediated degradation of I-° B± [43]. Maity. identified an alternate NF-° B signaling axis; they determined that in resting A549 cells NF-° B in the form of c-Rel/p50 heterodimers is held in complex with I-° Bμ and thus inactivated [44]. However, the administration of aqueous CSE enables IKK^2 mediated phosphorylation and degradation of I-° Bμ which allows the c-Rel/p50 heterodimer to translocate to the nucleus and transcribe NF-° B target genes [44]. Discrepancies between the IKK±- I-° B±- p50/p65 and IKK^2 - I-kBμ-c-Rel/p50 NF-° B signaling cascades as reported by Anto *et al.* and Maity. respectively, may be due to differences in cigarette smoke exposure preparation; Anto *et al.* utilized the particulate phase of CS which collected on a Cambridge filter and dissolved into DMSO while Maity *et al.* examined the role of aqueous CSE in mediating NF-° B signaling [43,44]. Together, these data suggest that CS can induce the modulation of several different pathways involved in modulating tumor associated immune responses.

DISCUSSION AND CONCLUSION

Data suggest that the exposure of cancer cells to CS increases proliferation, migration, invasion, metastasis, angiogenesis, and leads to activation of immunomodulatory pathways. Data also suggest that CS modulates cell death leading to a pro-survival phenotype. The effects of CS appear to ubiquitously affect cancer independent of cell type. However, interpretation is limited in part by studies using a variety of CS preparations. Given the strong clinical correlates showing that continued smoking by cancer patients is associated with increased mortality, toxicity,

and recurrence, it is critical to further delineate the specific effects of CS on cancer biology.

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